Validation of major QTL conferring salt stress tolerance in winter wheat

Dissertation

Zur Erlangung des Grades

Doktorin der Agrarwissenschaften

(Dr. agr.)

der Landwirtschaftlichen Fakultät

der Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Maisa Ahmed Hussin Mohamed

aus

Qena, Ägypten

Bonn 2023

Dedicated to Farida and Mohamed

In loving memory of my mother

Acknowledgments

First and foremost, praise and thanks to **the Almighty God** for His blessings throughout my life and for giving me the strength, ability, and opportunity to undertake this research study.

I want to express my deepest appreciation, respect, and love to my late mother. I will never forget her advice and encouraging words. May peace be upon her.

I deeply thank **Prof. Dr. Jens Léon** for the opportunity to work in the Plant Breeding group, for the trust and confidence in my abilities to carry out this research, and for all his wisdom in facing research problems. He did not only give me guidance and support throughout this research but was also there when I needed paternal advice.

I would like to give a special thanks to my second supervisor, **Prof. Dr. Mathias Becker**, for his insightful comments and contributions to this thesis. I much appreciate the time he spent reading my thesis despite his busy schedule.

Besides my supervisors, I would like to thank **Dr. Thomas Gaiser** and **Prof. Dr. Florian Grundler** for agreeing to serve on my PhD thesis committee. I have no doubt that I will benefit from your knowledge and experience.

I am also very grateful to **Dr. Agim Ballvora** and **Dr. Benedict Oyiga** for their continuous support, motivation, and guidance during my research. They taught me genetics and opened my eyes to new and undiscovered horizons.

I especially want to express my greatest gratitude to my colleague and dear friend, **Dr. Patrice Koua,** for helping me with statistical analysis. I cannot forget the assistance and support **Prof. Dr. Ali Naz** provided me during the time I worked in the laboratory, and always had the time to discuss scientific issues with me. I also sincerely thank **Prof. Dr. Annaliese Mason** for providing me with laboratory facilities.

I am most grateful to **Dr. Md. Nurealam Siddiqui** for his invaluable contribution to this project and his precious friendship.

My gratitude is also extended to all my colleagues and friends (**Karola Müller**, **Dr. Asis Shresta**, **Dr. MK Newton**, **Carolyn Kambona,** and **Dr. Nazanin Afsharyan**). They helped me during my data collection in both the greenhouse and the laboratory.

I am deeply honored and thankful to know **Karin Woitol**, who is one of the most generous and friendly people I have ever met. I am thankful for her companionship during my research.

My thanks go to the technicians including **Anne Reinders**, **Martina Brodeßer**, and **Jan Schoenenbach**.

I also want to thank **Mr. Josef Bauer**, who supported my experiments in the growth chamber.

My final words of gratitude are for my dear brother Mohamed and my daughter Farida, who have been my staunchest supporters over the years. Their enduring encouragement made this thesis possible.

CONTENTS

iii

LIST OF ABBREVIATIONS

ANOVA   Analysis of Variance

PCA   Principal Component Analysis

QTL   Quantitative Trait Loci

SNP   Single Nucleotide Polymorphism

SPAD   Soil Plant Analysis Development

FAO   Food and Agriculture Organization of the United Nations

TOL   Tolerance Index

MP   Mean Productivity

GMP   Geometric Mean Productivity

SSI   Stress Susceptibility Index

STI   Stress Tolerance Index

HM   Harmonic Mean

PSII   Photosystem II

FAA   Free Amino Acids

DNA   Deoxyribonucleic acid

RNA   Ribonucleic acid

mRNA   messenger RNA

PCR   Polymerase Chain Reaction

RT-qPCR   Real-Time quantitative PCR

RNA-seq   RNA sequencing

cDNA   complementary DNA

SWL   Shoot Water Loss

K   Potassium

Na   Sodium

NaCl   Chloride Sodium

DAP   Days After Planting

RWC   Relative Water Content

WL   Water Loss

FWC   Fresh Weight under Control

DWC   Dry Weight under Control

FWS   Fresh Weight under Stress

DWS   Dry Weight under Stress

SFW   Shoot Fresh Weight

SDW	Shoot Dry Weight
RFW	Root Fresh Weight
RDW	Root Dry Weight
CCi	Chlorophyll Content index
Fo	fluorescence intensity at 50µs
Fj	fluorescence intensity at J-step (at 2 ms)
Fi	fluorescence intensity at i-step (at 60 ms)
Fm	maximal fluorescence intensity
Fv	maximal variable fluorescence
Fo/Fm	non-photochemical loss in PSII
Fv/Fo	efficiency of the water-splitting complex on the donor side of PSII
Fv/Fm	quantum yield of PSII
PI(ABS)	performance index on absorption
MTAs	Markers Trait Associated
MAFFT	Multiple Alignment using Fast Fourier Transform
BLUE	Best Linear Unbiased Estimate
REML	Restricted maximum likelihood
H^2	broad-sense heritability

LIST OF FIGURES

LIST OF TABLES

Abstract

Wheat is the third most important crop grown extensively worldwide, with global production and use estimated at 775.6 and 755.8 million tons, respectively. Despite its importance for global food security, its productivity has been severely reduced due to multiple abiotic stresses, including salinity, drought, cold, and ion toxicity. Salt stress is one of the most destructive abiotic stressors causing yield losses in wheat worldwide. Although there are several strategies to increase wheat production in salt-affected areas, such as leaching and drainage, the cultivation of salt-tolerant genotypes is estimated to be the most effective way to increase wheat production in agroecological regions with high soil salinity. A prerequisite for improving salt tolerance is the identification of adaptive traits for screening genotypes and uncovering causative genes. In this study, two populations of 274 and 277 F_3 lines developed from crosses between Bobur (sensitive)*Altay2000 (tolerant) and Bobur*UZ-11CWA08 (tolerant) were tested for salinity tolerance at the seedling stage. As the progenies possessed high phenotypic and genetic variabilities in the measured traits, the genotypes could be classified as sensitive and tolerant. Under saline conditions, genotypes were considered tolerant when they had lower Na^+ and proline contents, higher K^+ and chlorophyll contents, a higher K^+/Na^+ ratio, higher rates of PSII activity, and higher photochemical efficiency compared to the sensitive ones. In the tested biparental populations, more than forty marker-trait associations have been tested, which have previously been described in a Genome Wide Association Study with a broad panel of cultivars. Of these marker-trait associations, five of them were polymorphic in the contrasting progenies, and one of them, namely Q-1DS on the short arm of chromosome 1D, showed a large quantitative trait locus (QTL) effect on dry root weight under salt stress. Several putative candidate genes underlying these five QTLs were identified. Expression analysis of the putative candidate genes showed that *TraesCS1D02G052200* and *TraesCS5B02G368800* had the highest expression levels in most of the organs and tissues, whereas they play important roles during development, growth, and grain filling. Additionally, the candidate genes that were validated in the parents were further validated in the contrasting lines for both segregating populations. This analysis revealed that *ZIP-7* exhibited higher differential expressions compared to *KefC*, *AtABC8*, and *6-SFT* under saline conditions. This study provides information on the genetic and molecular basis of salt tolerance that might be useful in developing salt-tolerant wheat cultivars.

Zusammenfassung

Weizen ist die drittwichtigste Nutzpflanze, die weltweit in großem Umfang angebaut wird. Die weltweite Produktion und Nutzung werden auf 775,6 bzw. 755,8 Millionen Tonnen geschätzt. Trotz seiner Bedeutung für die globale Nahrungsmittelsicherheit ist seine Produktivität durch zahlreiche abiotische Stressfaktoren wie hohe Salzgehalte, Trockenheit, Kälte und Ionentoxizität stark eingeschränkt. Salzstress ist dabei einer der schädlichsten abiotischen Stressfaktoren, der weltweit erheblichen bei Weizen führt. Obwohl es mehrere Strategien zur Steigerung der Weizenproduktion in salzbelasteten Gebieten gibt, wie z. B. Versickerung und Entwässerung, gilt der Anbau salztoleranter Genotypen als die effektivste Methode zur Steigerung der Weizenproduktion in agrarökologischen Regionen mit hohem Salzgehalt im Boden. Eine Voraussetzung für die Verbesserung der Salztoleranz ist die Identifizierung von adaptiven Merkmalen für das Screening von Genotypen und die Aufdeckung der ursächlichen Gene. In dieser Studie wurden zwei Populationen von 274 und 277 F_3 Linien, die aus Kreuzungen zwischen Bobur (empfindlich)*Altay2000 (tolerant) und Bobur*UZ-11CWA08 (tolerant) hervorgegangen sind, im Keimlingsstadium auf Salztoleranz getestet. Da die Nachkommenschaft eine hohe phänotypische und genetische Variabilität in den gemessenen Merkmalen aufwies, konnten die Genotypen als empfindlich und tolerant eingestuft werden.Unter salzhaltigen Bedingungen wurden Genotypen als tolerant eingestuft, wenn sie im Vergleich zu den empfindlichen Genotypen niedrigere Na^+ und Prolin-Gehalte, höhere K^+ und Chlorophyll-Gehalte, ein höheres K^+/Na^+ Verhältnis, höhere PSII-Aktivitätsraten und eine höhere photochemische Effizienz aufwiesen. In den getesteten biparentalen Populationen wurden mehr als vierzig Marker-Eigenschafts-Assoziationen getestet, die zuvor in einer Genom-Weiten-Assoziations-Studie mit einem breiten Panel von Weizensorten beschrieben wurden. Von diesen Marker-Eigenschafts-Assoziationen waren fünf in den kontrastierenden Nachkommen polymorph, und einer von ihnen, nämlich Q-1DS auf dem kurzen Arm vom Chromosom 1D, zeigte einen großen quantitativen Trait-Locus (QTL) Effekt auf das Wurzeltrockengewicht unter Salzstress. Es wurden mehrere mutmaßliche Kandidatengene identifiziert, die diesen fünf QTLs zugrunde liegen. Die Expressionsanalyse der mutmaßlichen Kandidatengene zeigte, dass *TraesCS1D02G052200* und *TraesCS5B02G368800* die höchsten Expressionswerte in den meisten Organen und Geweben aufwiesen, während sie während der Entwicklung, des Wachstums und der Kornfüllung eine wichtige Rolle spielen. Darüber hinaus wurden die Kandidatengene, die bei den Eltern validiert worden waren, in den kontrastierenden Linien für beide segregierenden Populationen weiter validiert. Diese Analyse ergab, dass *ZIP-7* im Vergleich zu *KefC*, *AtABC8* und *6-SFT* unter salzigen Bedingungen eine höhere

differentielle Expression aufweist. Diese Studie liefert Informationen über die genetischen und molekularen Grundlagen der Salztoleranz, die für die Entwicklung salztoleranter Weizensorten nützlich sein könnten.

1. Introduction

1.1. Wheat and its importance as a crop

Wheat (*Triticum aestivum* L.) is an allohexaploid (6x) plant consisting of three sub-genomes (A, B, and D) due to its polyploid nature. It consists of three sets of seven chromosomes, for a total of 21 chromosomes (Oyiga, 2017). The size of the wheat genome is 17 Gbp (Bennett and Leitch, 1995), which is bigger than the genome size of rice (~430 Mb in 12 chromosomes) and barley (~5.3 Gbp in 7 chromosomes). Sequencing the wheat genome has been a complex task due to its size, complexity, and repetitive nature. This makes the genome study and complete sequencing of wheat a very unnerving task.

Wheat (*Triticum aestivum* L.) is considered one of the world's main cereal crops and a staple food, grown under both irrigated and rain-fed conditions (Yadawad *et al.,* 2015). It plays a strategic role in global production, food, economy, and nutrition (Jahan *et al.,* 2019; Barutcular *et al.,* 2017; Darwish *et al.,* 2018). Wheat is the third most essential cereal grown extensively across the globe, and with global production and utilization now pegged at 794 and 791million metric tons (U.S. Wheat Associates 2021), respectively (**Figure 1**). This strongly indicates that there is a need to increase its production. Wheat accounts for more than 15% of the total cultivated area on earth and covers more of the earth's surface than any other food crop (FAOSTAT 2017). Shiferaw *et al.* (2013) revealed that wheat has been playing an outstanding role in improving global food security and feeding a hungry world. This crop accounts for approximately 20% of global proteins and total dietary calories (Shiferaw *et al.,* 2013).

Wheat faces significant grain yield losses when grown in moderately saline soil conditions, therefore, is considered a moderately saline-tolerant crop (Quayyum and Malik, 1988; Shahbaz *et al.,* 2011, 2012). Although wheat is important to global food security, its productivity has been severely constrained due to multiple abiotic stresses, including salinity, drought, cold, ion toxicity, etc. Among these stressors, salinity is regarded as one of the most important environmental factors threatening global nutritional balance and reducing crop yields (Abd El-Mohsen *et al.,* 2015).
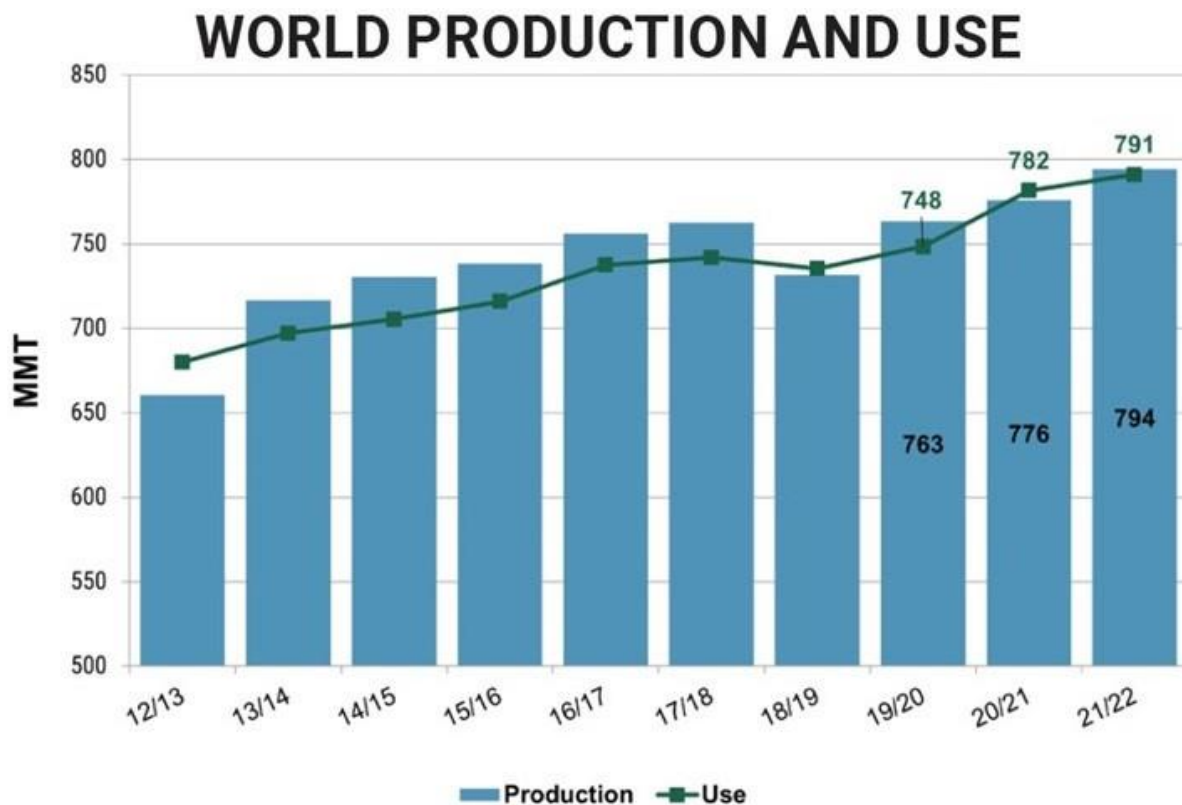
Figure 1. USDA's latest forecast continues the trend of record world wheat supply and demand (U.S. Wheat Associates 2021).

