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Water Relations and Drought Tolerance of Different *Zea mays* Cultivars as Influenced by Nitrogen Form and Application

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ABSTRACT

“Water Relations and Drought Tolerance of Different *Zea mays* Cultivars as Influenced by Nitrogen Form and Application”

This study was conducted to describe the physiological and morphological factors controlling the water use efficiency of maize (*Zea mays* L.) plants under drought conditions. The effects of localized ammonium (CULTAN) and broadcasted nitrate fertilization, two different forms of nitrogen nutrition on differently drought tolerant maize varieties were investigated. Greenhouse and field experiments were carried out in 2007 and 2008 using a randomised complete block design with eight and three repetitions in the greenhouse and the field, respectively. In 2007, three cultivars from Nicaragua and the European cultivar *Permanent* were used, while in 2008 four European cultivars (including *Permanent*) were chosen, according to different properties communicated by the breeder. Two water regimes were applied in the greenhouse. In the field, irrigation was installed on half of the plots. Due to abundant rainfall in 2007, no drought stress developed. This was different in 2008, enabling the establishment of two different water regimes in the field experiment.Growth and dry matter partitioning were monitored, gas exchange was regularly assessed by the measurement of light curves, and water status parameters (water potential, osmotic potential, turgor pressure and relative water content) were measured. The hydraulic conductance of the shoot was assessed by the measurement with a High Pressure Flow Meter (HPFM).

The results demonstrate different adaptability of the cultivars to NH_4^+ and NO_3^- nutrition and to drought conditions in both, greenhouse and field experiment. The NH_4^+ plants showed higher photosynthetic rates compared to NO_3^- plants when the photosynthetic photon flux density increased. The well-watered plants also showed higher photosynthetic rates compared to the drought-stressed plants in both, 2007 and 2008.

The parameters of leaf water relations were generally affected by the water regime, but not by the nitrogen form. Hydraulic conductivity was influenced by nitrogen form, water regime, and cultivar. Nutrader, Vitaminado and Permanent (greenhouse, 2007), Nutrader, Vitaminado and Mazorca de oro (field, 2007), and Permanent, Abakus and Mazurka (greenhouse and field, 2008) showed higher hydraulic conductance with CULTAN compared to nitrate, whereas higher hydraulic conductance with nitrate compared to CULTAN was only found for Aalvito in the greenhouse experiment of 2008.

Under well-watered conditions, all cultivars showed higher above-ground dry matter production with ammonium compared to nitrate in the greenhouse experiment of 2007, while no significant differences were found in the field experiment. In 2008 and under well watered conditions, the total dry matter production of the NH_4^+ plants was 19% higher compared to the NO_3^- plants. Dry matter production under well-watered conditions was superior by 34% for NH_4^+ plants and by 11% for NO_3^- plants, respectively, compared to drought-stressed conditions. Under well-watered conditions, the grain yield of NH_4^+ treated plants of the cultivars Permanent, Aalvito and Mazurka was higher (18-22%) compared to NO_3^- fertilization. The yield reduction under drought, however, was more pronounced (20-36%) for the NH_4^+ plants than for the NO_3^- plants (16-18%), with Aalvito and Abakus having higher yields with nitrate compared to ammonium. There was no yield reduction by drought for the Abakus cultivar fertilized with nitrate. The superiority of the NH_4^+ plants in grain yield was attributable to higher numbers of grain per ear.

Protein content was not affected by the water regime. Aalvito had higher corn protein content with CULTAN (20%) compared to nitrate. Abakus and Mazurka showed the highest corn protein content of all cultivars, but no difference between CULTAN and nitrate fertilization.

The results give a differentiated view of the ecophysiological adaptations of *Zea mays* cultivars to drought and the possibilities to improve their water-use efficiency by nitrogen fertilization.

ZUSAMMENFASSUNG

“Wasserhaushalt und Trockentoleranz verschiedener Mais-(*Zea mays*) Sorten in Abhängigkeit von Form und Einbringung der Stickstoffdüngung”

Ziel der vorgestellten Untersuchungen war die Beschreibung physiologischer und morphologischer Faktoren von Mais (*Zea mays* L.) unter Trockenheitseinfluss, unter besonderer Berücksichtigung der Wassernutzungseffizienz. Der Einfluss lokalisierter Ammoniumdüngung (CULTAN) und breitwürfiger Nitratdüngung auf unterschiedlich trockenheitstolerante Maissorten wurde verglichen. Hierzu wurden in den Jahren 2007 und 2008 Topfversuche im Gewächshaus sowie Feldversuche durchgeführt, unter Verwendung eines vollständig randomisierten Blockdesigns mit acht bzw. drei Wiederholungen. Im Jahr 2007 wurden drei Sorten aus Nicaragua sowie die europäische Sorte Permanent verwendet. 2008 wurden drei weitere, nach den Sortenbeschreibungen des Züchters ausgewählte europäische Sorten und wieder Permanent verwendet. In den Topfversuchen wurden zwei Wasserversorgungsstufen eingestellt. Im Freiland wurde auf der Hälfte der Versuchsplots eine Bewässerung installiert, es stellte sich allerdings 2007 aufgrund ausgiebiger Regenfälle keine Trockenheit ein. Dagegen trat 2008 gelegentlich Trockenstress auf, so dass auch im Feldversuch zwei Wasserversorgungsstufen verglichen werden konnten. Neben der Erfassung von Wachstumsparametern und Trockenmasseverteilung wurde regelmäßig der Gasaustausch bei Anwendung von Lichtkurven sowie weitere Wasserhaushaltsparameter (Gesamtwasserpotential, osmotisches und Turgorpotential, relativer Wassergehalt) gemessen. Die hydraulische Leitfähigkeit des Sprosses wurde durch Messung mit einem High Pressure Flow Meter (HPFM) ermittelt.

Die verschiedenen Sorten zeigten unterschiedliche Reaktionen auf Ammonium- und Nitraternährung und Unterschiede in der jeweiligen Anpassungsfähigkeit an Trockenheit, sowohl im Topf- als auch im Feldversuch. Die Photosyntheseraten bei ansteigendem Licht waren generell bei CULTAN-Pflanzen höher als bei Pflanzen mit Nitratdüngung, ebenso waren sie bei guter Wasserversorgung generell höher als in den Trockenvarianten.

Die Wasserhaushaltsparameter wurden allgemein durch die Wasserversorgung beeinflusst, nicht hingegen durch die verwendete Stickstoffdüngung. Sortenabhängig kam es zu Unterschieden der hydraulischen Leitfähigkeit bei unterschiedlicher Stickstoffdüngung und Wasserversorgung. Die hydraulische Leitfähigkeit bei guter Wasserversorgung war signifikant höher bei CULTAN-Pflanzen der Sorten Nutrader, Vitaminado und Permanent (Topfversuch 2007), Nutrader, Vitaminado und Mazorca de oro (Feldversuch 2007), sowie Permanent, Abakus und Mazurka (Topf- und Feldversuch 2008), jeweils verglichen zur Nitratbehandlung. Dagegen zeigte nur Aalvito 2008 im Topfversuch höhere hydraulische Leitfähigkeit mit Nitrat als mit CULTAN.

Bei guter Wasserversorgung zeigten im Gewächshausexperiment 2007 alle Sorten mit CULTAN-Versorgung höhere Trockenmasseproduktion als mit Nitratdüngung, dagegen traten hierzu im Feldversuch keine signifikanten Unterschiede auf. 2008 war die Trockenmasse der CULTAN-Pflanzen bei guter Wasserversorgung im Feldversuch um 19% höher als bei Nitratdüngung. Bei guter Wasserversorgung war die Trockenmasse für CULTAN-Pflanzen um 34%, bei Nitratdüngung um 11% höher als in der Variante mit gelegentlichem Trockenstress. Die CULTAN-Pflanzen der Sorten Permanent, Aalvito und Mazurka hatten bei guter Wasserversorgung einen gegenüber Nitratdüngung um 18 – 22% höheren Kornertrag als die nitratgedüngten Pflanzen. Bei Trockenheit war jedoch die Ertragsreduktion stärker bei den CULTAN-Pflanzen jedoch stärker (20-36%) als bei nitratgedüngten Pflanzen (16-18%), so dass zwei Sorten (Aalvito, Abakus) mit Nitratdüngung höhere Erträge zeigten als die CULTAN-Varianten. Die Nitratvariante der Sorte Abakus zeigte als einzige unter Trockenheit keinen Rückgang des Kornertrags gegenüber Bewässerung. Die bei guter Wasserversorgung höheren Kornerträge der CULTAN-Pflanzen ergaben sich durch höhere Kornzahlen pro Kolben.

Die Wasserversorgung hatte keine Auswirkungen auf die Proteingehalte. Die Sorte Aalvito hatte mit CULTAN einen gegenüber Nitratdüngung um 20% höheren Proteingehalt. Abakus und Mazurka hatten insgesamt die höchsten Proteingehalte, zeigten allerdings keinen Unterschied zwischen CULTAN und Nitratdüngung.

Somit ergibt sich ein differenziertes Bild, in welchem Maße die natürliche ökophysiologische Anpassung von Maispflanzen an Trockenheit durch Anwendung von Stickstoffdüngern zur Verbesserung der Wassernutzungseffizienz unterstützt werden kann.

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ABBREVIATIONS

ABA	Abscisic acid	
ATP/NADPH	Adenosine-triphosphate/Nicotinamide Adenine Dinucleotide Phosphate Hydrogen	
A_{net}	net photosynthetic rate	µmol m^{-2} s^{-1}
ANOVA	analysis of variance	
Ci	partial pressure of intercellular CO_2	µmol mol^{-1}
CV	coefficient of variation	%
E	transpiration	mmol m^{-2} s^{-1}
g_s	stomatal conductance	mol m^{-2} s^{-1}
g	gram	
HI	harvest index	%
HD	ammonium and drought-stressed	
HW	ammonium and well watered	
IS	intermediate stress	
kg	kilogram	
Kstem	Stem hydraulic conductance	kg s^{-1} m^{-2} Mpa^{-1}
cm	centimeter	
DCD	dicyandiamide	
m	meter	
mm	millimeter	
md	midday	
ml	mililiter	
MPa	MegaPascal	
Nis	nitrification inhibitor	
n.s.	no significant	
OD	nitrate and drought-stressed	
OW	nitrate and well watered	
PAR	Photosynthetically active radiation	µmol photons m^{-2} s^{-1}
pd	predawn	
PPFD	photosynthetic photon flux density	µmol photons m^{-2} s^{-1}
ρ	turgor potential MPa	
PS II	photosystem II	
PS I	photosystem I	
π	osmotic potential Mpa	
QPM	Quality Protein Maize	
RWC	relative leaf water content %	
Sd	stem diameter	mm

SD	standard deviation	
SS	drought stress	
WUE	water use efficiency	mmol mol^{-1}
WW	well water	

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1. INTRODUCTION

1.1 Background and problem statement

Maize is the most important cereal crop for food in sub-Saharan Africa and Latin America, and a key feed crop in Asia. Declining soil fertility and environmental stresses affect crop production and health in less developed regions. Climate change and degraded soils threaten the food security of millions, especially in the development countries. (Cimmyt, 2008).

Global warming is likely to increase the incidence of drought in many established maize growing areas (Zaidi 2002). Crosson and Anderson (1992) concluded that certainly maize production productivity will be affected by doubling of CO_2. Allen (1990) concluded that with increase in level of CO_2 concentration to 800 ppm canopy temperatures would rise by 4^0C leading to a decline in water use efficiency in C_4 species like maize. Rosenzweig *et al.* (1995) findings revealed that crop production would decline by 9-10% in tropic and subtropics. However, there are large uncertainties as to when and where climate change will impact on agriculture production and food security (IPCC, 2007). It is generally agreed that agricultural impacts will be more adverse in tropical areas than in temperate areas (IPCC, 2007). Due to the prediction of global climatic change with its serious impacts on water resources and agriculture (IPCC 2007); Significant yield losses in maize (*Zea mays* L.) from drought are expected to increase as temperatures rise and rainfall distribution changes in key traditional production areas (Campos et al. 2004). El-Niño effects will further increase, as realized in recent past. El-Niño is defined as a “large scale shift in water currents and winds of the equatorial and tropical Pacific, resulting in extreme climatic changes characterized by excessive rains and strong winds in some areas and drought in others” (Tibig, 1995).

Drought is one of three abiotic factors, most responsible for limiting maize production and productivity in the developing world; other two are the problems of waterlogging and low soil fertility. The tendency of growing maize in poor & marginal areas subject to the vagaries of rainfall and abnormal rainfall distribution pattern is thought to be a major reason for slow diffusion of improved varieties and crop management practices. Keeping in view the fact that global climate change is underway, intensity and frequency of disturbances like (Zaidi, 2002).

Global estimate about annual losses of maize production due to problem of drought in early 1990s across the non-temperate maize areas totaled about 19 million tons, representing about 15% reduction in production (Edmeades *et al.*, 1992). Such losses can be far more extreme; a devastating situation of drought stress in South Africa in 1991-92 reduced maize production by about 60% (Rosen and Scott, 1992) and in Nicaragua by about 70% in some regions (MAG-FOR; 2006). A number of abiotic factors such as drought, low soil fertility, excess moisture, soil acidity, persistent of weed problems, soil acidity or salinity, calcareous soils, step slopes etc. has been observed widely in maize growing areas on these regions (Zaidi, 2002).

Most maize in developing countries is produced under low N conditions because of low N status of tropical soils, low N use efficiency in drought-prone environments, high price ratios between fertilizer and grain, limited availability of fertilizer, and low purchasing power of farmers. One of the factors influencing physiological responses of plants to water stress is mineral nutrition. A significant role of nitrogen in regulating plant responses to water stress was established in a number of plant species. In some plants high nitrogen caused higher sensitivity towards drought (Bennet et al., 1986; Morgan, 1986; Tesha and Kumar, 1978). The mechanism by which nitrogen modifies plant responses to drought is still unclear. It has been suggested that nitrogen affects osmotic regulation, cell wall elasticity, carbohydrate metabolism and synthesis of drought-induced signal substances in roots (Morgan, 1986; Ogren, 1985). However, there is no evidence of an influence of nitrogen form on plant response to water stress. It is well known that plant metabolism depends to some extent on the ionic form in which nitrogen is absorbed. The uptake process and the sites of assimilation of NH_4 and NO_3 ions are different (Bloom, 1988). The two ions also have different influences on transport of other ions, as well as on metabolism and transport of amino- and organic acids (Allen and Raven, 1987; Arnozis and Findenegg, 1986; Van Beusichem et al., 1988). Ammonium and nitrate as different forms of nitrogen nutrients impact differently on some physiological and biochemical processes in the plants (Guo et al. 2006). The effect of nitrogen form on water uptake and transportation in plants is dependent both on leaf area or shoot parameter, and on the root activity (i.e., root hydraulic conductivity, aquaporin activity). Considering the photosynthetic processes associated with nitrogen form, according to Guo et al. (2006) the water use efficiency of the plants my be increase by nitrogen management, as the localized ammonium applications by CULTAN-technique or the releasing fertilizers in combination with nitrification inhibitors (Gerendás et al., 1997).

For improved production and productivity under drought-affected areas it is necessary to not to lose any option (Waddington and Heisey, 1997), therefore an integrated approach considering all technological option available both genetic and crop management practices could be more beneficial and effective, rather than either of them (Zaidi, 2002).

CULTAN (Controlled Uptake Long Term Ammonium Nutrition) is one cropping system, where the nitrogen nutrition source for the plants is ammonium instead of nitrate as common. Ammonium is injected in liquid form 4-10 cm deeper, depending on the plant system, than the furrow at the root level (Kuecke, 2001). This relatively new plant nutrition method is believed to create more vital plant development in addition to its benefit hindering nitrate leaching and reducing plant nutrition costs.

Based on the possible increased water use efficiency and the reported better nitrogen use efficiency (Sommer, 2000) of ammonium nutrition compared to nitrate (Guo et al. 2006), the aim of this study was to highlight the drought tolerance mechanisms of maize and to evaluate the inputs of CULTAN nutrition on the water relations of maize.

1.2 HYPOTHESIS AND OBJECTIVES

Hypothesis

Different nitrogen forms have different effects on gas exchange parameters, such as stomatal conductance, intercellular CO_2 partial pressure, and transpiration rate; therefore, ammonium nutrition positively influences water relations of maize cultivars under drought conditions.

Objectives

General objective

The overall objective of this study was to describe the physiological and morphological factors that control the water use efficiency in the maize under drought conditions, comparing different drought tolerant and non-tolerant varieties and different N-fertilization form: Localized ammonium and nitrate nitrogen nutrition.

Specific objectives

1. To elucidate the reaction of different maize cultivars to drought stress by measuring the leaf gas exchange parameters, hydraulic conductance of stem and roots, and the plant growth.

2. To compare ammonium and nitrate nutrition in different maize cultivars, focussing on: water use, hydraulic architecture and C allocation.

3. To relate the behaviour of the different varieties based on the results from the evaluations of the morphological and physiological properties with the purpose of generating adequate production alternatives for zones subject to drought, especially in Nicaragua and other Central American countries.

1. LITERATURE REVIEW

2.1 Growth and development of maize crop *(Zea mays L).*

Maize growth and development happen during a year or part of a year depending on ambient conditions and the maturity group of the cultivated variety (Salvador, 1997, FAO, 1993). Maize is a plant prefering high temperature plant, so this crop needs enough temperature for good development. This phenological phase starts at the appearance of the first leaf and end at the appearance of the tassel (FAO, 1993), this phase is fast enough and needs enough nitrogen and water (FAO, 1993). The development of the plant may be divided into two physiological stages. In the first or the vegetative stage, different tissues develop and differentiate until the flower structures appear. The vegetative stage is made up of two cycles. In the first cycle the first leaves are formed and development is upward. Dry matter production in this cycle is slow. It ends with the tissue differentiation of the reproductive organs. In the second cycle the leaves and reproductive organs develop. This cycle ends with the emission of the stigmas (FAO, 1993).

The second stage, also known as the reproductive stage, begins with the fertilization of the female structures, which will develop into ears and grains. The initial phase of this stage is characterized by an increase in the weight of leaves and other flower parts. During the second phase, the weight of the kernels increases rapidly (Tanaka and Yamaguchi, 1972).

Tassel is the male reproductive part of the maize plant. Tassel development needs water and nutrient supply more than vegetative growth, but less than ear development. Tassel is not the most important part of the maize plant, the maize can develop a good yield without a tassel, but without pollen, born from the tassels there is no yield (FAO, 1993).

The ear is the most important part of the maize. It solves the yield of the plant. Ear development begins in the last quarter of the vegetative growth (FAO, 1989). The potential number of ears is genetically determined; the actual number depends on environmental circumstances (Wang and Fields, 1978). Ear development and blossoming are the most nutrient and water-demanding processes. The blossom needs more water, so the most water-demanding period in the life of the plant is the ear blossom (Aguirre et al. 1953). After the ear blossom, the maize plant fills the kernels and its water content slowly decreases. While filling the kernels proceeds, strength development depends on the summer meteorological conditions. When the summer is too hot and there is no water, the plant dies before kernels are filled and the yield reduces seriously. When the water content is low enough, it is the best way for harvest (FAO, 1993).

2.2 Influence of drought on the different crop stages on maize crop

Alhough, every crop stage of maize has some susceptibility to drought, three stages, i.e.- early growth stage (when plant stand are established), flowering and mid-to-late grain filling stage, are considered critical stages for maize plant to drought (Zaidi, 2002). By affecting the plant stand at the beginning of the growing season, drought can strongly curtail yield. Drought at flowering can be devastating since maize is particularly susceptible to drought at this stage. Grain yield reduction by drought during mid- to late grain filling is relatively less compared to the other two stages. Domingo (1953) first quantified the large reductions that occur when drought stress coincides with the flowering period. A decrease in water availability to permanent wilting point during pre-flowering, flowering and post-flowering stages reduced grain yield by 25, 50 and 21%, respectively (Denmead and Shaw, 1960). Yield reduction as high as 90% and an incidence of barrenness reaching 77% were recorded by Nesmith and Retchie (1992) when plants were stressed in the interval from just prior to tassel emergence to the beginning of grain filling. According to Westgate (1997) maize is thought to be more susceptible to drought than other crops during three weeks bracketing flowering stage because:

i-Its florets develop virtually simultaneously and are usually borne on a single ear on a single stem. ii-Drought affects rate of photosynthesis, therefore reduced supply of current assimilate. ii-Drought at flowering also reduces the capacity of developing kernels to use available assimilates, because the functioning of a key enzyme, acid invertase, is impaired.

Once the kernels enter the linear phase of biomass accumulation about 2-3 week after pollination, they develop the capacity to access reserve assimilates in different plant parts. Therefore they can normally grow to at least 30% of the weight of kernels on unstressed plants, even though drought may become more severe (Bolaños and Edmeades, 1996). In general maize needs at least 500 to 700 mm distributed affluent precipitation during the cycle of culture, but this amount can vary depending on the quality of the soil. Cultivars differ in the time needed for ripening, which implies different water requirements depending on the duration of their growth cycle. As a rule of thumb the maize demands water in the order of 5 mm to the day. (Reta and Faz 1999).

During vegetative growth, more water is required and it is recommended to give irrigation about 10 to 15 days before the flowering. The flowering phase is the most critical period as already outlined above, reason why irrigations are advised to maintain soil humidity and alloweffective pollinization. Finally, for kernel growth and cob maturation, the applied amount of water may be reduced. Reta and Faz (1999) found that the best response of maize in terms of grain yield and use of the evapotranspired water was obtained when the crop had was kept well watered in the beginning of the differentiation phases of reproductive organs (35 to 51 days after the emergence), beginning cob growth (52 to 65 dap), growth of stigmata (65 to 69 days after the emergence), and milky grain (85 to 120 days after the emergency).

2.3 Physiology of drought tolerance

A plant experiences drought when demand from above ground plant parts for water exceeds the supply from the roots (Zaidi, 2002). Water uptake by plants is explained by the concept of water potential within a soil-plant-atmosphere continuum (SPAC), where the water flux depends on respective gradients of potential between these three components. At the time of water scarcity drought stress may therefore develop differently in different plant organs along this gradient. It is essential for any crop improvement program focusing on dry zones to understand the basic mechanisms for drought tolerance on individual plant and crop level. (Zaidi, 2002).

Maize responds to progressive drought with a reduction in assimilate production (Jones et al., 1986; Hirasawa and Hsiao, 1999), a reduction in leaf extension (Ben and Tardieu, 1997) and ultimately with a reduction in grain yield (Balaños and Edmeades, 1993). Variations in soil moisture can change root physiology and thereby enable plants to sense soil water status and adapt to decreasing soil moisture content by reducing growth, transpiring leaf surface and stomatal aperture (Hartung and Jeschke, 1999; Dodd et al., 1996). Those responses reflect decreasing plant available soil moisture and the plant's need to control and decrease its water use. Increasing soil moisture deficit was accompanied by relative changes in xylem nitrate concentration, xylem ABA concentration and early morning leaf water potential and stomatal conductance (Bahrung et al. 2001). Additionally, withholding irrigation immediately caused a small but significant increase in xylem pH. Xylem pH of drought-stressed plants was on average 0.2 units more alkaline than that of control plants throughout the drying cycle, but it was not significantly correlated with increasing soil moisture deficit (Bahrung et al, 2001).

Transpiration and stomatal conductance are sometimes taken almost synonymously in the literature. This is problematic under tropical conditions with often prevailing low wind speeds. Porometric estimates of transpiration, obtained as the product of the measured stomatal conductance and the leaf-bulk air vapour pressure deficit, without taking the boundary layer into account, may be up to 300% higher than the actual transpiration determined from sap flow measurements (Meinzer et al., 1995).

Two factors most efficiently control plant water losses: (1) the actual transpiring surface and (2) stomatal aperture (Kramer and Boyer, 1995). It has been shown that increasing drought rapidly leads to an inhibition of leaf extension (Ali et al., 1998; Bacon et al., 1998; Tardieu et al., 2000); thus, an active reduction in the transpiring leaf surface. Stomatal closure as a response to decreasing soil moisture availability has been observed even before the plant water status was affected (Tardieu et al., 1992; Ali et al., 1999).

Abiotic and biotic stresses cause alterations in the normal physiological processes of all plant organisms, including the economically important crops. Plant damage and decrease in their productivity take place most often due to naturally occurring un-favourable factors of the environment (natural stress factors) - extreme temperatures; water deficit or abundance; increased soil salinity; high solar irradiance; early autumn or late spring ground frosts; pathogens etc (Lichtenthaler 1998, Levitt 1980). Recently, along with these factors plant organisms are imposed to a large scale of new stressors related to human activity (anthropogenic stress factors) – toxic pollutants such as pesticides, noxious gasses (SO_2, NO, NO_2, O_x O_3 and photochemical smog); photo oxidants; soil acidification and mineral deficit due to acid rains; overdoses of fertilizers; heavy metals; intensified UV-B irradiation etc. All these stresses decrease the biosynthetic capacity of plant organisms, alter their normal functions and cause damages, which may lead to plant death (Lichtenthaler 1998, Levitt 1980).

2.4 Effects of drought at the cellular level in maize

2.4.1 Abscisic acid (ABA) accumulation: ABA is generated mainly in the roots, where it stimulates growth. It passes to leaves (and grain to a much lesser degree) where it causes leaf rolling, stomatal closure and accelerates leaf senescence. This happens even before hydraulic mechanisms reduce leaf turgor (Zhang et al. 1987). It seems likely that it is this "root signal" that causes the plant to reduce water loss. Thus, ABA is a plant growth regulator that helps the plant to survive drought stress but it does help to maintain grain yields (Zaidi, 2002). ABA is translocated to the grain as well, where it contributes to the abortion of tip grains during grain filling and may accelerate the ripening process (Goldbach 1975, Goldbach & Michael 1976, Goldbach & Goldbach 1977) Under mild to moderate stress, cell expansion is inhibited. This manifests itself in reduced leaf area expansion, followed by reduced silk growth, then reduced stem elongation, and finally reduced root growth, as stress intensifies (Zaidi, 2002).

Abscisic acid (ABA), proposed as a common mediator of plant stress responses (Quarrie and Jones, 1977), is associated with cold (Chen and Li, 1983), salt (La Rosa et al., 1985), drought (Innes et al., 1984) and heat tolerance (Hiron and Wright, 1973). According to Skriver and Mundy (1990) plant stress can also be induced by exogenous ABA.

A family of dehydrins accumulates in a wide range of plant species in response to dehydration stresses and ABA treatment. A novel property of some ABA-responsive proteins (Jacobsen and Shaw, 1989) and dehydrins (Close et al., 1989) is their resistance to heat induced coagulation. It has been hypothesized (Robertson et al., 1994) that the role of these unique proteins is to function in combination with cell osmolytes, such as sucrose, to prevent denaturation and coagulation of cellular proteins and membrane under stress conditions. Ivanov et al. (1995) showed that exogenous ABA induced protection of PS2 against photoinhibition in barley seedlings and this effect was accompanied by higher photochemical quenching.

As with many other stresses, growing plants at high temperatures increased their ABA content and often conjugated ABA as well (Daie et al., 1981). It was shown by Robertson et al. (1994) that the bromegrass cell suspension culture in medium, containing 75µM ABA, without prior heat treatment had a 87% survival rate, as determined by regrowth analysis following exposure to 42°C for 120min. In contrast, less than 1% of the control cells survived during this heat treatment. It was also shown that sucrose (8%), in combination with ABA-responsive heat stable proteins, is most effective in conferring heat stability (Yordanov, 1995). Under severe drought stress, cell division is inhibited, so even if the stress is alleviated, the affected organs lack the cells for full expansion (Zaidi, 2002).

Abscisic acid (ABA) is a phytohormone regulating several important plant processes of varying time response (Zeevaart et al. 1991, Mäntylä et al. 1995, Leung and Giraudat 1998), especially plant adjustment to water stress. Abscisic acid concentration [ABA] is higher and stomatal conductance is lower in water-stressed plants than in well-watered plants (Blake and Ferrell 1977, McMichael and Hanny 1977, Radin 1984, Tardieu et al. 1996). Also, stomata generally close faster after a decrease in leaf water potential in drought-exposed plants than in well-watered plants (Ackerson 1980, Hartung and Davies 1991, Chandler and Robertson 1994, Comstock and Mencuccini 1998). Although there have been few studies on the response of stomata to an increase in leaf water potential, evidence suggests that, compared with well-watered plants, stomatal opening is slower after rewatering in water-limited plants (Cowan and Farquhar 1977, Ludlow et al. 1985, Saliendra et al. 1995, Fang et al. 1996). Differences among species in stomatal conductance (e.g., Körner et al. 1979, Körner 1994) and stomatal response kinetics (Woods and Turner 1971, Davies and Kozlowski 1975) are well documented. However, because most studies of ABA have been carried out with drought-exposed and well-watered plants of the same species, the relationship between interspecific variation in foliar [ABA] and species differences in maximum stomatal conductance and the dynamics of stomatal response has not been clarified (Henson et al. 1989, Liang et al. 1997). Quarrie (1991) compared several lines of the same species and reported that stomatal conductance was lower in the variety with the highest leaf [ABA]. In maize the leaf ABA content in drought stressed plants increased by 100-200 ng g^{-1}, coinciding with a decrease in stomatal conductance before any significant decrease in midday leaf water potential (Bahrun et al. 2001). Understanding the determinants of the rate of stomatal opening is important when simulating foliar carbon gain in fluctuating environmental conditions, especially in fluctuating light environments. Because the activation of the biochemical reactions of photosynthesis is faster than stomatal opening, foliar lightfleck-use efficiency primarily depends on the kinetics of stomatal movements (Tinoco-Ojanguren and Pearcy 1993, Pearcy 1994). Aasamaa et al. (2001) demonstrate that leaf [ABA] is correlated with species-specific values of stomatal conductance, stomatal sensitivity to an increase in leaf water potential, shoot hydraulic conductance and photosynthesis. Drought-tolerant plants of several maize cultivars accumulate more ABA or increase production of ABA more rapidly than drought-sensitive cultivars (Larque-Saavedra and Wain 1974).

2.4.2 Osmotic adjustment: Most species are able to form osmotically active substances in the cytoplasm and vacuole, in response to drought stress. This allows the plant to take up more soil water and maintains turgor and cell function for a longer time under drought. Osmotic adjustment is particularly apparent in sorghum, wheat and rice [the increase in negativity in ψ_s (osmotic or solute potential) is from 1 to 1.7 MPa], but is much less in maize i.e.-0.3-0.5 MPa (Bolaños and Edmeades 1991).

Accumulation of proline has often been observed under severe drought. It may act as an osmolyte and/or protect protein structures, as turgor is lost. Drought affects photosystem II more than photosystem I in the photosynthetic mechanism (Zaidi, 2002). They become uncoupled, resulting in free, high-energy electrons in the leaf. Uncoupled electron transport leads to photo-oxidation of chlorophyll and loss of photosynthetic capacity (Westgate 1997). A very obvious bleaching of leaves exposed directly to the sun under drought stress can be observed. The enzyme activity is in general reduced under drought. For example, the conversion of sucrose to starch in the grain decreases because the activity of acid invertase, a key enzyme that converts sucrose to hexose sugars, diminishes (Westgate 1997).

2.4.3 Effects at whole plant level: According to Zaidi (2002), the changes at the cellular level sum up and result in the following drought responses of maize at the whole plant level:

- When drought ensues after initial rains, seeds germinate but the soil dries out, so that subsequent establishment and plant stand are badly affected.
- Drought leads to reduced leaf > silk > stem > root > grain expansion (in that order). Incomplete ground cover results from reduced leaf area expansion. Leaf senescence is accelerated (from the bottom of the plant first, but in conditions of high potential evapotranspiration it can also occur at the top of the plant), and this further reduces radiation interception.
- Stomatal closure occurs and photosynthesis and respiration decline from photo-oxidation and enzyme damage. Osmotic adjustment, especially in growing meristems, represent the plant's attempts to maintain cell division but does not seem to play a major role in maintaining growth when stress is severe.
- Assimilate fluxes to growing organs are reduced. Retarded silk growth gives rise to delayed silking and an increased anthesis-silking interval. Ear abortion and kernel abortion increase and plants may become barren. Barrenness can lead to a complete loss of grain yield. Female reproductive structures are more susceptible than tassels, though tassel blasting can occur if temperatures exceed 38°C.
- The root/shoot ratio increases slightly. When stress becomes more severe, root

growth also decreases, and nutrient uptake by mass flow/diffusion in dry soil is sharply reduced.

