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# Plant parameters for the marker and trait assisted selection of drought stress tolerance in barley *(Hordeum vulgare* ssp. *vulgare*)

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„Die Kunst besteht darin, einmal mehr aufzustehen, als man umgeworfen wird.“

Winston Churchill

## Abstract

The scarcity of water resources is becoming one of the most devastating stress factors for plants, which elicits a variety of responses. Therefore, effective screening techniques are necessary to understand the mechanism underlying drought stress tolerance. The main objectives of this thesis were (1) to examine effects of drought stress applied at different growth stages on crop growth and grain yield of spring barley (*Hordeum vulgare* ssp. *vulgare*), (2) to indentify major traits which are suited as selection criteria for phenotyping drought stress tolerance and (3) to evaluate spring barley cultivars for their drought tolerance and yield stability in response to different phenotyping environments (pot and field experiments). In 2012 and 2013, spring barley was grown under well-watered and terminal drought conditions in pot experiments, which were arranged in a split-plot design with four replications. The effects of temporary water shortage at different growth stages were studied among four spring barley cultivars. Drought treatments (DT) started at the end of the leaf development stage (DT1), tillering stage (DT2) and anthesis (DT3). Compared to well-watered plants, decreasing water availability at DT1 impaired the plant productivity by reducing the leaf number, leaf dry matter and plant nutrient concentrations. Water deficit imposed at DT2 and DT3 caused significant decreases in tiller formation and plant water content, while yellow leaf area and leaf senescence increased. The largest yield reductions (55%) were observed when drought occurred at anthesis (DT3). In addition, pot experiments were carried out to evaluate the suitability of nine morphological, five physiological and five yield-related traits as selection criteria for drought stress tolerance among 24 spring barley cultivars. Correlation analysis between traits, under stress and non-stress conditions, revealed that tiller number, leaf number, leaf and stem dry matter, leaf senescence and stem water content are vital parameters for phenotyping drought stress tolerance. None of these secondary traits were significantly correlated with the grain yield under water stress. Crop performance of the 24 spring barley cultivars was further studied in field trials of eight location-year combinations across Germany. Field experiments were laid out as complete randomized block designs with four to six replications, depending on the location. The soil moisture content and the soil temperature were the major weather parameters which determined the yield formation. Hence, mean grain yields varied between 41.6 and 83.5 dt $ha^{-1}$. Correlation analysis indicated that plant dry matters at anthesis as well as crop growth rates between anthesis and ripening stage are vital selection criteria for evaluating breeding material across contrasting environments. In accordance with examined pot experiments, none of the secondary traits were correlated with the grain yield under water deficit conditions in the field. A comparison of the genotypic performance of the 24 spring barley cultivars evaluated in pot and field experiments revealed that the drought stress tolerance varied depending on the phenotyping environment. The study provides new information about phenotyping drought stress tolerance in a wide range of phenotyping environments and during different developmental stages. Findings of this research have shown that parameters which are related to biomass accumulation and plant water status affect the plant growth and grain yield. Hence, phenotyping cereals for these parameters might result in the development of improved cultivars for drought-prone environments.

## Zusammenfassung

Die zunehmende Verknappung von Wasserressourcen gehört zu den verheerendsten, abiotischen Stressfaktoren, denen Pflanzen ausgesetzt sind. Da dieser Stressfaktor eine Vielzahl an pflanzlichen Reaktionen hervorruft, gilt es zukünftig effektive Phänotypisierungsmethoden zu entwickeln, so dass Aussagen zu Trockenstress-Toleranzmechanismen möglich werden. Ziele der vorliegenden Arbeit waren (1) die Beschreibung der Auswirkungen von Trockenstress zu unterschiedlichen Entwicklungsstadien auf das Wachstum und die Ertragsbildung von Sommergerste (*Hordeum vulgare* ssp. *vulgare*), (2) die Erfassung von Pflanzenparametern welche als Selektionskriterium zur Phänotypisierung von Trockenstresstoleranz geeignet sind und (3) die vergleichende Bewertung der Trockenstresstoleranz und Ertragsstabilität von Sommergerste in diversen Phänotypisierungsversuchen (Gefäß- und Feldversuche). In den Versuchsjahren 2012 und 2013 wurden unter zwei Bewässerungsstufen – Kontroll- und Trockenbehandlung – Gefäßversuche durchgeführt. In speziell angelegten Versuchen mit vier Sorten wurde geprüft welche Auswirkung Trockenstress zu unterschiedlichen Entwicklungsstadien auf die pflanzliche Produktivität hat. Die Applikation des Trockenstresses erfolgte am Ende der Blattentwicklung (DT1), zur Bestockung (DT2) und zum „Grannenspitzen"/Blüte (DT3). Im Vergleich zur Kontrollbehandlung bewirkte die Abnahme der Wasserverfügbarkeit am Ende der Blattentwicklung (DT1) eine Reduktion der Blattzahl, der Trockenmasse im Blatt und der Konzentration an Pflanzeninhaltsstoffen. Trockenstress zur Bestockung (DT2) und Blüte (DT3) führte zu einer signifikanten Reduktion der Triebzahl und des Wassergehalts in der Pflanze. Gleichzeitig nahm unter Trockenheit die gelbe Blattfläche und Blattseneszenz zu. Trockenheit zur Blüte (DT3) verursachte die größten Kornertragseinbußen (55%). Um Selektionskriterien für die Phänotypisierung von Trockenstresstoleranz detektieren zu können wurden neun morphologische, fünf physiologische und fünf ertragsrelevante Merkmale evaluiert. Signifikante Korrelationen unter Kontroll- und Trockenbehandlung belegen, dass die Triebzahl, die Blattzahl, die Blatt- und Stängelmasse, die Blattseneszenz und der Wassergehalt im Stängel für die Phänotypisierung von Trockenstresstoleranz geeignet sind. Unter Trockenbehandlung korrelierte jedoch keines dieser Merkmale mit dem Kornertrag. In einem Sommergersten-Set von 24 Sorten wurden, in zweijährigen Feldversuchen auf acht Standorten in Deutschland, Pflanzenparameter hinsichtlich ihrer Trockenstress-detektionsfähigkeit bewertet. Als Versuchsanlage diente eine randomisierte Blockanlage mit 4 bzw. 6 Wiederholungen, entsprechend des Versuchsstandorts. In Abhängigkeit von der Bodenfeuchte und der Bodentemperatur wurden Kornertragsschwankungen zwischen 41.6 und 83.5 dt $ha^{-1}$ festgestellt. Sowohl die oberirdische Biomasse zum Zeitpunkt der Blüte als auch die Wachstumsrate zwischen der Blüte und der Fruchtentwicklung waren signifikant mit dem Kornertrag korreliert. Sie werden daher als ein vielversprechendes Selektionskriterium unter variierenden Umweltbedingungen angesehen. Identisch zu den durchgeführten Gefäßversuchen zeigten die hier vorgestellten Parameter keine signifikante Korrelation mit dem Kornertrag unter Trockenheit. Ein Vergleich des Sortensets hinsichtlich der Trockenstresstoleranz und Ertragsstabilität in Gefäß- und Feldversuchen verdeutlichte, dass Trockentoleranz entsprechend der Versuchsbedingungen variiert. Die Ergebnisse dieser Dissertation geben einen fundierten Einblick in die Phänotypisierung von Trockenstresstoleranz. Die Arbeit zeigte, dass Parameter welche in Beziehung zur Biomasseakkumulation und Regulation des pflanzlichen Wasserhaushalts stehen einen signifikanten Einfluss auf das Wachstum und die Ertragsbildung haben. Dementsprechend stellen diese Merkmale vielversprechende Selektionskriterien für die Phänotypisierung von Trockenstresstoleranz dar.

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## List of abbreviations

| | |
|---|---|
| $\partial$ | Carbon isotope composition |
| $\Delta^{13}C$ | Carbon isotope discrimination |
| ‰ | Per mill (thousand) |
| Vol.% | Percent by volume |
| AAS | Atom absorption spectroscopy |
| ABA | Abscisic acid |
| ATP | Adenosine triphosphate |
| BBCH | Plant growth stage |
| C | Carbon |
| DF | Degree of freedom |
| dt | Decitonne |
| DT | Drought treatment |
| EEA | European environment agency |
| ha | Hectare |
| HI | Harvest index |
| HSD | Honest significant difference |
| HSI | Hue, Saturation, Intensity |
| K | Potassium |
| KCL | Potassium chloride |
| kg | Kilogram |
| kW | Kilo watt |
| m² | Square metre |
| MFVD | Membership function of drought tolerance |
| mg | Milligram |
| ml | Millilitre |
| mM | Millimolar |
| N | Nitrogen |
| NA | Not available |
| No | Number |
| P | Phosphor |
| REML | Restricted maximum likelihood |
| SII | Stress intensity index |
| ssp. | Subspecies |
| STI | Stress tolerance index |
| VWC | Volumetric water content |
| WU | Water use |
| WUE | Water user efficiency |

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# Chapter 1: General introduction

## 1.1 Introduction

Global warming is one of the major issues in the present century and affects various climate variables such as precipitation, solar radiation and air temperature. As a consequence of climate change, modifications in climatic parameters will affect evapotranspiration so that in future drought incidence increases (Goyal 2004; Long and Ort 2010). Several scientists projected that the land area which is affected by drought events increases and that decreases in precipitation will exceeding 25 to 30%, especially in Mediterranean region (Giorgi and Lionello 2008; Long and Ort 2010).

Globally, drought can be defined as a meteorological event which limits the crop productivity by the absence of rainfall over a period of time, long enough to decrease the soil moisture content and to cause a decline of water potential in plant tissues (Mitra 2001; Araus et al. 2002). Nezhadahmadi et al. (2013) pointed out that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the human population will live under water deficit conditions. Despite the forecasted increase in occurrence and severity of drought episodes, the decline of available arable lands and the multiple uses of crops for biofuels and food production emphasise the importance of intensive research to improve the yield stability of crops under drought conditions (Araus et al. 2002; Araus et al. 2008; Tomlinson 2011; Tardieu 2012).

In the recent years plant breeding has contributed to a large extent in tackling the challenge of breeding high-yielding crops which are adapted to future climate conditions. Thereby, the understanding of drought stress tolerance is a complex trait and progress towards this breeding aim is still very slow. In view of above considerations knowledge about plant responses to drought stress during the crop life circle as well as the investigation of secondary traits which are related to a higher yield potential and/or yield stability can contribute to improve cultivars for drought-prone environments.

## 1.2 Barley (Hordeum vulgare ssp. vulgare L.)

### 1.2.1 Origin, taxonomy and distribution

Barley (*Hordeum vulgare* ssp. *vulgare*) is an ancient cereal grain crop which was domesticated ~ 10 000 years ago from the wild progenitor, *Hordeum spontaneum* C. Koch (Forster 2000). Archaeological and genetic research indicated that the Fertile Crescent of the Middle East as well as Tibet and Ethiopia are the centre of the origin of barley (Harlan and Zohary, 1966; Newman and Newman, 2006; Feuillet et al., 2008).

In the Fertile Crescent, barley is besides einkorn wheat and emmer wheat one of the founder cereals of the Old World agriculture (Feuillet et al. 2008). *Hordeum vulgare* ssp. *vulgare* belongs to the tribe *Triticeae* of the grass family *Poaceae* (Badr et al. 2000). The genus *Hordeum* comprise 32 species and approx. 45 taxa (Bothmer et al. 2003). However, the wild ancestor of barley *Hordeum vulgare* ssp. *spontaneum* C. Koch is considered to be a subspecies of cultivated barley and can be found in its original habitat in the Middle East (Badr et al. 2000; Feuillet et al. 2008). In the recent years, the usage of wild barley species and primitive landraces was discussed as a vital source of genetic variation for crop improvements, especially for the improvement of abiotic stress tolerance (Nevo et al. 1986; Ceccarelli et al. 1987; Ellis 2000).

Cultivated barley plants are monocotyledonous, self-pollinating and diploids cereals with a basic chromosome number of 2n=2x=14 (Forster 2000; Bothmer et al. 2003). Furthermore, barley can be classified as spring or winter type, two-row or six-row, hulled or hulless type (Baik and Ullrich 2008). Due to its high natural diversity and wide adaptability with growing areas at higher latitudes and altitudes, barley is still a principal food source in extreme environments such as Himalayan nations, Ethiopia and semi-arid regions of North Africa (Baik and Ullrich 2008). It is also a model crop for phenotyping experiments and genetic studies because of its short life circle, diploid nature, wide diversity in morphology, physiology and genetics, its tolerance to drought, alkalinity and salinity, and its well-defined genetic maps (Forster 2000; Bothmer et al. 2003).

### 1.2.2. Barley production and uses

In 2013, 59.3% of the worldwide barley production was realized in Europe. Here, it was ranked as the fifth most important crop in dry matter production (85.8 Mt) after wheat (225.5 Mt), sugar beet (167.7 Mt), maize (117.4 Mt) and potatoes (113 Mt) (FAO 2015). Globally, the five top producers of barley are Russia (15.4 Mt), Germany (10.3 Mt), France (10.3 Mt), Canada (10.2 Mt) and Spain (10.1 Mt). Since 1970's the world barley cultivation is declining in favour of growing sugar cane, maize, rice and wheat (FAO 2015). In the recent times, approximately three-quarters of global production was used for animal feed, 20% was malted in breweries (beer and distilled beverages such as whisky) and 5% was used as ingredient in food products, including flour, oatmeal and grits (Mayer et al. 2012).

## 1.3 Drought and drought resistance

From the agronomical point of view, Tuberosa (2012) defined the term 'drought' as a condition in which the amount of water available through rainfall and/or irrigation is insufficient to meet the transpiration needs of the crop. Thus, one of the effects of the decreasing availability of soil water is that the rate of transpiration exceeds the water uptake by plants and will therefore result in dehydration of plants and a declining crop productivity (Bray 1997). The plant response to drought depends on the genotype, the length and severity of water deficit and developmental stage (Nezhadahmadi et al. 2013). Thus, adaptation to drought includes a diversity of mechanism which enables plants to withstand drought and to produce grains. According to the originally terminology of Levitt (1980) which was cited by Mitra (2001), drought resistance can be classified into three categories: drought escape, drought avoidance and drought tolerance.

The ability of a plant to complete its life circle prior to soil water deficit is considered to be a drought escape strategy. The drought escape strategy encompasses several mechanism including a rapid phenological development, an early flowering and maturity, developmental plasticity and remobilization of pre-anthesis assimilates (Turner, 1982; Mitra, 2001).

Drought avoidance is defined as the plant ability to maintain a relatively high plant water status or cellular hydration despite water shortage, so that plant functions are relatively unexposed to tissue dehydration (Mitra 2001; Blum 2005). Here, plants avoid dehydration and maintain their turgidity through a deep and efficient root system, an increased hydraulic

conductance by a reduced epidermal conductance, a reduced absorption of radiation due to leaf rolling and a reduced evaporative surface by a reduced leaf area.

The ability to withstand water deficit with low tissue water potential and to sustain plant functions in a dehydrate state is defined as drought tolerance. Mechanism of drought tolerance includes maintenance of turgidity through osmotic adjustment, an increase in cell elasticity and a decrease in cell size. Blum (2005) emphasized in his review paper that an efficient drought tolerance mechanism in plants is the sustained stem reserve utilization for grain filling under drought stress. Nevertheless, drought tolerance is rare in plants. Mitra (2001) pointed out that crop adaptation must reflect more than one mechanism at a time (escape, avoidance, tolerance) to resist water shortage and maintain an adequate productivity.

## 1.4 Breeding strategies for drought tolerance

Generally, it is accepted that lack of water during the vegetation period is one of the major environmental limitations to crop yields (Richards 1991). In the recent years, plant breeding has contributed to a large extent in examination of morphological, physiological and molecular plant response to drought. Thereby, previous studies and reviews have discussed in detail in what way drought stress affect the crop productivity (e.g. by earliness, decrease in the activity of photosynthesis, closure of stomata, reduction of water potential in leaves, decrease in stomatal conductance, leaf rolling, reduction of leaf area, reduced tillering and plant height, wilting, increased storage of carbohydrate, and accumulation of proline) and which breeding strategies can contribute to improve yield potential and stability under drought (Blum 1996; Passioura 1996; Richards 1996; Bray 1997; Richards 2000; Mitra 2001; Araus et al. 2002; Lafitte et al. 2003; Araus et al. 2003a; Condon et al. 2004; Blum 2005; Cattivelli et al. 2008; Ashraf 2010; Tuberosa 2012; Passioura 2012).

During the last century, three conventional breeding strategies have been developed. The first breeding approach suggest to select genotypes with an increased productivity under favourable environmental conditions (absence of water stress) where genetic variance, heritability and thus breeding progress for grain yield is greatest (Richards 1996; Mitra 2001; Bänziger et al. 2006). However, there is still a large distance between grain yields in optimal and stress environments (Cattivelli et al. 2008). Thus, the second strategy relies on the direct selection for grain yield in a target environment. Unfortunately, a direct selection for grain yield under water-stress conditions is complicated by the year-to-year variability of climatic variables (intensity and duration of water stress), the low heritability of grain yield under

drought and the high genotype-by-environment interaction (Araus et al. 2002; Cattivelli et al. 2008). Several researchers postulated that selection should be based on genotypes that yield well in non-stress and stress environments (Fernandez 1992). However, the drought stress tolerance of a plant is not a unified abiotic stress resistance mechanism at the level of the whole plant or a single gene (Blum and Toriyama 2005). The plant response to stress is determined by several morpho-physiological traits which interact and differ in their individual response according to the intensity and duration of water deficit (Witcombe 2008). Therefore, the third approach suggest to improve drought resistance through incorporation of morphological and physiological mechanism of drought resistance (Mitra 2001).

Araus and Slafer (2008) reported that progress in breeding for drought stress tolerance is based on the understanding of the crop at physiological and molecular biology levels. Thus, secondary traits may contribute to further crop improvements. Ideally, secondary traits which are considered as selection criteria in breeding programs should satisfy the following prerequisites: exhibit genetic variation, be causally and genetic correlated with grain yield under water-stress, have a greater heritability than yield itself and be easy, fast and inexpensive to evaluate (Ceccarelli et al. 1991; Richards 1996; Araus et al. 2002; Tuberosa 2012).

## 1.5 Plant parameters as selection criteria in breeding programs for drought tolerance

In the recent years many studies were dedicated to the improvement of yields under water deficit (Richards 1996; Ceccarelli et al. 1998; Richards 2000; Araus et al. 2002; Condon et al. 2004; Blum 2005; Passioura 2006; Araus et al. 2008; Blum 2009; Tuberosa 2012). According to Passioura (1996) the grain yield of crops in water limited environments is determined by three components, which often interact and simultaneously are independent from each other: (1) water use (amount of water transpired by crops), (2) water use efficiency (WUE, efficiency in producing biomass per unit of water used) and (3) the harvest index (HI, ratio between grain yield and total biomass). Araus et al. (2003) argued that three variables are not fully independent and the modification of a given trait may affect more than one variable. Plant traits which are considered to improve the yield performance under water limited conditions are summarized in Table 1.1.

**Table 1.1** Summary of plant parameters as selection criteria for studying the plant response to drought.

| Component | Plant trait for phenotyping | Effect | Reference |
|---|---|---|---|
| **Water use (W)** | Phenology/ Flowering time | drought escape strategy | Worland (1996)<br>Araus et al. (2002)<br>Sadras et al. (2009)<br>Bogard et al. (2011) |
| | Sty green/ Chlorophyll content | delayed senescence | Borrell et al. (2000) |
| | Dry matter accumulation/ Biomass | maintenance of growth | Blum (1998)<br>Richard (2000) |
| | Root architecture | increase in water uptake | Lampurlanés et al. (2001)<br>Lynch (2007)<br>Trachsel et al. (2011) |
| | Osmotic adjustment | increase in water uptake | González et al. (1999)<br>Blum and Toriyama (2005)<br>González et (al. 2008) |
| | Remobilization of water-soluble carbohydrates | increase in water uptake | Teulat et al. (2001) |
| | Abscisic acid concentration (ABA) | increase in water uptake | Tuberosa (2012) |
| | Canopy temperature depression/ Leaf temperature | regulation of transpiration | Turner and Begg (1981)<br>Lawlor and Cornic (2002)<br>Araus et al. (2008) |
| | Carbon isotope discrimination ($\Delta^{13}$ C) | regulation of transpiration | Araus et al. (1997)<br>Araus et al. (2003)<br>Condon et al. (2004) |
| **Water use efficiency (WUE)** | Early vigour/ Phenological adjustment | increase in WUE | Richards (1996) |
| | Leaf area | photosynthetic capacity, stomatal conductance | Richards (2000) |
| | Stay green/ Chlorophyll content | photosynthetic capacity, stomatal conductance | Richards (2000)<br>Tardy et al. (1998) |
| | Abscisic acid concentration (ABA) | regulating cell dehydration, acclimation of defence system | Cattivelli et al. (2008) |
| | Carbon isotope discrimination ($\Delta^{13}$ C) | transpiration efficiency | Araus et al. (2003)<br>Condon et al. (2004) |
| | Canopy temperature depression/ Leaf temperature | transpiration efficiency | Turner and Begg (1981)<br>Lawlor and Cornic (2002)<br>Araus et al. (2008) |
| **Harvest index (HI)** | Dry matter accumulation/ Biomass | converting biomass into grain | Blum (1998)<br>Richards (2000)<br>Villegas et al. (2001)<br>Slafer et al. (2005) |

## 1.6 Research objectives

The whole plant response to abiotic stress involves a wide range of morphological, physiological and yield-related traits which complicate breeding for drought stress tolerance. Research to improve phenotyping techniques is crucial in terms of identifying genetic variation of drought stress tolerance in varieties, landraces and wild species and, moreover, to investigate traits which contributes to drought stress tolerance.

The present study was undertaken to examine the presence of drought stress tolerance in twenty-four spring barley cultivars (*Hordeum vulgare* ssp. *vulgare*) through a combination of phenotyping experiments, under semi-controlled conditions in pot experiments and under rainfed conditions in the field. In particular, the thesis aimed to:

1. investigate the effect of drought stress at different growth stages on the crop development and grain yield;
2. identify major traits which are useful as effective selection criteria for phenotyping *Hordeum vulgare* ssp. v*ulgare* under drought conditions;
3. evaluate spring barely for grain yield, morphological and physiological traits across a range of field environments in Germany;
4. evaluate spring barley cultivars for their drought stress tolerance and yield stability in response to different phenotyping environments.

## 1.7 Structure and outline of the thesis

The overall of this thesis takes the form of seven chapters, including this introductory chapter.

Chapter 2 describe the methodological development and results of preliminary phenotyping experiments, which aimed to evaluate morphological, physiological and yield-related traits in terms of measuring effort and applicability.

In Chapter 3, the impact of water deficit during the crop life circle was investigated on the crop development and grain yield formation of twenty-four spring barely cultivars grown under semi-controlled environmental conditions in pot experiments.

The fourth chapter is concerned with the analysis of morphological, physiological and yield-related parameters as useful selection criteria for identifying drought stress tolerance in spring barley.

Chapter 5 presents results of phenotyping experiments of spring barley, evaluated under natural field conditions in eight environments in Germany.

Chapter 6 is focused on the comparison of plant performance and yield stability of twenty-four spring barely cultivars which were examined in pot and field experiments by using Shukla's stability variance, stress tolerance index and membership function of drought stress tolerance.

In general, the Chapters 2 to 6 have been organized in the following way: introduction, materials and methods, results, discussion and conclusion.

Chapter 7 draws up an overall discussion of findings. In the final Chapter 8, a brief summary and overall conclusion is given.

# Chapter 2: Preliminary experiments - evaluation and optimization of phenotyping experiments

## 2.1 Introduction

Abiotic stress factors such as drought have a major impact on crop growth, development and yield formation (Passioura 1996; Blum 2005; Passioura 2007; Whitmore and Whalley 2009; Nezhadahmadi et al. 2013). Thus, mechanisms of abiotic stress tolerance are very complex and phenotyping experiments dedicated to drought stress are vital to explore components which influences abiotic stress tolerance (Roy et al. 2011). Progress in breeding for novel traits is based on the accurate phenotyping of large numbers of genotypes and plant parameters. At the same time the utilization of suitable experimental designs is crucial to control the within-replicate variability and to reduce or remove spatial trends (Tuberosa 2012; Fiorani and Schurr 2013). Field experiments designed to evaluate the genotypic response to drought and the underlying complex genetic control of different drought tolerance mechanism are often hampered by additional environmental factors, including wind speed, irradiance, and variations in soil composition which masking important genetic variation for key traits (Cattivelli et al. 2008; Araus and Cairns 2014) . The comprehensive and careful evaluation of genotypes under repeatable and representative growing conditions is the fundamental basis of phenotyping experiments. Properly designed phenotyping experiments are pivotal for reducing the gap between genotype and phenotype, especially for quantitative traits, which are major determinates of drought resistance (Tuberosa 2012). In a controlled environment, pot experiments have the advantage that the water supply and thus the onset and intensity of water stress can be clearly defined (Berger et al. 2010). In view of the above considerations, well defined phenotyping environments under repeatable growing conditions will increase our understanding of plant growth and yield formation under water shortage. The objectives in the present chapter were (1) to evaluate the morphological and physiological response of spring barley to drought conditions at different growth stages and (2) to indentify valuable and manageable traits which allow the accurate phenotyping of larger numbers of genotypes under varying drought conditions.

## 2.2 Materials and Methods

In 2011, pot experiments took place in polyethylene-covered tunnels (poly tunnels) at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany. In the following section scored morphological, physiological and yield-related plant parameters are elucidated. Furthermore, this section provides a detailed description of the experimental setup in 2011.

### 2.2.1 Plant material

Four spring barley cultivars, including modern and old varieties, were used for phenotyping experiments in 2011. The spring barley cultivars, which are commonly used in spring barley breeding programs, were selected to represent the genetic variability within Central European breeding material. Plant material was provided by plant breeding companies which are listed in Table 2.1.

**Table 2.1** Spring barley cultivars used for phenotyping experiments in 2011 and names of plant breeding companies who have provided the plant material.

| Cultivar | Breeding company | Year of release |
|---|---|---|
| Bojos | Limagrain GmbH | 2006 |
| Henrike | Nordsaat Saatzucht GmbH | 2007 |
| Morex | RWTH | 1978 |
| Scarlett | Saatzucht Breun GmbH | 1995 |

### 2.2.2 Experimental setup

Phenotyping experiments were carried out under semi-controlled environmental conditions in polyethylene-covered tunnels (poly tunnels) which enable natural growth behaviour and protect plants against receiving precipitations. The experiments were conducted in 22 x 22 x 26 cm plastic pots containing a mixture of top soil, silica sand, milled lava and peat dust (Terrasoil®, Cordel&Sohn, Salm, Germany). Four spring barley cultivars (Table 2.1) were sown at the 17$^{th}$ March 2011 and arranged in a split-plot design. The two irrigation treatments (well-watered and drought treatment) were the main-plot factor, laid out in four complete randomized blocks. Spring barley cultivars were the sup-plot factor. To simulate a micro plant stock, 18 seeds per pot were sown. After emergence, seedlings were thinned to 12 plants per pot. For thermal insulation and sun protection pots were covered with

extruded polystyrene foam panels. Furthermore, an automated drip irrigation system has been installed which allowed the irrigation of potted plants to a desired soil moisture level. Thus, the amount of water applied to plants was easier to control and facilitate the supply of plants under controlled conditions with the amount of water required for normal plant growth. Plants under drought treatments were drip irrigated in order to guarantee limiting soil water conditions. Each pot had one dripper which provided plants with 33.3 ml water per minute. Both treatment levels were irrigated three times per day at 6:30 am, 00:30 pm and 6:30 pm. Further details concerning the watering time per irrigation treatment are specified in the Appendix. On the basis having a balanced ratio of macro- and micronutrients, plants were fertilized with KRISTALON®, a highly water-soluble chelate fertilizer. Water delivered to the system contained nitrogen (49.9 mg $l^{-1}$), phosphor (49.9 mg $l^{-1}$), potassium (49.9 mg $l^{-1}$), boron (2.08 mg $l^{-1}$), copper (0.83 mg $l^{-1}$), iron (5.83 mg $l^{-1}$), manganese (3.33 mg $l^{-1}$), molybdenum (0.33 mg $l^{-1}$) and zinc (2.08 mg $l^{-1}$). Detailed information regarding fertilization can be found in the Appendix. An optimum water supply was set up at a level of 30% volumetric water content (VWC). During the vegetation period two different drought stress scenarios have been realized: (1) drought treatment at BBCH 24 (before anthesis) and (2) drought treatment at BBCH 49 (after anthesis). Before starting the drought treatments, pots were saturated with water and kept on field capacity. In order to understand how plants response to drought, irrigation frequency for plants under drought treatment was reduced or rather stopped. Thus, water content for plants under drought treatment decreased over 21 days from the field capacity to the permanent wilting point (5-10% VWC). After 21 days pots under drought treatment were re-watered. Over the whole vegetation period pots under control treatment were well-watered (30% VWC). In the first year of experimentation, the nutrient supply was stopped during the time period of drought treatments for both, well-watered and drought treated pots. After the 21-day period of drought treatment, pots under control treatment and drought treatment were again supplied with KRISTALON® fertilizer. Generally, fertilizer and pesticides were used in accordance to agriculture practice. Plant growth regulators and pesticides referring to strobilurine and sulfonylurea were not applied.

Considering an accurately recording of weather data, the DL2e Data Logger from Delta-T Devices Ltd. collected every five minutes the following environmental parameters:

- volumetric water content with EC-5 soil moisture sensors
- soil temperature with TH2-f soil temperature sensors

- air temperature and relative humidity with RFT-2sensors
- solar radiation with PYR solar radiation sensor

### 2.2.3 Phenotypic data collection

For phenotyping experiments a total of 31 plant parameters were scored under well-watered and terminal drought conditions, in two drought stress scenarios: (1) drought treatment before anthesis and (2) drought treatment after anthesis (Fig. 2.1). Based on a decimal code principal growth stages were scored using the extended BBCH scale of Hess et al.(1997). After the 21-day period of drought treatment, the below described parameters were recorded. Grain yield and yield components were measured when spring barley was fully ripe. Table 2.2 provides a summary of recorded traits and their abbreviations.

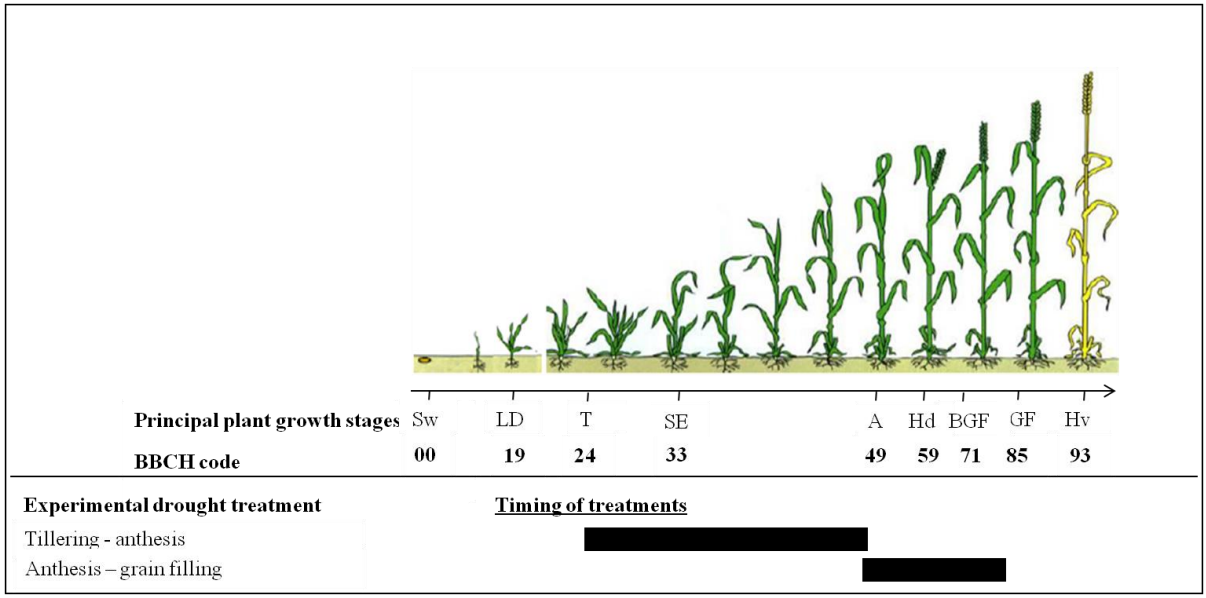


**Fig. 2.1. Timing of experimental drought treatments in 2011 at the research station in Bonn, Germany.** Presented developmental stages are sowing (Sw), leaf development (LD), tillering (T), stem elongation (SE), anthesis (A), heading (Hd), begin grain filling (BGF), grain filling/ development of fruit (GF), harvest (Hv). Based on a decimal code, principal growth stages were assigned using the extended BBCH scale of Hess et al. (1997). Figure adapted from Ugate et al. (2007). Draft available from: https://www.landwirtschaftskammer.de/landwirtschaft/ackerbau/getreide-ec-pdf.pdf [Accessed 9 March 2015]

### *Morphological plant parameters*

#### Plant Height

Plant height (PH) was scored by measuring the average distance from the soil surface to the tip of the spike, recorded to the nearest cm.

#### Tiller number per plant

The tiller number per plant (TNP) was determined by counting the total number of tillers from four plants per pot and averaged then.

#### Leaf number per plant

The total number of leaves per plant (LNP) was counted and averaged from four plants per pot.

#### Leaf area per plant

*Green/ yellow leaf area per plant*

The calculation of the green and yellow leaf area per plant was performed using the analysis software package APS-Asses by L. Lamari (Imaging Analysis Software for Disease Quantification of the American Phytophatological Society, University of Manitoba, Winnipeg, Canada, 2002). Images of leaves were captured with a Canon EOS 350D digital camera fixed 140 cm from a 62 x 78 cm translucent glass screen. Images were stored in high resolution-JPEG format and analyzed as described in the user manual. In short, leaves of four or respectively two plants per pot were photographed on a translucent glass screen. In order to calculate the whole leaf area, each image included a reference area (7.5 x 4.5 cm, 33.75 $cm^2$). Thresholds for the reference area were set in the HSI (hue, saturation and intensity) using intensity values between 16 and 150. Thresholds for leaf area were established in the HSI using saturation values between 12 and 215. For computing lesions thresholds were set with hue values between 31 and 100. The leaf area per plant (LAP) was calculated by using the following equation:

$$LAP\ (cm^2) = [(Area\ Pixel * Size\ Reference\ Area) / Reference\ Pixel]$$

Yellow leaf area per plant (YLA) was then calculated as:

YLA ($cm^2$) = [(Lesion Pixel * Size Reference Area) / Reference Area]

Afterwards the green leaf area per plant (GLA) was computed by subtracting the leaf area per plant (LAP) from the yellow leaf area per plant (YLA): GLA ($cm^2$) = LAP – YLA.

**Plant fresh matter**

Eight plants per pot were sampled and immediately separated into leaves and stems. Fresh matters of leaves (LFM) and stems (SFM) were recorded in grams (g). Thereby, fresh matters excluded the roots. Total plant fresh mater (PFM) per pot was computed by summing up the leaf fresh weight and the stem fresh weight.

**Plant dry matter**

Leaves and stems of eight plants per pot were packed into crisp bags and dried in the drying chamber at 50°C. After 72 hours leaves and stems were weight. Plant dry matter was scored in grams (g). The total plant dry mater (PDM) per pot was computed by summing up the leaf dry matter (LDM) and the stem dry matter per plant (SDM).

**Root dry matter**

In each pot the above ground biomass was cut and removed. The pots, containing soil and roots, were transferred into a basket and washed out in water tanks. Fine washing was done with a hand spray. Roots were packed into crisp bags and dried over 72 hours at 50°C.

**Root length**

Root length was recorded from the stem base to the root tip in cm.

### *Physiological plant parameters*

#### SPAD value

Leaf greenness present in a plant was determined with the Minolta-SPAD® Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). The SPAD-502 chlorophyll meter measures the chlorophyll absorbance in the red and near-infrared regions and calculates a numeric SPAD value which is proportional to the amount of chlorophyll in the leaf (Markwell et al. 1995). SPAD values were determined for each pot, using the upper, young fully expanded leaves of four plants.

#### Leaf senescence

Degree of leaf senescence was scored visually, assessing a value from 1 to 9 for each pot, where 1 = up to 10% yellow/slacking leaf area; 2 = up to 20% yellow/slacking leaf area; 3 = up to 30% yellow/slacking leaf area; 4 = up to 40% yellow/slacking leaf area, 5 = up to 50% yellow/slacking leaf area; 6 = up to 60% yellow/slacking leaf area; 7 = up to 75% yellow/slacking leaf area; 8 = over 75% yellow/slacking leaf area; 9 = over 95% yellow/slacking leaf area. For later data analysis scorings were transformed in their respective percentages.

#### Leaf temperature

Surface leaf temperature was recorded with a hand-held infrared thermometer (Model IR-365 FR, VOLTCRAFT ®). The IR-thermometer was held so that the sensor viewed the plant at the same distance and angle. Measurements of the upper, young fully expanded leaves were made four plants per pot and averaged. Measurements were taken in the late morning from 11 am to 2 pm.

#### Plant water content

The plant water content was determined by weighting the plant fresh matter immediately after sampling and re-weighting the samples after drying them at 50°C for 72 hours in the drying camber. The water content is based on the fresh matter.

The following equations were used to calculate the leaf water content (LWC), stem water content (SWC) and the plant water content (PWC):

LWC (%) = [(LFM – LDM)/ LFM] x 100

SWC (%) = [(SFM – SDM)/ SFM] x 100

PWC (%) = [(PFM – PDM)/ PFM] x 100

**Carbon isotope discrimination ($\Delta\,^{13}C$)**

For carbon isotope discrimination analysis leaf nodes from four upper, young fully expended leaves were sampled and bulked. Samples were dried in the drying chambers at 50°C for 72 h and ground to fine powder. 0.7 mg of leaf dry matter was weight in 5x9 mm tin capsules. The isotope composition was determined at the technical university of Munich, chair of grassland science, using an elemental analyzer which was attached to an isotope ratio mass spectrometer (Delta Plus; Finnigan; MAT). Each sample was measured against a laboratory working standard flour. Carbon isotope composition7 ratio ($^{13}C/^{12}C$) were expressed as $\partial\,^{13}C$ (‰) = ($R_{sample}/ R_{reference}$ -1) x 1000, where R is the ratio of $^{13}C/^{12}C$. The reference is a standard flour. According to Hubick and Farquhar (1989) carbon isotope discrimination was calculated as: $\Delta\,^{13}C = [(\partial a - \partial p)/ (1+\partial p)]$ x1000, with $\partial$ p is the $\partial\,^{13}C$ of the leaves and $\partial$a is the $\partial\,^{13}C$ of the atmosphere (- 8 ‰).

**Plant nitrogen content**

Leaves and stems from eight plants per pot were used to analyze the nitrogen content in plants. Samples were dried in the drying chambers at 50°C for 72 h and ground to fine powder using a cross beater mill (Cross Beater Mill SK1, Retsch GmbH, Haan, Germany) and a vibrating tube mill. The total nitrogen content of each sample was measured on 4 mg of plant powder with an elemental analyzer (Carlo Erba Instruments, Milano, Italy) after the method of Colombo et al. (1988). Determination of plant nitrogen content was done by using a gas analyzer (CARLO-ERBA type 1500, Milan, Italy).

### *Grain yield and yield components*

Plants were harvested at crop maturity and dried in the drying chambers at 50°C for 72 hours. The total dry matter was determined and the number of ears per sample was recorded. Ear samples were threshed using a trashing machine (SAATMEISTER, Allesdrescher – K35). Kernel samples were weighted and counted with a seed counter (Condator "E", Pfeuffer GmbH, Kitzingen, Germany).

#### Number of ears

The average number of ears per plant (NEP) was calculated by dividing the number of ears per pot by the number of plants per pot.

#### Number of kernels per ear

The amount of kernels per ear (NKE) was computed as:

NKE (No.) = No. of kernels per pot / No. of ears per pot

#### Number of kernels per plant

The number of kernels per plant (NKP) was calculated from the number of kernels per pot and the number of plants per pot. NKP (No.) = No. of kernels per pot / No. of plants per pot.

#### Thousand kernel weight

The thousand kernel weight (TKW) is defined as the weight of 1000 kernels in grams. For each sample 1000 kernels were counted and weight. Moisture content of grain was adjusted to 14%.

#### Grain yield

The total grain weight per plant (YLD), which was adjusted to 14% grain moisture, was calculated by using the following equation:

YLD (g/plant) = kernel weight of eight plants per pot (g) / No. of plants per pot.

**Table 2.2** Summary of plant parameters, their abbreviations and unit measured evaluated in preliminary experiments 2011.

| Trait | Abbreviation | Unit | Trait | Abbreviation | Unit |
|---|---|---|---|---|---|
| **plant growth stage** | BBCH | decimal code with two digits | | | |
| | | | | | |
| **morphological plant parameters** | | | **physiological plant parameters** | | |
| plant height | PLH | cm | SPAD value | SPAD | number |
| number of tillers per plant | TNP | No./plant | leaf senescence | LS | 1-9 |
| number of leaves per plant | LNP | No./plant | plant leaf temperature | PLT | °C |
| leaf area per plant | LAP | $cm^2$/plant | plant water content | PWC | % |
| yellow leaf area per plant | YLA | $cm^2$/plant | leaf water content per plant | LWC | % |
| green leaf area per plant | GLA | $cm^2$/plant | stem water content per plant | SWC | % |
| plant fresh matter | PFM | g/plant | plant nitrogen content | PNC | % |
| leaf fresh matter | LFM | g/plant | leaf nitrogen content | LNC | % |
| stem fresh matter | SFM | g/plant | stem nitrogen content | SNC | % |
| plant dry matter | PDM | g/plant | $^{13}C$-Discrimination | $^{13}C$ | % |
| leaf dry matter | LDM | g/plant | | | |
| stem dry matter | SDM | g/plant | **grain yield and yield components** | | |
| root dry matter | RDW | g/plant | number of ears per plant | NEP | No./plant |
| root length | RL | cm | numbers of kernels per ear | NKE | No./ear |
| | | | number of kernels per plant | NKP | No./plant |
| | | | thousand kernel weight | TKW | g |
| | | | grain yield per plant | YLD | g/plant |

### 2.2.4 Statistical analyses

Phenotyping experiments were arranged in a split-plot design with four replications. The split-plot design involved two experimental factors, the irrigation treatment and the genotype. The irrigation as main-plot factor was laid out in four randomized blocks, while genotypes as sub-plot factor were completely randomized within main-plots (Piepho et al. 2003). The statistical analysis of variance for each drought stress scenario was performed via PROC ANOVA using the SAS statistical software package version 9.2 (SAS Institute 2008). The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***. Significant differences between treatments were tested by TUKEY-test at probability level 0.05. Individual analysis of variance for each drought stress scenario was done with the following model:

$$Y_{ijk} = \mu + T_i + G_j + T_i*G_j + r_k + b_{ik} + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $T_i$ is the main effect of *i*-th treatment; $G_j$ is the main effect of *j*-th genotype; $T_i*G_j$ is the fixed interaction effect of *i*-th treatment with *j*-th genotype, $r_k$ is the effect of the *k*-th block, $b_{ik}$ is the error of *i*-th main plot within the *k*-th block and $e_{ijk}$ is random errors. All effects are considered as fixed, while the error terms $b_{ik}$ and $e_{ijk}$ are random.

The combined analysis of variance across the two drought stress scenarios was performed using the PROC MIXED procedure. In order to reliable compare data gathered from diverse phenotyping experiments, raw data were standardized to have a mean of 0 and a standard deviation of 1 by using the PROC STANDARD procedure in SAS. By default, PROC MIXED procedure computed the "Type 3 Test of Fixed Effects" to test the significance of each of the fixed effects. The model used for analysis of variance:

$$Y_{ijk} = \mu + S_i + T_j + S_i*T_j + G_k + G_k*S_i + G_k*T_j + G_k*S_i*T_j + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $S_i$ is the fixed effect of the *i*-th drought stress scenario, $T_j$ is the fixed effect of *j*-th treatment; $S_i*T_j$ is the fixed interaction effect of *i*-th drought stress scenario with the *j*-th treatment; $G_k$ is the fixed effect of the *k*-th genotype; $G_k*S_i$ is the fixed interaction of the *k*-th genotype with the *i*-th drought stress scenario, $G_k*T_j$ is the fixed interaction effect of *k*-th genotype with *j*-th treatment, $G_k*S_i*T_j$ is the fixed interaction effect of the *k*-th genotype with *i*-th drought stress scenario and the *j*-th treatment and $e_{ijk}$ are random errors.

## 2.3 Results

This section presents the major findings of preliminary experiments, focusing on the analysis of variance and the resultant parameters for further phenotyping experiments.

### 2.3.1 Growing conditions

Growing conditions in the first year of experimentation are summarized in Figure 2.2. The blue line represent the soil moisture content under well-watered conditions, the red line under drought treatment before anthesis (BBCH 24 - BBCH 49) and the orange line under drought treatment after anthesis (BBCH 49 - BBCH 76). For both scenarios, the automatic drip irrigation system allowed an accurate reduction of the soil moisture from 24% volumetric water content (VWC) to 6% VWC over a 21-day period. Plants under drought treatment were hold at least three days close to the wilting point (6 % VWC). After 21 days plants under drought treatment were re-watered and soil moisture rise again to 24% VWC. The average monthly air temperature during April, May and June was about 14.8, 17.1 and 19.1 °C. Throughout the whole vegetation period the average air temperature was 17°C and the average.2 air humidity 64%.

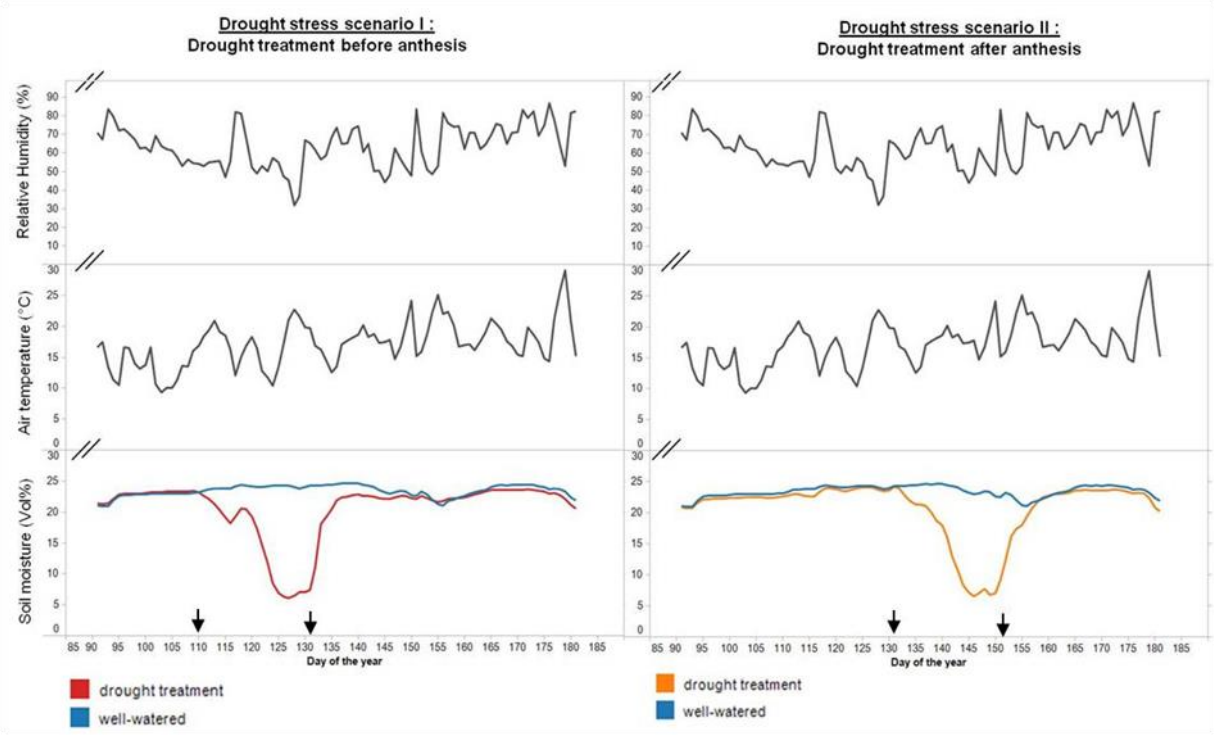


**Fig. 2.2. Mean daily relative humidity, air temperature and soil moisture content in poly tunnel experiments 2011 under well-watered (blue line) and drought conditions (red line and orange line).** Vertical arrows mark the beginning and the end of the drought treatment of each drought stress scenario.

### 2.3.2 Analysis of variance

In terms of measuring effort and applicability eleven morphological, ten physiological and five yield-related plant parameters were evaluated. For both drought stress scenarios, results of analysis of variance are summarized and listed in Table 2.3, 2.4 and 2.5.

***Analysis of variance for drought stress before anthesis***

Within main effects, significant variations between genotypes were noted for nine morphological, nine physiological and four yield-related parameters. Furthermore, analysis of variance revealed significant differences among treatment levels for morphological, physiological and yield-related traits such as: plant dry matter (PDM), stem dry matter (SDM), root dry matter (RDM), chlorophyll content (SPAD), leaf senescence (LS), plant water content (PWC), leaf water content (LWC), stem water content (SWC), plant nitrogen content (PNC), leaf nitrogen content (LNC), stem nitrogen content (SNC), carbon isotope discrimination (Δ 13C), number of ears per plant (NEP), number of kernels per plant (NKP), thousand kernel weight (TKW) and grain yield (YLD). Genotype-by-treatment interactions were detected for PLH, SPAD, LS and LWC.

***Analysis of variance for drought treatment after anthesis***

ANOVA analysis of variance revealed significant genotype differences for seven morphological (PLH, TNP, LNP, LAP, GLA, LDM and RL), six physiological (SPAD, LS, PWC, LWC, SNC and Δ13C) and four yield-related parameters (NEP, NKE, TKW and YLD). In contrast to the first drought stress scenario (drought treatment before anthesis), significant variation among treatment levels were only detected for three morphological and six physiological plant parameters including: PDM, SDM, RDM, SPAD, LS, LWC, SWC, PNC and SNC. Genotype-by-treatment interactions were non-significant for all evaluated parameters.

**Table 2.3** ANOVA analysis of variance for evaluated morphological plant parameters in two drought stress scenarios: (1) drought treatment before anthesis (DT-BBCH 24) and (2) drought treatment after anthesis (DT-BBCH 49).

| Trait | Source of variation | DF | DT-BBCH 24 | DT-BBCH 49 |
|---|---|---|---|---|
| | | | p value | p value |
| PLH | Treatment | 1 | 0.1163 | 0.4749 |
| | Genotype | 3 | <0.0001 | <0.0001 |
| | Genotype*treatment | 3 | 0.0004 | 0.8067 |
| TNP | Treatment | 1 | 0.7262 | 0.7487 |
| | Genotype | 3 | <0.0001 | <0.0001 |
| | Genotype*treatment | 3 | 0.1716 | 0.5577 |
| LNP | Treatment | 1 | 0.1730 | 0.4514 |
| | Genotype | 3 | <0.0001 | 0.0027 |
| | Genotype*treatment | 3 | 0.4646 | 0.9116 |
| LAP | Treatment | 1 | 0.5247 | 0.2158 |
| | Genotype | 3 | 0.0005 | 0.0086 |
| | Genotype*treatment | 3 | 0.3048 | 0.6334 |
| YLA | Treatment | 1 | 0.4645 | 0.5246 |
| | Genotype | 3 | 0.3943 | 0.2659 |
| | Genotype*treatment | 3 | 0.2642 | 0.6415 |
| GLA | Treatment | 1 | 0.3920 | 0.0962 |
| | Genotype | 3 | 0.0005 | 0.0002 |
| | Genotype*treatment | 3 | 0.3042 | 0.6313 |
| PDM | Treatment | 1 | 0.0201 | 0.0084 |
| | Genotype | 3 | 0.0462 | 0.3686 |
| | Genotype*treatment | 3 | 0.5648 | 0.9200 |
| LDM | Treatment | 1 | 0.7002 | 0.7765 |
| | Genotype | 3 | 0.0116 | 0.0010 |
| | Genotype*treatment | 3 | 0.7269 | 0.6723 |
| SDM | Treatment | 1 | 0.0067 | 0.0080 |
| | Genotype | 3 | 0.0006 | 0.1686 |
| | Genotype*treatment | 3 | 0.1905 | 0.9203 |
| RDM | Treatment | 1 | 0.0297 | 0.0300 |
| | Genotype | 3 | 0.0280 | 0.5218 |
| | Genotype*treatment | 3 | 0.3627 | 0.7548 |
| RL | Treatment | 1 | 0.3217 | 0.3148 |
| | Genotype | 3 | 0.3724 | 0.0138 |
| | Genotype*treatment | 3 | 0.3515 | 0.1387 |

Where: p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Trait: PLH: plant height, TNP: number of tillers per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, RDM: root dry matter, RL: root length.

**Table 2.4** ANOVA analysis of variance for evaluated physiological plant parameters in two drought stress scenarios: (1) drought treatment before anthesis (DT-BBCH 24) and (2) drought treatment afteranthesis (DT-BBCH 49).

| Trait | Source of variation | DF | DT-BBCH 24 | DT-BBCH 49 |
|---|---|---|---|---|
| | | | p value | p value |
| SPAD | Treatment | 1 | 0.0002 | 0.0203 |
| | Genotype | 3 | <0.0001 | 0.0002 |
| | Genotype*treatment | 3 | 0.0003 | 0.0940 |
| LS | Treatment | 1 | 0.0305 | 0.0354 |
| | Genotype | 3 | 0.0765 | 0.0054 |
| | Genotype*treatment | 3 | 0.0211 | 0.6382 |
| PLT | Treatment | 1 | 0.7405 | 0.1246 |
| | Genotype | 3 | 0.0135 | 0.2115 |
| | Genotype*treatment | 3 | 0.3445 | 0.2406 |
| PWC | Treatment | 1 | 0.0125 | 0.0871 |
| | Genotype | 3 | <0.0001 | 0.0326 |
| | Genotype*treatment | 3 | 0.2093 | 0.9351 |
| LWC | Treatment | 1 | 0.0094 | 0.0095 |
| | Genotype | 3 | 0.0345 | 0.0022 |
| | Genotype*treatment | 3 | 0.0045 | 0.6043 |
| SWC | Treatment | 1 | 0.0350 | 0.0244 |
| | Genotype | 3 | <0.0001 | 0.0724 |
| | Genotype*treatment | 3 | 0.24030 | 0.8994 |
| PNC | Treatment | 1 | 0.0176 | 0.0375 |
| | Genotype | 3 | 0.0010 | 0.0649 |
| | Genotype*treatment | 3 | 0.4671 | 0.3621 |
| LNC | Treatment | 1 | 0.0186 | 0.2252 |
| | Genotype | 3 | 0.0003 | 0.6460 |
| | Genotype*treatment | 3 | 0.4915 | 0.4191 |
| SNC | Treatment | 1 | 0.0190 | 0.0013 |
| | Genotype | 3 | <0.0001 | 0.0001 |
| | Genotype*treatment | 3 | 0.0855 | 0.9160 |
| Δ13C | Treatment | 1 | 0.0005 | 0.1535 |
| | Genotype | 3 | 0.0398 | 0.0256 |
| | Genotype*treatment | 3 | 0.0797 | 0.8086 |

Where: p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Trait: SPAD: SPAD value, LS: leaf senescence, PLT: plant leaf temperature, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, LNC: leaf nitrogen content, SNC: stem nitrogen content, Δ13C: $^{13}$C-Discrimination.

**Table 2.5** ANOVA analysis of variance for yield and yield-related plant parameters in two drought stress scenarios: (1) drought treatment before anthesis (DT-BBCH 24) and (2) drought treatment after anthesis (DT-BBCH 49).

| Trait | Source of variation | DF | DT-BBCH 24 | DT-BBCH 49 |
|---|---|---|---|---|
| | | | p value | p value |
| NEP | Treatment | 1 | 0.0224 | 0.8288 |
| | Genotype | 3 | <0.0001 | <0.0001 |
| | Genotype*treatment | 3 | 0.2385 | 0.7417 |
| NKE | Treatment | 1 | 0.3771 | 0.4470 |
| | Genotype | 3 | <0.0001 | <0.0001 |
| | Genotype*treatment | 3 | 0.1203 | 0.6049 |
| NKP | Treatment | 1 | 0.0057 | 0.5375 |
| | Genotype | 3 | 0.1924 | 0.2607 |
| | Genotype*treatment | 3 | 0.3588 | 0.7088 |
| TKW | Treatment | 1 | 0.0223 | 0.2856 |
| | Genotype | 3 | <0.0001 | <0.0001 |
| | Genotype*treatment | 3 | 0.2056 | 0.5770 |
| YLD | Treatment | 1 | 0.0079 | 0.2856 |
| | Genotype | 3 | 0.0002 | <0.0001 |
| | Genotype*treatment | 3 | 0.3949 | 0.5770 |

Where: p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Trait: NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 2.3.3 Effects of drought treatments on evaluated plant parameters

Table 2.6 provides genotypic mean values of 26 plant parameters evaluated under well-watered and drought conditions. In 2011, drought treatment before anthesis (DT-BBCH24) caused preliminary a significant increase of RDM (29.1%), SPAD (13.9%), LS (22.2%) and PNC (10.7%). In addition, the decreasing availability of water in DT-BBCH 24 induced a significant decrease of the plant dry matter, plant water content and Δ13C. Grain yield was reduced by 18.9%. Furthermore, the 21-day lasting reduction of the water supply caused a negative impact on grain yield components. Hence, number of ears per plant declined by 15.9% and number of kernels per plant decreased by 21.7%.

It is apparent from Table 2.6 that the genotypic performance under drought treatment after anthesis (DT-BBCH 49) was characterized by a decline in PDM (-18.6%) and LWC (-7.9%). Contrary to the 1$^{st}$ drought stress scenario (DT-BBCH 24) the increase of root dry matter and plant nitrogen content amounted 20.2% and 6.5%, respectively. The reduction of

the water supply between BBCH 46 and BBCH 76 caused no significant decreases for the grain yield and grain yield components.

### 2.3.4 Comparison of the phenotyping experiments

In order to assess differences between examined drought stress scenarios, combined analysis of variance was performed by using PROC MIXED. Results from analysis of variance for morphological and physiological traits are shown in Table 2.7. Findings from analysis of variance for grain yield and yield component are presented in Table 2.8.

Significant differences between the irrigation treatments were recorded for four morphological (GLA, PDM, SDM, and RDM), seven physiological (LS, LWC, SWC, PNC, LNC, SNC, and Δ13C) and three yield related traits (NEP, NKP, and YLD). With exception of the yellow leaf area, the root dry matter, the leaf nitrogen content and the number of kernels per plant, analysis of variance revealed significant differences among genotypes for 22 plant parameters. Main effects of the drought stress scenario were non-significant for all investigated traits. However, analysis of variance detected significant interactions between irrigation treatments and drought stress scenarios for SPAD, PWC, SWC, NEP, NKP, TKW, and YLD. Among all analysed parameters PLH, TNP, SPAD, LS, LWC, SWC, PNC and LNC showed significant interactions between the genotypes and examined drought stress scenarios. Overall, significant genotype-by-treatment interactions were found for PLH, LWC, and NKE.

**Table 2.6** Means values for morphological, physiological and yield-related plant parameters of four spring barley genotypes grown under well-watered and drought conditions in two drought stress scenarios: (1) drought treatment before anthesis (DT-BBCH 24) and (2) drought treatment after anthesis (DT-BBCH 49).

| | | DT-BBCH 24 | | | | DT-BBCH 49 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Trait** | **WET** | **DRY** | **Δ %[a]** | | **WET** | **DRY** | **Δ %[a]** | |
| **Morphological parameter** | **PLH** | 68.5 | 65.3 | -4.7 | ns | 90.8 | 91.4 | 0.7 | ns |
| | **TNP** | 3.4 | 3.3 | -2.3 | ns | 3.1 | 3.2 | 1.5 | ns |
| | **LNP** | 26.0 | 28.2 | 8.2 | ns | 25.8 | 27.3 | 6.0 | ns |
| | **LAP** | 249.0 | 229.4 | -7.8 | ns | 181.1 | 166.8 | -7.9 | ns |
| | **YLA** | 48.2 | 51.7 | 7.2 | ns | 77.2 | 79.9 | 3.5 | ns |
| | **GLA** | 200.7 | 177.8 | -11.5 | ns | 104.0 | 86.9 | -16.4 | ns |
| | **PDM** | 3.6 | 3.1 | -15.2 | * | 7.3 | 5.9 | -18.6 | * |
| | **LDM** | 1.1 | 1.1 | 1.8 | ns | 1.0 | 1.0 | -0.6 | ns |
| | **SDM** | 2.5 | 2.0 | -22.6 | * | 6.3 | 5.0 | -21.3 | * |
| | **RDM** | 0.6 | 0.8 | 29.1 | * | 0.7 | 0.8 | 20.2 | * |
| | **RL** | 37.3 | 36.6 | -1.8 | ns | 33.8 | 35.6 | 5.4 | ns |
| **Physiological parameter** | **SPAD** | 34.6 | 39.4 | 13.9 | *** | 33.0 | 29.1 | -11.8 | * |
| | **LS** | 2.8 | 3.4 | 22.2 | * | 4.6 | 5.0 | 9.6 | * |
| | **PLT** | 16.4 | 15.2 | -7.4 | ns | 12.3 | 10.5 | -14.8 | ns |
| | **PWC** | 79.5 | 77.8 | -2.1 | * | 51.9 | 54.7 | 5.4 | ns |
| | **LWC** | 78.8 | 75.8 | -3.9 | * | 62.8 | 57.8 | -7.9 | * |
| | **SWC** | 79.8 | 78.8 | -1.2 | * | 49.5 | 53.9 | 9.0 | * |
| | **PNC** | 2.9 | 3.2 | 10.7 | * | 1.3 | 1.4 | 6.5 | * |
| | **LNC** | 2.0 | 2.2 | 9.5 | * | 1.0 | 1.0 | 3.7 | ns |
| | **SNC** | 0.9 | 1.0 | 13.3 | * | 0.3 | 0.4 | 15.2 | ** |
| | **Δ 13C** | 20.6 | 18.9 | -8.4 | ** | 20.6 | 20.4 | -1.2 | |
| **Grain yield and yield components** | **NEP** | 3.1 | 2.6 | -15.9 | * | 3.1 | 3.1 | -1.0 | ns |
| | **NKE** | 27.9 | 26.1 | -6.7 | ns | 27.9 | 27.1 | -3.0 | ns |
| | **NKP** | 79.9 | 62.5 | -21.7 | * | 79.9 | 77.6 | -2.8 | ns |
| | **TKW** | 51.8 | 53.1 | 2.6 | * | 51.8 | 51.3 | -1.0 | ns |
| | **YLD** | 4.1 | 3.4 | -18.6 | * | 4.1 | 4.0 | -4.2 | ns |

Where DT-BBCH 24:drought treatment before anthesis, DT-BBCH 49: drought treatment after anthesis, WET: well-watered, DRY: drought treatment, Δ%[a] (relative difference in percent): (Dry-Wet)/ Wet *100; and *,**,** are significant at p value ≤ 0.05, ≤0.01 and ≤0.001, ns: none significant at P = 0.05, Traits: PLH: plant height, TNP: number of tillers per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, RDM: root dry matter, RL: root length, SPAD: SPAD value, LS: leaf senescence, PLT: plant leaf temperature, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, LNC: leaf nitrogen content, SNC: stem nitrogen content, Δ13C: $^{13}$C-Discrimination, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 2.7** Analysis of variance across two different drought stress scenarios for morphological parameters and physiological parameters of four spring barely genotypes.

| | | p value | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **TNP** | **LNP** | **LAP** | **YLA** | **GLA** | **PDM** | **LDM** | **SDM** | **RDM** | **RL** |
| Treatment | 1 | 0.3321 | 0.8669 | 0.2023 | 0.1266 | 0.3133 | 0.0299 | <0.0001 | 0.7544 | <0.0001 | <0.0001 | 0.7665 |
| Genotype | 3 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.7654 | <0.0001 | 0.0060 | <0.0001 | <0.0001 | 0.1448 | 0.0189 |
| Genotype*Treatment | 3 | 0.0218 | 0.1077 | 0.7092 | 0.5959 | 0.5647 | 0.6513 | 0.5206 | 0.9372 | 0.2935 | 0.7440 | 0.1579 |
| Scenario | 1 | 1.000 | 1.000 | 0.9734 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Scenario*Treatment | 1 | 0.1760 | 0.5763 | 0.7957 | 0.9276 | 0.7279 | 0.8003 | 0.9234 | 0.6030 | 0.7718 | 0.3065 | 0.3283 |
| Scenario *Genotype | 3 | 0.0011 | 0.0286 | 0.1879 | 0.1332 | 0.0666 | 0.0566 | 0.8684 | 0.7857 | 0.7230 | 0.2599 | 0.4616 |
| Genotype*Treatment* Scenario | 3 | 0.0060 | 0.7843 | 0.9162 | 0.4953 | 0.2870 | 0.3201 | 0.9946 | 0.5370 | 0.9691 | 0.6273 | 0.4667 |

**Table 2.7** (continued)

| | | p value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **SPAD** | **LS** | **PWC** | **LWC** | **SWC** | **PNC** | **LNC** | **SNC** | **Δ13C** |
| Treatment | 1 | 0.4919 | <0.0001 | 0.7647 | <0.0001 | 0.0177 | 0.0026 | 0.0314 | <0.0001 | 0.0005 |
| Genotype | 3 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0003 | 0.2071 | <0.0001 | <0.0001 |
| Genotype*Treatment | 3 | 0.1883 | 0.2164 | 0.5421 | 0.0151 | 0.6367 | 0.2385 | 0.2386 | 0.2495 | 0.1348 |
| Scenario | 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Scenario*Treatment | 1 | <0.0001 | 0.3026 | 0.0001 | 0.2864 | <0.0001 | 0.2877 | 0.2551 | 0.7307 | 0.0513 |
| Scenario *Genotype | 3 | <0.0001 | 0.0185 | 0.5142 | 0.0195 | 0.0059 | 0.0024 | 0.0002 | 0.9255 | 0.2212 |
| Genotype*Treatment* Scenario | 3 | 0.0020 | 0.0076 | 0.7125 | 0.1147 | 0.5524 | 0.2375 | 0.2229 | 0.8074 | 0.5986 |

Where: p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Scenario: drought treatment before anthesis (DT-BBCH 24) and drought treatment after anthesis (DT-BBCH 49), Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, LNC: leaf nitrogen content, SNC: stem nitrogen content, Δ13C: $^{13}$C-Discrimination.