1.2. Abiotic stresses, particularly salt stress

Environmental stresses, for instance, such as cold, heat, drought, and salinity, cause adverse influences on plant growth and crop productivity (Rodríguez *et al.,* 2005). They indicated that abiotic stress is the principal cause of crop loss worldwide, decreasing average yields for most major crop plants by more than 50%. Among the abiotic factors that have formed and continue to shape plant evolution, water availability is the most essential (Rodríguez *et al.,* 2005). Water stress in its widest sense encompasses both salinity and drought stresses. These stresses considered particularly widespread in many regions and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Bray 2000).

Abiotic stress leads to a series of morphological, biochemical, physiological, and molecular changes that dangerously affect plant growth and productivity (Wang *et al.,* 2000). Oxidative stress, salinity, drought, and extreme temperatures are often interconnected and may cause similar cellular destruction (Rodríguez *et al.,* 2005). They are very complex motivations with many different but related characteristics, each of which may provide the plant cell with very different information (Rodríguez *et al.,* 2005). High salt stress, for example, disrupts homeostasis in water potential (osmotic homeostasis) and ion distribution (Rodríguez *et al.,* 2005). This disorder of homeostasis happens at both the cellular and whole plant levels

2

(Rodríguez *et al.,* 2005). Radical changes in ion and water homeostasis lead to molecular destruction, growth stoppage, and even death (Rodríguez *et al.,* 2005). To attain salt tolerance, three interconnected phases of plant activity are important. First, the damage must be prevented or alleviated. Second, homeostatic conditions must be reproduced in the new, stressful environment. Third, growth must continue, albeit at a reduced rate (Zhu, 2001).

Salinity presents a tremendous threat to food security and is a major danger to agricultural productivity worldwide (Ahmad et al., 2013). Dadshani (2018) indicated that the area affected by salinity is more than one billion hectares of land, accounting for approximately 25% of the global land area. Due to improper irrigation practices or natural salinization, this area is increasing by up to 10 million hectares of land every year (Luo et al., 2017). Salinization is expected to cause global yearly yield (economic) losses in agricultural production worth more than US$ 12 billion (Shabala, 2013). Besides economic losses, salinity is a major concern for global food production, especially when considering the projected increase in demand by the year 2050 (Shabala, 2013). Further to this, soil degradation and urbanization are dramatically reducing the obtainability of arable land per capita (Godfray et al., 2010).

1.3. Arable lands salinization

Salinity is known as one of the most essential abiotic stresses, restricting crop production in arid and semi-arid regions where soil salt content is naturally high and precipitation can be lacking for leaching (Zhao *et al.,* 2007). The natural soil salinization is produced by either the weathering of parent rock materials or the shallow saline water table, which frees salts in the soil. on the other hand, the human-made soil salinity arises from human activities and improper irrigation or poor cultural practices, such as deforestation, overgrazing, the use of saline water for irrigation, and poor drainage of watered fields (Yadav *et al.,* 2011). Munns and Gilliham (2015) concluded that salinity is becoming more widespread due to unsustainable irrigation practices, land clearing, and pressures to bring marginal land into production.

Salinity continues to spread in many regions due to the changing climate. About 950 million ha of salt-affected lands occur in arid and semi-arid regions, which is expected to be about 33% of the arable land area of the world. Universally, 20% of irrigated land (450,000 km^2) is suffering from salinity, with 2,500-5,000 km^2 of production land lost every year (UNEP, 2008). The distribution of saline land worldwide, with the affected areas predominantly placed in the wheat-producing countries containing Northern Africa, Australia, Central and West Asia, and some parts of South and Northern America, is shown in **Figure 2**. Jamil *et al.* (2011) have predicted that by 2050, more than 50% of the arable land would be salinized. Approximately US$12 billion in global annual losses in agricultural production from salt-affected land are

estimated and are expected to increase in the future (Qadir *et al.,* 2008; Flowers *et al.,* 2010). To reduce the impact of salinity on crop yield, it is critical to consider adopting practices that limit further soil degradation, and coordinated efforts must be made to manage arable lands, particularly those prone to salinity.
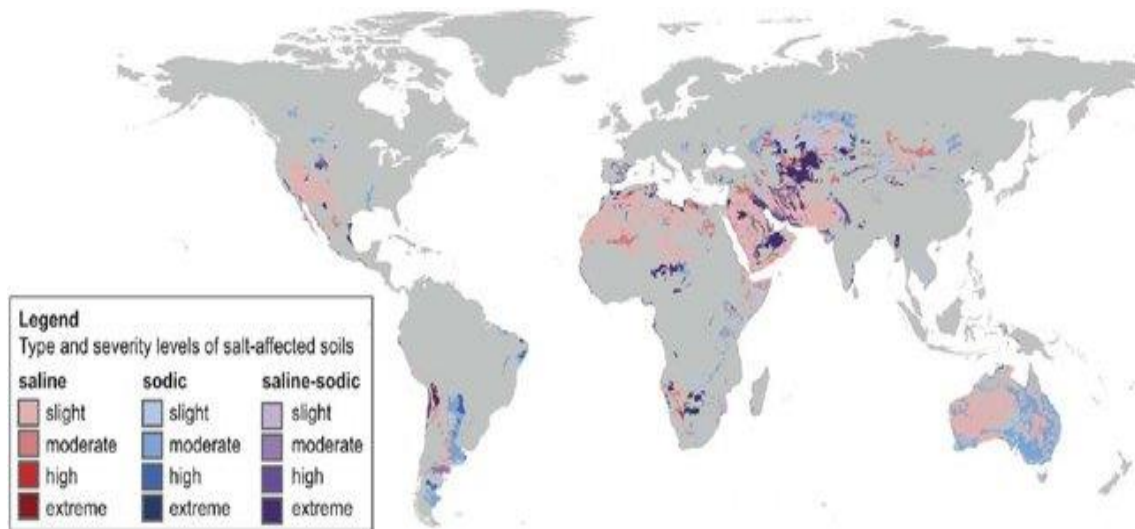
Figure 2. Global distribution of salt-affected soils, classified by type and severity. Reproduced from Wicke *et al.* (2011).

1.4. Effect of salt stress on plant growth

The capability of plants to survive under saline conditions differs between different species of halophytes and glycophytes (Oyiga, 2017). By adopting various tolerance mechanisms, the halophytes have acclimatized to live, support growth, and reproduce in soils that contain high concentrations of salt (above 200 mM NaCl) (Bose *et al.,* 2014). In contrast, the glycophytes cannot tolerate more than 25% of the salinity levels of seawater without short-changing their growth and yield (Oyiga, 2017). He indicated that unfortunately, most of the modern crops, including barley, wheat, and rice, are glycophytes.

The growth response of glycophytes to salinity (>40 mM NaCl) occurs in two stages as presented in **Figure 3**. The first stage; a rapid response to an increase in external salt known as the "osmotic phase," and the second stage; a slower response with an accumulation of Na^+ ions in vacuoles referred to as the "ionic phase.". Both stages have a negative influence on the growth and yield of crops (Munns and Tester 2008). The osmotic phase of growth decline is influenced by the salt concentration outside the plant rather than the salt in the plant tissues and by water deficit (drought stress) or osmotic stress, which is mostly responsible for growth inhibition with little genotypic difference. Nevertheless, usually between 2 and 4 weeks is the time in which the ionic phase of growth decline takes to develop because of an inner salt injury caused by an excessive build-up of toxic Na^+. At this phase, plants close their stomatal apertures

and consequently reduce the photosynthetic rate because of the harmful effect of toxic Na^+ that accumulates in the thylakoid membranes of the chloroplasts. The increasing oxidative stress and ROS formation results in leaf injury and damage to the photosynthetic capability of the plants.

Previous reports indicated that increased salt loadings in the leaf to levels that exceeded the capacity of salt compartmentation in the vacuoles cause salt to accumulate in the cytoplasm to toxic levels, leading to leaf injury and death (Munns, 2002; 2005; Munns *et al.,* 2006).

The tolerance status of the plant under salt stress would be determined by the balance between the rate at which the leaves die and the rate at which new leaves are produced. Plants are incapable of coping with, tolerating, and living in saline conditions long enough if the former process progresses faster than the latter to give enough photosynthate to the reproductive organs and produce viable seeds.

Based on the osmotic phase and ionic phase concepts, the osmotic influence applied by salts in the medium around the roots would cause the initial growth decline in both salt-tolerant and salt-sensitive genotypes as described in **Figure 4a** (i.e., the Osmotic Phase). However, at the ionic phase, the salt-sensitive genotypes are much more affected because of their inability to inhibit Na^+ accumulation in transpiring leaves to toxic levels (Munns *et al.,* 2006). Due to this development, crops have been classified into two categories: (i) salt-excluders and (ii) salt-includers. The salt-excluders adapt to saline stress by avoiding Na^+ uptake, whereas the salt-includers take up Na^+ and translocate it to the shoot, where it is isolated and used as vacuolar osmoticum (tissue tolerance) (Mian *et al.,* 2011). At the ionic phase, the salt-sensitive genotypes can be differentiated from the salt-tolerant ones, as indicated in **Figure 4b** and in **Figure 4c.** The effect of salinity on crops may also be a result of the mixture of osmotic and ionic salt influences. Several studies have found that the ionic phase is linked to lower stomatal conductance, photosystem II efficiency, photosynthesis capacity, biomass, and yield in plants (Isla *et al.,* 1998; Tester and Davenport, 2003; Netondo *et al.,* 2004; Tavakkoli *et al.,* 2011).
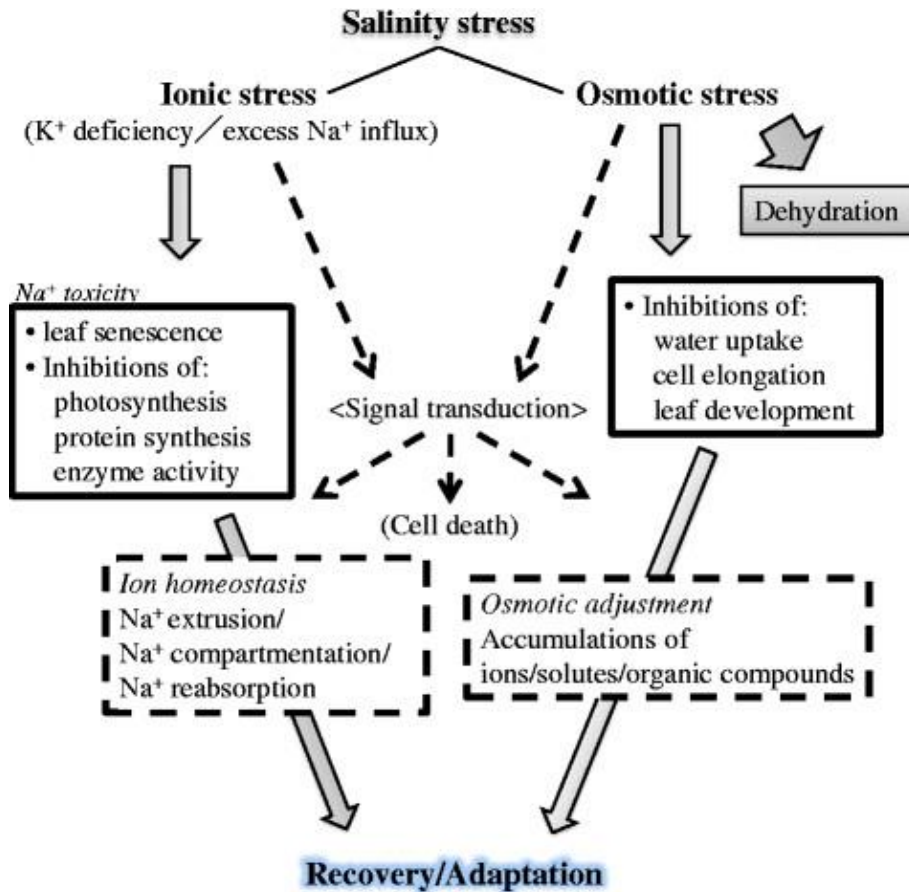
Figure 3. Adaptive responses of plants to the osmotic and ionic phases of salt stress, modified from De Oliveira *et al.* (2013).
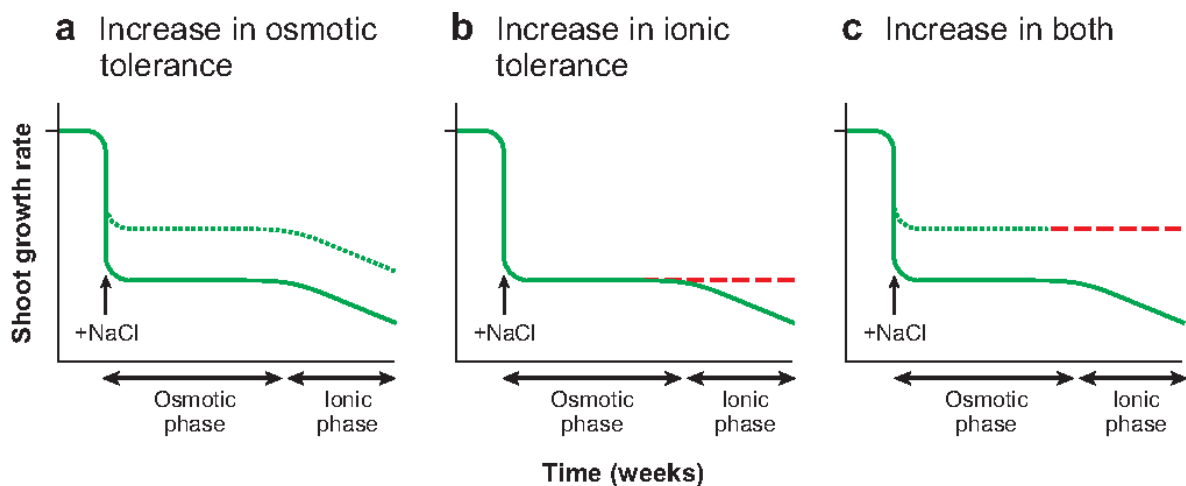
Figure 4. Model showing plant response phases of salt stress (Source: Munns and Tester, 2008). The thick green line represents the change in the growth rate after salt application. (a) The broken green line represents the hypothetical response of a plant because of increased tolerance to the osmotic component of salt stress. (b) The broken red line represents the response of a plant with an increased tolerance to the ionic component of salinity stress. (c) The green-and-red line represents the response of a plant with increased tolerance to both the osmotic and ionic components of salinity stress.