- Remobilization of stem reserves can occur, when stress coincides with the phase of linear grain growth. In extreme cases this can result in premature lodging. Drought can affect maize production by decreasing plant stand during the seedling stage, by decreasing leaf area development and photosynthesis rate during the pre-flowering period, by decreasing ear and kernel set during the two weeks bracketing flowering, and by decreasing photosynthesis and inducing early leaf senescence during grain-filling. Additional reductions in production may come from an increased energy and nutrient consumption of drought adaptive responses, such as increased root growth under drought.

2.5 Water relations and nutrients

Whereas xylem has been seen for a long time as a system of inert pipes, evidence was recently provided that plants may regulate their internal flow regime. Increasing concentrations of ions were found to produce rapid, substantial, and reversible increases of hydraulic resistance (Zwieniecki et al., 2001). This creates a so far not recognized relation between plant nutrition and water relations, which may also be relevant for the maize crop.

Application of N and K fertilizers increased the drought tolerance of trees (Akunda et al., 1990). This might be a consequence of reduced un-productive water losses and/or higher hydraulic conductivity caused by the ion content of the xylem. Xylem pH, as well, may be influenced by the ionic composition of the xylem sap. Gollan *et al.* (1992) found in sunflower plants subjected to drought that a reduction in xylem nitrate concentration correlated with an increase in xylem pH, and an excess of cations in the xylem sap. Bahrun et al. (2001) observed that the xylem concentrations of macro nutrients in maize decreased under progressive drought. However, the cation:anion ratio remained almost unaffected.

90% of the K used in maize are consumed in the V8 period (eight leaves) until more or less 15 days before or after flowering (Desvignes, 1978). After silk emergence, the K absorption is stopped. To cope with the maximum demand of K even under drought, an adequate supply of K is of high importance. Generally maize should receive a K supplement at the time of the first weeding (30 or 35 days after emergency) to assure high K levels during flowering.

The hydraulic resistance of the roots is an important factor in the water relations of plants.

To a large extent, the root resistance will determine the water status of the shoot because, next to the stomata, the root usually offers the highest resistance to water within the soil-plant-atmosphere continuum. (Steudle et.al 1987).

Studies of whole-plant water use have increased with the development of reliable and inexpensive methods for directly measuring sap flow in intact plants over a wide range of soil water availabilities (Granier 1987, Breda et al. 1993, Gutierrez et al. 1994, Becker 1996, Meinzer et al. 1999). Much attention has also been paid to the effects of drought on xylem function, because severe drought can induce cavitation, thereby reducing water transport. Large differences in xylem vulnerability to water-stress-induced cavitation, some relevant to plant performance in nature, have been found among species (Sperry et al. 1988, Sperry and Tyree 1990, Tyree et al. 1992, Sperry and Saliendra 1994, Kavanagh and Zaerr 1997). So far, no experiments have been carried out on the hydraulic conductivity in maize, although this is a subject of high importance. As potassium can improve the hydric relations in maize crop (Herrera and Lezcano, 2000) and coffee (Bustamante, 2002), an increase in the potassium concentrations resulted in an increase of the hydraulic conductivity. Similar results are to be expected for maize. The hydric relations in the crops are directly related to the hydraulic conductivity, which may be further affected by cavitations in the xylem under drought (Reich and Hinckley 1989, Sperry 1995).

The hydraulic conductivity depends on the path length, the degree of branching, the effective flow cross section of the xylem, the number and size distribution of xylem vessels, soil water potential, and the extent of cavitation. The hydraulic properties of the leaf, however, are poorly understood at present, although this organ has been (and still is) widely studied for gas exchange, water status and, of course, photosynthesis. Leaf hydraulics are intrinsically difficult to study because: (i) the extreme morpho-anatomical heterogeneity of this organ, even within one individual, generates analogous heterogeneity in the data and makes them difficult to generalize; (ii) liquid and gaseous water flow in a leaf are hard to discriminate from each other using the techniques presently available for measuring hydraulic variables, like the vacuum chamber (Kolb *et al.*, 1996; Nardini *et al.*, 2001) or the high pressure flow meter (Tyree *et al.*, 1995; Sack *et al.*, 2002); (iii) a typical leaf of an angiosperm consists of a highly branched xylem system connected to the photosynthetic tissues through the vein living cells (the bundle sheath) about whose functional features very little is known at present. In addition, liquid water flows within the leaf lamina both in the vascular compartment and through the mesophyll living cells (the extra-vascular compartment). Among the environmental factors that may influence leaf hydraulics, water stress can be safely expected to be a major one. Water stress is well known to impair the conductive efficiency of the plant vascular system through xylem embolism (Tyree and Sperry, 1989) and it is now known that the leaf xylem also undergoes cavitation-induced hydraulic failure (Salleo *et al.*, 2001).

The effects of macronutrients on the growth of different plant parts are variable. But the presence of a macronutrient in the growth medium did not affect the concentration of a micronutrient (Mn, Zn, Fe and B) in different plant parts under saline conditions (Hu and Schmidhalter, 1997; 2001, consulted by Banaras et. al. 2004). The synthesis of growth hormones decreases under salt stress or they may undergo degradation (Kuiper et al., 1988, consulted by Banaras et. al. 2004).

A decrease in chlorophyll content has been observed in salinity sensitive rice. However, tolerant lines show a slight increase in the presence of hormones (Krishnamarthy et al., 1987, consulted by Banaras et. al. 2004). An antagonistic effect of abscisic acid and kinetine has been reported in a number of physiological responses that are light- dependent (Hose et al., 2002, consulted by Banaras et. al. 2004). The role of growth hormones in plant growth is suppressed due to their degradation and lower rate of synthesis under saline soil environments. Application of plant growth hormones like kinetine and abscisic acid to the seeds at the time of sowing or directly to the seedlings may make up their deficiency. Moreover, information is lacking regarding the concentration of ions as affected by the application of growth hormones to fodderbeet and seabeet plants grown under saline conditions. (Banaras et. al. 2004.

2.6 Nitrogen and water relations in maize (*Zea* *mays* L.)

Of the fertilizer nutrients applied to crops in tropics and subtropics countries, nitrogen is the most important in terms of amounts applied; it constitutes slightly more than 50% of all nutrients used (Bumb, 1989). However, nitrogen is also one of the most difficult fertilizer nutrients to manage efficiently. Plant-available N is water soluble and therefore easily translocated in the soil by infiltrating water. In addition, the amounts of N in the soil solution are constantly changing because of soil adsorption, microbial immobilization, and mineralization. Thus, the amount of soil N that is available for crop growth can change dramatically over a short distance or period of time; this phenomenon makes it very difficult to test the soil of a particular field and develop a recommendation for N fertilizer requirements.

The dynamic nature of N and its properties leading to the loss from the cropping system has made effective management of N fertilizer one of the most difficult challenges in agriculture. As a result, many researchers have focused on developing production management practices that allow N fertilizer to be utilized more effectively. Since N is lost from the cropping system by a number of pathways, a single solution to the N management dilemma is unlikely. The most logical approach to increasing N fertilizer use efficiency is to supply N as it is needed by the crop (Keeney, 1982). This reduces the opportunity for N loss because the plant is established and rapidly taking it up. Many producers use this management practice by applying N at side dress or through irrigation systems later in the growing season.

The production system of maize, consisting of high-yielding cultivars and high inputs of fertilizers, agrochemicals and irrigation, contributed to great yield increases in developed and developing countries during the past decades (Conway, 1991; Evans, 1993). However, adverse effects of high input agriculture have been recognized, as fertilizers (nitrate) and agrochemicals discharged from agriculture cause surface water pollution (National Research Council, 1989). Breeding maize cultivars with large production under N deficiency could reduce environmental pollution and increase the economic efficiency of N fertilizer use. McCullough *et al.* (1994) reported that new maize hybrids were more tolerant than earlier hybrids to limited N supply during the early vegetative phase with respect to rate of leaf appearance, photosynthesis, stomatal conductance (g_s) and chlorophyll content.

Maize begins to rapidly take up N during the middle vegetative growth period with the maximum rate of N uptake occurring near silk (Hanway, 1963).Thus, applying N at side dress (V8–V10) should be one of the best ways of supplying N to meet this high demand. This appears to be substantiated in studies where side dressing N results in grain yields and fertilizer use efficiencies greater than that produced by applying preplant N (Miller et al., 1975; Olson et al., 1986). Delaying N application too long, however, may reduce yield and N fertilizer recovery (Jung et al, 1972). It seems that the soil N status would affect how late N application could be delayed without reducing yield.

Low soil N status early in the season caused the maximum rate of N uptake to be delayed (Russelle et al, 1983). Thus, it would seem that highly N deficient maize would be able to respond to N applied late in the season. There is little data to show how soil N status early in the season affects maize response to delayed N application. Jokela and Randall (1989) assumed high soil residual nitrate as the reason for a lack of N response by maize when applied at the V8 stage.

Previous studies have demonstrated positive correlations between nitrogen content and photosynthetic capacity (e.g., Yoshida and Coronel 1976, Evans and Terashima 1987), though the relationships between nitrogen nutrition, carbon partitioning, photochemical capacity and growth are less clear and likely differ between C-3 and C-4 plants, and may even differ significantly between species or genotypes.

It is not surprising that changes in nitrogen supply can have profound effects on chloroplast features as these organelles can account for more than 50% of the total leaf nitrogen (Hageman 1986). Indeed, nitrogen limitation during leaf expansion generally leads to leaf chlorosis, impairment of photosynthesis and ultimately reduced growth in most plants. Huber et al. (1989) suggested that increased growth of maize plants at high light and optimal nitrogen nutrition is related to greater capacity for photosynthesis and translocation in mature leaves, and possibly increased capacity for sucrose metabolism in expanding leaves.

Recent investigations have shown that the use of an ammonium fertilizer supplement to nitrate fertilizer regimes can result in a considerable increase in growth rate and yield in a number of plants. This observation also applies to the growth of maize (Murphy and Lewis, 1987). In Germany, Sommer (2000) introduced a cropping system for agriculture and horticulture named Controlled Uptake Long Term Ammonium Nutrition (CULTAN). This System consists of injecting the entire amount of N (ammonium, urea, or both) in a single dose at the beginning of the growing season locally to the root zone. Thus, highly concentrated ammonium depots are created at the injection sites, which are stable against nitrification due to the toxicity of ammonium (Schittenhelm and Menge-Hartmann, 2006). In CULTAN cropping systems, plants cover their N demand mainly as ammonium during the entire growing period (Sommer, 2000, 2003, cited by Schittenhelm and Menge-Hartmann, 2006). The adsorption of ammonium to the cation exchange sites and the dense root network around the depots reduce the risk of N losses. Schittenhelm and Menge-Hartmann (2006) found that the yield of spring barley was positively affected by ammonium applied as CULTAN compared with nitrate nutrition.

The effect of N form on photosynthesis is also associated with stomatal conductance (gs) and intercellular CO_2 partial pressure. Raven and Farquhar (1990) showed that ammonium resulted in higher carbon isotope discrimination than nitrate.

Plants supplied with ammonium had a higher assimilation rate, stomatal conductance, and carbon isotope discrimination than those supplied with nitrate (Høgh-Jensen and Schjoerring, 1997; Guo et al., 2002). These results suggested that nitrogen forms have different effects on gas exchange parameters, such as stomatal conductance, intercellular CO_2 partial pressure, and transpiration rate. According to Guo et al. (2006) the ammonium nutrition is a nutritional factor regulating leaf photosynthesis rate and water relations.

2.7 Water use efficiency

Drought is an important environmental factor limiting the productivity of wheat and other crops worldwide. As irrigation water sources have become scarcer, development of crop cultivars with improved adaptation to drought is a major goal in many crop-breeding programs. Of the physiological factors that affect drought tolerance in crops, the most important ones seem to be moderate water use through reduced leaf area and shorter growth duration, rooting ability to exploit deep soil moisture, the capacity for osmotic adjustment, and control of non-stomatal water loss from leaves (Nguyen et al. 1997). Breeding for drought tolerance might be facilitated by marker-assisted selection in the near future based on the development of molecular linkage maps for crop species (Nguyen et al. 1997).

When breeding for drought tolerance, biomass productivity and water use efficiency (WUE) are considered important agronomic characters (Boyer, 1996). There is increasing interest in improving WUE of crop cultivars so that plants can grow and yield well under water deficient conditions (Boyer, 1996; Ehdaie, 1995). Water use efficiency (WUE) is defined as a ratio of biomass accumulation, expressed as carbon dioxide assimilation (A), total crop biomass (B), or crop grain yield (G), to water consumed, expressed as transpiration (T), evapotranspiration (ET), or total water input to the system (I). The time-scale for defining water use efficiency can be instantaneous (i); daily (d), or seasonal (s). Water use efficiency is written symbolically as a function of these three variables. For example, we use WUE to refer to water-use efficiency expressed as the ratio of carbon dioxide assimilation to transpiration for an instantaneous observation (Sinclair et al. 1983). Water-use efficiency according to Lambers *et al.* (1998) refers to the quantity of water lost during the production of biomass or the fixation of CO_2 in photosynthesis.

The estimation of the WUE can be based either on the evapotranspiration or on crop transpiration; however, the transpiration based WUE provides a more useful indication of plant performance (Tanner and Sinclair 1983; Davies and Pereira 1992). Moreover, for forage crops that cover the ground most of the year and have abundant litter, the evaporation is generally very small and can be ignored as a variable (Hanks 1983; Thornley 1996).

Due to increase in atmospheric temperature the relative humidity (RH) will certainly decrease and my substantiate water use efficiency in crop plants. Moreover, high CO_2 concentration is known to cause partial stomata closure, thereby reducing stomata conductance. This may help in water saving by plants to conserve water and increase their water use efficiency. However, low transpiration will affect the evaporative cooling system of plants and under high temperate conditions this will increase heat load on plant leaves, which will accelerate senescence and reduce photosynthesis (Allen, 1990).

3. MATERIAL AND METHODS

3.1. Experimental site and climate

The experiment was conducted in a greenhouse and in a field at the Institute of Plant Nutrition of the Rheinische Friedrich-Wilhelm-Universität, Bonn in Germany.

The Figure 1 reports the temporal course of the temperature during the period of the experiment at the greenhouse; monitored by Tynitag data loggers (Gemini Data Loggers company) placed in the greenhouse in 2007 and 2008.

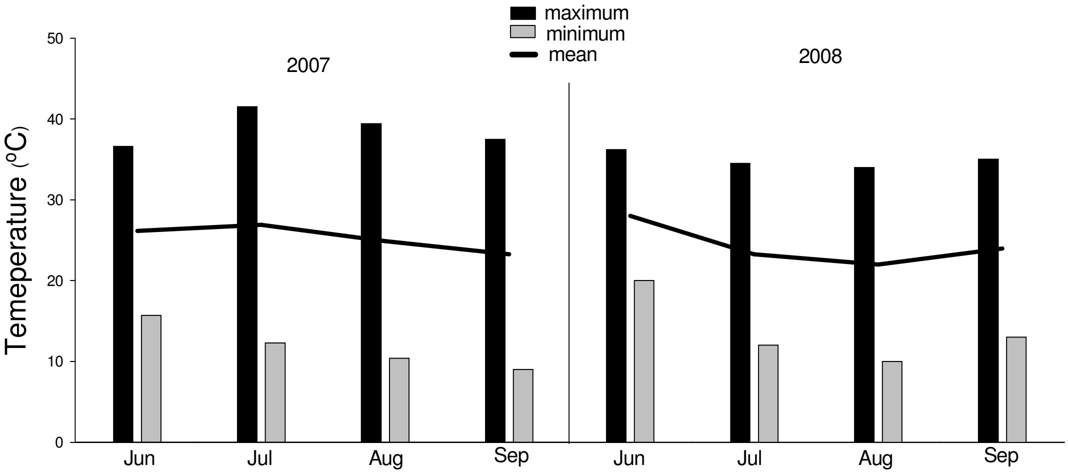


Figure 1. Temporal course of the temperature during the experiment at the greenhouse in 2007 and 2008.

3.2. Experimental set-up

The experiment was conducted in a greenhouse and in a field in 2007 and 2008, using four cultivars, respectively, under different water and nitrogen supply.

3.2.1 Plant material

In 2007 the seeds used for the present investigation were: Vitaminado, Nutrader, Permanent and Mazorca de oro. Seeds of maize cultivars Vitaminado, Nutrader and the hybrid Mazorca de oro were obtained from the Nicaraguan Institute of Agricultural Technology (INTA-USDA program). The cultivars Vitaminado and Nutrader were developed from the populations S03TLWQ AB-04 and S03TLWQ AB 02 respectively.

The hybrid Mazorca de oro is a triple hybrid generated from three endogamy lines. All the Nicaraguan cultivars are quality protein maize (QPM) and they are adapted to the Pacific and North dry zone of Nicaragua and have shown a high productive potential in the small farming system. The cultivar permanent / DKc 3660 was used in 2007 and 2008, which is adapted to European climate conditions. Permanent has a very good grain yield stability under various environmental conditions and is gut resistance to Fusarium and Helminthosporium.

In 2008 seeds of maize cultivars adapted to the European climate conditions were used, which were donated by the German company DSV-Saaten. Seeds of the variety Permanent / DKc 3660, Aalvito, Abakus and Mazurka were sown at the greenhouse and in the field. Aalvito is an early maturity variety adapted to the dry-cool regions. Mazurka is a drought tolerant hybrid used. Abakus has a very fast and impressive growth, very rapid youth development and it is a Stay Green Type. All cultivars adapted to the European climate conditions are used as grain and silage maize.

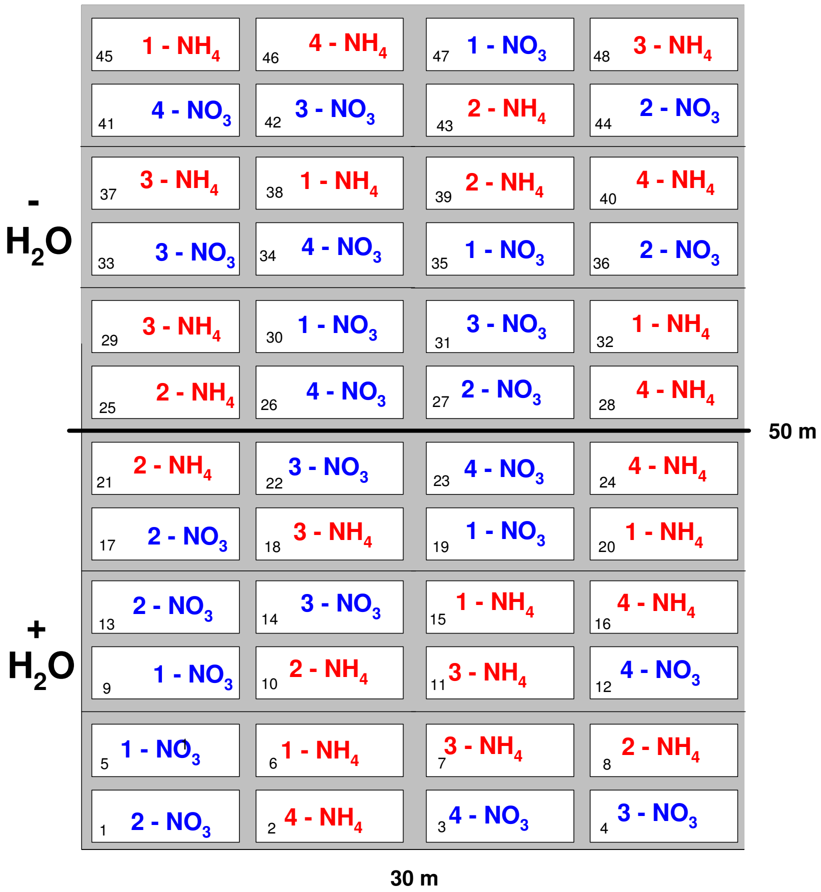
3.2.2 Experimental design at the greenhouse

At the greenhouse one hundred and twenty eight (128) pots were used. Four treatments with four cultivars were established: Two water regimes (well watered and stressed) and Two-nitrogen forms (ammonium through "CULTAN-technique" and nitrate) were arranged in a randomized complete block design with eight repetitions.

Each cultivar had thirty two (32) pots, eight pots (8) with ammonium and well watered (HW), eight (8) with ammonium and stressed (HD), eight (8) with nitrate and well watered (OW) and eight (8) with nitrate and stressed (OD).

3.2.3 Experimental design in the field

A randomized complete block design was established (see Figure 2), with three repetitions. Each plot repetition had 24 m^2 area approximately. The treatments were: Two nitrogen forms, ammonium as depots (CULTAN-technique) and nitrate, and two water regimes, well watered and stressed plots. In total forty-eight (48) plots were established, 24 corresponding to the well-watered area and 24 to the stressed area. The same cultivars as described above were used in the field experiment.



45 1 - NH_4	46 4 - NH_4	47 1 - NO_3	48 3 - NH_4
41 4 - NO_3	42 3 - NO_3	43 2 - NH_4	44 2 - NO_3
37 3 - NH_4	38 1 - NH_4	39 2 - NH_4	40 4 - NH_4
33 3 - NO_3	34 4 - NO_3	35 1 - NO_3	36 2 - NO_3
29 3 - NH_4	30 1 - NO_3	31 3 - NO_3	32 1 - NH_4
25 2 - NH_4	26 4 - NO_3	27 2 - NO_3	28 4 - NH_4
21 2 - NH_4	22 3 - NO_3	23 4 - NO_3	24 4 - NH_4
17 2 - NO_3	18 3 - NH_4	19 1 - NO_3	20 1 - NH_4
13 2 - NO_3	14 3 - NO_3	15 1 - NH_4	16 4 - NH_4
9 1 - NO_3	10 2 - NH_4	11 3 - NH_4	12 4 - NO_3
5 1 - NO_3	6 1 - NH_4	7 3 - NH_4	8 2 - NH_4
1 2 - NO_3	2 4 - NH_4	3 4 - NO_3	4 3 - NO_3

Figure 2: Field experiment lay out in 2007 and 2008.

Figure 3: Sowing of the different maize cultivar (left) and the experimental site two months after the sowing in 2007(right).

3.3 Cultural practices

The Maize cultivars were sown in April 2007 and 2008, respectively at the greenhouse. Four seeds per pot per pot four seeds were sown, and the plant density was, later reduced two plants per pot.

In the field, the experiment was established in April 2007 and 2008, respectively. The total experimental area was 1500 m^2 with forty-eight small plots (Figure 2). Each small plot included five rows with an interface of 0.75 m. Each row was 6.72 m long allowed for 28 holes, with a distance of 0,20 m and one seed sown in each hole. This resulted in a sowing density of 67,210 seeds ha^{-1} approximately.

3.3.1 Nutrient supply

3.3.1.1 Nutrient supply and depot preparation at the greenhouse

The nutrient supply at the greenhouse was made according to the nitrogen form (ammonium and nitrate).

The 64 pots with ammonium as depot (HW and HD) treatment were fertilized at sowing with P and K as NaH_2PO_4 and K_2SO_4 respectively. Quantities of 216.2 g NaH_2PO_4 and 473.6 g K_2SO_4 were diluted into 6.4 liter distilled water and each pot received 50 ml of this solution.

For the depot preparation in NH_4^+ treatments, a mixture of 20.78 g $(NH_4)_2SO_4$ and 0.44 g of nitrification inhibitor (Nis) dicyandiamide (DCD) were used. This depot was placed into each pot to 10 cm depth (Figure 4). To obtain the desired depths and to prepare the condition to place the depots, a glass cylinder was introduced into each pot one week before sowing. After that, the soil was moistened.

The 64 pots with nitrate as N-treatment (OW and OD) received 50 ml of a solution containing Ca $(NO_3)_2$ and DCD at sowing and when the plants were approximately 6 cm high. This solution was prepared with 1.650 kg Ca $(NO_3)_2$ and 14.08 g DCD diluted into 3.2 liter distilled water. Each plot received 50ml of this solution. At the same time, 50 ml per pot of a solution prepared with 216.2 g NaH_2PO_4 and 473.6 g K_2SO_4 and diluted in 6.4 liter distilled water was applied. To ensure that other mineral nutrients would not limit the crop response to N-treatments, all the 128 pots received at sowing 20 ml of a solution containing 256 g of fully water-soluble trace element fertilizer "Ferty 10" with the mineral nutrients, MgO, B, Cu, Fe, Mn, Mo and Zn diluted in 5.12 liter distilled water.

Figure 4: Ammonium depot in the greenhouse.

3.3.1.2 Nutrient supply and depot preparation in the field

In the two years of experiment the nutrient supply at the field was made according to the nitrogen form, ammonium and nitrate.

Phosphorus and potassium fertilizers were applied before sowing. An amount of 32.78 kg (219 kg ha^{-1}) of granular triple super phosphate (TSP) and 100 kg of Patentkali (666 kg ha-1) (Potassium sulfate with magnesium) were manually and uniformly broadcasted on the experimental plot (0,15ha^{-1}) (Figure 5a). Hereby, the experimental area (0,15 ha^{-1}) was fed with 200 kg K2O ha^{-1} ha^{-1}, 67 kg MgO and 114 kg S ha^{-1}. One day after this application, each plot with nitrate treatment (24 plots) were fertilized with a mixture containing 64.5 kg calcium nitrate Ca $(NO_3)_2$ and 1000 g DCD. In this case, DCD was used for reasons of comparability with CULTAN-plots. Each small plot received 2.69 Kg Ca $(NO_3)_2$ and 41.7 g DCD approximately; this application was made manually.

The ammonium depot was applied five days after the sowing. The depot was prepared with a mixture containing 100-liter ammonium sulfate ($(NH_4)_2 SO_4$) (25% N) and 1 Kg DCD. Each plot received approximately 4.17 liter of this solution (0,80 liter/pot). The depot was then incorporated manually to the depth of 5 cm in the soil, with a distance of 5 to 7 cm away in parallel to the row, where seeds were sown (Figure 5b). The depot was covered with soil immediately afterwards.

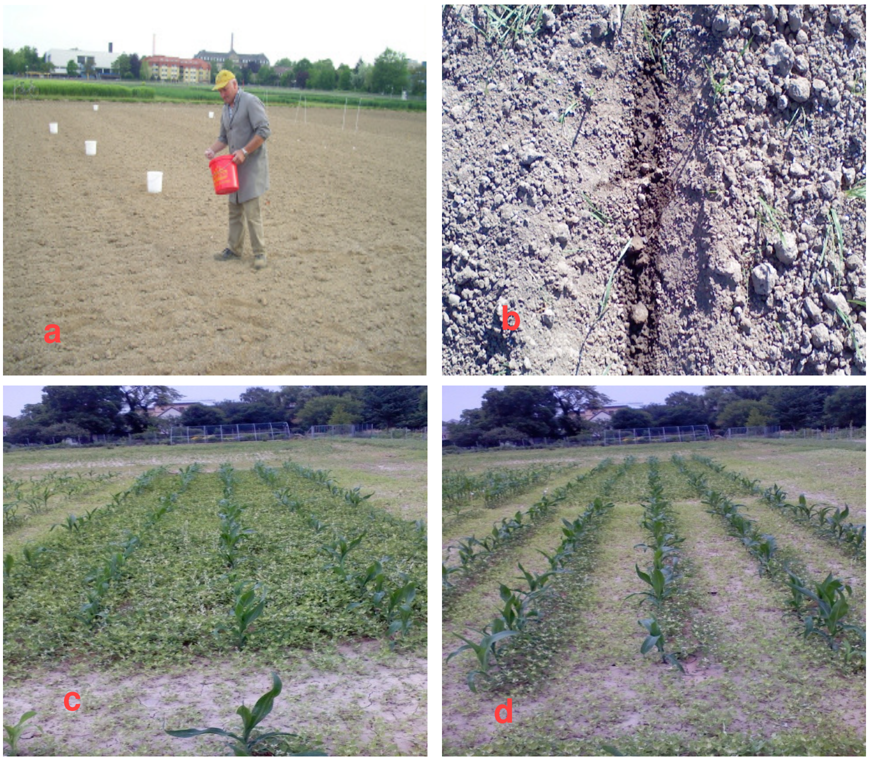


Figure 5: (a) Nitrate application in the plots, (b) Ammonium depot application in the row (CULTAN-method) (c) weed growth in nitrate broadcasted plot (d) weed growth in CULTAN-technique.

3.3.2 Irrigation at the greenhouse experiment

All pots (128) were weighed daily and soil moisture losses due to evapotranspiration were replenished according to the water regimes.

Two water regimes were used during the evaluation: First one was with well-watered (WW) condition; where water was applied every day. The second one was with intermediate stress (IS) or grain-filling stress, where irrigation was suspended at 50 days after sowing, approximately 3-4 week prior to the date of 50% anthesis, according to Bolaños and Edmeades (1992).

3.3.3 Irrigation in the field experiment

It was difficult to divide the field into different stress regimes. Because of this, in 2008, a half of the experimental field was managed with one stress level only (i.e. well watered). 24 plots were irrigated as needed to avoid water stress with tap water through a drip irrigation system. Other 24 plots were not irrigated and the water stress would be in dependency upon weather condition.

3.3.4 Diseases and weeds control

No applications of chemicals for diseases and insects control were made, both at the greenhouse and in the field. However, a herbicide with the equivalent of 50g/ha of Cato was applied for weed control a month after sowing in the field.

3.3.5 Harvest, grain Yield and total nitrogen

The harvest was made by hand at 120 days after sowing, approximately, considering the three central rows per plot only (number 2,3 and 4). The harvest area was 1-1,2 m^{-2}. The first and last plants in the rows were discarded in order to minimize border effects. At harvest, the following data were collected: plant and ear height, tassel height, stem diameter and number of plants per unit ground area.

The 10 hand-harvested plants (without roots) were collected from each plot and separated into stover (stem, leaves and tassel) and ears and immediately weighed (fresh weight). Thereafter, both parts (stover and ears) were dried at 70°C for 72 hours in a oven to reach constant weight and then they were weighed again (dry weight). The grain samples, representative of the different treatments, were stored for later analysis. These samples were used for dry matter, total nitrogen and yield components (cobs per plant, rows per cob, number of kernels per row, kernel weight) determination. The cobs per plant were calculated by dividing the total cobs by the total harvested plants per plot. The number of grains per ear was determined by dividing the total number of grains by the total number of ears. The weight per grain was determined by dividing the weight of 500 grains by 500.

The final biomass was estimated according to Zaidi (2002) multiplying all yield components as follows: [No. of plants/ha x RPP x GPR x WPG]; where : *RPP* = reproductive units (ear) per plant, GPR = no. of grains per reproductive unit adjusted to 14% moisture, WPG = weight per grain. At same time, the grain yield (GY) was calculated multiplying the final biomass by the harvest index (HI).

The harvest index was measured according to Dobermann and Walters (2006) as the ratio of grain weight to total plant to as follows:

Harvest index (HI) = $DWG_B/(DWV_B + DWG_B + DWC_B)$, where, DWG_B=Dry weight of

grain, DWV_B= Dry weight of whole vegetative organs and DWC_B=weight of cob.
For the analysis of total nitrogen, the micro-Kjeldahl method was used.