**Table 2.8** Analysis of variance across two different drought stress scenarios for yield-related parameters of four spring barely genotypes.

| | | p value | | | | |
|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| Treatment | 1 | 0.0023 | 0.1506 | 0.0001 | 0.0524 | <0.0001 |
| Genotype | 3 | <0.0001 | <0.0001 | 0.0851 | <0.0001 | <0.0001 |
| Genotype*Treatment | 3 | 0.4311 | 0.0226 | 0.6831 | 0.1914 | 0.5357 |
| Scenario | 1 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Scenario*Treatment | 1 | 0.0069 | 0.5544 | 0.0075 | 0.0021 | 0.0163 |
| Scenario *Genotype | 3 | 0.1978 | 0.3290 | 0.3941 | 0.3920 | 0.8514 |
| Genotype*Treatment* Scenario | 3 | 0.2695 | 0.2146 | 0.4753 | 0.2313 | 0.6722 |

where: p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Scenario: drought treatment before anthesis (DT-BBCH 24) and drought treatment after anthesis (DT-BBCH 49), Trait: NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

## 2.4 Discussion

Results of the current study indicate that the 21-day lasting drought treatment between tillering and anthesis ("drought stress before anthesis") caused significant reductions of the plant dry matter, root dry matter, plant water content, leaf water content, $\Delta\ ^{13}C$-Discrimination, number of ears per plant, number of kernels per plant and thousand kernel weight (Bayoumi et al. 2008; Chen et al. 2012). Compared to well-watered conditions, mean values of physiological parameters including chlorophyll content (SPAD value), leaf senescence, and plant and stem nitrogen content (PNC and SNC), remarkably increased under drought. Under water shortage, grain yield was significantly reduced by reductions of NEP (-15.9%), NKE (-6.7%) and NKP (-21.7%). Findings observed for the 1st drought stress scenario ("drought stress before anthesis") accord with ideas of Farooq et al. (2009) who emphasized that plant growth is achieved through cell division, cell enlargement and differentiation, and involves genetic, physiological and morphological events.

However, in the 2nd drought stress scenario ("drought stress after anthesis"), water shortage remarkably reduced the plant and stem dry matter, as well as the chlorophyll content. Surprisingly, genotypes of the 2nd drought stress scenario revealed no significant differences for grain yield and yield components. These results are in contrast to earlier findings of Samarah (2005) who showed that drought stress during the grain filling highly declined the number of tillers and grains, and thus the grain yield. Findings furthermore indicate that yield formation integrates many morphological and physiological processes, which are very complex and difficult to interpret (Farooq et al. 2009).

Globally, drought induced reductions of NEP, NKP and YLD observed in the 1st drought stress scenario were 14.4 to 18.9% higher than those of the 2nd drought stress scenario, suggesting that plants were subjected to drought during tillering and anthesis. In accordance to present results, Villegas et al. (2001) demonstrated that reserves (e.g. carbohydrates) which are accumulated before anthesis were a main source for grain filling under rainfed Mediterranean conditions. The non-significant effect of reduced water supply on grain yield and yield components in the 2nd drought stress scenario was might be attributable to the lack of an adequate NPK-fertilizer during the 21-day lasting drought treatment. Nitrogen (N), phosphor (P) and potassium (K) are essential macronutrients for crop growth and yield development (Przulj and Momcilovic 2001; Hu and Schmidhalter 2005; Pettigrew 2008). The stop of fertilization for plants under well-watered conditions accompanied by a constant watering of pots has lead to a leaching of nutrients. Thus, it seems possible that the

insufficient nutrient supply of plants under well-watered conditions has resulted in an inadequate plant development and yield formation. At the same time, irrigation stop under drought treatment probably reduced the leaching effect of plant nutrients. Hence, the nutrient concentration in drought treated pots increased, compared to fully irrigated pots. Mean values for plant nitrogen concentration presented in Table 2.6 support this assumption. Sinebo et al. (2002) found that insufficient nitrogen and phosphor fertilization reduced the grain yield of spring barley by 79%. Hence, under optimal growing conditions a balanced nutrient supply is of particular importance. Among these already discussed factors, it is important to note that the 2$^{nd}$ drought stress scenario started at BBCH 49 and ended at BBCH 76. One effect of this late starting drought treatment was probably that plants had successfully completed major growth stages which are of particular importance for yield formation. Thus, the timing of drought treatments during the vegetation period contributed to the non-significant differences between irrigation levels. Another possible explanation might be that pre-anthesis assimilates stored in vegetative tissues alleviate the effect of water shortage and contributed to the final grain yield (Blum 2005). Yang et al. (2001) studied the impact of water shortage during the grain filling in rice and reported that early senescence in rice plants might enhanced the remobilization of stored assimilates under drought stress which again accelerate the grain filling. In addition, Blum (2005) emphasized that water stress, which decrease the plant water status and photosynthesis during the grain filling, induces the mobilization of soluble sugars into the grain.

Another important aspect in this study was the evaluation of plant parameters in terms of measuring effort and applicability. With respect to the quality of results, phenotyping experiments in 2011 showed that the evaluation of the root dry matter (RDM) and the root length (RL) was labour intensive and difficult to phenotype regarding the homogeneity of the soil. The used Terrasoil ® mixture contained, besides top soil and silica sand, milled lava which made the root washing difficult and increased the risk of losing fine roots during washing. However, measuring the root dry matter and root length did not revealed the finer details of root architecture and anatomy (Fenta et al. 2014). Thus, phenotyping root systems under contrasting conditions should be focused on specific root phenotyping approaches such as ‘shovelomics’ (Araus and Cairns 2014).

The determination of the plant leaf temperature (PLT) proved to be extremely complicated. Due to changing weather conditions (wind and clouds) during the sampling day, accurate measurements of the leaf temperature (PLT) were difficult to realize and very time consuming.

The analysis of leaf and stem nitrogen content (LNC and SNC) revealed no additional information. Nevertheless, results of preliminary phenotyping experiments demonstrated the significant effect of nutrient supply on plant growth and yield formation. To understand the impact of drought on nutrient accumulation in plants, the relationship between drought stress and plant nitrogen, phosphor and potassium concentration will be investigated in phenotyping experiments 2012 and 2013. With regard to the time spend on sampling leaves for the $\Delta^{13}$C-Discrimination analysis and the gain of information, material for $\Delta^{13}$C-Discrimination analysis will be collected in the following experimentation years for only one drought stress scenario (drought treatment at tillering stage). The five parameters RDM, RL, PLT, LNC and SNC are excluded from phenotyping experiments 2012 and 2013. All other presented parameters from preliminary phenotyping experiments will be retained.

To ensure the collection of meaningfully phenotypic data, a combined analysis of variance was performed. An initial objective of this variance analysis was to identify if the type and timing of irrigation treatments are adequate for phenotyping drought stress tolerance at different time points during the vegetation period (Table 2.7 and 2.8). Non-significant differences between the examined drought stress scenarios were probably related to the similar availability of water in both phenotyping experiments and under both irrigation levels. Additionally, a higher number of genotypes and/or replicates within each treatment level could contribute to generate significant differences between drought stress scenarios. However, current study found significant treatment-by-scenario interactions for SPAD, PWC, SWC, NEP, NKP, TKW and YLD, suggesting that the effect of drought treatment differed between drought stress scenarios. In general, these findings agree with previously presented results in Table 2.6.

## 2.5 Conclusion

The purpose of the preliminary phenotyping experiments was to (1) to evaluate morphological and physiological plant parameters in terms of measuring effort and (2) to improve the application of drought treatments at different growth stages. This study has shown that out of 31 evaluated traits five parameters, including RDM, RL, PLT, LNC and SNC, had to be discarded. Based on results of this study it is clear that properly managed plant nutrition is essential for achieving a maximum plant growth. Thus, a well balanced fertilization with primary macronutrients is of particular importance and enables plants under well-watered conditions to display their full yield potential. To avoid a nutrient deficiency in future phenotyping experiments, fertilization will be connected to the automated irrigation system over the whole vegetation period. Studies of Christen et al. (1995) suggested that the performance of spring barley under drought depend the developmental stage in which water stress occurs. In this connection, phenotyping experiments in 2012 and 2013 will investigate the sensitivity of spring barley to water stress then drought occurs at three different growth stages: (1) the end of the leaf development, (2) the tillering stage and (3) at anthesis.

# Chapter 3: Effects of water shortage at different developmental stages on the plant performance of spring barley

## 3.1 Introduction

The currently and future global agriculture faces serious challenges: an increase of the global human population with more than 9 billion inhabitants by 2050, a declining availability of water, a decrease in crop-growing areas and the multiple use of crops for biofuels and food production (Tomlinson 2011; Tardieu 2012). With respect to the continuously growing human population, the food productions need to increase by 70% until 2050 (Tilman et al. 2011). Beside these considerations, future climate change is expected to increase in climate variability and extreme weather events. According to EEA Report in (2012), climate change in Central and East Europe is characterized by shifts in weather patterns, a reduced summer precipitation as well as an increased risk of high temperatures and droughts. The projected climate change will make it even harder to achieve food security in 21$^{st}$ century. Thereby, water shortage and droughts are major abiotic factors which limits the grain yield and yield stability (Cattivelli et al. 2008; Szira et al. 2008). Hence, it is necessary to develop cultivars which are able to cope with future climate conditions. In the recent years numerous of studies have been realized to understand the crop response to water deficits. Several studies emphasised that the plant response to drought stress varies depending on the genotype, the severity and duration of drought stress and the developmental stage in which drought occurs (Mogensen et al. 1985; Christen et al. 1995; Çakir 2004; Estrada-Campuzano et al. 2008). For spring wheat, Christen et al. (1995) reported that water stress between stem elongation and flag leaf stage caused significant reductions in plant dry matter and tiller number. They pointed out that the highest yield reduction was observed then drought occurred during ear emergency and anthesis (Christen et al. 1995). Moreover, extensive research has been conducted with the aim of detecting drought stress tolerant genotypes by analysing stress tolerance indicies (Sio-Se Mardeh et al. 2006; Nazari and Pakniyat 2010; Schittenhelm et al. 2014). However, the study primary aimed to quantify the spring barley response to water shortage during the crop life circle. Thereby, the evaluation morphological and pyhsiological plant parameters can be a useful tool to detect and explore genotypic variations in drought tolerance. In view of above considerations the purpose of the present study was (1) to evaluate the effect of drought stress at different growth stages on the crop development and grain yield

of spring barley and (2) to investigate specific growth stages which are most sensitive to drought.

## 3.2 Materials and Methods

A variety of methods are used to evaluate the effect of drought stress on the crop development and grain yield formation. The following section provides information about used plant material, measured parameters, experimental setup and growing conditions over two years of phenotyping experiments.

### 3.2.1 Plant material

In order to capture a representative range of genetic variability within Central European breeding material, four spring barley cultivars (Table 3.1) were selected for phenotyping experiments in 2012 and 2013. Plant material was provided by several plant breeding companies which are listed in the following table.

**Table 3.1** Spring barley cultivars used for phenotyping experiments in 2012 and 2013 and names of plant breeding companies who have provided the plant material.

| Cultivar | Breeder company | Year of release |
|---|---|---|
| Bojos | Limagrain GmbH | 2006 |
| Henrike | Nordsaat Saatzucht GmbH | 2007 |
| Morex | RWTH | 1978 |
| Scarlett | Saatzucht Breun GmbH | 1995 |

### 3.2.2 Experimental setup

In 2012 and 2013, pot experiments were conducted in polyethylene-covered tunnels (poly tunnels) at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany. The experiments were carried out in 22 x 22 x 26 cm plastic pots containing a soil mixture of top soil, silica sand, milled lava and peat dust (11.5 l Terrasoil®, Cordel&Sohn, Salm, Germany). To simulate a micro crop stand 18 seeds per pot were sown. After emergence, seedlings were thinned to 12 plants per pot. For thermal insulation and sun protection pots were covered with extruded polystyrene foam panels. Through automatic drip irrigation system water was supplied three times per day at 6:30 am, 00:30 pm and 6:30 pm. Information regarding the watering time per treatment are presented in the Appendix.

Nutrients were added to the drip irrigation in form of KRISTALON®, which is a fully water soluble NPK fertilizer. An optimum water supply was set up at a level of 30% volumetric water content (VWC). In the two consecutive years of experimentation the experimental design was a split-plot design with irrigation treatments as main-plot factor and genotypes as sub-plot factor. Main-plots and sub-plots within the main-plots were completely randomized. In general, there were two irrigation treatments (well-watered and terminal drought) and four replications per treatment. During the vegetation period three individual drought stress scenarios have been realized, whereas drought treatments started: (1) at the end of the leaf development (BBCH 19), (2) at tillering stage (BBCH 24) and (3) at anthesis (BBCH 49). In order to understand how plants response to drought, irrigation frequency for plants under drought treatment was reduced or rather stopped. Timings of drought treatments and specific growth stages are shown in Figure 3.1. Fertilizer and pesticides were used in accordance to agriculture practice. Growth regulators and pesticides referring to strobilurine and sulfonylurea were not applied. Details concerning the general experimental setup are described in Chapter 2 of this thesis. Further information about drought stress scenarios in 2012 and 2013 are presented in the Appendix.

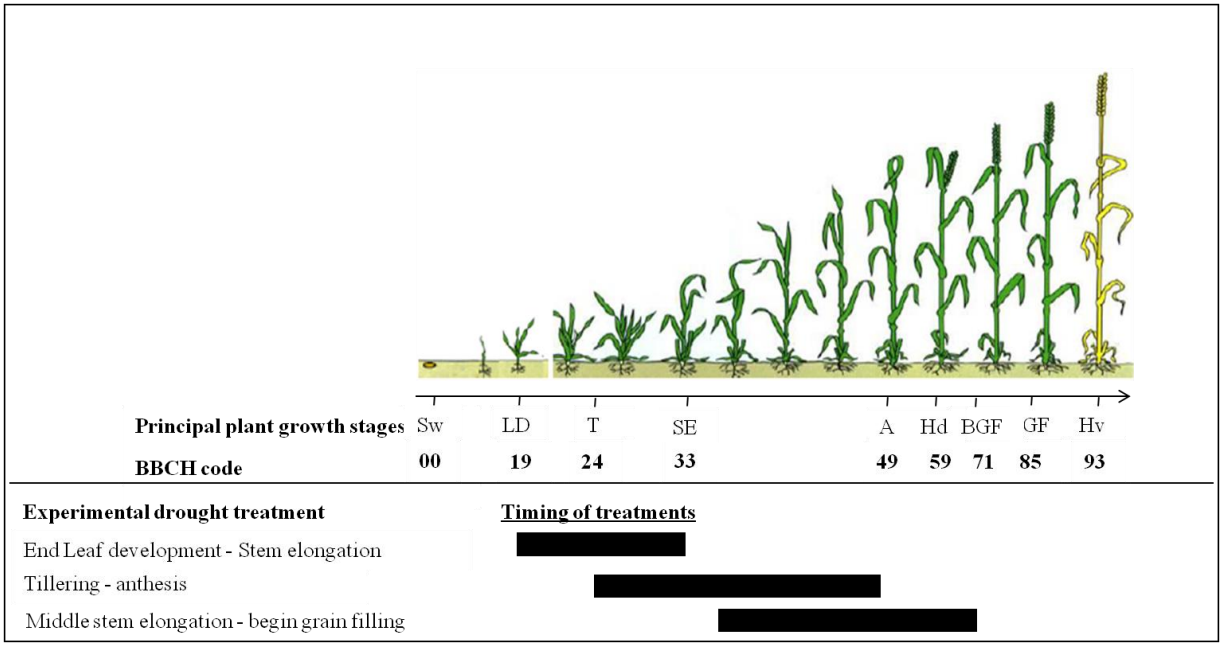


**Fig. 3.1. Timing of experimental drought treatments between 2012 and 2013 at the research station in Bonn, Germany.** Presented developmental stages are sowing (Sw), leaf development (LD), tillering (T), stem elongation (SE), anthesis (A), heading (Hd), begin grain filling (BGF), grain filling/ development of fruit (GF), harvest (Hv). Based on a decimal code, principal growth stages were assigned using the extended BBCH scale of Hess et al. (1997). Figure adapted from Ugate et al. (2007). Draft available from: https://www.landwirtschaftskammer.de/landwirtschaft/ackerbau/getreide-ec-pdf.pdf [Accessed 9 March 2015].

### 3.2.3 Environmental conditions

***Air temperature and relative humidity***

Daily weather parameters like the air temperature and relative humidity were measured with a DL2e Data Logger from Delta-T Devices Ltd.. Over two years of experimentation Figure 3.2 present the air temperature and relative humidity between April and July (85 day of the year - 185 day of the year). The average monthly air temperature in May and June 2012 was 16.2 and 17.1 °C, respectively. In 2013, the average air temperature was in May 13.5°C and in June 18.1°C. Throughout the whole vegetation period the average air temperature was almost equal, 14.7°C in 2012 and 15.3°C in 2013. In both years of experimentation the relative air humidity was on average 71%.

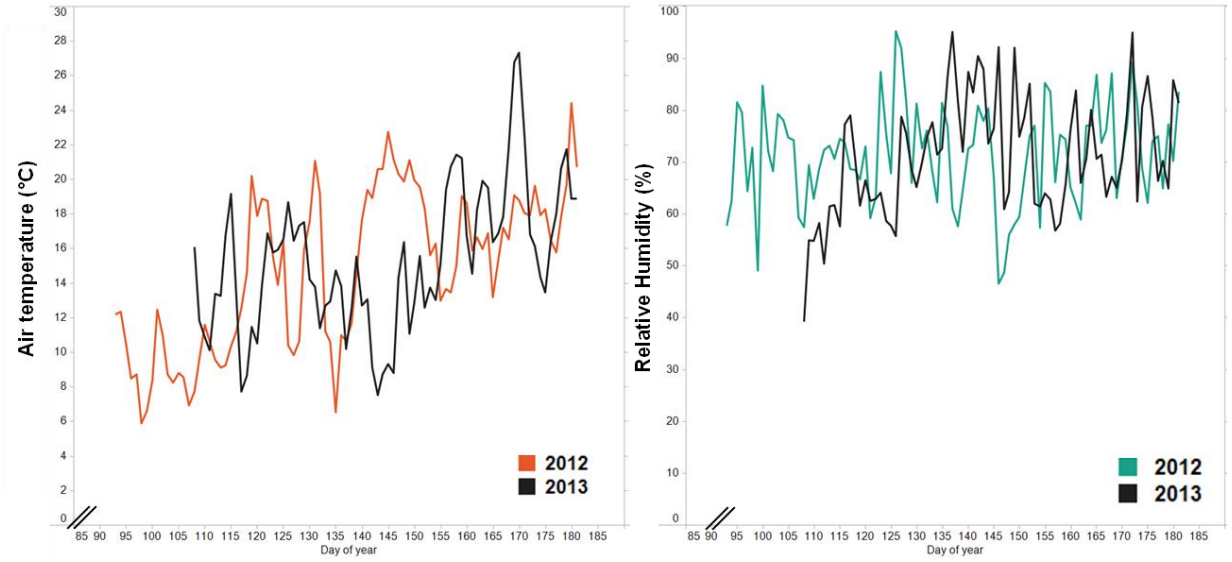


**Fig. 3.2. Mean daily air temperature and air humidity for the experimental years 2012 and 2013 at the research station of Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany.**

***Soil moisture and water treatments***

In both years of experimentation and for each drought stress scenario it was targeted to decrease the water content over 21 days from the field capacity to the permanent wilting point (5-10% VWC). Irrigation was withheld at three different developmental stages: at the end of the leaf development (1$^{st}$ drought stress scenario), at tillering (2$^{nd}$ drought stress scenario) and at anthesis (3$^{rd}$ drought stress scenario). Over the whole vegetation period pots under control treatment were fully irrigated. Soil moisture conditions for each drought stress scenario are summarized in Figure 3.3. The blue line represent the soil moisture content under well-watered conditions, the yellow line under drought treatment starting at the end of leaf

development stage, the red line under drought treatment beginning at tillering stage and the orange line under drought treatment getting started at anthesis. For all scenarios the automatic drip irrigation system allowed the reduction of the soil moisture from 25% VWC to 10% VWC over a 21-day period. After 21 days plants under drought treatment were re-watered and soil moisture rise again to 25% VWC. In 2013, the reduction of the soil moisture content has been more difficult to implement. This is due to the fact that the air temperature differed between the years. In 2013, lead the 9°C lower air temperature between day 140 and day150 along with a relative air humidity of 71% to a slower reduction of the soil moisture content, compared with environmental conditions in 2012.

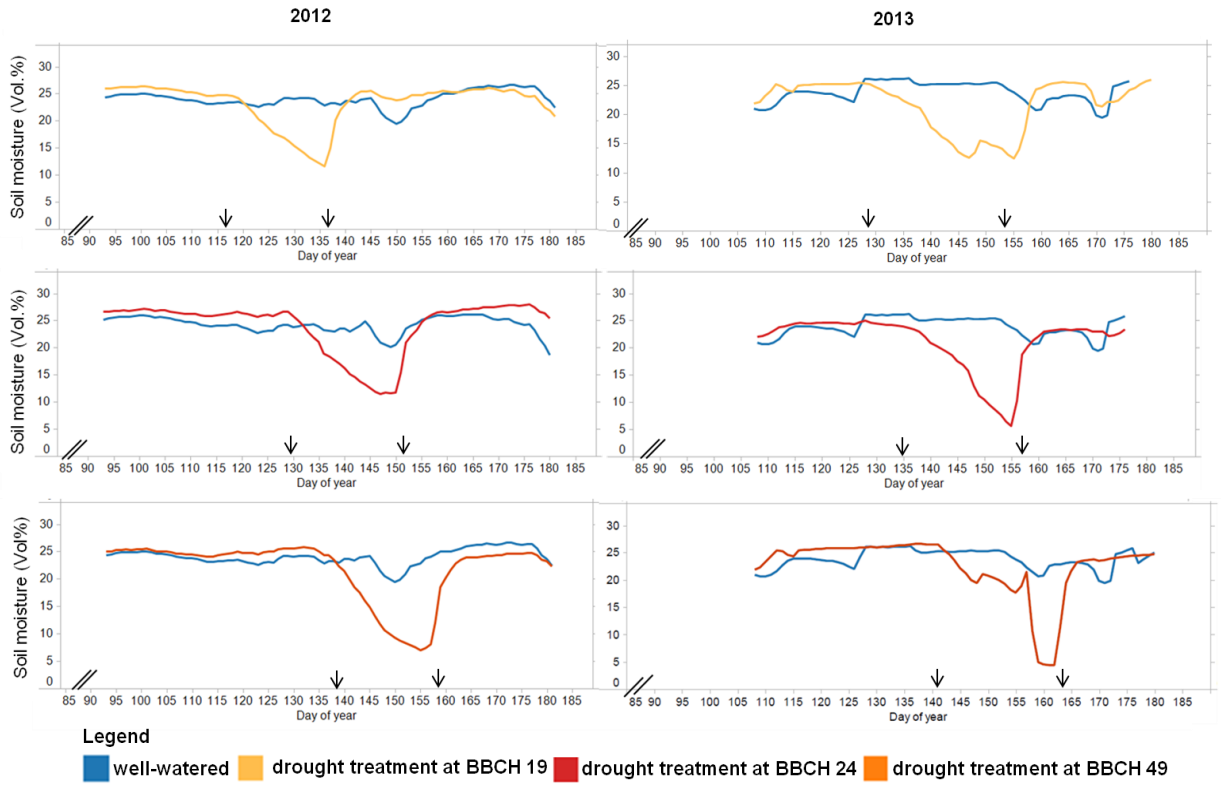


**Fig.3.3. Mean soil moisture content recorded for poly tunnel experiments in 2012 and 2013.** Vertical arrows mark the beginning and the end of the drought treatment of each drought stress scenario.

### 3.2.4 Phenotypic data collection

In 2012 and 2013, a total of 23 parameters were investigated under well-watered and drought conditions. Evaluated plant parameters included nine morphological, nine physiological and five yield-related plant parameters. A short definition and description of measured parameters is given in Table 3.2. Further, detailed specifications of taking measurements are described in detail in Chapter 2. Principal growth stages of leaf development, tillering, stem elongation, and anthesis were recorded using the extended BBCH

scale of Hess et al. (1997). Details, concerning the analysis of nitrogen, phosphor and potassium content in plant tissues are given in Chapter 6 of this thesis.

**Table 3.2** Classification, abbreviations and description of evaluated plant parameters.

| Plant parameter | Abbreviation | Unit | Description |
|---|---|---|---|
| **plant growth stage** | BBCH | number | decimal code with two digits |
| **morphological plant parameters** | | | |
| plant height | PLH | cm | average distance from the soil surface to the tip of the spike |
| number of tillers per plant | TNP | No./plant | average number of tillers per plant |
| number of leaves per plant | LNP | No./plant | average number of leaves per plant |
| leaf area per plant | LAP | $cm^2$/plant | total plant leaf area measured at different growth stages |
| yellow leaf area | YLA | $cm^2$/plant | yellow leaf area of a plant measured at different growth stages |
| green leaf area | GLA | $cm^2$/plant | green leaf area of a plant measured at different growth stages |
| plant dry matter | PDM | g/plant | total dry mass of above-ground plant |
| leaf dry matter | LDM | g/plant | total dry mass of leaves per plant |
| stem dry matter | SDM | g/plant | total dry mass of stems per plant |
| **physiological plant parameters** | | | |
| SPAD value | SPAD | number | estimate of leaf chlorophyll content with SPAD-502 chlorophyllmeter |
| leaf senescence | LS | 1-9 | visually scored degree of leaf senescence |
| plant water content | PWC | % | water content of above-ground plant mass |
| leaf water content per plant | LWC | % | water content of leaves per plant |
| stem water content per plant | SWC | % | water content of stems per plant |
| plant nitrogen content | PNC | % | nitrogen content of above-ground plant |
| plant potassium content | PKC | % | potassium content of above-ground plant |
| plant phosphor content | PPhC | % | phosphor content of above-ground plant |
| **grain yield and yield components** | | | |
| number of ears per plant | NEP | No./plant | average number of ears per plant |
| number of kernels per ear | NKE | No./ear | average number of kernels per ear |
| number of kernels per plant | NKP | No./plant | average number of kernels per plant |
| thousand kernel weight | TKW | g | weight of 1000 kernels |
| grain yield per plant | YLD | g/plant | average grain weight per plant |

### 3.2.5 Statistical analyses

Morphological, physiological and yield-related plant parameters were analyzed under the usage of the SAS software version 9.2 (SAS Institute 2008). In each year the statistical analysis of variance for each drought stress scenario was performed via PROC ANOVA. The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***.

***Individual analysis of variance***

For each drought stress scenario analysis of variance was conducted by using the model:

$$Y_{ijk} = \mu + T_i + G_j + T_i*G_j + r_k + b_{ik} + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $T_i$ is the main effect of i-th treatment; $G_j$ is the main effect of j-th genotype; $T_i*G_j$ is the fixed interaction effect of i-th treatment with j-th genotype, $r_k$ is the effect of the k-th block, $b_{ik}$ is the error of i-th main plot within the k-th block and $e_{ijk}$ is random errors. All effects are considered as fixed, while the error terms $b_{ik}$ and $e_{ijk}$ are random.

***Combined analysis of variance***

Across years, analyses of variance were performed by using the SAS PROC MIXED procedure. Thereby, years were considered as a fixed factor because two years of experimentation are not representative for the wide range of annual and environmental variation. To test the significance of each fixed effect, the PROC MIXED procedure computed the "Type 3 Test of Fixed Effects". The LSMEANS statement calculated least-squares means (LS-means) of fixed effects. The model used for analysis of individual drought stress scenarios across two years is:

$$Y_{ijk} = \mu + Y_i + T_j + Y_i*T_j + G_k + G_k*Y_i + G_k*T_j + G_k*Y_i*T_j + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $Y_i$ is the fixed effect of i-th year; $T_j$ is the fixed effect of j-th treatment; $Y_i*T_j$ is the fixed interaction effect of the i-th year with the j-th genotype, $G_k$ is the fixed effect of k-th genotype; $G_k*Y_i$ is the fixed interaction effect of k-th genotype with i-th year, $G_k*T_j$ is the fixed interaction effect of k-th genotype with j-th treatment; $G_k*Y_i*T_j$ is the fixed interaction effect of k-th genotype with j-th treatment and with i-th year and $e_{ijk}$ is random errors.

***Correlation analysis***

Based on genotypic means, genetic correlations between examined parameters were analyzed. The SAS procedure PROC CORR calculated the Spearman's rank correlation coefficient. The software package CIRCOS (Krzywinski et al. 2009) was applied to visualize significant correlations between evaluated plant parameters.

***Stress tolerance index (STI)***

Fernandez (1992) suggested the usage of stress tolerance indices (STI) as an overall index to identify genotypes with a superior performance in non-stress and stress environments. The stress tolerance index was determined from genotypic means using the following equation:

$$\text{STI} = \left(\frac{Y_{ww}}{\overline{Y_{ww}}}\right)\left(\frac{Y_{dt}}{\overline{Y_{dt}}}\right)\left(\frac{\overline{Y_{dt}}}{\overline{Y_{ww}}}\right) = \frac{(Y_{ww})(Y_{dt})}{\left(\overline{Y_{ww}}\right)^2}$$

Where $Y_{ww}$ and $Y_{dt}$ are the genotype mean for given plant parameter under well-watered conditions and drought treatment, and $\overline{Y}_{ww}$ and $\overline{Y}_{dt}$ are the overall means under both irrigation levels.

Genotypes with higher STI values were considered as drought tolerant. Based on the calculated STI values, analysis of variance (ANOVA) was used to compare effects of different drought stress scenarios. In order to determine differences among drought stress scenarios and genotypes, TUKEY'S test at significance level of 5% ($HSD_{5\%}$) was set. Ranking of genotypes were performed based on computed STI values. Genotypes with the highest performance level were given a rank value of 1, while a genotype with the lowest performance level was assigned a rank value of 4.

## 3.3 Results

In order to determine differences among and within different drought stress scenarios, several statistical analyses were carried out and results are presented thereafter.

### 3.3.1 Analysis of variance

#### *1st Drought stress scenario: drought treatment at the end of the leaf development stage*

Results of analysis of variance for nine morphological parameters, eight physiological and five yield related plant parameters are shown in the Appendix. The analysis of variance revealed highly significant effects of the treatment (p value $\leq 0.0001$) for all 22 investigated traits. Genotypic variation was significant for 16 parameters, including plant height (PLH), number of tillers per plant (TNP), number of leaves per plant (LNP), green leaf area (GLA), plant dry matter (PDM), leaf dry matter (LDM), stem dry matter (SDM), SPAD-value (SPAD), plant water content (PWC), stem water content (SWC), plant nitrogen content (PNC), plant potassium content (PKC), number of ears per plant (NEP), number of kernels per ear (NKE), number of kernels per plant (NKP) and 1000 kernel weight (TKW). Among all analyzed parameters five parameters (PLH, PNC, PKC, NKE, and TKW) showed significant genotype-by-treatment interactions. With exception of LS and TKW, significant differences among years were found.

#### *2nd Drought stress scenario: drought treatment at tillering stage*

Main effects of the treatment were significant for all 22 evaluated parameters. Here, analysis of variance indicated significant variations among genotypes for five morphological parameters (PLH, TNP, LNP, PDM, and SDM), five physiological parameters (SPAD, PWC, LWC, SWC and PKC) and three yield related parameters (NEP, NKE and TKW). Furthermore, significant genotype-by-treatment interactions were detected for five parameters, including PLH, TNP, PKC, NKE and NKP. With exception of PDM, SPAD, and TKW analysis of variance revealed significant differences among the year. Further results are presented in the Appendix.

### *3rd Drought stress scenario: drought treatment at anthesis*

The analysis of variance revealed significant main effects of the treatment for all 22 evaluated plant parameters (see Appendix). Significant genotypic variation existed for 17 plant parameters, in particular for five morphological (PLH, TNP, LNP, LDM and SDM), seven physiological (SPAD, PWC, LWC, SWC, PNC, PPhC and PKC) and five yield related plant parameters (NEP, NKE, NKP, TKW and YLD). In the 3rd drought stress scenario significant genotype-by-treatment interactions were observed for PLH, TNP, LWC, NKE, NKP, TKW and YLD. Except for PLH, TNP, PDM, and SPAD significant differences among years were found.

Overall, analysis of variance revealed for each drought stress scenario significant main effects of the year and genotype-by-year interactions. The magnitude of variation attributable to the year can be explained by different weather conditions between 2012 and 2013. Among the observed two-way interactions the genotype-by-treatment interaction was the most important one and will be presented thereafter.

## 3.3.2 Environmental conditions

To characterize environmental conditions and to regulate the irrigation of pots, weather stations continuously monitored meteorological data such as soil temperature, soil moisture, air temperature, relative humidity and solar radiation. In 2012, during the critical period of plant development, the average air temperature in May was 16.2 °C and in June 17.1 °C. Furthermore, the relative humidity was about 71%. Contrary to 2012, the average air temperature in May 2013 amounted only 13.5 °C. In both years, main plant growth period was between the beginning and the end of May (Day of year 130 - Day of year 150). In terms of air temperature, air humidity and solar radiation, climatic conditions differed during this period (Table 3.3). Thus, growing conditions in 2013 was characterized by a 5 °C lower air temperature, a 300 kW/m² lower solar radiation and an 11% higher humidity.

**Table 3.3** Climatic conditions between in 2012 and 2013 at the experimental research station Bonn, Germany. Recorded time period in both years: Day of year 130 - Day of year 150.

| Meteorological parameter | Unit | 2012 | 2013 |
|---|---|---|---|
| Air temperature | °C | 17 | 12 |
| Air humidity | % | 67.7 | 78.5 |
| Solar radiation | kW/m² | 954.1 | 654.3 |

### 3.3.3 Annual differences between evaluated plant parameters

Annual mean values of 22 traits evaluated in three contrasting drought stress scenarios revealed a clear differentiation between the two years of research (Table 3.4). Compared with annual means in 2012, plant performance in 2013 was characterized by an increase of TNP, LNP, LAP, LDM, PWC, PNC, NEP, NKP and finally YLD. Computed mean values of PKC in the 1st and 3rd drought stress scenario were under one percent in 2012, displaying a poor potassium supply. Data from Table 3.4 should be considered with the data in Table 3.3, which revealed that the advantageous plant growth in 2013 was attributable to favourable climatic conditions. Differences among years and treatments are shown in the Appendix. The following parts of the result section refers to the general means of morphological, physiological and yield-related parameters under control (well-watered) and drought treatment in two years of experimentation.

### 3.3.4 Effects of water shortage at different developmental stages on morphological plant parameters

Table 3.5 summarizes genotypic mean values of evaluated morphological plant parameters at different growth stages. Drought treatment at the end of the leaf development stage (DT-BBCH 19) caused primarily a reduction of LNP (25%), GLA (47%) and PDM (38%). The decrease of the above ground plant dry matter was mainly due to the reduction of the leaf dry matter (45%). Drought treatment at tillering stage (DT-BBCH 24) decreased TNP by 30%, PDM by 36% and GLA by 44%. At the same time, YLA increased by 53%. Genotypes in the 3rd drought stress scenario (DT-BBCH 49) produced under both irrigation levels the highest means for all morphological parameters. As part of the reduced watering in the 3rd drought stress scenario, mean values of LAP, GLA, PDM and SDM decreased over 35%, relative to genotypic means under well-watered conditions.

In summary, water deficit at various growth stages significantly reduced plant growth and differentiation processes. With the exception of the yellow leaf area (YLA), mean values of morphological parameters decreased under drought. The highest increase of YLA was noticed for genotypes in the 2nd drought stress scenario (DT-BBCH 24). Among nine evaluated plant parameters LAP, GLA, LDM and SDM decreased under drought treatment over 25%. Thereby, the observed decline of LNP, LAP, PDM and LDM in 1st drought stress scenario exceeded decreases of the 2nd and 3rd scenario. In all three drought stress scenarios TNP decreased under water deficit by one.

### 3.3.5 Effects of water shortage at different developmental stages on physiological plant parameters

It is apparent from Table 3.5 that drought treatment at the end of the leaf development sage (DT-BBCH 19) caused a decrease of the SPAD value by 29%, accompanied by a reduction of SWC by 7%, PNC by 56%, PPhC by 33% and PKC by 38%. Contrary to the 1st drought stress scenario, the decline of the SPAD value in the 3rd drought stress scenario amounted only 17%. At the same time, PNC decreased by 24%, PPhC by 19% and PKC by 16%. Furthermore, leaf water content (LWC) in 3rd drought stress scenario was reduced by 18% and exceeded LWC losses in the 1st and 2nd drought stress scenario. As shown in Table 3.5, drought between tillering and stem elongation significantly reduced the SPAD value and plant nutrient content. In contrast, water deficit during anthesis (DT-BBCH49) was characterized by high increases of leaf senescence and decreases in plant and leaf water content (PWC and LWC). Results showed that the plant nutrient content varied depending on the time period in which drought occurs. During the crop life circle and under well-watered conditions, the concentration of nitrogen and potassium decreased. Furthermore it is striking, that the plant potassium content examined in the 2nd drought stress scenario was generally higher than in 1st and 3rd drought stress scenario. In summary, drought between tillering and anthesis caused a decrease of PNC over 45 percent.

### 3.3.6 Effects of water shortage at different developmental stages on the grain yield and yield components

Data concerning grain yield and yield components are presented in Table 3.5. Globally, water deficit during the vegetation period resulted in serious grain yield reductions. Drought treatments between anthesis and grain filling (DT-BBCH 49) significantly decreased the grain yield by 55%. Thereby, the 21-day lasting drought treatment had a negative impact on grain yield components. Thus, drought conditions during this time period resulted in a reduction of numbers of ears per plant (NEP) by 29%, accompanied by a decline of number of kernels per ear (NKE) by 21% which led finally to severe losses in number of kernels per plant (NKP). TKW wasn't able to compensate losses of yield components. If drought occurs at the end of leaf development stage (DT-BBCH 19), observed decreases of NEP, NKE and NKP were lower. Hence, grain yield (YLD) losses were occasionally 39%. In the 2nd drought stress scenario YLD was reduced by 44%. Experiments in 2012 and 2013 showed that drought treatments between tillering and anthesis reduced the grain yield by decreasing the number of

kernels per ears. Late drought treatments (DT-BBCH 49) decrease the grain yield by decreasing the number of ears and the number of kernels per plant. In summary, drought treatments between the stem elongation and the beginning of the grain filling period decreases the grain yield over 40%.

**Table 3.4** Annual mean values for morphological, physiological and yield-related plant parameters of four spring barley cultivars grown under well-watered and drought conditions in three different drought stress scenarios.

| Trait | DT-BBCH 19 | | | | DT-BBCH 24 | | | | DT-BBCH 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012 | 2013 | Δ % [a] | | 2012 | 2013 | Δ % [a] | | 2012 | 2013 | Δ % [a] | |
| **PLH** | 36.6 | 45.0 | 22.9 | *** | 69.8 | 55.3 | -20.7 | ** | 78.5 | 80.4 | 2.5 | ns |
| **TNP** | 3.0 | 3.9 | 28.1 | *** | 2.6 | 4.0 | 51.1 | *** | 3.4 | 3.7 | 8.4 | ns |
| **LNP** | 13.8 | 19.7 | 43.5 | *** | 18.3 | 23.1 | 26.5 | ** | 22.8 | 25.4 | 11.6 | * |
| **LAP** | 105.3 | 262.7 | 149.4 | *** | 120.0 | 287.9 | 139.9 | *** | 152.3 | 313.1 | 105.7 | *** |
| **YLA** | 14.3 | 33.5 | 134.1 | *** | 30.1 | 46.0 | 52.8 | * | 38.0 | 61.6 | 62.1 | *** |
| **GLA** | 91.0 | 229.1 | 151.8 | *** | 89.9 | 241.9 | 169.1 | *** | 114.3 | 251.6 | 120.2 | *** |
| **PDM** | 0.7 | 1.2 | 78.0 | *** | 2.1 | 2.0 | -5.7 | ns | 3.5 | 3.7 | 4.6 | ns |
| **LDM** | 0.4 | 0.6 | 73.3 | *** | 0.4 | 0.8 | 84.9 | *** | 0.6 | 0.9 | 64.6 | *** |
| **SDM** | 0.3 | 0.6 | 82.7 | *** | 1.7 | 1.2 | -28.4 | * | 3.0 | 2.7 | -7.1 | * |
| **SPAD** | 28.4 | 37.7 | 32.5 | *** | 39.2 | 40.4 | 3.2 | ns | 41.9 | 42.3 | 1.0 | ns |
| **LS** | 29.4 | 29.4 | 0.0 | ns | 32.7 | 37.2 | 13.8 | * | 43.4 | 41.9 | -3.5 | * |
| **PWC** | 82.0 | 86.3 | 5.3 | *** | 72.9 | 83.4 | 14.4 | *** | 69.5 | 76.5 | 10.1 | *** |
| **LWC** | 81.8 | 86.5 | 5.8 | *** | 72.9 | 82.9 | 13.7 | *** | 69.4 | 75.5 | 8.8 | *** |
| **SWC** | 82.2 | 86.1 | 4.8 | *** | 72.9 | 83.7 | 14.8 | *** | 69.5 | 76.8 | 10.6 | *** |
| **PNC** | 1.9 | 2.8 | 47.6 | *** | 1.3 | 2.4 | 81.6 | *** | 1.4 | 2.0 | 39.4 | *** |
| **PPhC** | 0.6 | 0.6 | 3.6 | * | 0.5 | 0.6 | 21.3 | *** | 0.5 | 0.6 | 10.4 | *** |
| **PKC** | 0.3 | 4.2 | 1123 | *** | 2.4 | 3.9 | 60.8 | *** | 0.2 | 3.3 | 1432 | *** |
| **NEP** | 3.5 | 4.7 | 36.8 | *** | 3.3 | 5.3 | 60.0 | *** | 3.3 | 4.5 | 37.8 | *** |
| **NKE** | 21.6 | 24.1 | 11.3 | *** | 19.1 | 21.4 | 11.7 | ** | 20.7 | 22.3 | 7.7 | * |
| **NKP** | 70.9 | 103.8 | 46.4 | *** | 61.1 | 105.8 | 73.1 | *** | 63.0 | 91.1 | 44.5 | *** |
| **TKW** | 45.6 | 46.0 | 0.9 | ns | 45.8 | 47.9 | 4.5 | ns | 48.0 | 43.3 | -9.7 | *** |
| **YLD** | 3.2 | 4.8 | 48.8 | *** | 2.8 | 5.1 | 79.2 | *** | 3.0 | 4.0 | 34.0 | *** |

Where: Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis, Genotype: Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year = 2012, 2013, Δ % [a] (relative difference in percent):(Mean 2013 -Mean 2012)/ Mean 2013 *100; and significant at P value ≤ 0.05, ≤ 0.01, ≤ 0.001, ns: none significant, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 3.5** Mean values across two years for morphological, physiological and yield-related plant parameters of four spring barley cultivars grown under well-watered and drought conditions in three different drought stress scenarios.

| Trait | DT-BBCH 19 | | | | DT-BBCH 24 | | | | DT-BBCH 49 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet | Dry | Δ % [a] | | Wet | Dry | Δ % [a] | | Wet | Dry | Δ % [a] | |
| **PLH** | 43.6 | 38.0 | -12.9 | *** | 65.8 | 59.0 | -10.0 | * | 85.1 | 73.8 | -13.2 | *** |
| **TNP** | 3.9 | 3.0 | -23.4 | *** | 3.9 | 2.7 | -30.1 | *** | 4.1 | 3.0 | -26.1 | *** |
| **LNP** | 19.1 | 14.0 | -24.8 | *** | 23.0 | 18.0 | -20.1 | * | 26 | 22.2 | -14.6 | ** |
| **LAP** | 230.4 | 138.0 | -40.3 | *** | 242.6 | 165.0 | -31.8 | ** | 285.5 | 180.0 | -37.0 | *** |
| **YLA** | 21.1 | 27.0 | 26.7 | *** | 30.1 | 46.0 | 52.6 | * | 42.9 | 56.6 | 31.8 | *** |
| **GLA** | 209.3 | 111.0 | -47.0 | *** | 212.4 | 119.0 | -43.8 | *** | 242.5 | 123.0 | -49.2 | *** |
| **PDM** | 1.2 | 0.7 | -38.2 | *** | 2.5 | 1.6 | -35.8 | *** | 4.4 | 2.8 | -37.6 | *** |
| **LDM** | 0.63 | 0.4 | -44.5 | *** | 0.7 | 0.5 | -37.1 | *** | 0.9 | 0.6 | -31.8 | *** |
| **SDM** | 0.57 | 0.4 | -31.4 | *** | 1.8 | 1.1 | -35.2 | ** | 3.5 | 2.2 | -39.1 | *** |
| **SPAD** | 38.6 | 28.0 | -28.5 | *** | 43.9 | 36.0 | -18.7 | *** | 45.9 | 38.3 | -16.6 | *** |
| **LS** | 10.0 | 48.8 | 388 | *** | 13.1 | 61.6 | 370 | *** | 14.4 | 70.9 | 392 | *** |
| **PWC** | 86.5 | 82.0 | -5.3 | *** | 81.1 | 75.0 | -7.4 | *** | 77.2 | 68.9 | -10.7 | *** |
| **LWC** | 85.5 | 83.0 | -3.1 | *** | 81.1 | 75.0 | -7.9 | *** | 79.5 | 65.3 | -17.9 | *** |
| **SWC** | 87.3 | 81.0 | -7.3 | *** | 81.4 | 75.0 | -7.7 | *** | 76.5 | 69.8 | -8.8 | *** |
| **PNC** | 3.3 | 1.4 | -56.0 | *** | 2.4 | 1.3 | -46.0 | *** | 2.0 | 1.5 | -24.3 | *** |
| **PPhC** | 0.7 | 0.5 | -32.9 | *** | 0.7 | 0.5 | -29.2 | *** | 0.6 | 0.5 | -18.6 | *** |
| **PKC** | 2.8 | 1.7 | -38.4 | *** | 3.7 | 2.6 | -30.0 | *** | 1.9 | 1.6 | -15.9 | *** |
| **NEP** | 4.5 | 3.7 | -17.0 | ** | 4.7 | 3.9 | -15.4 | * | 4.5 | 3.2 | -29.0 | *** |
| **NKE** | 25.6 | 20.0 | -21.5 | *** | 23.7 | 17.0 | -29.0 | *** | 24.1 | 19.0 | -21.1 | *** |
| **NKP** | 103.8 | 71.0 | -31.7 | *** | 102.1 | 65.0 | -36.6 | *** | 100.9 | 53.2 | -47.3 | *** |
| **TKW** | 48.5 | 43.0 | -11.0 | *** | 49.8 | 44.0 | -11.7 | *** | 48.6 | 42.7 | -12.1 | *** |
| **YLD** | 5.0 | 3.1 | -38.7 | *** | 5.1 | 2.8 | -44.1 | *** | 4.9 | 2.2 | -55.0 | *** |

Where: Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis, Genotype: Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013, Δ % [a] ( relative difference in percent): (Dry-Wet) / Wet *100; and *, **, *** are significant at P value ≤0.05 ≤0.01 ≤0.001, ns: none significant, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 3.3.7 Multiple mean comparison of significant genotype-by-treatment interactions

In our study, drought treatments decreased in all examined drought stress scenarios the genotypic performance level. The combined analysis of variance exhibited significant genotype-by-treatment interactions, which are depicted in Figure 3.4.

In general, analysis of variance revealed in all three examined drought stress scenarios significant genotype-by-treatment interactions for plant height (PLH) and number of kernels per ear (NKE). In the 1st and 2nd drought stress scenario significant genotype-by-treatment interactions were observed for plant potassium content (PKC), while significant genotype-by-treatment interactions for tiller number per plant (TNP) were found in the 2nd and 3rd drought stress scenario.

Drought at the end of leaf development stage (1st drought stress scenario) reduced the plant height (PLH), tiller number per plant (TNP) and plant potassium content (PKC) by 13%, 23%, and 38%, respectively. Genotypic losses in number of kernels per ear ranged between 11% and 33%. Comparing the four genotypes of the 1st drought stress scenario it is striking, that 'Henrike' showed the lowest decrease for NKE (11%) and PKC (32%). In contrast, 'Morex' was characterized as drought sensitive spring barley genotype with high reductions of PLH (17%), TNP (30%), PKC (37%), and NKE (33%). The impaired performance level of 'Morex' increased under drought treatment between tillering stage and anthesis (2nd drought stress scenario). Here, water deficit caused for 'Morex' a decline in PLH of 13%, in TNP of 50% and in NKE of 44%. Contrary to 'Morex', 'Bojos' was able to maintain tillers. Thus, the TNP decrease of 'Bojos' amounted only 14%. If drought occurred between stem elongation and ripening (3rd drought stress scenario), water stress had a negative effect on the numbers of kernels per ear. Here, 'Morex' exhibited the greatest reductions with 27%, while 'Henrike' revealed the lowest sensitivity for water deficit with a decline in NKE of 10%. In general, the spring barley genotype 'Morex' displayed through all examined drought stress scenarios the highest sensitivity to water deficit. Contrary, the three genotypes 'Bojos', 'Scarlett' and 'Henrike' were able to achieve high means of PLH, TNP and NKE under water deficit conditions, which distinguished them from 'Morex'. Consequently, the observed significant genotype-by-treatment interactions can be attributed to the low genotypic performance level of 'Morex' under drought. Comparing the three drought stress scenarios, the widest range between treatment levels occurred in the 2nd drought stress scenario (DT-BBCH24).

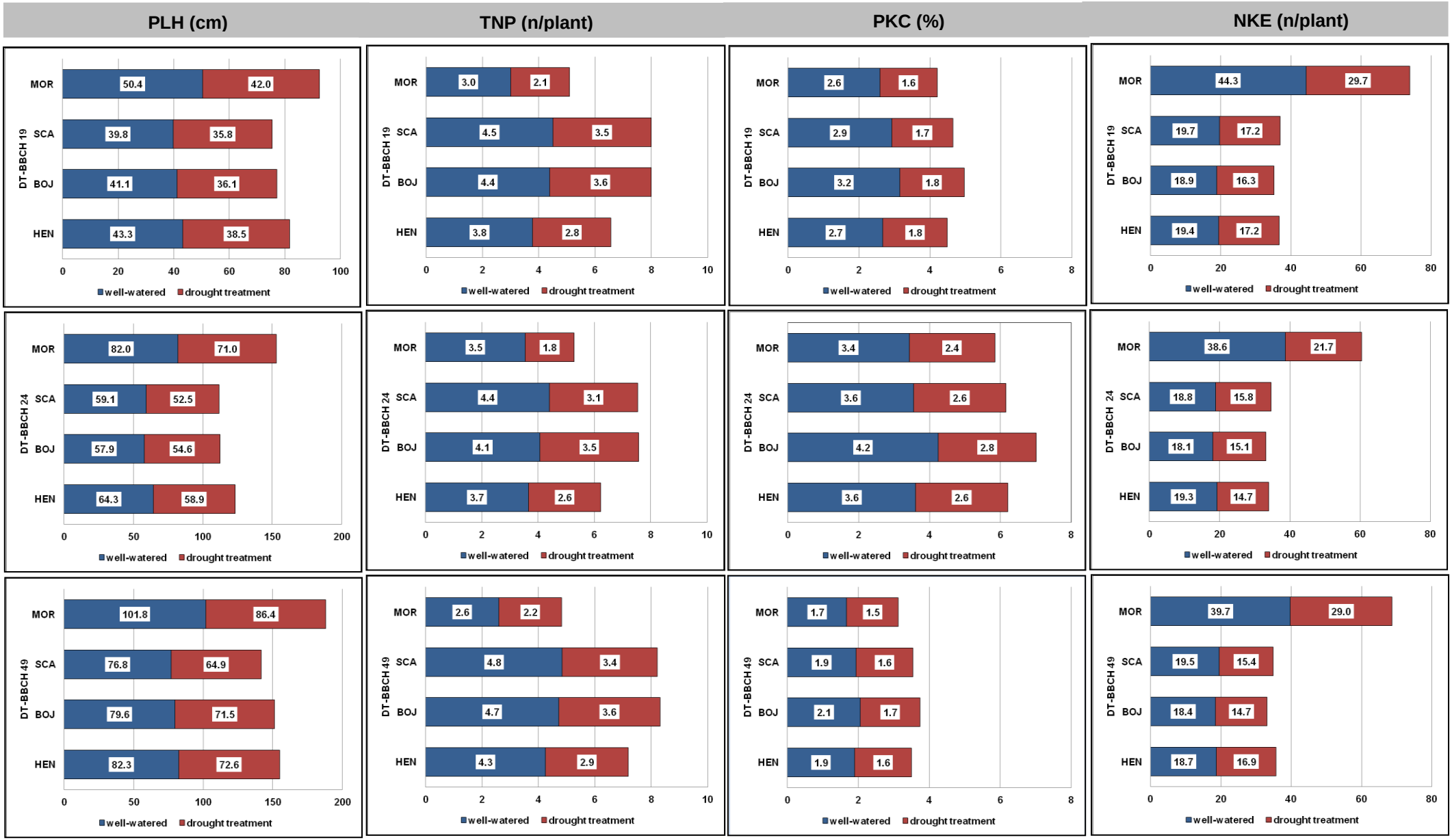


**Fig. 3.4. Mean values over two years of number of plant height (PLH), tillers per plant (TNP), plant potassium content (PKC), and number of kernels per plant (NKE) for four sping barley genotypes (MOR: Morex, SCA: Scarlett, BOJ: Bojos, HEN: Henrike) after 21-days of drought treatment.** DT-BBCH 19: drought treatment end of leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis.

### 3.3.8 Correlation analysis among evaluated plant parameters

Simple correlation analysis was used to assess the association between evaluated parameters. For three drought stress scenarios genetic correlation coefficients between evaluated pant parameters are summarized in the Appendix. Across examined drought stress scenarios the direction of association (positive or negative correlation coefficients) followed more or less the same trend.

In the 1st drought stress scenario (DT-BBCH 19) genotypic correlation analysis revealed significant, positive associations of the plant dry matter with LDM (0.76*), SDM (0.95***) and SPAD (0.74*). In contrast, PDM correlated negatively with LS (-0.84**). Furthermore, the tiller number per plant correlated highly with LNP (0.98***) and weak to moderate with plant nutrients, such as PNC (0.81*), PPhC (0.71*) and PKC (0.86**). Genotypic correlation coefficients of YLA with plant development traits such as PDM and SDM as well as physiological plant parameters (SPAD, PWC, LWC, SWC, PNC and PPhC) were negative, whereas the correlation of YLA with LS was positive.

Genotypic correlations coefficients between investigated traits of the 2nd drought stress scenario showed significant, positive correlations for TNP with physiological plant parameters including SPAD (0.81*), PWC (0.88**), SWC (0.88**), PNC (0.81*), PPhC (0.81*) and PKC (0.90**). Moreover, leaf number per plant exhibited significant correlations with PNC (0.71*) and PKC (0.76*). Leaf area per plant (LAP) was highly associated with GLA (0.98***) and only weakly with LDM (0.86*), SPAD (0.83*) and PKC (0.76*). PDM was mainly correlated with SPAD (0.79*) and LWC (0.83*), whereas negative associations were detected between PDM and LS (-0.80*).

Finally, correlation analysis of the 3rd drought stress scenario indicated highly, significant correlations of LDM with PWC (0.95***), SWC (0.95***), PNC (0.95***) and PKC (0.98***). Furthermore weak associations between LDM and TNP (0.83*), LNP (0.74*), LAP (0.83*) and GLA (0.83*) were observed. Except for YLA, negative correlations were detected between LS and three morphological parameters (LAP, GLA and LDM) and six physiological parameters (PWC, LWC SWC, PNC, PPhC and PKC). Relatively weak associations were found between green leaf area and PDM (0.76*), LDM (0.83*), LWC (0.81*), SWC (0.83*) and PKC (0.79*).

Overall, genotypic correlation analysis revealed that parameter which are related to biomass accumulation and productivity, e.g. TNP, LAP, GLA, PDM, are positive associated with leaf water content (LWC) and plant potassium content (PKC), whereas negative correlations were detected for leaf senescence (LS). The majority of significant correlations were detected when drought stress occurred between tillering and anthesis (DT-BBCH 24). Exceedingly few significant correlations were observed in the 3rd drought stress scenario.

### 3.3.9 Correlation analysis among evaluated plant parameters and grain yield components

Sperman's rank correlation coefficients ($r_g$) between genotypic mean values of grain yield components and morphological plant parameters as well as physiological plant parameters are summarized and presented thereafter (Table 3.6 – Table 3.8).

For the 1st drought stress scenario associations between evaluated plant parameters and grain yield components are displayed in Figure 3.5. Positive and highly significant genetic associations were detected for NEP with TNP (0.95***) and LNP (0.93***). NEP associated weekly with LDM (0.71*) and PKC (0.83*). The number of kernels per plant was mainly correlated with physiological plant parameters. Here, NKP was highly significant associated with PWC (0.98***) and LWC (0.98***) and moderate with YLA (-0.90**), LS (-0.84**), SWC (0.90**) and PPhC (0.83*), while weekly correlations were detected with LAP (0.71*), GLA (0.81*), SDM (0.76*) and PNC (0.81*). Furthermore, correlation analysis revealed moderate significant correlations between YLD and three morphological plant parameters, including LAP (0.86**), PDM (0.86**) and LDM (0.90**) (Table 3.6). Among investigated physiological plant parameters weekly genetic associations were found for YLD with SPAD (0.79*), LS (-0.79*) and PKC (0.74*). Overall, correlation analysis for the 1st drought stress scenario showed that NEP and YLD are mainly associated with morphological plant parameters, whereas physiological plant parameters are primary correlated with NKP.

For the 2nd drought stress scenario genotypic coefficients of correlations are given in Table 3.7. Figure 3.6 visualize significant correlations between evaluated traits. Here, NEP correlated highly significant with LNP (0.95***), but only weekly with TNP (0.81*) and PKC (0.76*). Moderate to high significant correlations were found for NKP with physiological parameters, such as PPhC (0.98***), PNC (0.88**) and SWC (0.86**). The grain yield showed positive genetic associations with morphological and physiological parameters,

including: LDM (0.90**) and LS (-0.86**). Furthermore, week correlations were ascertained for TNP (0.74*), LAP (0.71*), GLA (0.76*), PDM (0.81*), SDM (0.81*), LWC (0.76*), PNC (0.79*) and PKC (0.74*).

Finally, in the 3rd drought stress scenario positive and significant correlations were observed between NEP and LNP (0.83*), TNP (0.90**), LDM (0.90**) and PKC (0.95***). It is apparent from Table 3.8 that NKP was significant associated with LAP (0.83*), GLA (0.83*), LDM (0.83*), PWC (0.86**), LWC (0.83*) and SWC (0.86**). As shown in Figure 3.7, correlations were significant between the grain yield and morphological parameters such as: LAP (0.86**), YLA (-0.79*), GLA (0.86*), PDM (0.76*), LDM (0.79*) and SDM (0.74*). Seven physiological plant parameters showed significant associations with the grain yield, including: LS (-0.90**), PWC (0.71*), LWC (0.76*), SWC (0.71*), PPhC (0.76*) and PKC (0.71*).

Overall, genetic correlation analysis for different drought stress scenarios indicated that grain yield was differently associated with morphological and physiological plant parameters during the crop life circle. Regarding physiological plant traits, genotypic correlations were significant for LWC, PNC, PPhC and PKC in the time period between tillering and beginning of the grain filling. If drought occurs at the end of the leaf development stage (DT-BBCH 19), grain yield was mainly positive correlated with morphological plant parameters. With later developmental stages, weaker correlations of grain yield with morphological plant parameters were found. Simultaneously, the amount of detected significant correlations between grain yield and physiological plant parameters increased.