1.5. Mechanisms of plant response to salt stress

Jones and Gorham (1983) as well as Munns (1993) have shown a wide range of responses to salinity in plants that secure a wide range of adaptations at the whole plant level. Under high salinity conditions and over the years, plants have developed several mechanisms that permit them to adapt, grow, and reproduce. Roy *et al.* (2014) revealed that these mechanisms are grouped into three main categories: (i) tolerance of tissue to accumulated Na^+ or Cl^-, where Na^+ or Cl^- that succeed in getting into the plants is compartmentalized in the leaf vacuole to prevent salt injury to the sensitive thylakoid membrane of the chloroplasts, (ii) Na^+ or Cl^- exclusion, which tends to prevent Na^+ and Cl^- uptake and transport processes in roots to reduce the accumulation of these ions to toxic concentrations within leaves; and (iii) osmotic stress tolerance, which is controlled by long-distance signals that reduce shoot growth and is triggered before shoot Na^+ accumulation (**Figure 5**) (Munns, 2002; Tester and Davenport, 2003; Kumari *et al.*, 2014; Roy *et al.,* 2014).

Even if the information available for the plant tolerance to the "osmotic phase" remains shadowy, Mittler *et al.* (2011) have recommended that this process may be linked to Ca^{2+} waves, rapid, long-distance signaling via processes such as ROS waves, or long-distance electrical signaling (Maischak *et al.,* 2010). Due to the differences in long-distance signaling and/or in the initial salt stress perception and/or in the response to the signals present among plants, there are differences in plants' osmotic tolerance (**Figure 5**). However, to achieve a clearer understanding of osmotic tolerance in plants, further studies are required. The ionic phase is the most researched aspect of the salt tolerance mechanism, which is due to Na^+ and Cl^- accumulation in the leaf blade. In plants, the ion toxicity during the ionic phase can be reduced by increasing crops' capability to cope with salts that succeeded in entering the shoot (tissue tolerance) via compartmentation in the vacuoles and/or by decreasing the build-up of toxic ions (Na^+ and Cl^- exclusion) in the leaf blades. Sodium may play a role in both osmo-protection and osmotic adjustment in the synthesis of compatible solutes and higher-level controls to coordinate transport and biochemical processes (Munns and Tester, 2008; Flowers and Colmer, 2008). In plants, tissue tolerance, which involves Na^+ exclusion from the cytosol and compartmentalization in the vacuole has a harmful effect on cellular processes (Roy *et al.,* 2014).

These three mechanisms of salt tolerance are not mutually exclusive (Munns *et al.,* 2012; Roy *et al.,* 2014). This is because the occurrence of one does not inhibit the other. However, it might be possible that each of these tolerance mechanisms is more effective depending on genotype and/or a particular circumstance or growth stage. For example, "osmotic tolerance"

7

may be more noticeable at moderate salinity, whereas Na^+ exclusion may be more effective at higher salinity (Munns *et al.,* 2012).
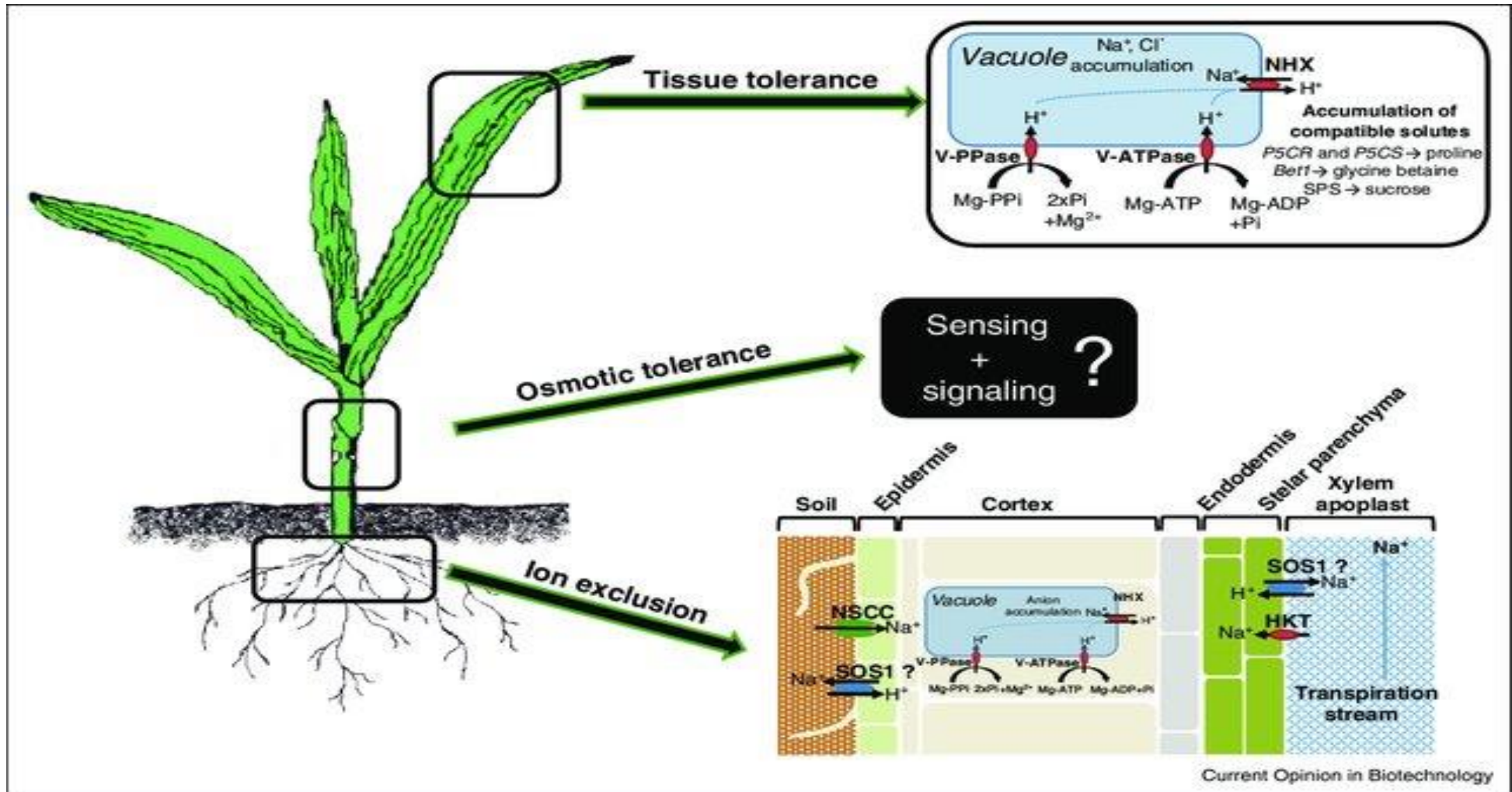
Figure 5. The three main mechanisms of a crop plant's salinity tolerance (Source: Roy *et al.,* 2014).

Tissue tolerance, where the accumulated toxic Na^+ in the leaves is compartmentalized in the vacuole, is a process involving ion transporters, proton pumps, and the synthesis of compatible solutes. Osmotic tolerance, associated with a reduction in shoot growth and related to drought stress, may be related to yet unknown sensing and signalling mechanisms. Ion exclusion is associated with the exclusion of toxic Na^+ and Cl^- from getting into the plant (primarily from the roots). This mechanism may include retrieval of Na^+ from the xylem, compartmentation of Na^+ and Cl^- in vacuoles of cortical cells, and/or efflux of Na^+ and Cl^- back to the soil.

9

1.6. Plants' morphological, physiological, and biochemical responses to salinity stress

The morphological, biochemical, and physiological responses of plants are changed due to salt stress (Amirjani, 2010). Salinity causes many physiological disorders resulting from osmotic stress, ion toxicity, and the variance of nutrient elements in the cytoplasm of plant cells (Flowers *et al.,* 1977; Muranaka *et al.,* 2002). Photosynthetic pigments absorbe active radiation, which migrated to the reaction centers of photosystems (PS) I and II, where the quantum conversion process occurr (Horton *et al.,* 1996). Hence, analysis of chlorophyll fluorescence parameters and chlorophyll content is considered an important method to assess the PSII activity during the photosynthetic process and the integrity of the internal apparatus (Clark *et al.,* 2000). These factors provide an accurate and rapid technique for identifying and quantifying plant tolerance to salt stress (Allel *et al.,* 2018).

To study the integrity and activity of the photosynthetic apparatus, the FluorPen FP100 for chlorophyll-a fluorescence detection (OJIP assay) is a highly sensitive technique for evaluating PSII photochemistry in addition to electron transport efficiency (Siddiqui *et al.,* 2021). Screening for chlorophyll fluorescence characteristics has added to the increasing interest in plant abiotic stress research (Oyiga *et al.,* 2016). They revealed that salinity stress has a negative effect on photosynthesis by inhibiting photosystem II (PSII) activity and damaging chlorophyll pigments because of the accumulation of toxic ions. The relationship between CO^2 assimilation in leaves and the PSII operating efficiency permits fluorescence to be used to discover differences in the response of plants to environmental challenges and, therefore, to screen for tolerance to environmental stresses (Baker and Rosenqvist, 2004).

Measuring the chlorophyll (Chl) content is considered one way to determine salt tolerance (Dong *et al.,* 2019). Kiani-pouya and Rasouli (2014) indicated that salinity affects Chl content in many crops by imposing negative effects on its synthesis or quickening its degradation, which reduces the photosynthetic capacity. In wheat, the ability to keep Chl content under salt stress is proposed as a salt-resistance trait (Cuin *et al.,* 2010). Therefore, in breeding programs, it can be used to choose salt-tolerant genotypes (El-Hendawy *et al.,* 2007; Din *et al.,* 2008; Azizov and Khanisheva, 2010).

Proline, which is considered a very effective class of compatible solutes and is found in a wide range of crop plants, has been reported to accumulate under abiotic stress (Singh *et al.,* 2018). Amirjani (2010) clarified that the production and accumulation of Free Amino Acids (FAA), especially proline, by plant tissue during salt stress, water stress, and drought, is an adaptive

response. Proline has been considered to perform an essential role in plant response to salt stress (Gaspar *et al.,* 2002) and proposed to perform as a compatible solute that regulates the osmotic potential in the cytoplasm (Arshi *et al.,* 2005; Bartels and Sunkar, 2005). Further proline contributes to many protective roles in stressed cells, including redox balance, (ROS) detoxification, and plasma membrane integrity. Under salt stress, the concentrations of numerous metabolites, including glycinebetaine and proline, rise, providing defense against osmotic challenges by utilizing compatible solutes (Sanchez *et al.,* 2008; Wu *et al.,* 2013). Under stress conditions, proline accumulation is preserved in unicellular to multicellular organisms (Hu *et al.,* 1992). Consequently, proline is considered one of the most widely studied molecules in the field of plant science (Kishor *et al.,* 2005; Szabados and Savouré, 2010).

Salinity tolerance is considered a complex trait controlled by many physiological factors and genes. As a result, Flowers and Yeo (1995) demonstrated that the multi-genic nature of salt stress tolerance in plants is likely a reason why traditional breeding for salt tolerance has not been successful (as measured by the lack of commercial products). With the improvement of cultivars with low Na^+ in the shoot or a high K^+/Na^+ ratio, the development of salt tolerance in glycophytic crops has been achieved (Tester and Davenport, 2003; Ren *et al.,* 2005; Munns and Tester, 2008; Thomson *et al.,* 2010; Munns *et al.,* 2012). For normal cellular functions and ion homeostasis, the capability of plants to keep high K^+/Na^+ is a key feature of salt tolerance. The K^+/Na^+ ratio in the plant is meaningfully decreased, when the plant roots are exposed to salinity (Tester and Davenport, 2003), because under saline conditions, Na^+ aggressively competes with K^+ uptake via K^+: Na^+ co-transporters, which may prevent the K^+ specific transporters of root cells (Zhu, 2003) and result in the buildup of Na^+ to toxic levels in the plant tissues. This means that through preferential absorption of K^+ over Na^+, the salt tolerance status of any plant mainly depends on its ability to exclude the Na^+ ions. Amtmann and Sanders (1998) have demonstrated that glycophytes show poor Na^+ exclusion potentials, which would disrupt ion homeostasis and prevent cellular growth and functions.

Zhu (2003) indicated that salt stress leads to distorted cytoplasmic K^+/Na^+ homeostasis by reducing the K^+/Na^+ ratio. Excess Na^+ and Cl^- cause an ionic imbalance, which can harm the selectivity of root membranes and cause K^+ deficiency in plants (Kibria *et al.,* 2017).

Keeping out toxic ions from the shoots is considered one of the important phases of the salt tolerance ability of a plant (Saqib *et al.,* 2012). It has been found that genotypic variation for salt tolerance is related to low rates of Na^+ transport and high selectivity for K^+ over Na^+ in wheat (Schachtman and Munns, 1992; Husain *et al.,* 2004). However, in maize, a positive

correlation between toxic ion exclusion and salt tolerance has also been observed (Cramer *et al.,* 1994). In rice plants under salt stress, the concentration of Na^+ and K^+/Na^+ was associated with seedling growth and grain yield (Lutts *et al.,* 1996). Consequently, Saqib *et al.* (2012) concluded that it is required to evaluate whether genotypes differing in salt tolerance use ion exclusion as a tolerance mechanism.

1.7. Salt tolerance response in wheat

Wheat is moderately tolerant to salt, with a yield loss of 50% at 13 dS m^{-1} (Mass and Hoffmann, 1977), but no yield loss at 6 dS m^{-1} (Mass and Hoffmann, 1977; Munns *et al.,* 2006). According to Yassin *et al.* (2019), the negative effects of salinity on plant growth may be due to osmotic stress and ion cytotoxicity. The major factor in maintaining wheat production is the ability of plants to cope with salinity stress (Guellim *et al.,* 2019). Although there are several strategies, such as drainage and leaching, to increase wheat production in salt-affected areas, the most effective way to overcome this limitation is the cultivation of salt-tolerant genotypes (Oyiga *et al.,* 2016).