3.4 Plant water relations and morphology

3.4.1 Leaf gas exchange parameters

The leaf gas exchange parameters were measured weekly from the onset of drought treatments until the last sampling was done. Stomatal conductance (gs) Transpiration (E), Photosynthesis rate (A_{net}), intercellular CO_2 concentration (Ci), vapour pressure deficit (VPD) and the photosynthetic active radiation (PAR) were measured on intact leaves from 3 plant per treatment in both the greenhouse and the field with a portable gas exchange system model Licor-6400 (LI-COR, Inc., NE. USA). The measurement parameters were: 400 µmol CO_2 mol^{-1}, 500 µmol s^{-1} flow rate to the sample cell, 500 µmol m^{-2} s^{-1} PAR. Water use efficiency (WUE) was calculated as the ratio of net assimilation (A) to transpiration (E). The measurements were restricted to the hours between 9 and 14h on sunny days to minimize diurnal effects on photosynthesis.

Photosynthetic light response curves were also measured weekly on leaves from 2-4 plants leaves per treatment. Measurements were made with a photosynthetic rate of 0 to 2000 µmol m^{-2} s^{-1}. The leaves had approximately three minutes to adjust to any light intensity setting.

3.4.2 Water relations

3.4.2.1 Hydraulic conductivity of the stem

The hydraulic conductivity was measured two times after the imposition of water stress with a High Pressure Flow Meter (HPFM) (Dynamax, Houston, TX 77099 USA). The method is described in detail elsewhere (Tyree et al., 1995). Briefly, the stem was cut 30–50mm above the soil surface, while the whole shoot was held under water.

The HPFM was connected to the sample plant stem and was perfused with water at a constant pressure until the flow rate or shoot conductance became stable. After collecting transient flow data, a regression analysis of the data was performed using the HPFM software to determine the hydraulic conductance of the sample. The slope of the line of increasing pressure and the flow was the hydraulic conductance of the stem (K_{Stem}). The plants used for these measurements ranged between 2 and 3 months in age and the measurements were taken from four plants per treatment. The stems were 10 cm long. The segments varied in diameter from 1 to 2 cm and immediately after cutting it, the stem was weighted and the fresh and dry weight recorded for dry matter determination. The segments diameters were measured with a precision vernier calliper (Kraffixx).

3.4.2.2 Leaf water content

The leaf water content (RWC) was determined by the standard method (Barrs and Weatherley, 1962), two times during the drought period on four pots per treatment in the greenhouse and five plants per plot in the field. Each sample represented a different plant and they were taken from between the 5th and 8th leaf of each plant. The youngest and the most fully expanded leaves were sampled. Each sample had about 1x7 cm and was taken from the area between the mid-vein and the edge. The samples were weighed to obtain fresh sample weight (FW). Thereafter the samples were placed in a beaker (25ml) filled with water for 4h under normal room light and temperature. After 4 hours the samples were taken out of water and were well dried of surface moisture with filter paper and immediately weighed to obtain fully turgid weight (TW), then they were oven dried at 80^0C for 24h and weighed to determine dry weight (DW). The RWC of the leaves was calculated as follows:

$$\text{RWC (\%)} = \frac{\text{W-DW}}{\text{TW-DW}} \times 100 \qquad (1)$$

3.4.2.3 Leaf water and osmotic potential

Two times after the imposition of stress, the leaf water potential (Ψ) was measured (both at the greenhouse and in the field) using an Scholander-pressure chamber (Scholander et al.1965). (Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Measurements were made on 4 plants per treatment with 2 leaves per plant (between the 5th and the 8th leaves) prior to sunrise (Ψpd: 04:00 to 06:00 h Mean European time, M.E.Z). The measurements were performed within 40–60 seconds after collecting the samples.

Pressure was applied to the chamber at a rate of 0.05 MPa s^{-1} (Turner 1981).

For the osmotic potential ($\Psi\pi$) determination, samples from the same leaves used for the water potential, were placed immediately in plastic bags and frozen at –20 °C for later measurement. The method used for the $\Psi\pi$ was the freezing point depression using an Osmometer (OSMOMAT 010; Gonotec GmbH; Germany).

The pressure potential (turgor pressure) (P) was calculated as the difference between the water potential and the osmotic potential. The same plants and leaf stages taken for the RWC measurements were used for the leaf water and osmotic potential determination.

3.4.2.4 Determination of soil water content

The soil moisture content in the field was measured weekly using a time–domain reflectometry (TDR IMKO-system) equipment (Topp et al., 1980). Six plastic tubes (PTL) were installed at 30 and 60 cm depths and properly distributed over the whole experimental area to monitor the soil moisture.

In the greenhouse, the pots were weighed daily and soil moisture losses due to evapo-transpiration were replenished accordingly with their water regimes.

3.4.3 Dry matter analysis

The total aboveground biomass in greenhouse was measured two times after the imposition of water stress according to Ritchie and Hanway (1993). Four plants per treatment were taken and the whole plant structure was dried at 70°C for 72 hours in an oven to reach constant weight. Dry Matter was calculated using the following equation:

%Total DM= Wo/W1 * 100, where: Wo is the weight of the dried sample and W1 is the weight before drying.

In The field, the dry matter was estimated three times after sowing; two times before the harvest and third time at harvest. The whole biomass of the 10 harvested plants was processed immediately to avoid long exposure of the samples to the sun. The dry matter percentage was determined using the same method and formula mentioned above.

3.4.4 Morphological characteristics

The morphological characteristics were measured two times after the imposition of drought at the greenhouse. In the field they were measured two times, at 60 days after sowing and at harvest. The following characteristics were measurement: Plant height (m), stem diameter (cm) as well as the ear size (cm) in the field. Furthermore, because in the field experiment in 2007 some plants showed more than one tillers, the tillers per plant was also measured.

3.4.4.1 Plant height and ear size: The plant height was defined as the distance from the ground to the ligule of the uppermost leaf and was measured on 8 pots per treatment at the greenhouse and on 18 plants per plot in the field. The ear size was measured directly using a ruler. At harvest (in the field) plant height, ear size and stem diameter were measured on the 10 harvested plants only.

3.4.4.2 Stem diameter: The stem diameter was measured on intact stems with a precision 1500 mm vernier calliper (Kraffixx). The measurements were made about 30 to 50 cm above the ground.

3.5 Statistical analysis

Statistical analysis was carried out with the SPSS software (version 14 Inc., Chicago, IL USA). The data was subjected to an analysis of variance (ANOVA) ($P < 0.05$) and the means were compared using Duncan's test (multiple comparison) at 95% confidence. Levene tests were done to check normality of data and homogeneity of variance, respectively. All graphs were made with Sigma plot 10 (Systat software, Inc). The same letters indicate that averages are not significantly different, and the alphabetic order corresponds to decreasing values. Correlation analysis between treatments was also made. Significance was as follows: ns, not significant; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$); ***, extremely significant ($P < 0.001$).

4 RESULTS

4.1 Greenhouse experiment

4.1.1 Gas exchange measurements

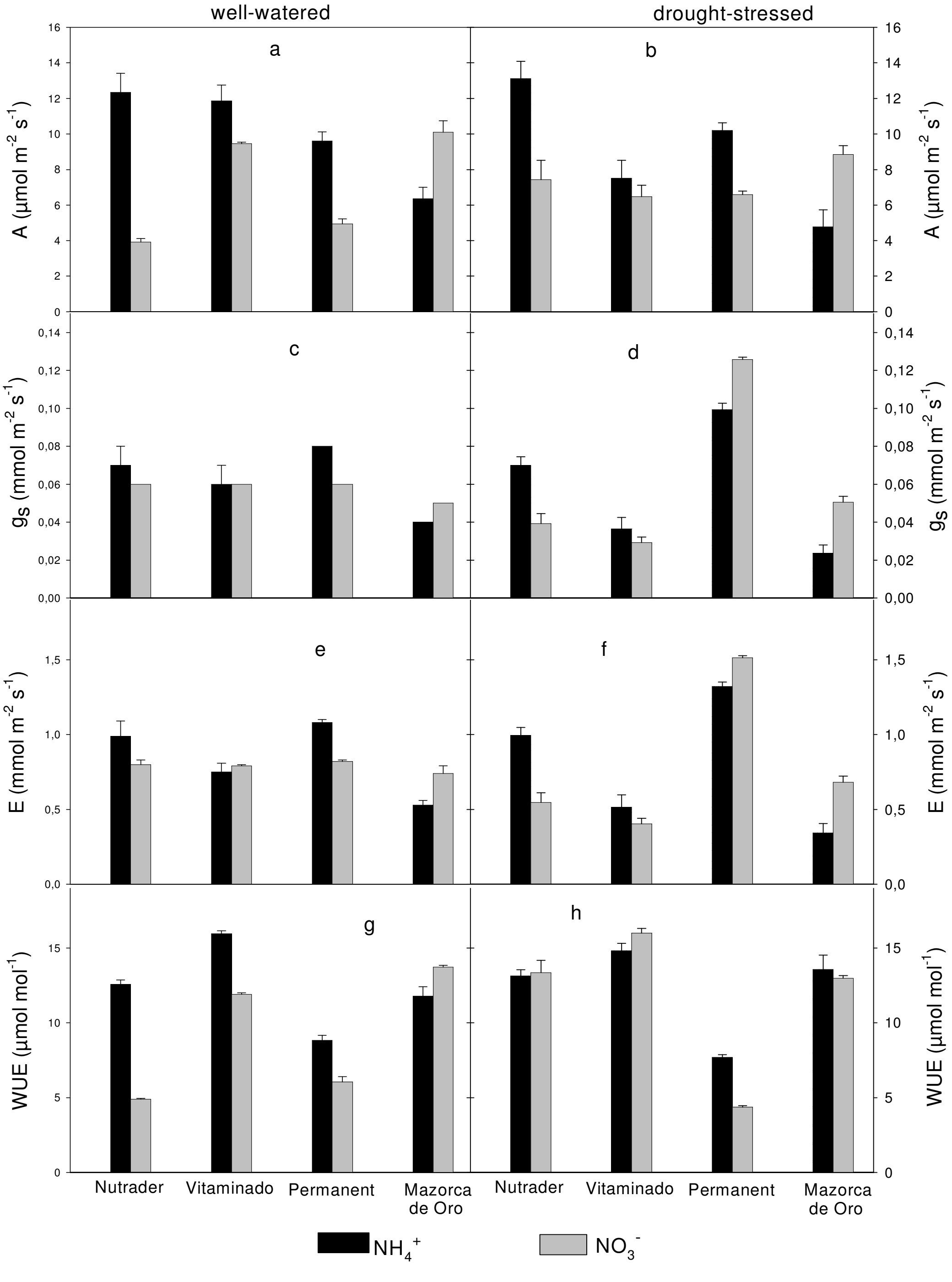


Figure 6: Gas exchange parameters in different maize cultivars under well-watered and drought conditions and different nitrogen forms (NH_4^+ and NO_3^-) in the greenhouse experiment (2007): (a and b) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (c and d) stomatal conductance (g_s, mol m^{-2} s^{-1}), (e and f) Transpiration rate (E, mmol m^{-2} s^{-1}) and (g and h) water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}).

The leaf gas exchange characteristics of the different maize cultivars under well-watered conditions and different nitrogen fertilization evaluated in 2007 are presented in the Figure 6. The ANOVA depicted that the differences in photosynthesis rate were significant ($p<0.001$) among cultivars. The plants treated with different nitrogen source also varied significantly ($p<0.001$). While the water regime did not significantly (ns) influence A_{net}, the interaction cultivars*nitrogen form, cultivar*water regime, nitrogen form*water regime were significant (at $p<0.001$ and $p<0.05$). On the other hand, stomatal conductance (g_s) and photosynthesis rate (A_{net}), differed significantly among cultivars. However the nitrogen form and water regime had no significant influence. Although, the interactions cultivars*nitrogen form, cultivar*water regime, nitrogen form*water regime and cultivars*nitrogen form*water regime were significant ($p<0.001$), similar behavior exhibited the transpiration (E), with the exception that the interaction nitrogen forms*water regime was not significant (ns). Consequently, water use efficiency varied among cultivars, between nitrogen forms and between water regimes.

When ammonium was used as nitrogen source instead of nitrate, the cultivar Nutrader increased (+68%) the photosynthesis rate (A_{net}), (+25%) stomatal conductance (g_s), (+20%) transpiration rate (E) and (+60%) water use efficiency (WUE).

As compared to nitrate fertilization, in Vitaminado the ammonium fertilization also increased (+20%) A_{net}. However, g_s and E did not vary with either ammonium or nitrate fertilization. Therefore, the WUE of the NH_4^+ treated plants was higher (+24%) than the NO_3^- treated plants.

In Permanent, the ammonium fertilization has also increased (+49%) A_{net}, (+25%) g_s, (+24%) E and (+32%) WUE than nitrate fertilization. In contrast, the NO_3^- treated plants in Mazorca de oro has increased (+49) A_{net}, (+20%) g_s, (28%) E and (13%) WUE than the NH_4^+ treated plants.

Drought slightly increased A_{net} in Nutrader with ammonium fertilization. This increase, however, was even more pronounced in the NO_3^- treated plants (+47%). Drought did not affect g_s and E with ammonium fertilization. However, with nitrate fertilization, drought has decreased (-33%) g_s and (-31%) E. At the same time, under drought condition, WUE increased only about 5% in the NH_4^+ plants, but 63% in the NO_3^-treated plants.

In Vitaminado, A_{net}, g_s and E were higher under well-watered conditions compared to the drought-stressed treatments with either ammonium or nitrate fertilization. The Figure 6 also shows an increase of WUE in both N-treatment under drought conditions than under well-watered conditions.

In permanent, drought has increased 6%, 20% and 82% A_{net}, g_s and E with ammonium fertilization and 25%, 54% and 56% A_{net}, g_s and E with nitrate fertilization respectively. However, WUE decreased 13% and 28% with ammonium and with nitrate fertilization, respectively.

In Mazorca de oro, drought has reduced (-25%) A_{net}, (-50%) g_s and (-36%) E in the NH_4^+ treated plants. However, drought has reduced (-12%) A_{net}, when nitrate was used as nitrogen source. Under drought conditions, WUE increased with (+13%) in NH_4^+ treated plants. However, WUE was not affected by drought in the NO_3^- treated plants.

The trend of stomatal conductance (g_s) versus vapor pressure deficit (VPD) in 2007 for all treatments is given in Figure 7a and 8b.

Stomatal responsiveness to changes in vapor pressure varied considerably according to the nitrogen form and water regime. In all treatment, g_s decreased with increasing VPD.

The NO_3^- and NH_4^+ plants under drought shows slightly higher stomatal conductance to a raised VPD compared to NH_4^+ and NO_3^- under sufficient soil moisture conditions (Figure 7a an 8b). Over a wide range of VPD, between 1.2 to 1.5 kPa, g_s of the well-watered and plants under drought with ammonium fertilization has decreased from 0.08±3E-3 to 0.04±2E-3 mol m^{-2} s^{-1} and from 0.1 to 0.02 mol m^{-2} s^{-1}, respectively, while g_s of the well watered and plants under drought with nitrate fertilization declined slightly from 0.06 to 0.05 mol m^{-2} s^{-1} and from 0.13 to 0.03 mol m^{-2} s^{-1}.

A similar response was observed also in the transpiration rate. The Figure 8a and 8b show the responses of transpiration rate versus vapor pressure deficit. The effect of VPD on stomatal conductance might also have affected the transpiration rate. Over a wide range of VPD, between 1.2 and 1.5 kPa, E ranged from 1.08 to 0.53 mol m^{-2} s^{-1} in well watered NH_4^+ plants, whereas 0.80 to 0.74 mol m^{-2} s^{-1} in well-watered NO_3^- plants. On the other hand, E ranged from 1.32 to 0.34 mol m^{-2} s^{-1} in plants under drought with ammonium fertilizations and from 1.51 to 0.41 mol m^{-2} s^{-1} in plants under drought with nitrate fertilization.

Under drought and with the same VPD conditions, both g_s and E were higher for NH_4^+ plants than for NO_3^- plants, respectively.

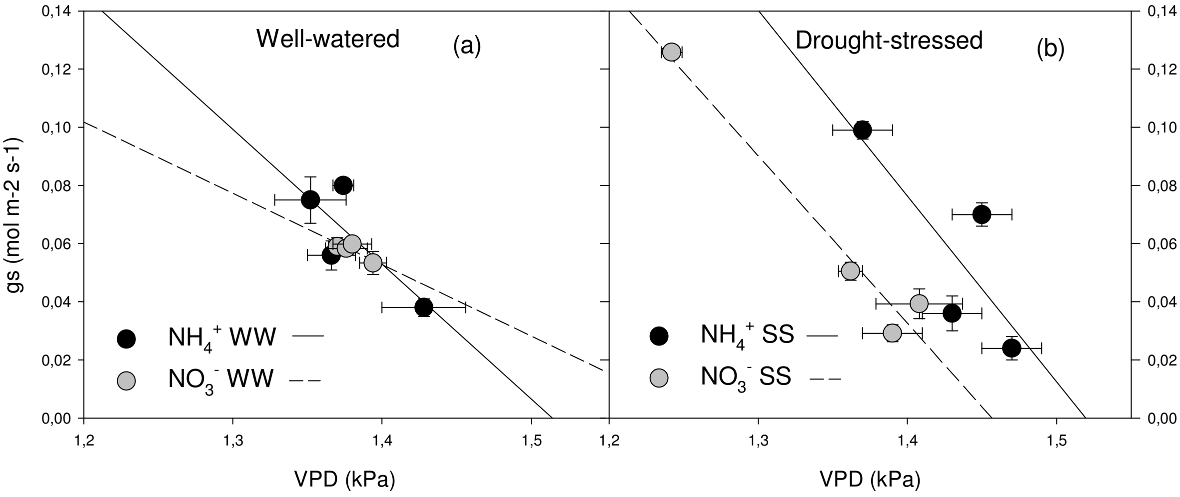


Figure 7: Change in stomatal conductance (g_s, mol m^{-2} s^{-1}) as a function of leaf-to-air vapour-pressure deficit (VPD, kPa) in different maize cultivars under different nitrogen fertilization and water regime: well watered (a) and drought-stressed (b) in the greenhouse experiment (2007).

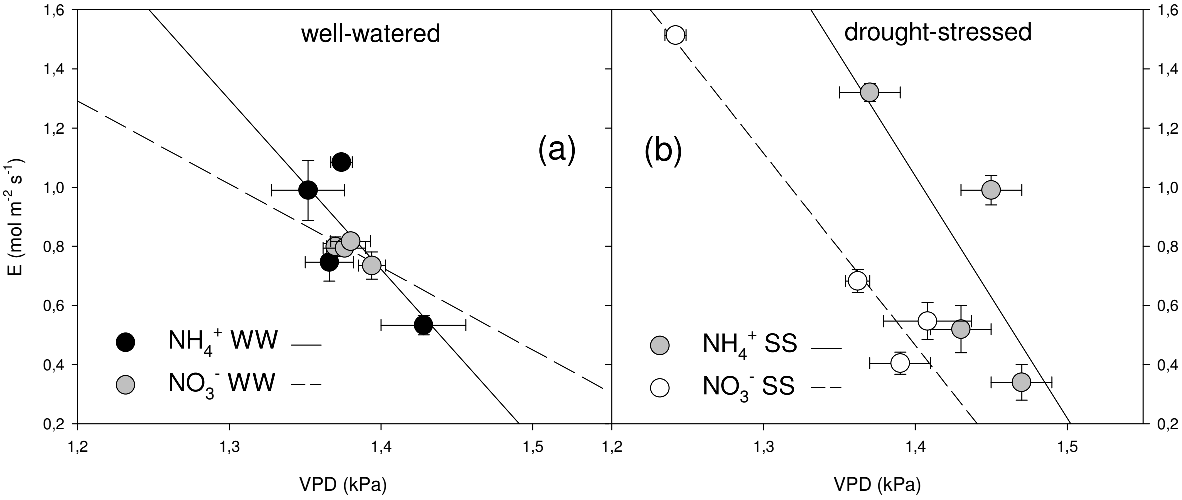


Figure 8: Change in transpiration rate (E, mmol m^{-2} s^{-1}) as a function of leaf-to-air vapour-pressure deficit (VPD, kPa) in different maize cultivars under different nitrogen fertilization and water regime: well watered (a) and drought-stressed (b) in the greenhouse experiment (2007).

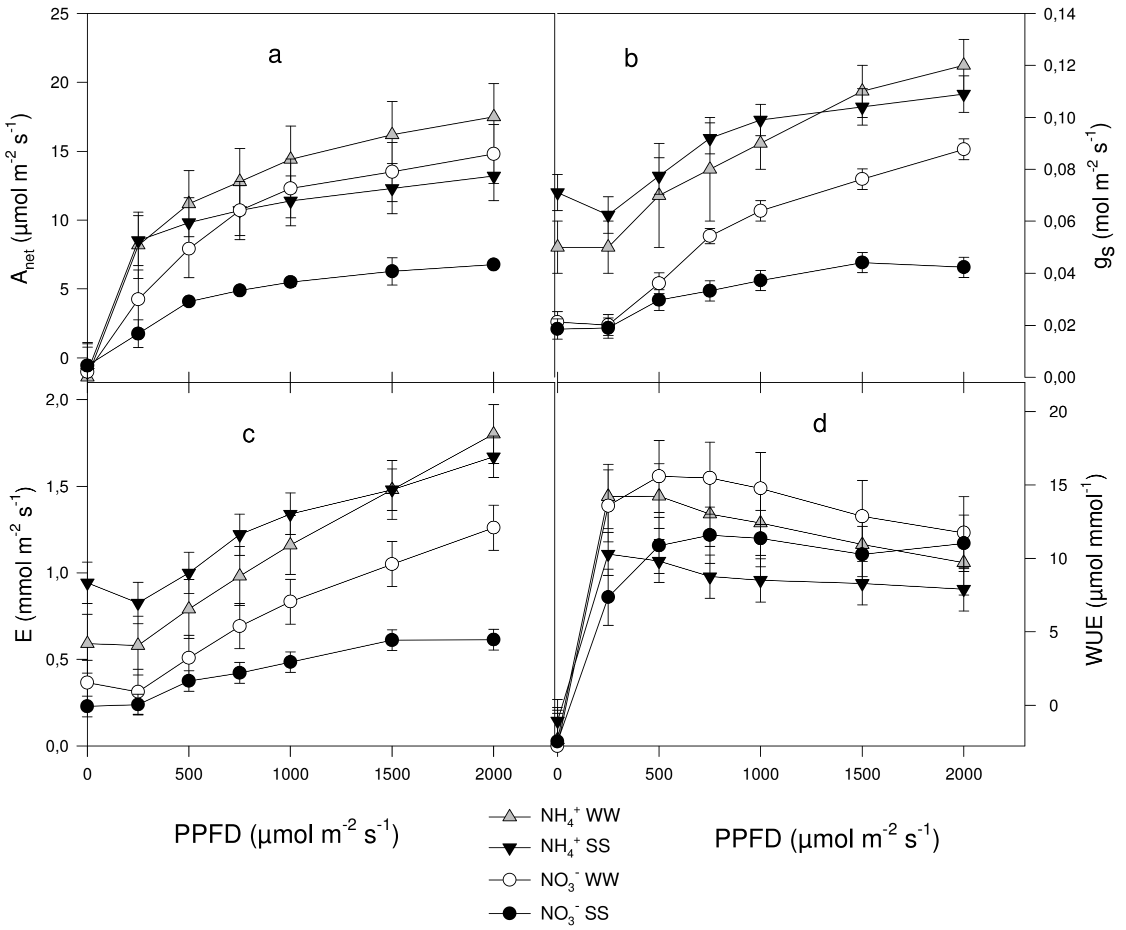


Figure 9: Change in (a) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (b) stomatal conductance (g_s, mol m^{-2} s^{-1}), (c) transpiration rate (E, mmol m^{-2} s^{-1}) and (d) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}) as a function of the photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) in different maize cultivars under different nitrogen fertilization and water regime in the greenhouse experiment (2007).

The light response curves of different treatments (different N-forms and water regimes) plotted in 2007 are presented in Figure 9.

All treatments showed a rapid increase of net photosynthetic rate (A_{net}) with increasing light intensity. Within the well-watered treatments, the ammonium supplied-plant showed higher photosynthetic capacity compared to nitrate plant when the light ability was increased from 0 to 2000-µmol m-^{2} s-^{1} (Figure 9a).

The NH_4^+ fertilized plants reached maximum A_{net} of 17.5 µmol m^{-2} s^{-1} at a PPFD of 2000 µmol m-^{2} s-^{1} compared to 14.8 µmol m^{-2} s^{-1} of nitrate plants. Under drought, the NH_4^+ plants showed higher A_{net} than the NO_3^-plants.

Stomatal conductance and transpiration rate also increased with increased light intensity (Figure 9b and 9c). The results also reveal that the light availability has a major influence on g_s and E of the treatments under favorable soil moisture conditions (Figure 9b and 9c).

However, the NO_3^- well watered plants has higher g_s and E values at a PPFD of about 1000 µmol m^{-2} s^{-1} compared to NH_4^+ well watered plants.

The WUE reached about 14.00 µmol m^{-2} s^{-1} at 250 mmol m-^{2} s-^{1} in both ammonium and nitrate treatment, but from this light intensity the WUE was reduced to 9.72 and 11.75 µmol m^{-2} s^{-1} for ammonium and nitrate supplied plants, respectively.

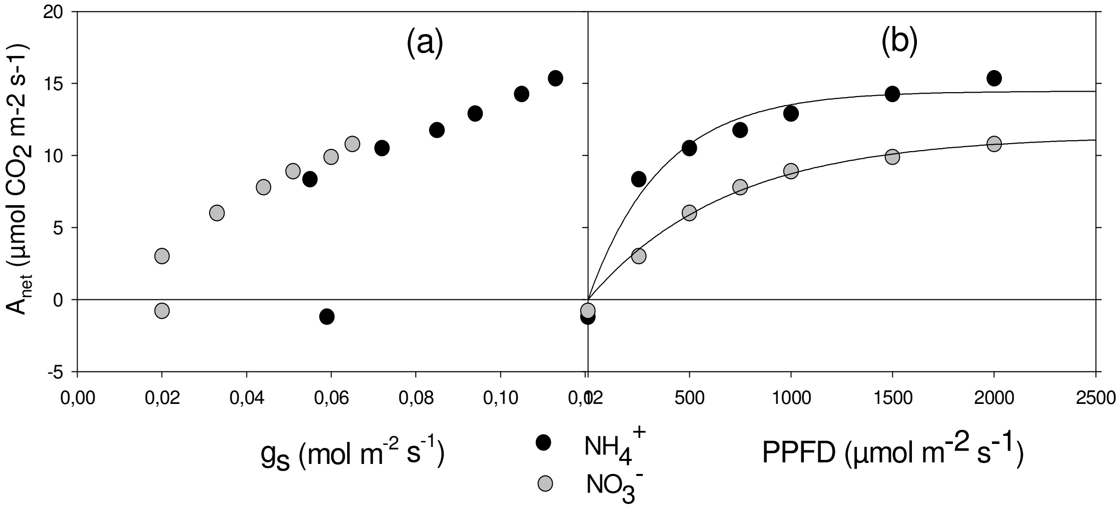


Figure 10: Change in net photosynthetic rate (A, µmol m^{-2} s^{-1}) as a function of: (a) stomatal conductance (g_s, mol m^{-2} s^{-1}) and (b) photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) in different maize cultivars under different nitrogen fertilization in the greenhouse experiment (2007).

Figure 10 shows the co-relation of A_{net} and g_s (a) and A_{net} vs. PPFD (b). There was a strong linearly relations between photosynthesis rate (A_{net}) and stomatal conductance (g_s)(Figure 10a). The NH_4^+ plants showed higher A_{net} than the NO3- plants at same values of g_s, suggesting than the assimilation was reduced in the leaves because of stomatal limitation in the NO3- treated plants. Furthermore, there was a curvilinear relation between photosynthesis rate (A_{net}) and light intensity (PPFD), in both treatments, NH_4^+ and NO_3^- plants (Figure 10b). However, the light availability had major influence on the photosynthetic capacity of the plants with ammonium fertilization than with nitrate fertilization.

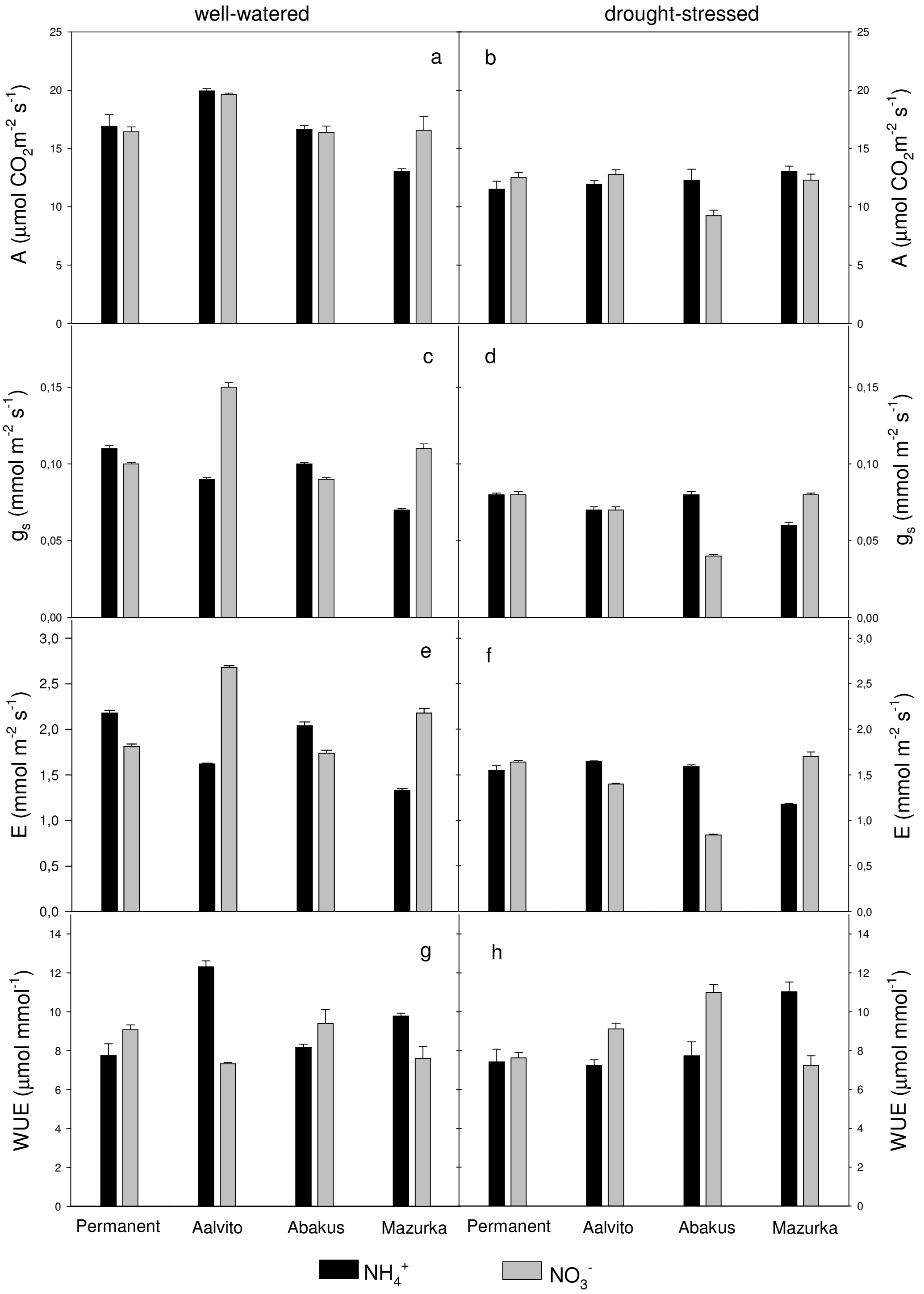


Figure 11: Gas exchange parameters in different maize cultivars under well-watered and drought conditions and different nitrogen forms (NH_4^+ and NO_3^-) in the greenhouse experiment (2008): (a and b) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (c and d) stomatal conductance (g_s, mol m^{-2} s^{-1}), (e and f) Transpiration rate (E, mmol m^{-2} s^{-1}) and (g and h) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}).