**Table 3.6** Spearman's correlation coefficient ($r_g$) among evaluated plant parameters of four spring barley genotypes of the 1st drought stress scenario (DT-BBCH 19).

| | NEP | | NKE | | NKP | | TKW | | YLD | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | -0.17 | | 0.81 | * | 0.74 | * | 0.07 | | 0.60 | |
| **TNP** | 0.95 | *** | -0.10 | | 0.43 | | 0.60 | | 0.48 | |
| **LNP** | 0.93 | *** | -0.05 | | 0.40 | | 0.64 | | 0.50 | |
| **LAP** | 0.57 | | 0.26 | | 0.71 | * | 0.52 | | 0.86 | ** |
| **YLA** | -0.19 | | -0.76 | * | -0.90 | ** | -0.21 | | -0.71 | * |
| **GLA** | 0.43 | | 0.52 | | 0.81 | * | 0.38 | | 0.76 | * |
| **PDM** | 0.21 | | 0.69 | | 0.76 | | 0.48 | | 0.86 | ** |
| **LDM** | 0.71 | * | 0.14 | | 0.57 | | 0.79 | * | 0.90 | ** |
| **SDM** | 0.21 | | 0.76 | * | 0.76 | * | 0.48 | | 0.81 | * |
| **SPAD** | 0.38 | | 0.60 | | 0.69 | | 0.62 | | 0.79 | * |
| **LS** | -0.47 | | -0.64 | | -0.84 | ** | -0.47 | | -0.79 | * |
| **PWC** | 0.38 | | 0.57 | | 0.98 | *** | 0.10 | | 0.64 | |
| **LWC** | 0.26 | | 0.69 | | 0.98 | *** | 0.10 | | 0.69 | |
| **SWC** | 0.48 | | 0.57 | | 0.90 | ** | 0.24 | | 0.57 | |
| **PNC** | 0.67 | | 0.43 | | 0.81 | * | 0.40 | | 0.62 | |
| **PPhC** | 0.52 | | 0.52 | | 0.83 | * | 0.36 | | 0.64 | |
| **PKC** | 0.83 | * | 0.12 | | 0.69 | | 0.60 | | 0.74 | * |

Where: *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001

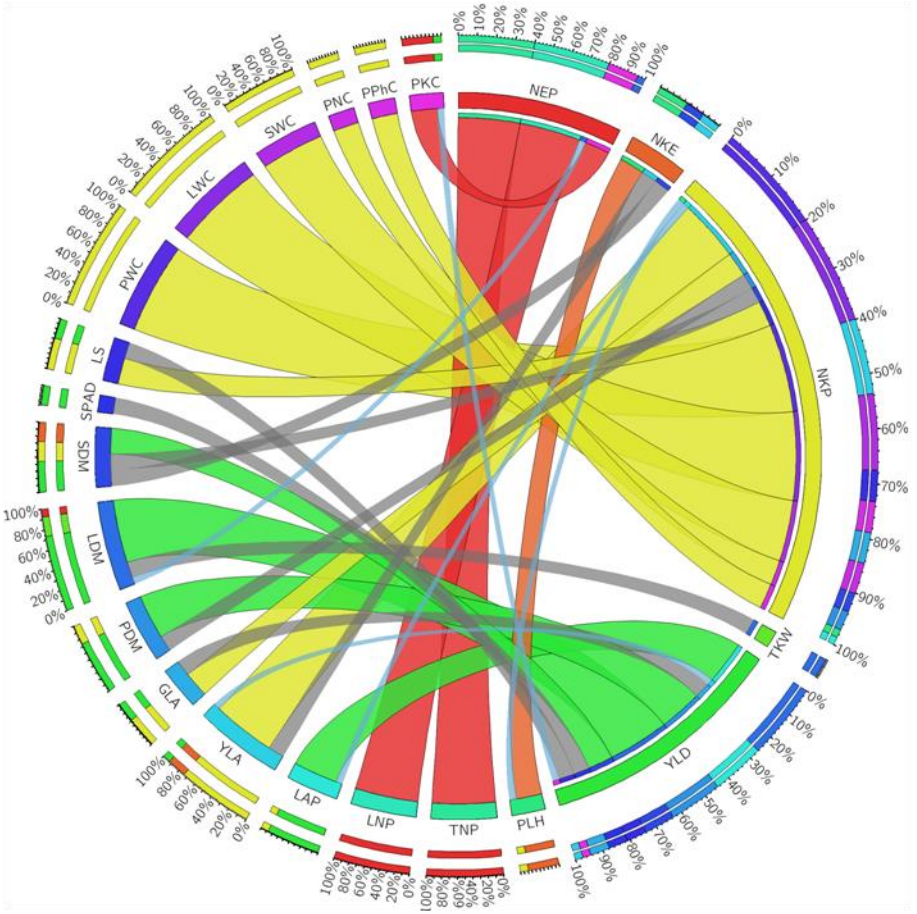


**Fig.3.5. Summary of the results obtained by correlation analysis for the 1st drought stress scenario.** The circos plot summarizes all significant coefficient of variation for morphological, physiological and yield related plant parameters. Thickness of the lines corresponds with correlation strength. Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 3.7** Spearman's correlation coefficient ($r_g$) among evaluated plant parameters of four spring barley genotypes of the 2nd drought stress scenario (DT-BBCH 24).

| | NEP | | NKE | | NKP | | TKW | | YLD | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | -0.45 | | 0.83 | * | 0.26 | | 0.17 | | 0.33 | |
| **TNP** | 0.81 | * | 0.19 | | 0.79 | * | 0.67 | | 0.74 | * |
| **LNP** | 0.95 | *** | -0.05 | | 0.60 | | 0.57 | | 0.60 | |
| **LAP** | 0.43 | | 0.52 | | 0.67 | | 0.67 | | 0.71 | * |
| **YLA** | -0.29 | | -0.76 | * | -0.74 | * | -0.48 | | -0.83 | * |
| **GLA** | 0.40 | | 0.55 | | 0.64 | | 0.69 | | 0.76 | * |
| **PDM** | 0.12 | | 0.79 | * | 0.71 | * | 0.52 | | 0.81 | * |
| **LDM** | 0.43 | | 0.38 | | 0.71 | * | 0.76 | * | 0.90 | ** |
| **SDM** | 0.12 | | 0.79 | * | 0.71 | * | 0.52 | | 0.81 | * |
| **SPAD** | 0.52 | | 0.62 | | 0.79 | * | 0.52 | | 0.83 | * |
| **LS** | -0.58 | | -0.58 | | -0.75 | * | -0.59 | | -0.86 | ** |
| **PWC** | 0.60 | | 0.26 | | 0.76 | * | 0.60 | | 0.60 | |
| **LWC** | 0.40 | | 0.45 | | 0.60 | | 0.74 | * | 0.76 | * |
| **SWC** | 0.60 | | 0.45 | | 0.86 | ** | 0.38 | | 0.57 | |
| **PNC** | 0.67 | | 0.43 | | 0.88 | ** | 0.43 | | 0.79 | * |
| **PPhC** | 0.50 | | 0.40 | | 0.98 | *** | 0.40 | | 0.81 | * |
| **PKC** | 0.76 | * | 0.14 | | 0.71 | * | 0.74 | * | 0.74 | * |

Where: *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001

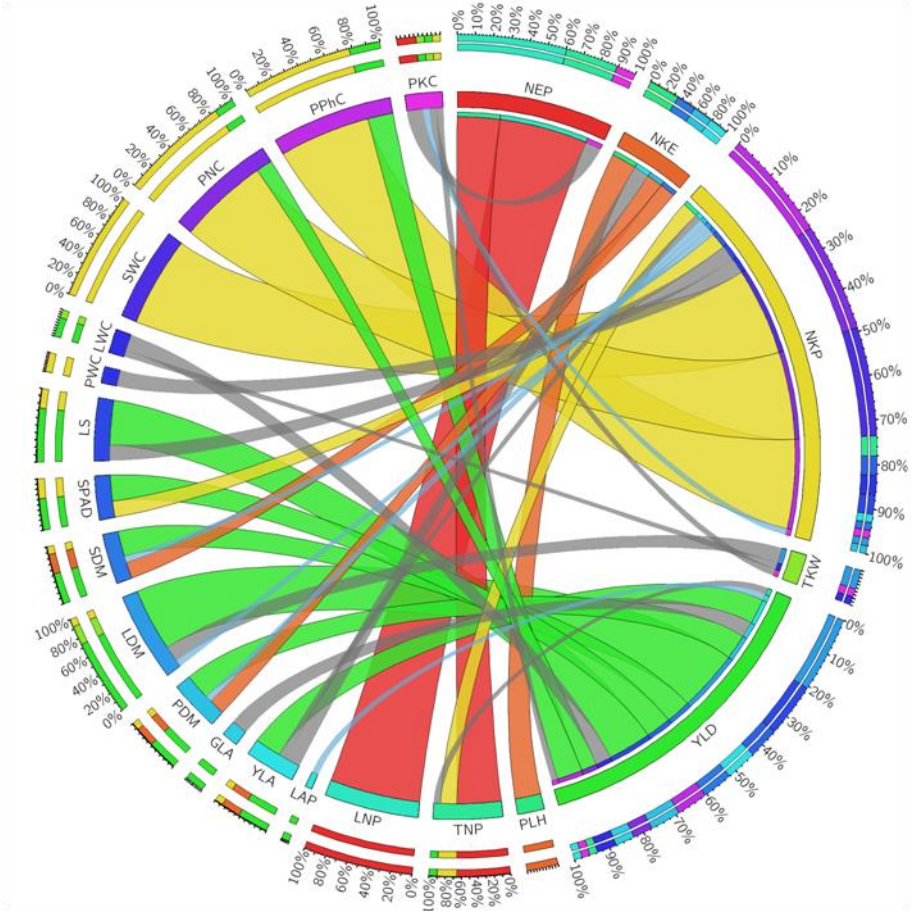


**Fig.3.6. Summary of the results obtained by correlation analysis for the 2nd drought stress scenario.** The circos plot summarizes all significant coefficient of variation for morphological, physiological and yield related plant parameters. Thickness of the lines corresponds with correlation strength. Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 3.8** Spearman's correlation coefficient ($r_g$) among evaluated plant parameters of four spring barley genotypes of the 3rd drought stress scenario (DT-BBCH 49).

| | NEP | NKE | NKP | TKW | YLD |
|---|---|---|---|---|---|
| **PLH** | -0.12 | 0.90 ** | 0.40 | 0.33 | 0.43 |
| **TNP** | 0.90 ** | -0.26 | 0.43 | 0.36 | 0.48 |
| **LNP** | 0.83 * | -0.48 | 0.36 | 0.02 | 0.26 |
| **LAP** | 0.71 * | 0.38 | 0.83 * | 0.48 | 0.86 ** |
| **YLA** | -0.38 | -0.60 | -0.79 * | -0.48 | -0.79 * |
| **GLA** | 0.71 * | 0.38 | 0.83 * | 0.48 | 0.86 * |
| **PDM** | 0.29 | 0.79 * | 0.74 * | 0.55 | 0.76 * |
| **LDM** | 0.90 ** | 0.12 | 0.83 * | 0.38 | 0.79 * |
| **SDM** | 0.24 | 0.83 * | 0.69 | 0.57 | 0.74 * |
| **SPAD** | 0.26 | 0.83 * | 0.69 | 0.38 | 0.64 |
| **LS** | -0.74 * | -0.26 | -0.81 * | -0.50 | -0.90 ** |
| **PWC** | 0.79 * | 0.29 | 0.86 ** | 0.19 | 0.71 * |
| **LWC** | 0.43 | 0.62 | 0.83 * | 0.33 | 0.76 * |
| **SWC** | 0.79 * | 0.29 | 0.86 ** | 0.19 | 0.71 * |
| **PNC** | 0.83 * | 0.19 | 0.76 * | 0.38 | 0.71 * |
| **PPhC** | 0.81 * | 0.21 | 0.74 * | 0.45 | 0.76 * |
| **PKC** | 0.95 *** | -0.05 | 0.74 * | 0.33 | 0.71 * |

Where: *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001

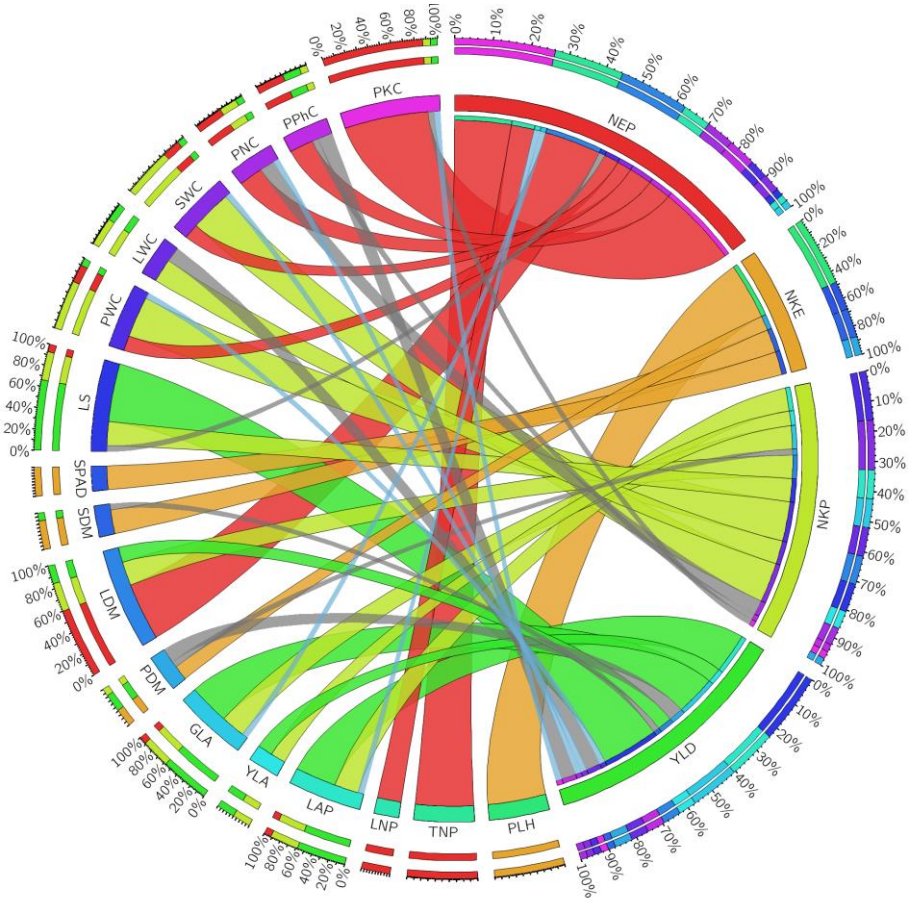


**Fig.3.7. Summary of the results obtained by correlation analysis for the 3rd drought stress scenario.** The circos plot summarizes all significant coefficient of variation for morphological, physiological and yield related plant parameters. Thickness of the lines corresponds with correlation strength. Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 3.3.10 Evaluation of investigated drought stress scenarios

Concerning morphological, physiological and yield related plant parameters, the stress tolerance index (STI) was used to assess the drought stress tolerance of four spring barley genotypes. ANOVA analysis of STI-values for 22 evaluated plant parameters revealed for LNP, SPAD and PNC significant variations among the scenarios (see Appendix). Here, STI-values of LNP, SPAD and PNC increased steadily from the 1st drought stress scenario to the 3rd drought stress scenario (Fig. 3.8). Nevertheless, differences between the drought stress scenarios were non-significant for the majority of plant parameters. Highly significantly variations ($P \leq 0.001$) among genotypes were observed for six morphological parameters (PLH, TNP, LNP, PDM, LDM, and SDM), five physiological parameters (SPAD, PWC, LWC, SWC, and PKC) and three yield-related parameters (NEP, NKE, and TKW). These results agree with data obtained from analysis of variance for individual drought stress scenarios, which showed significant genotype-by-treatment interactions for PLH, TNP, PKC and NKE (Fig. 3.4). Significant genotype-by-scenario interactions were observed for LDM and NKE.

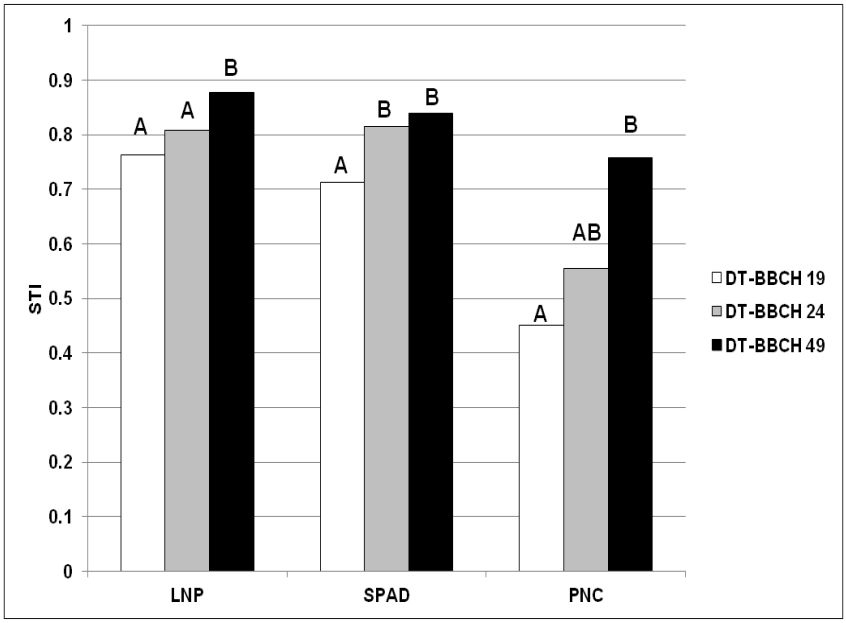


**Fig. 3.8. Effects of drought treatments at different growth stages on stress tolerance index (STI) of numbers of leaves per plant (LNP), SPAD value (SPAD), and plant nitrogen content (PNC).** The values presented are mean values for each drought stress scenario. Scenario means followed by different letters within a given plant parameter are significantly different (p-value ≤ 0.05; Tukey's HSD).

***Ranking of genotypes for stress tolerance (STI)***

Ranking of genotypes were performed according to calculated STI-values of 22 evaluated traits. A genotype with the highest stress tolerance was given a rank value of one and considered as drought tolerant, while a genotype with the lowest stress tolerance was assigned a rank value of four. Table 3.9 display the rankings of four spring barley genotypes for six morphological, five physiological and three yield related parameters. In particular, the table below presents those parameters which showed in the earlier elucidated analysis of variance for STI-values significant differences among genotypes. Globally, the genotype 'Bojos' exhibited the highest stress tolerance for two morphological plant parameters (TNP and LNP ), four physiological parameters (PWC, LWC, SWC and PKC) and one yield related trait (NEP). By contrast, 'Morex' was ranked as the most sensitive genotype for drought stress. Comparing the assigned rank values through all drought stress scenarios 'Morex' was given a rank value of 4 for TNP, LNP, LDM, PKC, NEP and TKW.

With exception of the leaf dry matter and number of kernels per ear, changes in ranking of genotypes in response to drought treatments at different growth stages were none significant. Especially, morphological parameters such as PLH, TNP, LNP and PDM revealed a nearly constant ranking of genotypes over all three drought stress scenarios (Table 3.9). Nevertheless, significant genotype-by-scenario interactions for leaf dry matter and number of kernels per ear suggest that not all genotypes respond similar to drought at different developmental stages. As can be seen from the data in Table 3.9, the genotype 'Henrike' showed in the 1$^{st}$ and 2$^{nd}$ drought stress a fairly constant and superior drought stress tolerance on leaf dry matter. However, in the 3$^{rd}$ drought stress scenario 'Henrike' demonstrate higher drought stress sensitivity for LDM. Through all three investigated drought stress scenarios rankings of 'Bojos' for LDM ranged between one and three, while 'Morex' showed the highest drought sensitivity. In addition, statistically significant variations between genotypic STI-values were observed for number of kernels per ear (NKE). Thus, 'Scarlett' and 'Morex' tend to have a higher stress tolerance for NKE, whereas 'Henrike' and 'Bojos' were more sensitive to drought.

**Table 3.9** Ranking of four spring barley genotypes based on stress tolerance indices (STI) for morphological, physiological and yield- related parameters. Ranking order from 1 to 4 assigned according to the performance level from high to low.

| Trait | Henrike | | | Bojos | | | Scarlett | | | Morex | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st DT | 2nd DT | 3rd DT | 1st DT | 2nd DT | 3rd DT | 1st DT | 2nd DT | 3rd DT | 1st DT | 2nd DT | 3rd DT |
| **PLH** | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 1 | 1 | 1 |
| **TNP** | 3 | 3 | 3 | 1 | 1 | 1 | 2 | 2 | 2 | 4 | 4 | 4 |
| **LNP** | 3 | 3 | 3 | 2 | 2 | 1 | 1 | 1 | 2 | 4 | 4 | 4 |
| **PDM** | 1 | 2 | 2 | 4 | 4 | 4 | 3 | 3 | 3 | 2 | 1 | 1 |
| **LDM** | 1 | 1 | 3 | 2 | 3 | 1 | 3 | 2 | 2 | 4 | 4 | 4 |
| **SPAD** | 3 | 3 | 3 | 4 | 4 | 4 | 1 | 1 | 2 | 2 | 2 | 1 |
| **PWC** | 4 | 4 | 4 | 2 | 1 | 1 | 3 | 2 | 2 | 1 | 3 | 3 |
| **LWC** | 3 | 1 | 3 | 2 | 2 | 1 | 4 | 4 | 4 | 1 | 3 | 2 |
| **SWC** | 4 | 4 | 4 | 1 | 1 | 1 | 3 | 2 | 2 | 2 | 3 | 3 |
| **PKC** | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 3 | 2 | 4 | 4 | 4 |
| **NEP** | 3 | 3 | 3 | 1 | 2 | 1 | 2 | 1 | 2 | 4 | 4 | 4 |
| **NKE** | 4 | 3 | 2 | 2 | 4 | 4 | 3 | 2 | 3 | 1 | 1 | 1 |
| **TKW** | 1 | 1 | 1 | 3 | 3 | 2 | 2 | 2 | 3 | 4 | 4 | 4 |

Where: 1st DT: drought treatment at the end of the leaf development stage, 2nd DT: drought treatment at tillering stage, 3rd DT: drought treatment at anthesis, Genotype: Scarlett, Morex, Bojos, Henrike, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, PDM: plant dry matter, LDM: leaf dry matter, SPAD: SPAD value, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, TKW: thousand kernel weight.

## 3.4 Discussion

The main concern of this study was to investigate the impact of growth stage specific water deficit on plant development and yield formation. Across three drought stress scenarios, nine morphological, eight physiological and five yield-related parameters were analyzed. Additionally, current study aimed to identify valuable traits which are useful to compare individual drought stress scenarios. The discussion in this chapter is structured in four parts. First, the effect of water deficit on crop growth and development across three droughts stress scenarios will be clarified. The second part is focused on the grain yield and yield performance under different drought treatments during the plant life circle. Afterwards the relationship between investigated plant parameters and yield formation will be further discussed. Finally, the fourth part debates in general the examined drought stress scenarios and genotypic differences in drought stress tolerance at different growth stages.

### 3.4.1 Effect of water shortage at different developmental stages on crop growth and development

***Morphological plant parameters***

Mean values of nine morphological plant parameters were calculated for three contrasting drought stress scenarios (Table 3.5). Compared to well-watered conditions, PLH, TNP, LNP, LAP, GLA, PDM, LDM and SDM remarkable decreased under drought at different growth stages. The results are in good agreement with other studies which have shown that drought caused reductions in plant height, number of tillers and leaf area (Blum et al. 1990; Çakir 2004; Bayoumi et al. 2008). Specific traits which are related to biomass accumulation, such as number of tillers per plant (TNP), leaf area per plant (LAP), leaf dry matter (LDM) and stem dry matter per plant (SDM), showed under water shortage a decrease over 20%, suggesting that these traits are very sensitive to water deficit during the vegetation period and can provide useful information concerning drought tolerance in spring barley. Interestingly, decreases in green leaf area remained relatively constant across the drought stress scenarios, whereas the yellow leaf area increased according to the growth stages in which drought treatments occurred. A closer look at gathered data revealed that water stress applied at different growth stages reduced the leaf area, leaf expansion and photosynthetic activity of plants through various ways (Blum 1996). Decreasing water availability under drought treatment at the end of the leaf development stage (1$^{st}$ drought stress scenario) limited

leaf expansion and photosynthesis by reductions in leaf number per plant, green leaf area per plant and finally plant dry matter (Farooq et al. 2009). According to Passioura (1996), leaf extension can be reduced under drought conditions in order to get a balance between water absorbed by roots and the water status of plant tissue. Chaves (2002) reported that water stress caused large decreases in rate of photosynthesis at leaf levels due to stomatal closure. Furthermore, Chaves (2002) pointed out that the stomatal response to water deficit is often more closely related to the soil moisture content than to the leaf water statutes, which indicates that stomata responding to chemical signals (e.g. ABA). A further study with focus on biochemical pathways under drought conditions is therefore suggested. Water deficit between tillering and anthesis (2$^{nd}$ drought stress scenario) resulted in impaired leaf expansion and photosynthetic reductions by declines in tiller formation and green leaf area, while yellow leaf area and thus, premature leaf senescence increased (Blum et al. 1990; Farooq et al. 2009). The present findings accords with research of Blum (Blum et al. 1990; Blum 1996) who reported that leaf area of wheat was determined under drought by the degeneration of existing tillers and the total termination of the formation of new tillers. Results of this study clearly demonstrate that water deficit during vegetative and reproductive growth stage suppressed the development of leaf area and plant dry matter. Similar findings were reported in previous studies in spring wheat and other crops (Blum et al. 1990; Christen et al. 1995; Teulat et al. 1997; Bayoumi et al. 2008). Çakir (2004) who studied the effect of water stress imposed at different growth stages on growth and yield formation in corn (*Zea mays* L.) argued that decreases in plant dry matter due to water stress were induced by a decline in plant extension growth, delayed leaf tip emergency and limited leaf size.

Overall, the present work demonstrated that water deficit at the end of the leaf development (1$^{st}$ drought stress scenario) caused the greatest reduction in LNP and LDM. It can therefore be assumed that in early stages of the plant development the leaf number preliminary modifies the leaf area, which is of particular importance to sustain a high photosynthesis under water shortage. Contrary, genotypes exposed to decreasing water availability during stem elongation and anthesis (2$^{nd}$ and 3rd drought stress scenario) showed the general tendency to reduce their leaf area through the degeneration of existing tillers and/ or the inhibition of the appearance of new tillers (Blum et al. 1990; Blum 1996). Hence, the most important finding in this study was that the leaf area and hence photosynthetic capacity is determined by the plant plasticity in leaf area formation (Blum 1996). To assess the impact on how plants cope with water deficit leaf number, tiller number and leaf area provides valuable information on drought adaption strategies.

***Physiological plant parameters***

The adequate water supply of plants is important for many physiological processes and therefore for the maintenance of plant growth and productivity. Decreasing availability of water under drought conditions has a considerable impact on photosynthesis, nutrient uptake by roots and nutrient transport from roots to shoots due to the restricted transpiration rates (Hu and Schmidhalter 2005; Farooq et al. 2009).

A major effect of the decreasing water availability in the 1st drought stress scenario was the impairment of the plant productivity by decreasing the nutrient availability, uptake and leaf chlorophyll content, which is closely linked to the SPAD value (Markwell et al. 1995; Netto et al. 2005; Farooq et al. 2009; del Pozo et al. 2012; Ahmed et al. 2013). In particular, water deficit during the 1st drought stress scenario (BBCH 19 - BBCH 32) had a significant negative effect on the plant nitrogen, phosphor and potassium content. In contrast, genotypes which were exposed to drought treatments at tillering stage (2nd drought stress scenario) and anthesis (3rd drought stress scenario) showed a lower decline in plant nutrient and chlorophyll content. Simultaneously, drought treatments imposed at tillering stage and anthesis reduced the transpiration rate by a decrease in relative plant water content (Blum 1996). These findings agree with previous research of Farooq et al. (2008) and Siddique et al. (2000) who observed that the relative water content of wheat leaves was higher during the leaf development and declined as plants accumulated dry matter and leaves matured. Teulat et al. (1997) who studied the relationship between relative water content and growth parameters in barley reported that water stress applied at the beginning of tillering stage decreased the relative water content of leaves. According to Teulat et al. (1997) the relative water content is a suitable criterion for drought tolerance improvement.

The present study showed that mean values of plant nitrogen, phosphor and potassium content considerably decreased under water deficit, especially if drought occurs during the leaf development (1st drought stress scenario). In general, potassium is required for protein synthesis, photosynthesis and maintaining of the turgor pressure in plants under water stress (Hu and Schmidhalter 2005). Currently, it is emphasized by several researchers that potassium increases the plant drought resistance by its function in stomatal regulation, osmoregulation, maintenance of photosynthesis and protection of chloroplasts from oxidative damage (Cakmak 2005; Hu and Schmidhalter 2005; Aown et al. 2012). According to Leigh et al. (1984), the potassium concentration in well supplied plants is about 200 mM or rather 6% in plant dry matter. Nevertheless, Leigh et al. (1984) emphasis that for near maximum plant

growth a potassium concentration of about 2% in dry matter is required, which cooperates well with our findings presented in Table 3.5. Jensen and Tophøj (1985) who investigated the interaction of different potassium levels and water stress on barley yield response found that that an increase in potassium application cause a significant rise of tissue water content in plants which was again highly and positively correlated with the final grain yield. They furthermore pointed out that potassium improved the plant water status during soil water stress. The positive effect of potassium fertilization on plants was also reported by Jouany et al. (1996) who have demonstrated that an insufficient potassium supply depressed grain yields in cereals-oilseeds-legumes rotations. Unlike the published results of Schittenhelm et al. (2014) dealing with drought stress in cereals from tillering to harvest, potassium concentration wasn't increasing under drought conditions. Taken together, present findings emphasise the importance of potassium for turgor regulation and thus for cell extension and growth (Jensen and Tophøj 1985). Results of this study underline the importance of a balanced potassium fertilization to increase the efficiency of crops in utilization available water (Farooq et al. 2009). The potassium availability in soils varies depending on the soil type and environmental conditions. Since readily available potassium is dissolved in soil water or rather held on surfaces of clay particles most of the sandy soil types with low clay content and buffer capacity are characterized by potassium leaching (Kolahchi and Jalali 2007). Additionally, losses of soil potassium increase in response to irrigation of sandy soils. Consequently, plants grown on sandy soils require higher rates of potassium. The soil used in phenotyping experiments 2011, 2012 and 2013 contained a mixture of top soil, silica sand, milled lava and peat dust. In view of the above considerations, results of the current study led to the assumption that sandy soils require a higher level of plant available potassium. From agronomical point of view high-potassium fertilizers such as potassium chloride are recommended for future phenotyping experiments. Potassium-magnesium sulphates are recommended when there is a higher need for magnesium. Besides potassium, the application of nitrogen (N) and phosphor (P) fertilizer might improve the plant growth under drought conditions (Hu and Schmidhalter 2005). Nielsen and Halvorson (1991) reported that increasing N applications stimulate the biomass production and root growth of winter wheat under mild water stress. Nevertheless, Nielsen and Halvorson (1991) showed that increasing amounts of N fertilizer under moderate to serve drought conditions increased levels of water stress caused by the excessive transpirational demand of the resulting larger leaf area and the increased root volume. Under drought stress, positive effects of P application on chlorophyll content, photosynthesis, biomass accumulation and grain yield have been reported by

Ackerson (1985) and Garg et al. (2004). However, research studies of Masoni et al. (2007) showed that durum wheat growth on clay-loam soils favoured the plant dry matter, N and P accumulation as well as the grain yield, compared to the durum wheat growth on sandy-loam soils. Graciano et al. (2005) investigated the response of *Eucalyptus grandis* to water deficit under different soil types and N and P fertilizer. They reported that water stress tolerance strategies are related to the fertilization and soil properties, and that P fertilization on sandy soils is only recommended under adequate water supply.

Among three examined drought stress scenarios water deficit at different growth stages decreased the SPAD value and hence the chlorophyll content in the flag leaf. Arunyanark et al. (2008) reported that the decline in chlorophyll content is accompanied by reduction in photosynthesis and might be responsible for decreases in the plant dry matter. The greatest decrease in SPAD value (-28.5%) was observed between the end of the leaf development stage and stem elongation (1$^{st}$ drought stress scenario) suggesting that the SPAD value is associated with leaf photosynthetic capacity and that plants with a higher SPAD values usually have a higher nitrogen content (see correlation matrix in the Appendix). However, although observed reduction of SPAD, PNC, PPhC and PKC were the greatest in the first and second drought stress scenario, the highest yield reductions were recorded for water deficit occurred after anthesis (3$^{rd}$ drought stress scenario).

### 3.4.2 Effect of water shortage at different developmental stages on grain yield and yield components

Drought treatments imposed at different growth stages reduced significantly the grain yield and yield components. In accordance to previous studies, the formation of the grain yield depends on number of ears per plant, number of kernels per ear, number of kernels per plant and thousand kernel weight (Blum et al. 1990; Christen et al. 1995; Samarah 2005).

While Christen et al. (1994) reported that water deficit between stem elongation (BBCH 31) and anthesis (BBCH 49) slightly increased the grain yield, data obtained in the present study indicated that water deficit during the mentioned time period caused a negative impact on the number of kernels per ear (-29%) and the number of kernels per plant (-36.6%), resulting in a grain yield decline of 44.1% (Table 3.5). Gathered data of this study are in good agreement with published results of Ugate et al. (2007) who analyzed the effect of thermal treatments on grain yield at different pre-anthesis stages. Here, heat stress implied between stem elongation and booting caused a yield reduction of 41%. Moreover, it is apparent from

the 2nd drought stress scenario that the number of kernels per ear was more reduced during stem elongation and anthesis. These findings are in agreement with the findings of Mogensen et al. (1985).

Additionally, realized experiments demonstrated that water deficit between leaf development (BBCH 19) and stem elongation (BBCH 32) resulted in a reduction of NEP (-21.5%) and NKP (-31.7%) which finally lead to a yield decline of 38.7%. The later plants were exposed to drought treatments, the greater the yield losses (DT-BBCH 49). In fact, the reduction of NEP was almost 50% higher than NEP reductions in prior drought stress scenarios. In addition decreases of NKP caused by water deficit resulted in a grain yield decline of 55%. Similar yield decreases were found by Mogensen et al. (1985).

Comparing the examined drought stress scenarios it is obvious that water deficit in the 1st drought stress scenarios produced relatively low yield losses (-38.7%). Interestingly, Blum et al. (1990) studied the effect of drought and reported that yield recovery after stress basically depends on NEP and NKE. Blum et al. (1990) explained that yield recovery was less successful for drought treatments occurred in later tillering stages. From the results presented in this work and previous findings of several scientists it can be assumed that the sensitivity of grain yield to water shortage relies on the growth stage in which is water stress occurs (Christen et al. 1995; Çakir 2004; Ugarte et al. 2007). In conformity with research of Mogensen et al. (1985) drought sensitivity of plants was greatest when water stress occurred between anthesis and heading. An important issue from these findings is that drought stress at various growth stages influenced the grain yield components differently. Therefore, a detailed analysis of the grain yield and grain yield components is fundamental for the characterization of the plant response to drought stress.

### 3.4.3 Relationship between investigated plant parameters and yield formation

Correlation analyses of investigated traits are useful to understand the plant response to drought stress and to detect valuable traits which are necessary for crop improvements. Genetic correlation analysis among 22 evaluated traits of four spring barley genotypes were performed for each drought stress scenario. The study revealed that the grain yield was significant correlated with the leaf area per plant (LAP), the green leaf area per plant (GLA), the plant dry matter (PDM), the leaf senescence (LS) and the plant potassium content (PKC). Similar findings were reported by Borrell et al. (2000) who showed that the grain yield of sorghum was positively associated with the green leaf area and negatively with the leaf

senescence under drought conditions. Interestingly, the positive correlation between the green leaf area (GLA) and the grain yield was higher for plants of the 3rd drought stress scenario (DT-BBCH49), which indicates that the maintenance of a functional green leaves during the grain filling is crucial for achieving high yields. Furthermore, positive associations of GLA with LDM, LWC and PKC underlying the fact that stay-green mechanism of plants are related to several morphological and physiological processes. Nevertheless, observed negative associations between LS and YLD increased throughout the life circle suggesting that chlorophyll content and photosynthetic activity decreased as part of plant senescence.

Nitrogen, phosphor and potassium are principle plant nutrients which are involved in several key plant functions such as photosynthesis, energy transfer and plant growth. In fact, potassium play an essential role for protein metabolism, stomatal activity and osmoregulation (Bednarz et al. 1998; Pettigrew 2008). As a consequence, it can be expected that potassium is involved in a wide range of physiological processes which influences the cell extension, photosynthesis and efficiency of crops to in utilization available water (Cakmak 2005; Hu and Schmidhalter 2005; Farooq et al. 2009). The observed significant correlations of PKC with a wide range of morphological, physiological and yield related parameters support the assumption of Hu and Schmidhalter (2005) that potassium increases the plant drought resistance. In general, significant reductions of leaf growth in terms of leaf area and plant dry matter, caused by water deficit, as well as significant associations of PKC with LAP and PDM confirm that drought reduced the mineral nutrient relations in plants and thus physiological mechanism of cell extension and growth. A decrease of LAP result in a reduced solar radiation interception and photosynthesis which finally led to a reduced assimilate transport and therefore to a reduced grain yield (Bednarz et al. 1998). Gathered data are in agreement with studies of Pettigrew (2008), who reported that potassium deficiency caused a decrease in plant biomass accompanied by a reduction of the leaf area. Significant and positive correlations of PKC with TNP and SWC underline the fact that potassium has a major impact on plant water relations and plays a central role in plants to cope with drought stress (Kusaka et al. 2005). Among examined drought stress scenarios, the observed associations between PKC and SWC increased in strength (0.70* - 0.90*). Present findings seems to be consistent with other studies and suggest that the reduced potassium uptake due to water deficit was might be attributable to the decreased transpiration rate and the low diffusion of potassium from roots to shoots (Kuchenbuch et al. 1986; Farooq et al. 2009).Cooper et al. (1987) demonstrated in their research the positive effect of fertilizer applications on water use in barley. According to their study, barley plants who received

fertilizers were characterized by a greater root growth and thus an increased ability to extract more moisture from the drying soil profile. Current study also showed that plant potassium content positively affected the number of ears per plant and the grain yield (Haeder and Beringer 1981). It was reported by Jensen and Tophøj (1985) that increases of potassium applications in barley plants increased the number of ears and grain yield. Zhang et al. (2011) emphasised that balanced potassium fertilization could assist to maintain the crop productivity and soil quality. Significant improvements of plant growth, grain yield and yield components by increasing the potassium fertilization under salt stress were reported by Endris and Mohammad (2007) and Akram et al. (2009). Aown et al. (2012) showed in their study that foliar application of potassium at different growth stages of wheat improved the grain yield and drought tolerance of plants. In view of these results and present findings, it is suggested that fertilizer applications increase the efficiency of plants in utilization available water (Farooq et al. 2009). Considering the high decrease in plant potassium content under drought treatment at the end of the leaf development stage (1$^{st}$ drought stress scenario), the application of potassium in early stages of plant development are recommended in order to increase the efficiency of nutrient utilization and plant growth. Moreover, the maintenance of soil fertility is beneficial to maintain a sufficient vegetation cover and to protect soils against wind and water erosion (Mengel 1997).

Nitrogen (N) is an important trait for vegetative plant growth because it is an essential component of plant cell components like amino and nucleic acids. Hence, N has a strong effect on plant growth and function (Chaves et al. 2003; Hu and Schmidhalter 2005). Among all drought stress scenarios PNC was negatively correlated with YLA and LS indicating that nitrogen deficiency enhance the premature wilting of plants and making the maintenance of a functional green leaf area difficult. Thus, a reduced plant nitrogen content caused by water deficit reduce the photosynthesis (Evans 1989; Farooq et al. 2009). Observed positive associations of PNC with LDM in the 2$^{nd}$ and 3$^{rd}$ drought stress scenario as well as significant correlations between SPAD and PDM over all three scenarios underline the detrimental effect of a reduced nitrogen content on chlorophyll content and photosynthetic activity (Shangguan et al. 2000). A possible explanation of the positive relationship between leaf dry matter and nitrogen content is that the higher nutrient availability in plants increased the photosynthesizing area and the volume of roots per unit soil surface (Arduini et al. 2006). Number of ears per plant was positively correlated with PNC which demonstrate the existence of a causal correlation between nitrogen content and final grain yield. Similar results regarding increased aboveground biomass, leaf area index, root depth and grain yield with

increasing levels of nitrogen fertilization under moderate water stress in winter wheat have been reported by Nielsen and Halvorson (1991).

Phosphor is an integral component of the complex nucleic acid structure and involved in several plant functions, including energy transformation in plants and the regulation of metabolic pathways (Schachtman 1998). The results of pot experiments exhibited significant associations between plant phosphor content and tiller number per plant (TNP), leaf dry matter (LDM) and plant water content (PWC). The present findings indicate that phosphor in plants is required for plant growth and water regulation (Radin 1984; Hu and Schmidhalter 2005). In our study plant phosphorus content revealed significant associations with TNP, LNP, NKP and YLD. Similar trends were found by Prystupa et al. (2004) who showed that phosphorus and nitrogen deficiencies limited the grain number in barley by decreasing the spike biomass around anthesis. In additions, Dordas (2009) reported that the dry matter production of durum wheat is directly related to the nitrogen and phosphor supply. Hence, deficiencies in nitrogen and phosphor resulted in a declining dry matter production, especially in leaves (Dordas 2009). Ercoli et al. (2008) examined the influence of nitrogen fertilizers on the dry matter assimilation in durum wheat plants, which were subjected to water stress during the grain filling. Ercoli et al. (2008) found that a higher N availability had, in general, a positive effect on the dry matter accumulation and grain yield. Nevertheless, Ercoli et al. (2008) pointed out that plants how received higher rates of N fertilizers were, compared to unfertilized plants, more sensitive to water stress. Zhao et al. (2009) found that water stress during the grain filling decreased the mineral contents (P, K, Ca and Mg) in grains of wheat. Thus, if spring barley genotypes are affected by water scarcity and droughts, low soil water availabilities might effects the mineral contents in barely grains and have perhaps a detrimental effect on the malting quality of barley.

In summary, genetic correlation analysis for three contrasting drought stress scenarios indicated that associations of the grain yield with morphological and physiological plant parameters was changing over the vegetation period. Water deficit during early stages of plant development (1$^{st}$ drought stress scenario) revealed primarily significant correlations for grain yield components with morphological plant parameters. Whereas significant associations of grain yield with physiological parameters were found between the tillering stage and anthesis (2$^{nd}$ drought stress scenario) indicating the importance of plant nutrients and water contents in plants for photosynthesis and carbon assimilation. The majority of significant correlations were detected in the 2$^{nd}$ drought stress scenario suggesting that this drought stress scenario revealed major genetic variation for certain traits.

### 3.4.4 Evaluation of investigated drought stress scenarios

***Genetic variation among genotypes***

The three contrasting drought stress scenarios, which were carried out during 2012 and 2013, were able to simulate drought stress (Figure 3.3) at different growth stages during the crop life circle. In addition, drought stress scenarios revealed considerable differences between evaluated spring barley genotypes. Besides the analysis of significant genotype-by-treatment interactions (Fig. 3.4), stress tolerance indices of 22 traits were calculated in order to identify drought sensitive and/ or drought tolerant genotypes (Table 3.9). Thereby, the effect of drought stress on individual barley genotypes was assessed based on the assumption that a drought tolerant genotype is characterized by low genotype-by-treatment interactions (Rizza et al. 2004; Cattivelli et al. 2008). Overall, the spring barley genotypes 'Scarlett', 'Morex',' Bojos' and 'Henrike' showed distinctive differences for the majority of investigated plant parameters. Among examined drought stress scenarios and evaluated genotypes, 'Morex' revealed the lowest stress tolerance for tiller number, leaf number, leaf dry matter, potassium content, number of ears and thousand kernel weight. A possible explanation might be that water deficit impaired the crop growth and dry matter accumulation which is essential to maintain the photosynthesis and thus to achieve high yields. Data of this study furthermore suggest that the high drought sensitivity of 'Morex' was independent from the timing of drought treatments during the vegetation period. Compared to the other investigated genotypes, the spring barley genotype 'Bojos' showed by far the highest drought stress tolerance among investigated scenarios. Regardless of the growth stage in which drought occurred, 'Bojos' was able to cope with water stress. Thus, the ability to maintain productive tillers and leaves, as well as the maintenance of a high plant water content (PWC) and a high potassium content (PKC) might be responsible for the capability of 'Bojos' to cope with water deficit. The genotypes 'Scarlett' and 'Henrike' showed intermediate values for these traits. Despite these findings it is important to note that the two genotypes 'Henrike' and 'Bojos' differed their leaf dry matter production corresponding to the applied water stress. 'Henrike' was characterized by a higher leaf dry matter under water shortage at the end of the leaf development stage (1$^{st}$ drought stress scenario) and tillering stage (2$^{nd}$ drought stress scenario). Water stress at anthesis (3$^{rd}$ drought stress scenario) diminished the leaf dry matter accumulation. A possible explanation might be that water shortage at anthesis significantly decreased the plant water content of 'Henrike', which reduced the transpiration rate and nutrient uptake, leading to a reduced photosynthesis and biomass accumulation (Farooq et al.

2009). Regarding STI-rankings for leaf dry matter, 'Bojos' revealed by far the broadest response to water deficit. 'Bojos' showed the highest drought susceptibility when water stress was applied at tillering stage. In contrast, leaf dry matter of 'Bojos' was less affected under water deficit at anthesis (3rd drought stress scenario). Present results lead to the assumption that leaf dry matter productivity of 'Bojos' is relatively high between tillering and anthesis.

Results in the present study clearly showed that genotypes differed in drought stress susceptibility. Findings in this study indicate that the individual drought response of the four evaluated spring barley genotypes remained fairly constant over the three drought stress scenarios. Nevertheless, significant genotype-by-scenario interactions for leaf dry matter indicate that 'Henrike' and 'Bojos' varied in their drought susceptibility, depending on the time point in which drought occurred. In future, pot experiments with a higher number of genotypes are necessary to explore genotypic differences in drought tolerance.

***Evaluation of different drought stress scenarios***

During the recent year's intensive research have been implemented in understanding the mechanism of drought stress tolerance. To our knowledge only a few studies were realized which evaluate and compare morphological, physiological and yield-related plant parameters at different crop developmental stages (Christen et al. 1995; Çakir 2004; Ugarte et al. 2007; Szira et al. 2008). Thus, a crucial aspect in this study was the assessment of different drought stress scenarios and the answering of relevant questions such as: 'Which stress scenario is qualified to investigate drought stress tolerance?' and 'Are there changes in drought stress tolerance during the vegetation period?'. In general, drought indices are used to identify superior genotypes that perform well under both, drought and well-watered conditions (Mitra 2001). In the current study, evaluation of genotypes based on stress tolerance index (STI) has facilitated the comparison of the three presented drought stress scenarios. Analysis of variance of STI values for 22 plant parameters showed that differences between drought stress scenarios were non-significant for most of the evaluated traits. Hence, these findings indicate that general plant response to water stress was similar, regardless of the growth stage in which drought occurred. However, concerning the leaf number, chlorophyll content and plant nitrogen content significant differences between stress scenarios were found. In particular, STI values for leaf number, chlorophyll content and plant nitrogen content increased throughout the crop life circle. It can therefore be assumed that an increased drought stress tolerance in later growth stages was probably attributable to the maturation of plants,

including the yellowing and drying of leaves under both, stress and non-stress conditions (Szira et al. 2008). Nevertheless, when comparing the results of examined drought stress scenarios, it must be noted that the majority of significant correlations were detected for the 2nd drought stress scenario. Thus, findings of the 2nd drought stress scenario suggest that this drought stress scenario is particularly useful to analyze a diverse set of traits for their applicability as indirect selection criteria. The study demonstrated that the leaf area and hence the photosynthetic capacity of plants was determined by the plant ability in leaf area formation. According to this, leaf number, tiller number, green leaf area and leaf dry matter are valuable traits which have a considerable effect on the plant response to drought (Blum 1996). Along with the importance of a functional leaf area and tiller formation under drought conditions, the present research showed the notable impact of plant nutrients, especially potassium, to the dry matter accumulation and grain yield. Overall, presented and discussed results of this Chapter indicate that future experiments on drought stress tolerance should be focused on drought treatments between tillering and anthesis. In this connection, leaf number, tiller number, green leaf area, plant dry matter, plant potassium content and relative water content are crucial to explore drought stress tolerance mechanism in plants. Nevertheless, the difficulty to identify reliable traits which revealed significant differences between drought stress scenarios was probably due to the year-to-year variability of environmental factors such as air humidity, air temperature and solar radiation (Cattivelli et al. 2008). The lack of significant genotype-by-treatment interaction within each drought stress scenario was mainly attributable to the fact that only four genotypes were evaluated. A reasonable approach to tackle this issue could be to increase the number of evaluated genotypes. In order to reduce the impact of climatic conditions on crop development and plant productivity it is necessary to realize pot experiments over several years.

## 3.5 Conclusion

Current study revealed that water stress applied at different growth stages reduce the leaf area, leaf expansion and thus photosynthetic activity of plants through various ways (Blum 1996). Decreasing water availability under drought treatment at the end of the leaf development stage (1st drought stress scenario) suppressed the leaf expansion by reductions in leaf number, green leaf area and plant dry matter, which finally diminished the grain yield. Water deficit at tillering stage and anthesis (2nd and 3rd drought stress scenario) resulted in an impaired leaf expansion by declines in tiller formation and green leaf area.

The effect of water deficit applied at different growth stages was furthermore determined by evaluating physiological parameters, including chlorophyll content (SPAD-value), plant nutrient content (PNC, PPhC and PKC) and plant water content (PWC, LWC, SWC). Here, water stress at early stages of plant development (1st drought stress scenario) preliminary decreased the chlorophyll content and plant nutrient concentrations. Higher decreases in plant water relations were noticed in the 3rd drought stress scenario. Significant correlations of the plant nitrogen, phosphor and potassium content with the leaf area, plant dry matter and yield components confirmed the negative effect of water shortage on mineral nutrient relations in plants. The significant relationship between plant potassium content and stem water content emphasise the importance of a balanced potassium fertilization to increase the efficiency of crops in utilization available water (Farooq et al. 2009). In accordance with previous studies, the present results leads to the conclusion that plants suffering from drought stress have a large requirement for potassium and that applications of potassium fertilizer might mitigates the effects of drought on crop growth (Cakmak 2005; Hu and Schmidhalter 2005). Analysis of yield components showed that higher grain yields were associated with a higher number of kernels and ears per plant. Among drought stress scenarios grain yield declined in the order of the 1st drought stress scenario > 2nd drought stress scenario > 3rd drought stress scenario. Although differences between drought stress scenarios were non-significant for most of the evaluated traits, the comparison of individual drought stress scenarios showed that the majority of significant correlations were detected for the 2nd drought stress. Thus, findings of the 2nd drought stress scenario suggest that this drought stress scenario is particularly useful to analyze the drought stress tolerance mechanism of plants. Results of the phenotyping experiments indicate that tiller number, leaf number, leaf area, plant dry matter, plant nitrogen and potassium content as well as yield components should be considered as selection criteria for drought stress tolerance.

# Chapter 4: Phenotyping of spring barley for drought stress tolerance using secondary trait*s*

## 4.1 Introduction

It is generally agreed that water deficit during the crop life circle is one of the most devastating stress factors, which causes serious yield reductions (Hlavinka et al. 2009). Previous studies indicated that drought stress affect the crop productivity by decreasing the plant height, number of tillers, photosynthetic active leaf area, plant dry matter and grain yield (Bolaños et al. 1993; Ober et al. 2005; Schittenhelm et al. 2014). Thus, current breeding programs are mainly focused on the development of improved cultivars for drought-prone environments. During the recent years several breeding strategies have been discussed to select genotypes with an acceptable performance level under abiotic stress. The first approach rely on the assumption that phenotyping programs for drought stress tolerance should be realized under the prevailing field conditions in the target population of environments (TPE) (Ceccarelli et al. 1998; Monneveux and Ribaut 2011). However, there is evidence that the direct selection of high yielding genotypes in drought-prone environments is often complicated by a lower heritability and large environmental variances, which increase the difficulties in relating the phenotype to the genotype (Ceccarelli et al. 1991; Rajaram et al. 1996). In contrast, the second strategy is focused on the selection of high yielding genotypes in non-stress environments, characterized by a maximized genetic variation and low genotype-by-environment interactions (Richards 1996; Ceccarelli et al. 1998). The third strategy assumes that selection should be based on genotypes that yield well in non-stress and stress environments (Fernandez 1992). However, progress in breeding for drought tolerance might be realized by targeting specific secondary traits, which have a high heritability and a reasonable genetic association with the grain yield in water-limited environments (Monneveux et al., 2008; Chen et al., 2012). Several studies identified specific traits such as leaf area/expansion, leaf senescence, plant height, nitrogen content per unit leaf and transpiration efficiency which contributes to drought tolerance (Condon et al. 2004; Ober et al. 2005; Tambussi et al. 2005; Lu et al. 2011; Chen et al. 2012). The primary objectives of the present chapter were (1) to assess the genetic variance and heritability for morphological, physiological and yield-related traits under well-watered and drought conditions, (2) to evaluate the relationship between the primary trait (grain yield) and secondary traits and (3) to evaluate the applicability of investigated secondary traits for identifying drought stress tolerance in spring barley.

## 4.2 Materials and Methods

This section outlines the used plant materials, experimental setup, data collection of phenotypic traits and statistical analyses of phenotyping experiments for drought stress tolerance in spring barley.

### 4.2.1 Plant material

Twenty-four spring barley cultivars were assumed to be a representative sample from a wide range of Central European breeding material and include both, old and modern varieties (Table 4.1). Plant material was provided by several plant breeding companies which are listed in Table 4.1.

**Table 4.1** Spring barley cultivars used for phenotyping experiments in 2012 and 2013 as well as names of plant breeding companies who have provided the plant material.

| Cultivar | Breeding company | Year of release |
|---|---|---|
| Barke | Saatzucht Breun GmbH | 1996 |
| Bojos | Limagrain GmbH | 2006 |
| Braemar | Syngenta Seeds GmbH | 2002 |
| Calcule | Limagrain GmbH | 2009 |
| Grace | Ackermann Saatzucht GmbH & Co. KG | 2008 |
| Henrike | Nordsaat Saatzucht GmbH | 2007 |
| Kangoo | Limagrain GmbH | 2007 |
| KWS Aliciana | KWS Lochow GmbH | 2009 |
| LFL24727 | Landesanstalt für Landwirtschaft Bayern (LFL) | NA[1] |
| Morex | RWTH | 1978 |
| NFC Tipple | Syngenta Seeds GmbH | 2004 |
| Prestige | Erhardt Eger | 2001 |
| Primadonna | Saatzucht Firlbeck | 2006 |
| Propino | Syngenta Seeds GmbH | 2009 |
| Quench | Syngenta Seeds GmbH | 2006 |
| Scarlett | Saatzucht Breun GmbH | 1995 |
| Sebastian | Saatzucht Streng-Engelen GmbH&Co. KG | 2005 |
| Streif | Saatzucht Streng-Engelen GmbH&Co. KG | 2007 |
| SuLilly | Nordsaat Saatzucht GmbH | NA[2] |
| Sunshine | Saatzucht Breun GmbH&Co. KG | 2009 |
| Tatum | Nordsaat Saatzucht GmbH | 2010 |
| Wiebke | Nordsaat Saatzucht GmbH | NA[2] |
| Wisa | Saatzucht Breun GmbH | 1951 |
| Xanadu | Nordsaat Saatzucht GmbH | 2003 |

Where NA[1]: experimental breeding line, NA[2]: no official registered variety in Germany

### 4.2.2 Experimental setup

During 2012 and 2013 phenotyping experiments were performed in transparent polyethylene plastic tunnels at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany. In each year, the experimental design was a split-plot design with four replications. The two irrigation treatments (well-watered and drought treatments) were the main-plot factor, while genotypes were the sub-plot factor. The sub-plots consisted of 24 spring barley cultivars which were completely randomized within main-plots. In general, phenotyping experiments were carried out in 22x22x26 cm plastic pots, containing 11.5 l of a silica sand soil mixture (Terrasoil ®, Cordel & Sohn, Salm, Germany). To simulate a micro crop stand, 18 seeds per pot were sown and after germination thinned out to 12 plants per pot. Pots were watered through an automatic drip irrigation system at three times per day (6:30 am, 00:30 pm and 6:30 pm). Further details regarding the watering time per irrigation treatment are presented in the Appendix. Nutrients were added to the drip irrigation in form of KRISTALON®, which is a fully water soluble NPK fertilizer (see Appendix). Herbicides and insecticides were applied in accordance to agriculture practice. Growth regulators and pesticides referring to strobilurine and sulfonylurea were not utilized. Spring barley genotypes were evaluated under two contrasting water regimes:

(1) Well-watered conditions: Genotypes were fully irrigated over the whole vegetation period to maintain volumetric moisture content (VWC) of 30%.

(2) Drought treatment from tillering to anthesis (BBCH 24 - BBCH 49): 21-day lasting reduction of the water supply to decrease the soil moisture content from field capacity to permanent wilting point (5 - 10% VWC). After three weeks pots were re-watered.

Spring barley was sown on March 28$^{th}$ in 2012 and on April 17$^{th}$ in 2013. Harvest was done manually at full maturity (14% seed moisture). During the vegetation period air temperature (°C), air humidity (%), solar radiation (W/m²), soil moisture (Vol.%) and soil temperature (°C) were recorded every five minutes with a DL2e Data Logger from Delta-T Devices Ltd. Further explanations of the general experimental setup have been explained in Chapter 2.

### 4.2.3 Phenotypic data collection

In this study nine morphological, six physiological and five yield-related plant parameters were investigated. Table 4.2 provides a short definition and description of measured parameters. Further specifications of taking measurements have been elucidated in Chapter 2. In terms of measuring effort and analysing costs, plant nitrogen, phosphor and potassium content haven't been analyzed. Principal growth stages were recorded using the extended BBCH scale of Hess et al. (1997).

**Table 4.2** Classification, abbreviations and description of evaluated plant parameters in 2012 and 2013.

| Plant parameter | Abbreviation | Unit | Description |
|---|---|---|---|
| **plant growth stage** | BBCH | number | decimal code with two digits |
| **morphological plant parameters** | | | |
| plant height | PLH | cm | average distance from the soil surface to the tip of the spike |
| number of tillers per plant | TNP | No./plant | average number of tillers per plant |
| number of leaves per plant | LNP | No./plant | average number of leaves per plant |
| leaf area per plant | LAP | cm²/plant | total plant leaf area measured at different growth stages |
| yellow leaf area | YLA | cm²/plant | yellow leaf area of a plant measured at different growth stages |
| green leaf area | GLA | cm²/plant | green leaf area of a plant measured at different growth stages |
| plant dry matter | PDM | g/plant | total dry mass of above-ground plant |
| leaf dry matter | LDM | g/plant | total dry mass of leaves per plant |
| stem dry matter | SDM | g/plant | total dry mass of stems per plant |
| **physiological plant parameters** | | | |
| SPAD value | SPAD | number | estimate of leaf chlorophyll content with SPAD-502 chlorophyllmeter |
| leaf senescence | LS | 1-9 | visually scored degree of leaf senescence |
| plant water content | PWC | % | water content of above-ground plant mass |
| leaf water content per plant | LWC | % | water content of leaves per plant |
| stem water content per plant | SWC | % | water content of stems per plant |
| carbon isotope discrimination | $\Delta\ ^{13}C$ | ‰ | $^{13}C/^{12}C$ ratio in plant tissue relative to the atmosphere |
| **grain yield and yield components** | | | |
| number of ears per plant | NEP | No./plant | average number of ears per plant |
| number of kernels per ear | NKE | No./ear | average number of kernels per ear |
| number of kernels per plant | NKP | No./plant | average number of kernels per plant |
| thousand kernel weight | TKW | g | weight of 1000 kernels |
| grain yield per plant | YLD | g/plant | average grain weight per plant |

### 4.2.4 Statistical analyses

Statistical analyses for morphological, physiological and yield-related parameters were performed using the SAS software version 9.2 (SAS Institute 2008). In each year, analysis of variance was conducted by using the SAS procedure PROC ANOVA. The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***.

***Individual analysis of variance***

Analysis of variance (ANOVA) was performed by using the model:

$$Y_{ijk} = \mu + T_i + G_j + T_i*G_j + r_k + b_{ik} + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $T_i$ is the main effect of i-th treatment; $G_j$ is the main effect of j-th genotype; $T_i*G_j$ is the fixed interaction effect of i-th treatment with j-th genotype, $r_k$ is the effect of the k-th block, $b_{ik}$ is the error of i-th main plot within the k-th block and $e_{ijk}$ is random errors. All effects are considered as fixed, while the error terms $b_{ik}$ and $e_{ijk}$ are random.

***Combined analysis of variance***

Across years, analysis of variance was performed by using the SAS PROC MIXED procedure. Here, years were considered as a fixed factor because two years of experimentation are not representative for the wide range of annual and environmental variation. To test the significance of each fixed effect, the PROC MIXED procedure computed the "Type 3 Test of Fixed Effects". The LSMEANS statement calculated least-squares means (LS-means) of fixed effects. The model used for analysis of variance across two years is:

$$Y_{ijk} = \mu + G_i + T_j + Y_k + G_i*T_j + G_i*Y_k + Y_k*T_j + G_i*Y_k*T_j + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $G_i$ is the fixed effect of i-th genotype; $T_j$ is the fixed effect of j-th treatment; $Y_k$ is the fixed effect of k-th year; $G_i*T_j$ is the fixed interaction effect of i-th genotype with j-th treatment; $G_i*Y_k$ is the fixed interaction effect of i-th genotype with k-th year; $T_j*Y_k$ is the fixed interaction effect of j-th treatment with k-th year; $G_i*T_j*Y_k$ is the fixed interaction effect of i-th genotype with j-th treatment and with k-th year and $e_{ijk}$ is random errors.

***Correlation analysis***

Based on genotypic means, Spearman's rank correlation analysis was performed by using the SAS procedure PROC CORR.

***Stress tolerance index (STI)***

Stress tolerance index (STI) has been used to identify genotypes that perform well, under control (well-watered) and drought treatment. According to the equation defined by Fernandez (1992), STI was calculated for each trait based on genotypic means:

$$\text{STI} = \left(\frac{Y_{ww}}{\overline{Y_{ww}}}\right)\left(\frac{Y_{dt}}{\overline{Y_{dt}}}\right)\left(\frac{\overline{Y_{dt}}}{\overline{Y_{ww}}}\right) = \frac{(Y_{ww})(Y_{dt})}{(\overline{Y_{ww}})^2}$$

Where $Y_{ww}$ and $Y_{dt}$ are the genotype mean for given plant parameter under well-watered conditions and drought treatment, and $\overline{Y}_{ww}$ and $\overline{Y}_{dt}$ are the overall means of genotypes under both irrigation levels.

***Membership function value of drought tolerance (MFVD)***

A drought tolerant genotype is defined as one that achieves a high grain yield relative to other genotypes under drought stress (Atlin 2003). Based on the computation of 19 stress tolerance indices, the membership function value of drought tolerance was calculated to assess the impact of drought on plant performance. The membership function describes the membership of the element X to a membership value which range between 0 and 1. However, the membership function allows a generalization of input data in specific sets. It represents the possibility of being a member of a specific set. Thus, a membership value of zero is assigned to those elements which don't belong to the set. Contrary, elements with a membership value of one are classified as a member of the specific set.