Although it is crucial to consider the entire life cycle of the plant and conduct comprehensive assessments to accurately evaluate its salinity tolerance, the seedling stage can provide preliminary screening or initial insights into salinity tolerance (Shannon et al., 1998; Rao and McNeilly, 1999; Soloviev et al., 2003; Khan et al., 2003; Oyiga et al., 2016). At the seedling stage, genetic variations for salt tolerance have been exploited successfully in the identification of salt tolerance genotypes (Tiwari *et al.,* 2013; Oyiga *et al.,* 2016; Puram *et al.,* 2017) and in revealing genetic mechanisms of salt tolerance in wheat (Oyiga *et al.,* 2018, 2019; Wang and Xia, 2018). Kiani-pouya and Rasouli (2014) revealed that plant breeders favour assessing their genetic material under controlled conditions, like a greenhouse, sand cultures, hydroponics, or growth chambers. Also, they indicated that to understand different mechanisms of salt tolerance among genotypes, screening genotypes for salinity tolerance under such conditions is necessary. Although some researchers, such as Richards (1996), Rajaram and Van Ginkle (2001), and Betran *et al.* (2003), favored selection under suitable conditions, others, such as Ceccarelli (1987), Ceccarelli and Grando (1991), and Rathjen (1994), recommended selection under stress conditions. Furthermore, several researchers (Fischer and Maurer, 1978; Clarke et al., 1992; Ud-Din *et al.,* 1992; Fernandez, 1992; Byrne *et al.,* 1995; Rajaram and Van Ginkle, 2001) have preferred the middle path and trust in selection under both favorable and stressful conditions.

Several selection indices have been recommended based on a mathematical relationship between stress and non-stress conditions to discriminate tolerant genotypes of bread wheat (Clarke *et al.,* 1984; Huang, 2000). Tolerance (TOL; Clarke *et al.,* 1992), mean productivity (MP; Mccaig and Clarke, 1982), stress susceptibility index (SSI; Fischer and Maurer, 1978), geometric mean productivity (GMP), and stress tolerance index (STI; Fernandez, 1992). To identify stress-tolerant genotypes, the stress tolerance index could be convenient (Mitra, 2001). Fischer and Maurer (1978) indicated that cultivars are stress-tolerant with an SSI of less than a unit since, of all cultivars, their yield reduction under stress conditions is smaller than the mean yield reduction (Bruckner and Frohberg, 1987). Favored criteria in the selection of drought-tolerant barley genotypes are STI, harmonic mean (HM), mean productivity, and GMP by Baheri *et al.* (2003).

A screening-based method is essential to improve salinity tolerance and better exploit convenient stress tolerance traits (El-Hendawy *et al.,* 2009). Munns and James (2003) concluded that a useful standard for screening salt tolerance is biomass yield because it permits the direct assessment of economic return under saline conditions.

1.8. Strategy to validate marker-trait associations (MTA) in bi-parental populations

Historically, breeding has contributed to radical increases in cereal production, particularly using dwarf genes as well as the exploitation of heterosis (Ain *et al.,* 2015). The absence of a completely sequenced reference genome in wheat has limited gene discovery in bread wheat in the last decade, and recent advancements in the field of functional genomics have given breeders a new push to achieve their goals (Pingault *et al.,* 2015). But at present, the best alternative for the wheat breeder is the use of high-density single nucleotide polymorphism (SNP) assays to define genomic regions associated with quantitative traits either in bi-parental mapping experiments or in genome-wide association studies (GWAS) (Bordes *et al.,* 2014; Edae *et al.,* 2014; Zanke *et al.,* 2014). GWAS in wheat is challenging because of the large genome, incomplete genome sequence, and polyploidy, which make it difficult to assign the markers to highly similar homoeologous chromosomes (Sukumaran and Yu, 2014). So, a combined meta-genomic approach using comparative analyses of cereals and GWAS may provide an opportunity in wheat to quicken the identification of genes controlling quantitative traits (Quraishi *et al.,* 2011).

For QTL fine mapping, the recombination-derived progeny test strategy is considered a powerful and widely used method, which can narrow down the genomic region of the target QTL through trait-marker association testing in recombination-derived progenies (Ding *et al.,*

2012; Liu *et al.,* 2016). A QTL qMrdd8 associated with maize rough dwarf disease resistance was fine-mapped to an interval of 347 kb by using the recombinant-derived progeny test, and two candidate genes, CG1 and CG2, were identified (Liu *et al.,* 2016). In addition, a major QTL, RppCML496 conferring resistance to Puccinia polysora in maize, was fine mapped to an interval of 128 kb, and the NBS-LRR gene was the most likely candidate gene (Lv *et al.,* 2021). Using QTL mapping in two mapping populations, the genomic regions linked with charcoal rot resistance identified in tropical maize were validated, apart from identifying population-specific QTLs (Rashid *et al.,* 2021). Validated regions/markers will be studied more in breeding populations for possible deployment in the breeding pipelines (Rashid *et al.,* 2021).

In addition to the seed oil and protein content in soybeans, and to making the crop attractive to growers, several other agronomic traits and yield components are important (Sonah *et al.,* 2015). Therefore, improved understanding of the genomic regions and genes that control such important traits is key to the further genetic improvement of soya beans (Sonah *et al.,* 2015). With the development of quantitative trait locus (QTL) mapping approaches, identification of genomic loci governing complex traits has been facilitated (Sonah *et al.,* 2015). Conventionally, using segregating biparental populations, QTL mapping is performed (Sonah *et al.,* 2015). Despite numerous QTL studies, knowledge of the gene(s) underlying important agronomic traits remains limited (Sonah *et al.,* 2015). Typically, these roughly estimated QTL intervals extend over several cM, a genetic distance that translates into large genomic regions with dozens, if not hundreds, of candidate genes (Sonah *et al.,* 2015). This limited resolution results mainly from the small number of recombination events that are accumulated over the few generations needed to develop a biparental mapping population (Balasubramanian *et al.,* 2009). Genome-wide association studies (GWAS) have proved useful for the identification of candidate loci associated with numerous traits in animal and plant species (Appels *et al.,* 2014; Korte and Farlow, 2013). The GWAS approach followed by candidate gene identification has been found to be successful in several plant species, including maize (Li *et al.,* 2013), rice (Zhao *et al.,* 2011), and Arabidopsis (Verslues *et al.,* 2014). In Arabidopsis, GWAS and QTL mapping made together have been found to be complementary by mitigating each other's limitations (Brachi *et al.,* 2010; Zhao *et al.,* 2007).

Thousands of associations between SNPs and diseases/traits have been detected (Bosse and Amos, 2018; Gallagher and Chen-Plotkin, 2018; Horwitz *et al.,* 2019; Liang *et al.,* 2020). Despite using a strict genome-wide statistical significance threshold ($p < 5 \times 10^{-8}$ or equivalently $-\log(p) > 7.3$), a significant number of detected SNP-phenotype associations fail independent

validation (Brzyski *et al.,* 2017; Marigorta *et al.,* 2018). Identifying SNP characteristics predicting validation success (true positives) is important for prioritizing SNPs for targeted validation and downstream functional studies (Gorlova *et al.,* 2022). Gorlov *et al.* (2014), Merelli *et al.* (2013), and Xu and Taylor (2009) identified several SNP characteristics associated with validation success. Here we present the results of an updated analysis of associations between SNP characteristics and validation success in wheat.

Compared with the application of cultivars containing only a qualitative resistance gene, breeding new wheat varieties with quantitative Powdery Mildew (PM) resistance genes/quantitative trait loci (QTLs) has been shown to be more effective in controlling itsi solates (Grimmer *et al.,* 2015). Before the 21st century, in common wheat, the discovery of PM resistance genes was focused mainly on bred cultivars and their fundamental parents (Du *et al.,* 2021). Due to the similar genetic basis of these cultivars, it is becoming increasingly difficult to discover new PM resistance genes (Du *et al.,* 2021). However, landraces have relatively extensive genetic diversity, and they carry abundant PM resistance genes (Lu *et al.,* 2020). To date, at least 18 PM resistance genes have been identified in Chinese landraces (Du *et al.,* 2021). At present, almost all wheat PM resistance gene mining in Henan Province has been conducted in biparental segregation populations, but disease resistance genes in wheat germplasms have not been fully explored (Du *et al.,* 2021).

Another example is that numerous studies have been conducted to identify wheat chromosome regions associated with grain yield components and drought-related traits (Zhang *et al.,* 2018). Multiple QTL have been identified in all wheat chromosomes, and results have been summarized in a meta-QTL analysis for drought and heat stress (Acuña-Galindo *et al.,* 2015; Gupta *et al.,* 2017). Most of the initial QTL studies were performed using biparental mapping populations, but GWAS have become common in recent years (Breseghello and Sorrells, 2006; Wang *et al.,* 2012; Edae *et al.,* 2014; Ain *et al.,* 2015; Sukumaran *et al.,* 2015; Zanke *et al.,* 2015). Compared to biparental mapping populations, GWAS populations can be developed faster and provide access to a wider range of alleles (Zhu *et al.,* 2008). However, GWAS can exhibit higher rates of false positives than biparental populations (Yu and Buckler 2006). In young polyploid inbreeding species such as wheat, where linkage disequilibrium (LD) extends over long distances (Chao *et al.,* 2010), GWAS can have limited resolution. By contrast, large biparental populations can generate high resolution genetic maps and have been used effectively in wheat to map several genes (Uauy *et al.,* 2006; Fu *et al.,* 2009; Zhang *et al.,* 2017).

Approaches that combine GWAS and biparental populations (e.g., nested association-mapping populations, NAM) (Yu *et al.,* 2008) can bring together the best of both methods.

Due to the scarcity of Wheat dwarf virus (WDV) resistant wheat genotypes, combining genotyping and phenotyping, followed by an association analysis, will allow the identification of potential quantitative trait loci (QTL) for resistance (Pfrieme *et al.,* 2022). For example, the Wheat Infinium iSelect Bead chip offers thousands of single nucleotide polymorphism markers (SNPs) for genotyping (Wang *et al.,* 2014). GWAS has already been successfully applied to detect QTL for virus resistance in wheat and other cereals. For example, Soil-borne wheat mosaic virus (SBWMV) resistance in wheat (Liu *et al.,* 2014), Barley yellow dwarf virus (BYDV) resistance in maize (Horn *et al.,* 2014), BYDV resistance in oat (Foresman *et al.,* 2016), and Wheat spindle streak mosaic virus (WSSMV) in wheat (Hourcade *et al.,* 2019).

2. Hypothesis and objectives of this study

1. Marker trait associations (MTA) observed in an association panel can be validated in the biparental crosses of selected contrasting parents.

2. Identified salt stress-related traits found in an association panel distinguish salt-tolerant and salt-sensitive genotypes in crossbred offspring.

3. Differential gene expression of allelic variation at candidate gene loci under salt-stress and non-stress conditions modulates the salt-stress response in wheat.

The objectives of the thesis

The overall goal of this study was to dissect the genetic and the underlying molecular mechanisms of salt tolerance in wheat. The genetic dissection of the quantitative traits controlling the salt tolerance in wheat is a prerequisite to allowing for the application of cost-effective genomics-based approaches in breeding high- yielding wheat genotypes for saline conditions. In particular, the objectives were:

(i) A salt-sensitive parent was crossed to two different salt-tolerant lines, respectively, to characterize the salinity tolerance of F_3 lines of two connected biparental crosses, whereas we described the ionomic, biochemical, and physiological responses of salt-sensitive and salt-tolerant lines.

(ii) To validate the effects of salt- tolerant candidate genes identified in the prequel wheat study.

(iii)To analyze the gene expression of putative candidate genes for the salinity response of the lines.

3. Materials and Methods
3.1. Plant Material

The tested plant material consisted of 274 and 277 segregating F_3 lines arising from crosses between Bobur and Altay2000, and between Bobur and UZ-11CWA08, respectively. Bobur is the salt-sensitive parent, while Altay2000 and UZ-11CWA08 are the salt-tolerant parents. These three genotypes exhibited a consistent response to salt stress in three growth stages including germination, seedling, and mature field plant (Oyiga *et al.,* 2016). For the establishment of the segregating F_3 lines of winter wheat population, the initial cross was between Bobur and Altay2000, and Bobur and UZ-11CWA08 (**Figure 6**). The resulting F_1 plants (maternal) were selfed twice to get (F_3 generation). To ensure that pure seeds were used and to minimize heterogeneity and contamination, the multiplication step and cleaning were performed at the greenhouse of Crop Science and Resource Conservation Institute (INRES), University of Bonn, Germany. The harvested seeds were then used for the ST evaluation in the hydroponic system at the seedling stage. At the seedling growth stage, the salt tolerance indices (STI) of the F_3 lines were determined to identify contrasting lines (salt-tolerant and salt-sensitive). The contrasting lines for both segregating populations were selected using the ST rank consisting of the selected indices that include STI (stress tolerance index), SSI (stress susceptibility index), TOL (tolerance index), and SWL (shoot water loss).
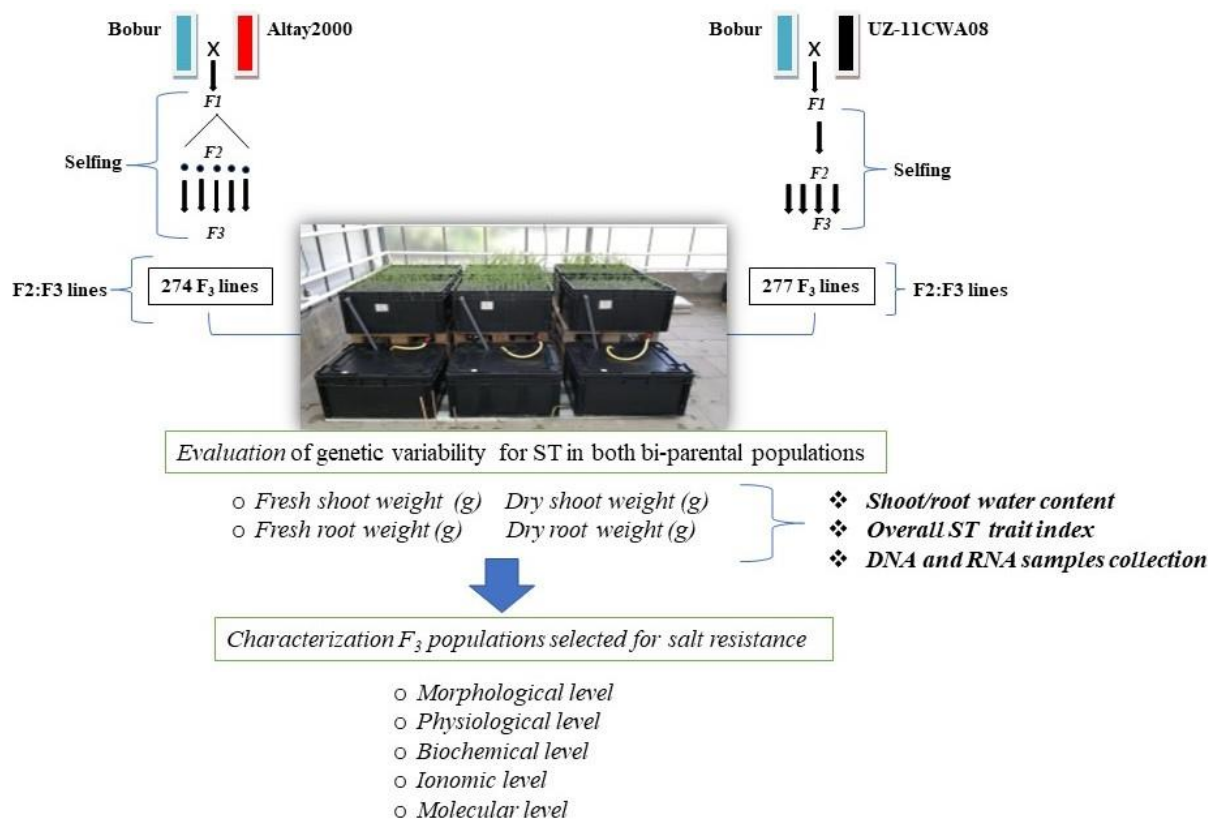
Figure 6. Setup of the F$_3$ lines from Bobur* Altay2000 and Bobur* UZ-11CWA08 crosses, as well as a schematic presentation of the working steps.