The results of ANOVA demonstrate that the cultivars, N-form and water regime influence net photosynthesis rate (A), stomatal conductance to water vapour (g_s), transpiration rate (E) and instantaneous water use efficiency (WUE: A/E) in the different maize cultivars evaluated in this work (Figure 11).

A specific analysis of each leaf exchange parameters evaluated in 2008 revealed that A_{net} changed with cultivars ($p<0.01$). It also interprets that the response of the leaves to the different N-forms and water regime was also significant ($p<0.001$). The interactions cultivars*nitrogen form*water regimes were also significant ($p<0.001$).

With regards to g_s and E, the ANOVA shows significant differences ($p<0.001$) between cultivars, N-forms and water regimes. Similarly, significant different ($p<0.01$) in WUE were found between varieties, N-forms ($p<0.001$) and water regimes ($p<0.05$) respectively. Moreover, the correlation between varieties*N-form*water regime was also significant ($p<0.001$).

Under well-watered conditions, in Permanent, Aalvito and Abakus cultivars, A_{net} was not affected by the N-form. However, A_{net} was higher with nitrate fertilization than with ammonium fertilization in Mazurka cultivar. Stomatal conductance (g_s) and transpiration rates (E) has increased in Permanent and Abakus cultivars with ammonium fertilization than the nitrate fertilization. On the other hand, Aalvito and Mazurka had higher g_s and E with nitrate than with ammonium. Despite the trend of higher transpiration rate and stomatal conductance with either ammonium or nitrate fertilization, the Aalvito cultivars had the highest water-use efficiency (WUE).

In general, the restricted water decreased A_{net}, g_s and E in all cultivars. However, drought did not affect A_{net} in Mazurka cultivar with ammonium fertilization. At same time, the transpiration rate slightly increased with drought in Aalvito with ammonium fertilization.

When nitrate was used as nitrogen source instead of ammonium, Aalvito and Mazurka increased the water-use efficiency as a response to the drought. Mazurka was the unique cultivar that increased the water-use efficiency (WUE) under drought conditions with ammonium fertilization. Hence, the cultivars differences in WUE were mainly due to their specific ability in reducing transpirational water loss rather than their capability to maintain net photosynthesis during the drought period.

4.1.2 Water potential and hydraulic conductivity

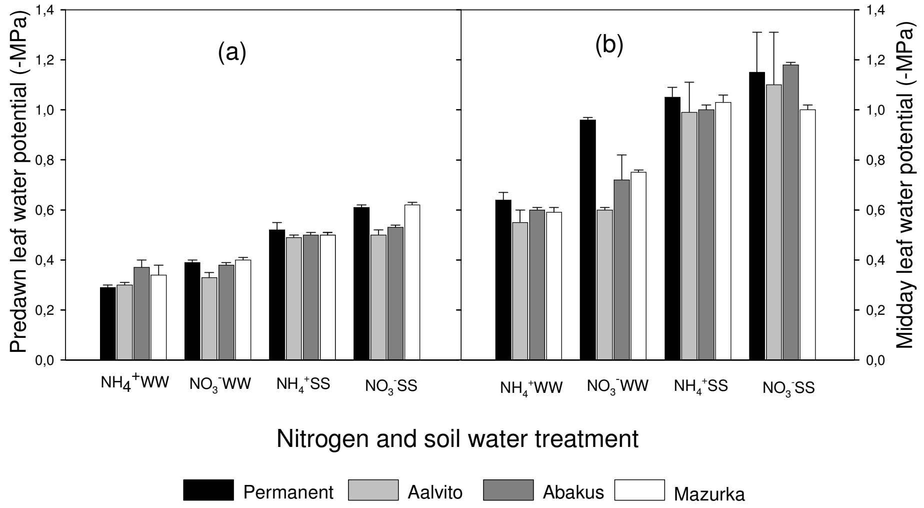


Figure 12: Predawn (a) and midday (b) leaf water potential of maize cultivars under different soil water regime and two different nitrogen fertilizers, NH_4^+ and NO_3^-.

Figure 13 shows the predawn and midday leaf water potential of four maize cultivars under two soil moisture levels and two nitrogen sources: ammonium and nitrate.

Diurnal leaf water potential was significantly ($p<0.05$) changed due to cultivar, N-forms and water regimes.

The well-watered NH_4^+ fed plants of cultivars Permanent and Aalvito showed an increase in diurnal leaf water potential (LWP) compared to the well-watered NO_3^- fed plants. Under well-watered conditions, the NH_4^+ plants of Permanent and Aalvito had 26% and 9% higher Ψ_{pd} compared to NO_3^- plants, respectively. With respect to the treatments under drought conditions, the NH_4^+ plants of Permanent and Mazurka showed 15% and 19% higher Ψ_{pd} (more positive) that those fertilized with nitrate. Similar results were seen in all cultivars, except Abakus, at midday leaf water potential measurements. All cultivars of all treatments have shown the lower midday Ψ values compared with the predawn (Figure 12a and 12b).

Drought had an influence (more negative) on the predawn and midday leaf water potential in all cultivars (Figure 12a and 12b). The response of all cultivars to drought was variable. However, the predawn leaf water potential in Permanent was more affected by drought with either ammonium or nitrate fertilization compared to the other cultivars. At same time, Abakus shows less effect by drought (27%) of predawn leaf water potential (Figure 12a). The results show maximum Ψ_{pd} (-0.29 MPa) and Ψ_{md} (-0.55) in well watered plants as compared to those in drought conditions with means of predawn and midday values of -0.62 and -1.18; respectively.

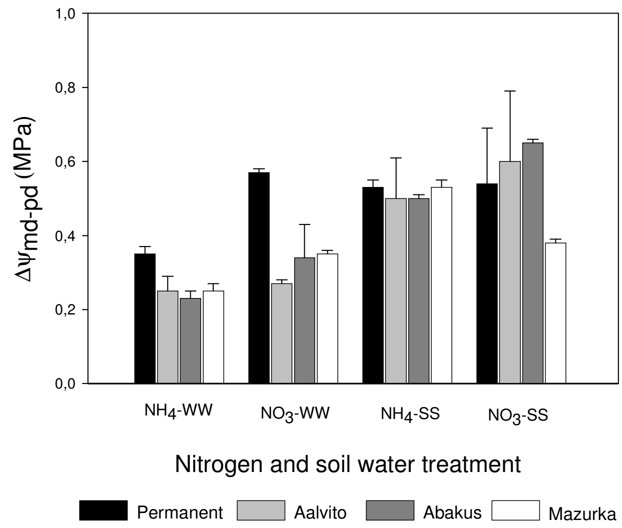


Figure 13: Comparison of predawn and midday leaf water potential of maize cultivars under different soil water regime and two different nitrogen fertilizations, NH_4^+ and NO_3^-.

The comparison of the midday and predawn leaf water potential ($\Delta\Psi_{md\text{-}pd}$) (Figure 13) shows decreasing values when the different cultivars were under drought conditions, as well as among nitrogen treatments, i.e., the NH_4^+-fed plants, except in Aalvito cultivar, showed the higher $\Delta\Psi_{md\text{-}pd}$ than those fertilized with NO_3^- under well watered conditions. This trend was also shown by drought treatment, where the NH_4^+-fed plants of different cultivars, except Mazurka, also demonstrated an increasing (more positive) $\Delta\Psi_{md\text{-}pd}$ compared to NO_3^--fed plants.

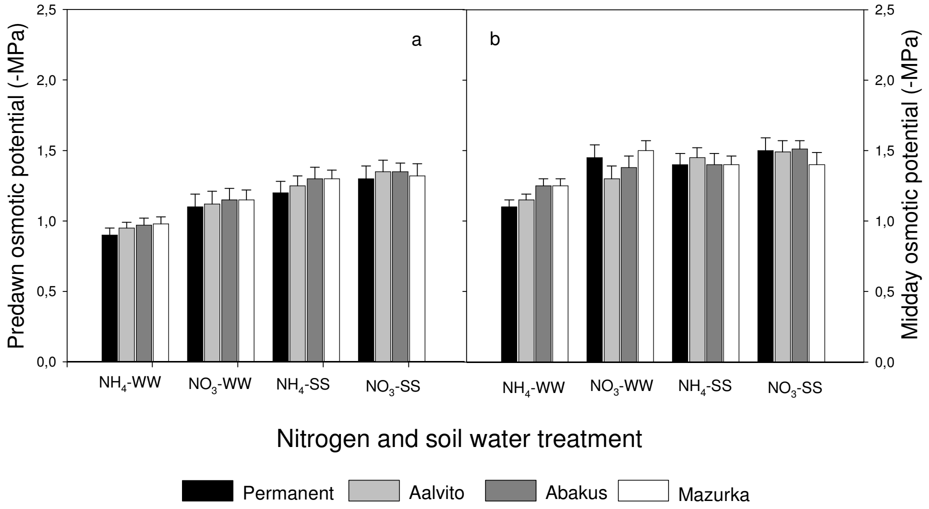


Figure 14: Predawn (a) and midday (b) osmotic potential of different maize cultivars under two nitrogen fertilizations, NH_4^+ and NO_3^-.

The predawn and midday osmotic potential (π_{pd}, π_{md}) is given in Figure 14. When ammonium or nitrate were used as nitrogen source, predawn and midday leaf water potential were not significant; furthermore, there were no differences between water regimes.

The differences between the π_{pd} and π_{md} values were small, compared to the difference between the midday and predawn leaf water potential. This difference might be attributable to a reduction of osmotic potential of drought stressed treatments to maintenance the turgor (ρ) presented in Figure 15.

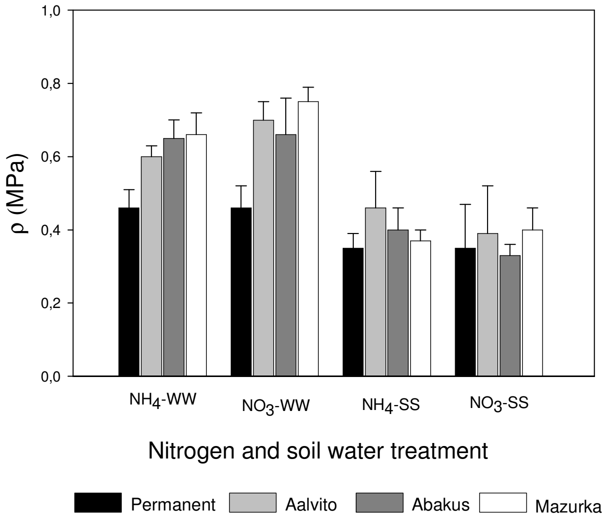


Figure 15: Turgor pressure of maize cultivars under different soil water regime and two different nitrogen fertilizations, NH_4^+ and NO_3^-.

The turgor pressure of the different treatments supplied with ammonium or nitrate showed no a significant differences. On the other hand, the well watered treatments had a higher turgor compared with drought stressed treatments.

Drought has decreased turgor in NH_4^+ treatments from 0.46 to 0.35 MPa, from 0.60 to 0.46 MPa, from 0.65 to 0.40 MPa and from 0.66 to 0.37 MPa in Permanent, Aalvito, Abakus and Mazurka, respectively. On the other hand, when nitrate was used as nitrogen source, drought has decreased turgor in Permanent, Aalvito, Abakus and Mazurka, from 0.46 to 0.35 MPa, from 0.70 to 0.39 MPa, from 0.66 to 0.33 MPa and from 0.75 to 0.40 MPa, respectively. Cultivar Permanent shows the lowest turgor values of all cultivars in the well-watered treatments.

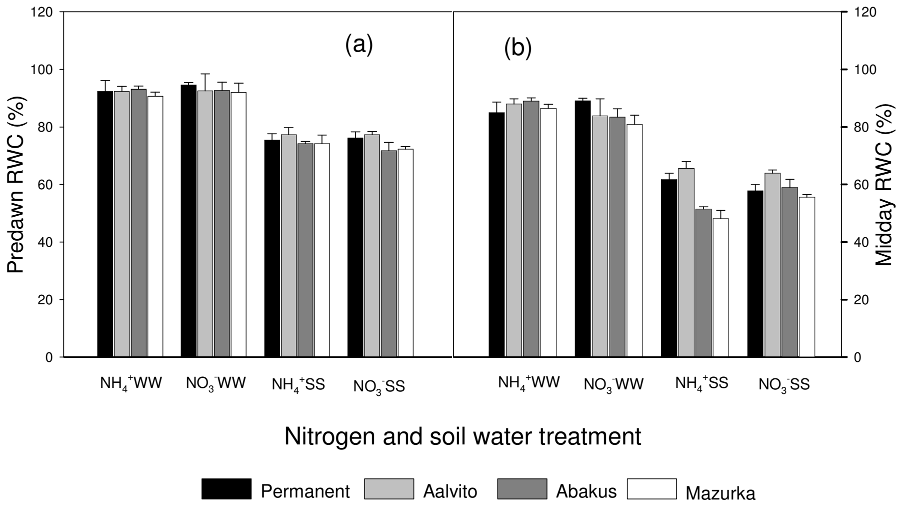


Figure 16: Predawn (a) and midday (b) relative water content of maize cultivars under different soil water regime and two different nitrogen fertilizers, NH_4^+ and NO_3^-.

There were significant differences ($p<0.001$) in the diurnal relative leaf water contents (RWC) of the different maize cultivars due to water regime (Figure 16a and 16b). The Figure 16 illustrates that the RWC decreased under soil drought conditions, in both, predawn and midday measurements. The results show no predawn RWC difference between nitrogen forms and between cultivars.

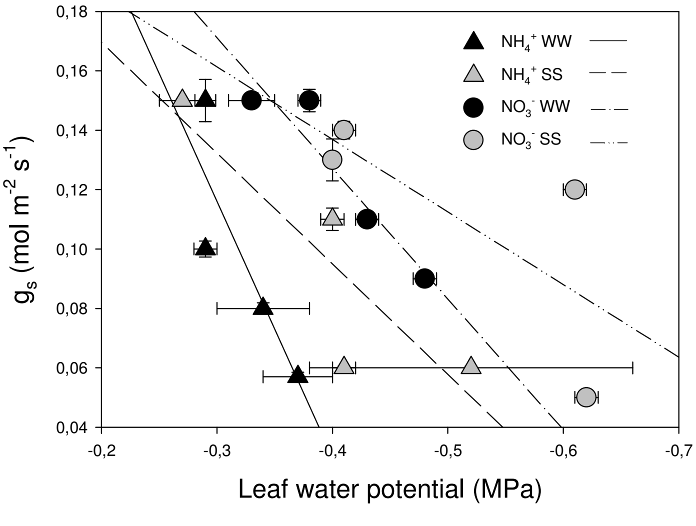


Figure 17: Relationship between stomatal conductance (g_s, mol m^{-2} s^{-1}) and leaf water potential in different maize cultivars under two different nitrogen fertilizations (NH_4^+ and NO_3^-) and two-water regime (well-watered and water stress).

Figure 17 shows the g_s behavior as a function of leaf water potential. There was a direct linear regression between leaf water potential and stomatal conductance in all treatment. The NO_3^- plants under drought stress had the lowest LWP values compared with the other treatments. Though the well-watered treatments had the highest LWP, g_s maximum values of all treatments did not vary much but at same values of leaf water potential, the NH_4^+ well watered plants had a slightly g_s increased compared to the NO_3^- well watered plants.

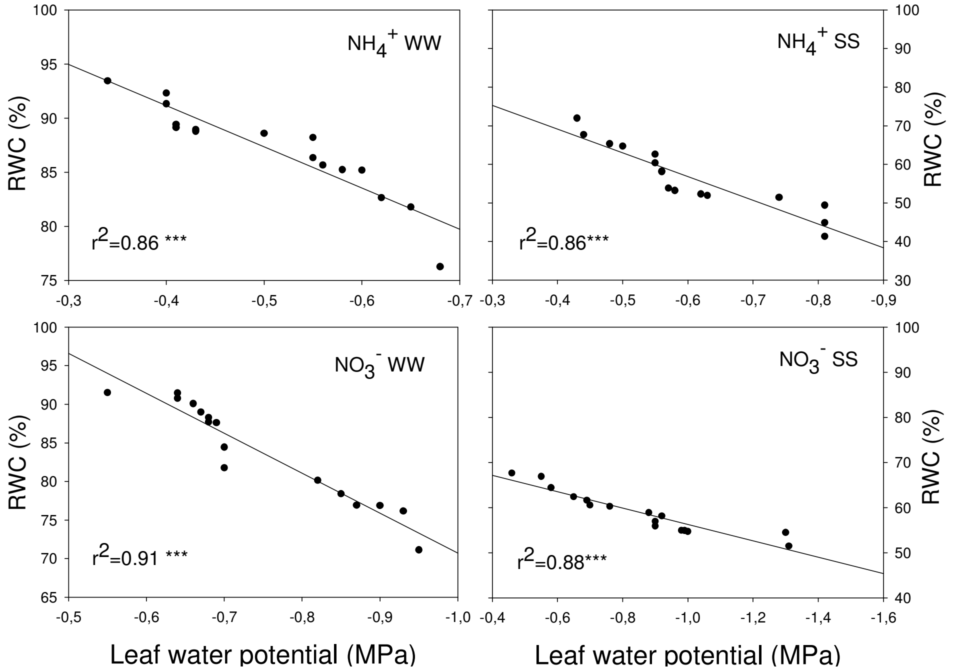


Figure 18: Relation between relative water content and leaf water potential of different maize cultivars under different nitrogen fertilizations and water regime in the greenhouse experiment (2008). (All regressions are statistically significant at p≤0.05).

An increase of leaf water potential resulted in an increase of the relative leaf water content in all treatments (Figure 17). Relative water content (RWC) plotted in Figure 18 against leaf water potential (LWP) shows significant differences not only between the water regimes, but also between the plants treated with different nitrogen forms. Thus, we can say that the plants supplied with ammonium under the same values of water potential, contain more water than those supplied with nitrate. The plants under well watered conditions and treated with ammonium had RWC average of 87 % compared with 83 % for the NO_3^- treated ones. The decreasing pattern of RWC was also clear when comparing the two water regimes. Thus, the well-watered plants treated with different nitrogen ionic forms exhibited higher RWC than drought-stressed plants. Drought has decreased RWC in NH_4^+ plants from 87 to 56 % at average LWP values of -0.51 and -0.60 MPa, respectively. Similarly, RWC was decreased in NO_3^- from 83 to 59% due to drought.

The stem hydraulic conductance (K_{stem}) of the four maize cultivars measured in the greenhouse (2007) is given in Figure 19.

Generally, the different maize cultivars under different water regime and N-form reveal significant differences in stem hydraulic conductance (Figure 19) ($P<0.001$). There were also a significant cultivars*nitrogen form, cultivar*water regime, nitrogen form*water regime and cultivar*nitrogen form*water regime effect.

Under well watered and drought conditions, the cultivar Nutrader and Permanent showed higher of K_{stem} with ammonium fertilization than with nitrate fertilization (Figure 19a and 19b).

Nutrader, Vitaminado and Permanent under well watered conditions with ammonium fertilization, showed higher hydraulic conductance than the drought-stressed plants ($P<0.001$). In the well-watered NH_4^+-supplied plants, the cultivars Nutrader and Permanente showed the highest stem hydraulic conductance. Average of K_{stem} of Nutrader and Permanent were 9.16 and 9.04 $x10^{-4}$ kg m^{-2} MPa^{-1}, respectively, and 3.5 and 3.22 $x10^{-4}$ kg m^{-2} MPa^{-1} for the cultivars Vitaminado and Mazorca de oro, respectively. The cultivar Vitaminado had the lowest K_{stem} values (Figure 19a).

Under well-watered conditions with nitrate fertilization, the cultivars Nutrader and Mazorca de oro had highest K_{stem} (4.30 and 3.16 $x10^{-4}$ kg m^{-2} MPa^{-1}, respectively) (Figure 19a).

In the stressed NH_4^+ and NO_3^- -supplied plant, the cultivars Nutrader showed the highest K_{stem} of 8.52 and 3.76 $x10^{-4}$ kg m^{-2} MPa^{-1}, respectively. However, the cultivars Vitaminado showed the lowest K_{stem} values (1.53 $x10^{-4}$ kg m^{-2} MPa^{-1}) (Figure 19b).

In order to evaluate the relationship between the hydraulic conductance and growth, the Figure 20 shows the linear relation between hydraulic conductance and stem diameter. The correlation has no significance (ns) between the stem diameter and hydraulic conductance; between the water and nitrogen treatment, in other words, between well watered and water stressed plants and between NH_4^+ and NO_3^- plants ($r^2=0.27$). Since the hydraulic conductance was not affected by stem diameter, the difference as seen in K_{stem} may be due to water regime, nitrogen form or by the difference between cultivars.

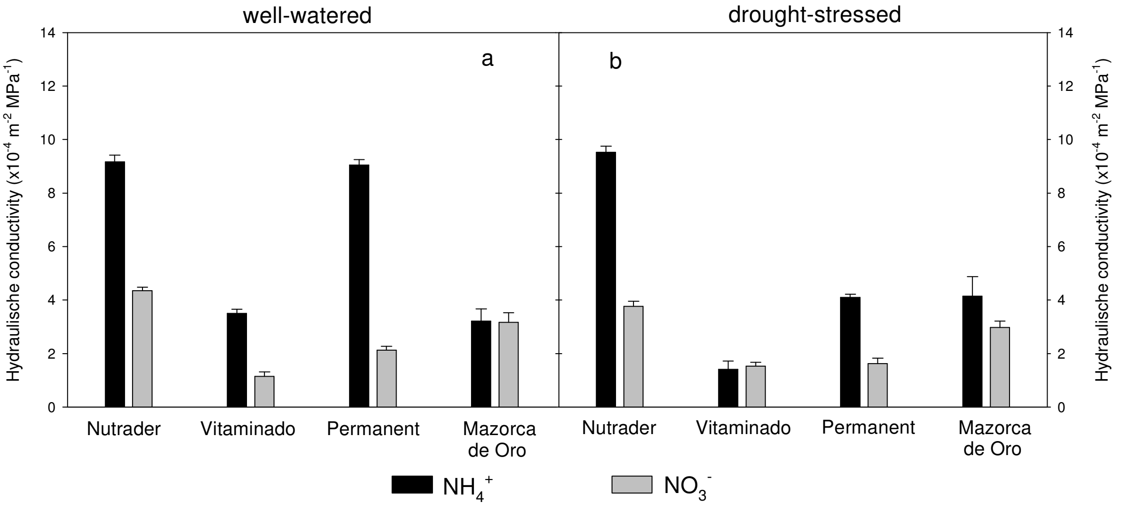


Figure 19: Hydraulic conductance in stem cut of different maize cultivars under two different nitrogen forms and two water regimes in the greenhouse experiment (2007).

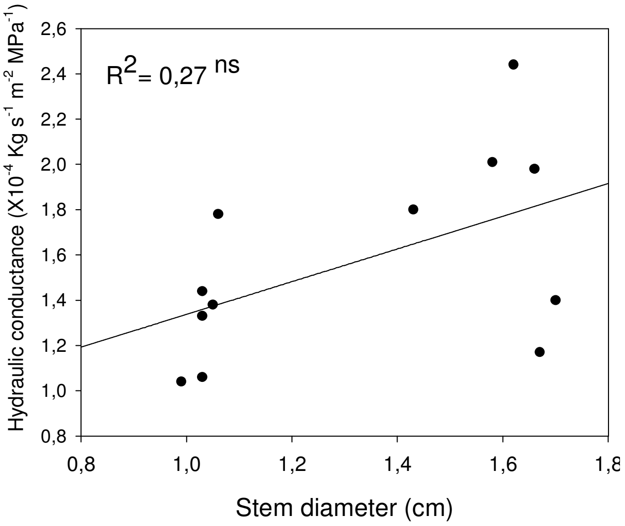


Figure 20: Relationship between stem cut (10 cm) and hydraulic conductance of maize (*Zea mays* L) under different nitrogen fertilization (NH_4^+ and NO_3^-) in the greenhouse experiment (2007).

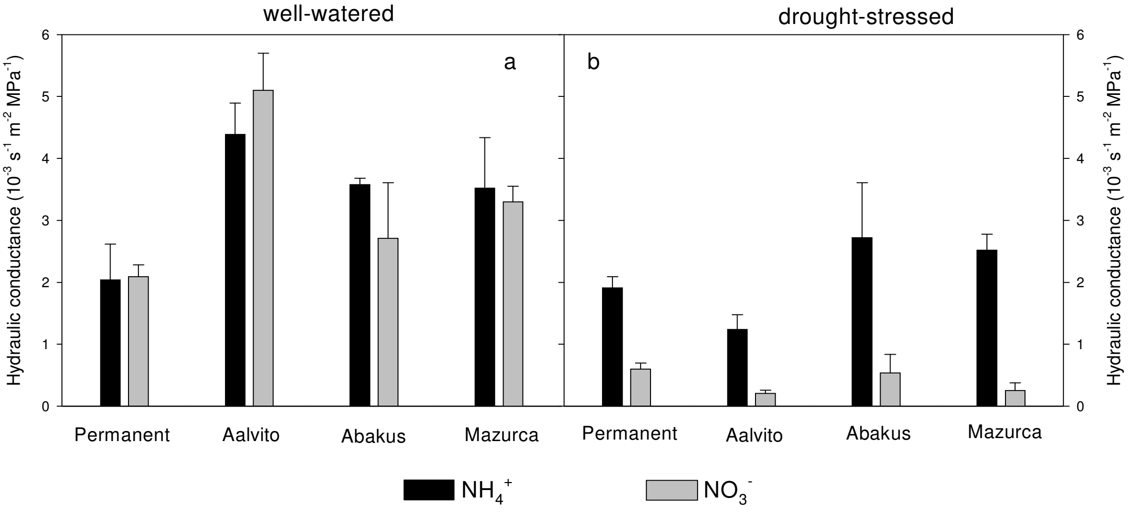


Figure 21: Hydraulic conductance in stem cut of different maize cultivars under two different nitrogen forms and two water regime in the greenhouse experiment (2008).

The results of stem hydraulic conductance (K_{stem}) in different maize cultivars under two nitrogen forms (NH_4^+ and NO_3^-) and two water regimes (well watered and drought-stressed) in the greenhouse 2008, are showed in Figure 21.

The stem hydraulic conductance was affected by cultivar ($p<0.001$), nitrogen form ($p<0.001$) and by water regime ($p<0.001$).

Under well-watered conditions Aalvito showed a clearly increasing of hydraulic conductance (+19%) with nitrate fertilization than with ammonium fertilization. Aalvito also had the highest K_{stem} when ammonium or nitrate was used as nitrogen source.

Generally, all cultivars with ammonium or nitrate fertilization showed higher K_{stem} under well-watered conditions than under drought conditions (Figure 21a and 21b).

Under drought conditions, sole ammonium supply provoked an increasing of hydraulic conductivity against sole nitrate supply. Abakus and Mazurka with ammonium and under drought conditions had the highest K_{stem} (Figure 21b).

In order to know the relationship between stem hydraulic conductance and growth, the Figure 22 shows the behavior of K_{stem} against stem diameter. The results reveal that the hydraulic conductance was positively and significantly correlated with stem diameter (r^2=0.42**).

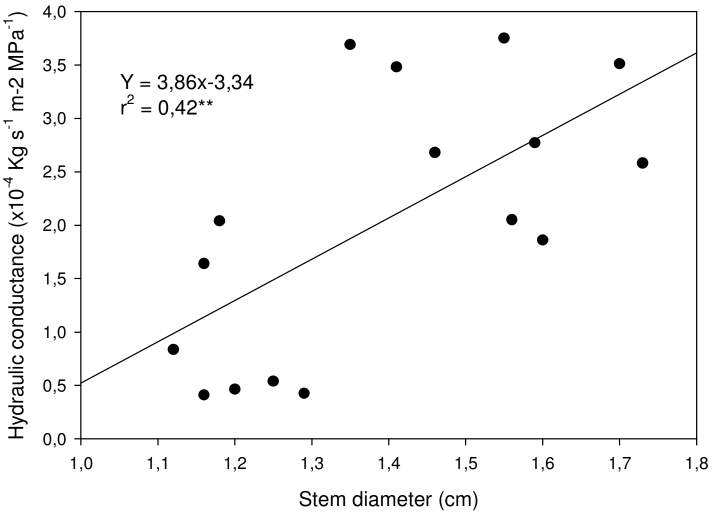


Figure 22: Relationship between stem cut (10 cm) and hydraulic conductance of maize (*Zea mays* L) under different nitrogen fertilization (NH_4^+ and NO_3^-) in the greenhouse experiment (2008).

4.1.3 Morphological traits and dry matter

Plant height is one of the important agronomic traits. Variance analysis indicated that plant height among cultivars was significantly different ($p<0.001$) (Figure 23a). When ammonium or nitrate was used as nitrogen source, the plant height was significantly different ($p<0.001$).

Vitaminado and Permanent showed a clear increase of plant height when ammonium was used as nitrogen source instead of nitrate (Figure 23a). The tallest plants (1.7 m) were produced when ammonium was applied instead of nitrate and the cultivar Permanent was the highest between cultivars.

The Figure 23b shows the dry matter of different maize cultivars grown in 2007. According to Figure 23a, it can be seen that the dry matter was higher in NH_4^+ plants than in NO_3^- plants ($p<0.05$). In all cultivars, the above-ground dry matter production has increased with ammonium fertilization than with nitrate fertilization (Figure 23b). Nutrader, Vitaminado, Permanent and Mazorca de oro showed an increase of about 20%, 15%, 12% and 14% of above-ground dry matter production with ammonium respectively than with nitrate fertilization.

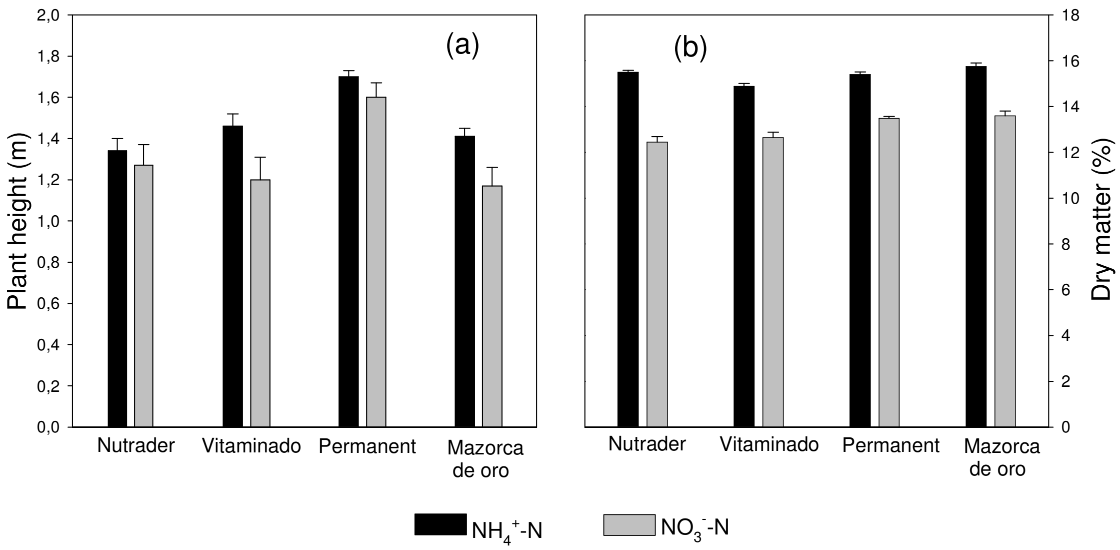


Figure 23: Dry matter (a) and plant height (b) of different maize cultivars grown in 2007 under two different nitrogen fertilizers, ammonium and nitrate.