In the present study the membership function value of drought tolerance (MFVD) was calculated as follows (Chen et al. 2012):

$$U_{ij} = \frac{STI_{ij} - STI_{j\min}}{STI_{j\max} - STI_{j\min}} \text{ , } U_i = \frac{1}{n}\sum_{j=1}^{n} U_{ij}$$

Where $U_{ij}$ is the membership function of the trait (*j*) for the genotype (*i*) for stress tolerance index; $STI_{jmax}$ is the maximum value of the stress tolerance coefficient for the trait (*j*); $STI_{jmin}$ is the maximum value of the stress tolerance coefficient for the trait; $U_i$ is the average value of the membership function of 19 traits for the genotype (*i*) for drought tolerance.

According to the average value and standard deviation of the MFVD, drought tolerance of spring barley was classified in four grades, whereas class I was drought susceptible, class II was moderate tolerant, class III was drought tolerant and class IV was highly tolerant.

***Calculation variance components, genetic coefficient of variation and heritability***

Variance components were estimated using SAS PROC VARCOMP procedure. Genetic coefficient of variation ($CV_g$) was computed as follows:

$$CV_g = \frac{\sqrt{\sigma_G^2}}{\overline{X}} * 100$$

Where $\sigma_G^2$ is the genotypic variance, $\overline{X}$ is the average value of the trait under well-watered or respectively drought conditions.

In order to calculate the heritability on a genotypic-mean basis of 24 spring barley genotypes, variance components were estimated for a model wherein all factors were considered as random. The computations were made by using the REML option of the SAS PROC VARCOMP procedure. The following formula was applied (Hoi et al. 1999):

$$h^2 = \frac{\sigma_G^2}{\left(\sigma_G^2 + (\sigma_{GE}^2 / e) + (\sigma_e^2 / re)\right)}$$

Where $\sigma_G^2$ is the estimate of genotypic variance, $\sigma_{GE}^2$ the estimate of genotypic-by-environment interactions variance, $\sigma_e^2$ is the estimate of residual variance, r is the number of replications per environment and e is the number of environments. In order to estimate the heritability a genotypic-mean basis, environments were defined as treatment level within each year.

## 4.3 Results

The result section of this chapter is organized as follows: (1) climatic conditions during two years of research, (2) analysis of variance, (3) crop performance under well-watered and drought conditions, (4) genetic correlations among investigated traits, (5) genetic variation and heritability of evaluated traits, (6) genetic correlation analysis among stress tolerance indices of investigated traits and (7) identification of drought stress tolerance among spring barley cultivars.

### 4.3.1 Climatic conditions and environmental differences between years

The automatic weather station from Delta-T Devices Ltd. monitored continuously meteorological data such as soil temperature, soil moisture, air temperature, relative humidity and solar radiation. Figure 4.1 presents the climatic data for 2012 and 2013. The average air temperature in May and June 2012 amounted 16.2°C and 17.1°C, respectively. In 2013, the DL2e Data Logger from Delta-T Devices Ltd. recorded average air temperatures of 13.5°C in May and 18.1°C in June. The relative humidity between May and June amounted to 71% in 2012 and 75% in 2013. In both years, the main growth period was between the beginning and end of May (day of year 130 - day of year 150). Comparing the climatic data during these time period it is striking that 2013 was characterized by a 5°C lower air temperature, an 11% higher humidity and a 300 kW/m² lower solar radiation. Thus, the environmental conditions in 2013 were generally favourable for the plant development. Additionally, Figure 4.1 shows the soil moisture content for spring barley genotypes under well-watered and drought conditions. The blue line represents the soil moisture content under well-watered conditions. The red line display the soil moisture content under drought treatment at tillering stage. Drought treatment started approximately at BBCH 24 by a gradually reduction of the water supply. Over 21 days the automatic drip irrigation system allowed an accurate decrease of the soil moisture from 26% volumetric water content (VWC) to 8% VWC. After three weeks of water deficit, pots were re-watered and soil moisture rise again to 26% VWC. Genotypes under well-watered conditions were fully irrigated over the whole vegetation period.

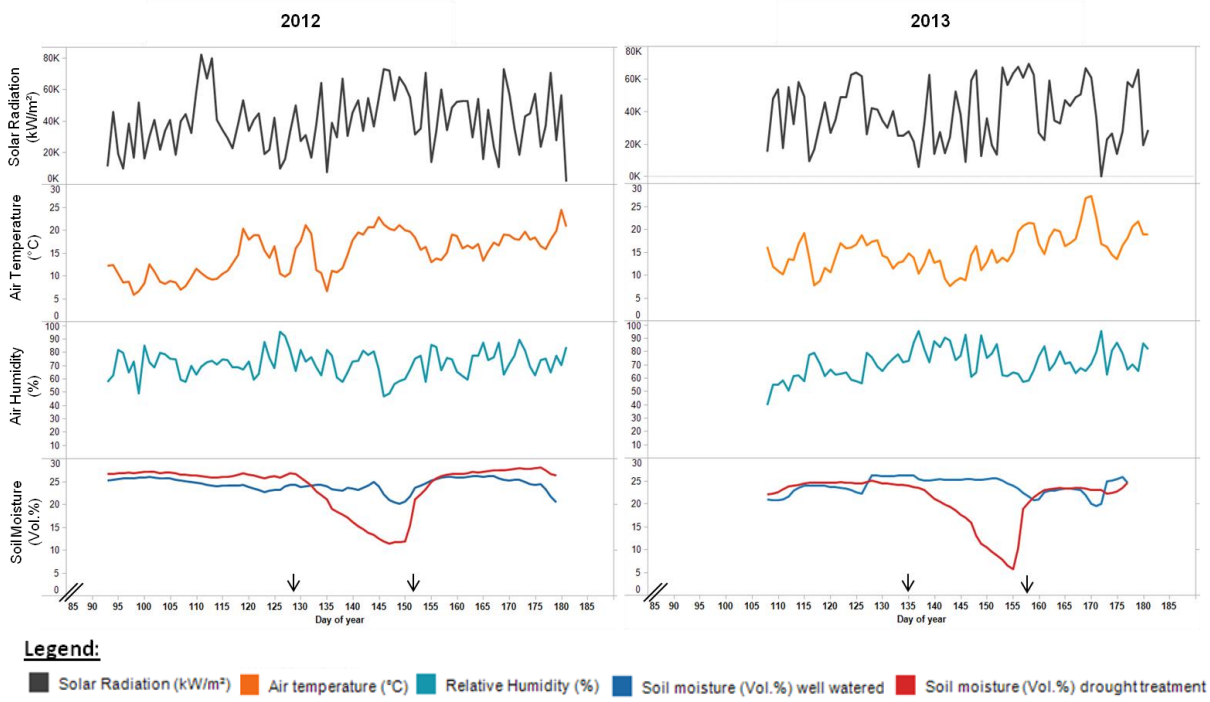


**Fig. 4.1. Sum daily solar radiation, mean daily air temperature, air humidity and soil moisture content recorded in poly tunnel experiments during 2012 and 2013 at the experimental research station of the Institute for Plant Breeding, University of Bonn, Germany.** Vertical arrows mark the beginning and the end of the drought treatment.

### 4.3.2 Analysis of variance

#### *Carbon isotope discrimination*

In 2012, carbon isotope analysis for 24 spring barley genotypes grown under two contrasting irrigation treatments revealed no significant variation among treatment levels, genotypes and genotype-by-treatment interaction (see Appendix). Therefore, carbon isotope discrimination was disregarded.

#### *Combined analysis of variance over two years*

Combined analysis of variance revealed for all evaluated plant parameters significant differences among treatment levels (see Appendix). Except for LDM, genotypic variation was significant for seven morphological (PLH, TNP, LNP, LAP, YLA, GLA, PDM, SDM), five physiological (SPAD, LS, PWC, LWC, SWC) and five yield-related traits (NEP, NKE, NKP, TKW, YLD). Furthermore, analysis of variance showed significant genotype-by-treatment interactions for yield-related parameters, including NEP, NKE, NKP and YLD. With exception of PDM and SPAD, significant main effects of the year were found for 17 traits. Further results are presented in the Appendix.

### 4.3.3 Annual differences between evaluated plant parameters

Table 4.3 summarizes mean values for 19 analyzed plant parameters. Differences among years and between treatments were highly significant (see Appendix). In 2013, mean values of TNP, LNP, LAP, GLA, LDM, PWC, NEP, NKP, TKW and YLD increased under both treatment levels, control (well-watered) and drought treatment. Data shown in this table should be considered with data in Figure 4.1, which demonstrate that the advantageous barley development in 2013 was attributable to favourable climatic conditions during the crop life circle. With regard to grain yield reductions, the effect of drought treatments were more pronounced in 2012.

The following parts of the result section refers to general means of morphological, physiological and yield-related parameters evaluated under control (well-watered) and drought treatment, across two years of experimentation.

### 4.3.4 Effects of water shortage on crop development and grain yield

It is apparent from Table 4.3 that water deficit between tillering stage and anthesis (BBCH 24 to BBCH 49) decreased the mean values of nine morphological, four physiological and five yield-related traits. Generally, drought treatments caused considerable morphological changes, including the decline in TNP (-27.8%), LNP (-22.2), GLA (-44.8%) and PDM (-38.5%). The decrease of the above ground plant dry matter (PDM) was preliminary due to the reduction of the leaf dry matter (-40.4%). Across years, the reduction of the chlorophyll content by 21.8% and the decrease of the plant water content by 6.9% triggered a negative impact on physiological processes. Additionally, drought treatments markedly increased the leaf senescence. Finally, the combined analysis across years revealed that water deficit during the crop life circle seriously reduced the grain yield by 42.7%. As show in Table 4.3, the reduction in grain yield was accompanied by reductions in all grain yield components. Here, water shortage remarkably reduced the number of ears per plant (-16.6%). Number of kernels per ear (NKE) decreased by 21.9%. Based on the fact that decreases in NKP are mainly due to the combined reductions in NEP and NKE, mean values of NKP declined under water deficit by 34.1%. In summary, the results showed that mean values of eleven parameters (TNP, LNP, LAP, GLA, PDM, LDM, SDM, NKE, NKP and YLD) decreased over 20% under drought treatment.

**Table 4.3** Mean values of 24 spring barley genotypes for morphological, physiological and yield related plant parameters studied under well-watered conditions (control treatment) and drought treatment in 2012 and 2013, as well as averaged means across years.

| Trait | Year 2012 | | | | Year 2013 | | | | Mean over years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet | Dry | Δ %[a] | | Wet | Dry | Δ %[a] | | Wet | Dry | Δ %[a] | |
| **PLH** | 67.0 | 61.4 | -8.4 | ** | 55.4 | 49.5 | -10.8 | * | 61.2 | 55.4 | -9.5 | *** |
| **TNP** | 3.3 | 2.1 | -35.9 | *** | 4.7 | 3.7 | -22.0 | *** | 4.0 | 2.9 | -27.8 | *** |
| **LNP** | 21.2 | 15.9 | -25.0 | ** | 26.6 | 21.2 | -20.0 | * | 23.9 | 18.6 | -22.2 | *** |
| **LAP** | 145.6 | 97.1 | -33.3 | ns | 359.6 | 226.3 | -37.1 | * | 252.6 | 161.8 | -35.9 | *** |
| **YLA** | 27.2 | 26.7 | -1.5 | ns | 31.2 | 49.5 | 58.5 | ** | 29.1 | 38.4 | 32.2 | *** |
| **GLA** | 129.4 | 59.3 | -54.2 | ns | 328.4 | 176.8 | -46.2 | * | 223.5 | 123.4 | -44.8 | *** |
| **PDM** | 2.7 | 1.4 | -47.0 | *** | 2.4 | 1.7 | -28.9 | * | 2.5 | 1.6 | -38.5 | *** |
| **LDM** | 0.6 | 0.3 | -43.5 | ** | 1.0 | 0.6 | -38.7 | * | 0.8 | 0.5 | -40.4 | *** |
| **SDM** | 2.2 | 1.1 | -47.9 | *** | 1.3 | 1.1 | -21.4 | ns | 1.8 | 1.1 | -37.7 | *** |
| **SPAD** | 43.8 | 33.0 | -24.6 | ** | 43.4 | 35.2 | -19.0 | *** | 43.6 | 34.1 | -21.8 | *** |
| **LS** | 14.2 | 51.2 | 261.8 | *** | 10.3 | 64.1 | 512.2 | *** | 12.2 | 57.7 | 371.0 | *** |
| **PWC** | 75.2 | 71.1 | -5.5 | ** | 86.9 | 79.8 | -8.1 | * | 81.1 | 75.5 | -6.9 | *** |
| **LWC** | 76.6 | 69.9 | -8.7 | ** | 86.2 | 80.9 | -6.1 | * | 81.4 | 75.4 | -7.3 | *** |
| **SWC** | 74.9 | 71.4 | -4.6 | ** | 87.4 | 79.0 | -9.6 | ** | 81.1 | 75.2 | -7.3 | *** |
| **NEP** | 4.7 | 3.1 | -33.9 | ** | 6.1 | 5.9 | -3.3 | ns | 5.4 | 4.5 | -16.6 | * |
| **NKE** | 18.3 | 14.7 | -19.5 | *** | 19.6 | 14.9 | -24.2 | *** | 18.9 | 14.8 | -21.9 | *** |
| **NKP** | 83.3 | 44.8 | -46.3 | *** | 115.4 | 86.1 | -25.4 | ** | 99.3 | 65.4 | -34.1 | *** |
| **TKW** | 48.8 | 42.3 | -13.3 | * | 52.1 | 46.0 | -11.7 | ** | 50.4 | 44.2 | -12.4 | *** |
| **YLD** | 4.0 | 1.8 | -55.3 | *** | 6.0 | 3.9 | -34.2 | ** | 5.0 | 2.9 | -42.7 | *** |

Where: Treatment = well-watered, drought treatment, Year = 2012,2013,Trait: Δ % [a] ( relative difference in percent): (Dry-Wet) / Wet *100; and *, **, *** are significant at P value ≤0.05, ≤0.01 ≤0.001, ns: none significant, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 4.3.5 Impact of water shortage on grain yield of 24 spring barley cultivars

Across years, drought treatments consistently lowered the grain yield of 24 spring barley genotypes. Mean yields under drought treatments ranged from 2.4 to 3.3 g plant$^{-1}$, representing a very narrow yield spectrum. Contrary, grain yields observed under well-watered conditions varied between 4.2 and 5.9 g plant$^{-1}$. Figure 4.2 display the grain yield performance of 24 spring barley genotypes under two contrasting irrigation levels. In particular, the genotypes 'Xanadu', 'Streif', 'Tatum', 'Primadonna', 'Barke', 'Grace' and 'Henrike' were characterized by high grain yields under both treatment-levels. In contrast, 'Sunshine', 'Wisa', 'Bojos' and 'Quench' were genotypes which revealed a poor yield performance under both irrigation treatments. Correlation analysis between grain yields of 24 spring barley genotypes under well-watered and drought conditions were non-significant.

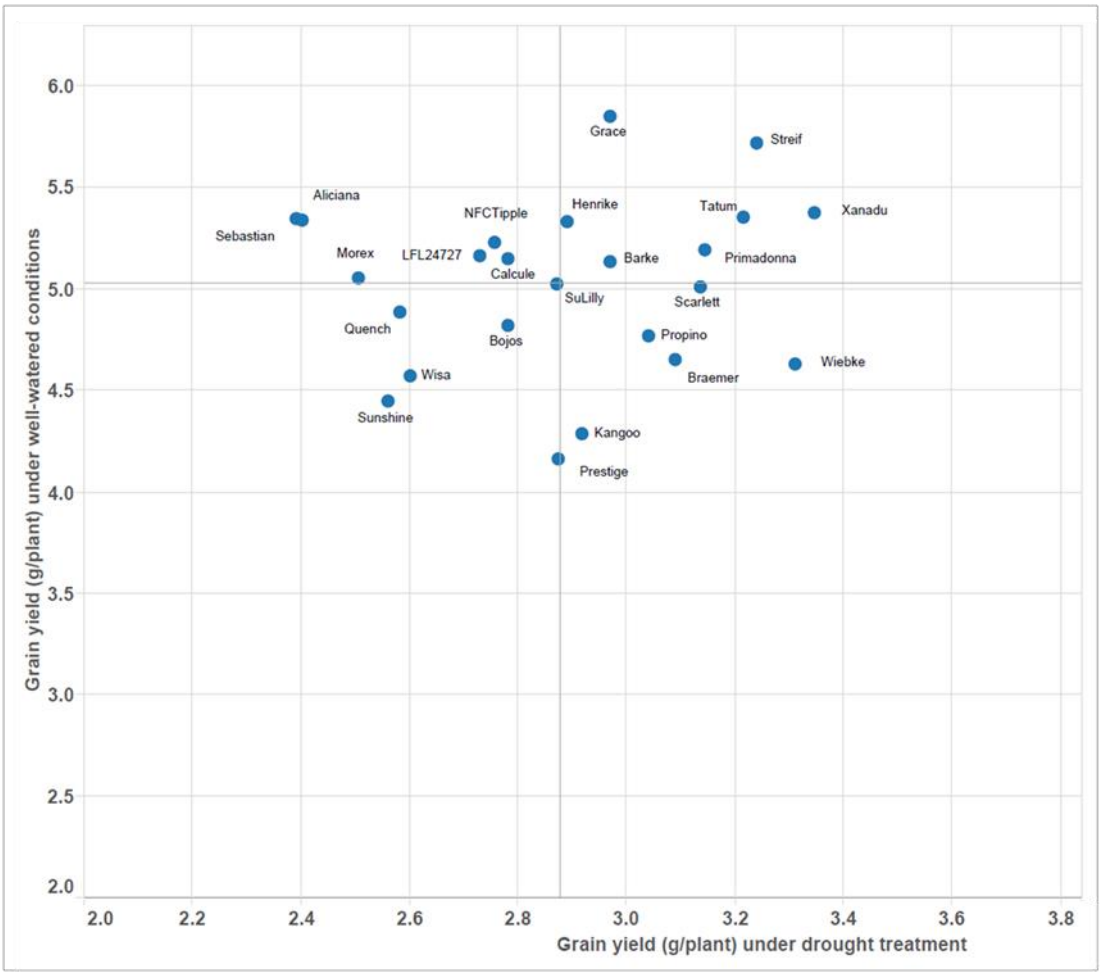


**Fig. 4.2. Scatter plot of the grain yield under well-watered and drought conditions for 24 spring barley genotypes.** The average grain yield was calculated over two years. The vertical and horizontal line represents the average grain yield under drought treatment and well-watered conditions.

### 4.3.6 Correlation analysis among investigated plant parameters

Spearman rank correlation analysis was used to assess the relationship between investigated traits. Based on computed LSMEANS values of 24 spring barley genotypes, genetic correlation analyses were performed for investigated traits, under well-watered and drought conditions. Results are presented in Table 4.4 and 4.5.

***Correlation analysis between investigated traits under well-watered conditions***

Under well-watered conditions a total of 34 significant correlations were detected. The number of tillers per plant (TNP) showed the most significant associations with evaluated traits. Hence, significant genetic correlations were observed between TNP and LNP (0.87***), LAP (0.43*), LDM (0.43*), PWC (0.60**), SWC (0.60**) and NEP (0.41*). The leaf number per plant (LNP) correlated positively with the plant leaf area, yellow leaf area, plant water content, stem water content and number of ears (Table 4.4). In contrast, significant, negative associations were found between the stem dry matter (SDM) and the plant water content (-0.53**) as well as the stem water content (-0.52**). The number of ears per plant (NEP) was weakly associated with TNP (0.41*) and LNP (0.47*), whereas moderate correlations were found with the SPAD value (0.54**). Number of kernels per plant (NKP) recorded significant correlations with the SPAD value (0.43*) and the leaf senescence (0.42*). No significant correlations were observed between the grain yield, as a primary trait, and morphological as well as physiological parameters. Nevertheless, grain yield correlated highly with NKP (0.70**) and moderate with NEP (0.59***).

***Correlation analysis between investigated traits under drought conditions***

Under drought treatment, 29 significant correlations were found. The tiller number per plant exhibited significant associations with the leaf number (0.71***), leaf dry matter (0.57**) and number of ears (0.41*). The plant dry matter (PDM) was highly correlated with the stem dry matter, while PWC and SWC were negative associated with PDM. In addition, leaf senescence was positively associated with the yellow leaf area, whereas negative correlations were detected with the leaf water content (-0.66***). No significant correlations were observed between the grain yield, and morphological as well as physiological

parameters. Overall, associations between traits were generally stronger under well-watered conditions (Table 4.5). Under water deficit conditions genetic correlations declined.

**Table 4.4** Spearman rank correlation coefficients ($r_g$) among evaluated plant parameters of 24 spring barley genotypes under well-watered conditions.

| | PLH | TNP | LNP | LAP | YLA | GLA | PDM | LDM | SDM | SPAD | LS | PWC | LWC | SWC | NEP | NKE | NKP | TKW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | 1 | | | | | | | | | | | | | | | | | | |
| **TNP** | -0.40 | 1 | | | | | | | | | | | | | | | | | |
| **LNP** | -0.16 | **0.87** *** | 1 | | | | | | | | | | | | | | | | |
| **LAP** | -0.17 | **0.43** * | **0.61** ** | 1 | | | | | | | | | | | | | | | |
| **YLA** | 0.02 | **0.47** * | **0.56** ** | **0.46** * | 1 | | | | | | | | | | | | | | |
| **GLA** | -0.23 | 0.28 | **0.44** * | **0.95** *** | 0.26 | 1 | | | | | | | | | | | | | |
| **PDM** | 0.36 | 0.02 | 0.08 | 0.19 | -0.05 | 0.17 | 1 | | | | | | | | | | | | |
| **LDM** | -0.17 | **0.43** * | 0.34 | **0.65** *** | 0.18 | **0.66** *** | **0.59** ** | 1 | | | | | | | | | | | |
| **SDM** | **0.50** * | -0.14 | -0.07 | -0.04 | -0.10 | -0.08 | **0.93** *** | 0.33 | 1 | | | | | | | | | | |
| **SPAD** | 0.22 | 0.17 | 0.21 | -0.21 | 0.22 | -0.30 | -0.04 | -0.14 | 0.05 | 1 | | | | | | | | | |
| **LS** | 0.28 | -0.17 | -0.02 | -0.06 | **0.44** * | -0.19 | -0.16 | -0.34 | -0.01 | 0.17 | 1 | | | | | | | | |
| **PWC** | -0.40 | **0.60** ** | **0.51** * | 0.33 | 0.09 | 0.31 | -0.38 | 0.19 | **-0.53** ** | -0.19 | -0.25 | 1 | | | | | | | |
| **LWC** | -0.14 | -0.05 | 0.04 | 0.11 | **-0.48** * | 0.24 | 0.01 | -0.01 | -0.04 | -0.35 | **-0.45** * | 0.23 | 1 | | | | | | |
| **SWC** | -0.39 | **0.60** ** | **0.51** * | 0.37 | 0.14 | 0.33 | -0.37 | 0.23 | **-0.52** ** | -0.16 | -0.14 | 0.98 *** | 0.11 | 1 | | | | | |
| **NEP** | -0.10 | **0.41** * | **0.47** * | 0.13 | 0.27 | 0.04 | -0.07 | 0.11 | -0.16 | **0.54** ** | 0.24 | 0.17 | -0.25 | 0.27 | 1 | | | | |
| **NKE** | 0.00 | -0.22 | -0.35 | -0.06 | 0.10 | -0.08 | 0.08 | -0.01 | 0.21 | -0.12 | 0.12 | -0.33 | -0.09 | -0.37 * | **-0.56** ** | 1 | | | |
| **NKP** | -0.04 | 0.13 | 0.11 | -0.04 | 0.30 | -0.10 | 0.04 | 0.04 | 0.06 | **0.43** * | **0.42** * | -0.07 | -0.40 | 0.03 | **0.68** *** | -0.04 | 1 | | |
| **TKW** | 0.01 | -0.15 | -0.23 | -0.08 | -0.05 | -0.01 | 0.05 | 0.07 | 0.09 | -0.06 | -0.09 | -0.30 | 0.14 | -0.35 | -0.23 | 0.30 | -0.3 | 1 | |
| **YLD** | -0.14 | 0.18 | 0.10 | 0.11 | 0.27 | 0.07 | 0.16 | 0.25 | 0.17 | 0.40 | 0.26 | -0.21 | -0.24 | -0.14 | **0.59** ** | 0.14 | **0.7** *** | 0.24 | 1 |

Where *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 4.5** Spearman rank correlation coefficients ($r_g$) among evaluated plant parameters of 24 spring barley genotypes under drought treatment.

| | PLH | TNP | LNP | LAP | YLA | GLA | PDM | LDM | SDM | SPAD | LS | PWC | LWC | SWC | NEP | NKE | NKP | TKW | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | 1 | | | | | | | | | | | | | | | | | | |
| **TNP** | **-0.49** * | 1 | | | | | | | | | | | | | | | | | |
| **LNP** | -0.36 | **0.71** *** | 1 | | | | | | | | | | | | | | | | |
| **LAP** | -0.22 | 0.24 | 0.26 | 1 | | | | | | | | | | | | | | | |
| **YLA** | 0.11 | 0.13 | **0.54** ** | **0.47** * | 1 | | | | | | | | | | | | | | |
| **GLA** | -0.36 | 0.27 | 0.01 | **0.83** *** | 0.01 | 1 | | | | | | | | | | | | | |
| **PDM** | **0.47** * | 0.03 | -0.16 | -0.32 | -0.03 | -0.32 | 1 | | | | | | | | | | | | |
| **LDM** | -0.14 | **0.57** ** | 0.26 | 0.07 | 0.04 | 0.21 | 0.38 | 1 | | | | | | | | | | | |
| **SDM** | **0.54** ** | -0.14 | -0.29 | -0.39 | -0.09 | -0.39 | **0.93** *** | 0.09 | 1 | | | | | | | | | | |
| **SPAD** | -0.05 | 0.20 | 0.33 | -0.02 | 0.19 | -0.13 | -0.02 | -0.19 | 0.01 | 1 | | | | | | | | | |
| **LS** | **0.41** * | -0.18 | 0.13 | 0.01 | **0.68** *** | -0.38 | 0.14 | -0.19 | 0.21 | 0.18 | 1 | | | | | | | | |
| **PWC** | -0.11 | 0.02 | -0.02 | **0.46** * | -0.03 | **0.51** * | **-0.63** *** | 0.02 | **-0.69** *** | -0.22 | -0.35 | 1 | | | | | | | |
| **LWC** | -0.08 | -0.19 | **-0.43** * | 0.16 | **-0.48** * | **0.47** * | -0.30 | -0.07 | -0.26 | -0.36 | **-0.66** *** | **0.65** *** | 1 | | | | | | |
| **SWC** | -0.18 | 0.22 | 0.26 | **0.48** * | 0.20 | 0.40 | **-0.61** ** | 0.14 | **-0.74** *** | -0.07 | -0.11 | **0.87** *** | 0.23 | 1 | | | | | |
| **NEP** | -0.15 | **0.41** * | 0.38 | 0.15 | 0.05 | 0.11 | 0.08 | 0.28 | -0.06 | -0.08 | -0.18 | 0.14 | -0.19 | 0.25 | 1 | | | | |
| **NKE** | 0.22 | -0.23 | -0.31 | -0.17 | -0.14 | -0.12 | 0.10 | -0.33 | 0.21 | 0.33 | -0.05 | -0.18 | 0.10 | -0.24 | **-0.52** ** | 1 | | | |
| **NKP** | -0.08 | 0.22 | 0.11 | 0.01 | -0.09 | 0.00 | 0.07 | 0.05 | 0.02 | 0.10 | -0.29 | 0.14 | -0.04 | 0.16 | **0.71** *** | 0.10 | 1 | | |
| **TKW** | 0.17 | -0.20 | -0.27 | -0.14 | -0.15 | 0.00 | 0.09 | 0.09 | 0.14 | -0.11 | -0.03 | -0.17 | 0.30 | -0.40 | **-0.45** * | 0.35 | -0.21 | 1 | |
| **YLD** | 0.00 | 0.18 | -0.02 | -0.09 | -0.23 | 0.01 | 0.19 | 0.10 | 0.18 | 0.07 | -0.34 | 0.00 | 0.10 | -0.11 | **0.49** * | 0.22 | **0.86** *** | 0.20 | 1 |

Where *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD-value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### *Genetic correlation analysis between plant parameters evaluated under well-watered and drought conditions*

Based on computed LSMEANS-values of 24 spring barley genotypes, genetic correlation analysis was performed to assess the relationship between traits under both, well-watered and drought conditions (Table 4.6). A total of 14 significant correlations were detected. The strongest positive correlations were found for thousand kernel weight (0.84***), stem water content (0.78***), leaf number per plant (0.77***), leaf senescence (0.69***) and tiller number per plant (0.66***). In the present study, correlation coefficients between irrigations treatments were not significant for five traits, including leaf area per plant, green leaf area per plant, number of ears per plant, number of kernels per plant and grain yield.

**Table 4.6** Spearman correlation coefficients ($r_g$) between plant parameters of 24 spring barley genotypes evaluated under well-watered and drought conditions.

| Correlation | $r_g$ | | Correlation | $r_g$ | |
|---|---|---|---|---|---|
| **PLH**$_{wet}$ x **PLH**$_{dry}$ | 0.63 | ** | **SPAD**$_{wet}$ x **SPAD**$_{dry}$ | 0.46 | * |
| **TNP**$_{wet}$ x **TNP**$_{dry}$ | 0.66 | *** | **LS**$_{wet}$ x **LS**$_{dry}$ | 0.69 | *** |
| **LNP**$_{wet}$ x **LNP**$_{dry}$ | 0.77 | *** | **PWC**$_{wet}$ x **PWC**$_{dry}$ | 0.62 | ** |
| **LAP**$_{wet}$ x **LAP**$_{dry}$ | 0.28 | ns | **LWC**$_{wet}$ x **LWC**$_{dry}$ | 0.62 | ** |
| **YLA**$_{wet}$ x **YLA**$_{dry}$ | 0.55 | * | **SWC**$_{wet}$ x **SWC**$_{dry}$ | 0.78 | *** |
| **GLA**$_{wet}$ x **GLA**$_{dry}$ | 0.31 | ns | **NEP**$_{wet}$ x **NEP**$_{dry}$ | 0.30 | ns |
| **PDM**$_{wet}$ x **PDM**$_{dry}$ | 0.46 | * | **NKE**$_{wet}$ x **NKE**$_{dry}$ | 0.44 | * |
| **LDM**$_{wet}$ x **LDM**$_{dry}$ | 0.43 | * | **NKP**$_{wet}$ x **NKP**$_{dry}$ | 0.00 | ns |
| **SDM**$_{wet}$ x **SDM**$_{dry}$ | 0.64 | ** | **TKW**$_{wet}$ x **TKW**$_{dry}$ | 0.84 | *** |
| | | | **YLD**$_{wet}$ x **YLD**$_{dry}$ | 0.14 | ns |

Where *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001, ns: none significant, Wet: well-watered conditions, Dry: drought treatment, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 4.3.7 Genetic variation and heritability of investigated plant parameters

Changes in the magnitude of genetic variance ($\sigma_G^2$), genetic coefficient of variation ($CV_g$) and heritability ($h^2$) of investigated parameters are presented in Table 4.7.

Under well-watered conditions, estimates of genetic variance ($\sigma_G^2$) ranged from 0 to 33 for 19 evaluated traits (Table 4.7). The greatest genetic variance was observed for plant height, leaf area, green leaf area and number of kernels per plant. Among investigated traits, $CV_g$ of plant height, yellow leaf area, number of ears, number of kernels per ear, number of kernels per plant and grain yield was higher than 5% with 8.6%, 10.6%, 13.2%, 21.4% and 5.7%, respectively. Under drought treatment, genetic variances for investigated traits ranged from 0 to 28.8. Here, genetic variance of plant height and yellow leaf area per plant were the greatest with 23.4 and 28.8, respectively. Genetic coefficients of variations (CVg) over 9% were observed for tiller number per plant (10.7%), yellow leaf area (14%), number of ears per plant (12%) and number of kernels per ear (10.5%). Globally, estimates of genetic variances for grain yield and grain yield components decreased under drought treatment. Thus, CVg for number of ears per plant, number of kernels per ear, number of kernels per plant and grain yield declined simultaneously with the reduction of the water supply. Nevertheless, the genetic coefficient of variation for tiller number per plant, leaf number per plant, yellow leaf area, leaf senescence and leaf water content showed the general tendency to increase under water deficit.

Estimates of heritability ($h^2$) for grain yield were 0.52 under well-watered conditions and 0.25 under drought treatment. The decrease in $h^2$ for grain yield and grain yield components corresponds with the observed grain yield reductions under water deficit (Table 4.3). Under well-watered conditions, plant height, chlorophyll content, stem water content, number of ears per plant and number of kernels per ear showed the highest heritability (Table 4.7). Under drought treatment, estimates of heritability for tiller number, leaf number, leaf water content and leaf senescence increased. The lowest heritability under water deficit was obtained for chlorophyll content (SPAD, 0.15) and grain yield (YLD, 0.25). Across irrigation levels, heritability of plant height, plant water content, stem water content, number of kernels per ear and thousand kernel weight remained fairly constant. Interestingly, estimates of genetic variance and heritability was zero for PDM, LDM and SDM under both, well-watered and drought conditions.

**Table 4.7** Estimation of genetic variance ($\sigma_G^2$), genetic coefficient of variation ($CV_g$) and heritability ($h^2$) across two years for 19 evaluated plant parameters of 24 spring barley grown under well-watered and drought conditions.

| Source of variation | | well-watered conditions | | | drought treatment | | |
|---|---|---|---|---|---|---|---|
| | | $\sigma_G^2$ | $CV_g$ (%) | $h^2$ | $\sigma_G^2$ | $CV_g$ (%) | $h^2$ |
| Morphological parameters | **PLH** | 27.59 | 8.58 | 0.86 ± 0.06 | 23.40 | 8.73 | 0.88 ± 0.05 |
| | **TNP** | 0.04 | 4.90 | 0.39 ± 0.25 | 0.10 | 10.71 | 0.78 ± 0.09 |
| | **LNP** | 0.61 | 3.27 | 0.22 ± 0.25 | 2.42 | 8.38 | 0.63 ± 0.16 |
| | **LAP** | 26.25 | 2.03 | 0.03 ± 0.41 | 0.00 | 0.00 | 0.00 |
| | **YLA** | 9.60 | 10.65 | 0.46 ± 0.23 | 28.76 | 13.95 | 0.34 ± 0.27 |
| | **GLA** | 33.47 | 2.56 | 0.04 ± 0.40 | 0.00 | 0.00 | 0.00 |
| | **PDM** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | **LDM** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | **SDM** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Physiological parameters | **SPAD** | 2.15 | 3.36 | 0.71 ± 0.09 | 0.45 | 1.98 | 0.15 ± 0.35 |
| | **LS** | 0.00 | 0.00 | 0.00 | 0.18 | 7.26 | 0.63 ± 0.15 |
| | **PWC** | 0.42 | 0.79 | 0.58 ± 0.18 | 0.59 | 1.02 | 0.58 ± 0.18 |
| | **LWC** | 0.00 | 0.00 | 0.00 | 1.42 | 1.58 | 0.56 ± 0.18 |
| | **SWC** | 0.90 | 1.17 | 0.69 ± 0.13 | 0.99 | 1.32 | 0.69 ± 0.13 |
| Grain yield and yield components | **NEP** | 0.51 | 13.24 | 0.82 ± 0.08 | 0.29 | 11.95 | 0.71 ± 0.12 |
| | **NKE** | 16.42 | 21.40 | 0.90 ± 0.04 | 2.39 | 10.45 | 0.85 ± 0.06 |
| | **NKP** | 30.97 | 5.60 | 0.39 ± 0.26 | 12.19 | 5.34 | 0.30 ± 0.29 |
| | **TKW** | 6.07 | 4.88 | 0.78 ± 0.09 | 5.93 | 5.51 | 0.75 ± 0.11 |
| | **YLD** | 0.08 | 5.74 | 0.52 ± 0.20 | 0.02 | 4.81 | 0.25 ± 0.31 |

Where: Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

### 4.3.8 Membership function value of drought stress tolerance (MFVD)

According to calculated stress tolerance indices of 19 evaluated plant parameters, the membership function value of drought tolerance was used to compare the drought tolerance of 24 spring barley genotypes (Table 4.8). Estimates of MFVD-values ranged from 0.33 (Prestige) to 0.57 (Streif). Based on the average value (0.44) and standard deviation (0.06) of MFVD, drought tolerance of spring barley was divided into four grades (ranging from drought susceptible to highly tolerant). Six genotypes were characterized as drought susceptible, nine genotypes showed a moderate drought tolerance, four genotypes were classified as drought tolerant and five genotypes showed a high drought tolerance.

**Table 4.8** Membership function value of drought tolerance (MFVD) for 24 spring barley genotypes used in phenotyping experiments during 2012 and 2013.

| Genotype | MFVD value | Grades | Classification[a] |
|---|---|---|---|
| Prestige | 0.33 | I | drought susceptible |
| Aliciana | 0.33 | I | drought susceptible |
| Sunshine | 0.34 | I | drought susceptible |
| Calcule | 0.34 | I | drought susceptible |
| SuLilly | 0.36 | I | drought susceptible |
| Propino | 0.37 | I | drought susceptible |
| Quench | 0.40 | II | moderate tolerant |
| Wiebke | 0.40 | II | moderate tolerant |
| Primadonna | 0.41 | II | moderate tolerant |
| Braemer | 0.42 | II | moderate tolerant |
| Henrike | 0.42 | II | moderate tolerant |
| Morex | 0.43 | II | moderate tolerant |
| Wisa | 0.44 | II | moderate tolerant |
| Sebastian | 0.45 | II | moderate tolerant |
| Bojos | 0.45 | II | moderate tolerant |
| Scarlett | 0.46 | III | drought tolerant |
| NFCTipple | 0.48 | III | drought tolerant |
| Tatum | 0.49 | III | drought tolerant |
| Xanadu | 0.49 | III | drought tolerant |
| Kangoo | 0.52 | IV | highly tolerant |
| Barke | 0.52 | IV | highly tolerant |
| LFL24727 | 0.55 | IV | highly tolerant |
| Grace | 0.56 | IV | highly tolerant |
| Streif | 0.57 | IV | highly tolerant |

Where a: classification of the spring barley set according to the calculated standard deviation (SD) of the mean MFVD-Value, SD = 0.06.

### 4.3.9 Relationship between membership function value of drought tolerance (MFVD) and stress tolerance indices

Drought tolerance in plants is a very complex phenomenon which involves various mechanism concerning morphological development, physiological and biochemical characteristics (Zhuang and Chen 2006). To identify genotypes with a superior performance in non-stress and stress environments, stress tolerance indices (STI) were calculated on the basis of evaluated traits over two years (Fernandez et al., 1992).Genotypes with a high STI values for the particular trait of concern were considered as drought tolerant. Furthermore, the membership function of drought tolerance (MFVD) was used to comprehensively evaluate the drought stress tolerance of investigated spring barley genotypes. Across all 24 spring barley

genotypes, spearman correlation coefficients ($r_g$) were calculated between membership function value of drought tolerance (MFVD) and stress tolerance indices of 19 traits (Table 4.9).

Membership function value of drought tolerance was positively correlated with stress tolerance indices of eight traits. The strongest positive correlation was found between MFVD and STI of LDM (0.75***). Highly to moderate associations were detected between MFVD and stress tolerance indices of TNP (0.52***), LNP (0.56**), LAP (0.56**) and NKP (0.60**). Among stress tolerance indices, significant positive correlations were noted between LNP, LDM, SWC, NEP and TNP. Genotypes with a high stress tolerance for leaf area (LAP) were positively associated with a high stress tolerance for yellow leaf area (0.55**), green leaf area (0.94***), leaf dry matter (0.47*), plant water content (0.51*) and stem water content (0.54**). Stress tolerance indices of plant dry matter (PDM) were negatively correlated with STI-values of plant water content (-0.62***) and stem water content (-0.60**), while significant and positive correlations were observed with STI values of grain yields (0.42*). In addition, stress tolerance indices concerning the leaf water content were negatively related to stress tolerance indices of the chlorophyll content (-0.43*) and leaf senescence (-0.50*). However, genotypes with a high stress tolerance for number of ears per plant (NEP) were positively associated with a high stress tolerance for tiller number (0.66**) and leaf number (0.51*) per plant, whereas negative correlations were found with STI values of plant height (-0.44*). Stress tolerance indices for grain yield (YLD) correlated with stress tolerance indices for plant dry matter (0.42*), number of ears per plant (0.55**) and number of kernels per plant (0.70***).

**Table 4.9** Spearman correlation coefficients for membership function value of drought tolerance (MVFD) and stress tolerance indices (STI) of 19 traits.

| | $STI_{PLH}$ | $STI_{TNP}$ | $STI_{LNP}$ | $STI_{LAP}$ | $STI_{YLA}$ | $STI_{GLA}$ | $STI_{PDM}$ | $STI_{LDM}$ | $STI_{SDM}$ | $STI_{SPAD}$ | $STI_{LS}$ | $STI_{PWC}$ | $STI_{LWC}$ | $STI_{SWC}$ | $STI_{NEP}$ | $STI_{NKE}$ | $STI_{NKP}$ | $STI_{TKW}$ | $STI_{YLD}$ | MFVD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $STI_{PLH}$ | 1 | | | | | | | | | | | | | | | | | | | |
| $STI_{TNP}$ | **-0.64*** ** | 1 | | | | | | | | | | | | | | | | | | |
| $STI_{LNP}$ | -0.38 | **0.79*** ** | 1 | | | | | | | | | | | | | | | | | |
| $STI_{LAP}$ | -0.19 | 0.29 | **0.52** ** | 1 | | | | | | | | | | | | | | | | |
| $STI_{YLA}$ | 0.19 | 0.23 | **0.60** ** | **0.55** ** | 1 | | | | | | | | | | | | | | | |
| $STI_{GLA}$ | -0.30 | 0.24 | 0.36 | **0.94*** ** | 0.23 | 1 | | | | | | | | | | | | | | |
| $STI_{PDM}$ | **0.42*** | -0.11 | -0.20 | -0.21 | -0.15 | -0.19 | 1 | | | | | | | | | | | | | |
| $STI_{LDM}$ | -0.35 | **0.55** ** | 0.37 | **0.47*** | 0.08 | **0.51*** | **0.45*** | 1 | | | | | | | | | | | | |
| $STI_{SDM}$ | **0.59** ** | -0.32 | -0.35 | -0.39 | -0.19 | -0.38 | **0.95*** ** | 0.15 | 1 | | | | | | | | | | | |
| $STI_{SPAD}$ | 0.05 | 0.12 | 0.31 | -0.10 | 0.16 | -0.20 | 0.05 | -0.05 | 0.07 | 1 | | | | | | | | | | |
| $STI_{LS}$ | 0.28 | -0.21 | -0.06 | -0.20 | 0.14 | -0.30 | 0.16 | -0.15 | 0.23 | 0.07 | 1 | | | | | | | | | |
| $STI_{PWC}$ | -0.31 | 0.36 | 0.29 | **0.51*** | 0.17 | **0.53** ** | **-0.62*** ** | 0.08 | **-0.71*** ** | -0.28 | **-0.49*** | 1 | | | | | | | | |
| $STI_{LWC}$ | -0.29 | -0.11 | -0.32 | 0.08 | **-0.58** ** | 0.33 | -0.22 | -0.01 | -0.24 | **-0.43*** | **-0.50*** | **0.46*** | 1 | | | | | | | |
| $STI_{SWC}$ | -0.25 | **0.46*** | **0.45*** | **0.54** ** | **0.41*** | **0.47*** | **-0.60** ** | 0.11 | **-0.70*** ** | -0.13 | -0.36 | **0.94*** ** | 0.14 | 1 | | | | | | |
| $STI_{NEP}$ | **-0.44*** | **0.66*** ** | **0.51*** | 0.14 | 0.07 | 0.11 | 0.11 | **0.44*** | -0.03 | 0.11 | 0.02 | 0.09 | -0.21 | 0.18 | 1 | | | | | |
| $STI_{NKE}$ | **0.74*** ** | **-0.61** ** | **-0.50*** | -0.12 | -0.08 | -0.11 | 0.27 | -0.25 | 0.39 | 0.14 | 0.12 | -0.20 | -0.09 | -0.16 | **-0.62** ** | 1 | | | | |
| $STI_{NKP}$ | 0.03 | 0.30 | 0.20 | 0.00 | -0.01 | -0.01 | 0.36 | 0.31 | 0.29 | 0.35 | 0.21 | -0.14 | -0.39 | 0.02 | **0.66*** ** | 0.12 | 1 | | | |
| $STI_{TKW}$ | -0.26 | -0.14 | -0.22 | -0.21 | -0.18 | -0.17 | 0.06 | 0.00 | 0.06 | -0.09 | -0.07 | -0.24 | 0.23 | -0.38 | -0.23 | -0.28 | **-0.46*** | 1 | | |
| $STI_{YLD}$ | -0.21 | 0.24 | 0.07 | -0.14 | -0.14 | -0.12 | **0.42*** | 0.36 | 0.33 | 0.30 | 0.16 | -0.30 | -0.21 | -0.24 | **0.55** ** | -0.15 | **0.70*** ** | 0.31 | 1 | |
| MFVD | -0.06 | **0.52*** ** | **0.56** ** | **0.56** ** | 0.39 | **0.48*** | **0.42*** | **0.75*** ** | 0.21 | 0.23 | 0.01 | 0.13 | -0.22 | 0.25 | 0.53 | -0.08 | **0.60** ** | -0.15 | **0.50** ** | 1 |

Where: *: 0.01 < P < 0.05, **: 0.001 < P < 0.01, *** P < 0.001 STI: stress tolerance index, PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield, MFVD: membership function value of drought tolerance.

### 4.3.10 Evaluation of drought tolerance among spring barley cultivars

Based on rank values of 24 spring barely genotypes for 20 computed stress tolerance indices, a multidimensional preference analysis was performed. In particular, the multidimensional preference analysis is a principle component analysis, which aimed to display the relationship between evaluated genotypes and stress tolerance indices (Fig. 4.3). Results of the principal component analysis showed that the first two components explained 74.1% of the total variation. Here, stress tolerance indices of stem water content, plant water content, leaf area per plant and green leaf area were the most contributing parameters for the first component, which explained 40.69% of the total variation. 33.8 percent of the total variability was explained by the second component, which showed high and positive loadings for the membership function value of drought tolerance and STI values of number of kernels per plant, number of ears per plant and the grain yield. Thus, the second component is preliminary characterized by yield-related traits. It is apparent from the biplot graph (Fig. 4.3) that genotype ‘Streif’ was associated with a high membership function value of drought tolerance and a high stress tolerance regarding the grain yield, number of kernels per plant, leaf dry matter and leaf number per plant. In contrast, ‘Sunshine’ was the one with the lowest loadings for the first and second component. Thus, ‘Sunshine’ was associated with a low average performance, especially for stress indices which involves YLD and MFVD.

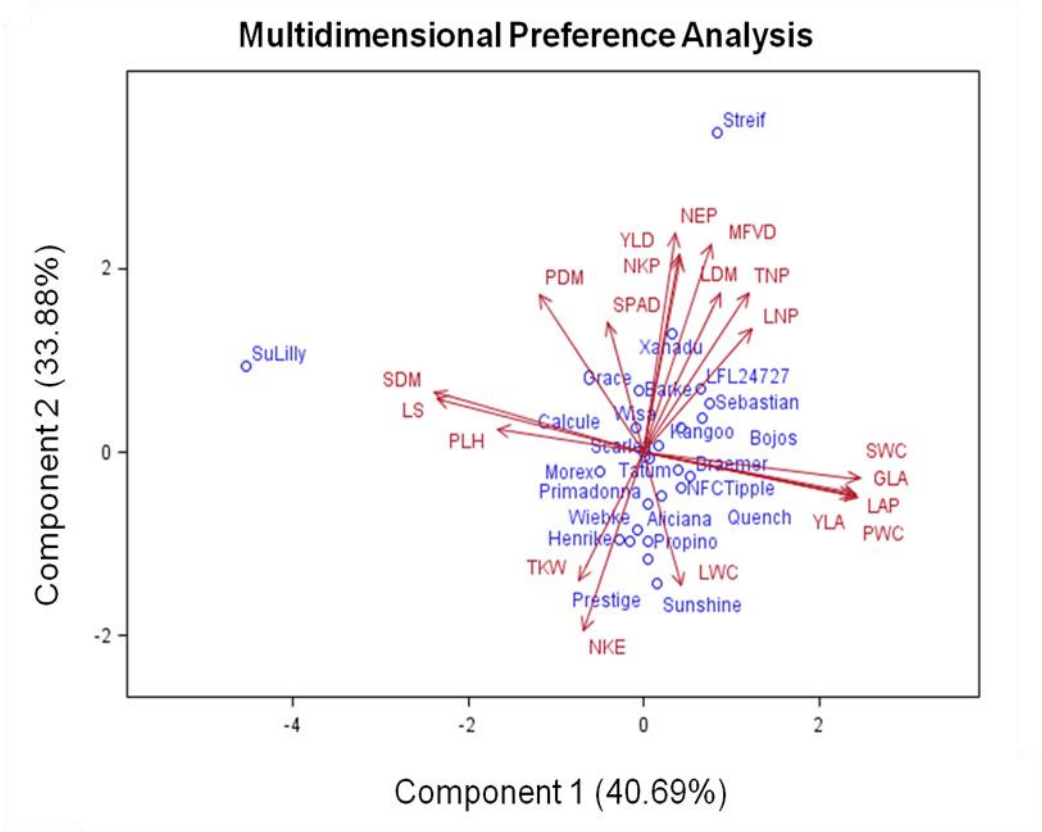


**Fig. 4.3. Biplot of 24 spring barley genotypes, 19 stress tolerance indices and MFVD-value based on the first and second component of multidimensional preference analysis.** The biplot represents genotypes by a point and stress tolerance indices by a vector.

## 4.4 Discussion

Breeding for drought stress tolerance is a difficult challenge and several strategies have been discussed in the context of increasing the genotypic performance under water-limited conditions (Richards, 1996; Tuberosa, 2012). Prior studies, for instance, have examined the usage of drought adaptive, secondary traits to improve crop yields in dry environments (Richards, 1996; Ober et al., 2005; Sinebo, 2005; Monneveux et al., 2008; Ziyomo and Bernardo, 2013). And, although many traits (e.g. leaf senescence, carbon isotope discrimination and yield components) have been proposed as selection criteria, breeding progress on drought stress tolerance is still very low. However, this study aimed (1) to assess the relationship between the grain yield and secondary traits and (2) to evaluate the applicability of investigated traits for identifying drought stress tolerance in spring barley. During 2012 and 2013, nine morphological, five physiological and four yield-related parameters have been examined in pot experiments at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES), Chair of Plant Breeding, University of Bonn, Germany. Investigated plant parameters were run for multiple statistical analyses and results will be discussed thereafter.

### 4.4.1 Effect of water shortage on crop development and grain yield

In current study, the reduction of the water supply from tillering to anthesis (BBCH 24 to BBCH 49) caused a serve drought stress so that the overall plant performance remarkably decreased. Genotypes under well-watered conditions were fully irrigated over the whole vegetation period. Thus, spring barley genotypes under well-watered conditions were able to display their yield potential. Based on 19 investigated traits, high significant effects for the irrigation treatment and the genotype were detected.

Compared to well-watered conditions, mean values of morphological parameters such as tiller number per plant (TNP), leaf number per plant (LNP), leaf area per plant (LAP), green leaf area (GLA), plant dry matter (PDM), leaf dry matter (LDM) and stem dry matter (SDM) decreased over 20% underlying the negative impact of water deficit on plant metabolism, growth and differentiation. Gathered data agree with other studies which have shown that plant height, leaf area, above dry mass, chlorophyll content, grain number, thousand kernel weight and grain yield per plant seriously declined under water limiting conditions (Borrell et al. 2000; Bayoumi et al. 2008; Chen et al. 2012). In fact, water deficit

between tillering and anthesis resulted in reductions of the leaf expansion and photosynthesis, which arise by decreases in the green leaf area (45%), leaf number per plant (22%), leaf water content (7%) and chlorophyll content (22%) (Araus et al. 2002; Farooq et al. 2009). At the same time, the yellow leaf area and thus premature leaf senescence increased. According to Passioura (1996), leaf extension can decline under water stress in order to get a balance between water absorbed by roots and the water status in plant tissues. In addition, fewer tillers under drought treatments accompanied by a lower leaf area and a higher leaf senescence support the idea that drought stress decreases the canopy leaf area due to the degeneration of existing tillers and the termination of the appearance of new tillers (Blum 1996). Furthermore, it seems possible that the reduction of the functional green leaf area under drought stress might had a negative influence on the expansion and division of cells, stomatal conductance, photosynthesis and therefore on the potential for assimilation (Blum 1996; Richards 2000; Araus et al. 2002; Arunyanark et al. 2008).

Globally, decreasing availability of water under drought conditions had a considerable impact on the photosynthesis and the transpiration rate due to reductions in chlorophyll content (SPAD), plant water content (PWC), leaf water content (LWC), stem water content (SWC) and grain yield components, which finally resulted in grain yield losses of 43% (Blum 1996; Farooq et al. 2009). These findings agree with previously presented and discussed results in Chapter 3. Drought treatments imposed between tillering stage and anthesis reduced the SPAD value and hence the chlorophyll content by 22%. Arunyanark et al. (2008) reported that chlorophyll losses due to drought stress were associated with reductions in photosynthesis and decreases in plant dry matter.

Overall, the grain yield depend on the number of ears per plant, number of kernels per ear, number of kernels per plant and thousand kernel weight (Blum et al. 1990; Christen et al. 1995; Samarah 2005; Bayoumi et al. 2008). Findings of this study indicate that drought significantly affected the grain yield and yield components. In particular, water deficit from tillering to anthesis decreased grain yields of spring barley by reducing the number of ears per plant and the number of kernels per plant (Blum et al. 1990; Samarah 2005).

### 4.4.2 Relationship between evaluated plant parameters and yield formation

Generally, correlation analyses are crucial to understand the overall plant response to water deficit and to assess the value of secondary traits as selection criteria for phenotyping drought stress tolerance (Edmeades and Bolaños 1996).

Under well-watered conditions, genetic correlation analysis among 19 examined traits indicated that the tiller number per plant (TNP) was positively associated with the leaf number per plant (LNP), leaf area per plant (LAP), yellow leaf area (YLA), leaf dry matter (LDM), plant water content (PWC) and number of ears per plant (NEP). The observed significant relationship of the tiller number (TNP) with a wide range of morphological, physiological and yield related parameters support the assumption that the formation and maintenance of tillers increased the photosynthesizing area and the volume of roots per unit soil surface, which again increase the efficiency of crops in utilizing available water and nutrients (Blum 1996; Arduini et al. 2006; Farooq et al. 2009). Positive correlations of the leaf number per plant (LNP) with the leaf area (LAP), plant water content (PWC) and number of ears per plant indicating the sensitivity of cell division and expansion of young leaves to soil water availability (Teulat et al. 1997).

Under water deficit conditions, the plant height (PLH) was negatively correlated with the tiller number, whereas positive associations were found with the plant dry matter (PDM) and the leaf senescence (LS). Hence, it seems possible that a high plant height appears to be very important to achieve a higher plant biomass under water shortage. Other researchers have also suggested that the semidwarf stature of plants is favourable under water stress conditions and aid in improving lodging resistance (Ginkel et al. 1998). The current study showed that the plant water content was positively correlated with the plant leaf area (LAP) indicating the susceptibility of cell division and leaf expansion to water stress, which additionally determines the plant ability to intercept light and convert it into biomass (Teulat et al. 1997; Lu et al. 2011). Furthermore, data revealed that leaf senescence (LS) was negatively related to leaf water content (LWC). Under water shortage, the observed association between LS and LWC increased in strength, indicating that leaves began earlier to die in genotypes with an insufficient water transport to leaves. Present findings seem to be consistent with other studies and suggest that genotypes with a greater access to water avoided or delayed leaf senescence. Hence, the degree of senescence might be a good indicator for the soil water content and the plant hydraulic conductance (Borrell et al. 2000; Ober et al. 2005).

Surprisingly, no significant associations were observed under drought conditions between grain yields and investigated morphological and physiological traits. Under well-watered and drought conditions, grain yield was found to be positively associated with the number of ears per plant (NEP) and the number of kernels per plant (NKP). With increasing water stress, correlations between the grain yield and the number of ears per plant decreased in strength. Contrary, observed associations between the grain yield and the number of kernels per plant increased under drought conditions indicating a greater genetic variation under water stress (Magorokosho and Tongoona 2004). In accordance with present results, previous studies of Leilah and Al-Khateeb (2005) showed that high yields of wheat plants could be obtained by selecting genotypes with high numbers of ears per square meter. Fussell et al. (1991) reported that the drought tolerance in pearl millet was preliminary expressed in traits which were related to the ability to maintain high grain numbers under water shortage. Additionally, Monneveux et al. (2008) claimed that a selection for a greater number of ears, bigger grains and smaller tassels in maize may be useful to increase the grain yield in water limited environments.

In the present study, attempt was made to explore the relationship between droughted and well-watered parameters. Under well-watered and drought conditions, significant positive correlations of seven morphological, five physiological and two yield-related parameters were observed (Table 4.6) indicating the possibility to select a genotypes with a high biomass and/or tiller number in non-stress environments. In general, non-stress environments are characterized by a maximized genetic variation and low genotype-by-environment interactions (Richards 1996; Ceccarelli et al. 1998). Despite these findings, associations between droughted and irrigated traits for leaf area per plant (LAP), green leaf area (GLA), number of ears per plant (NEP), number of kernels per plant (NKP) and grain yield (YLD) were non-significant. These findings suggest that genotypes selected for a high yield potential in non-stress environments will not immediately achieve high grain yields in stress environments. The results accords with findings of Ober et al. (2004) who highlighted the importance to differentiate between genotypes that show high yields under drought conditions due to their high inherent yield potential and those who have a greater drought tolerance *per se*.

Although a functional green leaf area, a delayed leaf senescence, a high plant dry matter and a high chlorophyll content have been proposed as vital secondary traits for drought stress tolerance, none of these traits showed an improved correlation under drought treatment. The present results are in good agreement with other studies which observed a poor genetic

correlation between secondary traits and grain yields under water deficit (Bolanos and Edmeades 1996; Gallais 2008; Kumar et al. 2008). Nevertheless, there were positive correlations between tiller number, leaf number, leaf dry matter and number of ears per plant suggesting that these traits might be a valuable indicator for drought stress tolerance in spring barley. As photosynthesis is closely related to the leaf expansion, water and nutrient supply, the present study demonstrated that the leaf senescence, stem dry matter and leaf area are related to dynamics of plant water use. Differences in correlation coefficients between individual traits and irrigation levels (well-watered and drought treatment) can be explained by the different amount of variation exhibited by traits under contrasting soil moisture conditions. Globally, the amount of variation decreased under water stress.

### 4.4.3 Genetic variation and heritability of evaluated plant parameters

Ideally, traits are suitable as potential selection criteria for drought stress tolerance if they show the presence of a sufficient genetic variability and if they have a greater heritability than the primary trait (Edmeades and Bolaños 1996; Tardieu and Tuberosa 2010).

The current study revealed under drought treatment a significant genetic variability for the majority of morphological parameters (PLH, TNP, LAP and YLA) and yield components (NEP, NKE, NKP and TKW). The findings suggest that variations in spring barley for these traits are mainly influenced by the genotype rather than environmental factors. Results of the present study showed that under drought conditions there is a efficient control of environmental variations and a better expression of genetic differences (Bouzerzour and Dekhili 1995). Thus, the tiller number per plant (TNP), leaf number per plant (LNP), yellow leaf area (YLA) and leaf senescence (LS) were characterized by a higher heritability. As expected, the grain yield and grain yield components showed a decrease in genetic variability and heritability under drought treatment. The results indicate that environmental variances generally increased under water deficit. Data obtained are in consistent with those of Bayoumi et al. (2008) who found that heritability values for biological yield, grain yield and thousand kernel weight in wheat decreased under stress conditions. Ziyomo and Bernardo (2013), Lu et al. (2011) and Magorokosho and Tongoona (2004) observed similar results where heritability estimates for grain yield in maize populations declined under drought. Moreover, lower estimates of heritability for chlorophyll content (SPAD), number of kernels per plant and number ear of ears per plant correspond with findings of Lu et al. (2011) who examined the heritability of multiple drought resistance criteria in maize. Although estimates of heritability

for the grain yield and yield components were moderate to high under drought, the observed decrease in genetic variability under water deficit indicate a careful selection of barley genotypes for these traits.

With regard to the plant height (PLH), chlorophyll content (SPAD value), grain yield and yield components, optimum growth conditions enabled genotypes to express their full range of phenotypic capacities (Ober et al. 2005). Surprisingly, the study revealed neither under control or under drought treatments a sufficient genetic variation and heritability for the plant dry matter (including leaf and stem dry matter). Since heritability captures the proportion of phenotypic variance, due to heritable genetic effect (Holland 2003), the lack of genetic variance could be explained by high phenotypic effects, or because of other environmental factors which interact with the genotypic performance and might make a genetic improvement through a selection of drought tolerant genotypes difficult (Bolaños et al. 1993; Bayoumi et al. 2008). Finally, higher heritability estimates and genetic variability for the tiller number per plant, the leaf number per plant and the leaf senescence led to the assumption that parameters which are linked to the biomass accumulation might be useful selection criteria under drought conditions.

### 4.4.4 Membership function value of drought tolerance in spring barley and its association with drought tolerance indices

Drought is a multidimensional stress and drought tolerance can only be determined if drought stress cause a considerable yield reductions (Blum 1996; Denčić et al. 2000). In the present study, drought treatments between tillering and anthesis significantly decreased the grain yield and yield components (Table 4.3). Additionally, significant genotype-by-treatment interactions for the grain yield were found suggesting that not all examined genotypes respond similar to water stress. Under these circumstances and in consideration of the non-significant relationship between droughted and well-watered yields, there is no guarantee that genotypes selected for high yields in non-stress environments will achieve a superior yield performance under water stress conditions (Ober et al. 2004). With regard to the yield performance, under well-watered and drought conditions (Fig. 4.2), and calculated MFVD-values (Table 4.8), the spring barley genotypes ‘Grace’ and ‘Streif” were the least sensitive genotypes to drought. In contrast, older varieties such as ‘Wisa’ and ‘Morex’ showed a low stress tolerance for the tiller number per plant, leaf number per plant, thousand kernel weight

and grain yield (Fig. 4.2 and 4.3). The results suggest that modern varieties are characterized by a higher yield potential and coped better with drought conditions (Denčić et al. 2000).