3.2. Hydroponic Experiment

The seedling stage screening was performed in a supported hydroponic system using the modified Hoagland solution as described by Tavakkoli *et al.* (2010) and summarized in **Table 1**. Two independent experiments, Experiment 1 (E1) and Experiment 2 (E2) using the two populations, were conducted. These experiments followed a randomized complete block design (RCBD), in which lines were assigned randomly within each hydroponic unit, with five replications for each line per each treatment combinations (control and salinity treatments). In E1 (which included 274 segregating F$_3$ lines of cross Bobur*Altay2000) (August–November 2019) and E2 (which included 277 segregating F$_3$ lines of cross Bobur* UZ-11CWA08) (December– March 2020), the lines were screened with non-saline (control) and saline (14.5 dS m^{-1}) nutrient solution. Supplementary Ca^{2+} as CaCl$_2$ was added to the saline nutrient solution in 20:1 molar ratio of NaCl (Haq *et al.,* 2010), to improve nutrient uptake and ameliorate the effects of salinity on the plant growth. In each replication, comparisons were made between saline and non-saline conditions. The electrical conductivity (EC) values for control and saline solutions ranged as follows for the E1 and E2 F$_3$ lines: 1.79–1.84, 14.24–15.44 and 1.79–1.84, 14.24–15.44 dS m^{-1}, respectively.

On each tub, 150 cylindrical PVC tubes (4.5 cm in diameter and 9.45 cm in depth) were placed and served by a separate tank containing 164 L of nutrient solution at 75-min intervals using an EHEIM Universal-pump 1046 (EHEIM GmbH and Co., Deizisau, Germany). Before the transfer into the hydroponic system, seeds were exposed to 40 °C for 24 h to remove the inherent differential dormancy. The seeds were sown and germinated in situ in the tubes filled with Aquagran filter quartz, 2–3.15 mm (Euroquarz GmbH, Dorsten, Germany) and tap water. Three days after planting (DAP), salt treatments were introduced together with the nutrient solution. The salt application was carried out on an equal incremental basis for 2 days to avoid osmotic shock. The stress was continued for 22 days after the final salt stress level was reached. The nutrient solutions were changed every 7 days, accompanied by an adjustment of the pH to 6.0 using a portable pH/ECmeter (Mettler Toledo SG2-FK SevenGO, Columbus, Ohio, United States) to measure pH and EC values every other day. HCl and NaOH were added to adjust the pH value. Thereafter, the solution pH was monitored daily and adjusted to 6.0. The nutrient solution temperature varied from 14.1 to 21.7 °C. The hydroponic boxes were placed during the testing period in the greenhouse with a 20°C day and 12°C night temperature, and a 12h/12h photo/dark period.

Figure 7. Hydroponic experiments were designed and supported for seedling screening under control (right) and salt (left) stress conditions.

Table 1. Composition of nutrient solutions for the hydroponic system

Chemical	Chemical formula	CAS number	Concentration	Supplier
Ammonium nitrate	NH_4NO_3	6484-52-2	0.2 mM / L	AppliChem GmbH
Potassium nitrate	KNO_3	7757-79-1	5 mM / L	AppliChem GmbH
Calcium Nitrate Tetrahydrate	$Ca(NO_3)_2.4H_2O$	10124-37-5	2 mM / L	AppliChem GmbH
Magnesium sulfate	$MgSO_4$	7487-88-9	2 mM / L	AppliChem GmbH
Potassium dihydrogen phosphate	KH_2PO_4	7778-77-0	0.1 mM / L	AppliChem GmbH
Di-Sodium Metasilicate	Na_2SiO_3	6834-92-0	0.5 mM / L	SIGMA-ALDRICH CHEMIE GmbH
Iron (III) monosodium salt	NaFe(III)- EDTA	15708-41-5	100 μM / L	Alfa Aesar GmbH
Boric acid	H_3BO_3	10043-35-3	12.5 μM / L	Pharmacia Biotech
Mangan chloride	$MnCl_2$	7773-01-5	2 μM / L	SIGMA-ALDRICH CHEMIE GmbH
Zinc sulfate	$ZnSO_4$	7733-02-0	3 μM / L	J.T.Baker Chemicals
Copper (II) sulfate	$CuSO_4$	7758-98-7	0.5 μM / L	AppliChem GmbH
Sodium molybdate	Na_2MoO_3	7631-95-0	0.1 μM / L	SIGMA-ALDRICH CHEMIE GmbH
Calcium chloride	$CaCl_2$	10043-52-4	5.75 mM / L	AppliChem GmbH

3.3. Data collection and estimation of salt tolerance indices

At harvest, 35 days after planting (DAP), the seedling shoot and root were separated and weighed to obtain the fresh shoot (SFW) and root weights (RFW). The harvested samples were dried at 60 °C for 4 days and weighed to obtain the shoot dry weight (SDW) and root dry weight (RDW).

Sharp *et al.* (1990) proposed a method for calculating the relative water content (RWC) of the shoot and root based on FW and DW in stress conditions (S) versus control conditions (C):

WL = [(FWC-DWC) - (FWS-DWS)]

where WL is water loss, FWC is fresh weight under control, DWC is dry weight under control, FWS is fresh weight under stress, and DWS is dry weight under stress.

3.3.1 Stress tolerance indices calculation

STI (Stress Tolerance Index) $= \dfrac{\text{Mean trait values stress}}{\text{Mean trait values control}}$ (Genc *et al.*, 2010).

SSI (Stress Susceptibility Index) $= \dfrac{1 - Ys/Yp}{1 - (\overline{Ys/Yp})}$ (Fischer and Maurer, 1978).

TOL (Tolerance Index) $= ((Yp - Ys)$ (Hossain *et al.,* 1990).

Mp (Mean Productivity) $= \dfrac{(Yp + Ys)}{2}$ (Hossain *et al.,* 1990).

GMP (Geometric Mean Productivity) $= \sqrt{(Ys x Yp)}$ (Fernandez, 1992).

Where $\mathbf{Ys}$ is the yield of a line under stress, $\mathbf{Yp}$ the yield of a line under non-stress condition, $\overline{\mathbf{Ys}}$ and $\overline{\mathbf{Yp}}$ are the mean yield of all lines under stress and non-stress conditions, respectively, and $\mathbf{1} - (\overline{\mathbf{Ys/\overline{Yp}}})$ is the stress intensity.

3.3.2 Lines ranking for salt tolerance

The lines were ranked for each of the salt tolerance indices, and the overall ST ranking for each genotype was calculated as follows:

$$\text{ST overall} = \sum_Z^M ST\ rankings$$

Where Z is the index of the ST estimates of lines for each measured trait, and M is the number of measured traits across replications. Lines with an extreme response to salt stress were identified as follows: tolerant (ST< 25th percentile) and sensitive (ST>75th percentile).

3.4 Ionic, biochemical, and physiological analyses in contracting wheat lines
3.4.1 Leaf Na^+ and K^+ content in contrasting wheat lines

To estimate Na^+ and K^+ content, leaf samples from the contrasting lines were oven-dried at 65°C for 3 days and ground for determination of mineral composition. Ash from leaf samples was dissolved in 5.1% HNO_3 and used to determine the Na^+ and K^+ contents with an atomic absorption spectrophotometer (Type 2380; Perkin Elmer, Wellesley, MA, USA), and then the K^+/Na^+ ratios were calculated.

3.4.2 Proline determination in contrasting wheat lines

Proline was measured from leaf samples using a microplate-based protocol adapted from Bates *et al.* (1973) (Ábrahám *et al.,* 2010). In short, leaves were homogenized in liquid nitrogen, and proline was extracted using 1 ml of 3% sulphosalicylic acid followed by centrifuging at 12,000 g for 5 minutes. The extract was incubated for 1 hour at 96°C with 2.5% ninhydrin and acetic acid at a 1:1:1 ratio. The reaction was stopped on ice, and the proline-ninhydrin reaction product was extracted with 1ml of toluene. The absorbance of a chromatophore containing toluene was measured at 520 nm using a microplate reader (TECAN Infinite 200 Pro, TECAN Group Limited, Switzerland). Leaf proline level was determined using a standard curve method and expressed as micrograms per gram of fresh weight.

3.4.3 Physiological analyses of contrasting wheat lines

The contrasting wheat lines were used to examine the effects of salt stress on some plant physiological and growth parameters, such as chlorophyll content using SPAD and chlorophyll a fluorescence (ChlF) using FluorPen FP100.

3.4.3.1 Plant chlorophyll contents index (CCi)

The chlorophyll content index (CCi) was measured using a portable SPAD-502 meter (Minolta, Osaka, Japan) (**Figure 8**). The leaf chlorophyll content index was measured from the

leaf tip to the leaf base of each line, and then the values were averaged between two different positions, including the leaf tip and leaf base. The age of the highly salt-tolerant and salt-sensitive wheat lines was 6 weeks under saline and non-saline conditions. The SPAD meter measures the chlorophyll absorbance in the red and near-infrared regions and calculates a numeric SPAD value that is proportional to the amount of chlorophyll in the leaf (Markwell *et al.,* 1995). SPAD values were determined for each plant using the third fully expanded leaf.

Figure 8. SPAD meter. SPAD-502 chlorophyll meter from Konica Minolta. Produced by Pham *et al.* (2019).

3.4.3.2 Chlorophyll a fluorescence (ChlF)

Chlorophyll-a fluorescence (ChlF) of leaf samples from 6-week-old highly salt-tolerant and salt-sensitive wheat lines under saline and non-saline conditions was measured with the FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic). The OJIP parameters were analyzed as follows: (i) fluorescence fast transients (F_o=fluorescence intensity at 50µs, F_j=fluo-rescence intensity at J-step (at 2 ms), F_i=fluorescence intensity at i-step (at 60 ms), F_m=maximal fluorescence intensity, F_v=maximal variable fluorescence); and (ii) PSII efficiencies (F_o/F_m=non-photochemical loss in PSII, F_v/F_o=efficiency of the water-splitting complex on the donor side of PSII, F_v/F_m=quantum yield of PSII, PI(ABS)=performance index on absorption). The light intensity reaching the leaf was 3000 mol (photons) $m^{-2}s^{-1}$, which was sufficient to generate maximal fluorescence.

Figure 9. FluorPen is a portable, battery-powered fluorometer that enables quick and precise measurement of chlorophyll fluorescence parameters in the laboratory, greenhouse, or field. It can be effectively used for studying photosynthetic activity, stress detection, herbicide testing, or mutant screening (Photon Systems Instruments, 2016).

3.5 Molecular analysis
3.5.1 Contrasting parents from the mapping populations

Bobur is the salt-sensitive parent, while Altay2000 and UZ-11CWA08 are the salt-tolerant parents, showing contrasting phenotypic traits at the seedling stage under salinity stress (Oyiga *et al.,* 2016). These genotypes were identified in an association mapping study that identified QTLs from salt stress-related traits measured at three developmental stages (Oyiga *et al.,* 2018).

3.5.2 DNA extraction

For the contrasting progenies, the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from dried plant tissues stored at room temperature following the manufacturer´s instructions. The quality of the extracted samples was checked by spectrophotometric analysis using nanodrop 2000 (Thermo Fisher, Rochester, USA) following the manufacturer´s instructions. The integrity of DNA was tested by applying 1% agarose gel electrophoresis at 100V for 30 min. Peqgreen (Peqlab, Fareham, UK) was utilized as a dye to visualize the nucleic acids and was added to melted agarose (4 – 6µl per 100 ml of agarose solution). In addition, a GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific) was loaded in the gel to estimate DNA size and approximate quantification. Easy visual tracking of DNA

migration during electrophoresis was possible by loading the gel with bromophenol blue (Thermo Fisher Scientific). Extracted DNA was stored at -20°C for later use.

3.5.3 MTAs (Markers Trait Association)

40 MTAs for salinity tolerance that have been identified (Oyiga *et al.,* 2018) were analyzed for their polymorphism between the groups of contrasting offspring. Specific primers around the associated SNP (single nucleotide polymorphism) were designed using the online program "Primer3" (http://primer3.wi.mit.edu/).

3.5.4 Amplification of DNA strands

The standard protocol for the polymerase chain reaction (PCR) was followed for amplification of DNA using locus-specific sequence primers. Thus, 100 ng of DNA template in 25 μl of 1x One Taq Standard Buffer (Biolabs, Ipswich, MA, USA), 0.2 mM dNTPs, and 0.2 μM of each primer were amplified with 0.5 units of One Taq DNA polymerase (Biolabs, Ipswich, MA, USA). Cycling conditions were established with an initial denaturation step at 95°C/2 min, followed by 40 cycles at 95 °C/45 s annealing temperature (Ta), extension at 72 °C/1 min per kbp, and a final extension step at 72°C/5 min.