Variety	N-form & water regime	Plant Height (m)	Stem Diameter (cm)
Permanent	NH_4^+WW	1.60±0.09	1.60±0.11
	NO_3^-WW	1.41±0.06	1.56±0.10
	NH_4^+SS	1.32±0.07	1.25±0.08
	NO_3^-SS	1.09±0.05	1.16±0.04
Aalvito	NH_4^+WW	1.55±0.03	1.59±0.04
	NO_3^-WW	1.36±0.08	1.35±0.06
	NO_3^-WW	1.17±0.07	1.29±0.07
	NO_3^-SS	1.11±0.01	1.12±0.08
Abakus	NH_4^+WW	1.54±0.03	1.73±0.03
	NO_3^-WW	1.43±0.09	1.70±0.11
	NO_3^-WW	1.36±0.09	1.55±0.06
	NO_3^-SS	1.20±0.06	1.20±0.06
Mazurka	NH_4^+WW	1.44±0.02	1.46±0.07
	NO_3^-WW	1.27±0.03	1.41±0.01
	NO_3^-WW	1.27±0.04	1.18±0.05
	NO_3^-SS	1.09±0.03	1.16±0.01
		ANOVA	
Variety (V)		*	***
N-form (N)		***	***
Water regime (W)		***	**
V*N		ns	ns
V*W		ns	***
N*W		ns	ns
V*N*W		ns	**

Ns, *,**,*** = Not significant, significant at $p<0.05$. at $p<0.01$. at $p<0.001$, respectively

Table 1: Morphological trait of different maize cultivars under two different soil water regimes and two different nitrogen fertilizers (NH_4^+ and NO_3^-) in the greenhouse experiment (2008).

There was a significant effect of cultivars ($p<0.05$), nitrogen form ($p<0.001$) and water regime ($p<0.001$) on plant height. On the other hand, the analysis of variance showed no significant (ns) V*N, V*W, N*W and V*N*W interaction (Table 1).

With ammonium fertilization, all cultivars were taller than with nitrate fertilization, in both well watered and drought conditions. Similar to 2007, Permanent was the tallest with plant height average of about 1.6 m.

The plant height was reduced in all cultivars with either ammonium or nitrate fertilization in drought conditions. In Permanent, Aalvito, Abakus and Mazurka with ammonium fertilization, drought reduced the plant height of 17%, 14%, 11% and 12%, respectively. However, in nitrate fed-plants, such reductions were 22%, 18%, 16% and 14% respectively (Table 1).

Changes in stem diameter (S_d) are frequently used to assess plant water status. The results from ANOVA analysis show a significant effect of cultivar ($p<0.001$), N-form ($p<0.001$) and water regime ($p<0.01$) in response to stem diameter. There were also significant V*W and V*N*W effect. Under well-watered conditions, the stem diameter has increased when nitrogen was supplied as ammonium instead of nitrate. However, in the Permanent cultivar, this increase was higher in the drought stressed plants than in the well-watered plants (Table 1).

Drought also reduced the plant height in all cultivars with either ammonium or nitrate fertilization (Table 1).

In the NH_4^+ treated plants, drought reduced S_d of about 12, 19, 10 and 19% in Permanent, Aalvito, Abakus and Mazurka, respectively. However, this increase was 26, 17, 29 and 18% in the NO_3^- treated plants, suggesting that Aalvito and Mazurka were less affected with ammonium fertilization than with nitrate fertilization (Table 1).

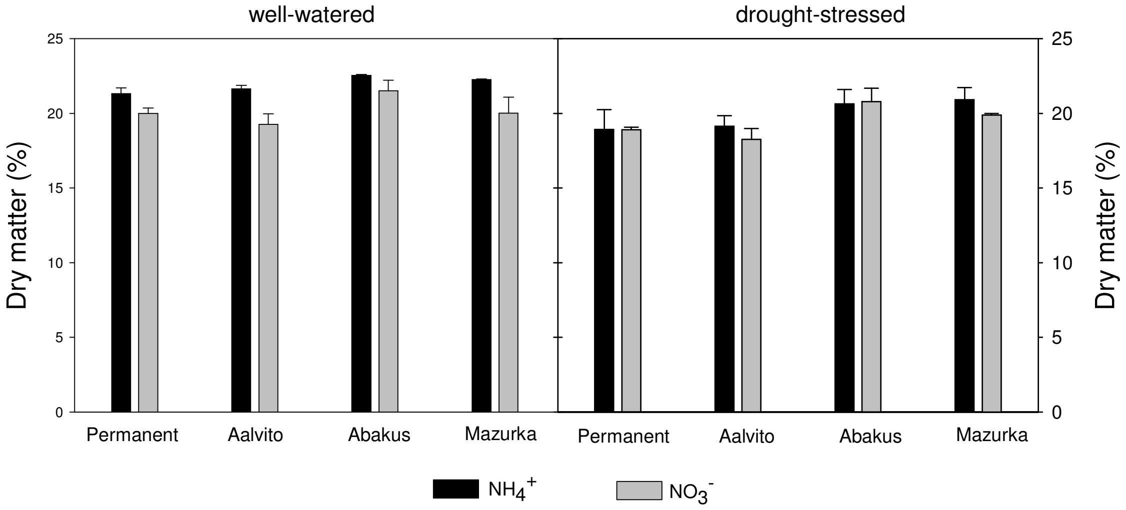


Figure 24: Dry matter in different maize cultivars under two water regimes and two-nitrogen fertilization (Ammonium and Nitrate) in the greenhouse experiment (2008).

There was a significant effect of cultivars ($p<0.001$), nitrogen form ($p<0.001$), and water regime ($p<0.001$) on dry matter. There was also were a significant N*W ($p<0.01$) effect. However, the analysis of variance shows no significant (ns) V*N, V*W and V*N*W interaction (Figure 24).

Under well-watered conditions, all cultivars showed higher above-ground dry matter production with ammonium fertilization than with nitrate fertilization. However, this was not the case under drought stressed conditions.

Drought reduced the above-ground dry matter production in all cultivars treated with ammonium. However, Aalvito and Mazurka cultivars were not affected by drought with nitrate fertilization. Between cultivars, Abakus and Mazurka had the highest dry matter (22%).

4.2 Field experiment

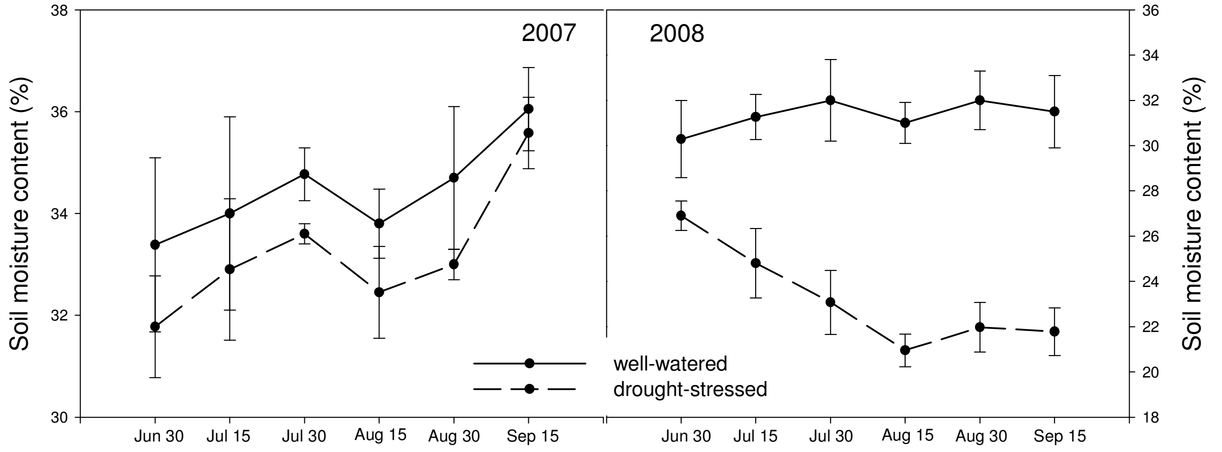


Figure 25: Soil moisture in the study site (2007 and 2008).

The moisture content in the field during the experiment in 2007 and 2008 is illustrated in Figure 25. Due to weather conditions, the soil moisture during 2007 showed no difference in the two water regimes (irrigated and drought-stressed), hence an analysis between well water and drought-stressed treatments were not possible. In 2008, the soil moisture content monitored in the drought-stressed plots was clear lower than in the well-watered plots (Figure 25).

During the experiment in 2007, a heavy rain occurred 3-5 day before the soil moisture measurements. Therefore, the differences in the soil water content between drought-stressed and well-watered plots were relatively low. However, during 2008 sporadic rains occurred, having differences in soil water content between drought-stressed and well-watered plots. Not surprisingly, the soil water content of the drought-stressed plots was not constant over the time in 2007 as well as in 2008.

4.2.1 Gas exchange measurements

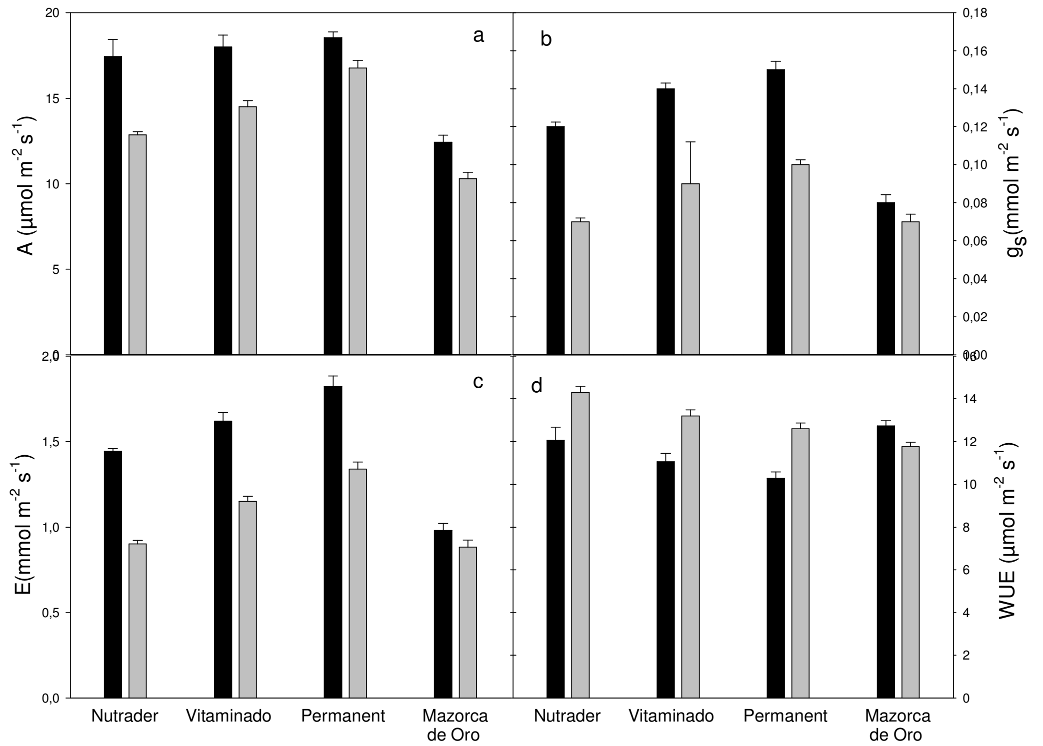


Figure 26: Gas exchange parameters in different maize cultivars under different nitrogen form (NH_4^+ and NO_3^-) in the field experiment (2007): (a) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (b) stomatal conductance (g_s, mol m^{-2} s^{-1}) (c) transpiration rate (E, mmol m^{-2} s^{-1}) and (d) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}).

The results of ANOVA for the gas exchange parameters (Figure 26) show that the photosynthesis (A) was significantly influenced by cultivars ($p<0.001$) and nitrogen form ($p<0.001$). Furthermore, the plants of the different cultivars and fertilized with the different nitrogen form showed a significant variety*nitrogen form effect.

An individual analysis of each cultivar shows that when the Nutrader cultivar was fertilized with ammonium instead of nitrate, the photosynthesis rate was 26% higher. Compared to NO_3^- plants, the NH_4^+ plants of the other three cultivars, showed also higher photosynthesis rate, being the increase of 19%, 9% and 17% for Vitaminado, Permanent and Mazorca de oro, respectively.

The stomatal conductance (g_s) was significantly ($p<0.001$) different between cultivars and between the plants under different nitrogen forms. There was also a significant variety*nitrogen form effect.

When ammonium was used as nitrogen form instead of nitrate, the stomatal conductance was clearly higher in Nutrader, Vitaminado and Permanent cultivars. However, the g_s

increase was slightly higher in Mazorca de oro. Compared to those fertilized with nitrate, the NH_4^+ plants had 41%, 35%, 33% and 12% higher g_s for Nutrader, Vitaminado, Permanent and Mazorca de oro, respectively (Figure 26b).

Plants fertilized with ammonium instead of nitrate showed also higher transpiration (E), hence cultivar and nitrogen form had a significant effect on transpiration rate ($p<0.001$) as well as the interaction variety*nitrogen form ($p<0.001$) (Figure26c).

The calculated water use efficiency (A/E) was significantly ($p<0.01$) different between cultivars and between nitrogen forms. Except cultivar Mazorca de oro, the other three cultivars demonstrated higher WUE when the plants were fertilized with nitrate instead of ammonium.

The NO_3^- plants of cultivars Nutrader, Vitaminado and Permanent had 14%, 16% and 18% higher WUE, respectively, compared to the NH_4^+ plants, while the NH_4^+ plants of Mazorca de oro showed 7% higher WUE compared to the NO_3^- plants (Figure 26d).

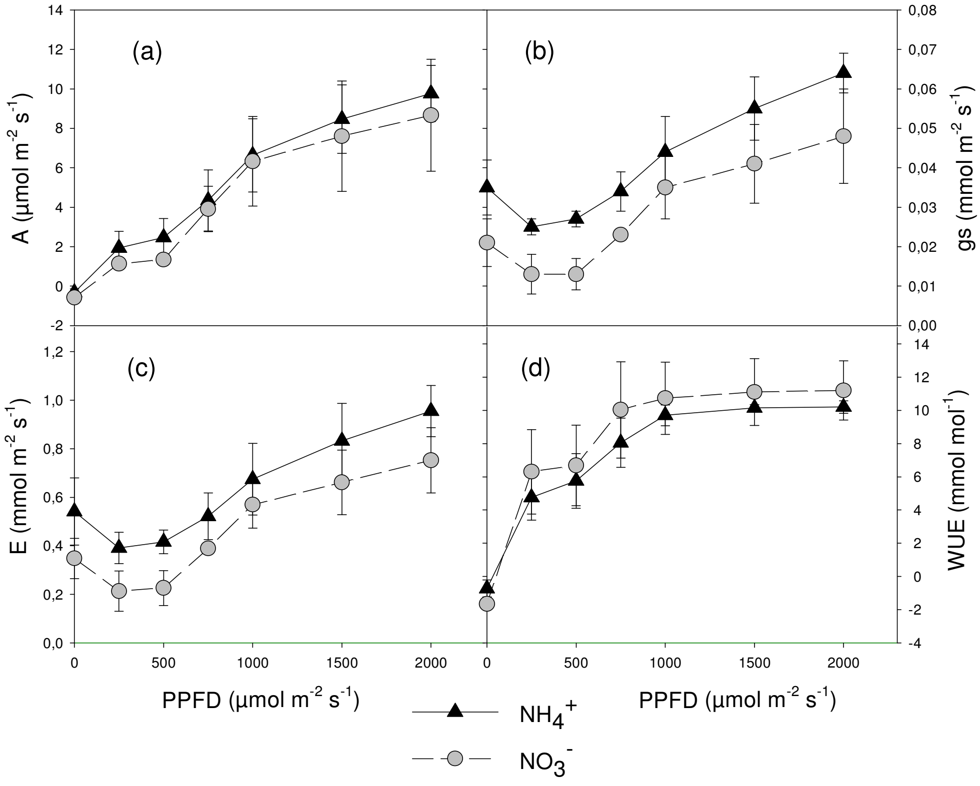


Figure 27: Change in (a) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (b) stomatal conductance (g_s, mol m^{-2} s^{-1}), (c), transpiration rate (E, mmol m^{-2} s^{-1}) and (d) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}) as a function of the photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) in different maize cultivars under different nitrogen fertilization in the field experiment (2007).

The light response curve of different maize cultivars under different nitrogen form in 2007 is shown in Figure 27.

With an increase of light intensity, the photosynthetic rate also increased. Similarly, stomatal conductance (g_s), transpiration rate (E) and water use efficiency (WUE) experimented an increased when the light intensity increased from 0 to 2000 (µmol m^{-2} s^{-1}). The NH_4^+fed plants reached maximum A_{net} of 9.77 µmol m^{-2} s^{-1} at 2000 µmol m^{-2} s^{-1} against 2.84 µmol m^{-2} s^{-1} of NO_3^-fed plants. In both treatments there were no any signs of photoinhibition.

An increase of light intensity from 0 to 2000 µmol m^{-2} s^{-1} led to an increase of g_s and E, although g_s and E decreased in the first 250 µmol m^{-2} s^{-1} of light intensity. When ammonium was applied as a nitrogen source, the plants showed higher g_s and E compared to nitrate. The NH_4^+ plants reached maximum g_s and E of 0.06 mmol m^{-2} s^{-1} and 0.96 mmol m^{-2} s^{-1}, respectively, compared to g_s and E values of 0.05 mmol m^{-2} s^{-1} and 0.75 mmol m^{-2} s^{-1} in NO_3^- plants, respectively.

The water use efficiency was higher in NO_3^- plants than NH_4^+ plants, although in the first 250 (mmol m^{-2} s^{-1}) of light intensity the WUE between the two nitrogen forms did not differ.

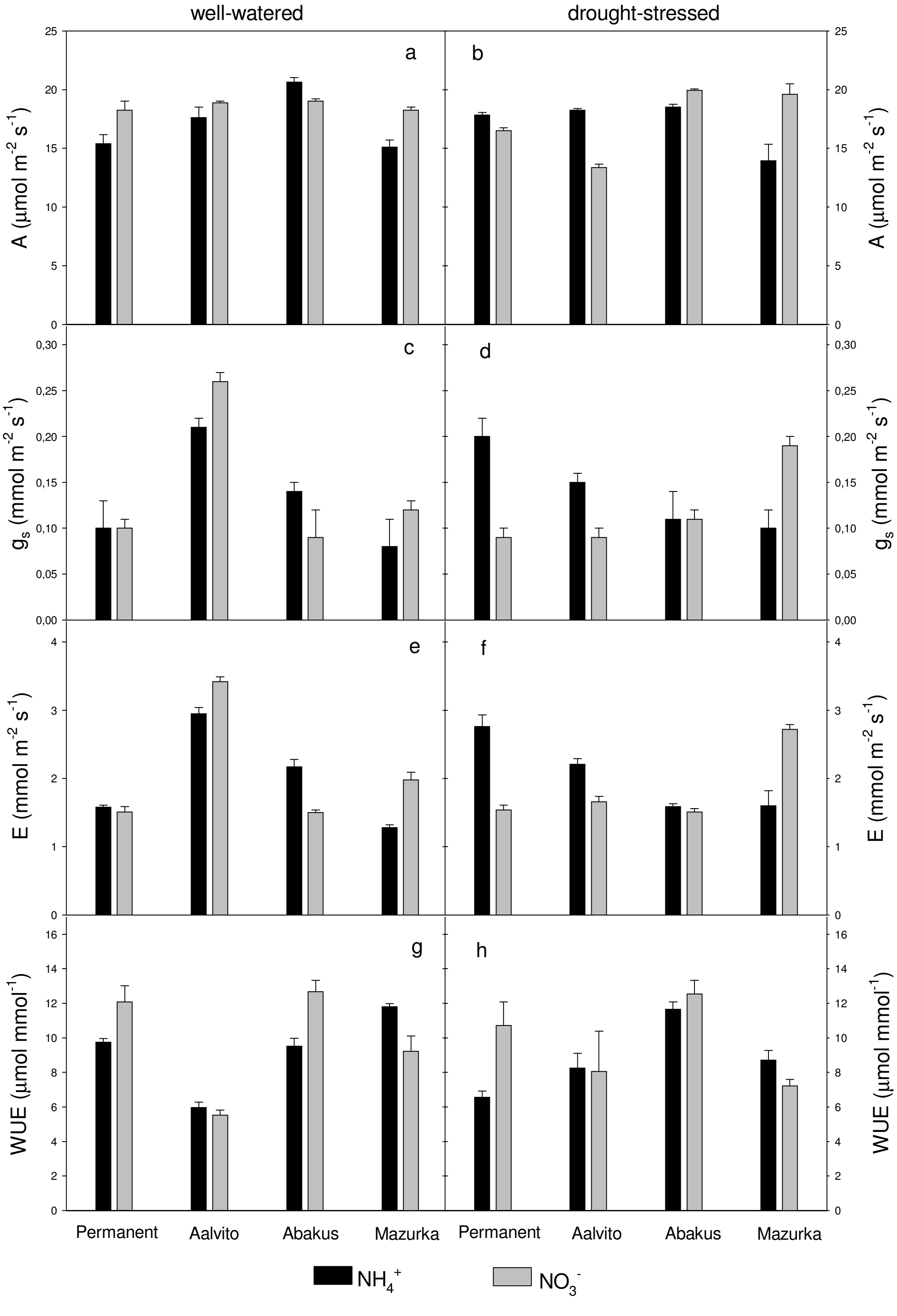


Figure 28: Gas exchange parameters in different maize cultivars under well-watered and drought conditions and different nitrogen forms (NH_4^+ and NO_3^-) in the field experiment (2008): (a and b) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (c and d) stomatal conductance (g_s, mol m^{-2} s^{-1}), (f and g) Transpiration rate (E, mmol m^{-2} s^{-1}) and (h and i) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}).

The result of ANOVA, for the leaf exchange parameters, show that the variety had significant influence on A_{net}, g_s, E and WUE ($p<0.001$). When the plants were fertilized with different nitrogen forms (NH_4^+ or NO_3^-), A_{net} was also significantly different ($p<0.01$), as well as g_s, E and WUE ($p<0.05$). When the water availability was reduced no significant difference was detected on A_{net} and g_s but E and WUE were significant differently ($p<0.001$) (Figure 28). Except the nitrogen form*water regime interaction effect on WUE, all other interactions were significant ($p<0.001$).

Except the Abakus, all cultivars fertilized with nitrate under well-watered conditions showed a higher A_{net} compared to NH_4^+ plants. Stomatal conductance (g_s) and transpiration rate (E) were also higher in the cultivars Aalvito and Mazurka with nitrate fertilization than with ammonium fertilization. However, g_s and E were higher in the cultivar Abakus with ammonium fertilization than with nitrate fertilization. The cultivar Permanent showed not increased in g_s and E with either ammonium or nitrate fertilization. The cultivar Aalvito showed the highest g_s and E, but the lowest water use efficiency (WUE). In the NO_3^--treated plants of the cultivars Permanent and Abakus WUE was 19 and 24% higher than the NH_4^+-treated plants, respectively, whereas, the cultivar Mazurka showed higher WUE (+22%) with ammonium fertilization than with nitrate fertilization.

Under drought conditions the NH_4^+-treated plants in the cultivars Permanent and Aalvito showed higher A_{net}, g_s, and E than the NO_3^--treated plants. However the WUE was higher with nitrate than with ammonium. Nevertheless, under drought conditions, Mazurka had higher A_{net}, g_s and E with nitrate than with ammonium. However, the WUE was higher with ammonium than with nitrate.

Drought increased A_{net}, g_s and E, but decreased WUE in the cultivar permanent when ammonium was used as nitrogen source than nitrate. Permanent had, therefore, the lowest WUE under drought conditions with ammonium fertilization. Drought decreased clearly g_s and E, but increased the WUE in the cultivar Aalvito with ammonium or with nitrate fertilization. Abakus had the highest WUE in both, well-watered and drought conditions with nitrate fertilization than with ammonium. This result suggests, therefore, that the ability of the plants, under different nitrogen form and/or water regime, in reducing transpiratinal water loss signified an increase in water WUE in all treatments.

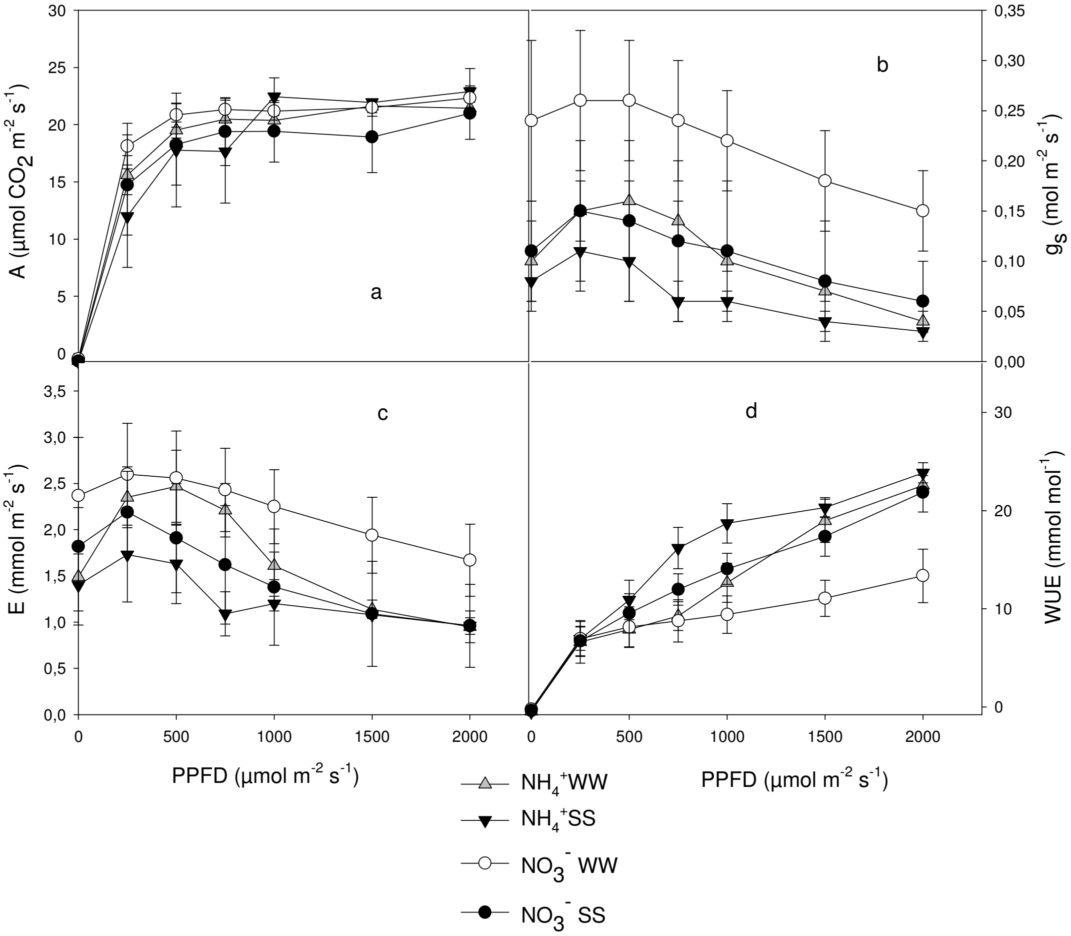


Figure 29: Change in (a) net photosynthetic rate (A, µmol m^{-2} s^{-1}), (b) stomatal conductance (g_s, mol m^{-2} s^{-1}) (c) transpiration rate (E, mmol m^{-2} s^{-1}) and (d) instantaneous water-use-efficiency (A/E) (WUE, µmol m^{-2} s^{-1}) as a function of the photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) in different maize cultivars under different nitrogen fertilization and water regime in the field experiment (2008).

The light response curve of different maize cultivars and fertilized with different nitrogen form are shown in Figure 29.

The photosynthesis (A_{net}) was directly proportional to light intensity. All cultivars increased A_{net} with an increase of light intensity from 0 to 2000 µmol m^{-2} s^{-1}. The well-watered treatments showed no any sin g_s of photoinhibition. However, the drought-stressed NH_4^+ and NO_3^- plants experimented slightly sin g_s of photoinhibition at about 500 and 1000 µmol m^{-2} s^{-1}, respectively; although A_{net} recovered to mach the other treatments.

All treatments showed a decrease of g_s and E from about 250-500 µmol m^{-2} s^{-1}. However, the g_s and E decrease was higher in the drought-stressed treatments than the well-watered treatments. Although, at about 1500 and 2000 µmol m^{-2} s^{-1}, except NO_3^- WW, all others treatment showed similar E values.

Stomatal conductance fell until 0.06, 0.004, 0.087 and 0.016 mmol m^{-2} s^{-1} in the NH_4^+ WW, NH_4^+ SS, NO_3^- WW and NO_3^- SS at about 2000 µmol m^{-2} s^{-1}, respectively. On the other hand, at about 2000 µmol m^{-2} s^{-1} E fell until 0.95, 0.96, 1.67 and 0.96 mmol m^{-2} s^{-1} in the NH_4^+ WW, NH_4^+ SS, NO3$^-$ WW and NO_3^- SS treatments, respectively.

The E fall of NH_4^+ WW plants possibly provoked a strong increase in WUE showing maximum values of 23.85 µmol m^{-2} s^{-1} at 2000 µmol m^{-2} s^{-1}.

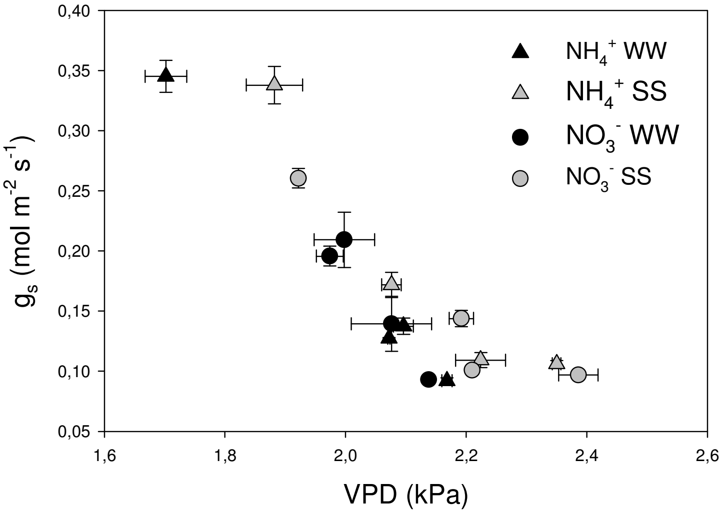


Figure 30: Change in stomatal conductance (g_s, mol m^{-2} s^{-1}) as a function of leaf-to-air vapour-pressure deficit (VPD, kPa) in different maize cultivars under different nitrogen fertilization and water regime in the Field experiment (2008).

The plots of stomatal conductance and transpiration rate versus vapor pressure deficit (VPD) referred to all treatments is given in Figures 30 and 31, respectively.

Stomatal responsiveness to changes in vapor pressure varied considerable according to nitrogen form and water regime. All treatments showed g_s decrease with an increase of VPD.

The NH_4^+ plants exhibited, over a wide range of VPD between 1.6 and 2.4 kPa, the higher g_s values compared to NO_3^- plants. At 1.7 kPa, the well-watered NH_4^+ plants had the highest g_s values of 0.35 mmol m^{-2} s$^{-1.}$ On the other hand, the drought-stressed NH_4^+ plants had at 1.88 kPa g_s values of 0.33 mmol m^{-2} s^{-1}.

Contrary to NH_4^+ treatments, the drought-stressed NO_3^- plants exhibited the higher g_s values of 0.26 mmol m^{-2} s^{-1} at 1.92 kPa against 0.21 mmol m^{-2} s^{-1} at 2 kPa for the well-watered NO_3^- plants.

The well-watered plants fell to the minimum g_s values of 0.09 mmol m^{-2} s^{-1} at 2.17 kPa for NH_4^+ plants and 0.09-mmol m^{-2} s^{-1} at 2.14 kPa for NO_3^- plants.