In the recent years, many studies analyzed intensively morphological, physiological and biochemical traits as well as drought tolerance indices in order to explore abiotic stress tolerance in plants (Ceccarelli et al. 1991; Ober et al. 2005; Leilah and Al-Khateeb 2005; Cattivelli et al. 2008; Chen et al. 2012; Ziyomo and Bernardo 2013). Since the membership function value of drought tolerance (MFVD) was proposed as a selection indicator, stress tolerance indices (STI) of 19 parameters were used to investigate the drought tolerance in spring barley (Chen et al. 2012). According to computed MFVD-values, five genotypes were classified as highly drought tolerant and only six genotypes were ranged as drought susceptible (Table 4.8). Thirteen genotypes were screened as moderate tolerant or drought tolerant, respectively. Correlation analysis between MFVD-value and stress tolerance indices of 19 traits suggest that tiller number per plant, leaf number per plant, leaf area per plant, yellow leaf area, plant dry matter, leaf dry matter, number of kernels per plant and grain yield are useful traits to asses drought tolerance. In contrast to genotypes with low MFVD-values, genotypes with a high overall drought stress tolerance might be able to maintain photosynthesis, carbon assimilation and leaf area formation which finally result in a better yield performance. Moderate to highly significant correlations between MFVD-value and $STI_{\mathbf{LNP}}$, $STI_{\mathbf{LAP}}$ and $STI_{\mathbf{LDM}}$ agree with findings of Lu et al. (2011), who evaluated drought resistance in 550 maize inbred lines. Lu et al. (2011) argued that measurements of the biomass and leaf expansion by normalized difference vegetation index (NDVI) are reliable drought resistance criteria because they are sensitive to water stress and determines the plant ability to intercept light and convert it into biomass. The observed negative correlations between STI of the leaf senescence and leaf water content support the idea that wilting is related to the plant water use and, moreover, that wilting was might be avoided or delayed by a greater access to water (Ober et al. 2005). Generally, positive correlations between stress tolerance indices of the grain yield and plant dry matter, number of ears per plant and number of kernels per plant support the hypothesis that drought tolerant genotypes are characterized by their ability to produce more biomass, ears and kernels under drought conditions. Surprisingly, physiological traits which are related to the plant water status (LS, PWC, LWC and SWC) appeared to have a little contribution for improving drought tolerance (Bolanos and Edmeades 1996).

Findings of the present study suggest that genotypes differ in their drought response. Drought tolerant genotypes might have some characteristics which prevent them from losses. However, when judging the drought tolerance of genotypes it is important to distinguish between the inherent genotypic performance level and drought tolerance *per se* (Ober et al. 2004). The distribution of genotypic performance in the biplot (Fig. 4.3) revealed that the drought stress tolerance of evaluated spring barley genotypes was almost equal. According to Ober et al. (2004) genetic improvements in drought stress tolerance is depending on identification of sources of germplasm with a greater drought tolerance than current varieties. Thus, studies on drought stress tolerance with introgression lines might be an important source of genetic variation and can make a valuable contribution for drought tolerance improvements in barley. Taken together, results indicate that traits which are directly linked to the grain yield, for example (NEP, NKP and YLD), and the biomass accumulation (PDM and TNP) are more valuable as selection criteria for drought tolerance than traits which are related to the plant water status (PWC, LWC and SWC).

## 4.5 Conclusion

In conclusion, findings of this chapter revealed that:

(1) water deficit between tillering and anthesis resulted in reductions of the leaf expansion and photosynthesis, which arise by decreases in tiller number, green leaf area, leaf number per plant, leaf water content and chlorophyll content (Araus et al. 2002; Farooq et al. 2009).

(2) the decreasing availability of water decreased spring barley grain yields by 43% through reductions of the number of ears per plant (-17%) and the number of kernels per plant (-34%).

(3) the tiller number, leaf number, leaf dry matter, stem dry matter, leaf senescence and stem water content were significantly associated under non-stress and stress conditions. Hence, these traits might be valuable selection criteria for phenotyping drought stress tolerance in spring barley.

(4) under drought conditions there is a better expression of genetic differences and thus a higher heritability of the tiller number per plant (TNP), leaf number per plant (LNP), yellow leaf area (YLA) and leaf senescence (LS).

(5) the indirect selection for grain yield under well-watered conditions is less efficient.

(6) the membership function values of drought tolerance (MFVD) is a useful, comprehensive selection index which combines information's of several to drought tolerance related traits.

In general, the absence of significant correlations between investigated secondary traits and the grain yield support the idea that breeding for drought stress tolerance should be based on the genotypic performance under well-watered and drought conditions. Results of this study showed that stress tolerance indices of the grain yield, number of ears per plant, tiller number per plant, leaf number per plant and leaf dry matter were valuable selection indices to identify drought stress tolerance in spring barley. Progress in breeding for drought tolerance can possibly obtained by selecting these traits in non-stress and stress environments. Generally, when judging the drought tolerance of genotypes it is important to distinguish between the inherent genotypic performance level and drought tolerance *per se* (Ober et al. 2004).

# Chapter 5: Phenotyping drought stress tolerance of spring barley under natural field conditions in Germany

## 5.1 Introduction

Water deficit is one the most important stress factors which represent a major challenge for plant breeders (Hlavinka et al. 2009). The importance of breeding crops with an improved drought stress tolerance is reinforced by the expected increase in climate variability and the continuously growing human population with more than 9 billion inhabitants by 2050 (Tomlinson 2011; Tilman et al. 2011; Tardieu 2012). So far, crop improvements under drought conditions involve complex mechanism due to the environmental variability and the co-occurrence of several types of abiotic stresses, including high temperatures, high irradiance, and nutrient deficiencies (Mittler 2006; Fleury et al. 2010; Araus and Cairns 2014). Thus, research programs especially for drought stress tolerance are slow in progress. However, in the recent years considerable effort have been made to understand drought tolerance mechanism by investigating agronomical and physiological traits (e.g. biomass accumulation, growth habits, and grain yield components) (Munoz et al. 1998; Francia et al. 2011; Lakew et al. 2011; Honsdorf et al. 2014). Munoz et al. (1998) assessed the adaptation of spring barley cultivars in Spain and reported that old barley cultivars were especially adapted to poorer field sites. Lakew et al. (2011) investigated the drought stress tolerance of fifty-seven barley lines derived from with *Hordeum spontaneum* C., ten barley cultivars and three landraces in field experiments. Here, Lakew et al. (2011) suggest that future studies should be realized across a wide range of environments. Despite the fact that drought is a climatologically event, drought tolerance which affect the grain yield can only be reliable assessed under field conditions (Campos et al. 2004; White and Andrade-Sanchez 2012). Nevertheless, there is still an insufficient knowlegde about the crop response to drought stress under natual field conditions. Hence, the objectives of this study were (1) to screen 24 spring barley cultivars for their crop development and yield performance in rainfed field environments, which encompasses well-watered and drought conditions, (2) to detect specific traits that improve the selection of genotypes with stable grain yields under various environmental conditions, (3) to assess the degree of genetic correlation, genetic variance and heritability for morphological, physiological and yield-related traits and finally (4) to investigate the yield performance and yield stability of spring barley in environments with contrasting amounts of available water.

## 5.2 Materials and Methods

The following section contains detailed information about the experimental setup, the used plant material, measured plant parameters and performed statistical analyses.

### 5.2.1 Experimental sites

Between 2012 and 2013, field experiments were carried out at five locations in Germany (Fig. 5.1). To determine the fertility (nutrient) status and chemical properties that affect the soil suitability as plant growth media, representative soil samples consisting of eight sub-samples per field were taken at 30 and 60 cm soil depth (Walworth 2006). The detail information concerning the field sites and the soil texture are summarized in the Appendix.


**Fig. 5.1. Location of experimental sites for phenotyping experiments in 2012 and 2013, in Germany.**

### 5.2.2 Monitoring meteorological data

In each environment (location-year combination), environmental parameters were recorded under the usage of a HOBO U30 Data logger (Onset®, Bourne, MA 02532, USA). The HOBO U30 Data logger is a remote monitoring weather station which transmitted every 20 minutes logged weather data to the web via cellular communications (72 connections per

day). Solar panels powered and recharged the batteries of the stations. Lightning conductors were installed to prevent the weather stations from damages by lightning strikes. The HOBO U30 station recorded the following parameters:

- volumetric water content with two HOBO soil moisture smart sensors ONS-S-SMA-M005
- soil temperature with two HOBO soil temperature smart sensors ONS-S-TMB-M006
- air temperature and humidity with HOBO smart sensor ONS-S-TMB-M002
- photosynthetically-active radiation with HOBO PAR sensor smart sensor ONS-S-LIB-M003
- rainfall with HOBO rain gauge sensor smart sensor ONS-S-RGB-M002
- wind speed with HOBO wind speed sensor smart sensor ONS-S-WSA-M003
- air pressure with HOBO barometric pressure smart sensor ONS-S-BPB-CM5

The two soil moisture and soil temperature sensors were installed in an undisturbed soil surrounding, in 20 cm depth.

### 5.2.3 Experimental design, plant material and crop management

In 2012 and 2013, field experiments were established as complete randomized block design with four to six replications, depending on the location (see Appendix). Twenty-four spring barley cultivars were allocated randomly to each block. The compilation of the spring barley set was carried out in close cooperation with relevant German breeding companies and includes both, old and modern cultivars. In particular, the 24 spring barley varieties were assumed to be a representative sample of a collection of varieties which is used in Central European breeding programs. Information concerning the names of spring barley cultivars and their year of release are presented in Table 4.1 of Chapter 4. In each year and for each location three sampling dates were set: (1) end of the tillering stage and begin of stem elongation (approx. BBCH 30), (2) between booting stage and anthesis (approx. BBCH 55) and finally (3) at the end of the fruit development and begin of the ripening stage (approx. BBCH 80). Plots were kept weed free and plant protection was applied as necessary to avoid the presence of pests and diseases. Fertilizers were applied according to the agriculture practice for malting barley. The application of growth regulators, strobilurine and sulfonylurea was avoided.

### 5.2.4 Phenotypic data collection

A total of 25 traits were investigated in phenotyping experiments (Table 5.1). Evaluated traits included eleven morphological, nine physiological and five yield and yield-related parameters. Recorded traits and their abbreviations are presented in Table 5.1. Principal growth stages were scored using the extended BBCH scale of Hess et al. (1997).

***Morphological plant parameters***

**Plant Height**

Plant height (PLH) was measured from the soil surface to the tip of the spike, recorded to the nearest cm.

**Above plant dry biomass**

The total above plant dry mass was sampled at (1) the end of the tillering stage and begin of stem elongation (BBCH 30), (2) between booting stage and anthesis (BBCH 63) and finally (3) at the end of the fruit development and begin of the ripening stage (BBCH 81). In particular, plant samples were obtained within a plot from 0.5 m of two rows. In each plot guard rows were excluded. Plants were cut to the ground level with manual shears. To determine the above plant dry matter (PDM), plant samples were dried in drying chambers at 50°C for 72 hours and weighted.

**Plant growth rates**

Crop growth rates (CGR), which are defined as the increases in plant dry matter per time, were determined using the following equations:

CGR 1 = $PDM_1$/ $DAS_1$

CGR 2 = ($PDM_2$ – $PDM_1$) / ($DAS_2$ – $DAS_1$)

CGR 3 = ($PDM_3$ – $PDM_2$) / ($DAS_3$ –$DAS_2$)

CGR 4 = ($PDM_3$ – $PDM_1$) / ($DAS_3$ – $DAS_1$)

Where $PDM_1$, $PDM_2$, $PDM_3$ are plant dry matters at BBCH 30, 63 and 81 and DAS are days after sowing until plants reached BBCH 30 (DAS1), BBCH 63 (DAS2) and BBCH 81 (DAS3). Crop growth rates are expressed as g $m^{-2}$ per day.

The mean growth rate (MWR) was calculated as: $MWR = \frac{CGR1 + CGR2 + CGR3}{3}$

Where CGR1, CGR2, CGR3 are the computed crop growth rates and expressed as g $m^{-2}$ per day.

***Physiological plant parameters***

Subsamples from plant dry matters (around 5g) were taken and ground to fine powder using a cross beater mill (Cross Beater Mill SK1, Retsch GmbH, Haan, Germany) and a vibrating tube mill. Afterwards subsamples were analyzed for nitrogen, phosphor and potassium concentration in plants.

**Plant nitrogen concentration**

Plant samples were dried in the drying chambers at 50°C for 72 h. The total nitrogen content was measured with an elemental analyzer (Carlo Erba Instruments, Milano, Italy) after the method of Colombo et al. (1988). For each sample 4 mg of plant powder were used.

**Plant phosphor and potassium content**

To determine the concentration of phosphor and potassium in plant material, 200 mg of fine powdered plant subsamples were wet digested in vessels using 5 ml of concentrated nitric acid (65% $HNO_3$) and 4 ml of hydrogen peroxide (35% $H_2O_2$). Wet digestion of samples was carried out in a pressurized system using the microwave oven CEM MARS 6 (CEM

Corporation, Matthews, NC, USA). A detailed description of the microwave digestion method has been described elsewhere (Swami et al. 2001). After full digestion samples were transferred to glass bulbs and diluted with distilled, deionized water to a final volume of 100 ml. Finally, solutions were analyzed for phosphor by filter photometer and for potassium by atomic absorption spectrometry (AAS).

### *Grain yield and yield components*

At maturity grain yield and grain yield components, e.g. number of ears and kernels per square meter, number of kernels per ear and thousand kernel weight were determined.

#### Number of ears per m²

Based on plant samples the number of ears per m² (NEM) was calculated as:
NEM = number of ears from 0.5 m of two rows * (1m/ row distance (m)).

#### Number of kernels per ear

At physiological maturity, plants of two rows with a length of 0.5 m were harvested. Spikes were counted. The number of kernels per ear (NKE) was calculated as:
NKE = number of kernels per m²/ number of ears per m².

#### Number of kernels per m²

Average single grain weight (g grain$^{-1}$) was calculated from the thousand kernel weight (TKW). Numbers of kernels per square meter (NKM) were calculated by dividing grain yield per square meter by single grain weight.

#### Thousand kernel weight

For thousand kernel weight (TKW), thousand grains were randomly selected and weighted (in grams).

#### Grain yield

In each year and at each location, plots were harvested at full maturity. Grain yield (YLD) was determined by weighing the grain samples before and after oven drying at 50°C for 72 hours. Grain yield was adjusted to 15% moisture content.

**Table 5.1** Summary of scored and calculated traits for field phenotyping experiments conducted during 2012 and 2013 at eight field sites in Germany.

| Plant parameters | Growth stage | Abbr. | Unit | Measured or calculated trait |
|---|---|---|---|---|
| **Morphological parameters** | | | | |
| **Plant height** | BBCH 30 | PLH1 | cm | measured |
| | BBCH 63 | PLH2 | cm | measured |
| | BBCH 81 | PLH3 | cm | measured |
| **Above plant dry matter** | BBCH 30 | PDM1 | g $m^{-2}$ | measured |
| | BBCH 63 | PDM2 | g $m^{-2}$ | measured |
| | BBCH 81 | PDM3 | g $m^{-2}$ | measured |
| **Crop growth rates** | | | | |
| Crop growth rate until BBCH 30 | | CGR1 | g $m^{-2}$ per day | calculated |
| Crop growth rate between BBCH 30 and BBCH 63 | | CGR2 | g $m^{-2}$ per day | calculated |
| Crop growth rate between BBCH 63 and BBCH 81 | | CGR3 | g $m^{-2}$ per day | calculated |
| Crop growth rate between BBCH 30 and BBCH 81 | | CGR4 | g $m^{-2}$ per day | calculated |
| **Mean growth rate** | | MWR | g $m^{-2}$ | calculated |
| **Physiological parameters** | | | | |
| **Plant nitrogen content** | BBCH 30 | PNC1 | % | measured |
| | BBCH 63 | PNC2 | % | measured |
| | BBCH 81 | PNC3 | % | measured |
| **Plant phosphor content** | BBCH 30 | PPhC1 | % | measured |
| | BBCH 63 | PPhC2 | % | measured |
| | BBCH 81 | PPhC3 | % | measured |
| **Plant potassium content** | BBCH 30 | PKC1 | % | measured |
| | BBCH 63 | PKC2 | % | measured |
| | BBCH 81 | PKC3 | % | measured |
| **Yield components** | | | | |
| Number of ears per square meter | BBCH 81 | NEM | No. $m^{-2}$ | measured |
| Number of kernels per ear | BBCH 81 | NKE | No./ear | calculated |
| Number of kernels per square meter | BBCH 81 | NKM | No. $m^{-2}$ | calculated |
| Thousand kernel weight | BBCH 99 | TKW | g | measured |
| **Grain yield** | BBCH 99 | YLD | dt $ha^{-1}$ | measured |

Abbr.=abbreviation

### 5.2.5 Statistical analyses

Across environments (location-year combination) each field experiment was laid out in a completely randomized block design with four to six replications (blocks), depending on the location (see Appendix). Out of the ten environments, eight environments were suitable for further statistical analyses. Due to serve weather conditions (storms and hail) in the beginning of July 2012, field experiments in Bavaria-Uffenheim had to be rejected. In 2013, field trials in Bavaria-Herzogenaurach were aborted due to the impact of soil compaction on plant development.

Statistical analyses for morphological, physiological and yield-related parameters were performed using the SAS software version 9.2 (SAS Institute 2008). Analysis of variance was conducted using SAS PROC MIXED with environments and genotypes having fixed effects. Replications (blocks) within each environment were considered as random effects. In order to test the significance of fixed effects, PROC MIXED procedure computed the "Type 3 Test of Fixed Effects". The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***. The LSMEANS statement in PROC MIXED procedure calculated least-squares means (LS-means) of fixed effects.

The model used for analysis of variance across eight environments is:

$$Y_{ijk} = \mu + E_i + B(E)_{j(i)} + G_k + G_k*E_i + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $E_i$ is the fixed effect of i-th Environment; $B(E)_{j(i)}$ is the random effect of the j-th block nested within i-th environment; $G_k$ is the fixed effect of k-th genotype; $G_k*E_i$ is the fixed interaction effect of ki-th genotype with i-th environment, and $e_{ijk}$ is the error.

#### *Correlation analysis*

Based on genotypic means, Spearman's rank correlation analysis was performed by using the SAS procedure PROC CORR.

***Calculation variance components, genetic coefficient of variation and heritability***

Variance components were estimated using SAS PROC VARCOMP procedure. Genetic coefficient of variation ($CV_g$) was computed as follows:

$$CV_g = \frac{\sqrt{\sigma_G^2}}{\bar{X}} * 100$$

Where $\sigma_G^2$ is the genotypic variance, $\bar{X}$ is the average value of the trait across eight environments.

In order to calculate the heritability on a genotypic-mean basis of 24 spring barley genotypes, variance components were estimated for a model wherein all factors were considered as random. The computations were made by using the REML option of the SAS PROC VARCOMP procedure. The following formula was applied (Hoi et al. 1999):

$$h^2 = \frac{\sigma_G^2}{\left(\sigma_G^2 + (\sigma_{GE}^2 / e) + (\sigma_e^2 / re)\right)}$$

Where $\sigma_G^2$ is the estimate of genotypic variance, $\sigma_{GE}^2$ the estimate of genotypic-by-environment interactions variance, $\sigma_e^2$ is the estimate of residual variance, r is the number of replications per environment and e is the number of environments.

***Genotype*environment interaction and stability analysis***

According to Anputhas et al. (2011), adaptability is defined as a function of mean productivity and production stability. In the recent years concepts to assess yield stability and statistical analysis of genotype-by-environment interactions (GEI) have been extensively studied by several researchers (Shukla, 1972; Becker and Leon, 1988; Boggini et al., 1997; Shafii and Price, 1998). In this chapter the yield stability of 24 spring barley genotypes across eight environments was examined by the Additive Main Effects and Multiplicative Interaction (AMMI) biplot analysis (Gauch 1988; Shafii and Price 1998).

The AMMI model is a multivariate method which analyses main effects with an analysis of variance (ANOVA) and their interaction with a principal component analysis (PCA). The biplot analysis of GEI, which allows the understanding and interpretation of the underlying structure, display PCA scores plotted against each other (e.g. component 1 vs. component 2) or PCA scores plotted against genotypic mean.

The following AMMI model was applied:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \sum_{k=1}^{N} \lambda_k \gamma_{ik} \delta_{jk} + \rho_{ij} + \varepsilon_{ijk}$$

Where $Y_{ijk}$ is the yield of genotype g in environment e, $\mu$ is the grand mean, $\alpha_i$ are the genotype mean deviations (genotype means minus the grand mean), $\beta_j$ are the environment mean deviations, $\lambda_k$ is the eigenvalue of principal component analysis (PCA) axis n, $\gamma_{ik}$ and $\delta_{jk}$ are the genotype and environment PCA scores for PCA axis n, N is the number of PCA axes retained in the model and $\varepsilon_{ijk}$ is the residual.

Thillainathan and Fernandez (2001) published a user friendly SAS program to perform stability analysis of genotype*environmental interaction (see Appendix).

## 5.3 Results

In the present study, screenings for drought stress tolerance under natural field conditions were accomplished by the evaluation of morphological, physiological and yield-related parameters in eight environments. In order to assess the applicability of investigated parameters for indentifying drought stress tolerance in spring barley, several statistical analyses were conducted and results are presented thereafter.

### 5.3.1 Climatic conditions and environmental differences between years

To characterize environmental conditions and detect drought stress, the HOBO U30 weather station monitored continuously the following meteorological data: soil temperature, soil moisture, air temperature, relative air humidity, solar radiation and daily precipitation. Environmental conditions were recorded over two years (2012 and 2013) in the time period between April and July. Gathered data showed that the examined field sites differed considerably in their range of climatic conditions (Table 5.2). Figure 5.2 and 5.3 present the soil moisture content at experimental sites in 2012 and 2013. On the experimental field site in Saxony-Anhalt the logging and transmission of recorded weather data proved to be difficult due to the damage or destruction of weather sensors. Thus, in both years, the observation period for Saxony-Anhalt was between the beginning of June and the middle of July (day of year 158 - day of year 195). Between the years, sum of rainfall and solar radiation were the main environmental factors that varied among the years and field sites. Compared to the growing season in 2012, the amount of rainfall in 2013 increased over 50% at Bavaria-Uffenheim, North Rhine-Westphalia (NRW) and Lower Saxony. Simultaneously, solar radiation at Bavaria-Uffenheim, NRW and Lower Saxony decreased in 2013 by 77.4%, 89.5% and 90.7%, respectively. Furthermore, field sites differed in their relative air humidity, air temperature, soil temperature and soil moisture content. In both years, the soil moisture content continuously decreased during the crop life circle in response to plant growth and development. At the same time the solar radiation and air temperature were relatively high. In 2013, climate conditions during vegetative growth phase were favourable mainly due to the higher amount of rainfall and the reduced irradiance. Comparing the climatic data, it is remarkable that in 2012 the field site Bavaria-Herzogenaurach was characterized by high sums of air and soil temperature (sum air temperature 2012: 1289°C, sum soil temperature 2013: 1373°C), a relatively low mean air humidity (70.7%) and a low mean soil moisture

content (12.1 Vol.%). According to this, the field site Bavaria-Herzogenaurach (Bavaria (1)) was characterized as a site with drought periods between the stem elongation and fruit development and/ or ripening (Fig. 5.2). Due to the relatively low total air temperature and the balanced water availability during the cropping season, the experimental sites in NRW were ranged as a non-stress environment, in 2012 and in 2013 (see Appendix). In summary, over two consecutive years 24 spring barley cultivars were exposed to a wide range of environmental conditions. The recorded soil moisture content showed a distinct differentiation of the eight environments, whereas Bavaria-Herzogenaurach (Bavaria (1)) was characterized as a field site the lowest soil moisture content during the crop life circle.

### 5.3.2 Analysis of variance

Results obtained from analysis of variance for 25 investigated plant parameters of 24 spring barley cultivars evaluated in eight environments are presented in the Appendix. Except for PNC-BBCH 30, PNC-BBCH 63, PPhC-BBCH 30, PPhC-BBCH63, PKC-BBCH 30, PKC-BBCH 63 and CGR2, analysis of variance revealed significant main effects of the genotype for ten morphological, three physiological and five yield related parameters. Significant differences between the environments were found for all examined traits. Furthermore, interactions between the genotype and the environment were significant for ten parameters, including PLH-BBCH 30, PLH-BBCH 63, PLH-BBCH 81, PDM-BBCH 30, CGR1, PNC-BBCH 63, PNC-BBCH 81, NKM, TKW and YLD.

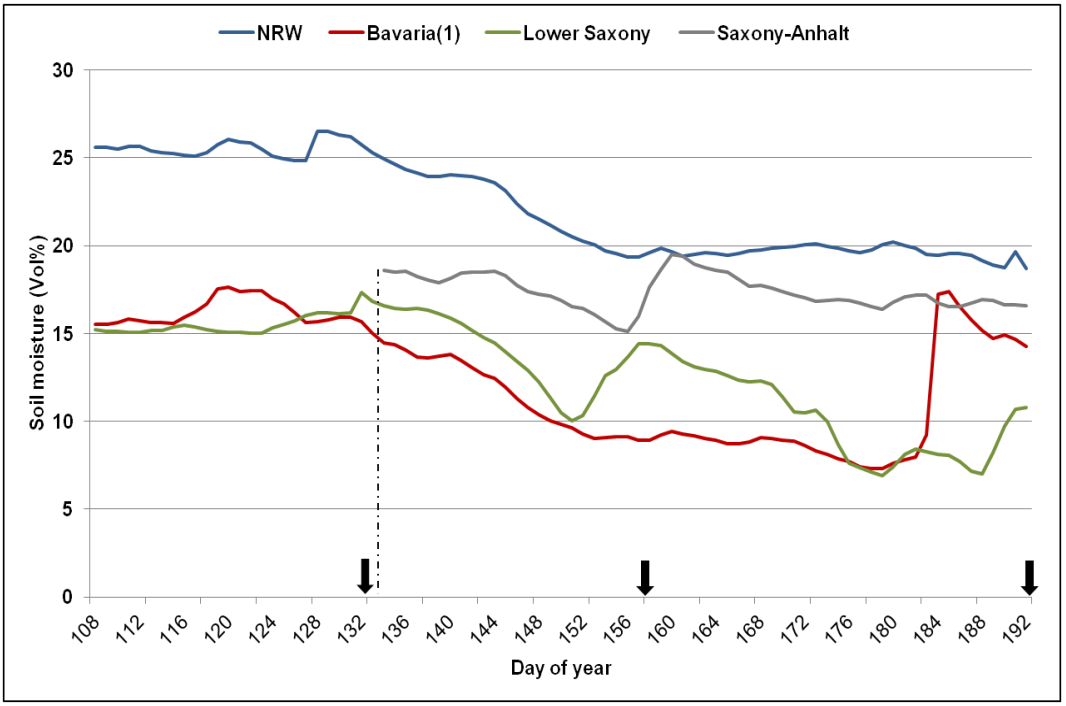


**Fig. 5.2. Soil moisture content of four experimental sites in Germany, 2012.** Arrows indicate the average growth stage: (1) end of tillering stage/ begin stem elongation, (2) end of anthesis/ begin heading and (3) fruit development and ripening stage.

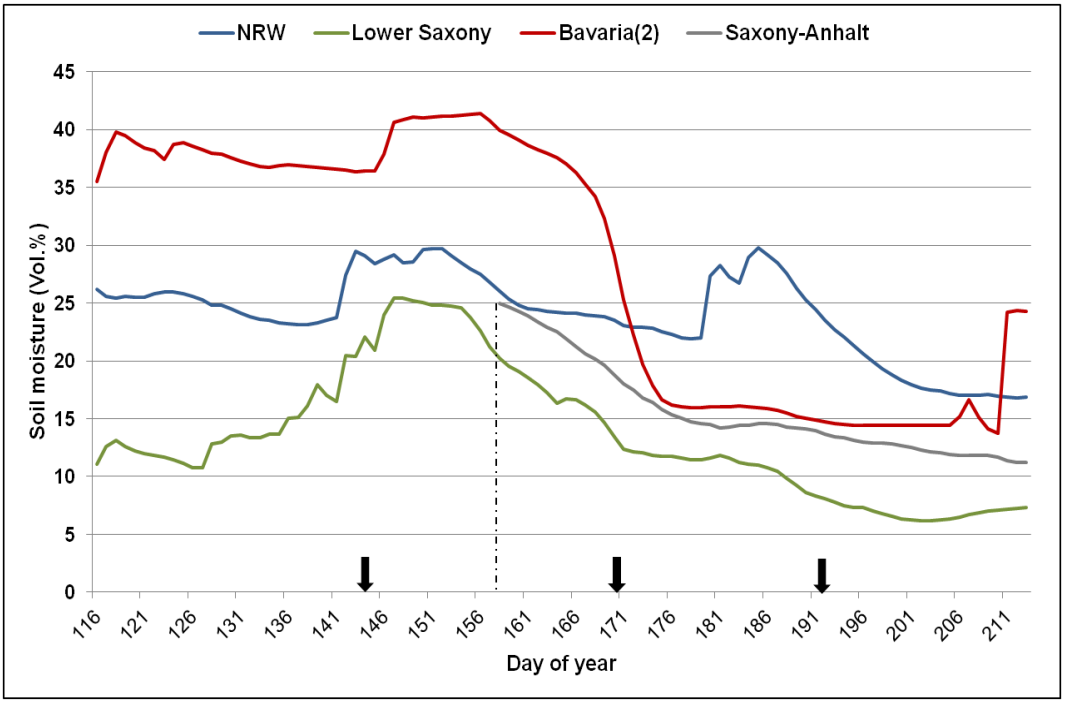


**Fig. 5.3. Soil moisture content of four experimental sites in Germany, 2013.** Arrows indicate the average growth stage: (1) end of tillering stage/ begin stem elongation, (2) end of anthesis/ begin heading and (3) fruit development and ripening stage.

**Table 5.2** Sum air temperature, sum soil temperature, sum solar radiation, sum rainfall, mean relative humidity and mean soil moisture recorded during the vegetation period in 2012 and 2013 at five filed sites in Germany.

| | **Bavaria** | | | **NRW** | | | **Lower Saxony** | | | **Saxony-Anhalt** [a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012 | 2013 | Δ% | 2012 | 2013 | Δ% | 2012 | 2013 | Δ% | 2012 | 2013 | Δ% |
| **Sum** Air temperature (°C) | 1289.5 | 1107.4 | -14.1 | 1198.1 | 1083.7 | -9.5 | 1170.0 | 1118.7 | -4.4 | 616.7 | 646.9 | 4.9 |
| **Sum** Soil Temperature (°C) | 1373.0 | 1127.4 | -17.9 | 1121.8 | 1097.4 | -2.2 | 1060.9 | 1060.8 | 0.0 | 614.8 | 731.4 | 19.0 |
| **Sum** Solar Radiation (MW/m²) | 23.1 | 5.2 | -77.4 | 19.1 | 2.0 | -89.5 | 23.5 | 2.2 | -90.7 | 9.3 | 1.2 | -86.9 |
| **Sum** Rainfall (l/m²) | 130 | 244 | 87.3 | 124 | 198 | 59.0 | 145 | 222 | 53.2 | 49 | 27 | -44.0 |
| **Mean** Relative Humidity (%) | 70.7 | 80.8 | 14.3 | 76.0 | 79.3 | 4.3 | 75.7 | 79.7 | 5.3 | 81.3 | 78.3 | -3.6 |
| **Mean** Soil Moisture (Vol. %) | 12.1 | 31.9 | 162.8 | 21.8 | 25.6 | 17.3 | 12.6 | 15.4 | 21.6 | 17.0 | 17.3 | 1.8 |

a = observation period for Saxony-Anhalt was in 2012 and 2013 between the beginning of June and the middle of July (day of year 158 - day of year 195)

Δ% : relative percentage difference between experimental years

Δ% = ((meteorological data 2012 - meteorological data 2013) / meteorological data 2013)*100

### 5.3.3 Phenotypic response of spring barley for grain yield and associated plant parameters across eight environments

Data concerning mean values of examined morphological, physiological and yield-related plant parameters, measured in eight environments, are summarized in Table 5.3.

Generally, the plant height and plant dry matter increased during the crop life circle, whereas the plant nitrogen, phosphor and potassium content declined from BBCH 30 to BBCH 81. Calculated crop growth rates (CGR) for each environment were lower during the early vegetative growth phase (until BBCH 30). Nevertheless, CGR increased sharply until BBCH 81. The mean crop growth rate (MGR) varied between 8.7 g $m^{-2}$ per day to 21.7 g $m^{-2}$ per day. Across eight environments a grain yield range was observed varying from 41.6 dt $ha^{-1}$ to 83.5 dt $ha^{-1}$.

Among all analyzed environments, spring barley genotypes in environment E5 (NRW 2013) produced maximum values for PDM, CGR, MGR, NEM, NKM and YLD. Contrary, evaluated genotypes in Bavaria-Herzogenaurach (E1) were characterized by the lowest means for PLH, PDM, CGR1, CGR2, CGR4, MGR, NEM, NKM, TKW and finally YLD. Compared to the genotypic performance at the field site in NRW 2013 (E5), PDM-BBCH 63, PDM-BBCH 81, MGR, NEM, NKM, TKW and YLD declined by 70%, 49%, 60%, 39%, 50%, 2% and 50% at the field site Bavaria-Herzogenaurach (E1). The grain yield reduction in E1 was accompanied by reductions of grain yield components.

**Table 5.3** Environmental means for morphological, physiological and yield related parameters of spring barley cultivars grown during 2012 and 2013 at eight field sites in Germany.

| | Environment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Trait** | **E1** | **E2** | **E3** | **E4** | **E5** | **E6** | **E7** | **E8** |
| **Growth stage** | | | | | | | | |
| 1st measuring date | 27.7 | 29.9 | 30.9 | 30.5 | 30.3 | 24.0 | 30.2 | 31.1 |
| 2nd measuring date | 55.5 | 65.4 | 64.8 | 60.1 | 71.7 | 52.9 | 61.5 | 67.8 |
| 3rd measuring date | 88.2 | 84.5 | 85.5 | 87.0 | 74.8 | 73.6 | 75.8 | 75.2 |
| **PLH** | | | | | | | | |
| BBCH 30 | 19.1 | 20.1 | 32.9 | 36.0 | 29.7 | 21.2 | 30.1 | 34.5 |
| BBCH 63 | 61.5 | 79.3 | 67.6 | 73.1 | 89.4 | 61.3 | 83.1 | 95.0 |
| BBCH 81 | 66.0 | 76.3 | 71.5 | 75.5 | 83.1 | 67.2 | 76.6 | 83.5 |
| **PDM** | | | | | | | | |
| BBCH 30 | 64.2 | 103.9 | 177.5 | 96.6 | 280.7 | 116.9 | 277.2 | 250.6 |
| BBCH 63 | 284.7 | 638.3 | 620.2 | 598.2 | 961.2 | 532.6 | 698.9 | 790.7 |
| BBCH 81 | 828.1 | 1614.3 | 1210.8 | 1104.5 | 1636.0 | 1083.9 | 1247.4 | 1225.6 |
| **CGR 1** | 1.5 | 2.1 | 3.0 | 1.9 | 5.7 | 3.2 | 5.2 | 5.1 |
| **CGR 2** | 9.1 | 19.8 | 22.1 | 25.1 | 27.2 | 16.0 | 16.2 | 20.8 |
| **CGR 3** | 15.2 | 28.7 | 14.4 | 13.0 | 32.1 | 26.3 | 27.4 | 20.7 |
| **CGR 4** | 12.8 | 24.8 | 16.9 | 17.1 | 29.5 | 20.5 | 21.1 | 20.7 |
| **MGR** | 8.7 | 16.9 | 13.1 | 13.3 | 21.7 | 15.1 | 16.2 | 15.5 |
| **PNC** [a] | | | | | | | | |
| BBCH 30 | 4.7 | 4.6 | - | 3.5 | - | - | 3.2 | - |
| BBCH 63 | 1.5 | 1.4 | - | 1.5 | - | - | 1.3 | - |
| BBCH 81 | 0.4 | 0.4 | - | 0.3 | - | - | 0.5 | - |
| **PPhC** [a] | | | | | | | | |
| BBCH 30 | 0.8 | 0.7 | - | 0.8 | - | - | 0.6 | - |
| BBCH 63 | 0.5 | 0.5 | - | 0.5 | - | - | 0.4 | - |
| BBCH 81 | 0.3 | 0.3 | - | 0.2 | - | - | 0.2 | - |
| **PKC** [a] | | | | | | | | |
| BBCH 30 | 5.1 | 4.0 | - | 6.8 | - | - | 0.4 | - |
| BBCH 63 | 2.4 | 2.1 | - | 2.8 | - | - | 0.2 | - |
| BBCH 81 | 1.3 | 1.4 | - | 1.7 | - | - | 0.2 | - |
| **NEM** | 622.8 | 1085.9 | 980.7 | 662.7 | 1025.7 | 735.8 | 695.5 | 725.0 |
| **NKE** | 15.2 | 15.2 | 15.4 | 19.5 | 17.7 | 16.3 | 17.8 | 17.9 |
| **NKM** | 8787.6 | 15020.0 | 14471.0 | 12408.0 | 17462.0 | 11539.0 | 12483.0 | 12501.0 |
| **TKW** | 47.4 | 53.6 | 50.5 | 50.8 | 48.2 | 51.9 | 50.6 | 50.5 |
| **YLD** | 41.6 | 80.6 | 73.0 | 62.5 | 83.5 | 59.5 | 63.2 | 63.0 |

Where a = genotypic means of eight spring barley genotypes, Environment: E1: Bavaria-Herzogenaurach 2012, E2: NRW 2012, E3: Saxony-Anhalt 2012, E4: Lower Saxony 2012, E5: NRW 2013, E6: Saxony-Anhalt 2013, E7: Bavaria-Uffenheim 2013, E8: Lower Saxony 2013, Trait: PLH: plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

### 5.3.4 Significant genotype-by-environment interactions

In this study, analysis of variance indicated the presence of significant genotype-by-environment interactions for the crop growth rate (CGR1) and the grain yield (YLD).

***Crop growth rate – CGR1***

Genotypic means of CGR1 in response to different environments are illustrated in Figure 5.4. In general, means for CGR1 varied noticeably across environments, ranging from 1.5 g $m^{-2}$ per day in environment E1 to 5.7 g $m^{-2}$ per day in environments E5. On average, the lowest values for CGR1 were obtained in E1 with 'Henrike', 'Primadonna' and 'Quench' showing the tendency to accumulate more biomass even under less favourable environmental conditions (Table 5.2 and 5.3). The environments E2, E3, E4 and E6 were intermediate with crop growth rates ranging from 1.9 g $m^{-2}$ per day to 3.2 g $m^{-2}$ per day. In environment E5, a field site which was characterized as a non-stress environment, CGR1 varied between 4.9 and 6.9 g $m^{-2}$ per day. Across all eight environments, the spring barley cultivars 'Henrike, 'Wisa', 'Tatum', 'Quench' and 'Sebastian' showed a high biomass accumulation with CGR1-values varying from 4.1 g $m^{-2}$ per day to3.6 g $m^{-2}$ per day.

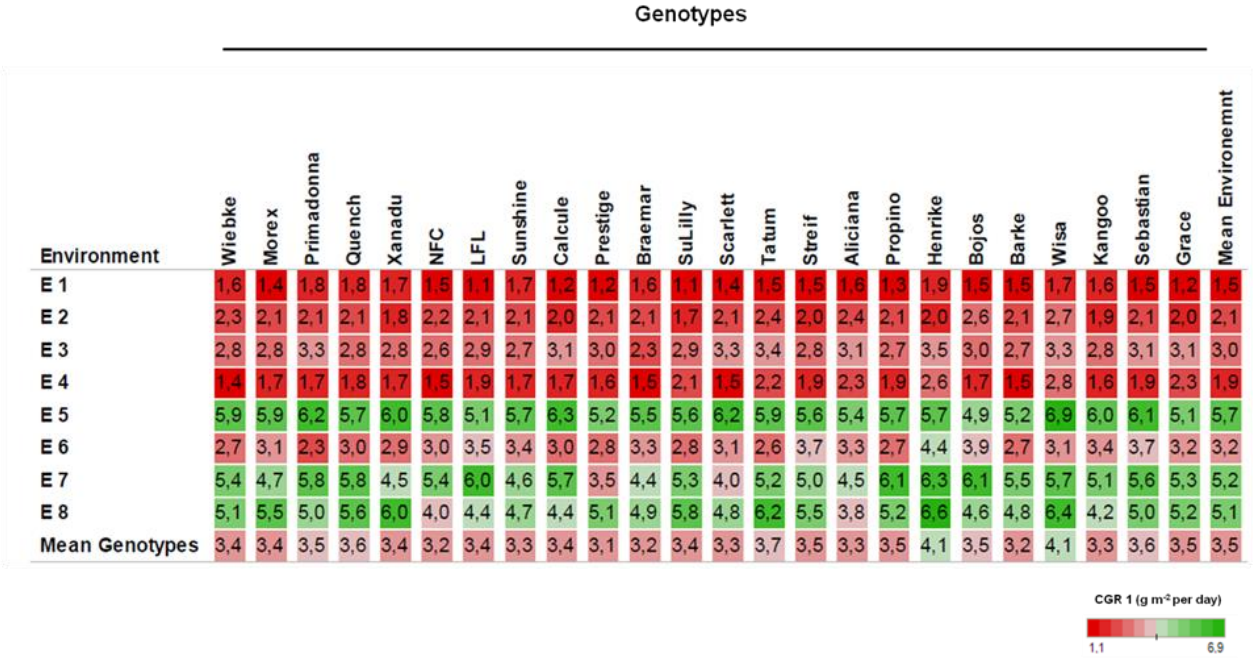


**Fig. 5.4. Heat map concerning the crop growth rate until plants reached BBCH 30 (CGR1) of 24 spring barley genotypes grown in eight environments during 2012 and 2013**. Red values represent low crop growth rates, whereas green values represents high crop growth rated. Environments: E1: Bavaria-Herzogenaurach 2012, E2: NRW 2012, E3: Saxony-Anhalt 2012, E4: Lower Saxony 2012, E5: NRW 2013, E6: Saxony-Anhalt 2013, E7: Bavaria-Uffenheim 2013, E8: Lower Saxony 2013.

### *Grain yield - YLD*

Grain yield means of the 24 spring barley cultivars, which were evaluated in eight environments, are presented in Figure 5.5. Average grain yield varied greatly between the environments and ranged from 42 dt $ha^{-1}$ to 83 dt $ha^{-1}$. The highest overall yields were obtained in E5 and E2, the field sites in NRW. The environments E3, E4, E7 and E8 were intermediate with yields varying from 73 dt $ha^{-1}$ to 63 dt $ha^{-1}$. The yield spectrum detected in environment E1 varied from 31 dt $ha^{-1}$ to 54 dt $ha^{-1}$. Nevertheless, results from analysis of variance and genotypic mean values for grain yield revealed that several genotypes had a superior yield performance under less favourable environmental conditions. Across all environments, the five cultivars 'Grace', 'Sebastian', 'Calcule', 'Tatum' and 'Bojos' were the highest yielding genotypes with 72.4 dt $ha^{-1}$, 71.4 dt $ha^{-1}$, 70.3 dt $ha^{-1}$, 70 dt $ha^{-1}$ and 69.9 dt $ha^{-1}$, respectively. Remarkably is the fact that 'Grace' and 'Sebastian' tended to outyield the investigated spring barley genotypes. According to Figure 5.5 it is striking that the spring barley cultivar 'Wiebke' was characterized by a higher yield performance in environments with sufficient precipitation, whereas the yield performance declined under warm and drought conditions (e.g. in environment E1and E7).

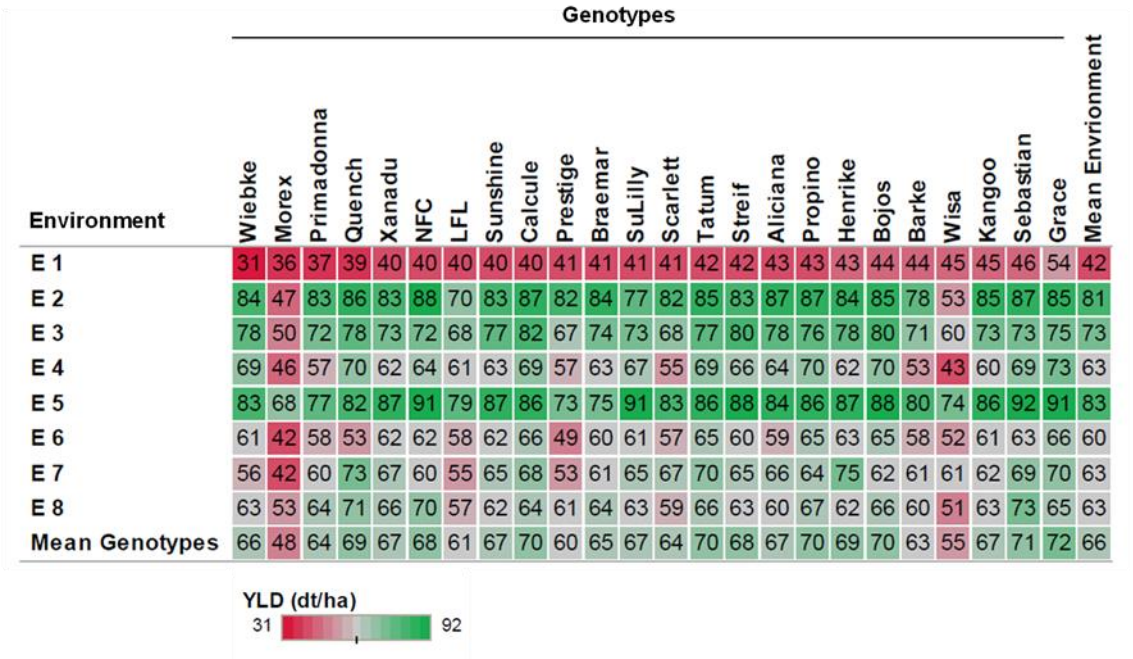


**Fig. 5.5. Heat map concerning the average grain yield (YLD) of 24 spring barley genotypes grown in eight environments during 2012 and 2013.** Red heat map values represent low grain yields, whereas green heat map values represent high grain yields. Environments: E1: Bavaria-Herzogenaurach 2012, E2: NRW 2012, E3: Saxony-Anhalt 2012, E4: Lower Saxony 2012, E5: NRW 2013, E6: Saxony-Anhalt 2013, E7: Bavaria-Uffenheim 2013, E8: Lower Saxony 2013.

### 5.3.5 Correlation analysis between climatic factors and grain yield components

The correlation coefficients between environmental parameters and grain yield components are shown in Table 5.4. Here, the correlation analysis was based on genotypic means of 24 spring barley cultivars evaluated in six environments. Due to destruction of weather sensors, recorded weather data at field site in Saxony-Anhalt were excluded from correlation analysis, in both years of the field experiments.

The number of kernels per ear (NKE) was highly, negative correlated with the soil temperature between May and June (-1.0***). In June and July, the time period between anthesis and ripening (T3), the number of ears per square meter (NEM) was significant and positive related to the soil moisture content (0.89*).

Interestingly, correlations between the grain yield, as a primary trait for drought tolerance, and environmental parameters were non-significant. The number of kernels per square meter (NKM) and the thousand kernel weight (TKW) were not significantly associated with meteorological data.

**Table 5.4** Correlation coefficients for grain yield, yield components and weather data recorded during 26th of April and 10th of July in 2012 and 2013 in six environments in Germany.

| Trait | Time point of measurement | Rainfall ($l/m^2$) | Air temperature (°C) | Soil temperature (°C) | Soil moisture (Vol. %) | Solar radiation ($W/m^2$) |
|---|---|---|---|---|---|---|
| **NEM** | T1 | 0.26 | 0.14 | 0.09 | 0.60 | - 0.42 |
| | T2 | 0.14 | 0.66 | 0.09 | 0.77 | - 0.09 |
| | T3 | - 0.37 | - 0.60 | - 0.49 | 0.89 * | - 0.54 |
| | T4 | - 0.03 | -0.14 | 0.54 | 0.77 | - 0.03 |
| **NKE** | T1 | 0.66 | 0.43 | 0.14 | - 0.54 | - 0.14 |
| | T2 | -0.09 | - 0.60 | - 1.00 *** | 0.03 | - 0.54 |
| | T3 | - 0.09 | - 0.20 | - 0.43 | - 0.26 | - 0.09 |
| | T4 | 0.43 | -0.09 | -0.09 | 0.03 | - 0.26 |
| **NKM** | T1 | 0.49 | 0.26 | 0.14 | 0.03 | - 0.49 |
| | T2 | - 0.09 | 0.09 | - 0.31 | 0.43 | - 0.37 |
| | T3 | - 0.26 | - 0.37 | - 0.60 | 0.71 | - 0.60 |
| | T4 | -0.09 | - 0.09 | 0.26 | 0.43 | - 0.26 |
| **TKW** | T1 | -0.03 | - 0.26 | - 0.43 | 0.49 | 0.09 |
| | T2 | - 0.09 | 0.43 | - 0.09 | 0.49 | 0.37 |
| | T3 | - 0.09 | - 0.31 | - 0.09 | 0.20 | 0.14 |
| | T4 | - 0.03 | - 0.09 | 0.14 | 0.49 | 0.54 |
| **YLD** | T1 | 0.49 | 0.26 | 0.14 | 0.03 | - 0.49 |
| | T2 | - 0.09 | 0.09 | - 0.31 | 0.43 | - 0.37 |
| | T3 | - 0.26 | - 0.37 | - 0.60 | 0.71 | - 0.60 |
| | T4 | - 0.09 | - 0.09 | 0.26 | 0.43 | - 0.26 |

Where p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001,Time point of measurement = T1: time period until genotypes reached BBCH 30, T2: time period between BBCH 30 and BBCH 63, T3: time period between BBCH 63 and BBCH 81, T4: time period until genotypes reached BBCH 81, Trait = NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

### 5.3.6 Correlation analysis among investigated plant parameters

Genetic correlation coefficients between the grain yield, yield component and evaluated morphological and physiological traits are presented in the Appendix.

Overall, the grain yield (YLD) showed across all three sampling dates (BBCH 30 to BBCH 81) significant and positive correlations with PLH, PDM, MGR and calculated crop growth rates (CGR 1 to CGR4). The strongest positive associations were found between the grain yield and measured plant dry matters, whereas the strength of correlation increased during the crop life circle. Thus, highly significant and positive correlations of YLD with PDM-BBCH 30 (0.39***), PDM-BBCH 63 (0.58***) and PDM-BBCH 81(0.82***) were found. Moreover, the grain yield associated positively with CGR1 (0.33***), CGR2 (0.60***), CGR3 (0.45***), CGR4 (0.68***) and MWR (0.64***), while moderate correlations were found for YLD with PLH-BBCH 63 (0.27***) and PLH-BBCH 81(0.21**). Genotypic correlation coefficients of YLD with physiological parameters such as PNC, PPhC and PKC were negative and weak. Only the plant phosphor content at BBCH 30 correlated moderately with YLD (-0.36*).

Results obtained from correlation analysis showed positive and significant associations of NEM and NKM with PDM-BBCH 30, PDM-BBCH 63, PDM-BBCH 81 and calculated crop growth rates (CGR1 to CGR4). Plant phosphor content at BBCH 81 was moderate related to NEM (0.49**). At each sampling date significant, positive correlations between NKE and PLH were determined. Remarkable is the fact that the genetic correlation coefficient between NKE and PLH decreased during the crop development. The thousand kernel weight (TKW) showed weak to moderate associations with PPhC (-0.45*), PLH-BBCH 81 (-0.16*) and MWR (0.42**). The grain yield was highly significant correlated with NEM (0.78***), NKM (0.96***) and TKW (0.73***).

### 5.3.7 Genetic correlation analysis of investigated traits of spring barley evaluate in North Rhine-Westphalia and Bavaria

Analysis of climatic conditions as well as results obtained from analysis of variance showed significant variations among environments for all investigated traits. According to the previously presented results in Chapter 5, the field site Bavaria-Herzogenaurach (E1) was characterized as an environment with drought periods during the crop life circle, whereas the field site in NRW 2013 (E5) was ranged as a non-stress environment. Spearman rank correlation analysis was used to evaluate the relationship between investigated plant parameters under both, non-stress and stress environments. Based on computed LSMEANS values, genetic correlation analysis was performed for two contrasting environments (E1 and E5). Results are presented thereafter in Table 5.5 and 5.6.

#### *Genetic correlation analysis of investigated traits of spring barley evaluated in North Rhine-Westphalia*

Table 5.5 provides the genotypic correlation coefficients between examined plant parameters in NRW 2013 (E5). A total of 31 significant correlations were detected.

The number of ears per square meter (NEM) showed highly significant and positive associations with PDM-BBCH 81 (0.70***), CGR3 (0.68***), CGR4 (0.75***), MGR (0.71***), NKM (0.83***) and YLD (0.69***), while NEM was negatively correlated with NKE (-0.48*) and TKW (-0.54*). Furthermore, correlation analysis revealed negative relationships between the number of kernels per ear (NKE) and PDM-BBCH 81 (-0.48*), CGR3 (-0.48*), CGR4 (-0.43*) and MGR (-0.45*). Significant and positive correlation coefficients were found for the number of kernels per square meter (NKM) with the plant dry matter at BBCH 81 (0.58*), CGR3 (0.56*), CGR4 (0.63**), MGR (0.59**) and YLD (0.84***), whereas negative associations were detected for TKW (-0.62**). The grain yield, as primary trait, showed moderate to high significant and positive correlations with PDM BBCH 81 (0.65**), CGR3 (0.60**), CGR4 (0.68***), and MGR (0.66**). Correlation analysis demonstrate that the plant height was, at each sampling dates (BBCH 30 to BBCH 81), negatively associated with the grain yield and yield components, such as NEM and NKM.

### *Genetic correlation analysis of investigated traits of spring barley evaluated in Bavaria-Herzogenaurach*

The genotypic correlations for investigated plant parameters in stress environments (E1) are presented in Table 5.6. Correlations between morphological traits and the grain yield as well as grain yield components were non-significant. As can be seen from Table 5.6 grain yield components were significantly correlated. Hence, the number of ears per square meter (NEM) was negatively correlated with NKE (-0.85***). In addition, significant and positive associations were found between NKE and NKM (0.69***) as well as YLD (0.45*). The number of kernels per square meter (NKM) was positively related to YLD (0.74***), while NKM was negative correlated with TKW (-0.59**).

**Table 5.5** Spearman's correlation coefficient ($r_g$) for evaluated plant parameters of 24 spring barley genotypes grown in 2013 in North Rhine Westphalia (E5).

| | **NEM** | | **NKE** | | **NKM** | | **TKW** | | **YLD** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | | | | | | | | | | |
| BBCH 30 | -0.59 | ** | 0.32 | | -0.55 | * | 0.16 | | -0.64 | ** |
| BBCH 63 | -0.55 | * | 0.28 | | -0.61 | ** | 0.14 | | -0.72 | *** |
| BBCH 81 | -0.53 | * | 0.29 | | -0.53 | * | 0.07 | | -0.69 | *** |
| **PDM** | | | | | | | | | | |
| BBCH 30 | -0.04 | | -0.08 | | -0.08 | | 0.03 | | -0.11 | |
| BBCH 63 | 0.05 | | -0.04 | | -0.01 | | -0.02 | | -0.08 | |
| BBCH 81 | 0.70 | *** | -0.48 | * | 0.58 | * | -0.21 | | 0.65 | ** |
| **CGR 1** | -0.04 | | -0.08 | | -0.08 | | 0.03 | | -0.11 | |
| **CGR 2** | 0.17 | | 0.03 | | 0.10 | | -0.17 | | -0.01 | |
| **CGR 3** | 0.68 | *** | -0.48 | * | 0.56 | * | -0.26 | | 0.60 | ** |
| **CGR 4** | 0.75 | *** | -0.43 | * | 0.63 | ** | -0.29 | | 0.68 | *** |
| **MGR** | 0.71 | *** | -0.45 | * | 0.59 | ** | -0.24 | | 0.66 | *** |
| **NEM** | 1 | | -0.48 | * | 0.83 | ** | -0.54 | * | 0.69 | *** |
| **NKE** | -0.48 | * | 1 | | -0.05 | | -0.26 | | -0.13 | |
| **NKM** | 0.83 | *** | -0.05 | | 1 | | -0.62 | ** | 0.84 | *** |
| **TKW** | -0.54 | * | -0.25 | | -0.62 | ** | 1 | | -0.25 | |
| **YLD** | 0.69 | *** | -0.13 | | 0.84 | *** | -0.25 | | 1 | |

Where p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001,Trait: PLH: Plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

**Table 5.6** Spearman's correlation coefficient ($r_g$) for evaluated plant parameters of 24 spring barley genotypes grown in 2012 in Bavaria-Herzogenaurach (E1).

| | **NEM** | | **NKE** | | **NKM** | | **TKW** | | **YLD** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | | | | | | | | | | |
| BBCH 30 | -0.19 | | 0.11 | | -0.08 | | 0.23 | | -0.07 | |
| BBCH 63 | -0.10 | | 0.07 | | -0.02 | | 0.14 | | 0.04 | |
| BBCH 81 | -0.14 | | 0.23 | | 0.11 | | -0.15 | | -0.05 | |
| **PDM** | | | | | | | | | | |
| BBCH 30 | 0.40 | | -0.29 | | -0.10 | | 0.06 | | -0.07 | |
| BBCH 63 | 0.24 | | -0.23 | | 0.00 | | -0.03 | | 0.16 | |
| BBCH 81 | 0.46 | | -0.41 | | -0.26 | | 0.22 | | -0.06 | |
| **CGR 1** | 0.40 | | -0.29 | | -0.10 | | 0.06 | | -0.07 | |
| **CGR 2** | 0.23 | | -0.20 | | 0.02 | | -0.01 | | 0.20 | |
| **CGR 3** | 0.06 | | -0.06 | | -0.05 | | 0.01 | | -0.16 | |
| **CGR 4** | 0.21 | | -0.21 | | -0.04 | | 0.13 | | 0.03 | |
| **MGR** | 0.20 | | -0.22 | | -0.07 | | 0.18 | | 0.04 | |
| **NEM** | 1 | | -0.85 | *** | -0.32 | | 0.05 | | -0.16 | |
| **NKE** | -0.85 | *** | 1 | | 0.69 | *** | -0.37 | | 0.45 | * |
| **NKM** | -0.32 | | 0.69 | *** | 1 | | -0.59 | ** | 0.74 | *** |
| **TKW** | 0.05 | | -0.37 | | -0.59 | ** | 1 | | -0.04 | |
| **YLD** | -0.16 | | 0.45 | * | 0.74 | *** | -0.04 | | 1 | |

Where p value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001,Trait: PLH: Plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

### 5.3.8 Genetic variation and heritability of investigated plant parameters

Values of genotypic variance, environmental variance, genotype-by-environment variance and heritabilities of investigated plant parameters are given in Table 5.7.

Across examined growth stages the environment was the most important source for the expression of traits. Previous presented results of analysis of variance revealed significant genotypic variations for all traits except for PNC-BBCH 30, PNC-BBCH 63, PPhC-BBCH 30, PPhC 63, PKC-BBCH 30, PKC-BBCH 63 and CGR2. The largest genotypic variation was found for PDM-BBCH 81. At ripening stage the greatest genetic variance was observed for NEM and NKM. Interestingly, the magnitude of genetic variance tended to increase during the crop life circle for PLH and PDM. Among all traits, the genetic coefficient of variation (CVg) of NKM, CGR3,YLD, PLH-BBCH 63, PLH-BBCH 81, PLH-BBCH 30, NEM and NKE were higher than 6%,with 6.9%, 7.6%, 8%, 9.9%, 10.3%, 10.8%, 11.2% and 15,1%, respectively. Overall, the results show a significant genetic variation among 24 spring barley genotypes during the vegetation period. Compared to presented genotypic and environmental variance in Table 5.7, estimates of genotype-by-environment variance, which were of similar importance as genotypic variance, showed lower values. The highest estimates for error variances were recorded PDM-BBCH81and NKM.

Mean heritabilities for the grain yield and yield components ranged from 0.85 to 0.94. The highest heritabilities across environments were observed for morphological and physiological parameters, such as PLH-BBCH 63 (0.97), PLH-BBCH 81 (0.97), PDM-BBCH 30 (0.64), PDM-BBCH 81 (0.69), PKC-BBCH 81 (0.63), CGR4 (0.68) and MGR (0.70). Nevertheless, the lowest heritability was calculated for physiological traits such as PNC and PKC.

**Table 5.7** Results of computed variance components, genetic coefficient of variation ($CV_g$) and heritability ($h^2$) with standard error for 24 spring barley cultivars grown in eight environments.

| Trait | $\sigma^2_G$ | $\sigma^2_E$ | $\sigma^2_{GE}$ | $\sigma^2_e$ | $CV_g$ | $h^2$ |
|---|---|---|---|---|---|---|
| **PLH** | | | | | | |
| BBCH 30 | 9.16 | 47.43 | 2.94 | 22.00 | 10.83 | 0.90± 0.03 |
| BBCH 63 | 56.57 | 154.18 | 7.50 | 29.55 | 9.86 | 0.97± 0.01 |
| BBCH 81 | 59.62 | 41.25 | 3.87 | 41.97 | 10.30 | 0.97± 0.01 |
| **PDM** | | | | | | |
| BBCH 30 | 94.93 | 7707.40 | 103.27 | 1068.40 | 5.70 | 0.64± 0.12 |
| BBCH 63 | 393.13 | 38312.50 | 0.00 | 9477.80 | 3.10 | 0.52± 0.14 |
| BBCH 81 | 2901.70 | 72633.00 | 178.94 | 33772.10 | 4.33 | 0.69± 0.10 |
| **PNC** | | | | | | |
| BBCH 30 | 0.0004 | 0.6055 | 0.0023 | 0.1104 | 0.51 | 0.07± 0.64 |
| BBCH 63 | 0.0001 | 0.0015 | 0.0022 | 0.0192 | 0.00 | 0.00± 0.00 |
| BBCH 81 | 0.0001 | 0.0045 | 0.0011 | 0.0039 | 2.69 | 0.20± 0.53 |
| **PPhC** | | | | | | |
| BBCH 30 | 0.0002 | 0.0063 | 0.0001 | 0.0025 | 1.68 | 0.45± 0.31 |
| BBCH 63 | 0.0000 | 0.0012 | 0.0000 | 0.0008 | 1.50 | 0.48± 0.37 |
| BBCH 81 | 0.0000 | 0.0009 | 0.0000 | 0.0003 | 2.11 | 0.56± 0.29 |
| **PKC** | | | | | | |
| BBCH 30 | 0.0000 | 7.3765 | 0.0097 | 0.1065 | 0.00 | 0.00± 0.00 |
| BBCH 63 | 0.0016 | 1.3258 | 0.0000 | 0.0624 | 2.14 | 0.38± 0.42 |
| BBCH 81 | 0.0038 | 0.4528 | 0.0026 | 0.0253 | 5.42 | 0.63± 0.25 |
| **CGR 1** | 0.04 | 2.79 | 0.04 | 0.44 | 5.61 | 0.63± 0.12 |
| **CGR 2** | 0.29 | 32.61 | 0.00 | 15.73 | 2.75 | 0.32± 0.20 |
| **CGR 3** | 2.89 | 53.69 | 0.47 | 65.70 | 7.65 | 0.51± 0.16 |
| **CGR 4** | 1.06 | 26.02 | 0.00 | 13.21 | 5.04 | 0.68 ±0.01 |
| **MGR** | 0.37 | 13.70 | 0.14 | 5.99 | 0.10 | 0.58± 0.13 |
| **NEM** | 8450.00 | 33205.60 | 81.29 | 22976.50 | 11.25 | 0.90± 0.03 |
| **NKE** | 6.53 | 2.19 | 2.14 | 24.03 | 15.14 | 0.85± 0.05 |
| **NKM** | 812357.20 | 6585479.60 | 506575.10 | 2128424.50 | 6.89 | 0.85± 0.05 |
| **TKW** | 4.95 | 3.61 | 2.80 | 5.04 | 4.41 | 0.94± 0.01 |
| **YLD** | 27.98 | 173.76 | 9.55 | 40.68 | 8.03 | 0.92± 0.02 |

Where $\sigma^2_G$: genotypic variance, $\sigma^2_E$: environmental variance, $\sigma^2_{GE}$: genotype*environment interaction variance, $CV_g$: genetic coefficient of variation, $h^2$: heritability, Trait: PLH: plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

### 5.3.9 AMMI analysis

Data used for AMMI analysis were obtained from field experiments conducted during the years 2012 and 2013 at eight sites in Germany. The initial analysis of variance indicated the presence of significant genotype-by-environment interactions for the grain yield (see Appendix). Here, the environment was the most important factor contributing to the yield variability. Applying the AMMI analysis, principal component analysis (PCA) was used to decompose the genotype-by-environment interaction. Thus, the first two principal components explained 62.8% of the variability (interaction sum of squares) with 46% and 16.8% for PCA1 and PCA2.