3.5.5 Sequencing for DNA fragments

The amplified fragments were purified using the QIAquick PCR purification kit (Cat.No.:FG-91302, Company name : Nippon Genetics Co., Ltd.). The purified template had a concentration of 20 to 80 ng/μl and that of the sequencing primers was 5 μM (5 pmol/μl). Sanger sequencing was performed by GATC Biotech AG (Konstanz, Germany).

4.5.6 Computer-assisted sequence analysis

Web-based blast servers such as the National Center for Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi), ViroBLAST in Unité de Recherche Génomique Info (URGI, https://urgi.versailles.inra.fr/blast/blast.php) and EnsemblPlants genome annotation system (http://plants.ensembl.org/Triticum_aestivum/Tools/Blast?db=core) were used to find regions of similarity between the biological sequences of barley, rice, maize, Arabidopsis , and detect homoeologous chromosomal locations (Deng *et al.,* 2007, Johnson *et al.,* 2008, Kersey *et al.,* 2016). MAFFT (Multiple Alignment using Fast Fourier Transform, http://mafft.cbrc.jp/alignment/server/) was conducted for additional sequence alignments (Yamada *et al.,* 2016).

4.5.7 *In silico* expression analysis

The expression profiles of all the putative candidate genes associated with the identified SNPs were obtained from the published RNA-seq expression database for wheat in the WheatGmap web tool (https://www.wheatgmap.org; Zhang *et al.,* 2021).

4.5.8 RNA extraction and qRT-PCR analyses

4.5.8.1 Ribonucleic acid (RNA) extraction

Total RNA was extracted from the harvested leaf samples after 42 days in saline and non-saline conditions using the RNeasy Plant Mini Kit (Cat# 74903 and 74904). RNA isolation consists of three steps that include sample lysis/homogenization, sample clearing/genomic deoxyribonucleic acid (gDNA) removal, and RNA purification. All steps were performed at room temperature (20–30 °C). RNA quality and quantity were determined spectrophotometrically by analyzing the absorption ratios A260/230 and A260/280 using a Nanodrop 1000 spectrophotometer (6305 JENWAY spectrometer). RNA was stored at − 80 °C.

4.5.8.2 Complementary DNA (cDNA) synthesis

cDNA synthesis was done using the LunaScript RT SuperMix Kit (NEB #E3010) (New England BioLabs). The cDNA synthesis reaction was prepared by adding 4 μl of LunaScript RT SuperMix (1×) to 10 μl of the RNA sample and making up to 20 μl with 6 μl of nuclease-free water. The reactions were then incubated. The primers were allowed to anneal for 2 min at 25°C, followed by cDNA synthesis, which lasted for 10 min at 55 °C. Lastly, heat inactivation lasted for 1 minute at 95°C.

4.5.8.3 Quantitative PCR (qPCR)

qPCR was done using the Luna Universal qPCR Master Mix Kit (NEB #M3003). Dye-based qPCR detection was prepared using 10 μl of Luna Universal qPCR Master Mix (1×), 0.5 μl of 10 μM forward primer (0.25 μM), 0.5 μl of 10 μM reverse primer (0.25 μM) (table 2), 3 μl of diluted cDNA (1:10), and 6 μl of nuclease-free water. Thermal cycling conditions were initial denaturation (1 cycle) for 60 s at 95 °C, denaturation (40 cycles) for 15 s at 95 °C, and extension (40 cycles) for 30 s at 60 °C. The melt curve (1 cycle) was determined at 72 °C for 60 s. The gene primers were designed around the associated SNPs using Primer3 (http://primer3.wi.mit.edu/). The gene expression data were analyzed with the standard methods of Livak &Schmittgen (2001), normalized with two internal control genes, TaEf-1a and TaEf-1b (Unigene accession: Ta659). The PCR reaction efficiencies of target and internal control genes are comparable (Oyiga *et al*., 2018). Melting curves of the amplified PCR products were generated using the following program: 95 °C for 10 s, 60 °C for 30 s, and 95 °C for 15 s (Oyiga *et al*., 2018).

Table 2. Sequences of the primers used in the qRT-PCR. The corresponding amplified fragment sizes are shown. *ZIP7*, putative zinc transporter; *KefC*, glutathione-regulated potassium-efflux system protein; *AtABC8*, putative ABC transporter B family member 8; *6-SFT*, sucrose: sucrose 1-fructosytransferase (Oyiga *et al.,* 2018).

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Product size (bp)
ZIP7	TCTCATTCCACCAGTTCTTCG	GATGCCTTCAACCACTAGAGC	191
KeFC	AGCAAAACTTCCAATGTCCG	ATCAATGGTGTCGCTCTCGT	175
AtABC8	CAACAAGACCACAATGCCTG	TCTCCCTCACATCCATACCA	177
6-SFT	CGTGGAGGAGATTGAGACCC	GCAGAAGCATCAAGGTGGA	141
Internal control			
TaEf-1a	CTGGTGTCATCAAGCCTGGT	TCCTTCACGGCAACATTC	151
TaEf-1b	CAGATTGGCAACGGCTACG	CGGACAGCAAAACGACCAAG	227

3.6. Statistical Analysis

For analysis, firstly, an adjusted best linear unbiased estimate (BLUE) was calculated for each entry for all the different traits to correct for errors due to planting positions (row-and-column effects) in the hydroponic tubes by including "Replication/Row∗Column" which means that rows crossed with columns were nested within replication (Gilmour *et al.,* 1995). The adjusted phenotypic values were analyzed population-wise and thereafter combined for both populations in ANOVAs using PROC GLM (SAS version 9.4) according to the following models:

Population wise: $Yiklr = \mu + S_i + g_k + b_l(S_i) + eijlr$

Combined: $Yijklr = \mu + S_i + P_j + S_{*}Pij + g_k(P_j) + b_l(S_i) + eijklr$

where Y_{ijklr} is the adjusted phenotype (trait value) of the kth genotype of the jth population grown in the ith salt treatment in the lth block in the hydroponic system; μ is the general mean, S_i is the fixed effect of the ith salt treatment, P_j is the fixed effect of the jth population, $S_{*}P_{jk}$ is the fixed effect of the jth population grown under ith salt treatment (interaction), $g_k(P_j)$ is the random effect of the kth genotype of the (nested in) population P_j, $b_l(S_i)$ is the random effect of the lth block within ith salt treatment, and e_{ijklr} represents the error term. Fixed effects are denoted by uppercase letters, random effects are denoted by lowercase letters, interaction is indicated by "∗", and nesting is indicated by "()".

The variance components due to genotypic (σ^2_g) and error (σ^2_e) effects for each treatment were estimated based on the adjusted BLUE values using the REML option in PROC VARCOMP (SAS Institute, 2015). Furthermore, we calculated broad-sense heritability H^2 for each hydroponic treatment and population using Proc varcomp's REML option:

$H^2 = \sigma^2_g / [\sigma^2_g + (\sigma^2_e/r)]$,

where σ^2_g is the genotypic variance, σ^2_e is the residual error variance, and r is the number of replications. The heritability was categorized as low, moderate, and high as given by Robinson *et al.* (1949).

A Pearson correlation analysis of genotypic means was performed to assess the correlation between RWC and other quantitative indices of salt tolerance using the package *Performance Analytics*. The PCA for some salt tolerance indices and SWL was done by *Factominer* and *Factoextra*, which are also implemented in R software (R Core Team 2020). Using R software (R Core Team 2020), a histogram of the measured traits was analyzed to determine the distribution of the measured traits.

4. Results

To assess the phenotypic and genotypic variability of the F_3 lines of crosses Bobur*Altay2000 and Bobur*UZ-11CWA08 towards salinity stress, hydroponic experiments were carried out at the seedling stage. Subsequently, marker-trait association analysis was performed to detect chromosomal regions associated with the traits of interest and detect candidate genes.

The following section is divided into four subsections:

1. Phenotyping of 274 and 277 segregating F_3 lines arising from crosses Bobur*Altay2000 and Bobur* UZ-11CWA08, respectively, at the seedling stage.
2. Identifying salt-tolerant and salt-sensitive progenies in segregating populations.
3. Ionic, biochemical, and physiological characterization for contrasting lines for both crosses
4. Validation of candidate genes in both segregating populations and by expression analysis.

4.1. Phenotyping of 274 and 277 segregating F_3 lines arising from crosses Bobur*Altay2000 and Bobur* UZ-11CWA08 respectively, at the seedling stage

The lines (F_3 generation) were tested for shoot and root traits under two salt treatments in hydroponic conditions. The populations were significantly different from each other in the tested traits and showed significant interactions with the salt treatments (**Table 3**). The ANOVAs showed that the salinity treatment resulted in a significant reduction in trait scores in both populations. For instance, a reduction of 61.82% in SFW, 18.31% in SDW, 28.57% in RFW, and 6.41% in RDW were observed in the Bobur*Altay2000 population, while the Bobur*UZ-11CWA08 population showed a reduction of 51.53% in SFW, 16.21% in SDW, 35.71% in RFW, and 10.52% in RDW (**Table 4**). Except for RDW for genotype effects, there were highly significant genotype and genotype by salinity interaction effects in all tested traits in both populations (**Table 4**).

Table 3. F-values and significance levels of the combined ANOVA. The seedling stage traits in the hydroponic tests are analyzed for both populations together. F-values are shown; significance levels p: *** p ≤0.001; ns not significant; SOV source of variance; DF degrees of freedom; MS mean squares; SFW shoot fresh weight; SDW shoot dry weight; RFW root fresh weight; RDW root dry weight.

SOV	DF	SFW (F-Value)	SDW (F-Value)	RFW (F-Value)	RDW (F-Value)
Salt-Treatment (ST)	1	3181.97 ***	1887.43 ***	1760.77 ***	1350.48 ***
Population (P)	1	118.41 ***	140.15 ***	57.81 ***	128.08 ***
ST*P	1	136.96 ***	12.70 ***	64.04 ***	74.13 ***
Genotypes within Population (G(P))	549	1.38 ***	1.83 ***	2.35 ***	3.09 ***
ST*G(P)	543	3.10 ***	1.16 *	0.81 ns	0.48 ns
		MS	MS	MS	MS
Error	1648	1.235858	0.033956	0.674057	0.016304

Table 4. F-tests of the main effects of salinity and genotypes and their interactions for the measured traits at the seedling stage. F-values are shown; significance levels p: *** p ≤0.001; ns not significant; SOV source of variance; DF degrees of freedom; SFW shoot fresh weight; SDW shoot dry weight; RFW root fresh weight; RDW root dry weight.

SOV	DF	SFW	SDW	RFW	RDW
F_3 lines of cross Bobur*Altay2000					
Treatments (T)	1	12343.8***	2172.64***	8817.58***	2117.06***
Genotypes (G)	273	8.01***	2.57***	6.60***	1.36^{ns}
TxG	271	6.10***	1.94***	10.10***	1.68***
Error (Mean sq)	548	1.67	0.03	0.19	0.007
F_3 lines of cross Bobur*UZ-11CWA08					
Treatments (T)	1	11679.6***	5825.92***	1440.62***	1915.45***
Genotypes (G)	276	7.88***	2.29***	1.68***	1.09^{ns}
TxG	272	12.07***	9.86***	1.71***	3.64***
Error (Mean sq)	554	1.22	0.02	0.75	0.02

In both populations, the genotype values for the traits under control and salt stress conditions were normally distributed (**Figure 10**). The coefficients of variation (CV) for all traits were lower under salinity compared to salinity-free treatment in both populations and parents, except parents UZ-11CWA08 and Bobur (for SFW and SDW), for which the coefficients of variation (CV) were higher under saline conditions (**Tables 5 and 6**).

The H² was calculated on a population basis and showed higher values for the shoot traits than for the root traits. Comparing fresh and dry weight H^2 values, the fresh weight values were always higher (**Table 6**). A perusal of the results on heritability revealed low to moderate heritability estimates for all the measured traits.
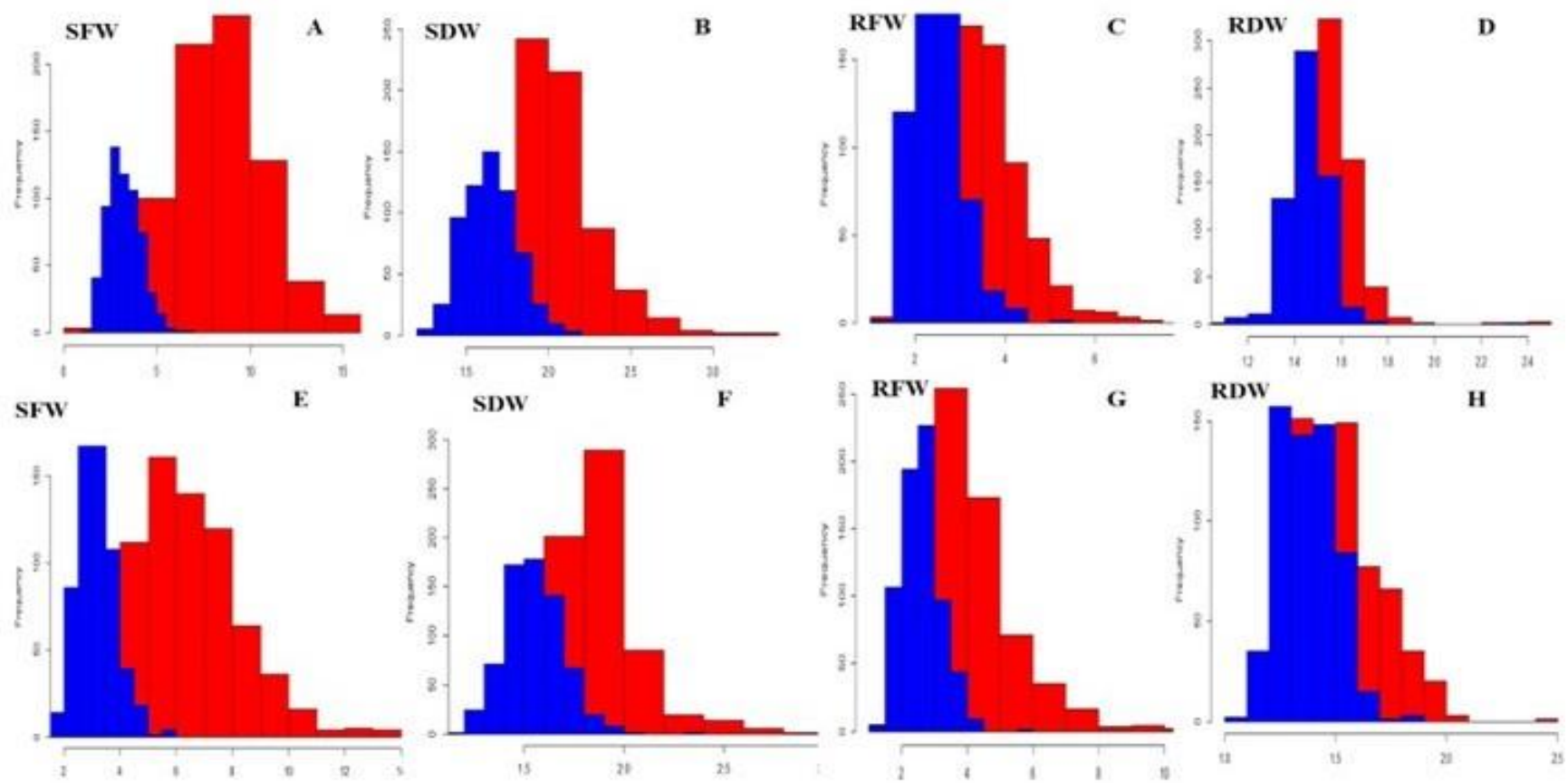
Figure 10. (A-D) Histogram analysis of the measured traits of the F₃ lines Bobur*Altay2000 under non-saline (Red) and saline (Blue) conditions, and **(E-H)** of the F₃ lines of cross Bobur*UZ-11CWA08.SFW shoot fresh weight; SDW shoot dry weight; RFW root fresh weight; RDW root dry weight.