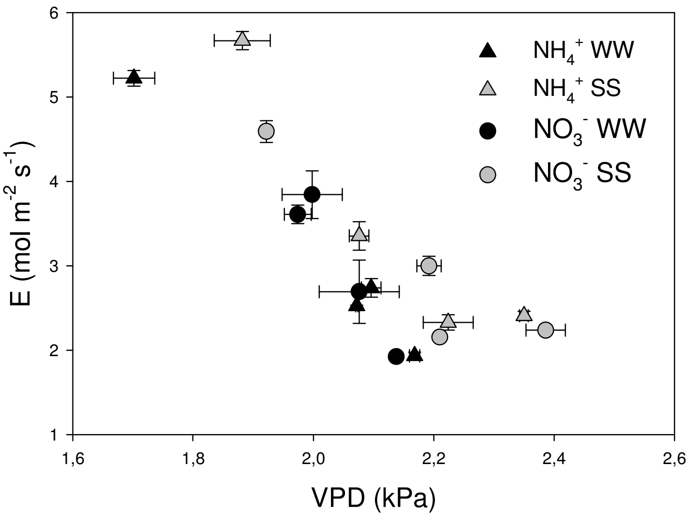


Figure 31: Change in transpiration rate (E, mmol m^{-2} s^{-1}) as a function of leaf-to-air vapour-pressure deficit (VPD, kPa) in different maize cultivars under different nitrogen fertilization and water regime in the field experiment (2008).

Generally, the drought-stressed plants exhibited the higher transpiration rate compared to the well-watered plants. The NH_4^+ drought-stressed treatments had maximum E values of 5.67 mmol m^{-2} s^{-1} at 1.88 kPa. However, the well-watered NH_4^+ treatments had E maximum values of 5.22 mmol m^{-2} s^{-1} at 1.70. The NO_3^- plants under drought conditions had the maximum E values of 4.6 mmol m^{-2} s^{-1} at 1.92 kPa compared to 3.84 mmol m^{-2} s^{-1} at 2 kPa for the well watered NO_3^- plants. Therefore, these results suggest that the plants under drought conditions transpired more than the plants under well-watered conditions.

4.2.2 Water potential and hydraulic conductivity

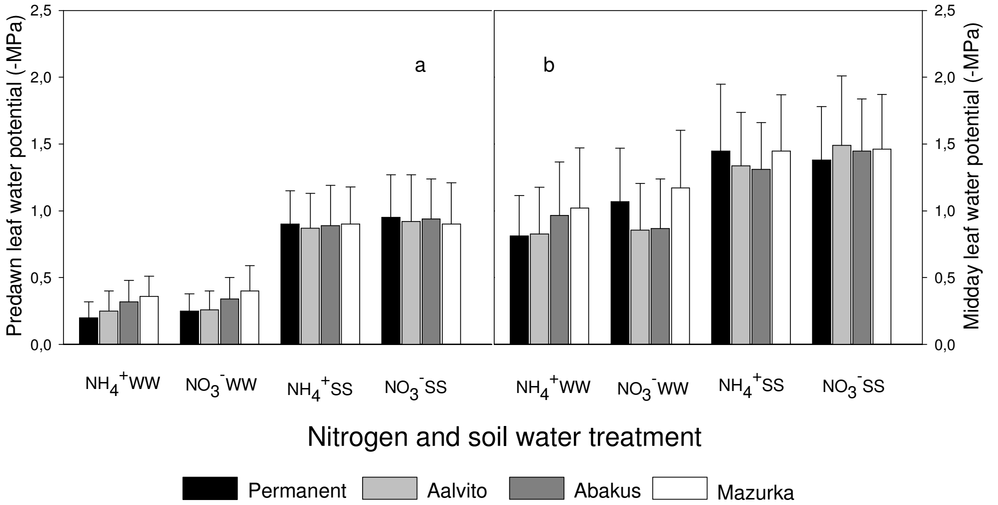


Figure 32: Predawn (a) and midday (b) leaf water potential of maize cultivars under different soil water regime and two different nitrogen fertilizers, NH_4^+ and NO_3^-.

When plants were supplied with NH_4^+ and NO_3^- and under well-watered conditions exhibited higher Ψ_{pd} and Ψ_{md} than the drought-stressed plants. Sole NH_4^+ or NO_3^- supply provokes not significant change in Ψ_{pd} and Ψ_{pd} among cultivars. (Figure 32 a and b).

Drought decreased Ψ_{pd} in both; NH_4^+ and NO_3^- treated plants in all cultivars. However, the difference in Ψ_{md} between well-watered and drought-stressed treatments was less pronounced than in Ψ_{pd}. All cultivars also exhibited consistently lower Ψ_{md} compared with $\Psi_{pd.}$

On the other hand, all cultivars under well-watered conditions had lower $\Delta\Psi_{pd-\ md}$ compared with the drought-stressed plants (Figure 33). This could explain why the drought-stressed plants had signs of wilting at the moment of take out the sample to Ψ_{md} measure.

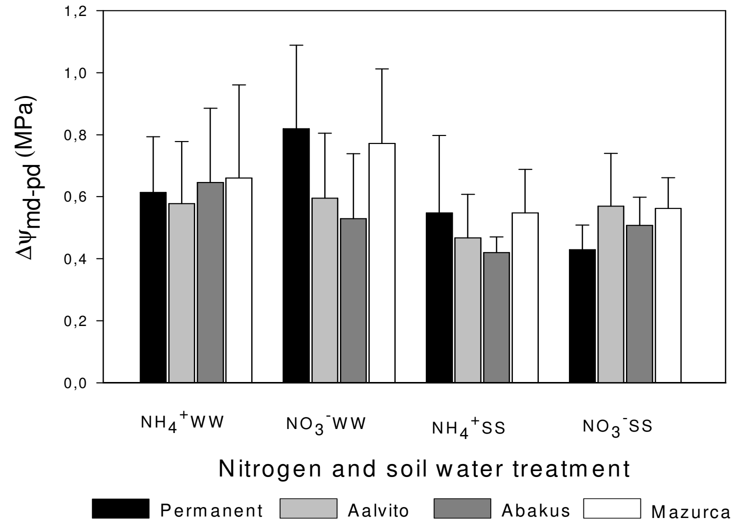


Figure 33: Comparison of predawn and midday leaf water potential of maize cultivars under different soil water regime and two different nitrogen fertilizations, NH_4^+ and NO_3^-.

In Figure 34 is showing the predawn and midday osmotic potential (π_{pd} and π_{md}). The osmotic potential was considerably higher in the well-watered plants than in drought-stressed plants. However, the Osmotic potential did not differ among nitrogen treatments. The Figure 34 showed that the predawn osmotic potential in the well-watered NH_4^+ plants are about 30, 24, 17 and 24% higher than the plants under drought conditions in the cultivars Permanent, Aalvito, Abakus and Mazurka, respectively. Similarly, the NO_3^- well-watered plants had about between 22 and 25% higher π_{pd} compared with the drought-stressed plants. The behavior of midday osmotic potential was similar as predawn osmotic potential. Thus, the NH_4^+ well-watered plants had 22, 17, 14 and 6 higher midday osmotic potential compared to the drought-stressed plants in Permanent, Aalvito, Abakus and Mazurka, respectively. On the other hand, the NO_3^- well watered plants had 15, 19, 17 and 10% higher midday leaf water potential compared to the drought-stressed plants in Permanent, Aalvito, Abakus and Mazurka, respectively.

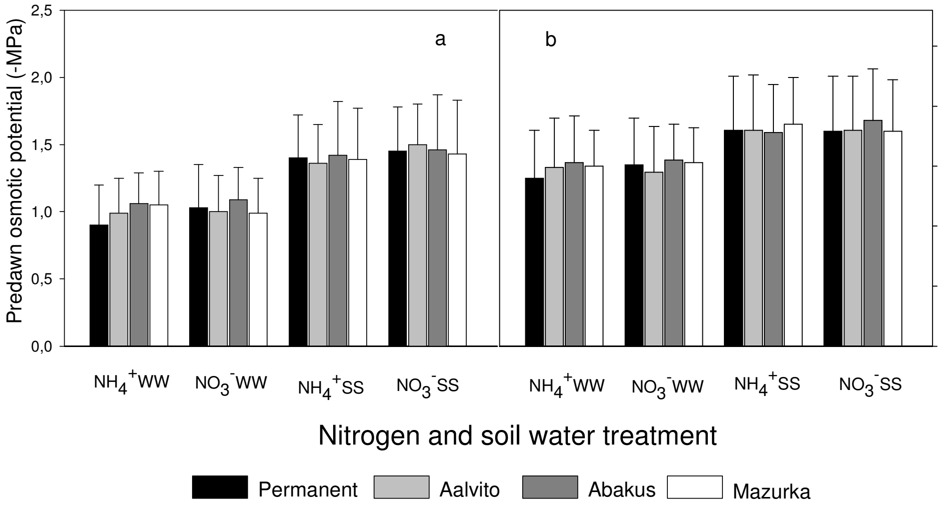


Figure 34: Predawn (a) and midday (b) osmotic potential of different maize cultivars under two nitrogen fertilizations, NH_4^+ and NO_3^-.

The pressure potential or turgor pressure is shown in Figure 35.

There was significance difference ($p<0.05$) in turgor pressure between the well-watered and drought-stressed plants with ammonium or nitrate fertilization.

The cultivars Permanent, Aalvito, Abakus and Mazurka with ammonium fertilization and under well-watered conditions had 40, 30, 16 and 16% higher turgor than with nitrate fertilization under drought conditions, respectively. Likewise, the NO_3^- well-watered plants exhibited higher turgor than the drought-stressed plants, being the Permanent cultivar lower affected by drought in comparison with the others cultivars.

Permanent and Aalvito showed difference between both nitrogen forms ($p<0.05$). Permanent cultivar had 20% higher turgor with ammonium fertilization than with nitrate fertilization. Likewise, Abakus had 17% higher turgor when nitrate was applied instead of ammonium.

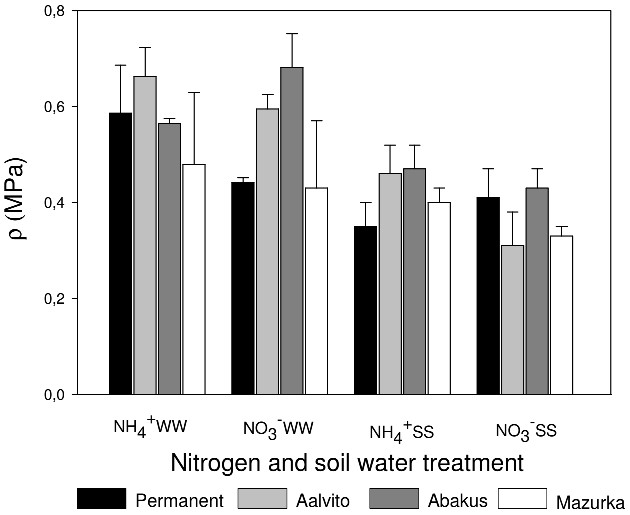


Figure 35: Turgor pressure of maize cultivars under different soil water regime and two different nitrogen fertilizations, NH_4^+ and NO_3^-.

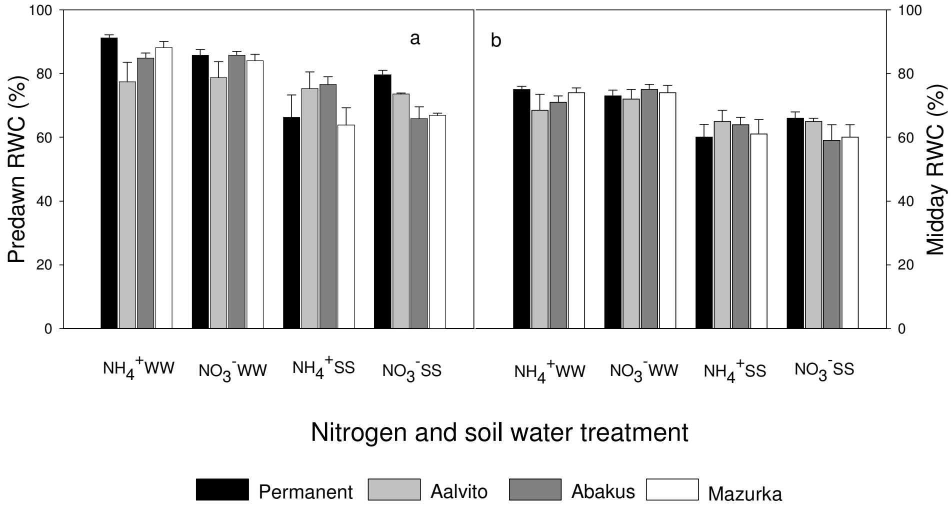


Figure 36: Predawn (a) and midday (b) relative water content of maize cultivars under different soil water regime and two different nitrogen fertilizers, NH_4^+ and NO_3^-.

The results observed in RWC (Figure 36) coincide with the results obtained in the other leaf water relations' parameters, as leaf and osmotic potential and turgor pressure.

Water regime had a significant ($p<0.01$) effect on predawn and midday leaf water content. However the cultivars and nitrogen form had no significance influence on RWC (Figure 36).

The Figure 36 illustrates that the RWC decreased in the drought-stressed plants, in both, predawn and midday measurements compared with well-watered plants. When the cultivars Permanent, Aalvito, Abakus and Mazurka under well watered conditions and treated with ammonium had 27, 3, 9 and 28% higher predawn RWC compared with the drought-stressed plants. On the other hand the NO_3^- well-watered plants had 7, 6, 23 and 21% predawn RWC than the drought-stressed plants of the cultivars Permanent, Aalvito, Abakus and Mazurka.

The midday RWC pattern were similar as the predawn and Aalvito treated with ammonium were the less affected cultivar with a decrease because drought of only 3%. The decrease in the other cultivars was between 10 and 21% in both, ammonium and nitrate treated plants. Additionally, the predawn RWC was slight higher than the midday measurements.

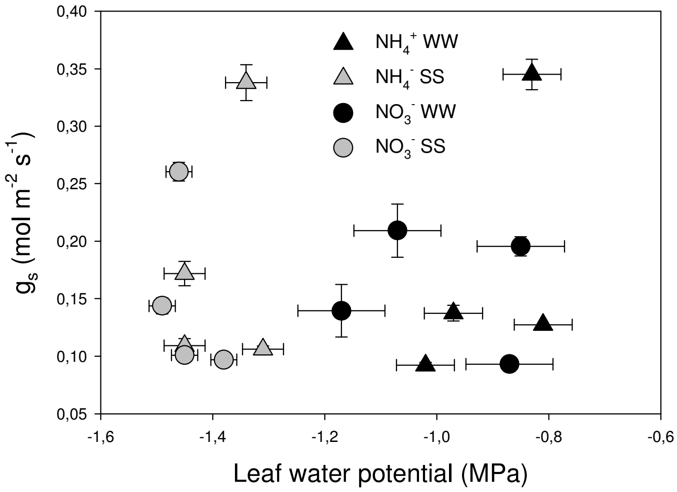


Figure 37: Relationship between stomatal conductance (g_s, mol m^{-2} s^{-1}) and leaf water potential in different maize cultivars under two different nitrogen fertilizations (NH_4^+ and NO_3^-) and two-water regime (well-watered and water stress).

The reduction in stomatal conductance was related with water stress for the NO_3^- fed plants, however a decrease of leaf water potential was not correlated to an increase of stomatal conductance for NH_4^+ fed plants, although the fluctuation of g_s was not clear in all treatments but it can be seen that the g_s values of well watered treatments are between a range of –0.8 and –1.2 of LWP, this represents about 50% higher LWP compared to drought-stressed treatments (Figure 37).

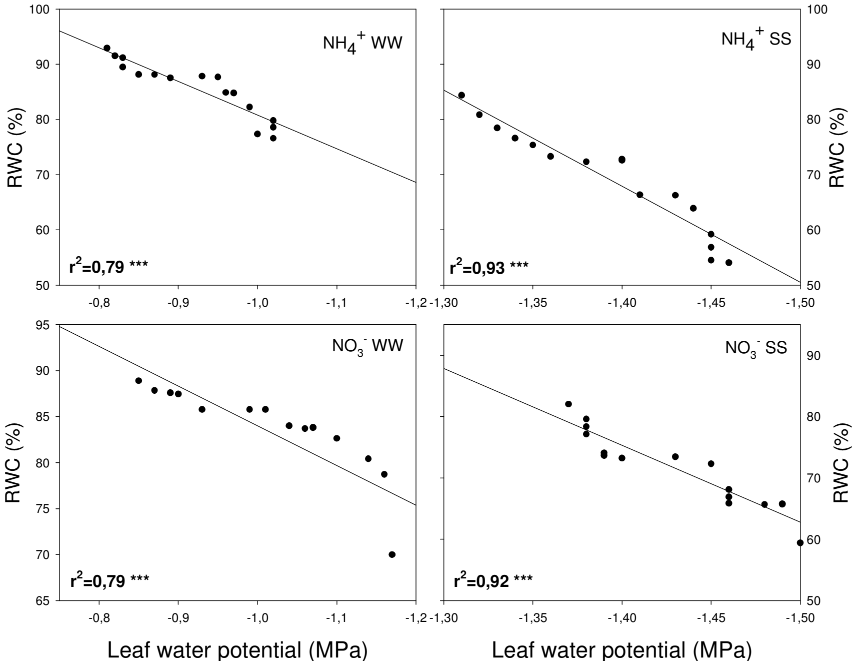


Figure 38: Relation between relative water content and leaf water potential of different maize cultivars under different nitrogen nutrition and water regimes in the field experiment (2008). (All regression are statistically significant at p≤0.05).

Reduction of RWC was associated to a decrease of leaf water potential and to water stress (Figure 38). The well-watered treatment exhibited, therefore, the highest RWC values at lower LWP than the drought-stressed treatments. The different between nitrogen forms was not significant. Although, the well-watered and drought-stressed plants of the NH_4^+ treatments showed the maximum (91%) and minimum (63%) RWC values, respectively.

Drought decreased RWC in NH_4^+ plants from about 85% to about 70(%). On the other hand, the NO_3^- well watered plants showed a decrease of RWC because drought from 83% to 70%.

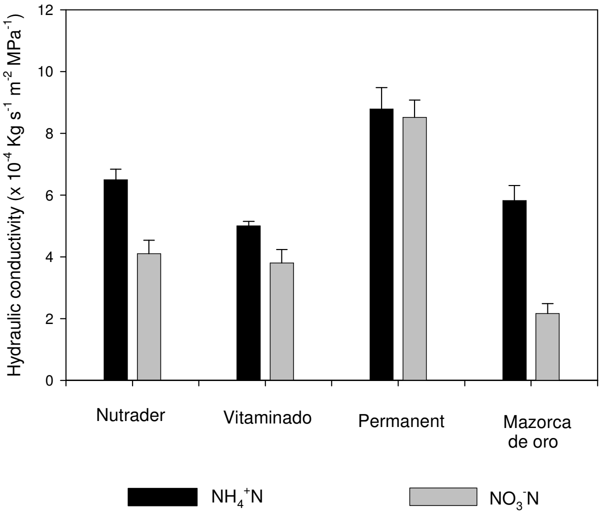


Figure 39: Hydraulic conductance in stem cut of different maize cultivars under two different nitrogen forms in the field experiment (2007).

The results of ANOVA for hydraulic conductance (K_{stem}) obtained in 2007 revealed variations due to effect of the cultivars and nitrogen forms ($p<0.001$). The results revealed also a significant variety*nitrogen form factor effect ($p<0.001$) (Figure 39).

The plants treated with ammonium instead of nitrate had 37% higher K_{stem} in Nutrader cultivars. Similarly results were showed in the plants fertilized with ammonium compared to nitrate plants in Vitaminado cultivars, showing the NH_4^+ plants 24% higher K_{stem} than the NO_3^- plants.

Among cultivars, Permanent exhibited the highest K_{stem}. Means K_{stem} values for the NH_4^+ treated plants in Permanent were 8.78 (x10^{-3} s^{-1} m^{-2} Mpa^{-1}) against 8.52 (x10^{-3} s^{-1} m^{-2} Mpa^{-1}) in NO_3^- plants, i.e. 3% lower than the NH_4^+ plants.

Mazorca de oro was the cultivars with the largest K_{stem} variation between the treatments fertilized with the different nitrogen form. Thus the NH_4^+ plants had about 63% higher K_{stem} than those fertilized with NO_3^-.

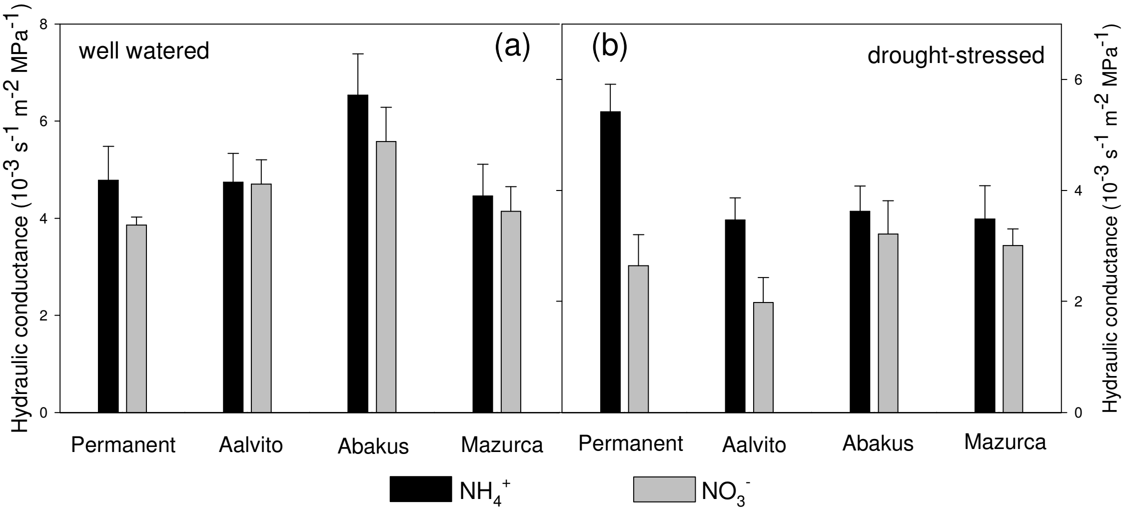


Figure 40: Hydraulic conductance in stem cut of different maize cultivars under two different nitrogen forms and two water regimes in the field experiment (2008).

The hydraulic conductance (K_{stem}) in 2008 (Figure 40) was significantly different among cultivars, between the plants fertilized with the different nitrogen form, as well as between the plants under the different water regime ($p<0.001$). The analysis of variance also revealed significant different in all interactions.

In the NH_4^+ plants under well-watered conditions, Abakus had the highest K_{stem} (4.78 $x10^{-3}$ s^{-1} m^{-2} Mpa^{-1}), followed by Permanent and Aalvito with 4.78 and 4.74($x10^{-3}$ s^{-1} m^{-2} Mpa^{-1}), respectively. Mazurka showed the lowest K_{stem} (4.46 $x10^{-3}$ s^{-1} m^{-2} Mpa^{-1}).

In the NO_3^- plants under well-watered conditions, Abakus also exhibited the highest K_{stem} values with 5.58±0.07 $x10^{-3}$ s^{-1} m^{-2} Mpa^{-1}.

Under well-watered conditions the NH_4^+ plants showed higher K_{stem} compared to those fertilized with NO_3^-. This increase was of 19, 14 and 7% in Permanent, Abakus and Mazurka cultivars, respectively.

The NH_4^+ plants under drought conditions exhibited also a higher K_{stem} than the drought-stressed NO_3^- plants. Therefore, Permanent, Aalvito, Abakus and mazurka treated with ammonium had 51, 43, 11 and 13% higher K_{stem} than those treated with nitrate.

It is interesting to see that the NH_4^+ drought-stressed plants in Permanent had about 11% higher K_{stem} than the NH_4^+ well watered plants. While the well watered plants of the other cultivars exhibited an increase of K_{stem} with respect to the drought-stressed plants.

The hydraulic conductance was positively and significantly correlated (Figure 41) with stem diameter (r^2=0.20**). Thus, an increase in hydraulic conductance in NH_4^+ plants compared with NO_3^- plants was attributable to the effect of nitrogen form on plant morphology, especially to the stem diameter.

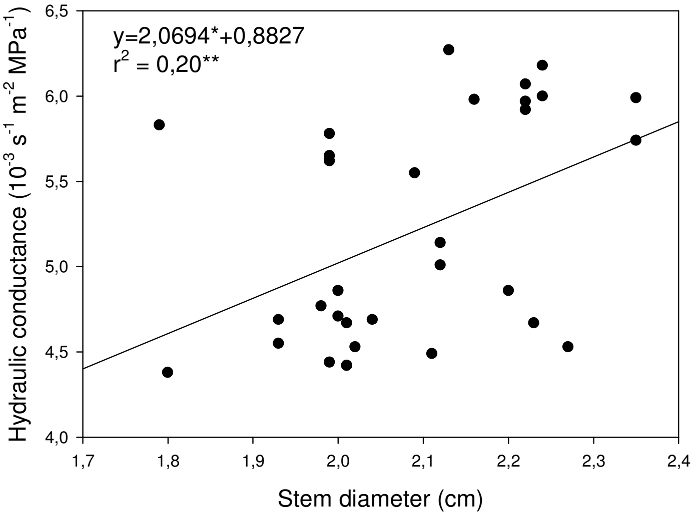


Figure 41: Relationship between stem cut (10 cm) and hydraulic conductance of maize (*Zea mays* L) under different nitrogen fertilization (NH_4^+ and NO_3^- in the field experiment (2008).

4.2.3 Morphological traits and dry matter.

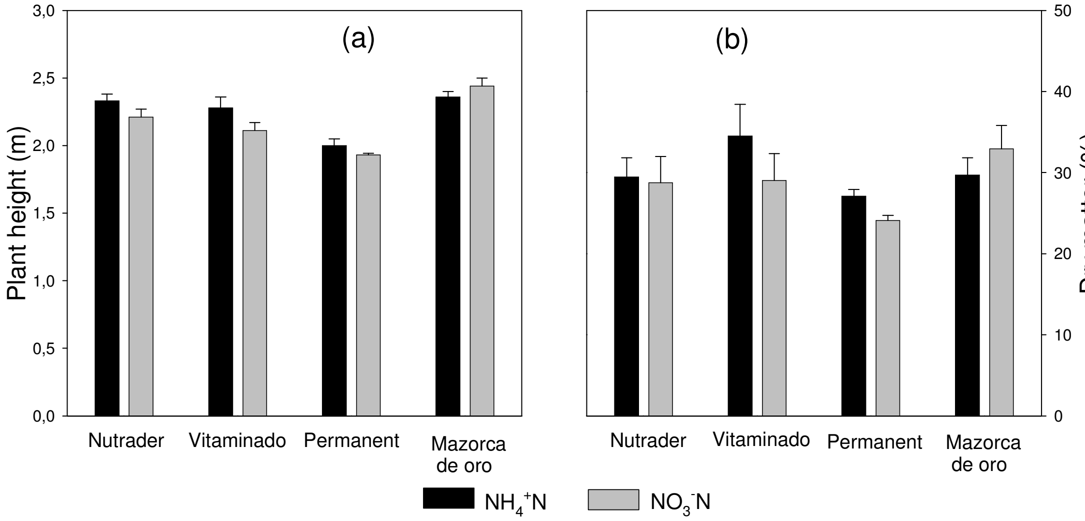


Figure 42: Dry matter (a) and plant height (b) of different maize cultivars under different nitrogen form in the field experiment (2007).

The plant height measured in the field (2007) is shown in Figure 42a.There was a significant effect of cultivars ($p<0.001$) and nitrogen form ($p<0.05$) on plant height.

Compared with NO_3^-, the NH_4^+ plants were tallest in Nutrader, Vitaminado and Permanent.

When the cultivars Permanent, Vitaminado and Mazorca de oro were fertilized with ammonium instead of nitrate, the plants were 5, 7 and 4% tallest, respectively. On the other hand, the NO_3^- plants in Mazorca de oro cultivar were 3% taller compared with NH_4^+plants. Between cultivars, Mazorca de oro was the tallest with 2.44 m approximately. Nutrader, Vitaminado and Permanent were 2.33, 2.28 and 2 m height, approximately.

The slightly increase of plant height in NH_4^+ plants compared to NO_3^- and between cultivars was not enough to have an increase in dry matter. In the Figure 42b is given the whole aboveground biomass. The results of ANOVA demonstrate, that cultivars and N-form not influenced on dry matter.

Variety	N-form and water regime	Plant Height (m)	Stem Diameter (cm)
Permanent	NH_4^+ WW	2.35±0.03	2.23±0.01
	NO_3^-WW	2.13±0.09	2.21±0.07
	NH_4^+ SS	1.71±0.03	1.77±0.07
	NO_3^-SS	1.84±0.10	1.92±0.06
Aalvito	NH_4^+ WW	2.24±0.03	2.04±0.08
	NO_3^-WW	2.05±0.03	1.93±0.07
	NH_4^+ SS	1.57±0.09	1.67±0.15
	NO_3^-SS	1.62±0.08	1.92±0.06
Abakus	NH_4^+ WW	2.27±0.07	2.05±0.09
	NO_3^-WW	2.27±0.05	2.24±0.08
	NH_4^+ SS	1.85±0.05	1.88±0.07
	NO_3^-SS	1.8±0.04	2.07±0.06
Mazurka	NH_4^+ WW	2.10±0.05	2.01±0.02
	NO_3^-WW	2.0±0.06	1.97±0.09
	NH_4^+ SS	1.61±0.11	1.78±0.07
	NO_3^-SS	1.51±0.05	1.72±0.10
		ANOVA	
Variety (V)		***	***
N-form (N)		NS	NS
Water regime (W)		***	***
V*N		NS	NS
V*W		NS	NS
N*W		*	NS
V*N*W		NS	NS

Ns, *,**,*** = Not significant, significant at p<0.05. at p<0.01. at p<0.001, respectively

Table 2: Morphological trait of different maize cultivars under two different nitrogen fertilization and two different soil water regimes in the field experiment (2008).

There was also significant effect of cultivars ($p<0.001$) and water regime ($p<0.001$) on plant height (Table 2).

It also was a significant nitrogen*water regime effect ($p<0.05$) (Table 2). In the ammonium treatments, drought reduced the plant height about 27, 30, 18 and 23% in Permanent, Aalvito, Abakus and Mazurka, respectively. On the other hand, in nitrate treatments, the plant height was reduced by drought about 13, 21, 20 and 24% for Permanent, Aalvito, Abakus and Mazurka, respectively. Between cultivars, Abakus and Permanent were the tallest compared to the other two cultivars.

With regard to stem diameter (S_d), the results of ANOVA showed significant differences ($p<0.001$) between cultivars. Similarly, significant differences ($p<0.001$) between water regimes were observed. The cultivars Abakus had the highest S_d were Abakus, followed by Permanent.

Drought reduced the S_d about 17, 9, 8 and 12% for Permanent, Aalvito, Abakus and Mazurka, respectively.

The dry matter measured in year 2008 is shown in Figure 43 and as in the year 2007, there were not significant differences between all treatment and interactions (Figure 43).