As shown in Figure 5.6, PCA scores of genotypes and environments are useful indicators for analyzing yield stability. However, spring barley cultivars like 'Kangoo', 'Streif', 'Xanadu' and 'Tatum' are located close to the biplot centre and were rated as stable due to their small genotype-by-environment interaction. In contrast, the varieties 'Morex', 'Wisa', 'Wiebke' and 'Henrike' were further away from the centre of the axes. Thus, these cultivars showed stronger interaction effects. Furthermore, it is apparent from Figure 5.6 that environment E3 showed a positive association with ten cultivars. 'Grace' and 'SuLilly' showed a specific adaptation to environment E5 and E6, while 'Morex' was negatively correlated environment E3 and E2.

Figure 5.7 present the main effect of each genotype and each environment (mean values) in relation to PCA scores of interaction effects. In general, PCA scores with values close to zero are characteristic for environments and genotypes which provide a small contribution to the genotype-by-environment interaction. These genotypes and environments are therefore ranged as stable. Referring to this, 'Kangoo', 'Xanadu', 'Sebastian' and 'Sunshine' were characterized as stable with a yield performance over the general mean. In contrast, 'Morex', 'Wisa' and 'Wiebke' were classified as unstable with a mean grain yields under the general mean of 65.9 dt $ha^{-1}$. Across eight environments, 'Grace' exhibited the highest yield performance with 72.4 dt $ha^{-1}$. Moreover, Figure 5.7 illustrate that analyzed environments exhibited a large yield range, whereas genotypes were more or less located around 66 dt $ha^{-1}$. However, environment E8 was outstanding with a small contribution to the genotype-by-environment interaction. On the other hand, environments E1 and E2, which were characterized by either the poorest or best field site, showed a high contribution to the genotype-by-environment interaction.

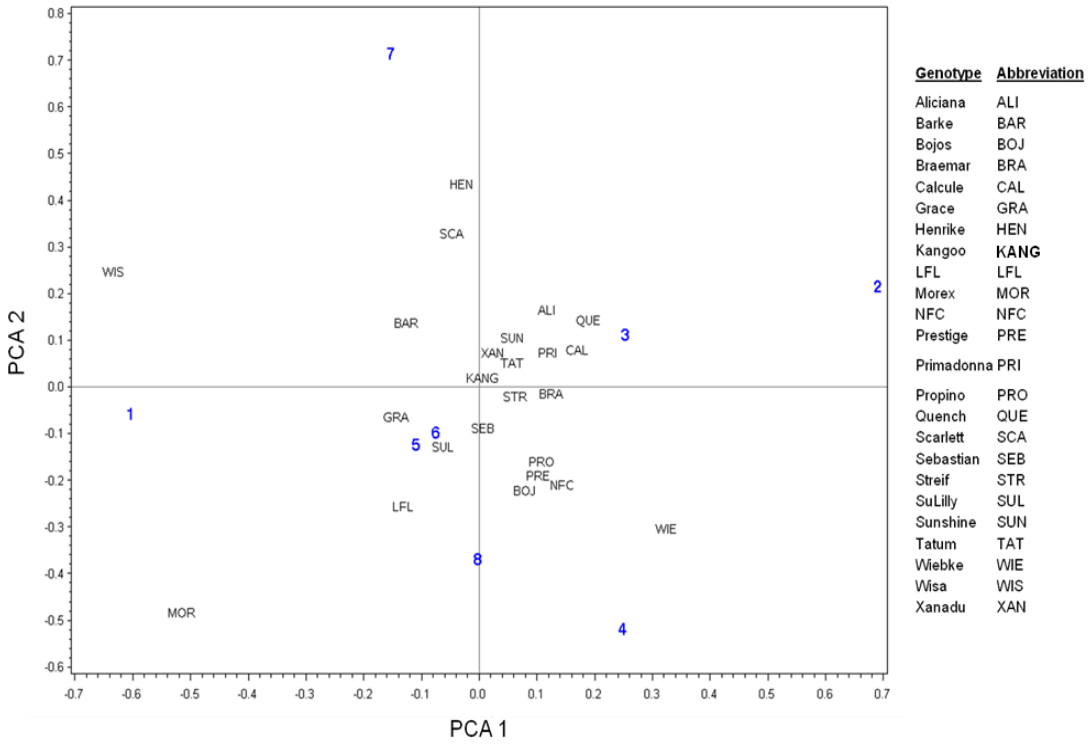


**Fig. 5.6. AMMI biplot presenting the first principal axes of interaction (PCA1 vs. PCA2) for YLD of 24 spring barley genotypes evaluated in eight environments.** Environment: E1: Bavaria-Herzogenaurach 2012, E2: NRW 2012, E3: Saxony-Anhalt 2012, E4: Lower Saxony 2012, E5: NRW 2013, E6: Saxony-Anhalt 2013, E7: Bavaria-Uffenheim 2013, E8: Lower Saxony 2013.

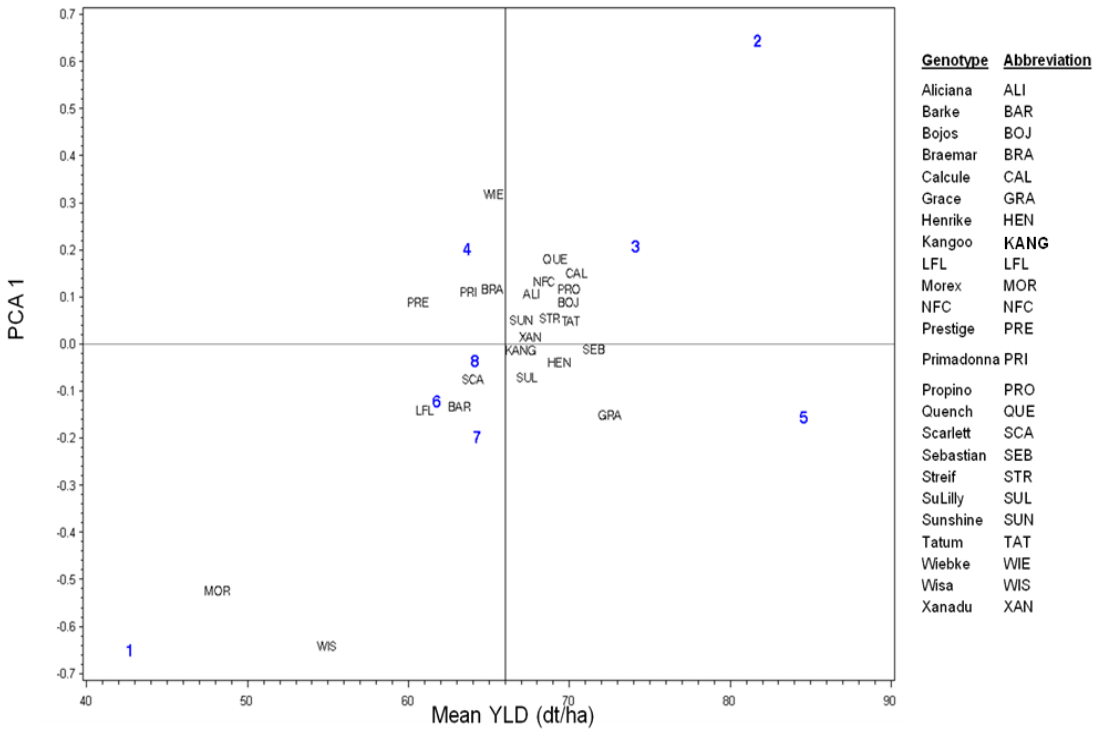


**Fig. 5.7. AMMI biplot presenting PCA1 scores vs. means for YLD of 24 spring barley genotypes evaluated in eight environments**. Environment: E1: Bavaria-Herzogenaurach 2012, E2: NRW 2012, E3: Saxony-Anhalt 2012, E4: Lower Saxony 2012, E5: NRW 2013, E6: Saxony-Anhalt 2013, E7: Bavaria-Uffenheim 2013, E8: Lower Saxony 2013.

## 5.4 Discussion

The present study aimed to investigate the crop development and grain yield of 24 spring barley cultivars under natural field conditions in Germany. In order to assess the applicability of secondary traits for indentifying drought stress tolerance in spring barley, the degree of genetic variance and heritability for morphological, physiological and yield-related traits was computed. Significant genotype-by-environment interactions were examined by using the AMMI model. The structure of the discussion section is as follows. First, the phenotypic response of spring barley for grain yield and associated plant parameters will be reconsidered. After analyzing the genetic association among examined plant parameters, the genetic variability and heritability of investigated traits will be clarified. Finally, significant genotype-by-environment interactions for the grain yield will be discussed.

### 5.4.1 Phenotypic response of spring barley for grain yield and associated plant parameters across eight environments

Phenotyping for drought stress tolerance is a major challenge in the 21$^{st}$ century because of the heterogeneity of field conditions and the inability to control environmental factors (Araus and Cairns 2014). The current study detected high environmental variations for morphological, physiological and yield-related parameters, which indicate that the manifestation of traits is strongly influenced by environmental factors. The degree of variation for investigated traits differed between the environments. High temperatures as well as the reduced soil moisture content triggered a negative impact on the crop development grain yield in Bavaria-Herzogenaurach 2012 (E1). On the other hand, the favourable climatic conditions in environment E2 and E5 enabled genotypes to demonstrate their yield potential. Compared to the field experiment conducted in NRW 2013 (E5), the measured plant dry matters, calculated crop growth rates and grain yields decreased over 50% on field site Bavaria-Herzogenaurach (E1). Gathered data agree with previous findings of Araus et al. (2003b) who studied the effect of environmental factors on 25 durum wheat genotypes in Spain. Araus et al. (2003b) indicate that sum of rainfall during the growing period explained about 70% of the total variability in durum wheat yield. Rizza et al. (2004) examined the diversity of barley yield performance under rainfed and irrigated conditions in field experiments. They found that the impact of climatic conditions on the barley development, plant height and grain yield was different between the experimental years and treatments.

However, the present study demonstrates that the crop growth rate varied between the analyzed environments. These findings led to the assumption that the effectiveness of photosynthesis is reduced under less favourable environmental conditions. So far, differences in the crop dry matter production and accumulation were particularly clear in the time interval between stem elongation and anthesis (CGR2), where CGR2 varied between environments by 18 g $m^{-2}$ per day. In addition, means of morphological, physiological and yield-related traits indicate that the crop development and finally the grain yield were sensitive to climatic conditions, especially in dealing with a high solar radiation, high soil temperatures and a reduced precipitation. One unanticipated finding was that the amount of solar radiation dramatically decreased in 2013 (Table 5.2). The solar radiation is the key energy resource for photosynthesis and a reasonable amount of studies have emphasized that solar radiation is a determining factor for the crop development and dry matter production (Daughtry et al. 1983). Savin and Slafer (1991) observed that shading treatments in wheat reduced the biological yield, grain yield and numbers of grains per square meter. Doehlert et al. (2001) reported that the grain yield of oats was positively correlated with the seasonal solar radiation. According to their findings Doehlert et al. (2001) suggest that warm, bright (high solar radiation) spring weather, and cooler summer weather without excessive precipitation during the grain filling generated the best oat yields. Although the photosynthetic active radiation decreased in 2013 over 50% compared to 2012, results of this study indicate that the productivity of examined spring barley genotypes (Table 5.3) was generally higher in 2013. A possible explanation for this result might be that the crop productivity depends on the plant ability to convert intercept incident solar radiation, which is again related to the available leaf area and the architecture of vegetation cover (Campillo et al. 2012). The overall lower yield level in 2012 could also be explained by deficiencies in water and nutrient inputs in 2012 (Fig. 5.2 and 5.3), and by higher air and soil temperatures which negatively modified the rate of leaf growth (Chmielewski and Köhn 1999). Cakmak (2005) has speculated that plants suffering from potassium deficiency are extremely sensitive to an increased light intensity which result in serve decreases in net photosynthesis and hence in the impairment of dry matter accumulation. In the recent years, research on the impact on weather conditions on yield components demonstrated that the timing and amount of precipitation as well as the temperature were determining factors for crop yields (Chmielewski and Köhn 1999; Lobell and Field 2007; Bannayan et al. 2011). Under these circumstances it is possible to speculate that the higher amount of air and soil temperature in 2012 as well as the lower amount of rainfall mainly promoted the formation of lower grain yields, especially in environment E1.

However, data must be interpreted with caution because only eight environments were considered in this study. Clearly further field experiments in divers environments are required to examine the impact of weather influences on the formation and differentiation on yield characteristics. In this context, Karimi and Siddique (1991) suggested that a selection of genotypes with a vigorous growth and the tendency to flower earlier might contribute to select genotypes with a higher grain yield in stress environments. In addition, lower grain yields under less favourable climate conditions were mainly due to the reductions in the number of ears per square meter (NEM) and the number of kernels per square meter (NKM). This observation is in agreement with previous discussed results in Chapter 3 and 4. Moreover, results corroborate with findings of Samarah (2005) who demonstrated that drought stress during the grain filling reduced the barley grain yield by decreasing the number of fertile ears and kernels per plant. Ehdaie et al. (2008) reported that drought decrease the amount of current assimilate and stem reserves as well as the grain yield due to reductions in the grain weight and number of grains. Additionally, Ehdaie et al. (2008) emphasized that the decrease in number of grains and grain weight was might be a result of the abortion of gains, which arise by a decreased supply of water-soluble carbohydrates and the decline in number of endoplasts cells and amyloplasts in the grain. Finally, field experiments revealed that grain yield ranges were larger in stress environments (E1) than in non-stress environments (Lakew et al. 2011).

### 5.4.2 Relationship between environmental factors and grain yield

Field conditions are by nature highly variable and the inability to control environmental factors result in the necessity to screen genotypes in multi-environmental trails, where plants experience a range of environmental stresses throughout the crop life circle (Araus and Cairns 2014). Thus, the observed variability of crop yields across different environments is the result of the complex interactions among different factors, including management practice, soil properties and weather conditions (Kravchenko et al. 2005). In the current study, correlation analysis for grain yield and yield components were calculated to assess the influence of environmental factors on spring barley yields. Overall, the number of ears per square meter (NEM) was positively correlated with the soil moisture content in June and July, the time period between anthesis and ripening (Table 5.4). The results indicate that majority of the investigated environments had an adequate water availability to sustain plant growth and development (Doehlert et al. 2001). From May to June, the time frame between stem

elongation and anthesis, soil temperatures were mainly negatively correlated with the number of kernels per ear (NKE). The results suggest that high temperatures during the grain development and grain filling decreased the grain yields through excessive respiration (Barnabás et al. 2008). These findings are in agreement with previous research of Amir and Sinclair (1991) who analyzed the effects of temperature and solar radiation on spring wheat. They pointed out that warmer temperatures decreases grain yields by an accelerated crop development, which resulted in a shorter growing season with less cumulative radiation interception. Voltas et al. (2002) defined drought as a function of rainfall, temperature and soil water holding capacity. They pointed out that variations in genotypic performance across different environments are mainly caused by differences in rainfall regimes. In general, water movements in soils are influenced by environmental factors, including precipitation intensity and frequency, air temperature, humidity, evaporation, vegetation and soil type (Hsieh et al. 1998; Knapp et al. 2002; Lee et al. 2007; Xu et al. 2012). Araus et al. (2003b) reported that the sum of rainfall during the grain filling period was strongly correlated with durum wheat yields, while the mean temperature was negatively correlated with the yield. However, previous studies found positive associations between the seasonal precipitation and crop yields (Rizza et al. 2004; Sinebo 2005; Francia et al. 2011; Cossani et al. 2011; Bannayan and Sanjani 2011). Lobell and Field (2007) observed that measures of growing season temperatures and precipitation explained around 30% of the year-to-year yield variability for rice, maize, soybeans, barley and sorghum, while the temperature provided the most explanatory power. With regard to results of the correlation analysis, it is possible to emphasise that a balanced precipitation accompanied by lower air and soil temperatures enabled genotypes to accumulate soil nutrients and to stimulate dry matter accumulation.

### 5.4.3 Relationship between investigated traits and grain yield

Generally, genetic correlation analysis across eight environments has conclusively determined positive correlations between the grain yield and the plant height, plant dry matter, crop growth rates, mean growth rate, number of ears per square meter, and number of kernels per square meter. During the crop life circle, correlations for the plant height and the plant dry matter increased in strength. Furthermore, the plant height was positively related to the number of kernels per and the number of kernels per square meter. The present findings accords with observations of Sinebo (2005) who reported that the harvest index, a rapid early vegetative shoot growth, a taller mature plant height, higher straws yields and a greater

number of living leaves enhance the grain yield of barley. Leilah and Al-Khateeb (2005) illustrated that the grain yield of wheat was positively associated with the plant height, number of ears per square meter, 100-grain weight and biological yield. Moreover, positive correlations between the number of kernels per square meter (NKM) and the post-flowering growth were found by Cossani et al. (2009). Karimi and Siddique (1991) reported that the crop growth rate at anthesis was highly correlated with the grain yield in wheat cultivars. Obtained results in this study confirmed the strong relationship between the plant dry matter at the beginning of stem elongation (PDM-BBCH 30), the dry matter accumulation at early stages of plant development (CGR1) and the grain yield. Hence, these parameters might be useful selection criteria under different soil moisture regimes. Taking into account that the plant dry matters at anthesis (PDM-BBCH 63) as well as the crop growth rate between stem elongation and anthesis (CGR2) were stronger correlated with and the final grain yield, the parameters PDM-BBCH 63 and CGR2 can be recommended as vital criteria for evaluating breeding material across contrasting environments. Findings of this research corroborate with earlier studies of Kandić et al. (2009) who have demonstrated that the early vigour, plant biomass and leaf senescence had a significant effect on the grain yield. Although the conventional measurement of plant biomass is laborious and destructive, the analysis of biomass and dry matter accumulation permit the evaluation and characterization of genotypes under varying environmental conditions. Remote sensing phenotyping methods which rely on digital RGB, spectral and/ or thermo infra-red cameras are non-destructive and allow the rapid and accurate measurement of multiple traits across a wide range of environments (Roy et al. 2011; White and Andrade-Sanchez 2012; Araus and Cairns 2014). Thus, the usage of sensor technology and digital eye is recommended for future field-based, high-throughput phenotyping experiments. Contrary to evaluated morphological traits, physiological parameters were mainly negatively associated with the grain yield. A possible explanation for these results might be that the plant nutrition is a complex process which involves numerous of physiological traits and reactions. In general, it is influenced by the soil fertility, the plant absorption efficiency and climatic conditions (Aerts and Chapin 1999; Asseng and Milroy 2006; Nikolić and Živanović 2011). Estrada-Campuzano et al. (2008) analyzed the responsiveness of time to anthesis to water shortage in wheat and triticale. They reported that the nitrogen availability had a non-significant effect on triticale development phases.

Due to the fact that drought is a major threat which seriously influence the crop production, one objective of the present study was to evaluate the relationship between plant parameters under both, non-stress and stress environments. In environment E5, correlations

between the grain yield and plant dry matter at repining stage (PDM-BBCH 81), crop growth rate between anthesis and ripening (CGR3) and mean growth rate (MGR) were significant, indicating that these traits are reliable selection criteria for the grain yield in non-stress environments. Correlation analysis under water deficit conditions in environment E1 (Table 5.6) revealed that none of the morphological traits were related to the grain yield and grain yield components. In accordance with the present results, previous studies of Kumar et al. (2008) have shown that a direct selection for grain yield in water-stress environments is more effective than the selection for secondary traits. Fukai et al. (1999) suggested that breeding for high yielding cultivars in drought areas should be based on the screening of genotypes initially for desirable phenological groups and the selection of genotypes with high yield potential under well-watered conditions.

In agreement with the ideas of Ober et al. (2005) measurements of the plant height and the plant dry matter are easy and inexpensive screening methods to assess drought stress in multi-environmental trails. The detected medium to high significant correlations between these traits and grain yield components suggest that genetic differences in grain yield are associated with differences in morphological and developmental traits (Ceccarelli et al. 1991). By contrast, the determination of plant nutrient concentrations was time consuming, expensive and required an accurate sample preparation.

### 5.4.4 Genetic variance and heritability of investigated plant parameters

As mentioned by Bouzerzour and Dekhili (1995), plant breeding aims to identify genotypes that perform well over a wide range of environments. Bouzerzour and Dekhili (1995) pointed out that an accurate heritability estimate and expected gains from selection are necessary to develop an optimum selection and evaluation strategy for barley. The current research analyzed across eight environments the components of variation and heritability of spring barley for the grain yield and associated plant parameters. Information regarding the genetic variation and heritability of traits are of particular importance to assess the breeding value and the usefulness of these traits (Ober et al. 2005; Chen et al. 2012). A significant genotypic variation was found for the plant height, plant dry matter, crop growth rates, gain yield and yield components. Heritability estimates and genetic coefficient of variation for PLH-BBCH 30, PLH-BBCH 63, PLH-BBCH 81, PDM-BBCH 30, PDM-BBCH 81, CGR3, NEM, NKE and NKM indicate that a sufficient variation for phenotyping of drought stress tolerance existed. Lakew et al. (2011) observed high variations for barley grain yields and

agronomic traits in various environments, which underlie the existence of genetic variability for drought tolerance. In this study, the comparison of variance components and heritability estimates indicate that morphological traits were controlled to a larger extent by genotypic effects. In particular, the observed high heritability and genetic variance for PDM-BBCH 63, PDM-BBCH 81 and CGR3 suggest that these traits are considerable selection criteria for phenotyping experiments across varying field conditions. As can be seen from Table 5.7, low estimates of genotypic variance and heritability for the plant nitrogen, phosphor and potassium content led to the assumption that the nutrient concentration in plants was greatly affected by environmental factors, including soil moisture content, soil fertility and temperature. These findings accords with earlier presented findings (data shown in the Appendix), which showed a lack of significant correlation between grain yield components and plant nutrient concentrations (PNC, PPhC and PKC). The data obtained are broadly consistent with findings of Chen et al. (2012) who studied the effectiveness of morphological, physiological and yield related traits in wheat. Chen et al. (2012) pointed out that the broad sense heritability of yield related and morphological parameters were relatively high, whereas the heritability of physiological traits, such as stomatal conductance, intercellular CO2 concentration and transpiration rate was lower. They concluded that physiological traits are easily influenced by the environment and could not be evaluated accurately. Furthermore, it is interesting to note that the plant potassium content revealed among all investigated physiological traits the highest environmental variance, indicating the presence of significant environmental differences between analyzed field sites and years. Potassium is an essential plant nutrient which is required in several key plant functions, such as photosynthesis, energy transfer, plant growth and yield development (Pettigrew 2008). In particular, potassium is considered to be essential for protein metabolism, production of adenosine triphosphate (ATP), osmoregulation and maintenance of the turgor pressure in plants (Bednarz et al. 1998; Pettigrew 2008). However, drought stress affect many physiological processes in plants, including the difficulty to maintain a nutrient uptake capacity, which results in reductions of tissue nutrient concentration, photosynthetic rate, growth rate and the increase in senescence of older leaves (Chapin 1980; Huang 2001; Pettigrew 2008). Due to its function in stomatal regulation and osmoregulation the assumption is made that potassium increases the plant drought resistance (Beringer and Trolldenier 1980; Andersen et al. 1992; Hu and Schmidhalter 2005; Marschner and Marschner 2012). Andersen et al. (1992) analyzed how potassium applied as KCL at rates of 50, 125 and 200 kg K/ha influenced the grain yield of barley grown under drought conditions in rainout shelters. According to their findings the

grain yield under well-watered conditions was not significantly affected by different levels of potassium. When water stress was imposed at early grain filling stages, the increase in potassium slightly increased the grain yield. Cakmak (2005) suggested that increases in severity of drought stress result in corresponding increases in potassium demand in order to maintain the photosynthesis and to protect chloroplasts from oxidative damages. In view of above considered findings and reports, it is seems that the potassium nutritional status of plants has a profound influence on the crop growth and development. Hence, the plant potassium concentration is important for achieving high yields under rainfed field conditions (Cakmak 2005; Pettigrew 2008). However, the differential effects of soil drying on plant nutrient concentration as well as the understanding of potassium accumulation might provide a valuable contribution to breeding programs for drought stress tolerance (Huang 2001). Regarding the fact that 24 spring barley cultivars were tested across eight environments, the variance of genotype-by-environment interaction was low. Nevertheless, the presence of genotype-by-environment interactions suggest that future studies for drought stress tolerance should be realized in diverse environments in order to explore yield variability.

### 5.4.5 AMMI analysis

In the recent years, methods of analyzing genotype-by-environment interactions (GEI) have been extensively discussed and several studies examined the usage of the additive main effect and multiplicative interaction analysis (AMMI model) as a technique to explore GEI in crops (Westcott 1986; Becker and Leon 1988; Piepho 1994; Shafii and Price 1998; Tarakanovas and Ruzgas 2006; Yan et al. 2007). In this study genotype-by-environment interactions could be explained by using the AMMI model (Fig. 5.6). The AMMI biplots showed that the spring barley cultivars ‘Kangoo’, ‘Xanadu’, ‘Sebastian’ and ‘Sunshine’ were stable suggesting that these varieties are suitable for the cultivation in a wider range of environments. In contrast, the varieties ‘Wisa’, ‘Wiebke’ and ‘Morex’ were ranged as unstable. The yield variability, which is displayed in the biplots, was mainly caused by the wide variation of environmental conditions and not by genotypic differences (Tariku et al. 2013). These findings are in agreement with research of Francia et al. (2011) who indicate that the crop performance under natural field conditions depends on the combined effect of the genotype, environmental conditions and their interaction. Furthermore, they reported that variations in grain yields were largely explained by variations in the environments. Voltas et al. (2002) stated that temperature and the rainfall are climatic factors, which affect the grain

yield. Hence, they play an important role in the occurrence of GEI. Nevertheless, it is interesting to note that the varieties ‘Morex’ and ‘Wisa’, which were released in 1950 and 1978, were more or less adapted to the environment E1, the field site which was characterized by drought periods during the crop life circle. This supports the hypothesis that older varieties may cope better with drought conditions.

## 5.5 Conclusion

The primary aims of the present research were (1) to screen 24 spring barley cultivars for their crop development and yield performance in rainfed field environments, which encompasses well-watered and drought conditions, and (2) to detect specific traits that improve the selection of genotypes with stable grain yields under various environmental conditions. In conclusion the study has shown that:

(1) the environmental variability was high due to the unpredictable timing and severity of weather conditions. The timing and frequency of precipitation as well as the temperature were determining factors for the yield formation.

(2) the means of plant dry matters, calculated crop growth rates and grain yields decreased over 50% under water deficit conditions in Bavaria-Herzogenaurach (E1).

(3) the number of ears per square meter was positively correlated with the soil moisture in June and July, the time frame between anthesis and ripening. The soil temperature between stem elongation and anthesis was negatively correlated with the number of kernels per ear suggesting that a balanced precipitation accompanied by lower air and soil temperatures were favourable for the dry matter accumulation and grain yield formation.

(4) the plant dry matter and dry matter accumulation until BBCH 30 was positively correlated with the grain yield. Nevertheless, the plant dry matter at anthesis (PDM-BBCH 63) as well as the crop growth rate between anthesis and ripening stage (CGR3) were stronger correlated with the final grain yield indicating that PDM-BBCH 63 and CGR3 are vital selection criteria.

(5) physiological parameters, such as PNC, PPhC and PKC, had due to their low heritability and genetic variation ($CV_g$) a little adaptive value for the selection of high yielding genotypes.

(6) physiological traits were mainly influenced by environmental conditions and therefore difficult to measure. The observed high environmental variance for the plant potassium content led to the assumption that the plant potassium demand varied depending on the environmental conditions.

(7) morphological traits were not significantly correlated with the grain yield and yield components under water stress (E1), indicating that a direct selection for grain yield in water-stress environments is more efficient than an indirect selection through secondary traits.

(8) the usage of the AMMI model in breeding programs make a valuable contribution to explore the yield stability across divers environments.

Evidence from this research suggests that phenotyping for drought stress tolerance should be realized under various environmental conditions in the field. Furthermore, the study extended our knowledge of environmental factors which contribute to the grain yield formation. Thus, a detailed recording and analysis of climatic conditions is required to select high yielding genotypes. Clearly, further research will be needed to investigate the capability of morphological and physiological parameters for identifying drought stress tolerance under natural field conditions. In this connection the usage of remote sensing phenotyping methods are recommended to obtain accurate and precise phenotypic data.

# Chapter 6: Phenotyping for drought stress tolerance - Evaluation of the grain yield and drought related traits in pot and field experiments

## 6.1 Introduction

Abiotic stress conditions such as drought, salinity and heat are primary causes of yield losses, which are in future expected to increase due to an increase in climate variability and extreme weather events. Here, water stress is one of the most important environmental factors limiting cereal yields. With regard to the continuously growing human population, crop production must double by 2050 (Tilman et al. 2011). As a consequence of this, crop yields have to increase at a rate of 2.4% per year (Araus and Cairns 2014). Barley (*Hordeum vulgare* L.), which is ranked as fifth important cereal grain crop worldwide, is a widely adapted species and known to be relatively tolerant to abiotic stresses (Baik and Ullrich 2008; Honsdorf et al. 2014). Thus, barley is an important model species for genetic studies. The response of barley to drought has been extensively examined under controlled environmental conditions in greenhouse experiments and under natural field conditions (Forster 2000). However, in the recent years considerable research effort has been made in breeding cereals for drought tolerance. The term 'drought tolerance' is thereby defined either by the ability of plants to survive serve stresses and complete their life circle, or to achieve acceptable yields under moderate stress conditions (Tardieu and Tuberosa 2010). Methods to investigate drought stress tolerance are wide and can broadly be divided into field, rainout shelter, greenhouse and poly tunnel experiments. Generally, phenotyping genotypes for drought tolerance in field experiments is complicated by the simultaneous presence of other stress factors and the diversity of plant mechanism which can be used by plants to tolerate each of these stresses (Roy et al. 2011). The complexity of environmental conditions makes it difficult to score genotypes for drought tolerance and to interpret results. Hence, it is necessary to evaluate genotypes over several years and in diverse environments. Contrary, the evaluation of genotypes under controlled conditions (e.g. greenhouse or growth chamber experiments) reduces the complexity of environmental effects and genotype-by-environment interactions. Several researchers pointed out that results obtained from controlled environments distort the real plant performance and underestimate the plasticity in plant response to drought (Roy et al. 2011; Araus and Cairns 2014). Future progress in breeding cereals for improved yield performance and yield stability requires specific screening procedures in greenhouse and field. To date, an appreciable part of scientific research has

been undertaken to explore and understand drought stress tolerance, but only few studies compared directly the results obtained from different screening methods. Thus, the present study aimed to compare the genotypic performance of 24 spring barley cultivars for their drought stress tolerance in pot and field experiments. In addition the study discusses the possibilities and limitations of different screening methods.

## 6.2 Materials and Methods

The following part provides information about the used plant material, experimental design, phenotypic data collection and performed statistical analyses.

### 6.2.1 Plant material

During 2012 and 2013, a total of twenty-four spring barley varieties (*Hordeum vulgare* subsp. *vulgare*) were used for phenotyping experiments in pot and field experiments. The spring barley cultivars were selected in order to capture the genetic variability within Central European breeding material. Detailed information regarding names of cultivars and their year of release are presented in Table 4.1 of Chapter 4.

### 6.2.2 Experimental design

**Pot experiments**

In 2012 and 2013, pot experiments were conducted in polyethylene plastic tunnels at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany. In order to study the effect of water shortage on the plant performance, spring barley genotypes were grown under two contrasting water regimes (Chapter 4; Fig.4.1):

(1) Well-watered conditions: Genotypes were fully irrigated over the whole vegetation period to maintain volumetric moisture content of 30%.

(2) Drought treatment from tillering to anthesis (BBCH 24 – BBCH 49): 21-day lasting reduction of the water supply to decrease the soil moisture content to the permanent wilting point. After three weeks genotypes were re-watered.

Experiments were carried out in a two-factorial split-plot design. The irrigation treatments as main-plot factor were laid out in complete randomized blocks, while genotypes as sup-plot factor were completely randomized within main-plots. In each year, genotypes were planted in 22x22x26 cm plastic pots containing 11.5 l of a silica sand and soil mixture (Terrasoil ®, Cordel & Sohn, Salm, Germany). Before sowing, pots were irrigated to saturate the soil and facilitate the germination of seeds. Through automatic drip irrigation system water and KRISTALON®, which is a fully water soluble NPK fertilizer, were supplied three times per day (6:30 am, 00:30 pm and 6:30 pm). Further details regarding the watering time per irrigation treatment are presented in the Appendix. Herbicides and insecticides were applied in accordance to agriculture practice. The application of growth regulators, strobilurine and sulfonylurea was avoided. During the vegetation period air temperature (°C), air humidity (%), solar radiation (W/m²), soil moisture (Vol.%) and soil temperature (°C) were recorded every five minutes with a DL2e Data Logger from Delta-T Devices Ltd. Further, detailed explanations of the experimental setup have been given in detailed in Chapter 2 and Chapter 4.

**Field experiments**

Details on the experimental design, sampling, analysis and climatic conditions have been described in the previous chapter 5. Out of the eight already presented and analyzed environments in Chapter 5, the two environments with the lowest and highest yield performance (E1: 41.6 dt/ha; E5: 83.5 dt/ha) were chosen for further analysis on genotypic drought stress tolerance. Hence, this section provides detailed information for the environments E1 and E5. The field experiments were conducted in 2012 at field site in Bavaria-Herzogenaurach (49° 34' 14 N,10° 52' 59 E) and in 2013 at field site in North-Rhine Westphalia (50° 36’48 N, 6° 59’ 38 E). Soil types in Bavaria-Herzogenaurach (E1) and North-Rhine Westphalia (E5) have been classified and summarized in the Appendix. The experimental design was a complete randomized block designs with four replications. For phenotypic data acquisition, spring barley cultivars were evaluated at three sampling dates: (1) end of the tillering stage and begin of stem elongation (BBCH 30), (2) between booting and anthesis (BBCH 55) and finally (3) at the end of the fruit development and begin of the ripening stage (BBCH 80). Evaluated genotypes correspond to those used in pot experiments (see Chapter 4, Table 4.1). Plots were kept weed free and plant protection was applied as necessary to avoid presence of pests and diseases. Corresponding to pot experiments the

application of growth regulators, strobilurine and sulfonylurea was avoided. Fertilizers were applied according to the agriculture practice for malting barley. Climatic conditions during the cropping season, including soil moisture content ($m^3/m^3$), soil temperature (°C), air temperature (°C), relative air humidity (%) and precipitation ($l/m^2$), were recorded under the usage of a HOBO U30 Data logger (Onset®, Bourne, MA 02532, USA). Details of the experimental setup and the field sites were described in Chapter 5.

### 6.2.3 Phenotypic data collection

In this section two morphological and five yield related plant parameters were investigated. Table 6.1 provides a short definition and description of measured parameters. Further specifications of taking measurements have been elucidated in Chapter 2 and in Chapter 5. Principal growth stages were recorded using the extended BBCH scale of Hess et al. (1997).

**Table 6.1** Summary of scored and calculated traits for pot and field phenotyping experiments in 2012 and 2013, their abbreviations and growth stage at the time of measurement.

| Pot experiments | | | | Field experiments | | | |
|---|---|---|---|---|---|---|---|
| **Trait** | **BBCH** | **Abbr.** | **Unit** | **Trait** | **BBCH** | **Abbr.** | **Unit** |
| **Plant height** | 49 | $PLH_P$ | cm | **Plant height** | 63 | $PLH_F$ | cm |
| **Plant dry matter** | 49 | $PDM_P$ | g/plant | **Plant dry matter** | 63 | $PDM_F$ | $g/m^2$ |
| **Number of ears per plant** | 91 | $NEP_P$ | No./plant | **Number of ears per square meter** | 81 | $NEM_F$ | No./ $m^2$ |
| **Number of kernels per ear** | 91 | $NKE_P$ | No./ear | **Number of kernels per ear** | 81 | $NKE_F$ | No./ear |
| **Number of kernels per plant** | 91 | $NKP_P$ | No./plant | **Number of kernels per square meter** | 81 | $NKM_F$ | No./$m^2$ |
| **Thousand kernel weight** | 91 | $TKW_P$ | g | **Thousand kernel weight** | 91 | $TKW_F$ | g |
| **Grain yield** | 91 | $YLD_P$ | g/plant | **Grain yield** | 91 | $YLD_F$ | dt/ ha |

Abbr.: abbreviation

### 6.2.4 Climatic conditions

**Pot experiments**

Daily air temperatures, the relative humidity and the soil moisture content were recorded with a DL2e Data Logger from Delta-T Devices Ltd. (Figure 4.1 in Chapter 4). In 2012, climate conditions were dry and sunny with an average air temperature in May and June of 16.2 °C and 17.1°C. In 2013, genotypes were exposed to average air temperatures of 13.5°C in May and 18.1°C in June. Between May and June the relative humidity amounted 71% in 2012 and 75% in 2013. Between the years, the mean temperatures, the relative humidity and the solar radiation differed during main growth period (day of year 130 - day of year 150). Thus, climate conditions in 2013 can be distinguished from climate conditions in 2012 by a 5°C lower air temperature, an 11% higher humidity and a 300 kW/m² lower solar radiation. In both years of experimentation the automatic drip irrigation system allowed an accurate reduction of the soil moisture content from 26% volumetric water content (VWC) to 8% VWC. The reduction of the water supply started approximately at BBCH 24 and lasted 21 days. After three weeks of water deficit genotypes were re-watered and soil moisture rise again to 26% VWC.

**Field experiments**

The daily air temperature, relative humidity, precipitation, solar radiation and soil moisture content were recorded at each field site using a HOBO U30 Data logger (Onset®, Bourne, MA 02532, USA). Spring barley genotypes in environment E1 and E5 were exposed to contrasting environmental conditions during the vegetation period. In particular, the amount of rainfall and distribution differed between the environments and showed a variability of 68 l/m² (Chapter 5, Table 5.2). Hence, environment E1 (location-year combination) was characterized by dry weather conditions, while genotypes in environment E5 received more water. In environment E1 rainfall was limited during stem elongation and anthesis (average approximately 4.1 l/m² per week). Contrary, genotypes in environment E5 experienced a balanced water supply over the whole vegetation period. Thus, soil water content remained constant over 20 Vol. % (Chapter 5, Fig. 5.3). Mean air and soil temperatures as well as solar radiation differed during the years and experimental sites. Compared to weather conditions in environment E5, climatic conditions in E1 were

characterized by a 19% higher sum of air temperature, a 26% higher sum of soil temperature, a 34% lower amount of rainfall and an 11% lower humidity (Chapter 5, Table 5.2). According to this, the field site in Bavaria-Herzogenaurach (E1) was characterized as a stress environment, whereas the field site in North-Rhine Westphalia was ranged as a non-stress environment.

### 6.2.5 Statistical analyses

Statistical analyses for morphological and yield-related traits were performed using the SAS software version 9.2 (SAS Institute, 2008).

#### *Pot experiments*

Analysis of variance for data obtained in pot experiments were conducted using the SAS procedure PROC MIXED with years, treatments and genotypes having fixed effects. By default, PROC MIXED procedure computed the "Type 3 Test of Fixed Effects". The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***. Years were considered as fixed effects because two years of experimentation are not representative for the wide range of environmental factors influencing the crop development. The LSMEANS statement in PROC MIXED procedure calculated least-squares means (LS-means) of fixed effects.

#### *Combined analysis of variance*

The model used for analysis of variance across two years is:

$$Y_{ijk} = \mu + Y_i + T_j + Y_i*T_j + G_k + G_k*Y_i + G_k*T_j + G_k*Y_i*T_j + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $Y_i$ is the fixed effect of *i*-th year; $T_j$ is the fixed effect of *j*-th treatment; $Y_i*T_j$ is the fixed interaction effect of the *i*-th year with the *j*-th genotype, $G_k$ is the fixed effect of *k*-th genotype; $G_k*Y_i$ is the fixed interaction effect of *k*-th genotype with *i*-th year, $G_k*T_j$ is the fixed interaction effect of *k*-th genotype with *j*-th treatment; $G_k*Y_i*T_j$ is the fixed interaction effect of *k*-th genotype with *j*-th treatment and with *i*-th year and $e_{ijk}$ is random errors.

***Field experiments***

Analysis of variance was conducted using SAS PROC MIXED with environments and genotypes having fixed effects. Replications (blocks) within each environment were considered as random effects. In order to test the significance of fixed effects, PROC MIXED procedure computed the "Type 3 Test of Fixed Effects". The test of significance was accepted at $P \leq 0.05$*, $P \leq 0.01$ **, and $P \leq 0.001$***. The LSMEANS statement in PROC MIXED procedure calculated least-squares means (LS-means) of fixed effects.

The model used for analysis of variance across eight environments is:

$$Y_{ijk} = \mu + E_i + B(E)_{j(i)} + G_k + G_k*E_i + e_{ijk}$$

Where $Y_{ijk}$ is response variable; $\mu$ is general mean; $E_i$ is the fixed effect of *i*-th Environment; $B(E)_{j(i)}$ is the random effect of the *j*-th block nested within *i*-th environment; $G_k$ is the fixed effect of *k*-th genotype; $G_k*E_i$ is the fixed interaction effect of k*i*-th genotype with *i*-th environment, and $e_{ijk}$ is the error.

***Statistical computations for pot and field experiments***

Despite the individual analysis of variance for pot and field experiments, the present study aimed to compare the results obtained from different experimental approaches. Thus, gathered data were exposed to several statistical procedures which are explained thereafter.

***Correlation analysis***

Based on genotypic means, Spearman's rank correlation analysis was performed by using the SAS procedure PROC CORR.

***Stress tolerance index (STI)***

Stress tolerance index (STI) has been used to identify genotypes that perform well, under control (well-watered) and drought treatment. According to the equation defined by Fernandez (1992), STI was calculated based on genotypic means:

$$\text{STI} = \left(\frac{\mathrm{Y}_{\mathrm{ww}}}{\overline{\mathrm{Y}_{\mathrm{ww}}}}\right)\left(\frac{\mathrm{Y}_{\mathrm{dt}}}{\overline{\mathrm{Y}_{\mathrm{dt}}}}\right)\left(\frac{\overline{\mathrm{Y}_{\mathrm{dt}}}}{\overline{\mathrm{Y}_{\mathrm{ww}}}}\right) = \frac{\left(\mathrm{Y}_{\mathrm{ww}}\right)\left(\mathrm{Y}_{\mathrm{dt}}\right)}{\left(\overline{\mathrm{Y}_{\mathrm{ww}}}\right)^2}$$

Where $Y_{ww}$ and $Y_{dt}$ are the genotype mean for given plant parameter under well-watered conditions and drought treatment, and $\overline{Y}_{ww}$ and $\overline{Y}_{dt}$ are the overall means of genotypes under both irrigation levels/ environments.

***Membership function value of drought tolerance (MFVD)***

Based on the computation of stress tolerance indices, the membership function value of drought tolerance was calculated to assess the impact of drought on the plant performance. Detailed explanations regarding the membership function value of drought tolerance was given in the previous Chapter 4. The membership function value of drought tolerance (MFVD) was calculated as follows (Chen et al. 2012):

$$U_{ij} = \frac{STI_{ij} - STI_{j\min}}{STI_{j\max} - STI_{j\min}} \quad U_i = \frac{1}{n}\sum_{j=1}^{n} U_{ij}$$

Where $U_{ij}$ is the membership function of the trait (*j*) for the genotype (*i*) for stress tolerance index; $STI_{jmax}$ is the maximum value of the stress tolerance coefficient for the trait (*j*); $STI_{jmin}$ is the maximum value of the stress tolerance coefficient for the trait; $U_i$ is the average value of the membership function of seven traits for the genotype (*i*) for drought tolerance.

***Shukla's stability variance ($\sigma_i^2$)***

In pot experiments and field experiments, significant genotype-by-environment interactions were detected. To identify high-yielding and stable genotypes, Shukla's procedure of stability variance was applied. Shukla's stability variance is an unbiased estimate of the variance of a genotype across two environments (Becker and Leon 1988).

Shukla's stability variance ($\sigma^2$) for the *i*-th genotype was calculated using the following equation (Stelluti et al. 2011):

$$\sigma_i^2 = \left[\frac{p}{(p-2)(q-1)}\right]\sum_{j=1}^{q}(X_{ij} - m_i - m_j + m)^2 - \left[\frac{SS(SxA)}{(p-1)(p-2)(q-1)}\right]$$

Where, p: number of compared genotypes, q: number of environments, $X_{ij}$: yield response of the *i*-th genotype in the *j*-th environment, $m_i$: yield averaged across environments of the *i*-th genotype, $m_j$: yield averaged across all tested genotypes of the *j*-th environment, m: grand mean, SS(SxA): sum of squares of the interaction "genotype-by-environment".

A stable genotype is defined as having a stability variance ($\sigma_i^2$) equal to zero. Negative estimates of $\sigma_i^2$ have been taken as equal to zero (Shukla 1972).

## 6.3 Results

This section seeks to present and explain results from phenotyping experiments of spring barley for drought stress tolerance under poly tunnel and field conditions. Statistical analyses were conducted and findings are elucidated thereafter.

### 6.3.1 Analysis of variance

***Analysis of variance - pot experiments***

Combined analysis of variance revealed significant variation among genotypes and treatments for all traits (see Appendix). Genotype-by-treatment interaction was significant for yield parameters, including $NEP_P$, $NKE_P$, $NKP_P$ and $YLD_P$.

***Analysis of variance - field experiments***

Analysis of variance indicated significant main effects of the environment for one morphological trait ($PLH_F$) and five yield related traits ($NEM_F$, $NKE_F$, $NKM_F$, $TKW_F$, $YLD_F$). Significant genotypic variations were detected for six plant parameters, in particular for two morphological traits ($PLH_F$, $PDM_F$) and four yield related traits ($NEM_F$, $NKE_F$, $NKM_F$ and $YLD_F$). Furthermore, interactions between genotype and environment were significant for $NKE_F$, $NKM_F$, $TKW_F$ and $YLD_F$. Genotype-by-environment interactions for $PLH_F$, $PDM_F$ and $NEM_F$ were non-significant.

### 6.3.2 Effects of water shortage on genotypic performance in pot experiments

Generally, water deficit between BBCH 24 and BBCH 49 caused a significant decline of the above ground plant dry matter ($PDM_P$) by 38.5% (Table 6.2). Moreover, drought treatments triggered a negative impact on the grain yield and yield components. Thus, water deficit during the crop life circle seriously reduced the grain yield by 42.7%. The significant decrease in grain yield was accompanied by reductions in all yield components. Under drought $NEP_P$, $NKE_P$, $NKM_P$ and $TKW_P$ declined by 16.6%, 21.9%, 34.1% and 12.4%, respectively. In summary, results showed that measured genotypic means of $PDM_P$, $NKE_P$, $NKP_P$ and $YLD_P$ decreased over 20%, under water deficit.

### 6.3.3 Effects of water shortage on genotypic performance in field experiments

Mean values of investigated morphological and yield-related parameters in field experiments are set out in Table 6.2. Considering environmental means, water shortage during the vegetation period reduced in environment E1 the above plant dry matter (77.1%), the grain yield (50.2%) and yield components. Across analyzed environments, genotypes on the field site in NRW in 2013 (E5) produced maximum mean values for $NEM_F$, $NKE_F$, $NKM_F$, $TKW_F$ and $YLD_F$. Furthermore, it is apparent from Table 6.2 that less favourable growing conditions in environment E1 remarkably decreased the number of ears per square meter (-39.3%) and the number of kernels per ear (-13.9%). Consequently, mean values of $NKM_F$ declined by 49.7%. The thousand kernel weight wasn't able to compensate losses of grain yield components (-1.7%).

**Table 6.2** Mean values for morphological and yield related plant parameters of 24 spring barley genotypes grown in pot and field experiments.

| Trait | Pot experiments | | | Trait | Field experiments | | |
|---|---|---|---|---|---|---|---|
| | Wet | Dry | Δ % [a] | | E5 | E1 | Δ %[b] |
| **$PLH_P$** | 61.2 | 55.4 | -9.5 | **$PLH_F$** | 89.47 | 61.5 | -32.2 |
| **$PDM_P$** | 2.5 | 1.6 | -38.5 | **$PDM_F$** | 961.2 | 284.7 | -70.4 |
| **$NEP_P$** | 5.4 | 4.5 | -16.6 | **$NEM_F$** | 1025.7 | 622.8 | -39.3 |
| **$NKE_P$** | 18.9 | 14.8 | -21.9 | **$NKE_F$** | 17.7 | 15.2 | -13.9 |
| **$NKP_P$** | 99.3 | 65.4 | -34.1 | **$NKM_F$** | 17462.0 | 8787.6 | -49.7 |
| **$TKW_P$** | 50.4 | 44.2 | -12.4 | **$TKW_F$** | 48.2 | 47.4 | -1.7 |
| **$YLD_P$** | 5 | 2.9 | -42.7 | **$YLD_F$** | 83.5 | 41.6 | -50.2 |

Where: Wet: well-watered, Dry: drought treatment, Δ% [a]: (Dry-Wet)/ Wet *100, E1: Bavaria-Herzogenaurach 2012, E5: NRW 2013, Δ %[b] : (E1-E5)/ E5 *100, Trait: $PLH_P$: plant height measured in pot experiments, $PDM_P$: plant dry matter measured in pot experiments, $NEP_P$: number of ears per plant measured in pot experiments, $NKE_P$: number of kernels per ear measured in pot experiments, $NKP_P$: number of kernels per plant measured in pot experiments, $TKW_P$: thousand kernel weight measured in pot experiments, $YLD_P$: grain yield per plant (g) measured in pot experiments, $PLH_F$: plant height measured in field experiments, $PDM_F$: plant dry matter measured in field experiments, $NEM_F$: number of ears per square meter measured in field experiments, $NKE_F$: number of kernels per ear measured in field experiments, $NKM_F$: number of kernels per square meter measured in field experiments, $TKW_F$: thousand kernel weight measured in field experiments, $YLD_F$: grain yield (dt/ha) measured in field experiments.

### 6.3.4 Correlation analysis among investigated plant parameters examined in pot and field experiments

This study set out with the aim of assessing drought stress tolerance in spring barley and identifying valuable traits, which can be used as selection criteria for drought stress tolerance. In this connection, correlation analysis is an appropriate method to analyze the association of promising traits under both, non-stress and stress conditions. Based on genotypic means correlation analysis was performed for pot and field experiments. The results are presented thereafter.

#### *Genetic correlation coefficients among investigated traits examined in pot experiments*

The relations of the plant height and plant dry matter with the grain yield and yield components are shown in Table 6.3. Under well-watered conditions, significant correlations were found between $NEP_P$ and $NKE_P$ (-0.56**), $NKP_P$ (0.68***) and $YLD_P$ (0.59**). The grain yield was significantly and positively associated with the number of kernels per plant (0.68***). The plant height and plant dry mater was not statistically significant correlated with the grain yield and yield components.

However, under water-stress conditions the plant height was moderate correlated with the plant dry matter (0.47*). Correlation analysis for the grain yield and yield components showed significant associations of $NEP_P$ with $NKE_P$ (-0.52*), $NKP_P$ (0.71*), $TKW_P$ (-0.45*), and $YLD_P$ (0.49*).The grain yield under water-stress was highly correlated with the $NKP_P$ (0.86***).

The results showed that the strength of association between $NKP_P$ and both, $NEP_P$ and $YLD_P$ increased under water-stress conditions. Under water-stress conditions a higher number of significant correlations were found.

**Table 6.3** Spearman correlation coefficients ($r_g$) among the plant height, plant dry matter, grain yield and yield components of 24 spring barley genotypes grown under in poly tunnels.

| Trait | $PLH_P$ | $PDM_P$ | $NEP_P$ | $NKE_P$ | $NKP_P$ | $TKW_P$ | $YLD_P$ |
|---|---|---|---|---|---|---|---|
| **$PDM_P$** | | | | | | | |
| wet | 0.36 | 1 | | | | | |
| dry | **0.47** * | | | | | | |
| **$NEP_P$** | | | | | | | |
| wet | -0.10 | -0.07 | 1 | | | | |
| dry | -0.15 | 0.08 | | | | | |
| **$NKE_P$** | | | | | | | |
| wet | 0.00 | 0.08 | **-0.56** ** | 1 | | | |
| dry | 0.22 | 0.10 | **-0.52** * | | | | |
| **$NKP_P$** | | | | | | | |
| wet | -0.04 | 0.04 | **0.68** *** | -0.04 | 1 | | |
| dry | -0.08 | 0.07 | **0.71** *** | 0.10 | | | |
| **$TKW_P$** | | | | | | | |
| wet | 0.01 | 0.05 | -0.23 | 0.30 | -0.34 | 1 | |
| dry | 0.17 | 0.09 | **-0.45** * | 0.35 | -0.21 | | |
| **$YLD_P$** | | | | | | | |
| wet | -0.14 | 0.16 | **0.59** ** | 0.14 | **0.75** *** | | 1 |
| dry | 0.00 | 0.19 | **0.49** * | 0.22 | **0.86** *** | 0.20 | |

Where: wet: well-watered conditions, dry: water-stress conditions, Trait: $PLH_P$: plant height measured in pot experiments, $PDM_P$: plant dry matter measured in pot experiments, $NEP_P$: number of ears per plant measured in pot experiments, $NKE_P$: number of kernels per ear measured in pot experiments, $NKP_P$: number of kernels per plant measured in pot experiments, $TKW_P$: thousand kernel weight measured in pot experiments, $YLD_P$: grain yield per plant (g) measured in pot experiments.

### *Correlation analysis among investigated traits examined in field experiments*

Table 6.4 gives the matrix of genetic correlations for investigated traits in well-watered and water-stress environments. In the well-watered environment, significant and negative correlations were noted between $PLH_F$ and $NEP_F$ (-0.55*), $NKP_F$ (-0.61**), and $YLD_F$ (-0.72***). The number of ears per square meter was significantly associated with yield components, including $NKE_F$ (-0.48*), $NKM_F$ (0.83***), $TKW_F$ (-0.54*), $YLD_F$ (0.69***). Further, significant correlations were observed between $NKM_F$ and both, $TKW_F$ (-0.62**) and $YLD_F$ (0.84***). In the stress environment E1, $NEM_F$ was negatively related to $NKE_F$ (-0.85***). Positive genetic correlations were found for $NKE_F$ with $NKP_F$ (0.69***) and $YLD_F$ (0.45*). Finally, the number of kernels per square meter exhibited significant correlations with $TKW_F$ (-0.59**) and $YLD_F$ (0.74***). From Table 6.4 it is evident that correlations under well-watered (non-stress) conditions in the field were generally stronger

than under stress conditions. No statistically significant correlations were found between the plant dry matter and evaluated traits under both, well-watered and water-stress conditions.

**Table 6.4** Spearman correlation coefficients ($r_g$) among the plant height, plant dry matter, grain yield and yield components of 24 spring barley genotypes grown under rainfed conditions in the field.

| Trait | $PLH_F$ | | $PDM_F$ | $NEM_F$ | | $NKE_F$ | | $NKM_F$ | | $TKW_F$ | $YLD_F$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$PDM_F$** | | | | | | | | | | | |
| wet | 0.29 | | 1 | | | | | | | | |
| dry | 0.19 | | | | | | | | | | |
| **$NEM_F$** | | | | | | | | | | | |
| wet | **-0.55** | * | 0.05 | 1 | | | | | | | |
| dry | -0.10 | | 0.24 | | | | | | | | |
| **$NKE_F$** | | | | | | | | | | | |
| wet | 0.28 | | -0.04 | **-0.48** | * | 1 | | | | | |
| dry | 0.07 | | -0.23 | **-0.85** | *** | | | | | | |
| **$NKM_F$** | | | | | | | | | | | |
| wet | **-0.61** | ** | -0.01 | **0.83** | *** | -0.05 | | 1 | | | |
| dry | -0.02 | | 0.00 | -0.32 | | **0.69** | *** | | | | |
| **$TKW_F$** | | | | | | | | | | | |
| wet | 0.14 | | -0.02 | **-0.54** | * | -0.25 | | **-0.62** | ** | 1 | |
| dry | 0.14 | | -0.03 | 0.05 | | -0.37 | | **-0.59** | ** | | |
| **$YLD_F$** | | | | | | | | | | | |
| wet | **-0.72** | *** | -0.08 | **0.69** | *** | -0.13 | | **0.84** | *** | -0.25 | 1 |
| dry | 0.04 | | 0.16 | -0.16 | | **0.45** | * | **0.74** | *** | -0.04 | |

Where: wet: well-watered conditions in environment E5, dry: water-stress conditions in environment E1,Trait: $PLH_F$: plant height measured in field experiments, $PDM_F$: plant dry matter measured in field experiments, $NEM_F$: number of ears per square meter measured in field experiments, $NKE_F$: number of kernels per ear measured in field experiments, $NKM_F$: number of kernels per square meter measured in field experiments, $TKW_F$: thousand kernel weight measured in field experiments, $YLD_F$: grain yield (dt/ha) measured in field experiments.

### 6.3.5 Comparative correlation analysis

In the comparative correlation analysis, the trait association between variables, evaluated in different phenotyping experiments (pot and field experiments), was performed based on mean values of 24 spring barley genotypes. Trait associations were analyzed independently for genotypic means under well-watered and water-stress conditions (Table 6.5 and 6.6).

***Trait association between plant parameters investigated in pot and field experiments under well-watered conditions***

Genetic correlations were studied for grain yield, morphological traits and yield components. Results are presented in Table 6.5. Interestingly, a significant and negative correlation was found between the plant height measured in field experiments and the grain yield examined in pot experiments. The plant dry matter at BBCH 63 determined in field experiments was negatively related to the thousand kernel weight of genotypes grown in pot experiments (-0.44*). However, the number of kernels per ear evaluated in pot and field experiments was positively correlated (0.42*). Under well-watered conditions no other significant correlations were detected.

**Table 6.5** Spearman correlation coefficient ($r_g$) between examined plant parameters of 24 spring barley genotypes grown under well-watered conditions in pot and field experiments (environment E5).

| | **PLH $_P$** | **PDM $_P$** | **NEP$_P$** | **NKE $_P$** | **NKP $_P$** | **TKW $_P$** | **YLD $_P$** |
|---|---|---|---|---|---|---|---|
| **PLH $_F$** | -0.06 | 0.13 | -0.30 | 0.00 | -0.11 | -0.39 | **-0.43** * |
| **PDM $_F$** | 0.22 | 0.16 | 0.05 | -0.04 | 0.03 | **-0.44** * | -0.23 |
| **NEM $_F$** | 0.36 | -0.21 | 0.27 | -0.07 | 0.03 | 0.08 | 0.18 |
| **NKE $_F$** | -0.09 | 0.15 | -0.24 | **0.42** * | 0.11 | 0.09 | 0.05 |
| **NKM $_F$** | 0.30 | -0.33 | 0.23 | 0.21 | 0.12 | 0.19 | 0.35 |
| **TKW $_F$** | -0.36 | 0.00 | -0.02 | -0.27 | -0.17 | -0.10 | -0.20 |
| **YLD $_F$** | -0.15 | 0.03 | 0.25 | -0.17 | 0.03 | 0.01 | 0.11 |

Where: Trait: $PLH_F$: plant height measured in field experiments, $PDM_F$: plant dry matter measured in field experiments, $NEM_F$: number of ears per square meter measured in field experiments, $NKE_F$: number of kernels per ear measured in field experiments, $NKM_F$: number of kernels per square meter measured in field experiments, $TKW_F$: thousand kernel weight measured in field experiments, $YLD_F$: grain yield (dt/ha) measured in field experiments, $PLH_P$: plant height measured in pot experiments, $PDM_P$: plant dry matter measured in pot experiments, $NEP_P$: number of ears per plant measured in pot experiments, $NKE_P$: number of kernels per ear measured in pot experiments, $NKP_P$: number of kernels per plant measured in pot experiments, $TKW_P$: thousand kernel weight measured in pot experiments, $YLD_P$: grain yield per plant (g) measured in pot experiments.

### *Trait association between plant parameters investigated in pot and field experiments under stress conditions*

Genotypic correlations for analyzed plant parameters under water-stress conditions are presented in Table 6.6. Under water-stress, the plant height measured under semi-controlled conditions in poly tunnels was positively associated with the plant height recorded under rainfed conditions in the field (0.56**). In addition, the thousand kernel weight determined in pot experiments revealed a positive correlation with the thousand kernel weight determined in field experiments (0.69***).

**Table 6.6** Spearman correlation coefficient ($r_g$) between examined plant parameters of 24 spring barley genotypes grown under well-stress conditions in pot and field experiments (environment E1).

| | **PLH $_P$** | **PDM $_P$** | **NEP$_P$** | **NKE $_P$** | **NKP $_P$** | **TKW $_P$** | **YLD $_P$** |
|---|---|---|---|---|---|---|---|
| **PLH $_F$** | 0.01 | 0.00 | 0.20 | 0.07 | 0.28 | 0.02 | 0.33 |
| **PDM $_F$** | 0.26 | **0.56** ** | -0.31 | 0.21 | -0.13 | 0.14 | -0.09 |
| **NEM $_F$** | -0.20 | -0.37 | -0.09 | -0.17 | -0.14 | 0.02 | 0.03 |
| **NKE $_F$** | 0.21 | 0.35 | 0.17 | 0.01 | 0.09 | -0.30 | -0.18 |
| **NKM $_F$** | 0.33 | 0.23 | 0.07 | -0.03 | -0.05 | -0.28 | -0.22 |
| **TKW $_F$** | -0.08 | 0.01 | -0.20 | 0.07 | -0.01 | **0.69** *** | 0.29 |
| **YLD $_F$** | 0.26 | 0.16 | 0.02 | -0.11 | -0.07 | 0.10 | -0.09 |

Where: Trait: $PLH_F$: plant height measured in field experiments, $PDM_F$: plant dry matter measured in field experiments, $NEM_F$: number of ears per square meter measured in field experiments, $NKE_F$: number of kernels per ear measured in field experiments, $NKM_F$: number of kernels per square meter measured in field experiments, $TKW_F$: thousand kernel weight measured in field experiments, $YLD_F$: grain yield (dt/ha) measured in field experiments, $PLH_P$: plant height measured in pot experiments, $PDM_P$: plant dry matter measured in pot experiments, $NEP_P$: number of ears per plant measured in pot experiments, $NKE_P$: number of kernels per ear measured in pot experiments, $NKP_P$: number of kernels per plant measured in pot experiments, $TKW_P$: thousand kernel weight measured in pot experiments, $YLD_P$: grain yield per plant (g) measured in pot experiments.