Table 5. Descriptive statistics were used to assess the phenotypic traits of parents in two salinity treatments (T). C, Control; S Stress; SD Standard deviation; CV% Coefficient of Variation; SFW shoot fresh weight**;** SDW shoot dry weight**;** RFW root fresh weight**;** RDW root dry weight.

	Altay2000								UZ-11CWA08							
	SFW		SDW		RFW		RDW		SFW		SDW		RFW		RDW	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
SD	0.8	0.5	0.1	0.1	0.3	0.2	0.09	0.08	0.8	0.7	0.07	0.06	0.4	0.4	0.02	0.05
Mean	3.7	3.2	1.7	1.6	2.2	2.3	1.4	1.5	7.1	2.9	2.05	1.5	3.3	2.3	1.5	1.3
Min	2.8	2.8	1.6	1.5	1.9	2.1	1.4	1.4	6.3	2.1	1.9	1.4	2.9	1.7	1.5	1.3
Max	4.5	3.9	1.9	1.7	2.6	2.6	1.5	1.5	7.9	3.5	2.1	1.6	3.8	2.7	1.5	1.4
Range	1.6	1.07	0.3	0.2	0.6	0.5	0.1	0.1	1.6	1.4	0.1	0.1	0.8	0.9	0.04	0.1
Variance	0.7	0.3	0.02	0.01	0.1	0.07	0.009	0.006	0.6	0.5	0.005	0.004	0.1	0.2	0.0004	0.002
CV%	22.3	17.1	9.03	6.7	15.8	11.9	6.6	5.3	11.4	24.4	3.6	4.2	13.01	20.6	1.2	3.9

	Bobur							
	SFW		SDW		RFW		RDW	
	C	S	C	S	C	S	C	S
SD	0.03	0.4	0.08	0.095	0.4	0.2	0.05	0.04
Mean	7.6	2.7	2.2	1.5	2.6	2.1	1.4	1.4
Min	7.5	2.3	2.1	1.4	2.1	1.9	1.4	1.4
Max	7.6	3.2	2.3	1.6	3.08	2.4	1.5	1.4
Range	0.07	0.9	0.1	0.1	0.8	0.5	0.1	0.08
Variance	0.001	0.2	0.007	0.009	0.1	0.08	0.002	0.001
CV%	0.4	17.7	3.8	6.1	16.8	13.7	3.3	2.7

Table 6. Descriptive statistics were used to assess the phenotypic traits of F$_3$ lines from both populations in two salinity treatments (T). C, Control; S Stress; SD Standard deviation; CV% Coefficient of Variation; h^2% BS Broad-sense heritability; SFW shoot fresh weight**;** SDW shoot dry weight**;** RFW root fresh weight**;** RDW root dry weight.

	F$_3$ lines of cross Bobur*Altay2000								F$_3$ lines of cross Bobur* UZ-11CWA08							
	SFW		SDW		RFW		RDW		SFW		SDW		RFW		RDW	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
SD	2.3	0.8	0.2	0.1	0.8	0.55	0.11	0.09	1.8	0.6	0.2	0.1	1.3	0.5	0.1	0.1
Mean	8.4	3.2	2.02	1.6	3.5	2.50	1.56	1.4	6.5	3.1	1.8	1.5	4.06	2.6	1.5	1.3
Min	1.5	1.4	1.3	1.2	1.2	1.37	1.17	1	2.2	1.8	1.2	1.1	1.7	1.05	1.0	1.04
Max	17.2	6.6	3.3	2.1	8.01	5.38	2.49	2.3	13.5	5.8	3.1	2.3	10.9	5.7	2.4	1.88
Range	15.6	5.1	2.07	0.9	6.7	4.01	1.32	1.3	11.3	4	1.9	1.1	9.2	4.6	1.3	0.84
Variance	5.7	0.7	0.07	0.02	0.7	0.30	0.01	0.008	3.4	0.4	0.05	0.02	1.7	0.3	0.03	0.01
CV%	28.3	27.07	13.1	9.6	24.7	22.16	7.34	6.2	28.3	20.5	12.2	9.2	32.4	22.4	12.1	9.2
h^2% BS	51.90		40.84		27.54		26.08		34.50		26.30		19.02		12.32	

4.2. Identifying salt-tolerant and salt-sensitive progenies in the segregating populations

To classify the progenies of the populations into salt-tolerant and sensitive lines, several indices were calculated from the relationships between the stress and control treatments. To identify the index that describes shoot water loss (SWL) (Oyiga *et al.,* 2016), the correlation between the respective index and the SWL was calculated (**Figures 11A, 12A**). TOL and SSI had highly significant correlations with SWL, whereas STI had a strong negative correlation with SWL. The biplot plot showed that the SSI and TOL indices were clustered together with SWL, while the STI index was opposite them. The MP and GMP clustered together but gave different information compared to SWL (**Figure 11B** for Bobur*Altay2000 and **Figure 12B** for Bobur*UZ-11CWA08). MP and GMP were neither highly correlated with SWL nor with each other. Consequently, STI, TOL, and SSI were the most appropriate indices among all evaluated indices, including SWL, to rank the progeny.

Using the selected indices simultaneously, including SWL, and based on the entire ST rank list (**Tables 7and 8**) (see details in **Appendix 1, Appendix 2, Appendix 3, and Appendix 4**), 52, 84, 83, and 51 lines were categorized as tolerant, moderately tolerant, moderately sensitive, and sensitive to salt stress for the 274 F_3 lines of the cross Bobur*Altay2000, and 49, 84, 84, and 45 lines were categorized as tolerant, moderately tolerant, moderately sensitive, and sensitive to salt stress for the 277 F_3 lines of the cross Bobur*UZ-11CWA08. Mean ST estimates ranged from 13.14 and 10.97 in salt-tolerant lines to 84.28 and 83.64 in salt-sensitive lines for lines 274 and 277 F_3 of the Bobur*Altay2000 and Bobur*UZ-11CWA08 cross, while the overall mean was 49.53 and 48.55, respectively (**Figures 13A, 13B**).

The ranking results showed that the contrasting lines were four (salt-tolerant lines) versus two (salt-sensitive lines) and two (salt-tolerant lines) versus two (salt-sensitive lines) for F_3 lines of cross Bobur*Altay2000 and F_3 lines of cross Bobur*UZ-11CWA08, respectively. These lines showed a consistent response to salt stress in five replications under a hydroponic system. These lines were P1G082, P1G119, P1G202, and P1G264 (salt-tolerant) and P1G132 and P1G253 (salt-sensitive) for the F_3 lines of the Bobur*Altay2000 cross, while lines were P2G076 and P2G243 (salt-tolerant) and P2G027 and P2G162 (salt-sensitive) in the contrasting F_3 lines of the Bobur*UZ-11CWA08 cross (**Appendix 3 and Appendix 4**).
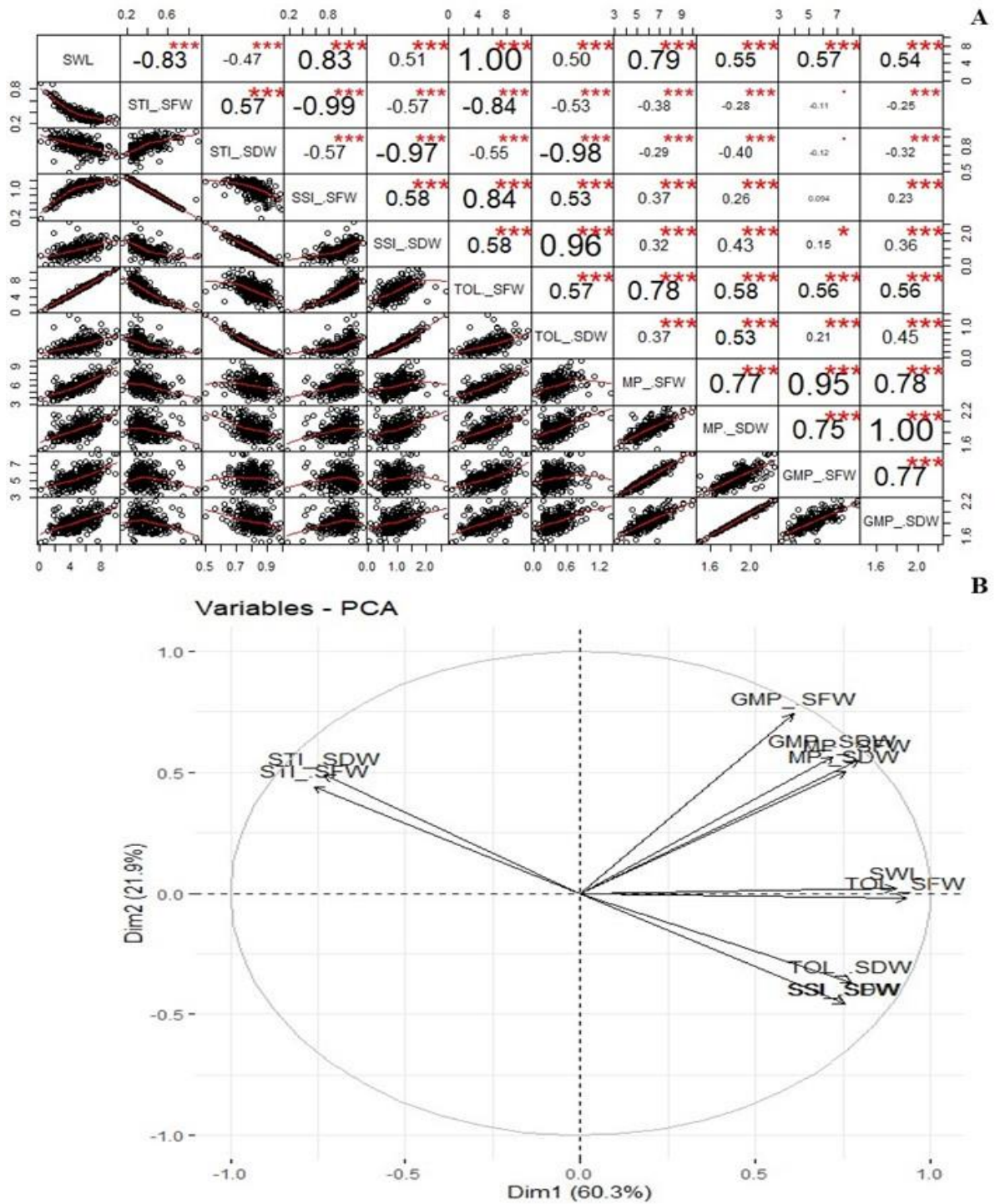
Figure 11. (A) Simple correlation coefficients between SWL and some salt tolerance indices of Bobur*Altay2000 F_3 lines. **(B)** Graphical representation of a biplot for some salt tolerance indices and SWL based on 274 F_3 cross Bobur*Altay2000 lines. significance levels p: * p ≤ 0.05; *** p ≤0.001; ns not significant. SWL shoot water loss; STI_SFW stress tolerance index for shoot fresh weight; STI_SDW stress tolerance index for shoot dry weight; SSI_SFW stress susceptibility index for shoot fresh weight; SSI_SDW stress susceptibility index for shoot dry weight; TOL_SFW tolerance index for shoot fresh weight; TOL_SDW tolerance index for shoot dry weight; MP_SFW mean productivity for shoot fresh weight; MP_SDW mean productivity for shoot dry weight; GMP_SFW geometric mean productivity for shoot fresh weight; GMP_SDW geometric mean productivity for shoot dry weight.
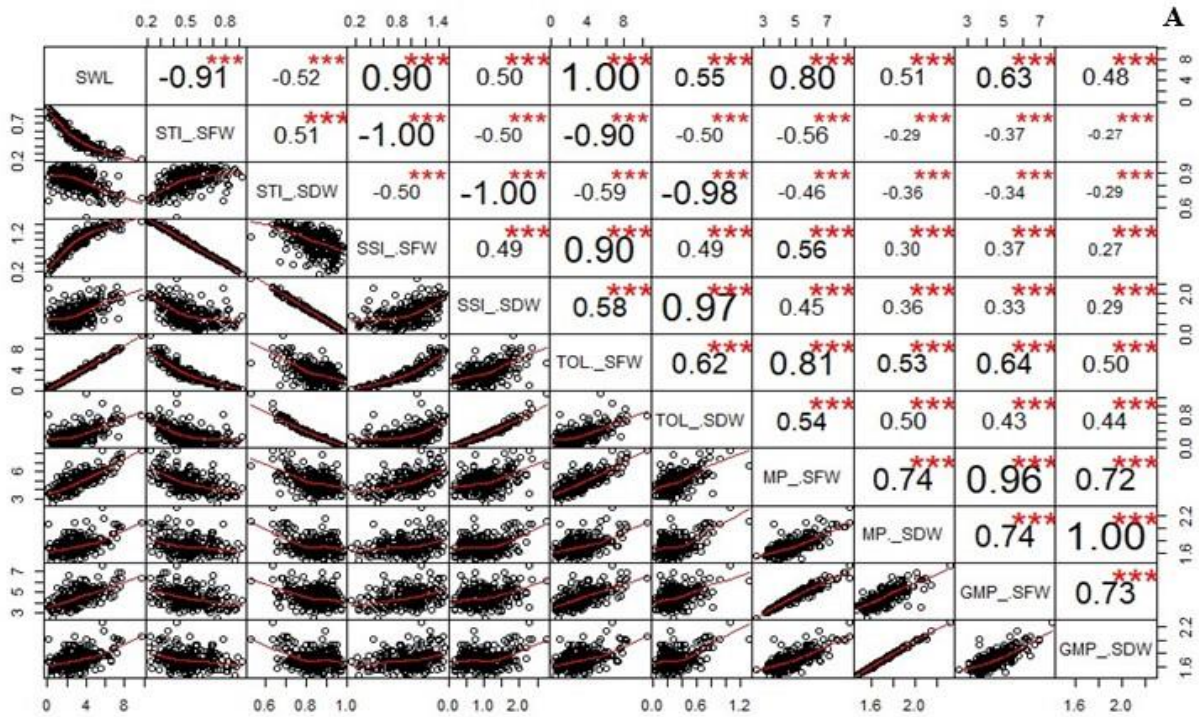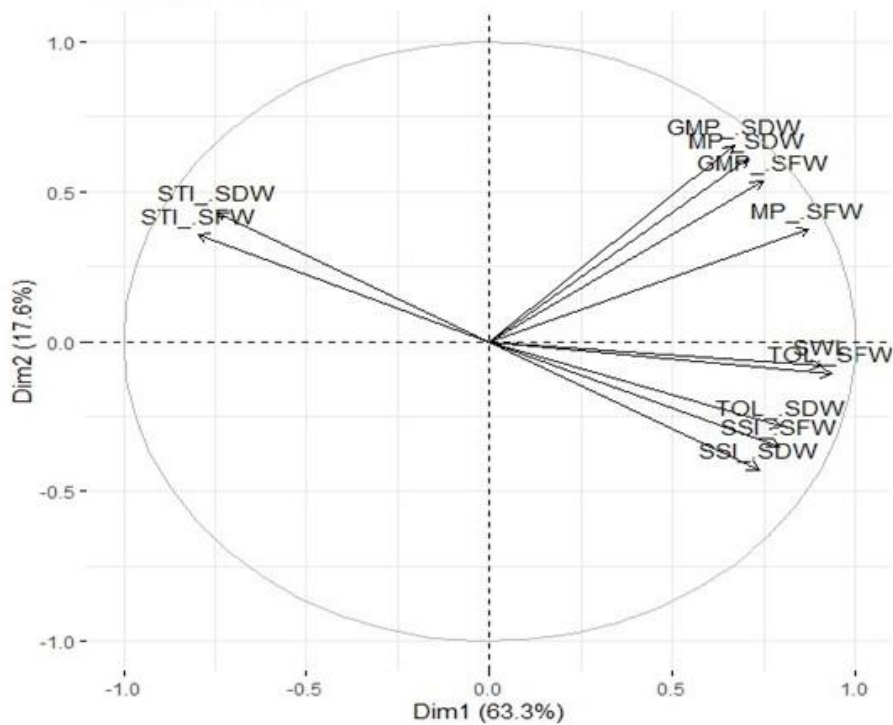
Figure 12. (A) Simple correlation coefficients between SWL and some salt tolerance indices of Bobur*UZ-11CWA08 lines. **(B)** Graphical representation of a biplot for some salt tolerance indices and SWL based on 277 F₃ cross Bobur*UZ-11CWA08 lines. significance levels p: *** p ≤0.001. SWL shoot water loss; STI_SFW stress tolerance index for shoot fresh weight; STI_SDW stress tolerance index for shoot dry weight; SSI_SFW stress susceptibility index for shoot fresh weight; SSI_SDW stress susceptibility index for shoot dry weight; TOL_SFW tolerance index for shoot fresh weight; TOL_SDW tolerance index for shoot dry weight; MP_SFW mean productivity for shoot fresh weight; MP_SDW mean productivity for shoot dry weight; GMP_SFW geometric mean productivity for shoot fresh weight; GMP_SDW geometric mean productivity for shoot dry weight.
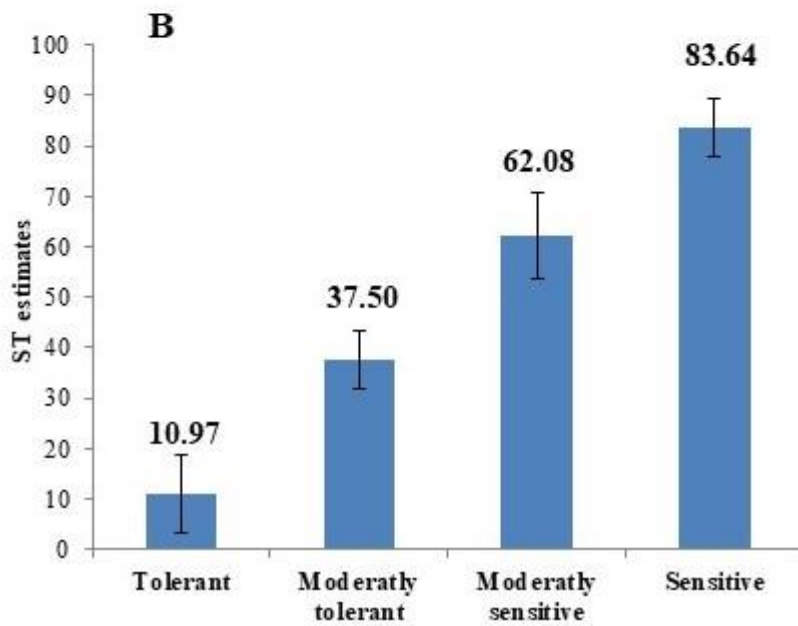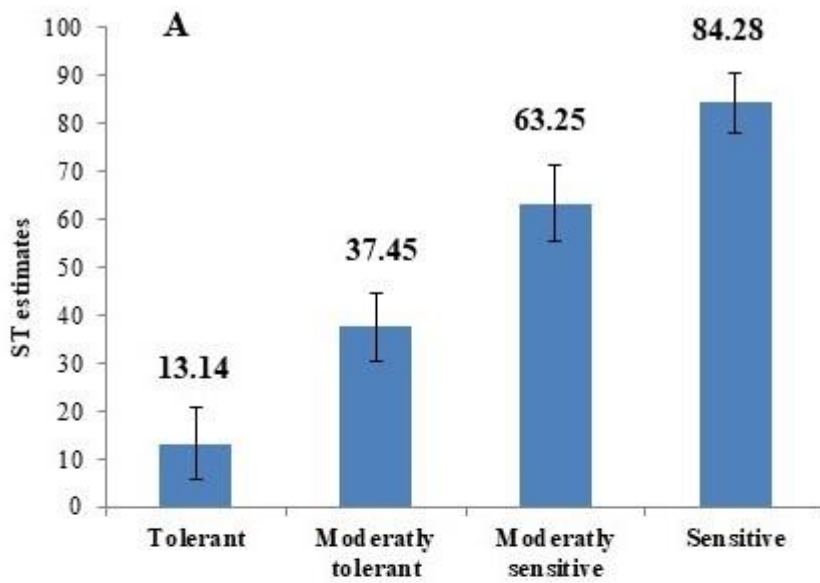
Figure 13. (A) Using the ST rankings, illustrated the representation of Bobur*Altay 2000 F_3 lines.ST status of all 274 genotypes. (**B)** Using ST rankings, illustrated the representation of Bobur*UZ-11CWA08 F_3 lines.ST status of all 277 genotypes.