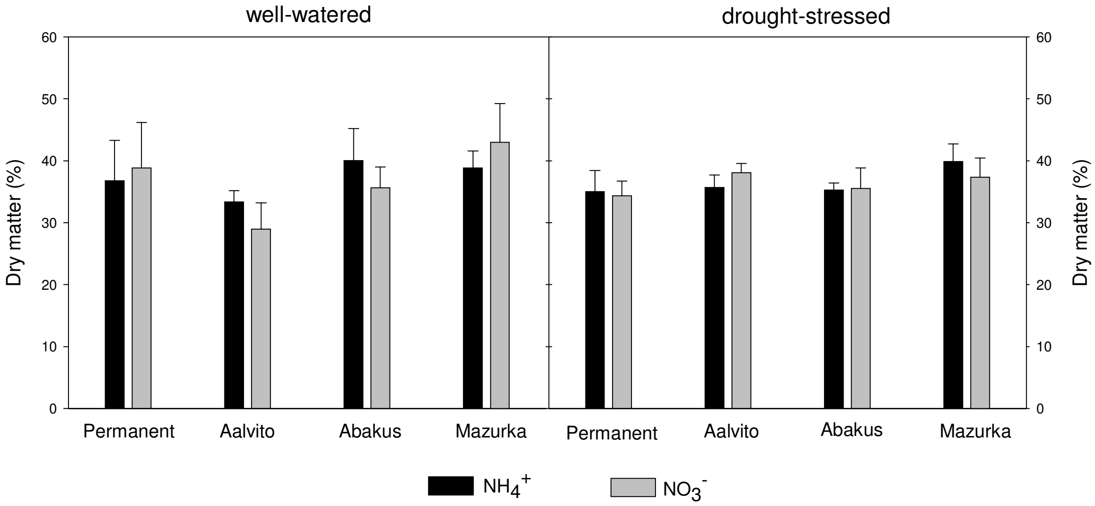


Figure 43: Dry matter in different maize cultivars under two water regimes and two-nitrogen fertilization (Ammonium and Nitrate) in the field experiment (2008).

4.2.5 Yield and yield components and corn protein content

Cultivar	N-form & Water regime	Ears/plant (n)	Grains/ear (n)	DW/grain (mg)	H.I (%)	Total dry matter (Ton ha^{-1})
Permanent	NH4-WW	1.13±0.07	460.02±3.50	327.49±9.72	0.41±0.00	27.81±1.42
	NO3-WW	1±0.00	395.76±15.78	334.41±22.48	0.41±0.01	21.51±2.05
	NH4-SS	1±0.00	343.26±8.49	315.43±14.16	0.41±0.01	17.52±0.09
	NO3-SS	1±0.00	345.56±31.98	319.58±9.80	0.41±0.01	17.99±0.95
Aalvito	NH4-WW	1.22±0.09	422.49±13.06	314.36±6.22	0.44±0.05	27.29±1.82
	NO3-WW	1.07±0.03	385.42±46	308.15±4.88	0.44±0.05	19.16±0.77
	NH4-SS	1±0.00	341.91±18.28	287.97±14.19	0.40±0.01	16.48±0.36
	NO3-SS	1.03±0.03	370.62±11.56	282.17±23.96	0.40±0.01	17.67±0.76
Abakus	NH4-WW	1.17±0.03	405.66±11.42	256.33±5.74	0.39±0.01	21.93±1.00
	NO3-WW	1±0.00	392.76±7.12	300.09±16.55	0.39±0.01	20.34±0.45
	NH4-SS	1±0.00	349.71±15.63	279.57±14.72	0.38±0.00	17.05±1.41
	NO3-SS	1±0.00	390.54±28.81	304.80±23.91	0.38±0.01	20.66±0.48
Mazurka	NH4-WW	1.23±0.01	428.15±1.39	283.96±6.08	0.38±0.00	25.76±0.96
	NO3-WW	1.17±0.22	376.73±29.40	285.96±4.67	0.38±0.00	21.59±2.64
	NH4-SS	1.±0.00	352.89±14.51	272.94±12.18	0.40±0.01	16.28±1.29
	NO3-SS	1±0.00	339.98±11.53	293.11±16.51	0.40±0.01	16.83±1.24
				ANOVA		
Variety (V)		NS	NS	***	**	NS
N-form (N)		NS	NS	NS	NS	**
Water regime (W)		***	***	NS	NS	***
V*N		NS	NS	NS	NS	NS
V*W		NS	NS	NS	NS	*
N*W		NS	**	NS	NS	***
V*N*W		NS	NS	NS	NS	NS

Ns, *,**,*** = Not significant, significant at $p<0.05$. at $p<0.01$. at $p<0.001$, respectively

Table 3: Effect of nitrogen form and water regime on yield components in the field experiment (2008).

Average number of ears per plant and grain per ear of the different treatments were affected by water regime ($p<0.001$). The analysis of variance shows also a significant nitrogen form*water regime effect on grain per ear ($p<0.01$) (Table3).

The results of ANOVA show variation due to cultivars in dry weight per grain (DWG)($p<0.001$) and harvest index (HI) ($p<0.01$).

The results also reveal that the yield varied with the water regime ($p<0.001$), as well as, with the interaction between nitrogen form and water regime (Figure 44).

The total dry matter was not only affected by water regime ($p<0.001$) but also by nitrogen form. Likewise, the variety*water regime interaction and the nitrogen form*water regime interaction were significant at $p<0.05$ and $p<0.001$, respectively.

Drought reduced the number of ear per plants from 1.07 to 1.0 in Permanent cultivars, from 1.15 to 1 in Aalvito, from 1.08 to 1 in Abakus and from 1.2 to 1 in Mazurka.

The NH_4^+ compared to NO_3^- plants of Permanent, Aalvito, Abakus and Mazurka had 14, 8, 3 and 12% higher grain per ear, respectively. On the other hand, drought decreased grains per ear in all treatment. However, cultivar Permanent showed the highest decrease of grains per ear (25%) and 12% in NH_4^+ and NO_3^- plants, respectively.

In the NH_4^+ fed plants grain yield was 22, 29 and 18% higher than NO_3^- fed plants in Permanent, Aalvito and Mazurka, respectively. However, Abakus showed a very low increase with ammonium fertilization than with nitrate fertilization. The well-watered plants also showed higher yield compared with the drought-stressed treatments (Figure 44). Thus, the NH_4^+ well watered plants had 36, 39, 19 and 35% higher grain yield compared to the drought-stressed NH_4^+ plants in Permanent, Aalvito, Abakus and Mazurka, respectively. On the other hand, the well-watered NO_3^- plants had 17, 16 and 18% higher grain yield compared to the drought-stressed NO_3^- plants in Permanent, Aalvito and Mazurka, respectively. Thereby, drought affected more intensive the NH_4^+ treatments than the NO_3^- treatments (Figure 44).

Average of total dry matter of all cultivars treated with NH_4^+ was 25.70 Ton ha^{-1} compared to 20.65 Ton ha^{-1} in the NO_3^--treated plants (+19%). On the other hand, the well-watered treatment compared to the drought-stressed treatments, showed also an increase of total dry matter of 34 and 11% for NH_4^+ and NO_3^- treatments, respectively. This total dry matter superiority of NH_4^+ over NO_3^- plants and of the well watered over the drought-stressed plants was attributed to an increase of grain yield.

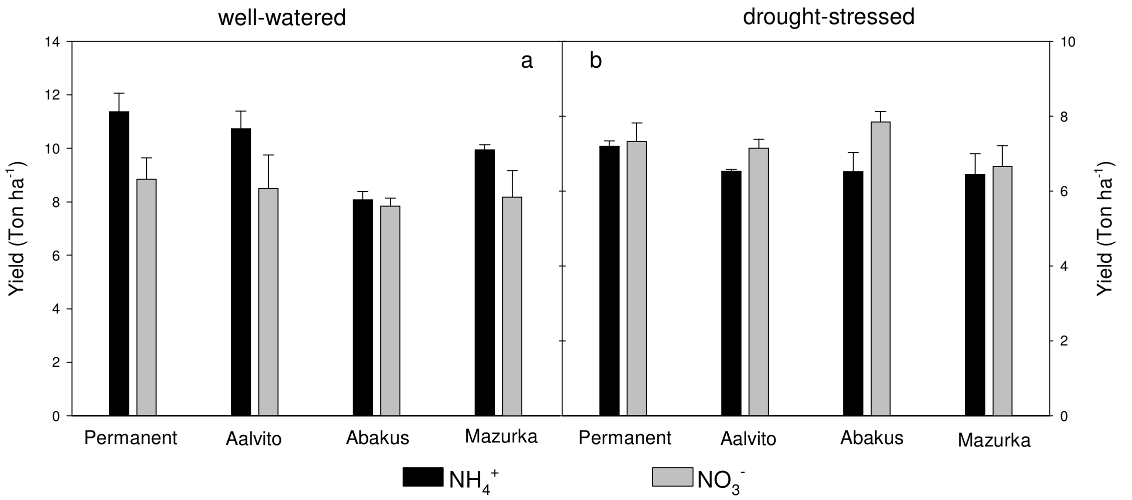


Figure 44: Effect of nitrogen form and water regime on yield in the field experiment (2008).

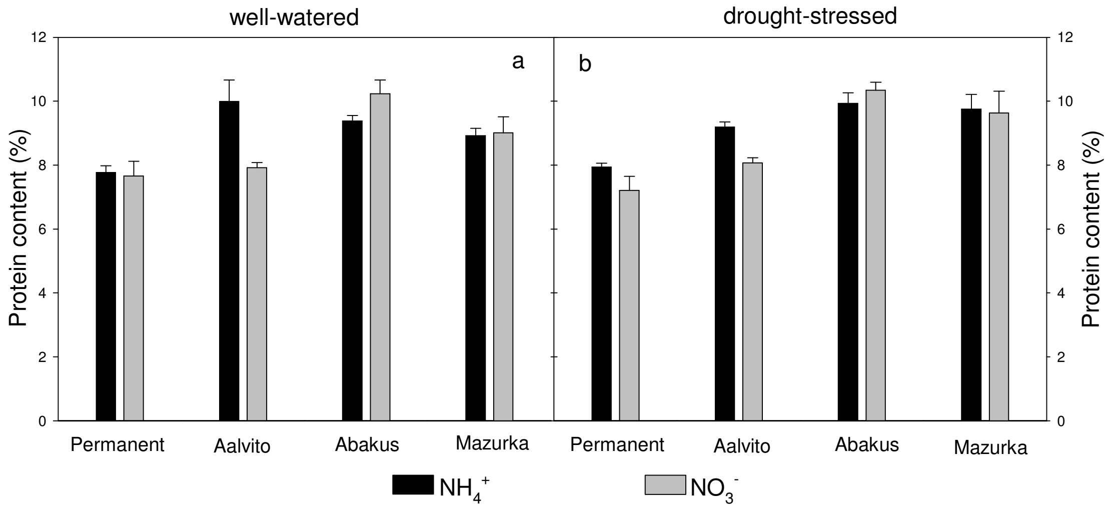


Figure 45: Effect of nitrogen form and water regime on corn protein content in the field experiment (2008).

There were significant variations between cultivars in the protein content (Figure 45). There was also significant Cultivar*N-forms effect (Figure 45). The cultivars Abakus and Mazurka had the highest corn protein content. When Abakus was treated with nitrate instead of ammonium, the protein content was 8% higher. When Aalvito was fertilized with ammonium had 20% higher protein content than with nitrate fertilization. The cultivars Permanent and Mazurka were not affected by the N-forms. Indeed, in the field experiment (2007) the cultivars permanent also showed no difference between the ammonium and nitrate treated plants (data no showed).

1. DISCUSSION

2. Leaf gas exchange parameters

In the greenhouse experiment (2007), the cultivars and N-form significantly influenced photosynthesis rate (A_{net}), stomatal conductance (g_s), transpiration rate (E) and instantaneous water use efficiency (WUE). Although the water regime had no significant influence on the leaf gas exchange parameters, the interactions cultivars*water regime and nitrogen form*water regime were significant. The effect of the N-forms, however, depended on the cultivars and climatic conditions. Thus, the same cultivars responded differently to the N-forms in the greenhouse and in the field experiment. Furthermore, the cultivar Permanent responded differently not only to the greenhouse and field conditions, but also between the two experimental years (2007 and 2008). Plant species vary in their sensitivity to ammonium nutrition (Barker and Mills, 1980). Therefore the cultivars Nutrader, Vitaminado and Permanent showed higher A_{net} when ammonium was used as nitrogen source instead of nitrate in the greenhouse experiment 2007 (Figure 6). Similarly all cultivars (Nutrader, Vitaminado, Permanent and Mazorca de oro) showed higher A_{net} with ammonium than nitrate in the field experiment (Figure 26).

In the greenhouse experiment 2008, the German cultivars Permanent, Aalvito and Abakus had slightly higher A_{net} with ammonium than nitrate under well-watered conditions (Figure11). However, in the field experiment 2008 Permanent, Aalvito and Mazurka showed higher A_{net} with nitrate than ammonium, being Abakus the only cultivar with higher A_{net} when ammonium was used as nitrogen source instead of nitrate (Figure 28).

The positive response of the different cultivars to ammonium nutrition, especially in the greenhouse and in the field experiment (2007) and in the greenhouse experiment (2008) could be due to an increase of CO_2 assimilation rate. Our results agree with Raab and Terry (1994); they observed a higher net photosynthetic CO_2 exchange rate in ammonium supplied sugar beet plants compared to nitrate plants. Similar results were found in French bean by Guo *et al.* (2005), and highbush blueberry (Claussen and Lenz, 1999), whereas in clover (Høgh-Jensen and Schjoerring, 1997), raspberry, and strawberry (Claussen and Lenz, 1999), no significant differences in CO_2 assimilation rates among N forms were found. Although ammonium nutrition may lead to a decrease in net photosynthesis (Takács and Técsi, 1992), even higher net photosynthetic rates per unit leaf area have sometimes been measured when plants were supplied with ammonium as the sole nitrogen source (Raab and Terry, 1994).

The effect of N- form on photosynthesis is associated with the differences in photo-energy consumption and reductant supply between nitrate- and ammonium grown plants (Guo et al, 2007). The photoenergy cost for nitrate supply is 145% higher than for ammonium supply (Raven, 1985) because the NH_4^+ does not have to be chemically reduced. The fundamental differences in energy requirements for N assimilation between NO_3^- and NH_4^+ -supplied plants should have consequences of leaf carbohydrate metabolism and ATP/NADPH balance, which are expected to affect the net CO_2 assimilation (Guo et al., 2007). Therefore ammonium-supplied plants had a high CO_2 assimilation rate under high CO_2 and/or high light supply compared to nitrate-supplied plants. Generally, an increase of the photosynthetic photon flux density (PPFD) from 0 to 2000 µmol m^{-2} s^{-1} meant an increase of the photosynthetic rate in all treatments and in both, greenhouse and in the field experiment (2007 and 2008) ($P < 0.05$ Figure 9a, 27a and 29a). However, this increase was significantly higher in plants receiving NH_4^+ instead of NO_3^- under well watered conditions in the greenhouse (2007-2008) and in the field experiment (2007), even if the initial slope of the A–Ci greenhouse curve was relatively unaffected by nitrogen treatment. This was not confirmed by the field experiment (2008), however, where no difference between ammonium and nitrate nutrition was found (Figure 29a). As light intensity increased, the production of ATP and NADPH is increased, which leads to increased regeneration of ribulose-1.5-bisphosphate (RuBP) and thus an increase in CO_2 assimilation rate (Guo et al., 2006). Thus, species such as *Lemna minor* L. (Fuhrer and Erismann,1984), *Beta vulgaris* L. (Raab and Terry, 1994), *Rubus ideaus* L. (Claussen and Lenz, 1999), *Nardus stricta* (Bowler and Press, 1996), *Phaseolus vulgaris* L. (Guo et al., 2001), and *Rhinanthus minor* L. (Seel et al., 1993), had a higher CO_2 assimilation rate on a leaf area basis under ammonium nutrition than under nitrate nutrition. This effect was even more pronounced under higher light intensity, due to a higher total chlorophyll content and total amount and activity of Rubisco (Warren et al., 2000). This further implies a higher rate of RuBP regeneration under higher light intensity in ammonium-supplied plants than in nitrate-supplied plants (Farquhar et al., 1980; Farquhar and von Caemmerer, 1982). It is important to highlight, that none of the different treatments treated with either ammonium or nitrate in the greenhouse and in the field experiment (2007) and in the greenhouse (2008) showed photoinhibition, which confirms that the NH_4^+ or NO_3^- photo-assimilation in maize (C_4)

was not inhibited by elevated light. In the greenhouse and in the field experiment (2007) an increased A_{net} caused by higher PPFD was accompanied by an increase of g_s and E (Figure 9b, 9c and 27b, 27c). This was not the case for the experiment conducted in 2008, where an increase of A_{net} was not always accompanied by an increase of g_s and E, especially for the NO_3^- WW in all treatments in the field experiment (Figure 29a, 29b). From the statements made the above it can be interpreted that the effect of N form on photosynthesis is also associated with stomatal conductance (g_s) (Guo et al., 2006). Plants supplied with ammonium had a higher assimilation rate, carbon isotope discrimination and stomatal conductance than those supplied with nitrate (Hogh-Jensen and Schjoerring, 1997; Guo et al., 2002). These results suggest that nitrogen forms have different effects on gas exchange parameters, such as stomatal conductance, intercellular CO_2 partial pressure, and transpiration rate (Guo et al., 2006).

The ammonium compared to the nitrate nutrition caused higher stomatal conductance (gs) in Nutrader and Permanent in the greenhouse experiment in year 2007 (Figure 6c). At the same time, all cultivars showed an increase of gs with ammonium compared to nitrate in the field experiment (Figure 26b). In contrast, Mazorca de oro showed lower gs with ammonium than with nitrate in the greenhouse experiment in year 2007 (Figure 6c).

In 2008, stomatal conductance also varied with the nitrogen form, showing clearly higher gs for Aalvito and Mazurka with nitrate as compared to ammonium fertilization in both, greenhouse and field experiment (Figure 28c). However, Abakus had greater gs with ammonium than nitrate in the field experiment (Figure 28c). It has been reported that NH_4^+ caused an increase in stomatal conductance in white clover (Høgh-Jensen and Schjoerring, 1997), and enhanced the transpiration rate in alfalfa (Khan et al., 1994) and tomatoes (Lugert et al., 2001). However, our results also agree with the observation that the use of NH_4^+ compared with nitrate decreased the stomatal conductance in *Phaseolus vulgaris* (Guo et al., 2002) and in wheat and maize (Cramer and Lewis, 1993). On the other hand, the nitrogen source had no effect on stomata1 conductance of *Beta vulgaris L.* (Raab and Terry, 1994). Thus, it can be considered that photosynthesis, stomatal conductance and transpiration rate change in response to the fertilization with different N-forms and/or to changes in ambient conditions.

In the greenhouse 2007, the transpiration rate (E), as well as A_{net} and g_s, were affected by N-form and cultivars. Nutrader and Permanent exhibited clear increases of E (20 and 24%, respectively) when ammonium was used as nitrogen source compared to nitrate (Figure

6e). However, the cultivar Mazorca showed higher E with NO_3^- than NH_4^+ (28%). On the other hand, in the field experiment (2007) Nutrader, Vitaminado, Permanent and Mazorca de oro increased E, g_s and A_{net} under ammonium nutrition compared to nitrate nutrition. The different behaviour between the greenhouse and the field grown plants was also clear in the experiment of year 2008. In fact, the cultivars Permanent and Abakus grown in the greenhouse showed higher E with NH_4^+ than NO_3^-, whereas Aalvito and Mazurka had about 39% higher E with NO_3^- fertilization than with NH_4^+ fertilization (Figure11e). At the same time, Permanent and Abakus also showed higher E with ammonium fertilization than with nitrate fertilization in the field experiment. Aalvito and Mazurka, however, showed higher E when nitrate was used as nitrogen form instead of ammonium (Figure 28e). Similar to our results, other authors found that the use of NH_4^+ increased the transpiration in alfalfa (Khan at el., 1994), Tomato (Lugert et al., 2001), maize and wheat (Lewis et al., 1989) but our results are also in agreement with Lu et al., (2005) who found that NH_4^+ caused a marked reduction in transpiration compared to those tobacco (*Nicotiana Tabaccum L.*) plants treated with either NO_3^- and $NH_4^+NO_3^{-.}$ However, the reduced leaf transpiration in the NH_4^+ treatment was due to reduced stomatal conductance (Guo et al., 2002, Wang and Zhang, 2003).

The transpiration rate is an important parameter that can determine the water use efficiency (WUE). Therefore, different nutrient levels may also influence WUE of plants.Under well-watered conditions the cultivars Nutrader, Vitaminado and Permanent increased the WUE with ammonium fertilization compared to nitrate fertilization in the greenhouse experiment in year 2007. However, when Mazorca de oro was fertilized with ammonium instead of nitrate, the WUE decreased (Figure 6g). On the other hand, Nutrader, Vitaminado and Permanent decreased the WUE with ammonium fertilization compared to nitrate fertilization in the field experiment. At the same time, the WUE of Mazorca de oro plants fertilized with ammonium was higher than the WUE of plants fertilized with nitrate (Figure 26d).

In 2008, Aalvito and Mazurka under controlled and well-watered conditions had the highest WUE (12.3 and 9.8 mmol mol^{-1}, respectively) with ammonium fertilization and not with nitrate fertilization (Figure 11g). However, in the field experiment, Mazurka and Permanent showed the highest WUE values (11.8 and 9.7 mmol mol^{-1}, respectively) (Figure 28g) compared to the other cultivars, under well-watered conditions and under ammonium fertilization compared to nitrate fertilization.

When ammonium was used as nitrogen form instead nitrate, the WUE also increased for wheat (*Triticum aestivum L.*) (Morgan, 1986; Yin and Raven, 1998) and white clover (*Trifolium repens L.*) (Høgh-Jensen and Schjoerring, 1997), but decreased for tomatoes (Lugert et al., 2001; Claussen, 2002), *Phaseolus vulgaris* and *Ricinus communis* (Raven et al., 1992). It seems that the influence of NH_4^+ on water relations varies depending on the plant species and the experimental conditions. We can suggest, therefore, that the leaf gas exchange parameters A_{net}, g_s and E determined the water use efficiency of the different maize cultivars in our experiment. Water-use efficiency (WUE) is an eco-physiological measure quantifying the ratio of net CO_2 uptake from the atmosphere during photosynthesis versus net H_2O loss (Larcher 2003). Stomata are vital to WUE, as stomata are the common channel for CO_2 and water; photosynthesis and transpiration are both subject to stomatal regulation. Therefore, the decline of stomatal conductance impacts transpiration more than photosynthesis, so that partial closure of stomata has a positive effect on the enhancement of leaf WUE (Plaut, 1995). However, plant WUE is not conservative as it can change between plant varieties, cultivation practices and environmental conditions (Zhang et al., 2005).

Since WUE depends on the ratio A/E, the vapour pressure deficit (VPD) can influence the leaf water potential through stomatal conductance. When vapour pressure deficit (VPD) increased, stomatal conductance (g_s) and transpiration rate (E) decreased in the greenhouse (2007) (Figure 7 and 8). However, the well watered plants fertilized with ammonium or nitrate did not show a difference. In contrast, the NH_4^+ plants under drought exhibited higher g_s and E compared to NO_3^- plants at the same VPD, indicating that the NH_4^+ plants can maintain relatively high photosynthetic activity, high transpiration rate and high WUE under drought, and high VPD compared to nitrate plants, which is associated with stomatal response. Above all, stomatal conductance (g_s) was the most sensitive parameter to varying VPD. Stomatal conductance is the dominant factor determining photosynthetic rate and its response to VPD change. Photosynthetic rate and stomatal conductance of different rice varieties decreased with VPD increase from 1 to 2.3 kPa (Oshumi et al. 2008). Under shade and open sun conditions, stomatal conductance and transpiration rate of wild *Coffea arabica* populations decreased due to high leaf-to-air vapour pressure deficit (VPD) and the response of both parameters to changing VPD varied depending on the soil moisture. Under shade conditions, transpiration rates (E) and photosynthetic rate (A_{net}) of C*offea arabica* followed the curves of stomatal conductance with a decrease at high VPD and under limited soil moisture supply (Beining, 2007). Wiebel et al. (1992) reported a decrease of stomatal conductance (g_s) and photosynthetic rate (A_{net}) with an increase of vapour pressure deficit (VPD) in mangosteen (*Garnicia mangostana L.*). The decline of A_{net} may be due to high transpiration rate (E) and reduced availability of CO_2 (Sharkey, 1984).

The combined effects of water status and vapour pressure deficit (VPD) affect the physico-chemical responses to drought. Under conditions of low VPD, plants adapt to the environmental changes through alterations in their physico-chemistry, including an increased level of abscisic acid in both xylem and leaves, which leads to a stable water status in the plant. Under conditions of high VPD a transient peak of xylem abscisic acid in maize plants was observed following the time course of VPD (Asch et al., 2002). ABA has numerous effects on plant growth and reproduction, which are expected to reduce plant productivity. ABA decreases general growth, photosynthesis, leaf initiation, germination, tillering in grasses, pollen viability and seed set. ABA promoted flower abscission. On the other hand, ABA accumulation has long been assumed to constitute an adaptive factor under drought stress (Blum, 1996).

Plants of maize did not adapt to the drought stress and had a negative water status, low stomatal conductance and reduced photosynthesis rates, despite the absence of increased levels of leaf or xylem abscisic acid (Asch et al., 2002). Stomatal control is the first, and perhaps most important, step in the response to drought, as decreased stomatal conductance reduces the rate of water loss, slows the rate of development of water stress and minimizes its severity (Raftoyannis and Radoglou, 2002).

On the other hand, drought reduces leaf area, if the stress occurs before flowering. At any time of crop development, stress reduces crop photosynthesis rate and therefore the total assimilates available to the crop. Thus, maize is very sensitive to stress at flowering and grain filling stages (Zaidi, 2002). In the greenhouse the drought was started two weeks before flowering in the year 2007. In contrast, in the field experiment drought was dependent on rainfall and in 2007 the drought was actually absent due to frequent rainfall. Although ANOVA did not discover any effect of the water regime, a detailed analysis of each variable shows that the effect of the drought depended on the cultivars and on N forms. Compared to well-watered plants, drought decreased A_{net}, g_s and E in the cultivars Vitaminado and Mazorca de oro fertilized with ammonium. On the other hand, drought decreased A_{net}, g_s and E in Vitaminado and treated with nitrate. However, drought decreased only A_{net} and E in Mazorca de oro and gs and E in Nutrader. In 2008, in the greenhouse experiment, drought decreased A_{net}, g_s and E in the Permanent, Aalvito, Abakus and Mazurka cultivars when either ammonium or nitrate was used as nitrogen source. However, drought did not affect E for ammonium fertilizated Aalvito and Mazurka plants. At same time in the field experiment, the response of the different cultivars and fertilized with either ammonium or nitrate was variable. Thus, drought decreased gs and E in Aalvito with ammonium or nitrate fertilization. However, gs and E increased in Mazurka under drought and with ammonium or nitrate fertilizations.

The response to drought of the different maize cultivars was different, among cultivars, between climatic conditions and different fertilizer source (ammonium or nitrate). However, our results suggest that the stomatal conductance and transpiration influenced the adaptability of maize to drought conditions through the enhanced water use efficiency. This depends, however, on the climatic conditions and nitrogen fertilization.

The relative role of stomata1 and non-stomatal factors in reducing photosynthesis under drought has been a continuously debated issue. Farquhar et al. (1989) concluded that stomata1 factors are considered more important than non-stomata1 factors in affecting photosynthesis under drought stress, mainly by way of leaf stomata1 heterogeneity, which allows perhaps considering ABA as a major modulator of effects. It is well documented that stomatal closure is also the main cause for transpiration decline as water stress develops (Hsiao, 1973).

Net photosynthetic rate (A_{net}), transpiration rate (E) and stomatal conductance (g_s) decreased as soil water content declined in an agroforestry treatment containing trees as *Grevillea robusta* (A. Cunn), *Alnus acuminata* or *Paulownia fortunei* (Hemsl.) with maize (Muthuri et al., 2008). CO_2 exchange rate (CER) and g_s values were strongly lower under drought conditions in wheat (Monneveux et al., 2006). Photosynthetic rate, stomatal conductance and transpiration rate decreased significantly by water stress in soybean plants (Ohashi et al., 2005) and in rice (Halder and Burrage, 2004), while the intercellular CO_2 concentration did not change. This indicated that the decrease in photosynthesis was due the effect of water deficit on the photosynthetic apparatus and on the biochemistry of the photosynthesis process. Plants regulate their diurnal water status at a favourable level by the control of stomatal aperture (Farquhar and Sharkey,1982). Stomatal closure contributes to maintain high leaf water content and high leaf water potential, but it leads to a decrease in leaf photosynthesis. Thus, variations in net photosynthesis (A_{net}) combined with an efficient stomatal control of the transpirational water loss during the drought period in the greenhouse and in the field experiment (2007-2008) could have led to an improvement in instantaneous water-use efficiency (WUE) in some of the cultivars tested (Figure 6h, Figure11h and 28h) as reported elsewhere (Chaves et al. 2003 cited in Beining 2006). In periods with optimal water supply, plants are predicted to maximize use of water by fully opening their stomata and assimilating as much carbon as possible, which leads to low WUE. However, when the water supply is limited, drought resistant plants should minimize water loss by greatly increasing WUE (Beining, 2006). The higher WUE of C_4 species occurs mainly because their efficient CO_2-fixation pathway, which allows them to maintain high A_{net} values concurrently with lower g_s and E values. Thus, it can been said that nitrogen form and water conditions could interact to influence WUE (Shangguan et al, 2000).

5.2 Plant water relations

Water is driven through the plant from the soil to the atmosphere by the difference in water potential between the atmosphere (very low potential) and the soil (relatively high potential when wet) and this flux is affected by hydraulic conductivity of all component (e.g. roots, xylem, and leaf) involved (Guo et al., 2006).

Mineral nutrient deficiency (e.g., N or phosphate) may reduce hydraulic conductivity (Rheinbott and Blevins, 1999; Clarkson et al., 2000), and it is thus tempting to speculate that N form does affect water uptake of roots via this regulatory mechanism. These findings indirectly suggest that water uptake is at least partly controlled by the shoot, namely the water potential of the leaves (Guo et al., 2006).

In the greenhouse experiment (2008), the differences in leaf water relation parameters (Ψ,π and p) between the stressed and well-watered plants during the drought were dependent on the cultivars, and nitrogen form (ammonium or nitrate). On the other hand, in the field experiment; these parameters depended on cultivars and water regime. Our results are in agreement with Mihailovic et al., (1992) who found that the NH_4^+-plants of two hybrids of maize (*Zea mays L.*) maintained higher turgor pressure during the drought by better osmotic adaptation. On the other hand, our results do not agree with Quebedeaux and Ozbun (1973), who found that ammonium (NH_4^+) nutrition inhibits water uptake and root exudation and decreases leaf water potential of tomato plants grown in solution culture. The leaf water relations were measured only in year 2008. In the greenhouse experiment of this year, the diurnal leaf water potential of the well-watered Permanent and Aalvito cultivars was higher for the ammounium fertilized plants (Figure 12) than for the well-watered NO_3^--treated plants (Figure 12). The well-watered NH_4^+ and NO_3^-- plants of all cultivars (Permanent, Aalvito, Abakus and Mazurka) had higher diurnal and midday leaf water potential compared with drought-stressed plants (Figure 12). However, in the field experiment (2008) (Figure 32) the leaf water potential did not differ in the plants either fertilized with ammonium or nitrate in the different evaluated cultivars. Because in the greenhouse (2008), the well-watered plant were superior to drought-stressed plants, therefore, it is possible to say, that the turgor ofammonium fertilized Permanent and Aalvito plants, could be sustained by keeping a high LWP through water uptake from the drying soil. However, in the field (2008), because the osmotic potential did not differ between the different nitrogen treatments, possibly the plant had to reduce the osmotic potential through solute accumulation (osmotic potential) to sustain the turgor (for review see plantstress web). Thus, our results do not agree with Mengel, (1984) or Magalhaes and Wilcox (1983), who considered that the NH_4^+-grown plants contain less low molecular solutes (cations, NO_3^- and carboxylates) which results in a higher osmotic potential, and therefore NH_4^+-grown plants often have a lower water (higher DM) content than NO_3^--grown plants.