### 6.3.6 Shukla's stability variance for grain yield

The analysis of variance revealed for the grain yield highly significant genotype-by-environment interactions suggesting that genotypic performance was inconsistent across treatments levels and environments. Shukla's stability variance ($\sigma_i^2$) was applied as a useful tool to indentify genotypes with a specific and wide adaptation to contrasting environments. Shukla (1972) defined a genotype as stable if its stability variance is equal to the environmental variance ($\sigma_E^2$) and thus, $\sigma_i^2$ is equal zero. Results of stability analysis, which were carried out for pot and field experiments, are presented in Figure 6.1. Colors for spring barley cultivars were assigned depending of the year of their release. According to Figure 6.1, computed stability variance ($\sigma_i^2$) of 24 spring barley cultivars can be divided into four groups: (I) genotypes with a high yield stability in pot and field experiments, (II) genotypes who have a high yield stability under field conditions, (III) genotypes with a high yield stability under semi-controlled environmental conditions in pot experiments and finally, (IV) genotypes who have a low yield stability in both, pot and field experiments.

Based on $\sigma_i^2$, the most stable cultivars in both, pot and field experiments were 'Primadonna', 'Tatum' and 'Quench'. By contrast, the most unstable varieties for grain yield were 'Morex', 'Wiebke', 'Prestige' and 'SuLilly'. Interestingly, the genotype 'Streif' was characterized as a cultivar with a low stability variance under field conditions. On the other hand, 'Streif' showed a higher yield stability in pot experiments. In summary, Figure 6.1 displays that modern barley cultivars were characterized by a lower stability variance indicating their higher yield stability across contrasting environmental conditions.

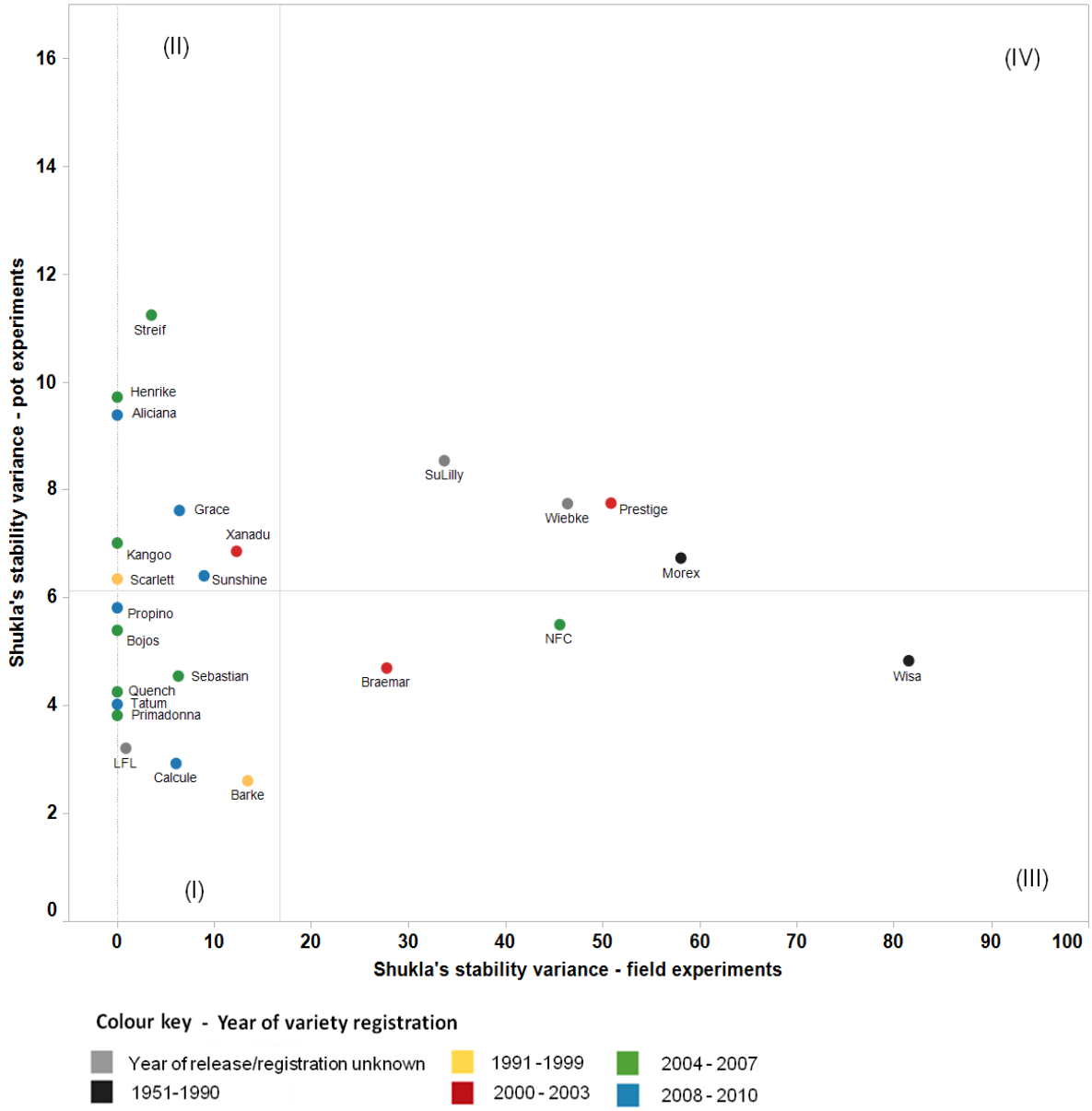


**Fig. 6.1. Scatter plot of Shukla's stability variance ( $\sigma_i^2$ ) for 24 spring barley genotypes grown in pot and field experiments during 2012 and 2013.**

### 6.3.7 Stress tolerance index (STI) of grain yield and membership function value of drought tolerance (MFVD) for 24 spring barley genotypes

The stress tolerance index (STI) for the grain yield and the membership function value of drought tolerance (MFVD) was calculated based on the genotypic means of examined spring barley genotypes. According to STI-values of two morphological (PLH and PDM) and five yield-related parameters (NEP, NKE, NKP, TKW and YLD), MFVD-values were computed. MFVD estimates were classified into five groups, based on the averaged MFVD value (0.40) and their standard deviation (0.07). Genotypes with low MFVD-values were ranged as drought susceptible, while genotypes with higher MFVD-values were classified as drought tolerant. Regarding the stress tolerance indices and estimates of MFVD, Table 6.7 shows that several spring barley genotypes were characterized by a superior drought stress tolerance. In response to different phenotyping environments (pot and field experiments), the spring barley genotypes 'Grace', 'Streif' and 'Henrike' showed a relatively high stress tolerance for the grain yield. In terms of average scores, the STI for the grain yield ranged from 0.96 to 1.76. MFVD-values varied between 0.24 and 0.54. On average, MFVD-values suggest that 'Grace', 'Streif' and 'Henrike' appears to be good performers under varying environmental conditions, especially under drought stress. By contrast, 'Prestige' and 'Sunshine' showed a limited yield potential under both, field and pot experiments. Furthermore, the experiments varied in terms of their stress intensity index (SII). The comparison of the stress intensity for pot and field experiments revealed that the water-stress intensity was slightly higher under natural field conditions (Table 6.7).

To determine the association between stress tolerance indices, correlation analysis between STI of YLD, MFVD and assigned ranks was performed (data not shown). The correlation analysis revealed that examined stress indices were not significantly correlated.

**Table 6.7** Stress tolerance index (STI) for the grain yield and membership function value of drought tolerance (MFVD) for 24 spring barley genotypes used in phenotyping experiments during 2012 and 2013.

| | Grain yield (YLD) in dt/ha - field experiments | | | Grain yield (YLD) in g/plant - pot experiments | | | Mean across trials | | Classification |
|---|---|---|---|---|---|---|---|---|---|
| **Genotype** | **STI** | **MFVD** | **Rank [a]** | **STI** | **MFVD** | **Rank [a]** | **STI** | **MFVD** | |
| Prestige | 1.71 | 0.28 | 21 | 0.47 | 0.20 | 22 | 1.09 | 0.24 | I |
| Sunshine | 2.02 | 0.41 | 13 | 0.45 | 0.19 | 24 | 1.24 | 0.30 | I |
| Quench | 1.84 | 0.39 | 18 | 0.50 | 0.25 | 20 | 1.17 | 0.32 | II |
| LFL24727 | 1.83 | 0.30 | 19 | 0.56 | 0.33 | 15 | 1.19 | 0.32 | II |
| Aliciana | 2.08 | 0.39 | 11 | 0.51 | 0.25 | 17 | 1.30 | 0.32 | II |
| Bojos | 2.21 | 0.43 | 4 | 0.53 | 0.24 | 16 | 1.37 | 0.34 | II |
| Braemar | 1.78 | 0.43 | 20 | 0.57 | 0.29 | 13 | 1.18 | 0.36 | II |
| Sebastian | 2.42 | 0.51 | 2 | 0.51 | 0.22 | 18 | 1.46 | 0.37 | II |
| NFCTipple | 2.11 | 0.39 | 9 | 0.57 | 0.35 | 12 | 1.34 | 0.37 | II |
| Wiebke | 1.49 | 0.35 | 23 | 0.61 | 0.40 | 8 | 1.05 | 0.38 | II |
| Propino | 2.14 | 0.42 | 8 | 0.57 | 0.35 | 10 | 1.36 | 0.38 | II |
| Scarlett | 1.96 | 0.39 | 16 | 0.62 | 0.38 | 6 | 1.29 | 0.38 | II |
| Kangoo | 2.23 | 0.47 | 3 | 0.50 | 0.31 | 21 | 1.37 | 0.39 | III |
| Calcule | 2.01 | 0.46 | 14 | 0.57 | 0.34 | 14 | 1.29 | 0.40 | III |
| Primadonna | 1.67 | 0.40 | 22 | 0.65 | 0.40 | 5 | 1.16 | 0.40 | III |
| Barke | 2.04 | 0.46 | 12 | 0.60 | 0.36 | 9 | 1.32 | 0.41 | III |
| Morex | 1.42 | 0.33 | 24 | 0.50 | 0.52 | 19 | 0.96 | 0.42 | III |
| Wisa | 1.91 | 0.51 | 17 | 0.47 | 0.36 | 23 | 1.19 | 0.43 | III |
| Tatum | 2.10 | 0.43 | 10 | 0.68 | 0.51 | 4 | 1.39 | 0.47 | IV |
| Xanadu | 1.99 | 0.42 | 15 | 0.71 | 0.53 | 2 | 1.35 | 0.47 | IV |
| SuLilly | 2.16 | 0.51 | 7 | 0.57 | 0.45 | 11 | 1.36 | 0.48 | IV |
| Henrike | 2.20 | 0.51 | 5 | 0.61 | 0.47 | 7 | 1.40 | 0.49 | IV |
| Streif | 2.16 | 0.44 | 6 | 0.73 | 0.63 | 1 | 1.45 | 0.53 | V |
| Grace | 2.84 | 0.52 | 1 | 0.69 | 0.56 | 3 | 1.76 | 0.54 | V |
| **YLD wet/ YLD dry** | | 83.5/ 41.6 | | | 5.0/2.9 | | | | |
| **SII** | | 0.50 | | | 0.42 | | | | |

Where STI: stress tolerance index for field and pot experiments, MFVD: membership function value of drought tolerance for field and pot experiments, Classification by standard deviation of MFVD value across trials, a: ranked by STI, SII: stress intensity index = 1- ($YLD_{dry}$/ $YLD_{wet}$) , $YLD_{wet}$: grain yield under well-watered conditions, $YLD_{dry}$: grain yield under drought treatment.



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### 6.3.8 Correlation analysis among stress tolerance indices, MFVD value and investigated traits examined in pot experiments

Based on genotypic means of 24 spring barley genotypes, correlation analysis among stress tolerances indices (STI), membership function value of drought tolerance and grain yields under well-watered and drought conditions was performed. Results are presented in the Appendix. A total of twelve significant correlations were detected. Among all analyzed parameters, significant and positive correlations were observed between $MFVD_P$ and STI of $PLH_P$ (0.47*), $PDM_P$ (0.77***), $NKP_P$ (0.56**) and $YLD_P$ (0.77***). The stress tolerance index for thousand kernel weight ($TKW_P$) was negatively associated with STI of $NEP_P$ (-0.46*) and $NKP_P$ (-0.47*). STI of $TKW_P$ was positive correlated with STI of $NKE_P$ (0.42*). No significant correlations were observed for the grain yield examined in field experiments (E1 and E5) and investigated stress tolerance indices. The grain yield examined under well-watered conditions in pot experiments ($YLD_P$) revealed weak correlations with STI of $PDM_P$ (0.50*) and the grain yield evaluated in E1 (0.41*).

### 6.3.9 Correlation analysis among stress tolerance indices, MFVD value and investigated traits examined in field experiments

Results obtained from correlation analysis for stress tolerance indices and $MFVD_F$ values are illustrated in the Appendix. Overall, a total of 18 significant correlations were found. The highest correlation was observed between STI of $YLD_F$ and $NKM_F$ with r =0.85***, respectively. In contrast to results obtained by correlation analysis for pot experiments, membership function value of drought tolerance showed fewer significant associations with STI of $PDM_F$ (0.47*) and $YLD_F$ (0.74***). However, significant correlations between $YLD_F$ in environment E1 (YLD-E1) and six parameters, including STI of $PLH_F$ (-0.58**), $NEM_F$ (0.66***), $NKM_F$ (0.78***), $YLD_F$ (0.84***), $MFVD_F$ (0.58**) and YLD-WW (0.41*), were found. Highly to moderate significant correlations were detected between the grain yield in environment E5 and STI of $NKM_F$ (0.56**), $YLD_F$ (0.77***) and $MFVD_F$ (0.75***).

## 6.5 Discussion

The comparison of results obtained from different drought stress experiments has been scarcely examined or even published. To understand the mechanism underlying drought stress tolerance, the study aimed to compare the genotypic performance of 24 spring barley cultivars for their drought stress tolerance in pot and field experiments. The remaining discussion part of the paper is structured as follows: (1) investigation of the crop performance and grain yield of spring barley grown in pot and field experiments, (2) elucidation of investigated traits as selection criteria in pot and field experiments, (4) analyzing correlations between examined traits and stress indices, and finally (5) discussion of the possibilities and limitations of different experimental approaches to explore drought stress tolerance.

### 6.5.1 Crop performance and grain yield of spring barley grown in pot and field experiments

In both phenotyping experiments, water stress caused significant reductions in plant height, plant dry matter, grain yield and yield components indicating that water shortage during the vegetation period had a negative impact on the biomass accumulation and crop productivity. Gathered data seem to be consistent with other studies which observed a decline in plant height, 1000 kernel weight, number of kernels per ear and grain yield under drought conditions (Rizza et al. 2004; Elhani et al. 2007; Bayoumi et al. 2008; Chen et al. 2012). Bayoumi et al. (2008) assumed that decreases in plant height under water stress were attributable to reductions in the relative turgidity and dehydration of the protoplasm, which is again associated with reductions in cell expansions and cell divisions. Comparing the mean values of evaluated parameters, it is striking that means for YLD were lower under less favourable climatic conditions. The decline in grain yield was attributable to reductions in numbers of ears and kernels per plant or rather kernels per square meter. Similar results were reported by Samarah (2005) who studied in greenhouse experiments the effect of drought on grain growth and grain yield of barley. Samarah (2005) reported that drought stress during the grain filling significantly decreased the grain yield by reducing the number of tillers, fertile ears and grains. In accordance with the present results, previous research of Schittenhelm et al. (2014) have shown that drought stress reduced the grain yield of barley by 62%. Among phenotyping experiments, the plant dry matter, number of kernels per unit area and grain yield displayed the highest sensitivity to water shortage. Compared to pot experiments, decreases of

plant height, plant dry matter and number of ears were two to four times higher under natural, rainfed conditions in the field. A possible explanation might be that crops were exposed to a wide range of additional environmental factors (e.g. heat and/or irradiance) which strongly influenced the manifestation of traits (Mittler 2006). Previously presented results in Chapter 5 suggest that the decreasing soil water availability in the stress environment E1 triggered a negative impact on the crop development and yield performance of spring barley genotypes. Contrary to field experiments, screening genotypes for drought stress tolerance under semi-controlled conditions in poly tunnels made it possible to control the duration and intensity of drought treatments during the vegetation period. Overall, findings of this research indicate that drought is a climatological event, which increase the complexity of environmental variations (Roy et al. 2011; White and Andrade-Sanchez 2012).

### 6.5.2 Trait association among plant parameters investigated in pot and field experiments

Improving cereals yields under various environmental conditions is a major challenge for the plant breeding research in the 21$^{st}$ century. The investigation of relationships among secondary traits defining the grain yield is essential to understand the plant responses to drought. The aim of this study was to analyse the association of recorded plant parameters (1) within each phenotyping experiment and (2) between phenotyping experiments. According to this, the subsequent discussion is initially focused on relevant findings for correlation analysis within each phenotyping experiment. Afterwards, the relationship between phenotyping experiments will be elucidated.

#### *The relationship among plant parameters evaluated in pot experiments*

Correlation analysis among examined traits under two contrasting water regimes (well-watered and water-stress conditions) revealed significant associations between the grain yield and grain yield components. However, the amount and strength of significant correlations depended on the water regime and the phenotyping environment.

Under well-watered conditions, the grain yield correlated with the number of ears and kernels per plant. The correlation analysis indicates that genotypes with the tendency to produce a higher number of ears tend to have a low grain number per inflorescence and, on the other hand, a higher grain number per plant. According to these findings, it is may be

worthwhile to enhance the number of ears per plant, when selecting for increased yields under well-watered and water-stress conditions. Under well-stress conditions, the plant height was positively correlated with the plant dry matter. It therefore seems possible that the stem elongation significantly contributes to the biomass accumulation under water deficit conditions. Under well-watered conditions other factors/traits rather than the plant height (e.g. leaf dry matter, leaf area) may have a greater influence on the biomass accumulation.

***The relationship among plant parameters evaluated in field experiments***

Under rainfed well-watered conditions in the field, the plant height was negatively related to the number of ears and kernels per square meter (NEM and NKM), and the grain yield. The results suggest that genotypes with a reduced height have a superior resistance to lodging and a higher yield potential (Ginkel et al. 1998). Richards (1992) confirmed that high grain yields in spring wheat were achieved by lines with a plant height varying between 70 and 100 cm. Furthermore, studies of Richards (1992) demonstrated that the kernel number was more sensitive to variations in plant height than the kernel weight. Since the plant height has a high heritability under various moisture regimes and contributes to high biomasses, the plant height is recommended as a vital selection criterion for drought stress tolerance (Sellammal et al. 2014).

Under well-watered conditions in the field, grain yield was significantly correlated with yield-related traits, including NEM and NKM. Present findings accords with previous studies of Cossani et al. (2009) who showed that grain yield was mainly determined by grain number per unit area. Fussell et al. (1991) reported that drought tolerance was found to be mainly expressed in traits relating to the ability to maintain grain numbers under stress.

In case of water-stress, the amount of significant correlations declined. The higher number of significant correlations, under well-watered conditions in environment E5, leads to the assumption that the spring genotypes were able to express their full range of phenotypic capacity under optimum growing conditions (Ober et al. 2005). Ober et al. (2005) argued in their study that “drought conditions decrease the level of phenotypic variation for certain traits, masking genetic sources of variation”. To improve the genotypic performance of cereals under water deficit conditions, it is may by worthwhile to select high yielding genotypes with specific stress-adaptive traits in non-stress environments, which are characterized by a maximized genetic variation and low genotype-by-environment

interactions (Richards 1996; Ginkel et al. 1998; Ceccarelli et al. 1998; Sio-Se Mardeh et al. 2006).

Taken together, the lack of significant correlations between plant dry matters in pot and field experiments was might be attributable to the measurement technique which was either too imprecise or too insensitive to detect differences in biomass accumulation between genotypes. In addition, the limited number of genotypes tested in this study may also contribute to non-significant associations of plant dry matter at anthesis and grain yield components. These findings are in contrast to studies of Villegas et al. (2001) who found a positive and significant relationship between biomass at anthesis and grain yield. Nevertheless, results of current study showed significant correlations between yield components and grain yield. Hence, grain yield improvements in spring barley varieties can be achieved by selection of genotypes with a higher number of ears.

***Correlation analysis among different phenotyping experiments***

In order to choose an appropriative selection strategy for drought stress tolerance, the phenotyping environment has to be carefully considered (Ginkel et al. 1998; Lakew et al. 2011). Results of correlation analysis between phenotyping environments showed an interesting aspect of the relationship among phenotyping experiments in pot and field experiments (Table 6.5 and 6.6).

Under water-stress conditions, the plant dry matter and thousand kernel weight investigated in pot experiments was significantly related to the dry matter and thousand kernel weight examined under rainfed conditions in the field. Under well-watered conditions, significant correlations between pot and field experiments were found for the number of kernels per ear.

The results of the present study points out the possibility of identifying genotypes producing high plant dry matters, kernel weights and number of kernels per ear in both phenotyping environments. Nevertheless, results presented in Table 6.5 and 6.5 revealed additionally the absence of significant relationships between grain yields. A possible explanation for the lack of adequate correlations might be high heterogeneity of different phenotyping environments and the limited number of environments-year combinations. Poor correlations between different phenotyping environments may also indicate the presence of different adaptive processes and responses to stress which occurs under artificial and natural conditions (Sio-Se Mardeh et al. 2006; Lakew et al. 2011). In this context, Lakew et al.

(2011) emphasized that the evaluation of drought stress tolerance should be realized in a sufficient large sample of real environments, which represent a relevant environmental variability. Lakew et al. (2011) concluded that simulated drought conditions are not suitable to substitute real drought conditions and should be therefore only used as an addition to real drought conditions. In summary, results of correlation analysis confirm for the plant dry matter, kernel weight and number of kernels per ear a positive response to selection in different phenotyping environments. Across varying phenotyping environments, the evaluation of breeding materials should focus on grain yield components, especially on the number of ears.

### 6.5.3 Drought stress tolerance and yield stability of spring barley in response to different phenotyping experiments

Yield stability and stress tolerance indices are crucial parameters which have to be considered when judging different phenotyping experiments for drought stress tolerance (Stelluti et al. 2011). In this study phenotyping experiments were conducted in pot and field trials. Water shortage significantly reduced the grain yield and evaluated spring barley genotypes were characterized by diverse yield stability. Findings of the present study indicate that a reasonable drought tolerance exist in the used spring barley set. According to stress tolerance indices for grain yield (Table 6.7), the genotypes 'Grace', 'Streif' and 'Henrike' were ranged as tolerant cultivars across various phenotyping experiments. In contrast, stability variances, which are depicted for the 24 spring barley genotypes in Figure 6.1, showed that 'Grace', 'Streif' and 'Henrike' were characterized by a stable yield performance under rainfed conditions in the field. However, under semi-controlled conditions in poly tunnels their stability variance increased suggesting that the assessment of yield stability depend on prevailing environmental conditions and the experimental setup. The same assumption was drawn by Szira et al. (2008) who indicate that the observed tolerance in drought related studies is specific to a given environment. Sio-Se Mardeh et al. (2006) reported that the effectiveness of selection indices in wheat cultivars varied with stress severity. Nevertheless, findings of this study suggest that STI, MFVD and $\sigma_i^2$ were suitable to identify genotypes with a specific adaptation or respectively wide adaptation to varying environmental conditions in different phenotyping experiments. In pot and field experiments, the spring barley genotype 'Grace' exhibited high grain yields and a high yield stability.

Thus, ‘Grace’ is recommended as useful model crop for further breeding programs on drought tolerance.

### 6.5.4 Relationship between stress tolerance indices and grain yield across different phenotyping environments

Crop grain yields are the result of crop development, differentiation processes and the growth of yield components during the crop life circle (Sadras and Slafer 2012). Therefore, scientist seeks to investigate the relationship between grain yield and growth parameters as well as yield components.

Within pot experiments, spring barley yields evaluated under well-watered and water-stress conditions were not or rather very low associated with stress tolerance indices. Contrary, correlation analysis for traits examined under rainfed conditions in the field revealed that the grain yield in environment E1 was moderate to highly significant correlated with the membership function of drought tolerance and stress tolerance indices. In both phenotyping experiments, the STI of the grain yield was highly significant associated with membership function of drought tolerance (MFVD), with coefficients of correlation ranging from 0.77 for pot experiments to 0.74 for field experiments.

Since the membership function of drought tolerance was previously defined in Chapter 4, present findings suggest that several mechanism operate in high yielding genotypes, which allow them to perform well under water deficit (Rizza et al. 2004; Chen et al. 2012). Rizza et al. (2004) claimed that the challenge in finding genotypes with a high yield potential and a low sensitivity to drought is might be attributable to the existence of distinct mechanism responsible for these traits. In general, the number of kernels per plant unit area (per plant or square meter) is based on two factors, (1) the number of ears and (2) the number of kernels per ears. A decline in number of kernels per unit area always reflects the effect of the combined reductions in number of ears per plant and number of kernels per ear. In both phenotyping experiments, STI of NKP and NKM were strongly associated with STI of grain YLD. Here, the correlation coefficient increased from pot to field experiments. These findings are consistent with those of Sadras and Slafer (2012) who reported that grain yield is more closely related to grain number than to grain size. Hence, it is possible to emphasize that an overall hierarchy between yield components exist. However, Blum (1998) argued that high correlations between grain yield and yield components are due to the lack of independency between them.

In this study the majority of significant correlations were determined within field experiments, especially in environment E1, the field site which was previously defined as a stress environment. Contrary, nearly none of the stress indices calculated within pot experiments were related with crop yields evaluated under semi-controlled conditions in poly tunnels. The lack of significant relationships between stress tolerance indices and crop yields indicate that drought stress tolerance varies depending on the experimental site. Hence, a low stress tolerance for traits like plant height and plant dry matter under semi-controlled conditions in pot experiments does not necessarily indicate a low stress tolerance under natural field conditions. However, gathered data must be interpreted carefully due to the fact that the beginning and duration of water stress under rainfed conditions is almost impossible to control. At the same time the co-occurrence of additional biotic and abiotic stress factors, which contribute to the overall plant performance, may lead to an over- or rather underestimation of genotypic performance in field experiments. Based on genotypic correlation analysis among varying stress trials with maize, Weber et al. (2012) suggest that results for managed drought stress screenings should be weighed in somewhat less heavily than results from other test environments.

### 6.5.5 Possibilities and limitations of different experimental approaches to explore drought stress tolerance

Phenotyping plants under semi-controlled conditions in pot experiments are an efficient way for selecting superior genotypes from large numbers of breeding materials. Furthermore, studies on drought stress tolerance in controlled environments have the advantage of defining the beginning, duration and intensity of water stress. In addition, the complexity of interactions between genetic and environmental effects and thus the variability in experimentation is reduced (Roy et al. 2011). Nonetheless, lack of significant relationships between genotypic means of plant height, plant dry matter and yield components, evaluated in pot and field experiments, suggest that selection decisions should be realized in a sufficient number of locations and years. The study indicates that the identification of genotypes which produce high biomasses, growth rates and yields in different screening environments is complicated by the heterogeneity of phenotyping environments (Lakew et al. 2011). Roy et al. (2011) pointed out that phenotyping experiments in controlled environments tend to underestimate the plasticity in plant response to abiotic stress in field conditions and will further fail to account for interactions with other environmental factors. Besides the above

presented findings, prior discussed results of phenotyping experiments in Chapter 3 demonstrated that number of tillers per plant, leaf area, leaf dry matter and plant potassium content were positively related to grain yields. In this context, the lack of significant interactions between secondary traits, evaluated in poly tunnels and field experiments, suggest that the lower soil volume in pots has a considerable effect on the amount of water and nutrients which is available to plants. The declining soil moisture content, which is associated with an increased mechanical impedance, is difficult to simulate in under controlled conditions within pots (Whitmore and Whalley 2009; Roy et al. 2011; Poorter et al. 2012). Field phenotyping experiments enabled plants to display their full yield potential. However, the wide range of environmental factors which are usually superimposed onto drought stress and the heterogeneity of field conditions make the interpretation of results related to drought fairly difficult (Roy et al. 2011; Araus and Cairns 2014).

## 6.5 Conclusion

This study set out to compare the genotypic performance of 24 spring barley cultivars for their drought stress tolerance in pot and field experiments. In addition the study discussed the possibilities and limitations of different screening methods. Despite the phenotyping environment (pot and field experiments), water stress caused significant reductions in plant dry matter around anthesis, number of kernels per unit area (per plant or per square meter) and grain yield, with decreases over 30%. In field experiments, declines in plant height ($PLH_F$), plant dry matter ($PDM_F$) and number of ears ($NEM_F$) were 3.7, 2.0 and 2.4 times than those in pot experiments. From correlation analysis the following conclusions can be drawn:

(1) In both phenotyping experiments (pot and field experiments) grain yield components were positive and significant correlated to the grain yield. Although time-consuming, yield components are relatively easy to measure and their interpretation enhanced our knowledge about the yield determination.

(2) Under rainfed conditions with naturally occurring droughts (Table 6.4), correlations between morphological traits, grain yield and yield components were non-significant. Findings of this study agree with the breeding philosophy which favours a selection of genotypes in non-stress environments (Richards 1996). Non-stress environments are characterized by higher genetic variances and heritabilities for traits which will serve as selection criteria (Edmeades and Bolaños 1996; Lafitte et al. 2003; Betrán et al. 2003).

(3) Under water-stress conditions, the plant dry matter and thousand kernel weight investigated in pot experiments was significantly related to the dry matter and thousand kernel weight examined under rainfed conditions in the field.

(4) The lack of significant correlations among phenotyping environments was might be attributable to the limited number of genotypes in this study. In further phenotyping experiments the linkage between poly tunnel and field experiments should be examined.

The current report provides comparative results for phenotyping drought stress tolerance in spring barley. Findings of this study suggest that STI, MFVD and $\sigma_i^2$ were suitable to identify genotypes with a specific adaptation or respectively wide adaptation to varying environmental conditions. In pot and field experiments, the spring barley cultivar ‘Grace’ was characterized by high grain yields and a high yield stability. Thus, ‘Grace’ is recommended as useful model crop for further breeding programs on drought tolerance.

# Chapter 7: General discussion

Along with the declining availability of crop-growing areas and the increasing demand of crops for biofuels and food production, plant adaptations to abiotic stresses such as drought are one of the most challenging tasks for plant breeding research in the 21st century (Roy et al. 2011; Tomlinson 2011; Tardieu 2012). Due to the dynamic and complex nature of drought, yield maintenance under water deficit is still poorly understood (Passioura 2006; Berger et al. 2010). Returning to the objectives posed at the beginning of this thesis, the study aimed (1) to examine the effect of drought stress imposed at different growth stages on crop development and grain yield of spring barley and (2) to identify major traits which are useful as effective selection criteria for phenotyping *Hordeum vulgare* ssp. v*ulgare* under drought conditions. Additionally, in the present study attempt was made to explore the drought stress tolerance and yield stability of twenty-four spring barley cultivars in response different phenotyping environments.

## 7.1 Effect of water shortage on crop development and grain yield across different phenotyping environments

Knowledge of environmental and plant specific factors affecting plant development and productivity is essential to understand the yield formation in cereals (Ugarte et al. 2007). Between 2011 and 2013 pot experiments were carried out in polyethylene-covered plastic tunnels at the experimental research station of the Institute of Crop Science and Resource Conservation (INRES) at the Chair of Plant Breeding, University of Bonn, Germany. Here, present study showed that water stress imposed at different growth stages limited the photosynthesis, leaf expansion, biomass accumulation and thus potential for assimilation through various ways. Most importantly, findings of pot experiments demonstrate that the yield performance under drought conditions relies on the growth stage in which water stress occurs (Christen et al. 1995; Çakir 2004). Drought sensitivity increased during the crop life circle, while the highest yield losses were recorded when drought occurred between anthesis and heading. In contrast, phenotyping for drought stress tolerance under natural field conditions is complicated by the heterogeneity of field conditions and the inability to control environmental factors (Roy et al. 2011; Araus and Cairns 2014). Genotypic improvements on drought stress tolerance are based on multi-location phenotyping experiments over several years in order to identify components of abiotic stress tolerance which may have been

overlooked or rather ranged as unimportant in greenhouse experiments (Roy et al. 2011; Araus and Cairns 2014). In this research, twenty-four spring barley cultivars were evaluated, between 2012 and 2013, in eight contrasting environments in Germany. Here, spring barley varieties showed a grain yield range of 42 to 83 dt $ha^{-1}$ (Chapter 5, Fig. 5.5).

A comparison of the soil moisture conditions in pot and field experiments (Fig. 3.3 in Chapter 3 and Fig. 5.2 and 5.3 in Chapter 5) reveals that the experimental setup and thus examined drought stress scenarios in pot experiments can be considered as representative for natural occurring drought stress events in rainfed environments. According to the climatic conditions in 2012, the field site Bavaria-Herzogenaurach (E1) was characterized as a site with drought periods between stem elongation and ripening stage (Fig. 5.2, Chapter 5). Due to the balanced soil water availability throughout the whole vegetation period, the field site in NRW in 2013 (E5) was characterized as a non-stress environment. Less favourable growing conditions in environment E1 decreased the number of ears per square meter by 39% and the number of kernels per square meter by 50% which finally resulted in grain yield losses of 50%, compared to the non-stress environment E5 (Table 5.3 in Chapter 5). These findings agree with gathered results of the 2$^{nd}$ drought stress scenario (Table 4.3 in Chapter 4). Here, the 21-day lasting decrease of the water supply reduced the soil moisture content from field capacity to permanent wilting point. Thus, water deficit between tillering stage and anthesis decreased the grain yield by 43%. In fact, the wide natural climate variability leading to changing precipitation patterns and changes in weather events are difficult to simulate under semi-controlled conditions in poly-tunnel experiments. However, the simulated soil drying and rewetting scenarios examined in pot experiments represents common natural occurring periods of water shortage in Germany. Soil moisture sensors used for field experiments in Saxony-Anhalt and Lower Saxony in 2012 represented such soil drying conditions during the vegetation period with relatively fast rewetting events (Fig. 5.2 in Chapter 5). In the recent years, numerous of studies have focused on the identification of single traits or genes that confer with drought stress tolerance (Tardieu 2012). But the majority of these studies were examined under controlled conditions. Only few studies have directly investigated and compared water shortage in contrasting phenotyping environments. Hence, this research makes several noteworthy contributions to explore drought tolerance in specific scenarios and field experiments.

## 7.2 Identification of useful traits as selection criteria for phenotyping spring barley under drought conditions

In recent studies several traits have been identified to be associated with plant productivity under dry conditions, including early maturity, plant size, leaf area, plant height, stomatal closure and transpiration rate (Lafitte et al. 2003; Ober et al. 2005; Cattivelli et al. 2008; Chen et al. 2012). In pot experiments, decreasing water availability of from tillering to anthesis (2$^{nd}$ drought stress scenario) had a considerable effect on plant growth, differentiation processes, photosynthesis and transpiration rate which arise by reductions of the plant height (PLH), functional green leaf area (GLA), tiller number (TNP), leaf number (LNP), plant dry matter (PDM), chlorophyll content (SPAD) and absolute plant water content (PWC) (Bednarz et al. 1998; Borrell et al. 2000; Chaves et al. 2003; Farooq et al. 2009; Araus et al. 2012). Under rainfed conditions in the field, the following traits displayed a maximum sensitivity to water shortage, with decreases over 35%: plant dry matter at stem elongation and anthesis, crop growth rate between stem elongation and anthesis as well as crop growth rate between anthesis and ripening stage, number of ears per square meter, number of kernels per square meter and grain yield. However, decreases of plant dry matter and yield components were two to four times higher under natural, rainfed conditions in the field. These findings suggests that additional environmental influences such as soil density, a declining soil moisture content which is again associated with an increased mechanical impedance in the field, water input, air temperatures and solar radiation can have a greater influence on crop development and grain yield formation than the effect of water stress evaluated under controlled conditions in poly tunnels (Cattivelli et al. 2008; Roy et al. 2011; Francia et al. 2013; Araus and Cairns 2014).

Based on genotypic means, Spearman's rank correlation analysis was performed to assess the relationship between investigated plant parameters, grain yield and yield components. Correlation analysis across examined drought stress scenarios (see Chapter 3) showed the positive relationship between the grain yield and leaf area, green leaf area, plant dry matter and plant potassium content. If drought occurred at early stages of plant development (1$^{st}$ drought stress scenario), morphological parameters such as leaf and plant dry matter were primary related to the grain yield and yield components. With maturation of plants significant associations for grain yield with physiological parameters were observed (2$^{nd}$ and 3$^{rd}$ drought stress scenario). Present results accords with observed genetic correlations under natural, rainfed conditions. Across eight field sites, current study confirmed

the presence of significant positive correlations between the grain yield and morphological and yield-related traits. Regardless of the phenotyping environment, our data suggest that factors contributing to the production and accumulation of biomass had a significant influence on yield formation. Villegas et al. (2001) who investigated the genetic variation in biomass accumulation of durum wheat under Mediterranean conditions found positive and strongly significant correlations between biomass at anthesis and grain yield. According to them, transient photosynthesis and translocation of reserves accumulated pre-anthesis have a favourable effect on biomass accumulation and grain filling. In pot and field experiments, ear and kernel number per unit area (plant or rather square meter) was significant related to the grain yield. Hence, the kernel number per unit area might be a good indicator for soil water conditions and drought. Bearing in mind that the seed number is a major yield component which is mainly determined at flowering time, results of this thesis suggest that a reduction in kernel abortion rate will have a positive effect on grain yield (Tardieu 2012). Fussell et al. (1991) reported that drought tolerance of cereals was mainly expressed in traits relating to the ability to maintain grain numbers under water deficit (e.g. grain number per panicle and per unit area, and grain yield per panicle). In addition, Cossani et al. (2009) observed that the grain yield was primary determined by grain number per unit area.

Contrary to pot experiments, the plant potassium content was not significantly correlated with examined plant parameters under rainfed conditions. These findings indicate that physiological factors affect the yield formation of spring barley to a different degree. According to Araus and Cairns (2014), the phenotyping environment plays a crucial role in the quality of data. Results of this study suggest that variability of weather parameters and field variations might increased error variances and masked the genetic variation for physiological traits (Araus and Cairns 2014).

Despite correlation analysis among examined plant parameters, it is noteworthy that none of the investigated morphological and physiological traits were correlated with the grain yield under water deficit conditions. The lack of association has been observed in both, pot and field experiments. Present findings seems to be consistent with research of Kumar et al. (2008) who emphasized that a direct selection of genotypes under water stress is more effective than a selection through secondary traits.

The genotypic yield performance of the 24 spring barley genotypes varied depending on the phenotyping experiment and drought treatment (Fig. 4.2 in Chapter 4 and Fig. 5.5 in Chapter 5). If drought occurred under natural rainfed conditions, the spring barley cultivar ‘Wiebke’ displayed a poor yield performance, whereas ‘Wiebke’ was able to achieve a

relatively high grain yield under semi-controlled conditions in poly tunnels. In general, findings of the present research show the difficulty to compare the crop performance of genotypes which were grown under contrasting environmental conditions.

Taken together, results of this thesis demonstrate: (1) that plant sensitivity to drought stress conditions varied depending on the duration and intensity of water deficiency and the crop developmental stage in which drought stress occurs (Cattivelli et al. 2008; Szira et al. 2008), (2) that higher levels of plant biomass (PDM) and dry matter accumulation between stem elongation and anthesis (CGR2) as well as dry matter accumulation between anthesis and ripening (CGR3) are of particular importance for the grain yield formation (Villegas et al., 2001), (3) that the number ears kernels per unit area is a vital parameters which is related to yield performance and thus drought tolerance under water deficit (Fussell et al. 1991).

## 7.3 Possibilities and limitations of different phenotyping environments

Gloablly, the development of improved varieties which are able to cope with water stress relies on phenotyping methods which are repeatable, applicable at a reasonable cost to large breeding populations, and predictive of grain yield under stress (Atlin and Lafitte 2004). Current study revealed that the phenotyping environment plays a crucial role regarding the gathered phenotypic data (Araus and Cairns 2014). Overall, the phenotyping experiments showed that a reasonable drought tolerance examined in pot/poly tunnel experiments does not indicate a higher drought stress tolerance under natural field conditions. Phenotyping for drought tolerance in pot experiments is a useful technique to screen a large number of genotypes for their response to drought and to control the timing, duration and intensity of water shortage. Contrary, such controlled environments tend to underestimate the plasticity in plant response to abiotic stress (Roy et al. 2011). Nonetheless, phenotyping experiments in the field are often frequently laborious and destructive due to removal of plant biomass during the vegetation period. In addition, field experiments are characterized by the complexity of environmental factors which additionally affect plants and might mask important genetic variations for secondary traits (Roy et al. 2011; Araus and Cairns 2014). Thus, successful phenotyping experiments refer to: (1) a precise recording of weather conditions, (2) knowledge about the field variability, (3) thoughtful experimental designs, which allow a better control of within-replicate variability and (4) precise phenotyping protocols and sets of methodologies to measure plant growth (Furbank and Tester 2011; Tuberosa 2012). Overall, the study concludes that the choose of an appropriative phenotyping environments depends on

the target traits and plant mechanism which should be analyzed (Tardieu 2012). Sensible biochemical and physiological processes that affect crop productivity should be studied in controlled greenhouse or growth chamber experiments. Contrary, a direct selection for grain yield should be realized under natural conditions in diverse environments. Indices such as stress tolerance index, membership function of drought stress tolerance and Shukla's stability variance allowed us to identify genotypes, such as 'Grace' and 'Streif', which showed a reliable yield performance under diverse phenotyping environments.

## Chapter 8: Summary and overall conclusions

The current study clearly demonstrates that plant productivity and yield formation of spring barley is strongly affected by temporary water stress during the vegetation period. Phenotyping experiments under semi-controlled conditions in poly tunnels showed that the sensitivity of spring barley to water shortage varied depending on the developmental stage in which drought occurred. Water stress started at the end of the leaf developmental stage (1$^{st}$ drought stress scenario) caused serve reductions in leaf number, green leaf area, leaf dry matter, chlorophyll content (SPAD value) and plant nutrient concentrations (PNC, PPhC and PKC) that finally resulted in yield losses of 39%. In contrast, water deficit at tillering and anthesis (2$^{nd}$ and 3$^{rd}$ drought stress scenario) decreased primarily the tiller number, plant dry matter and plant water status (PWC, LWC, SWC). Drought treatments of the 2$^{nd}$ and 3$^{rd}$ drought stress scenario reduced the grain yield o spring barley by more than 40%. If drought occurred at early stages of plant development (1$^{st}$ drought stress scenario), the grain yield was positively correlated with morphological parameters. On the other hand, a close linear relationship between physiological and yield-related parameters has been observed under water shortage between tillering and ripening stage (2$^{nd}$ and 3$^{rd}$ drought stress scenario).

Experiments which were described and discussed in Chapter 4 revealed a none-significant relationship between droughted and well-watered grain yields. Thus, an indirect selection for grain yield under well-watered conditions cannot be recommended (Kumar et al. 2008). The study has also shown that the genetic variability and heritabilities for plant height, tiller number, leaf number, leaf senescence and number or ears increased under water shortage. None of the analyzed morphological and physiological traits were correlated with the grain yield. However, stress tolerance indices of 19 traits indicate that the grain yield, leaf area, plant dry matter, stem water content, leaf number, leaf water content, chlorophyll content (SPAD value) and leaf senescence appear to be useful traits for further studies on drought stress tolerance and the complex interactions between plant growth and yield formation. Number of ears and kernels per plant were significantly related to the grain yield under well-watered and drought conditions. Hence, these parameters are associated with drought tolerance.

In the field, timing and severity of water stress are highly variable. At the same time drought periods are often associated with high temperatures and high light stresses (Chaves 2002). Field experiments conducted over two consecutive years at five field sites in Germany

revealed that the field site Bavaria-Herzogenaurach (E1) was characterized as a site with drought conditions during stem elongation and ripening stage. The field site in NRW 2013 was ranged as non-stress environment. In general, our results showed that a balanced precipitation in June and July accompanied by lower air and soil temperatures were favourable for the dry matter accumulation and grain yield formation. Correlation analysis between investigated traits and grain yield demonstrate that PDM-BBCH 81, CGR2 and CGR3 stage are valuable selection criteria for selecting genotypes across contrasting environments. Correlations between physiological traits and grain yield components were weak suggesting that physiological traits are easily influenced by varying environmental conditions. Likewise results obtained by correlation analysis in Chapter 4, the relationship between the grain yield and examined morphological and physiological traits was none-significant under water deficit conditions in environment E1. Lack of significant correlations between different phenotyping environments (comparison of poly tunnel and field experiments in Chapter 6) suggest that a reasonable drought tolerance for spring barley genotypes examined in pot/poly tunnel experiments does not indicate a higher genotypic drought stress tolerance under natural field conditions. However, computed stress tolerance indices (STI) and membership function of drought tolerance indicate a variability of drought tolerance within examined spring barley genotypes. The spring barley cultivars ‘Grace’ and ‘Streif’ were classified as drought tolerant, whereas ‘Prestige’ and ‘Sunshine’ performed poorly under water deficit conditions.

In summary, the present study provides comparative results for phenotyping drought stress tolerance in spring barley. The research has shown that parameters which are related to biomass accumulation and plant water status can affect plant growth, development and finally grain yield. Stress tolerance indices (STI) and membership function of drought tolerance (MFVD) are recommended for identifying drought tolerant genotypes. To our knowledge this study provides new information about phenotyping drought stress tolerance in a wide range of phenotyping environments and during different developmental stages. The usage of automatic weather stations, which continuously record meteorological data, facilitate the analysis of environmental effects on crop growth and yield formation across diverse environments.

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# Appendix

## Appendix A

**KRISTALON® 18-18-18 (N-$P_2O_5$-$K_2O$-Extra micro)**

**Primary macronutrients**

- Nitrogen (N) **18%**
  - 9.8% N as nitrate nitrogen
  - 8.2% N as ammonia nitrogen
- Phosphor (P) **18%**
  - phosphorous pentoxide ($P_2O_5$)
- Potassium (K) **18%**
  - water soluble potassium oxide ($K_2O$)

**Micronutrients / trace minerals**

- Boron (B) **0.05%**
- Copper (Cu) as chelate of EDTA **0.02%**
- Iron (Fe) as chelate of EDTA **0.14%**
- Manganese (Mn) as chelate of EDTA **0.08%**
- Molybdenum (Mo) **0.008%**
- Zinc (Zn) as chelate of EDTA **0.05%**

**EDTA** - Ethylenediaminetetraacetic acid

Supplied by
**YARA GmbH & Co.KG**
Hanninghof 35
D-48249 Dülmen, Gemany

## Appendix Chapter 2

**Table 8** Watering time in minutes for pot experiments conducted under semi-controlled conditions in poly-ethylene covered tunnels at the experimental research station in Bonn, Germany in 2011.

| **Date** | **Control** | | | **DT-BBCH 24** | | | **DT-BBCH 24** | | |
|---|---|---|---|---|---|---|---|---|---|
| | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm |
| 23.03.11 - 31.03.11 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 01.04.11- 10.04.11 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 |
| 11.04.11 - 13.04.11 | 5 | 4 | 5 | 5 | 4 | 5 | 4 | 4 | 4 |
| 14.04.11 - 17.04.11 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 |
| 18.04.11 - 19.04.11 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 3 |
| 20.04.11 | 4 | 4 | 4 | 2 | 2 | 2 | 3 | 4 | 4 |
| 21.04.11 - 25.04.11 | 4 | 4 | 5 | 2 | 2 | 2 | 3 | 4 | 4 |
| 26.04.11 - 27.04.11 | 4 | 5 | 5 | 3 | 3 | 3 | 3 | 4 | 5 |
| 28.04.11 | 4 | 5 | 4 | 2 | 3 | 2 | 3 | 4 | 4 |
| 29.04.11 - 30.04.11 | 4 | 5 | 4 | 2 | 2 | 2 | 3 | 4 | 4 |
| 01.05.11 | 4 | 5 | 4 | 2 | 1 | 1 | 3 | 4 | 4 |
| 02.05.11 | 4 | 5 | 4 | 0 | 0 | 0 | 3 | 4 | 4 |
| 03.05.11 | 4 | 5 | 5 | 0 | 0 | 0 | 4 | 4 | 4 |
| 04.05.11 | 4 | 5 | 5 | 1 | 0 | 1 | 4 | 4 | 4 |
| 05.05.11 | 4 | 5 | 5 | 1 | 1 | 1 | 4 | 4 | 4 |
| 06.05.11 | 5 | 5 | 5 | 1 | 2 | 2 | 5 | 5 | 4 |
| 07.05.11 | 5 | 5 | 6 | 2 | 2 | 3 | 5 | 5 | 5 |
| 08.05.11 | 5 | 5 | 6 | 2 | 3 | 3 | 5 | 5 | 5 |
| 09.05.11 - 10.05.11 | 5 | 5 | 6 | 2 | 2 | 2 | 5 | 5 | 5 |
| 11.05.11 | 5 | 5 | 6 | 2 | 1 | 2 | 5 | 5 | 5 |
| 12.05.11 | 5 | 5 | 6 | 11 | 10 | 11 | 2 | 2 | 2 |
| 13.05.11 - 15.05.11 | 5 | 5 | 6 | 8 | 7 | 8 | 2 | 2 | 1 |
| 16.05.11 - 18.05.11 | 5 | 5 | 6 | 6 | 5 | 6 | 1 | 1 | 1 |
| 19.05.11 | 5 | 5 | 5 | 5 | 6 | 5 | 1 | 1 | 1 |
| 20.05.11 - 21.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 1 | 0 |
| 22.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 1 | 1 |
| 23.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 2 | 1 |
| 24.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 2 | 1 |
| 25.05.11 - 28.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 2 | 2 |
| 29.05.11 - 31.05.11 | 4 | 5 | 4 | 4 | 5 | 4 | 1 | 1 | 2 |
| 01.06.11 - 04.06.11 | 4 | 4 | 4 | 4 | 4 | 4 | 7 | 9 | 7 |
| 05.06.11 | 4 | 4 | 3 | 4 | 4 | 3 | 5 | 6 | 5 |
| 06.06.11-08.06.11 | 4 | 4 | 3 | 4 | 4 | 3 | 6 | 7 | 6 |
| 09.06.11 | 4 | 4 | 3 | 4 | 4 | 3 | 7 | 6 | 8 |
| 10.06.11 - 13.06.11 | 4 | 4 | 3 | 4 | 4 | 3 | 8 | 6 | 8 |
| 14.06.11 | 3 | 4 | 3 | 3 | 4 | 3 | 5 | 6 | 6 |
| 15.06.11 | 3 | 4 | 3 | 3 | 4 | 3 | 4 | 6 | 4 |
| 16.06.11 - 19.06.11 | 3 | 4 | 3 | 3 | 4 | 3 | 4 | 5 | 4 |
| 20.06.11 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| 22.06.11 until harvest | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Where Date: date and time interval of watering, 6.30 am/ 0.30 pm/ 6.30pm: time of watering during the day Control: under control treatment, DT-BBCH 24: drought treatment before anthesis, DT-BBCH 49: drought treatment after anthesis.

## Appendix Chapter 3

**Table 2** Watering time in minutes for pot experiments conducted under semi-controlled conditions in poly-ethylene covered tunnels at the experimental research station in Bonn, Germany in 2012.

| **Date** | **Control treatment** | | | **DT-BBCH 19** | | | **DT-BBCH 24** | | | **DT-BBCH 49** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm |
| 28.03.12 - 29.03.12 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 30.03.12 - 09.04.12 | 3 | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 3 |
| 10.04.12 - 18.04.12 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 |
| 19.04.12 - 22.04.12 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 1 |
| 23.04.12 - 24.04.12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| 24.04.12 - 27.04.12 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| 28.04.12 - 07.05.12 | 1 | 2 | 2 | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 2 | 2 |
| 08.05.12 - 09.05.12 | 1 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| 10.05.12 - 15.05.12 | 2 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 2 | 2 |
| 16.05.12 - 18.05.12 | 3 | 3 | 2 | 7 | 7 | 8 | 0 | 0 | 0 | 0 | 1 | 0 |
| 19.05.12 - 21.05.12 | 3 | 3 | 2 | 4 | 4 | 4 | 0 | 1 | 0 | 0 | 1 | 0 |
| 22.05.12 - 25.05.12 | 3 | 4 | 3 | 3 | 4 | 3 | 0 | 1 | 1 | 0 | 1 | 0 |
| 26.05.12 - 28.05.12 | 4 | 4 | 3 | 4 | 4 | 3 | 1 | 1 | 1 | 1 | 1 | 1 |
| 29.05.12 - 31.05.12 | 4 | 4 | 4 | 4 | 4 | 3 | 7 | 7 | 8 | 1 | 0 | 1 |
| 01.06.12 - 05.06.12 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 5 | 1 | 1 | 1 |
| 06.06.12 - 07.06.12 | 4 | 5 | 4 | 4 | 5 | 4 | 4 | 4 | 3 | 5 | 5 | 6 |
| 08.06.12 - 10.06.12 | 4 | 5 | 4 | 4 | 5 | 4 | 4 | 4 | 5 | 4 | 5 | 4 |
| 11.06.12 - 19.06.12 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 |
| 20.06.12 - 26.06.12 | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 4 | 3 | 4 | 4 | 3 |
| 27.06.12 - 08.07.12 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 09.07.12 until harvest | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Where Date: date and time interval of watering, 6.30 am/ 0.30 pm/ 6.30pm: time of watering during the day Control: under control treatment, Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis.

**Table 3** Watering time in minutes for pot experiments conducted under semi-controlled conditions in poly-ethylene covered tunnels at the experimental research station in Bonn, Germany in 2013.

| Date | Control treatment | | | DT-BBCH 19 | | | DT-BBCH 24 | | | DT-BBCH 49 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm | 6.30 am | 0.30 pm | 6.30 pm |
| 17.04.13 - 07.05.13 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 08.05.13 - 09.05.13 | 4 | 5 | 4 | 2 | 0 | 2 | 4 | 5 | 5 | 4 | 5 | 4 |
| 10.05.13 - 13.05.13 | 4 | 5 | 4 | 0 | 0 | 0 | 4 | 5 | 5 | 4 | 5 | 4 |
| 14.05.13 | 4 | 5 | 4 | 0 | 0 | 0 | 4 | 5 | 5 | 4 | 5 | 4 |
| 15.05.13 - 20.05.13 | 4 | 5 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 | 4 |
| 21.05.13 - 28.05.13 | 4 | 5 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| 29.05.13 | 3 | 3 | 4 | 8 | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30.05.13 - 05.06.13 | 3 | 3 | 4 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 06.06.13 - 12.06.13 | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 7 | 6 | 1 | 1 | 0 |
| 13.06.13 - 23.06.13 | 5 | 5 | 6 | 5 | 5 | 6 | 6 | 7 | 7 | 6 | 6 | 7 |
| 24.06.13 - 14.07.13 | 6 | 7 | 7 | 6 | 7 | 7 | 6 | 7 | 7 | 6 | 7 | 7 |
| 15.07.13 - 20.07.13 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 1 |
| 21.07.13 until harvest | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Where Date: date and time interval of watering, 6.30 am/ 0.30 pm/ 6.30pm: time of watering during the day Control: under control treatment, Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis.

**Table 4** Description of drought stress scenarios realized in 2012 and 2013 in Bonn, Germany.

| Year | Drought stress scenario [a] | Sowing date | Begin of drought treatment (BBCH [b]) | End of the drought treatment (BBCH [b]) |
|---|---|---|---|---|
| 2012 | DT-BBCH 19 | 28.03.2012 | 20 | 32 |
| 2013 | DT-BBCH 19 | 17.04.2013 | 21 | 33 |
| 2012 | DT-BBCH 24 | 28.03.2012 | 30 | 53 |
| 2013 | DT-BBCH 24 | 17.04.2013 | 23 | 37 |
| 2012 | DT-BBCH 49 | 28.03.2012 | 33 | 63 |
| 2013 | DT-BBCH 49 | 17.04.2013 | 31 | 53 |

[a] Drought stress scenario: DT-BBCH 19:drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis

[b] principal plant developmental stages according to the extended BBCH scale of Hess et al. (1997)

**Table 5** Analysis of variance across two years for morphological parameters, physiological and yield related parameters of four genotypes under drought treatment - end of leaf development stage (1st drought stress scenario).

| | | p-value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **TNP** | **LNP** | **LAP** | **YLA** | **GLA** | **PDM** | **LDM** | **SDM** |
| Treatment | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0006 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Genotype | 3 | <0.0001 | <0.0001 | <0.0001 | 0.0948 | 0.1391 | 0.0359 | 0.0015 | <0.0001 | 0.0001 |
| Genotype*Treatment | 3 | 0.0381 | 0.8812 | 0.6370 | 0.6941 | 0.5078 | 0.4995 | 0.0659 | 0.0604 | 0.0842 |
| Year | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Year*Treatment | 1 | 0.2542 | 0.0139 | 0.2051 | 0.0503 | 0.2755 | 0.0130 | 0.1454 | 0.0007 | 0.9439 |
| Year*Genotype | 3 | 0.4605 | 0.9096 | 0.4299 | 0.2450 | 0.0864 | 0.2518 | 0.2869 | 0.1517 | 0.2019 |
| Genotype*Treatment*Year | 3 | 0.0521 | 0.4722 | 0.2230 | 0.4169 | 0.3300 | 0.1606 | 0.2901 | 0.1780 | 0.4398 |

**Table 5 (continued)**

| | | p-value | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **SPAD** | **LS** | **PWC** | **LWC** | **SWC** | **PNC** | **PPhC** | **PKC** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| Treatment | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0030 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Genotype | 3 | <0.0001 | 0.0601 | 0.0108 | 0.5647 | 0.0086 | <0.0001 | 0.0795 | <0.0001 | <0.0001 | <0.0001 | 0.0425 | <0.0001 | 0.7333 |
| Genotype*treatment | 3 | 0.4869 | 0.0601 | 0.5319 | 0.9038 | 0.3622 | 0.0080 | 0.4344 | 0.0003 | 0.3184 | <0.0001 | 0.3329 | 0.0176 | 0.9189 |
| Year | 1 | <0.0001 | 1.000 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0226 | <0.0001 | <0.0001 | 0.0002 | <0.0001 | 0.4739 | <0.0001 |
| Year*treatment | 1 | 0.9097 | 1.000 | 0.5242 | 0.5316 | 0.1631 | <0.0001 | 0.0002 | <0.0001 | 0.3331 | <0.0001 | 0.0024 | 0.7199 | 0.0013 |
| Year*genotype | 3 | 0.1760 | 0.0048 | 0.3095 | 0.1468 | 0.3291 | 0.0253 | 0.2301 | <0.0001 | 0.0651 | <0.0001 | 0.0891 | 0.2487 | 0.0467 |
| Genotype*treatment*year | 3 | 0.3600 | 0.0048 | 0.4895 | 0.6744 | 0.6604 | 0.1689 | 0.3540 | 0.0003 | 0.9873 | <0.0001 | 0.0789 | 0.0504 | 0.1603 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013 Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 6** Analysis of variance across two years for morphological parameters, physiological and yield related parameters of four genotypes under drought treatment at tillering stage (2nd drought stress scenario).

| | | p-value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **TNP** | **LNP** | **LAP** | **YLA** | **GLA** | **PDM** | **LDM** | **SDM** |
| Treatment | 1 | 0.0071 | <0.0001 | 0.0052 | 0.0035 | 0.0303 | 0.0004 | 0.0006 | 0.0001 | 0.0014 |
| Genotype | 3 | <0.0001 | <0.0001 | <0.0001 | 0.9306 | 0.4030 | 0.9906 | 0.0060 | 0.9364 | <0.0001 |
| Genotype*Treatment | 3 | 0.0382 | 0.0078 | 0.4996 | 0.4482 | 0.3700 | 0.6295 | 0.3776 | 0.4692 | 0.3392 |
| Year | 1 | 0.0030 | <0.0001 | 0.0039 | <0.0001 | 0.0300 | <0.0001 | 0.5491 | <0.0001 | 0.0076 |
| Year*Treatment | 1 | 0.4850 | 0.4753 | 0.8570 | 0.0808 | 0.0665 | 0.0169 | 0.0929 | 0.3206 | 0.0193 |
| Year*Genotype | 3 | 0.7601 | 0.8892 | 0.5080 | 0.9533 | 0.4369 | 0.7478 | 0.1487 | 0.2247 | 0.1307 |
| Genotype*Treatment*Year | 3 | 0.9953 | 0.9851 | 0.0325 | 0.0760 | 0.1138 | 0.1597 | 0.0485 | 0.0336 | 0.0798 |

**Table 6 (continued)**

| | | p-value | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **SPAD** | **LS** | **PWC** | **LWC** | **SWC** | **PNC** | **PPhC** | **PKC** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| Treatment | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0322 | <0.0001 | <0.0001 | 0.0001 | <0.0001 |
| Genotype | 3 | <0.0001 | 0.0954 | 0.0001 | 0.0108 | <0.0001 | 0.0717 | 0.4459 | <0.0001 | <0.0001 | <0.0001 | 0.1001 | <0.0001 | 0.2170 |
| Genotype*treatment | 3 | 0.1650 | 0.2009 | 0.1851 | 0.3080 | 0.1170 | 0.0631 | 0.1676 | 0.0290 | 0.1962 | <0.0001 | 0.0356 | 0.5652 | 0.2900 |
| Year | 1 | 0.1356 | 0.0087 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0027 | <0.0001 | 0.0769 | <0.0001 |
| Year*treatment | 1 | 0.0470 | 0.0004 | 0.0262 | 0.8063 | 0.0031 | 0.0037 | 0.3518 | 0.0006 | 0.0212 | 0.0064 | 0.6248 | 0.9621 | 0.9622 |
| Year*genotype | 3 | 0.3253 | 0.0459 | 0.0529 | 0.2211 | 0.0499 | 0.0524 | 0.6764 | 0.2814 | 0.4749 | 0.0059 | 0.1281 | 0.3846 | 0.9161 |
| Genotype*treatment*year | 3 | 0.0360 | 0.9691 | 0.7191 | 0.1145 | 0.8446 | 0.0678 | 0.6565 | 0.0948 | 0.1850 | 0.0010 | 0.0026 | 0.0249 | 0.1142 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013 Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 7** Analysis of variance across two years for morphological parameters, physiological and yield related parameters of four genotypes under drought treatment at anthesis (3rd drought stress scenario).

| | | p-value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **TNP** | **LNP** | **LAP** | **YLA** | **GLA** | **PDM** | **LDM** | **SDM** |
| Treatment | 1 | <0.0001 | <0.0001 | 0.0014 | <0.0001 | 0.0004 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Genotype | 3 | <0.0001 | <0.0001 | <0.0001 | 0.3170 | 0.3363 | 0.4074 | 0.2354 | <0.0001 | 0.0015 |
| Genotype*Treatment | 3 | 0.0116 | 0.0386 | 0.5728 | 0.9468 | 0.6949 | 0.9482 | 0.8122 | 0.6879 | 0.6595 |
| Year | 1 | 0.2360 | 0.1107 | 0.0137 | <0.0001 | <0.0001 | <0.0001 | 0.1252 | <0.0001 | 0.0262 |
| Year*Treatment | 1 | 0.1521 | 0.4984 | 0.9069 | <0.0001 | 0.0904 | <0.0001 | 0.0002 | <0.0001 | 0.0011 |
| Year*Genotype | 3 | 0.7062 | 0.1202 | 0.3499 | 0.5129 | 0.5696 | 0.3707 | 0.0317 | 0.1566 | 0.0104 |
| Genotype*Treatment*Year | 3 | 0.1204 | 0.6945 | 0.6436 | 0.8551 | 0.8611 | 0.8649 | 0.1600 | 0.4297 | 0.1221 |

**Table 7 (continued)**

| | | p-value | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **SPAD** | **LS** | **PWC** | **LWC** | **SWC** | **PNC** | **PPhC** | **PKC** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| Treatment | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Genotype | 3 | <0.0001 | 0.1304 | <0.0001 | 0.0360 | <0.0001 | 0.0049 | 0.0245 | <0.0001 | <0.0001 | <0.0001 | 0.0043 | <0.0001 | 0.0008 |
| Genotype*treatment | 3 | 0.4810 | 0.2189 | 0.4929 | 0.0399 | 0.9188 | 0.7642 | 0.6310 | 0.4532 | 0.0671 | <0.0001 | <0.0001 | 0.0129 | 0.0049 |
| Year | 1 | 0.6072 | 0.0062 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0412 | <0.0001 | <0.0001 | <0.0001 |
| Year*treatment | 1 | 0.0174 | 0.0003 | <0.0001 | 0.0017 | <0.0001 | 0.0619 | 0.8495 | 0.0002 | 0.2206 | <0.0001 | 0.0004 | 0.0002 | <0.0001 |
| Year*genotype | 3 | 0.0027 | 0.0780 | 0.4392 | 0.6587 | 0.3135 | 0.0350 | 0.4945 | <0.0001 | 0.1611 | <0.0001 | 0.0812 | 0.0139 | 0.0068 |
| Genotype*treatment*year | 3 | 0.7655 | 0.9146 | 0.4758 | 0.7068 | 0.6198 | 0.4015 | 0.8446 | 0.5233 | 0.0940 | <0.0001 | <0.0001 | 0.0419 | 0.0045 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Genotype = Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013 Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 8** Mean values of four spring barley genotypes in three different drought stress scenarios.

| Trait | Experiment | 2012 | | | 2013 | | | Mean | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Wet | Dry | Δ %[a] | Wet | Dry | Δ %[a] | Wet | Dry | Δ %[a] |
| **PLH** | DT – BBCH 19 | 39.9 | 33.4 | -16.3 | 47.4 | 42.6 | -10.0 | 43.6 | 38.0 | -12.9 |
| | DT – BBCH 24 | 73.0 | 66.5 | -8.9 | 58.6 | 52 | -11.3 | 65.8 | 59.3 | -10 |
| | DT – BBCH 49 | 82.9 | 74.1 | -10.7 | 87.3 | 73.6 | -15.6 | 85.1 | 73.8 | -13.2 |
| **TNP** | DT – BBCH 19 | 3.3 | 2.7 | -17.8 | 4.5 | 3.3 | -27.4 | 3.9 | 3.0 | -23.4 |
| | DT – BBCH 24 | 3.3 | 2.0 | -38.6 | 4.5 | 3.5 | -24.1 | 3.9 | 2.7 | -30.1 |
| | DT – BBCH 49 | 4.0 | 2.8 | -29.6 | 4.2 | 3.2 | -22.8 | 4.1 | 3.0 | -26.1 |
| **LNP** | DT – BBCH 19 | 15.7 | 11.8 | -24.7 | 22.5 | 16.9 | -24.8 | 19.1 | 14.4 | -24.8 |
| | DT – BBCH 24 | 20.4 | 16.1 | -21.4 | 25.5 | 20.7 | -19.1 | 23.0 | 18.4 | -20.1 |
| | DT – BBCH 49 | 24.7 | 20.8 | -15.8 | 27.3 | 23.6 | -13.5 | 26.0 | 22.2 | -14.6 |
| **LAP** | DT – BBCH 19 | 143.2 | 67.5 | -52.9 | 317.6 | 207.7 | -34.9 | 230.4 | 137.6 | -40.3 |
| | DT – BBCH 24 | 138.2 | 101.7 | -26.4 | 346.9 | 229.0 | -34.0 | 242.6 | 165.4 | -31.8 |
| | DT – BBCH 49 | 170.2 | 134.3 | -21.1 | 400.8 | 225.5 | -43.7 | 285.5 | 179.9 | -37.0 |
| **YLA** | DT – BBCH 19 | 12.3 | 16.3 | 32.0 | 29.9 | 37.2 | 24.5 | 21.1 | 26.7 | 26.7 |
| | DT – BBCH 24 | 28.7 | 31.5 | 9.80 | 31.6 | 60.5 | 91.5 | 30.1 | 46.0 | 52.6 |
| | DT – BBCH 49 | 33.8 | 42.2 | 24.9 | 52.1 | 71.0 | 36.3 | 42.9 | 56.6 | 31.8 |
| **GLA** | DT – BBCH 19 | 130.8 | 51.2 | -60.9 | 287.7 | 170.6 | -40.7 | 209.3 | 110.9 | -47.0 |
| | DT – BBCH 24 | 109.5 | 70.2 | -35.9 | 315.2 | 168.5 | -46.6 | 212.4 | 119.4 | -43.8 |
| | DT – BBCH 49 | 136.4 | 92.1 | -32.5 | 348.6 | 154.5 | -55.7 | 242.5 | 123.3 | -49.2 |
| **PDM** | DT – BBCH 19 | 0.90 | 0.50 | -44.3 | 1.50 | 0.98 | -34.6 | 1.20 | 0.70 | -38.2 |
| | DT – BBCH 24 | 2.70 | 1.50 | -45.6 | 2.30 | 1.7 | -23.9 | 2.5 | 1.6 | -35.8 |
| | DT – BBCH 49 | 4.10 | 2.95 | -28.0 | 4.80 | 2.60 | -45.8 | 4.40 | 2.80 | -37.6 |
| **LDM** | DT – BBCH 19 | 0.47 | 0.25 | -46.4 | 0.79 | 0.45 | -43.3 | 0.63 | 0.35 | -44.5 |
| | DT – BBCH 24 | 0.50 | 0.30 | 41.8 | 0.90 | 0.60 | -34.5 | 0.74 | 0.47 | -37.1 |
| | DT – BBCH 49 | 0.64 | 0.52 | -18.6 | 1.20 | 0.70 | -38.9 | 0.91 | 0.62 | -31.8 |
| **SDM** | DT – BBCH 19 | 0.43 | 0.25 | -42.0 | 0.70 | 0.53 | -24.9 | 0.57 | 0.39 | -31.4 |
| | DT – BBCH 24 | 2.19 | 1.2 | -46.5 | 1.30 | 1.10 | -16.3 | 1.80 | 1.10 | -35.2 |
| | DT – BBCH 49 | 3.47 | 2.44 | -29.8 | 3.6 | 1.87 | -48.1 | 3.54 | 2.16 | -39.1 |

where: Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis, Genotype: Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013, **Δ % [a]** ( relative difference in persent): (Dry-Wet) / Wet *100;Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter.