Since the relative water content (RWC) is proposed as an indicator of water status in tissue culture plants, their measurement was fundamental to know the response of maize to restricted soil water under different N-forms. When transpiration exceeds absorption, cell turgor (ρ) falls while RWC and cell volume decrease, the concentration of cellular contents increases, and osmotic (π) as well as water potential (ψ) fall (Lawlor and Cornic, 2002). In our experiment, the N-form (ammonium or nitrate) did not affect RWC, neither for the well-watered plants nor for the plants under drought. Thus, the well-watered NH_4^+or NO_3^- plants had about 15-20% higher RWC compared to the drought-stressed plants. Therefore itcan be said, that drought decreased the cell turgor (Figure 15 and 35) and RWC (Figure 16 and 36), while both the osmotic (Figure 14 and 34) and the leaf water potential (Figure 12 and 32) fell.

The relative water content in different maize cultivars decreased significantly with drought stress (Chen and Dai, 1994; Liu and He, 1995). In our study RWC changes plotted against water potential in the greenhouse and in the field experiment (2008) (Figure 18 and 38) show that the plants under well-watered conditions had more water than the drought-stressed plants in both, the greenhouse and in the field experiment. Furthermore, the RWC of plants with ammonium or nitrate fertilizations was not different, in both, in the greenhouse and in the field experiment (Figure 18 and 38). Therefore, our results do not agree with Mihailovic et al., (1992), who found that the maize plants treated with NH_4^+ contained more water at the same values of water potential, than the NO_3^- treated one. However, the difference of the root system of the maize plants influenced by the N-form availability could be due not only to the high concentrations of nitrogen in CULTAN plants, but also due to the water uptake from soil layers (Wiesler, 1997). In periods with restricted soil water, the CULTAN plants were more drought tolerant compared to those treated with nitrate (Sommer 1991). In wild C*offea Arabica* under drought conditions Kufa (2006) and Beining (2006) also found a decrease of RWC together with a decrease of leaf and osmotic potential and turgor potential. Because Permanent and Aalvito cultivars under well-watered conditions in the greenhouse experiment (2008) with ammonium fertilization showed higher water potential than with nitrate fertilization, one might assume that the NH_4^+ plants had greater ability to retain water during drought compared to NO_3^- supplied plants. Therefore, the difference in the water retained could be attributed to the efficient stomatal control of transpiration (Nunes 1976), exhibited by the NH_4^+ plants under drought. Since the NH_4^+ plants increased their transpiration rate in comparison with NO_3^- plants, it is also possible that the NH_4^+ plants, enhanced their water uptake, although it is normally believed that the plants treated with ammonium have low water consumption compared to those treated with nitrate, because of the smaller leaf area and lower leaf area ratio (Guo et al., 2006). However, the higher values of hydraulic conductance of $NH4^+$ fertilized plants also indicate higher water transport. On the other hand, a small water loss causes a shift in turgor so that the leaves tend to maintain high relative water content to retain a high symplast volume. According to our results, the same cultivars responded differently to the induced drought in the greenhouse and in the field, possibly because the maximization of water uptake by the deeper root system (Pinheiro et al., 2005), which is missing in the greenhouse. Thus, the interaction between nutrient and water uptake as affected by N-form is certainly a complex phenomenon that could involve many diverse regulatory sequences and adaptive mechanisms (Guo et al., 2007).

Although drought decreased the RWC in NH_4^+ and NO_3^- plants, it is possible that the NH_4^+

treated plants had higher nitrogen uptake than the NO_3^- treated plants under drought conditions, and though there is no evidence that supply of N (as NO_3^-) to plants is limiting even at small RWC, one should not forget that the flux of NO_3^- to the roots is by mass flow, so decreasing transpiration may decrease uptake. Probably the major limitation is reduction of NO_3^-, because small RWC decreases nitrate reductase (NR) in leaves substantially and rapidly, whereas rehydratation quickly re-establishes it (Kaiser & Foster 1989; Foyer et al. 1998; Ferrario-Méry et al. 1998). In our experiment a nitrate reductase (NR) assay was not possible but since the NO_3^- plants exhibited lower transpiration rates (see below), it can be hypothesised that the solute flow was affected.

The interrelationship between N form and water relations is further illustrated by the reduced water potential and lower water retention of NH_4^+-grown plants as compared to NO_3^- grown controls (Quebedeaux and Ozbun, 1973; Pill and Lambeth, 1980). However, in the greenhouse (2008), the cultivars Permanent and Aalvito treated with NH_4^+ had higher (26 and 9%, respectively) predawn water potential than the NO_3^- plants.

The greenhouse experiment in 2007, Nutrader, Vitaminado and Permanent showed higher hydraulic conductance with ammonium fertilization than with nitrate fertilization under well-watered conditions (Figure 19a). Furthermore, the hydraulic conductance of $NH4^+$ treated Permanent plants was higher when they were well watered than under drought (Figure 19a and b). In the field experiment, however, Nutrader, Vitaminado and Mazorca de oro had higher hydraulic conductance with ammonium fertilization than with nitrate (Figure 39).

In the greenhouse experiment of 2008, Permanent, Abakus and Mazurka under well watered conditions showed slightly higher hydraulic conductance with ammonium than with nitrate. When comparing the drought effect on the hydraulic conductance, Figure 21b shows clearly that the reduction of the hydraulic conductance was greater in the NO_3^- plants than in the NH_4^+ plants by drought. However, well-watered Aalvito plants had higher hydraulic conductance with nitrate fertilization than with ammonium fertilization. In the field experiment, Permanent, Abakus and Mazurka under well-watered conditions with ammonium fertilization showed higher hydraulic conductance than with nitrate fertilization, which was consistent with the results from the greenhouse. Generally, all cultivars were less affected by drought in the field experiment than in the greenhouse experiment (Figure 40).

Although the measurements of the gas exchange parameters and hydraulic conductance were not taken at the same time, same cultivars, as Nutrader and Permanent in the greenhouse (2007) and Nutrader, Vitaminado and Mazorca de oro in the field experiment (2007), showed higher transpiration rates and hydraulic conductances for ammonium fertilization compared to nitrate fertilization. Similarly, the increase of transpiration rate of Permanent and Abakus corresponded with an increase of the hydraulic conductance in the greenhouse and in the field experiment (2008), which was more pronounced for ammonium fertilization compared to nitrate fertilization. Therefore, the higher transpiration rates and stem hydraulic conductances in NH_4^+ plants compared with NO_3^- plants demonstrates that under optimum water regime, the maize crop fertilized with ammonium by CULTAN-method could enhance the root water uptake. Guo et al. (2007) compared water uptake rates of intact beans plants, which showed relatively small differences between both N-forms, but the relative increase in shoot water consumption under ammonium supply (+85%) was higher than under nitrate supply (+38). In well-watered plants, water absorption is limited either by shoot transpiration or root water uptake capacity. Water uptake rates of ammonium-supplied bean plants were also clearly higher when the metabolism of roots was de-activated. These data on high shoot water consumption further agree with high enzyme density and clearly higher rates of gas exchange under ammonium nutrition (Raab and Terry 1994). The difference in the intensity of light interception and photosynthesis rates between the plants treated with ammonium or nitrate could be the reason for the variation in growth, hydraulic architecture and hence, hydraulic characteristics of the maize crop. The plant hydraulic conductance may directly respond to transpiration rate (Liu et al, 2005). Plant transpiration responded linearly to temperature-induced changes in plant hydraulic conductivity (K_{plant}). Under drought soil conditions, a linear relationship has also been found between K_{plant} and E (transpiration) in *Quercus* (Cochard *et al.*, 1996). For instance, Sperry and Pockman (1993) demonstrated that, in *Betula*, stomata were closing when shoot hydraulic conductance was reduced by xylem embolism. Using partial defoliation, Meinzer and Grantz (1990) have also shown a co-ordination between stomatal and hydraulic conductances in sugarcane.

An increase in stem diameter corresponded with an increase of the hydraulic conductance in both, the greenhouse and the field experiment in the year 2008 (Figure 22 and 41). However, this correlation was not significant in the greenhouse experiment 2007 (Figure 20). In previous work with coffee plants (2003), we found similar results (Bustamante, 2003). Kufa (2006) also found that the main stem hydraulic conductance of wild coffee trees was significantly correlated with the stem diameter. Our results suggest, therefore, that the hydraulic conductance of the maize crop corresponded with the morphological growth parameters.

In the greenhouse in 2007, the cultivars Nutrader and Permanent fertilized with ammonium and under well-watered conditions had the highest hydraulic conductances (9.16 and 9.04 $X10^{-4}$ Kg m^{-2} Mpa^{-1}, respectively). Under well-watered conditions in 2008, Aalvito with nitrate fertilization had the highest hydraulic conductance. Under drought conditions, Abakus and Mazurka had the highest stem hydraulic conductance when ammonium was used as nitrogen form instead of nitrate. Because Mazurka is a drought tolerant cultivar (Trockels, personal communication), it might be better adapted to restricted soil water conditions than the non-tolerant cultivars. On the other hand, Abakus is a stay green cultivar and the photosynthesis rate could be maintained. Accompanied by an increase in hydraulic conductance, Mazurka and Permanent in the greenhouse (2008) showed high values of leaf water potential under drought conditions. In fact, stomatal conductance is a function of LWP. Stomatal conductance (g_s) and transpiration rates vary widely across plant species. Leaf hydraulic conductance (K_{leaf}) tends to change with g_s, to maintain hydraulic homeostasis and prevent wide and potentially harmful fluctuations in transpiration-induced water potential gradients across the leaf ($\Delta\Psi$leaf) (Augé et al. 2008). Similar results were found for wild coffee trees (Kufa, 2006), in rice (*Oryza sativa L.)* (Kobata and Murshidul, 1999), and in *maize* (Melkonian et al., 2004).

Another reason that could have influenced the hydraulic conductance increase of NH_4^+ compared to NO_3^- plants, could be the presence of aquaporins. It has been shown that the activity and abundance of water channel proteins (*or aquaporins*) in the root plasma membrane play a significant role in the cell-to-cell pathway for maize (Maurel and Chrispeels, 2001; Chaumont et al., 2001; Aroca et al., 2005). The aquaporins represent a trait of high plasticity, which together with plant root architecture result in a highly variable root hydraulic conductivity under different external environments (Mu et al., 2005). In a certain condition, such as a limited water or nutrition deprivation, the decreased ability of single root water uptake might be compensated for by the increase of the overall absorbing area of the root system (Mu et al., 2005). The N-form could influence the hydraulic conductance in maize through the aquaporins. However, in our work such an assay was not made.

On the other hand, drought can diminish the hydraulic conductance due to the presence of cavitations. Xylem vessels, composed of dead cells, well known for their low resistance in water transport, were usually ignored. However, under stress conditions, especially under water deficiency, cavitations occurring in xylem could significantly decrease the hydraulic conductivity (Zwieniecki et al., 2001; Stiller et al., 2003). On the other hand, Zwieniecki et al. (2001) also found that increasing concentrations of ions flowing through the xylem of plants produced a rapid, substantial, and reversible decrease in hydraulic resistance, and this ion-dependent mechanism allows plants to compensate for decrease in hydraulic conductivity induced by cavitations (Mu et al., 2005). Guo et al., (2007) also found a positive correlation between water uptake and N, Mg and K uptake, although in maize and tomatoes the cation content was reduced when NH_4^+ was applied (Gerendás et al., 1997). In previous work (Bustamante, 2003) with coffee plants, we found that an increase of K concentrations increased the stem hydraulic conductivity. However, an assay of the K uptake in CULTAN plants has not yet been made. On the other hand, the axial resistance in stem xylem also affects water transport under stress conditions, and in turn affects plant water relations.

5.3 Maize growth

Generally, ammonium was superior to nitrate in whole aboveground biomass in the greenhouse experiment (2007). There was an increase of about 12 to 20% of the whole aboveground biomass of the plants treated with ammonium compared to those treated with nitrate in the greenhouse experiment (2007) (Figure 23b). This corresponded with an increase in plant height (Figure 23a), with Permanent being tallest cultivar, even though it was not the cultivar with the highest aboveground biomass production. However, in the field experiment (2007), N-form had no influence on the whole aboveground biomass, although the NH_4^+ plants of all cultivars were about 4-7% taller than the NO_3^- plants (Figure 42a and b). These differences between plant height and dry matter could be because an increase of less than 10% in NH_4^+ plants height was not enough to produce significant dry matter differences.

In the 2008 greenhouse experiment all cultivars showed an increase in plant height when ammonium was used as a nitrogen source instead nitrate. The stem diameter was also slightly increased for ammonium plants compared to nitrate plants in Permanent, Abakus and Mazurka (Table 1). At the same time, Aalvito was the cultivar with the greater stem diameter (S_d) values compared to Permanent, Abakus and Mazurka. As it can be seen, the tallest plants were not necessarily the plants with the largest stem diameter. These growth increases affected the dry matter production of the maize cultivars, hence, when ammonium was used as nitrogen form instead nitrate, the cultivars Aalvito and Mazurka exhibited a slight increase of dry matter (Figure 24).

In the field experiment (2008), contrary to the greenhouse, the N-form did not affect the plant growth and therefore the dry matter for any of the cultivars.

In an experiment conducted in the greenhouse, Schittenhelm and Menge-Hartmann (2006) noticed that about 60 days after sowing the plants of spring barley treated with nitrate had lower aboveground dry matter and plant height compared to those plants treated with ammonium. Similar to our results, ammonium was also reported to be superior to nitrate for growth of rice (Qian et al., 2004) and maize (Lewis et al., 1989, Teyker and Hobbs, 1992; Gentry and Below, 1993). Plant height, leaf number and total fresh weight increased in plants of tomato grown with NH_4^+ (Hohjo et al.1995). There is also some evidence that growth of tea plants is improved with NH_4^+ as compared with NO_3^- nutrition (Ishigaki, 1974). In contrast, when ammonium was used as sole nitrogen source, growth was also reduced compared to nitrate fed plants (Greef, 1989; Walch-Liu et al., 2000; Guo et al., 2002).

The increase in growth and dry matter with NH_4^+ over the NO_3^- could be attributable to the absence of toxicity in the rooting medium. The poor growth of NH_4^+ plants compared to those fertilized with NO_3^- is mainly associated with toxicity and acidification of the root medium during NH_4^+ assimilation. According to Findenegg (1987), plant growth may be depressed by ammonium toxicity, particularly if NH_4^+ concentration is high. However, ammonium toxicity was not relevant in this work, because with the CULTAN cropping system the nitrification and phytotoxicity of ammonium are prevented (Sommer, 1998). With the CULTAN-technique, the deposits with high NH_4^+ concentration attract the roots of the plants but in these conditions the root contact with the deposits is low and phytoxicity does not have an effect on the plants. Feng et al. (1997) assumed that the occurrence of high NH_4^+ concentration in the soil in CULTAN fertilization might be toxic to broad bean but these results were different. However, the physiological response of the plants to different nitrogen sources, nitrate and ammonium, are assorted (Lasa, 1998). There is a controversy about the relative advantage of one form over the other in terms of various physiological processes or plant species, while according to Guo et al. (2006), the effect of ammonium supply on plant growth results also from its effects on regulatory processes by which plants adjust their metabolism to nitrogen assimilation. Furthermore, agricultural practices such as the application of ammonium fertilizers or urea along with nitrification inhibitors (NI) or the creation of soil zones with high ammonium concentration by specific application techniques (CULTAN technique: Sommer, 1994) may result in an enhanced ammonium supply.

Although in the field (2008) the N-form had no influence on the growth and dry matter of the well watered plants, an interaction between N-form and water regime shows that the drought reduced plant height more in the NH_4^+ plants than the NO_3^- plants in the Aalvito cultivar. I.e. compared with NH_4^+ plants, the NO_3^- plants of these cultivar adapted better to drought conditions in the field experiment. This superiority of NO_3^- plants over the NH_4^+ plants could be related to lack of rain immediately after the fertilization. In some plots of the drought treatments, the ammonium deposit in some rows were applied at distance greater than required (greater than 10 cm), thus, the initial growth was possibly inhibited. Following an application of ammonium-N or urea to the soil surface, the fertilizer should be moved into the soil profile through irrigation or mechanical incorporation if rainfall is not imminent. According to Schittenhelm and Menge-Hartmann (2006) the first reason for the plants in the NH_4^+ treatments to display slower early growth than the plants in NO_3^- treatments is the time needed by the roots to reach the soil zones containing the injected ammonium. There, they can take up the large amount of N necessary for rapid plant growth. The plants in the NO_3^- treatments, in contrast, can immediately start exploring the broadcasted nitrate, which is more evenly distributed in the soil moisture. This reasoning is normally valid only when the ammonium deposit is applied at long distance to the root or seed, but extremes of dry and wet weather will have less effect on N uptake by the plant. More generally, it has also been mentioned that the CULTAN plants are healthier than the NO_3^- plants and that for this reason, the dry matter can increase by about 10-15% (Sommer, 1991).

During growth a higher efficiency of nitrogen applied as "CULTAN" has to be taken assumed in comparison to common fertilization. For equal yields, the application of nitrogen with "CULTAN-technique" can be reduced up to 30 % in comparison to nitrogen topdressings. In locations where water is not the limiting factor of growth, higher yields can be expected by "CULTAN-technique" with the same amount of nitrogen in comparison to common nitrogen topdressings (Schumacher and Sommer , 2001).

One of the factors influencing the physiological response of plants to water stress is mineral nutrition. Due to water stress stem length reduces by inhibiting the elongation of internodes and also reducing the tillering capacity of plants (Aspinall et al. 1964).

Hence, the interactive effect of nitrogen nutrition and water stress on plant growth has received attention from several workers. Viets (1962) suggested that the growth response of crop plants to irrigation depends on the availability of nitrogen in the soil. Other experiments also suggest that the internal water relations of the rice crop plants during water stress depend on the supply of nitrogen (Lahring, 1980, Yambao and O´Toole, 1984). The NH_4^+ fed plants of two hybrids of maize exhibited better adaptation to drought than NO_3^- fed plants (Mihailovic, 1992).

In our work, drought decreased vegetative growth (plant height and stem diameter) in all cultivars with either ammonium or nitrate fertilization in both, greenhouse and in the field experiment in 2008. Although, when Aalvito was fertilized with nitrate, the drought did not reduce the stem diameter. However, the drought effect depended on cultivars and climatic conditions (Table 1 and 2).

Water deficit alters a variety of biochemical and physiological processes, ranging from photosynthesis to protein synthesis and solute accumulation (Larcher 1995). Variations in soil moisture can change root physiology and thereby enable plants to sense soil water status and adapt to decreasing soil moisture content by reducing growth (Hartung and Jescke, 1999). Those responses reflect decreasing plant available soil moisture and the plant's need to control and decrease its water use.

Plants depend on the availability of water for growth and development and have to tightly control the internal water balance to survive under drought stress (Maurel, 1997). Drought reduced whole aboveground biomass in Aalvito and in Abakus with ammonium fertilization in the greenhouse experiment (2008) (Figure 24), although in the field experiment the whole aboveground biomass was not reduced by drought (Figure 43). Sorghum responds to progressive drought with reductions in shoot dry matter and leaf area (Tsuji et al., 2003), similar to our results at the vegetative stage in some cultivars as Aalvito and Abakus with ammonium. In rice, total dry matter production as well as plant height were influenced by the moisture regime, increasing with soil moisture; they were greatest under field capacity conditions and smaller under progressive drought conditions (Abdulai, 2005). Generally, fresh and dry weights and yield were significantly reduced under water stress treatments in mungbean (*Vigna radiata* L.) (Tawfik, 2008).

In our experiment, different cultivars showed different response to drought when ammonium or nitrate as nitrogen sources were used. Thus, the Nicaraguan cultivars Nutrader, and Vitaminado responded better to ammonium nutrition than nitrate. In contrast, Mazorca de oro showed better growth with nitrate than ammonium. On the other hand, all German cultivars (Permanent, Aalvito, Abakus and Mazurka) responded differently under greenhouse and under field conditions, respectively. The Nicaraguan cultivars are well adapted to water restricted conditions and to lower nitrogen application (INTA; 2007). On the other hand, the German cultivars Mazurka and Abakus are drought tolerant and stay green type, respectively. Plant species originating from habitats, where adverse soil conditions hamper nitrification, often exhibit better or at least equal growth when nitrogen is supplied predominantly in the form of ammonium instead of nitrate alone (Haynes and Goh, 1978).

As a general rule, ammonium supplied at high concentrations caused severe reductions in growth. In a study of Moritsugu and Kawasaki (1983), different plants responded very sensitively to ammonium when grown in a nutrient solution of 5000 mM N supply. However, no detrimental effects of ammonium were observed when these species were grown under conditions of constant low (depending on species 50-250 pM) N supply in maize *(*Alfoldi et al., 1992). On the other hand, with the CULTAN technique the controlled N application increases the root distribution and with that the plants can take the N amount necessary for growth. In other words, the deposits cover the N demand for a long time, i.e. for about nine months (Sommer 1991).

The response of the different cultivars to the N-form, the method of nitrogen application and drought, could be influenced not only by the climatic conditions, but also by soil conditions and the root development. In the greenhouse experiment (in 2007 and 2008) the deposits were placed in the central part of the pot, which means that the distance between the deposit and the seeds was less in comparison with the field experiment (2007 and 2008). In contrast, in the field experiment, the deposits were placed of about 10± cm.

On the other hand, during the day, the temperature was about 20% higher in the greenhouse than in the field and this temperature difference could influence the root growth of the different cultivars. Several studies have shown that root zone temperature can affect plant growth (Malcolm et al., 2007). Top and root growth of maize was much greater at 28.2 than at 14.8°C (Moraghan and Porter, 1975). The root temperature, independently of air temperatures and light levels, influences growth, differentially affecting the partitioning of dry matter among roots, stems and leaves at different phenological stages in peaches (Malcolm et al., 2007). On the other hand, root cooling in beans, maize and wheat reduces leaf growth rates (Barlow et al., 1977; Milligan and Dale, 1988). At the other extreme, high root temperatures can affect dry weight, leaf area, leaf numbers and stomatal conductance in capsicums (Dodd et al., 2000) as well as photosynthesis and the shoot/root ratio in lettuce (He and Lee, 1998; Lee and Cheong, 1996). Although an increase in temperature can also increase the root growth, in our experiment in the greenhouse the root development was possibly inhibited because of the restricted pot space. Contrary, in the field grown plants, the root development could be higher than for the plants grown in the greenhouse and this would of course enhance the water uptake.

Another important element to highlight is the water regime. In the greenhouse, the pots of the different treatments were weighed daily and the water loss was replenished according to the water regime. On the other hand, in the field experiment, the water application was not controlled, although drip irrigation on half of the plots was installed.

5.3.1 Yield, total biomass and grain protein content

Generally, the NH_4^+ treated plants under well-watered conditions had higher yield (4-29%) than the NO_3^- treated plants. Obviously the relative superiority of either form of nitrogen was highly dependent on the cultivar and also on the year of the study (Wiesler, 1997) (Figure 44), with Permanent and Aalvito being the cultivars with the highest yield (11.37 and 10.74 Ton ha^{-1}, respectively). This grain yield increase was attributable to an increase of grain per ear. Many investigators found that the yield of maize increased with nitrogen fertilization (Muirhead et al., 1985, Baldwin, 1986, Sutton et al. 1986). Similarly to our study, the higher grain yield of the NH_4^+ plants compared to the NO_3^- plants was attributable to a significantly higher number of ears per plant and grain per ears in spring barley (Schittenhelm and Menge-Hartmann, 2006). Injecting the N sources resulted in higher yields when compared with broadcasting ammonium nitrate in maize (*Zea mays L*) (Howard and Tyler, 1989), in winter rye and wheat (Kücke, 2001). However, an increase of the yield in maize (*Zea mays*) with CULTAN-technique in comparison with nitrate fertilization has not been clearly demonstrated (Gruber, 2007). Mixed nitrogen nutrition generally increased the grain yield of wheat, with differences being most pronounced when plants were grown in nutrient solution. With regard to yield structure, mixed nitrogen nutrition generally increased the number of ears per plant but often reduced the number of grains per ear. Thousand-grain weight was hardly influenced by nitrogen form. Mixed nitrogen nutrition increased kernel yield of maize, as it did for wheat, when plants were grown in gravel- hydroponics systems (Wiesler, 1997). Plants fertilized with ammonium assimilate nitrogen as both nitrate derived from nitrification in the CULTAN depot zone and from ammonium (Blanke and Bacher, 2001). Potato plants fertilized by CULTAN showed an increase up to 25% of yield starch concentration compared to conventional fertilization (Leufen, 2008) and 15 to 30 % higher yields in different locations (Schumacher and Sommer, 2001). The increases in yields in general were based on bigger sizes of the potato tubers but not on increases in the number of tubers per plant. Repeated observations showed that in comparison to common fertilization the growth of stolons and tubers is promoted by "CULTAN".

The final biomass production was also increased (about 19%) with ammonium nutrition (CULTAN) compared to the broadcast nitrate applications (Table 3). While under well-watered conditions, the different cultivars responded better to the ammonium fertilization than to the nitrate fertilization. The NH_4^+ plants were more affected by drought in comparison with the NO_3^- plants and the yield was not affected by drought in Abakus with nitrate fertilization. Our results agree with Bolaños and Edmeades (1993), who found that the drought decreased yield and final biomass in different maize varieties. Grain yield reductions in maize (*Zea mays L*) resulting from drought stress depend upon numerous factors, such as the stage of plant development at which the drought stress occurs, the severity and durations of water deficiency, and the susceptibility of the hybrids examined (Lorens et al., 1987).

Moisture stress prior to silking reduced grain yield by 25%, moisture stress at silking reduced grain yield by 50% and moisture stress after silking reduced grain yield by 21% (Denmead and Shaw, 1960). In our work, drought at three weeks prior to flowering decreased yield in NH_4^+plants by about 20-36%, but in NO_3^- plants the decrease was about 16-18%. Drought reduced kernel yield to 60 and 80%, which was associated with a lower kernel weight (Chwen et al., 1993).

The imposed drought caused reductions in grain yield in the four maize lines with losses between 20 and 71%, but the level of the effect varied among the genotypes. Such reductions in yield are mainly due to decrements in the number of kernels per ear (Zarco-Perelló et al., 2005). On the other hand, the total biomass production of maize (*Zea mays L.*) at anthesis was reduced by drought from 7.3 to 4.9 Mg ha^{-1} (about 33%) (Bolaños and Edmeades, 1993).

Application of N fertilizer is used to increase both grain yield and protein content. Protein content was increased by N application. Forage production and protein percentage in maize increased significantly with N application (Soto et al. 2004). On the other hand, there was little difference among the various sources of N in their effects on protein content (Grant, 2000). There is little or no information over the corn protein content in maize under ammonium or nitrate nutrition. Our results show significant differences between cultivars. On the other hand, the interaction N-form*protein content was also significant (Figure 45). It is important to highlight that the cultivars Abakus and Mazurka had the greatest corn protein content (10.34 and 9.75%, respectively) compared to the other cultivars. On the other hand, the cultivar Aalvito showed an increase (20%) of protein content when ammonium was used as nitrogen source instead nitrate. Since the percentage of protein in maize corn depends on nitrogen concentration (Soto et al., 2004), the N-form and their application method (as CULTAN –technique) could play an important role in the nitrogen translocation from vegetative organs to the ear and enhanced the corn protein content in maize. Thus the stay-green Abakus and the rapid-growth and drought-tolerant Mazurka were superior to the other cultivars. Therefore, drought and nitrogen tolerant cultivars could enhance their growth and percent of protein content in corn, even under drought condition, i.e., a relation could exists not only between N-form and the water relations of maize, but also between N-form and corn protein accumulation. During ear development in maize (*Zea mays L.*), nitrogenous compounds are translocated from vegetative organs to the kernels and to the leaves and roots; the stalk contributes an important reservoir of nitrogen (Ta, 1991).

In general, as the grain yield of the maize increases in response to N, the concentrations of protein in the kernel also increase. However, in our experiment, an increase of yield of the plants treated with ammonium in Permanent, Aalvito and Mazurka did not corresponded with an increase in protein content. When protein concentrations are high, protein could continue to increase but at the expense of starch accumulation (Singletary and Below 1989) and terminate further increases in kernel weight and grain yield (Tsai 1983). If corn protein content increases in response to an increase of N and the availability of nitrogen and its efficiency increase by use of the CULTAN-technique, we could assume that the CULTAN-technique could enhance not only the grain yield in maize, but also the protein content in corn. These studies are, however, consistent with the observation that maize cultivars require different levels of N for their maximum yield and protein formation (Tsai et al., 1992).

6. CONCLUSIONS AND RECOMMENDATIONS

The finding of this study confirms the view that the more or less favorable effect of NH_4^+ in individual cases vary among species and cultivars and is dependent on the environmental conditions.

In both years, an increase of photosynthetic photon flux density led to an increase of photosynthetic rates for all treatments: NH_4^+ and NO_3^- plants under well watered and drought conditions. This increase was higher for the well-watered plants and for the plants fertilized by the CULTAN- technique, compared to the broadcasted nitrate fertilization. The well-watered plants also showed higher photosynthesis rate compared to the drought-stressed plants in both, the greenhouse and the field. In 2007, this led in 2007 to a higher above-ground dry matter production of the NH_4^+ plants than the NO_3^- plants of all cultivars tested in the greenhouse experiment. This finding suggests, therefore, that under high light conditions, ammonium nutrition by the CULTAN-technique has a positive effect on plant growth in dependence of the maize cultivars, water regime and climatic conditions.

The leaf water status parameters do not appear to have generally been affected by the N-form and application, but by the water regime. However, the cultivars Permanent and Aalvito had higher diurnal leaf water potential with ammonium fertilization than with nitrate under well-watered conditions in the greenhouse experiment.

In 2007, the hydraulic conductance never was higher with nitrate fertilization than with CULTAN-technique fertilization in both, in the greenhouse and in the field experiment. Whereas, it was significantly higher for Nutrader and Permanent cultivars of the NH_4^+ treatments, respectively. However, in the greenhouse experiment (2008), Aalvito had higher hydraulic conductance with nitrate fertilization than with ammonium fertilization by the CULTAN-technique. The same behavior showed Abakus in the field experiment (2008).

In the greenhouse experiment (2007), drought reduced clearly the hydraulic conductance in Permanent cultivar fertilized with the CULTAN-technique. Similarly, Aalvito decreased the hydraulic conductance of plants with CULTAN-technique in the greenhouse (2008). Drought also reduced clearly the hydraulic conductance in Aalvito, Abakus and Mazurka with nitrate fertilization in the greenhouse experiment (2008).

In 2008, the yield of the cultivars Permanent, Aalvito and Mazurka was 18 – 22 % higher with ammonium fertilization through the CULTAN-technique compared to the broadcast nitrate application under well-watered conditions, with CULTAN fertilized Permanent and Aalvito having the highest yield (11.37 and 10.74 Ton ha^{-1}, respectively). This increase is attributable to the increase of the grain per ear. However, the yield reduction by drought was more pronounced in the NH_4^+ (20-36%) plants than the NO_3^- (16-18%) plants. Therefore, under drought conditions Aalvito and Abakus had clearly higher yield when nitrate was used as nitrogen form instead of ammonium fertilization through the CULTAN-technique.

With nitrogen application through the CULTAN-technique the cultivar Aalvito had higher corn protein content (20%) compared to the broadcasted nitrate application under well-watered conditions. On the other hand, Abakus increase the protein content (8%) with nitrate fertilization than with ammonium fertilization through CULTAN-technique. Abakus (with nitrate fertilization) and Mazurka showed the highest corn protein content of all cultivars. Mazurka showed no difference between CULTAN and nitrate fertilization. Under drought conditions Permanent and Aalvito had higher protein content with ammonium fertilization through CULTAN-technique, compared to nitrate fertilization.

Summarizing, the different *Zea mays* cultivars are adapted to drought conditions through an efficient stomatal control of the transpirational water loss leading to an improvement in instantaneous water-use efficiency (WUE) depending of the N-form and applications as well as the climatic conditions.

The CULTAN-technique could be a viable alternative for maize smallholder growers in the drought-affected areas for improved production and productivity. However, further investigations should include an integrated approach considering all technological options available, as e.g. genetically improved cultivars, crop management practices as tillage practices and mulching as well as the nitrogen fertilization through the CULTAN-technique.

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