**Table 8 (continued)**

| Trait | Experiment | 2012 | | | 2013 | | | Mean | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Wet | Dry | Δ %[a] | Wet | Dry | Δ %[a] | Wet | Dry | Δ %[a] |
| **SPAD** | DT – BBCH 19 | 34.0 | 22.9 | -32.6 | 43.1 | 32.2 | -25.3 | 38.6 | 27.6 | -28.5 |
| | DT – BBCH 24 | 44.1 | 34.2 | -22.5 | 43.6 | 37.2 | -14.8 | 43.9 | 35.7 | -18.7 |
| | DT – BBCH 49 | 46.8 | 37.1 | -20.8 | 45.1 | 39.6 | -12.2 | 45.9 | 38.3 | -16.6 |
| **LS** | DT – BBCH 19 | 1.0 | 4.9 | 387.5 | 1.0 | 4.9 | 387.5 | 1.0 | 4.9 | 387.5 |
| | DT – BBCH 24 | 1.5 | 5.3 | 254.2 | 1.1 | 7.0 | 522.2 | 1.3 | 6.2 | 369.0 |
| | DT – BBCH 49 | 2.8 | 7.8 | 184.1 | 1.1 | 7.3 | 544.4 | 1.4 | 7.1 | 393.5 |
| **PWC** | DT – BBCH 19 | 84.1 | 79.9 | -5.1 | 88.8 | 83.9 | -5.5 | 86.5 | 81.9 | -5.3 |
| | DT – BBCH 24 | 74.9 | 70.9 | -5.3 | 87.4 | 79.4 | -9.1 | 81.1 | 75.1 | -7.4 |
| | DT – BBCH 49 | 71.6 | 67.4 | -5.9 | 82.7 | 70.3 | -15.0 | 77.2 | 68.9 | -10.7 |
| **LWC** | DT – BBCH 19 | 83.2 | 80.3 | -3.6 | 87.7 | 85.3 | -2.7 | 85.5 | 82.8 | -3.1 |
| | DT – BBCH 24 | 76.0 | 69.9 | -8.1 | 86.3 | 79.6 | -7.8 | 81.1 | 74.7 | -7.9 |
| | DT – BBCH 49 | 74.8 | 63.9 | -14.7 | 84.2 | 66.7 | -20.8 | 79.5 | 65.3 | -17.9 |
| **SWC** | DT – BBCH 19 | 84.8 | 79.5 | -6.3 | 89.8 | 82.4 | -8.2 | 87.3 | 81.0 | -7.3 |
| | DT – BBCH 24 | 74.6 | 71.2 | -4.6 | 88.2 | 79.2 | -10.2 | 81.4 | 75.2 | -7.7 |
| | DT – BBCH 49 | 70.9 | 68.0 | -4.0 | 82.2 | 71.5 | -13.0 | 76.5 | 69.8 | -8.8 |
| **PNC** | DT – BBCH 19 | 2.5 | 1.3 | -50.7 | 3.3 | 1.4 | -56 | 3.3 | 1.4 | -56.0 |
| | DT – BBCH 24 | 1.6 | 1.0 | -39.9 | 3.1 | 1.6 | -49.2 | 2.4 | 1.3 | -46.0 |
| | DT – BBCH 49 | 1.6 | 1.2 | -24.9 | 2.3 | 1.7 | -23.9 | 2.0 | 1.50 | -24.3 |
| **PPhC** | DT – BBCH 19 | 0.7 | 0.5 | -38.2 | 0.7 | 0.5 | -27.7 | 0.7 | 0.5 | -32.9 |
| | DT – BBCH 24 | 0.6 | 0.4 | -30.0 | 0.7 | 0.5 | -28.8 | 0.7 | 0.5 | -29.2 |
| | DT – BBCH 49 | 0.6 | 0.4 | -19.6 | 0.6 | 0.5 | -18.8 | 0.6 | 0.5 | -15.9 |
| **PKC** | DT – BBCH 19 | 0.4 | 0.3 | -29.9 | 5.2 | 3.2 | -39.1 | 2.8 | 1.7 | -38.4 |
| | DT – BBCH 24 | 2.7 | 2.2 | -19.0 | 4.7 | 3.0 | -36.2 | 3.7 | 2.6 | -30.0 |
| | DT – BBCH 49 | 0.2 | 0.2 | -12.5 | 3.5 | 3.0 | -16.1 | 1.9 | 1.6 | -15.9 |
| **NEP** | DT – BBCH 19 | 3.7 | 3.2 | -15.2 | 5.2 | 4.3 | -18.6 | 4.5 | 3.7 | -17.0 |
| | DT – BBCH 24 | 4.1 | 2.6 | -37.1 | 5.3 | 5.3 | 1.29 | 4.7 | 3.9 | -15.4 |
| | DT – BBCH 49 | 4.1 | 2.5 | -38.9 | 5.0 | 4.0 | -21.0 | 4.5 | 3.2 | -29.0 |
| **NKE** | DT – BBCH 19 | 23.1 | 19.9 | -13.7 | 28.3 | 19.9 | -29.7 | 25.6 | 20.1 | -21.5 |
| | DT – BBCH 24 | 21.6 | 16.7 | -22.7 | 25.8 | 17.0 | -34.3 | 23.7 | 16.8 | -29.0 |
| | DT – BBCH 49 | 21.0 | 20.5 | -2.7 | 27.1 | 17.5 | -35.3 | 24.1 | 19.0 | -21.1 |
| **NKP** | DT – BBCH 19 | 79.8 | 60.9 | -23.7 | 127.9 | 79.7 | -37.7 | 103.8 | 70.9 | -31.7 |
| | DT – BBCH 24 | 80.9 | 41.3 | -4.9 | 123.3 | 88.3 | -28.4 | 102.1 | 64.8 | -36.6 |
| | DT – BBCH 49 | 80.4 | 45.6 | -43.3 | 121.4 | 60.8 | -49.9 | 100.9 | 53.2 | -47.3 |
| **TKW** | DT – BBCH 19 | 48.1 | 42.9 | -10.9 | 48.8 | 43.3 | -11.3 | 48.5 | 43.2 | -11.0 |
| | DT – BBCH 24 | 48.8 | 42.9 | -12.1 | 50.8 | 45.0 | -11.4 | 49.8 | 44.0 | -11.7 |
| | DT – BBCH 49 | 49.2 | 46.7 | -5.1 | 47.9 | 38.7 | -19.2 | 48.6 | 42.7 | -12.1 |
| **YLD** | DT – BBCH 19 | 3.8 | 2.6 | -31.6 | 6.2 | 3.5 | -44.1 | 5.0 | 3.1 | -38.7 |
| | DT – BBCH 24 | 3.9 | 1.7 | -56.4 | 6.2 | 3.9 | -36.3 | 5.1 | 2.8 | -44.1 |
| | DT – BBCH 49 | 3.9 | 2.1 | -47.2 | 5.8 | 2.3 | -60.3 | 4.9 | 2.2 | -55.0 |

where: Drought stress scenario = DT-BBCH 19: drought treatment at the end of the leaf development stage, DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis, Genotype: Scarlett, Morex, Bojos, Henrike, Treatment = well-watered, drought treatment, Year= 2012,2013, **Δ % [a]** ( relative difference in persent): (Dry-Wet) / Wet *100;Trait: SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

**Table 9** Spearman's correlation coefficient ($r_g$) of four spring barley genotypes for evaluated plant parameters of the 1st drought stress scenario (DT-BBCH 19).

| | PLH | TNP | LNP | LAP | YLA | GLA | PDM | LDM | SDM | SPAD | LS | PWC | LWC | SWC | PNC | PPhC | PKC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | 1 | | | | | | | | | | | | | | | | |
| **TNP** | -0.07 | 1 | | | | | | | | | | | | | | | |
| **LNP** | -0.10 | **0.98 ***** | 1 | | | | | | | | | | | | | | |
| **LAP** | 0.64 | 0.60 | 0.52 | 1 | | | | | | | | | | | | | |
| **YLA** | **-0.83 *** | -0.38 | -0.36 | **-0.76 *** | 1 | | | | | | | | | | | | |
| **GLA** | **0.81 *** | 0.48 | 0.43 | **0.93 ***** | **-0.86 **** | 1 | | | | | | | | | | | |
| **PDM** | **0.88 **** | 0.29 | 0.31 | **0.81 *** | **-0.86 **** | **0.90 **** | 1 | | | | | | | | | | |
| **LDM** | 0.45 | **0.71 *** | 0.69 | **0.93 ***** | -0.62 | **0.81 *** | **0.76 *** | 1 | | | | | | | | | |
| **SDM** | **0.81 *** | 0.36 | 0.40 | **0.71 *** | **-0.90 **** | **0.83 *** | **0.95 ***** | 0.69 | 1 | | | | | | | | |
| **SPAD** | 0.48 | 0.55 | 0.62 | 0.57 | **-0.74 *** | 0.57 | **0.74 *** | 0.69 | **0.83 *** | 1 | | | | | | | |
| **LS** | -0.60 | -0.60 | -0.66 | -0.70 | **0.84 **** | **-0.78 *** | **-0.84 **** | **-0.73 *** | **-0.91 **** | **-0.89 **** | 1 | | | | | | |
| **PWC** | 0.64 | 0.52 | 0.48 | **0.76 *** | **-0.88 **** | **0.79 *** | 0.69 | 0.62 | 0.69 | 0.67 | **-0.80 *** | 1 | | | | | |
| **LWC** | **0.81 *** | 0.38 | 0.36 | **0.79 *** | **-0.93 ***** | **0.88 **** | **0.83 *** | 0.62 | **0.81 *** | 0.64 | **-0.84 **** | **0.95 ***** | 1 | | | | |
| **SWC** | 0.52 | 0.67 | 0.62 | 0.69 | **-0.86 **** | **0.71 *** | 0.62 | 0.62 | 0.69 | **0.76 *** | **-0.80 *** | **0.93 ***** | **0.83 *** | 1 | | | |
| **PNC** | 0.31 | **0.81 *** | **0.83 *** | 0.62 | **-0.71 *** | 0.62 | 0.57 | 0.67 | 0.67 | **0.83 *** | **-0.89 **** | **0.83 *** | **0.74 *** | **0.90 **** | 1 | | |
| **PPhC** | 0.38 | **0.71 *** | **0.74 *** | 0.60 | **-0.79 *** | 0.60 | 0.60 | 0.62 | **0.71 *** | **0.88 **** | **-0.89 **** | **0.86 **** | **0.76 *** | **0.93 ***** | **0.98 ***** | 1 | |
| **PKC** | 0.31 | **0.86 **** | **0.81 *** | **0.86 **** | -0.60 | **0.74 *** | 0.60 | **0.90 **** | 0.55 | 0.67 | **-0.73 *** | **0.76 *** | 0.67 | **0.79 *** | **0.83 *** | **0.76 *** | 1 |

where: *: 0.01 < P < 0.05, ** : 0.001 < P < 0.01, *** P < 0.001, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content.

**Table 10** Spearman's correlation coefficient ($r_g$) of four spring barley genotypes for evaluated plant parameters of the 2nd drought stress scenario (DT-BBCH 24).

| | PLH | TNP | LNP | LAP | YLA | GLA | PDM | LDM | SDM | SPAD | LS | PWC | LWC | SWC | PNC | PPhC | PKC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | 1 | | | | | | | | | | | | | | | | |
| **TNP** | 0.02 | 1 | | | | | | | | | | | | | | | |
| **LNP** | -0.31 | **0.90 **** | 1 | | | | | | | | | | | | | | |
| **LAP** | 0.50 | **0.83 *** | 0.60 | 1 | | | | | | | | | | | | | |
| **YLA** | -0.62 | -0.57 | -0.38 | -0.69 | 1 | | | | | | | | | | | | |
| **GLA** | 0.52 | **0.79 *** | 0.55 | **0.98 ***** | **-0.76 *** | 1 | | | | | | | | | | | |
| **PDM** | **0.79 *** | 0.55 | 0.26 | **0.76 *** | **-0.93 **** | **0.79 *** | 1 | | | | | | | | | | |
| **LDM** | 0.38 | **0.79 *** | 0.55 | **0.86 *** | **-0.74 *** | **0.90 **** | **0.76 *** | 1 | | | | | | | | | |
| **SDM** | **0.79 *** | 0.55 | 0.26 | **0.76 *** | **-0.93 **** | **0.79 *** | **1.00 ***** | **0.76 *** | 1 | | | | | | | | |
| **SPAD** | 0.38 | **0.81 *** | 0.71 | **0.83 *** | **-0.81 *** | **0.81 *** | **0.79 *** | **0.74 *** | **0.79 *** | 1 | | | | | | | |
| **LS** | -0.36 | **-0.80 *** | **-0.73 *** | **-0.80 *** | **0.86 *** | **-0.80 *** | **-0.80 *** | **-0.74 *** | **-0.80 *** | **-0.98 ***** | 1 | | | | | | |
| **PWC** | 0.24 | **0.88 **** | 0.64 | **0.81 *** | -0.57 | **0.76 *** | 0.62 | **0.74 *** | 0.62 | 0.62 | -0.62 | 1 | | | | | |
| **LWC** | 0.52 | 0.64 | 0.40 | **0.74 *** | **-0.86 *** | **0.81 *** | **0.83 *** | **0.81 *** | **0.83 *** | 0.62 | -0.70 | **0.76 *** | 1 | | | | |
| **SWC** | 0.24 | **0.88 **** | 0.69 | **0.81 *** | -0.64 | **0.76 *** | 0.62 | 0.67 | 0.62 | **0.74 *** | **-0.72 *** | **0.93 **** | 0.67 | 1 | | | |
| **PNC** | 0.12 | **0.81 *** | **0.71 *** | 0.64 | **-0.81 *** | 0.69 | 0.64 | **0.74 *** | 0.64 | **0.76 *** | **-0.80 *** | **0.74 *** | **0.74 *** | **0.83 *** | 1 | | |
| **PPhC** | 0.19 | **0.81 *** | 0.62 | 0.64 | -0.69 | 0.62 | 0.69 | **0.76 *** | 0.69 | **0.74 *** | **-0.72 *** | **0.79 *** | 0.62 | **0.81 *** | **0.86 *** | 1 | |
| **PKC** | 0.07 | **0.90 **** | **0.76 *** | **0.76 *** | -0.64 | **0.79 *** | 0.57 | **0.83 *** | 0.57 | 0.64 | -0.69 | **0.90 **** | **0.83 *** | **0.83 *** | **0.86 *** | **0.76 *** | 1 |

Where: *: 0.01 < P < 0.05, ** : 0.001 < P < 0.01, *** P < 0.001, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content.

**Table 11** Spearman's correlation coefficient ($r_g$) of four spring barley genotypes for evaluated plant parameters of the 3rd drought stress scenario (DT-BBCH 49.

| | PLH | TNP | LNP | LAP | YLA | GLA | PDM | LDM | SDM | SPAD | LS | PWC | LWC | SWC | PNC | PPhC | PKC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | 1 | | | | | | | | | | | | | | | | |
| **TNP** | -0.31 | 1 | | | | | | | | | | | | | | | |
| **LNP** | -0.50 | **0.90 **** | 1 | | | | | | | | | | | | | | |
| **LAP** | 0.57 | 0.50 | 0.36 | 1 | | | | | | | | | | | | | |
| **YLA** | -0.52 | -0.26 | -0.02 | -0.60 | 1 | | | | | | | | | | | | |
| **GLA** | 0.57 | 0.50 | 0.36 | **1.00 ***** | -0.60 | 1 | | | | | | | | | | | |
| **PDM** | **0.83 *** | 0.10 | -0.14 | **0.76 *** | **-0.88 **** | **0.76 *** | 1 | | | | | | | | | | |
| **LDM** | 0.14 | **0.83 *** | **0.74 *** | **0.83 *** | -0.62 | **0.83 *** | 0.55 | 1 | | | | | | | | | |
| **SDM** | **0.81 *** | 0.12 | -0.17 | 0.69 | **-0.90 **** | 0.69 | **0.98 ***** | 0.52 | 1 | | | | | | | | |
| **SPAD** | **0.71 *** | 0.19 | -0.05 | 0.62 | **-0.90 **** | 0.62 | **0.90 **** | 0.57 | **0.95 ***** | 1 | | | | | | | |
| **LS** | -0.26 | -0.67 | -0.40 | **-0.74 *** | **0.79 *** | **-0.74 *** | -0.64 | **-0.83 *** | -0.69 | -0.69 | 1 | | | | | | |
| **PWC** | 0.29 | 0.69 | 0.64 | **0.83 *** | -0.62 | **0.83 *** | 0.60 | **0.95 ***** | 0.57 | 0.67 | **-0.76 *** | 1 | | | | | |
| **LWC** | 0.62 | 0.36 | 0.21 | **0.81 *** | -0.69 | **0.81 *** | **0.76 *** | **0.74 *** | **0.79 *** | **0.81 *** | **-0.74 *** | **0.83 *** | 1 | | | | |
| **SWC** | 0.29 | 0.69 | 0.64 | **0.83 *** | -0.62 | **0.83 *** | 0.60 | **0.95 ***** | 0.57 | 0.67 | **-0.76 *** | **1.00 ***** | **0.83 *** | 1 | | | |
| **PNC** | 0.10 | **0.83 *** | 0.69 | 0.69 | **-0.71 *** | 0.69 | 0.55 | **0.95 ***** | 0.57 | 0.67 | **-0.86 *** | **0.90 **** | 0.69 | **0.90 **** | 1 | | |
| **PPhC** | 0.12 | **0.81 *** | 0.60 | 0.67 | **-0.76 *** | 0.67 | 0.57 | **0.90 **** | 0.62 | 0.69 | **-0.93 **** | **0.83 *** | 0.67 | **0.83 *** | **0.98 ***** | 1 | |
| **PKC** | 0.00 | **0.90 **** | **0.83 *** | **0.79 *** | -0.45 | **0.79 *** | 0.38 | **0.98 ***** | 0.36 | 0.40 | **-0.79 *** | **0.90 **** | 0.64 | **0.90 **** | **0.90 **** | **0.86 *** | 1 |

Where: *: 0.01 < P < 0.05, ** : 0.001 < P < 0.01, *** P < 0.001, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content.

**Table 12** Analysis of variance of STI stress tolerance indices for 22 evaluated plant parameters.

| Source of variation | DF | p-value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PLH $_{STI}$ | TNP $_{STI}$ | LNP $_{STI}$ | LAP $_{STI}$ | YLA $_{STI}$ | GLA $_{STI}$ | PDM $_{STI}$ | LDM $_{STI}$ | SDM $_{STI}$ |
| **Year (Y)** | 1 | 0.9101 | 0.7699 | 0.2972 | 0.3901 | 0.3799 | 0.7428 | 0.7283 | 0.7017 | 0.5516 |
| **Scenario (S)** | 2 | 0.6357 | 0.6081 | 0.0092 | 0.1805 | 0.8089 | 0.8481 | 0.9509 | 0.4529 | 0.8963 |
| **Genotype (G)** | 3 | <0.0001 | <0.0001 | <0.0001 | 0.1155 | 0.0880 | 0.2241 | 0.0017 | 0.0003 | 0.0009 |
| **G*S** | 6 | 0.0562 | 0.3506 | 0.1155 | 0.6523 | 0.4749 | 0.7010 | 0.0637 | 0.0022 | 0.2035 |

**Table 12 (continued)**

| Source of variation | DF | p-value | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SPAD $_{STI}$ | LS $_{STI}$ | PWC $_{STI}$ | LWC $_{STI}$ | SWC $_{STI}$ | PNC $_{STI}$ | PPhC $_{STI}$ | PKC $_{STI}$ | NEP $_{STI}$ | NKE $_{STI}$ | NKP $_{STI}$ | TKW $_{STI}$ | YLD $_{STI}$ |
| **Year (Y)** | 1 | 0.0030 | 0.1845 | 0.2182 | 0.5423 | 0.1145 | 0.2467 | 0.2508 | 0.1310 | 0.2733 | 0.0818 | 0.9955 | 0.4415 | 0.8477 |
| **Scenario (S)** | 2 | 0.0031 | 0.9471 | 0.4134 | 0.0633 | 0.8712 | 0.0354 | 0.1122 | 0.1003 | 0.7147 | 0.3140 | 0.4770 | 0.9874 | 0.5708 |
| **Genotype (G)** | 3 | 0.0003 | 0.1638 | 0.0084 | 0.0444 | 0.0026 | 0.0622 | 0.1073 | 0.0012 | <0.0001 | <0.0001 | 0.1363 | 0.0002 | 0.1395 |
| **G*S** | 6 | 0.0747 | 0.7208 | 0.3019 | 0.1099 | 0.2947 | 0.9005 | 0.4855 | 0.3342 | 0.1573 | 0.0007 | 0.2412 | 0.3963 | 0.3471 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P < 0.0001, DF = degree of freedom, Year: 2012 and 2013, Scenario= DT-BBCH 24: drought treatment at tillering stage, DT-BBCH 49: drought treatment at anthesis , Genotype = Scarlett, Morex, Bojos, Henrike, Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

## Appendix Chapter 4

**Table 13** Analysis of variance for carbon isotope discrimination for 24 spring barley genotypes in 2012.

| | | p-value |
|---|---|---|
| **Source of variation** | **DF** | **Δ 13C** |
| **Replication (Rep)** | 3 | 0.1187 |
| **Treatment (T)** | 1 | 0.1954 |
| **Genotype (G)** | 23 | 0.2478 |
| **T*G** | 23 | 0.4814 |

**Table 14** Combined analysis of variance across for 19 evaluated parameters of 24 genotypes under drought treatment at tillering stage.

| | | p-value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **TNP** | **LNP** | **LAP** | **YLA** | **GLA** | **PDM** | **LDM** | **SDM** |
| **Treatment** | 1 | 0.0003 | <0.0001 | <0.0001 | <0.0001 | 0.0002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Genotype** | 23 | <0.0001 | <0.0001 | <0.0001 | 0.0155 | <0.0001 | 0.0241 | 0.0140 | 0.0807 | <0.0001 |
| **Genotype*Treatment** | 23 | 0.1241 | 0.1269 | 0.9867 | 0.6099 | 0.0996 | 0.4460 | 0.8772 | 0.8204 | 0.8328 |
| **Year** | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.7681 | <0.0001 | 0.0003 |
| **Year*Treatment** | 1 | 0.8819 | 0.2553 | 1.000 | 0.0080 | 0.0003 | 0.0011 | 0.0181 | 0.0180 | 0.0010 |
| **Year*Genotype** | 23 | 0.0002 | 0.0290 | 0.2265 | 0.1402 | 0.0286 | 0.2160 | 0.3004 | 0.2559 | 0.1693 |
| **Genotype*Treatment*Year** | 23 | 0.5592 | 0.0910 | 0.5821 | 0.1341 | 0.0631 | 0.0843 | 0.5690 | 0.5250 | 0.5905 |

**Table 14 (continued)**

| | | p-value | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **SPAD** | **LS** | **PWC** | **LWC** | **SWC** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| **Treatment** | 1 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0122 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Genotype** | 23 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Genotype*treatment** | 23 | 0.8328 | 0.0569 | 0.2383 | 0.0866 | 0.3184 | 0.0007 | <0.0001 | 0.0001 | 0.8071 | 0.0004 |
| **Year** | 1 | 0.0665 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0019 | <0.0001 | 0.0036 | <0.0001 |
| **Year*treatment** | 1 | 0.0118 | <0.0001 | 0.0367 | 0.3815 | 0.0006 | 0.0411 | 0.0107 | 0.3410 | 0.8416 | 0.6645 |
| **Year*genotype** | 23 | 0.0253 | 0.4254 | 0.0775 | 0.0544 | 0.0051 | 0.0907 | <0.0001 | 0.0037 | 0.5269 | 0.0322 |
| **Genotype*treatment*year** | 23 | 0.0225 | 0.0368 | 0.5925 | 0.1385 | 0.9205 | 0.1397 | <0.0001 | 0.0162 | 0.0719 | 0.0988 |

Where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤0.001, DF = degree of freedom, Treatment = well-watered, drought treatment, Year= 2012,2013
Trait: PLH: plant height, TNP: tiller number per plant, LNP: number of leaves per plant, LAP: leaf area per plant, YLA: yellow leaf area per plant, GLA: green leaf area per plant, PDM: plant dry matter, LDM: leaf dry matter, SDM: stem dry matter, SPAD: SPAD value, LS: leaf senescence, PWC: plant water content, LWC: leaf water content, SWC: stem water content, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.

## Appendix Chapter 5

**Table 15** Experimental details for the field experiments conducted in 2012 and 2013 in Germany.

| Region | Location | Position | Soil texture | | | Soil type |
|---|---|---|---|---|---|---|
| | | Latitude/longitude/altitude a.s.l. | Sand (%) | Silt (%) | Clay (%) | |
| Bavaria | Herzogenaurach | 49° 34' 14/10° 52' 59/296 m | 54 | 31 | 16 | sandy loam |
| Bavaria | Uffenheim | 49° 32' 38/10° 13' 58/329 m | 3 | 65 | 32 | sandy loam |
| Lower Saxony | Müden an der Aller | 52° 31' 45/10° 21' 20/ 47 m | 93 | 4 | 3 | sand |
| Saxony-Anhalt | Böhnshausen | 51° 51' 11/10° 59' 15/171 m | 6 | 71 | 23 | sandy loam |
| North Rhine-Westphalia | Klein-Altendorf | 6° 59’ 38/50° 36’48/186 m | 7 | 70 | 23 | sandy loam |

**Table 16** Years and locations of field experiments including plot size, plant density and sowing date.

| Year | Region | Location | Abbreviation | Plot size ($m^2$) | No. Replications | Plant density (plants/$m^2$) | Sowing date |
|---|---|---|---|---|---|---|---|
| 2012 | Bavaria | Herzogenaurach | KL12BRE | 11.7 | 4 | 300 | 28.03.2012 |
| 2012 | Bavaria | Uffenheim | KL12STR | 6.9 | 6 | 330 | 16.03.2012 |
| 2012 | Lower Saxony | Müden an der Aller | KL12SYN | 16.6 | 4 | 300 | 21.03.2012 |
| 2012 | Saxony-Anhalt | Böhnshausen | KL12NOSA | 9.6 | 4 | 280 | 14.03.2012 |
| 2012 | North Rhine-Westphalia | Klein-Altendorf | KL12KA | 36 | 4 | 300 | 21.03.2012 |
| 2013 | Bavaria | Herzogenaurach | KL13BRE | 11.7 | 4 | 300 | 24.04.2013 |
| 2013 | Bavaria | Uffenheim | KL13STR | 6.9 | 6 | 330 | 02.04.2013 |
| 2013 | Lower Saxony | Müden an der Aller | KL13SYN | 16.6 | 4 | 300 | 03.04.2013 |
| 2013 | Saxony-Anhalt | Böhnshausen | KL13NOSA | 9.6 | 4 | 280 | 16.04.2013 |
| 2013 | North Rhine-Westphalia | Klein-Altendorf | KL13KA | 36 | 4 | 300 | 08.04.2013 |

**Table 17** Analysis of variance across for morphological parameters, physiological and yield related parameters of spring barley genotypes investigated in field experiments during 2012 and 2013.

| | | | p-value at | | |
|---|---|---|---|---|---|
| **Trait** | **Source of variation** | **DF** | **BBCH 30** | **BBCH 63** | **BBCH 81** |
| **PLH** | Genotype | 23 | <0.0001 | <0.0001 | <0.0001 |
| | Environment | 7 | <0.0001 | <0.0001 | <0.0001 |
| | Genotype*Environment | 161 | <0.0001 | <0.0001 | 0.0035 |
| **PDM** | Genotype | 23 | <0.0001 | 0.0107 | <0.0001 |
| | Environment | 7 | <0.0001 | <0.0001 | <0.0001 |
| | Genotype*Environment | 161 | 0.0190 | 0.6794 | 0.6062 |
| **PNC** | Genotype | 7 | 0.3082 | 0.1387 | 0.0091 |
| | Environment | 3 | <0.0001 | 0.0386 | 0.0010 |
| | Genotype*Environment | 21 | 0.3806 | 0.0450 | 0.0069 |
| **PPhC** | Genotype | 7 | 0.0679 | 0.1318 | 0.0160 |
| | Environment | 3 | 0.0002 | 0.0060 | <0.0001 |
| | Genotype*Environment | 21 | 0.2608 | 0.6824 | 0.2064 |
| **PKC** | Genotype | 7 | 0.5003 | 0.4755 | 0.0210 |
| | Environment | 3 | <0.0001 | <0.0001 | <0.0001 |
| | Genotype*Environment | 21 | 0.2269 | 0.9939 | 0.2782 |
| **NEM** | Genotype | 23 | - | - | <0.0001 |
| | Environment | 7 | - | - | <0.0001 |
| | Genotype*Environment | 161 | - | - | 0.2608 |
| **NKE** | Genotype | 23 | - | - | <0.0001 |
| | Environment | 7 | - | - | 0.0005 |
| | Genotype*Environment | 161 | - | - | 0.1331 |
| **NKM** | Genotype | 23 | - | - | <0.0001 |
| | Environment | 7 | - | - | <0.0001 |
| | Genotype*Environment | 161 | - | - | <0.0001 |
| **TKW** | Genotype | 23 | - | - | <0.0001 |
| | Environment | 7 | - | - | <0.0001 |
| | Genotype*Environment | 161 | - | - | <0.0001 |
| **YLD** | Genotype | 23 | - | - | <0.0001 |
| | Environment | 7 | - | - | <0.0001 |
| | Genotype*Environment | 161 | - | - | <0.0001 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Trait: PLH: plant height, PDM: plant dry matter, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEM: number of ears per square meter, NKE: number of kernels per ear, NKP: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

**Table 9** (continued)

| Trait | Source of variation | DF | p-value |
|---|---|---|---|
| **CGR1** | Genotype | 23 | <0.0001 |
| | Environment | 7 | <0.0001 |
| | Genotype*Environment | 161 | 0.0174 |
| **CGR2** | Genotype | 23 | 0.1390 |
| | Environment | 7 | <0.0001 |
| | Genotype*Environment | 161 | 0.6436 |
| **CGR3** | Genotype | 23 | 0.0478 |
| | Environment | 7 | <0.0001 |
| | Genotype*Environment | 161 | 0.7288 |
| **CGR4** | Genotype | 23 | <0.0001 |
| | Environment | 7 | <0.0001 |
| | Genotype*Environment | 161 | 0.7584 |
| **MGR** | Genotype | 23 | 0.0034 |
| | Environment | 7 | <0.0001 |
| | Genotype*Environment | 161 | 0.5851 |

where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001, DF = degree of freedom, Trait: CGR1: growth rate between sowing and BBCH 30, CGR2: growth rate between BBCH 30 and BBCH stage 63, CGR3: growth rate between BBCH 63 and BBCH 81, CGR4: growth rate between sowing and BBCH 81, MWR: mean growth rate.

## **SAS program for AMMI biplot analysis** (Thillainathan 2001)

URL "http://www.ag.unr.edu:80/gf/_sasmacro/ammi.ppt" ;

```
%display ammi;

%ammi
data yld2; set d;

goptions cback=white;
%LET ENV_N = 25; /*** Set the number of Environments here ***/
%LET VAR = x; /*** Set the name of the response variable here ***/
%LET ENV = trait;/*** Set the name of the environment variable here ***/
%LET GEN = genotype; /*** Set the name of the genotype variable here ***/

data yld2;
  set yld2;

proc sort;
  by &ENV &GEN ;

proc means noprint;
  var &VAR;
  output out=avgyld mean=ylda;
  by &ENV &GEN ;
```

```
proc glm noprint;
  class &GEN &ENV;
  model ylda = &ENV &GEN ;
  output out=resid r=res;

proc iml;
 use resid var{&ENV &GEN res};
 read all var{res} into r;

 e=shape(r,&ENV_N);

 call svd(envvec,val,genvec,e);
   val=val#val;

 propeig=val/(val`*j(nrow(val),1,1));

 print 'Percentages of each component', val propeig;
 print '','Eigenvectors' ,genvec envvec;

 labe={'env_1' 'env_2' 'env_3'};
 labg={'gen_1' 'gen_2' 'gen_3'};

 create vece from envvec (|colname=labe|);
 append from envvec;

 create vecg from genvec (|colname=labg|);
 append from genvec;
quit;
run;

proc means data=avgyld noprint;
 var ylda;
  by &ENV;
 output out=enva mean=ydoteye;

proc sort data=avgyld;
 by &GEN;

proc means data=avgyld noprint;
 var ylda;
  by &GEN;
 output out=vara mean=ydoteye;

data enva;
 merge enva vece;

data vara;
 merge vara vecg;

data envanno(keep=xsys ysys x y color function position size text style);
  length text $ 8;
 set enva;
 text=&ENV;
 style = 'SWISSB';
 xsys='2'; ysys='2'; color='blue'; position='5'; function='label';
 size=1.0;
 x=env_1;
 y=env_2;

proc print;
```

```
  var text x y;

data varanno(keep=xsys ysys x y color function position size text style);
  length text $ 8;
 set vara;
 text=&GEN;
 style = 'ZAPFB';
 xsys='2'; ysys='2'; color='black'; position='5'; function='label';
 size=0.8;
 x=gen_1;
 y=gen_2;

data vecann1;
 set envanno varanno;

data envanno(keep=xsys ysys x y color function position size text style);
  LENGTH TEXT $ 8;
 set enva;
 text=&ENV;
 style = 'SWISSB';
 xsys='2'; ysys='2'; color='blue'; position='5'; function='label';
 size=1;
 x=ydoteye;
 y=env_1;

proc print;
  var text x y;

data varanno(keep=xsys ysys x y color function position size text style);
  length text $ 8;
 set vara;
 text=&GEN;
 style = 'ZAPFB';
 xsys='2'; ysys='2'; color='black'; position='5'; function='label';
 size=1;
 x=ydoteye;
 y=gen_1;

data vecann2;
 set envanno varanno;

data vectors;
 set enva vara;

proc gplot data=vectors;
 symbol1 v=none i=none color=white;

 plot gen_2*gen_1=1 env_2*env_1=1/anno=vecann1 overlay vref=0 href=0 ;
title1 'Biplot of G*E Interaction';

proc gplot data=vectors;
 plot env_1*ydoteye=1 gen_1*ydoteye=1/anno=vecann2 overlay vref=0 ;
title1 'Biplots of First E-Vector vs Means';run;
```

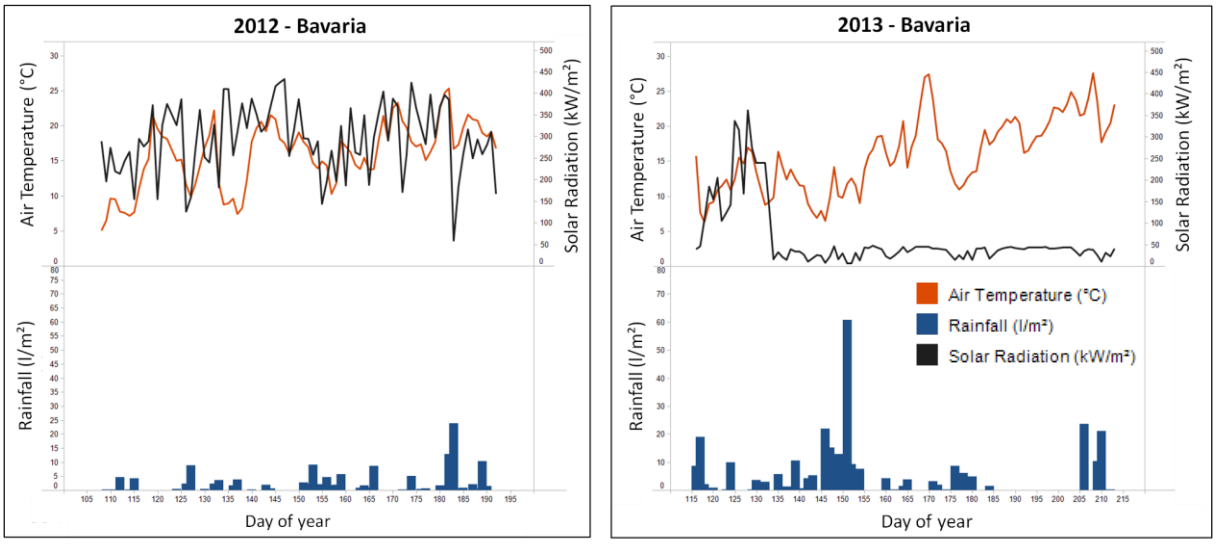


**Fig. 1.** Daily mean air temperatures, total rainfall and total solar radiation for growing season 2012 and 2013 in Bavaria, Location Bavaria (1): Bavaria-Herzogenaurach, Location Bavaria(2): Bavaria-Uffenheim**.**

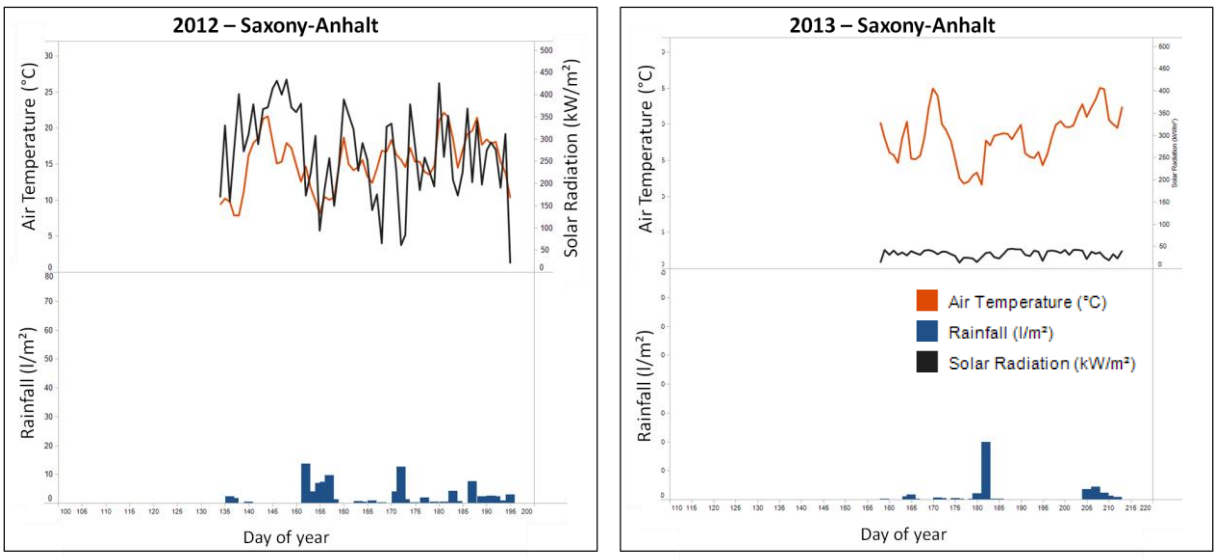


**Fig. 2.** Daily mean air temperatures, total rainfall and total solar radiation for growing season 2012 and 2013 in Saxony-Anhalt.

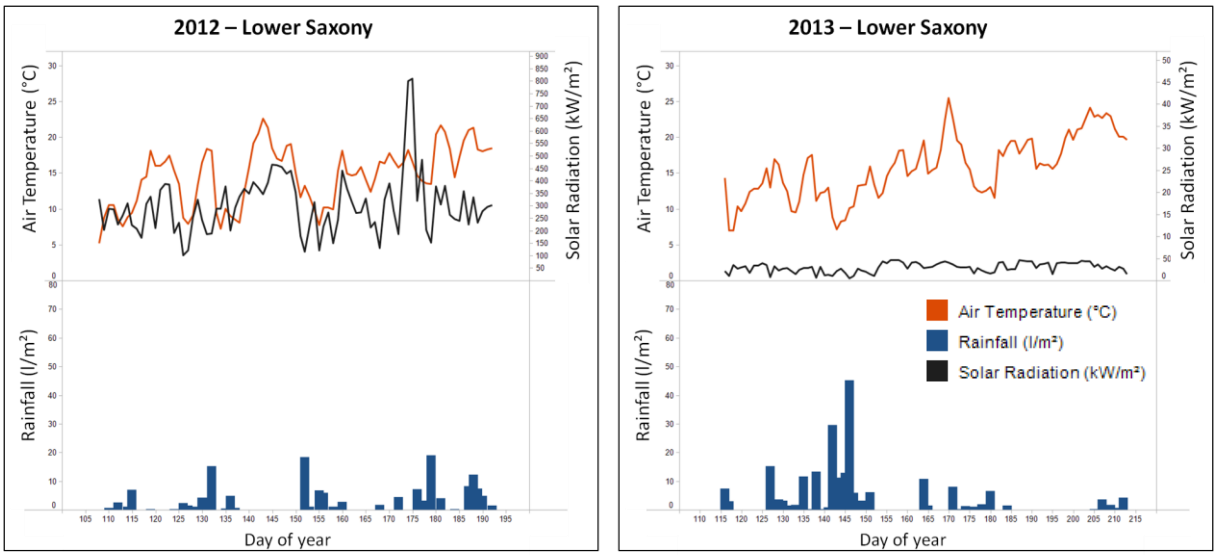


**Fig. 3.** Daily mean air temperatures, total rainfall and total solar radiation for growing season 2012 and 2013 in Lower Saxony.

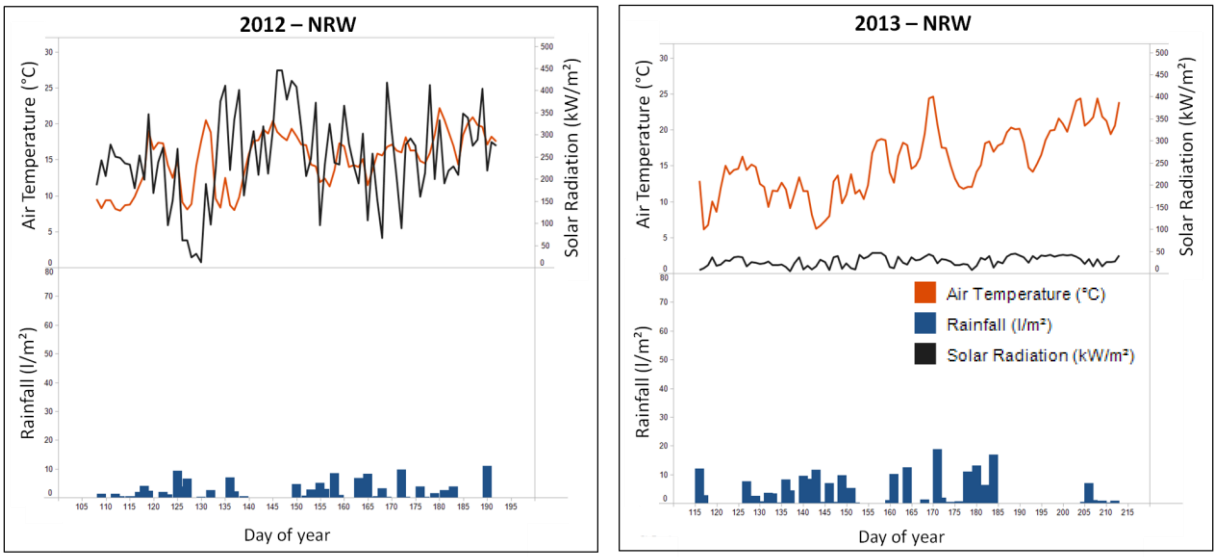


**Fig. 4.** Daily mean air temperatures, total rainfall and total solar radiation for growing season 2012 and 2013 in North Rhine-Westphalia (NRW).

**Table 18** Spearman's correlation coefficient (r) for evaluated plant parameters of 24 spring barley genotypes grown during 2012 and 2013 in eight environments

| | NEM | | NKE | | NKM | | TKW | | YLD | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | | | | | | | | | | |
| **BBCH 30** | - 0.16 | * | 0.44 | *** | 0.14 | | - 0.02 | | 0.07 | |
| **BBCH 63** | 0.09 | | 0.39 | *** | 0.32 | *** | - 0.07 | | 0.27 | *** |
| **BBCH 81** | 0.08 | | 0.38 | *** | 0.29 | *** | - 0.16 | * | 0.21 | ** |
| **PDM** | | | | | | | | | | |
| **BBCH 30** | 0.25 | *** | 0.27 | *** | 0.44 | *** | - 0.04 | | 0.39 | *** |
| **BBCH 63** | 0.40 | *** | 0.27 | *** | 0.63 | *** | - 0.03 | | 0.58 | *** |
| **BBCH 81** | 0.80 | *** | - 0.15 | * | 0.79 | *** | 0.17 | * | 0.82 | *** |
| **CGR 1** | 0.23 | ** | 0.27 | *** | 0.38 | *** | -0.04 | | 0.33 | *** |
| **CGR 2** | 0.39 | *** | 0.27 | *** | 0.66 | *** | -0.02 | | 0.60 | *** |
| **CGR 3** | 0.54 | *** | - 0.15 | * | 0.41 | *** | 0.14 | | 0.45 | *** |
| **CGR 4** | 0.67 | *** | - 0.03 | | 0.66 | *** | 0.17 | * | 0.68 | *** |
| **MGR** | 0.62 | *** | 0.002 | | 0.63 | *** | 0.14 | * | 0.64 | *** |
| **NEM** | 1 | | -0.58 | *** | 0.76 | *** | 0.49 | *** | 0.78 | *** |
| **NKE** | -0.58 | *** | 1 | | -0.14 | | -0.27 | | -0.19 | *** |
| **NKM** | 0.76 | *** | -0.14 | | 1 | | 0.56 | *** | 0.96 | *** |
| **TKW** | 0.49 | ** | -0.27 | | 0.56 | *** | 1 | | 0.73 | *** |
| **YLD** | 0.78 | *** | -0.19 | | 0.73 | *** | 0.73 | *** | 1 | |

Where p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001,Trait: PLH: Plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

**Table 19** Spearman's correlation coefficient (r) for evaluated plant parameters of 8 spring barley genotypes grown during 2012 and 2013 in 4 environments.

| | NEM | | NKE | | NKM | | TKW | | YLD | |
|---|---|---|---|---|---|---|---|---|---|---|
| **PLH** | | | | | | | | | | |
| **BBCH 30** | - 0.33 | | 0.56 | *** | - 0.05 | | 0.17 | | 0.03 | |
| **BBCH 63** | 0.06 | | 0.25 | | 0.12 | | 0.37 | * | 0.23 | |
| **BBCH 81** | - 0.09 | | 0.50 | ** | 0.12 | | 0.12 | | 0.15 | |
| **PDM** | | | | | | | | | | |
| **BBCH 30** | 0.28 | | 0.15 | | 0.39 | * | 0.47 | ** | 0.45 | * |
| **BBCH 63** | 0.37 | * | 0.02 | | 0.54 | ** | 0.52 | ** | 0.59 | *** |
| **BBCH 81** | 0.79 | *** | -0.20 | | 0.81 | *** | 0.69 | *** | 0.87 | *** |
| **PNC** | | | | | | | | | | |
| **BBCH 30** | 0.09 | | - 0.27 | | - 0.15 | | - 0.28 | | - 0.18 | |
| **BBCH 63** | - 0.17 | | 0.15 | | - 0.06 | | - 0.26 | | - 0.10 | |
| **BBCH 81** | 0.21 | | - 0.18 | | 0.05 | | 0.21 | | 0.07 | |
| **PPhC** | | | | | | | | | | |
| **BBCH 30** | - 0.17 | | - 0.22 | | - 0.30 | | - 0.45 | * | - 0.36 | * |
| **BBCH 63** | 0.08 | | - 0.36 | * | - 0.13 | | - 0.34 | | - 0.20 | |
| **BBCH 81** | 0.49 | ** | - 0.45 | * | 0.17 | | - 0.02 | | 0.15 | |
| **PKC** | | | | | | | | | | |
| **BBCH 30** | - 0.25 | | 0.11 | | - 0.16 | | - 0.25 | | - 0.18 | |
| **BBCH 63** | - 0.24 | | 0.10 | | - 0.17 | | - 0.31 | | - 0.20 | |
| **BBCH 81** | 0.13 | | - 0.01 | | 0.21 | | - 0.03 | | 0.22 | |
| **CGR 1** | 0.28 | | 0.07 | | 0.32 | | 0.43 | * | 0.38 | * |
| **CGR 2** | 0.19 | | 0.24 | | 0.50 | ** | 0.34 | | 0.53 | ** |
| **CGR 3** | 0.66 | *** | - 0.28 | | 0.62 | *** | 0.58 | *** | 0.65 | *** |
| **CGR 4** | 0.75 | *** | - 0.20 | | 0.80 | *** | 0.70 | *** | 0.86 | *** |
| **MGR** | 0.69 | *** | - 0.19 | | 0.79 | *** | 0.72 | *** | 0.83 | *** |

Where p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤ 0.001,Trait: PLH: Plant height, PDM: plant dry matter, CGR1: crop growth rate until BBCH 30, CGR2: crop growth rate between BBCH 30 and 63, CGR3: crop growth rate between BBCH 63 and 81, CGR4: crop growth rate until BBCH 81, MGR: mean growth rate, PNC: plant nitrogen content, PPhC: plant phosphor content, PKC: plant potassium content, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

## Appendix Chapter 6

**Table 20** Combined analysis of variance across for morphological and yield related parameters of 24 spring barley genotypes under drought treatment at tillering stage.

| | | p-value | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **PDM** | **NEP** | **NKE** | **NKP** | **TKW** | **YLD** |
| **Treatment** | 1 | 0.0003 | <0.0001 | 0.0122 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Genotype** | 23 | <0.0001 | 0.0140 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Genotype*Treatment** | 23 | 0.1241 | 0.8772 | 0.0007 | <0.0001 | 0.0001 | 0.8071 | 0.0004 |
| **Year** | 1 | <0.0001 | 0.7681 | <0.0001 | 0.0019 | <0.0001 | 0.0036 | <0.0001 |
| **Year*Treatment** | 1 | 0.8819 | 0.0181 | 0.0411 | 0.0107 | 0.3410 | 0.8416 | 0.6645 |
| **Year*Genotype** | 23 | 0.0002 | 0.3004 | 0.0907 | <0.0001 | 0.0037 | 0.5269 | 0.0322 |
| **Genotype*Treatment*Year** | 23 | 0.5592 | 0.5690 | 0.1397 | <0.0001 | 0.0162 | 0.0719 | 0.0988 |

Where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤0.001, DF = degree of freedom, Treatment = well-watered, drought treatment, Year= 2012,2013 Trait: PLH: plant height, PDM: plant dry matter, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield.



**Table 21** Analysis of variance for morphological and yield related parameters of 24 spring barley genotypes investigated in field experiments during 2012 and 2013.

| | | p-value | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Source of variation** | **DF** | **PLH** | **PDM** | **NEM** | **NKE** | **NKM** | **TKW** | **YLD** |
| **Environment** | 1 | <0.0001 | 0.7227 | <0.0001 | <0.0001 | 0.0004 | 0.0037 | <0.0001 |
| **Genotype** | 23 | 0.0001 | 0.0360 | 0.0009 | 0.0214 | <0.0001 | 0.1558 | <0.0001 |
| **Genotype*Environment** | 23 | 0.2444 | 0.9990 | 0.3862 | 0.0002 | 0.0041 | 0.0004 | 0.0394 |

Where: p-value with *: 0.01< P <0.05 level, **: 0.001< P <0.01, *** P ≤0.001, DF = degree of freedom, Treatment = well-watered, drought treatment, Year= 2012,2013 Trait: PLH: plant height, PDM: plant dry matter, NEM: number of ears per square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield.

**Table 22** Correlation analysis between stress tolerance indices (STI), membership function value of drought tolerance (MFVD) and grain yield (YLD) on 24 spring barley genotypes evaluated in pot experiments.

| | **STI $_{PLH}$** | **STI $_{PDM}$** | **STI $_{NEP}$** | **STI $_{NKE}$** | **STI $_{NKP}$** | **STI $_{TKW}$** | **STI $_{YLD}$** | **MFVD** | **YLD - E5** | **YLD - E1** | **YLD - WW** | **YLD - DT** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **STI $_{PLH}$** | 1 | | | | | | | | | | | |
| **STI $_{PDM}$** | 0.40 | 1 | | | | | | | | | | |
| **STI $_{NEP}$** | -0.05 | 0.06 | 1 | | | | | | | | | |
| **STI $_{NKE}$** | 0.19 | 0.29 | **-0.56 **** | 1 | | | | | | | | |
| **STI $_{NKP}$** | 0.05 | 0.24 | **0.63 ***** | 0.07 | 1 | | | | | | | |
| **STI $_{TKW}$** | 0.14 | 0.09 | **-0.46 *** | **0.42 *** | **-0.47 *** | 1 | | | | | | |
| **STI $_{YLD}$** | 0.05 | 0.36 | 0.32 | 0.32 | **0.54 **** | 0.38 | 1 | | | | | |
| **MFVD** | **0.47 *** | **0.77 ***** | 0.22 | 0.36 | **0.56 **** | 0.17 | **0.77 ***** | 1 | | | | |
| **YLD - E5** | 0.10 | 0.03 | 0.24 | -0.19 | -0.07 | 0.06 | -0.02 | -0.07 | 1 | | | |
| **YLD - E1** | -0.28 | 0.29 | 0.15 | 0.06 | -0.04 | 0.23 | 0.30 | 0.12 | 0.37 | 1 | | |
| **YLD - WW** | 0.15 | **0.50 *** | -0.29 | 0.13 | -0.29 | 0.31 | 0.10 | 0.28 | 0.13 | **0.41 *** | 1 | |
| **YLD - DT** | 0.12 | 0.11 | -0.03 | 0.17 | -0.20 | 0.24 | -0.04 | -0.04 | 0.10 | 0.15 | 0.14 | 1 |

Where STI: stress tolerance index, PLH: plant height, PDM: plant dry matter, NEP: number of ears per plant, NKE: number of kernels per ear, NKP: number of kernels per plant, TKW: thousand kernel weight, YLD: grain yield, MFVD: membership function value of drought tolerance, YLD – E5 grain yield in Environment 5, YLD - E1: grain yield in Environment 1, YLD - WW: grain yield in pot experiments under well-watered conditions, YLD - DT: grain yield in pot experiments under drought treatment.

**Table 23** Correlation analysis between stress tolerance indices (STI), membership function value of drought tolerance (MFVD) and grain yield (YLD) on 24 spring barley genotypes evaluated in field experiments.

| | **STI $_{PLH}$** | **STI $_{PDM}$** | **STI $_{NEM}$** | **STI $_{NKE}$** | **STI $_{NKM}$** | **STI $_{TKW}$** | **STI $_{YLD}$** | **MFVD** | **YLD - WW** | **YLD - DT** | **YLD - E5** | **YLD - E1** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **STI $_{PLH}$** | 1 | | | | | | | | | | | |
| **STI $_{PDM}$** | 0.12 | 1 | | | | | | | | | | |
| **STI $_{NEM}$** | -0.36 | 0.27 | 1 | | | | | | | | | |
| **STI $_{NKE}$** | 0.23 | **-0.46 *** | **-0.76 ***** | 1 | | | | | | | | |
| **STI $_{NKM}$** | -0.36 | -0.04 | **0.59 **** | 0.01 | 1 | | | | | | | |
| **STI $_{TKW}$** | 0.03 | 0.10 | -0.23 | -0.21 | **-0.43 *** | 1 | | | | | | |
| **STI $_{YLD}$** | -0.38 | -0.08 | 0.38 | 0.07 | **0.85 ***** | -0.02 | 1 | | | | | |
| **MFVD** | -0.11 | **0.47 *** | 0.32 | -0.02 | **0.63 **** | -0.07 | **0.74 ***** | 1 | | | | |
| **YLD - WW** | 0.03 | -0.27 | 0.01 | 0.16 | 0.30 | 0.21 | **0.43 *** | 0.13 | 1 | | | |
| **YLD - DT** | 0.28 | 0.06 | 0.21 | 0.01 | 0.40 | -0.13 | 0.28 | 0.23 | 0.14 | 1 | | |
| **YLD - E5** | -0.15 | 0.05 | -0.07 | 0.40 | **0.56 **** | 0.01 | **0.77 ***** | **0.75 ***** | 0.13 | 0.10 | 1 | |
| **YLD - E1** | **-0.58 **** | -0.02 | **0.66 ***** | -0.29 | **0.78 ***** | -0.02 | **0.84 ***** | **0.58 **** | **0.41 *** | 0.15 | 0.37 | 1 |

Where STI: stress tolerance index, PLH: plant height, PDM: plant dry matter, NEM: number of square meter, NKE: number of kernels per ear, NKM: number of kernels per square meter, TKW: thousand kernel weight, YLD: grain yield, MFVD: membership function value of drought tolerance, YLD – E5: grain yield in Environment 5, YLD - E1: grain yield in Environment 1, YLD - WW: grain yield in pot experiments under well-watered conditions, YLD - DT: grain yield in pot experiments under drought treatment.

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**Eidesstattliche Erklärung**

Hiermit erkläre, dass ich die vorliegende Arbeit selbständig von mir verfasst wurde und keine anderen als die gekennzeichneten Quellen verwendet wurden.

Die Arbeit wurde an keiner anderen Stelle als Prüfungsleistung vorgelegt.