4.3 Ionic, biochemical and physiological characterization for contrasting lines for both cross Bobur*Altay2000 and Bobur* UZ-11CWA08

4.3.1 Salt stress response of leaf ionic traits in contrasting lines

To investigate the effect of salt stress on different ion accumulations in contrast lines, Na^+ and K^+ contents in wheat leaves were determined under control and salt stress conditions. Significant differences in ion accumulation were evident in wheat lines after exposure to salt stress as compared to control conditions (**Figure 14**; **Table 7**). The application of salt stress led to a significant increase in Na^+ as well as a decrease in the K^+ contents of leaves in wheat lines (**Figure 14**). We found that leaf Na^+ content varied depending on the line. Bobur has higher leaf Na^+ contents (10.18) than Altay2000 (5.61) and UZ-11CWA08 (4.93) (**Figure 14A**). Analysis of the Na^+ and K^+ contents of the selected lines revealed that the salt-tolerant lines have higher K^+ and lower Na^+ contents when compared with the salt-sensitive lines (**Figures 14B, C,** and **Figures 14E, F**). For example, in F_3 lines of the cross Bobur*Altay2000, Na^+ content increased in the leaf with increasing salinity, with higher contents in P1G132 and P1G253 (salt-sensitive) (9.1 and 9.9, respectively), and lower contents in P1G082, P1G119, P1G202, and P1G264 (salt-tolerant) (4.76, 8.63, 4.86, and 7.70, respectively) (**Figure 14B**). While in F_3 lines of Bobur*UZ-11CWA08, higher contents of leaf Na^+ were noted in P2G027 and P2G162 (salt-sensitive) (4.63 and 5.20, respectively), while lower Na^+ contents were noted in P2G076 and P2G243 (salt-tolerant) (4.53 and 4.12, respectively) (Figure **14C**).
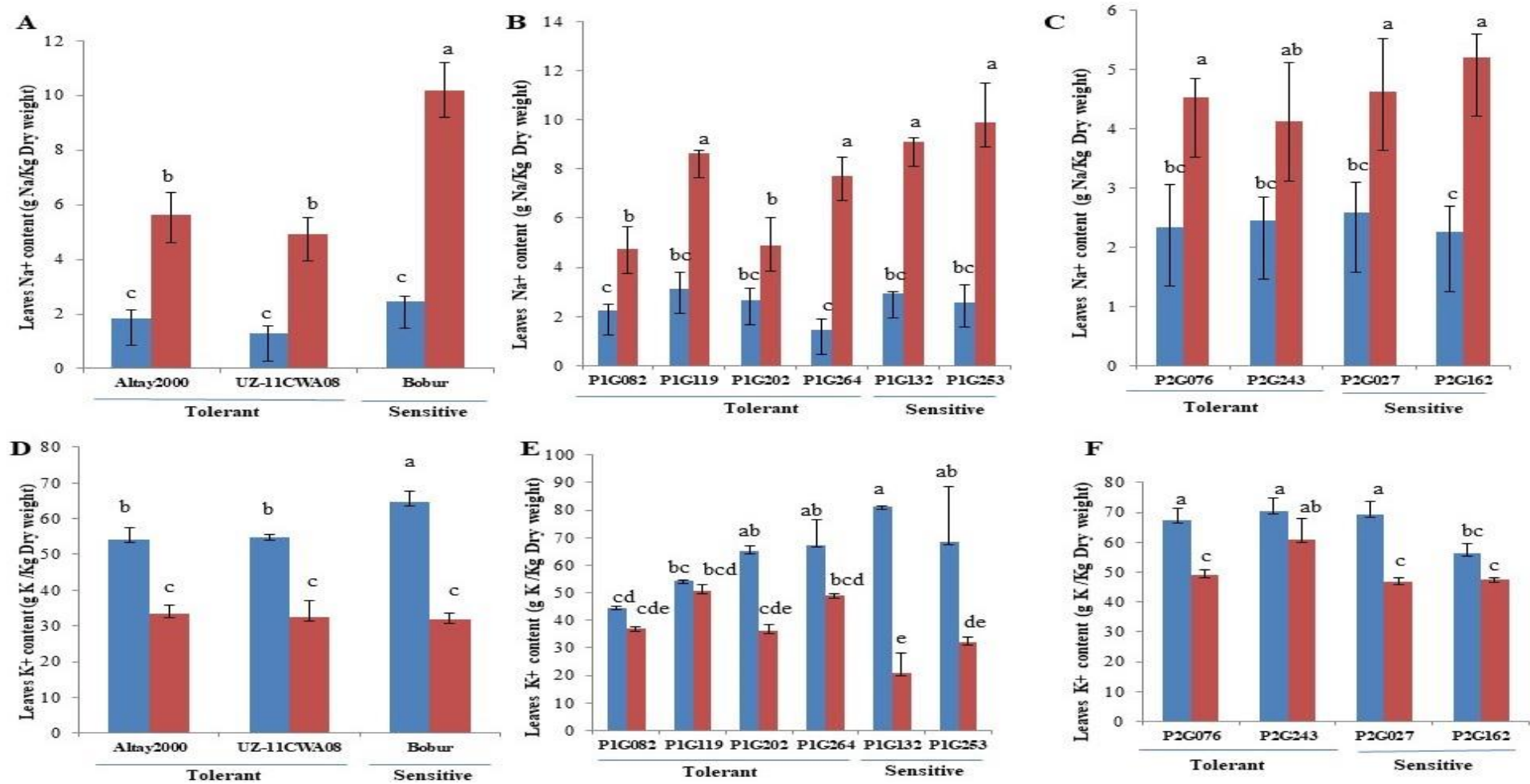
Figure 14. Effect of salinity on leaf Na$^+$ and K$^+$ content. (**A**), (**D**) Parents. (**B**), (**E**) Contrasting F$_3$ lines of cross Bobur*Altay2000, (**C**), (**F**) of cross Bobur* UZ-11CWA08. Means with the same letter are not significantly different from each other. non-saline (blue) and saline (red) conditions.

Table 7. F-values and significance levels of the two-way ANOVA. The seedling stage traits in the hydroponic tests are analyzed for contrasting genotypes of both segregating populations and parents. F-values are shown; significance levels p: * $p \le 0.05$; ** $p \le 0.01$; *** $p \le 0.001$; ns not significant; SOV source of variance; DF degrees of freedom; Chl. Content chlorophyll content; MS mean squares.

		Parents						Contrasting lines of F_3 lines of cross Bobur*Altay2000				
SOV	DF	Na^+ content	K^+ content	proline accumulation	Chl. Content	Fixed area	DF	Na^+ content	K^+ content	proline accumulation	Chl. Content	Fixed area
Genotypes (G)	2	45.84***	4.356*	0.532 ns	1.433 ns	0.722 ns	5	12.605***	4.099**	8.811***	0.578 ns	1.497 ns
Salt-Treatment (ST)	1	299.37***	331.66***	27.852***	45.33***	15.88**	1	324.704***	132.712***	62.889***	11.202**	16.254***
G*ST	2	21.06***	7.144**	13.035***	3.896*	2.066 ns	5	9.875***	14.235***	15.908***	0.266 ns	0.985 ns
		MS	MS	MS	MS	MS		MS	MS	MS	MS	MS
Error	12	0.38	8.8	8.01	0.00595	7.867e+12	24	0.69	46	13.1	0.0297	5.319e+12

		Contrasting lines of F_3 lines of cross Bobur*UZ-11CWA08				
SOV	DF	Na^+ content	K^+ content	proline accumulation	Chl. Content	Fixed area
Genotypes (G)	3	0.562 ns	12.54***	172.6***	4.244*	1.568 ns
Salt-Treatment (ST)	1	74.43***	87.72***	795.2***	95.45***	60.495***
G*ST	3	1.122 ns	4.673*	309.7***	13.40***	0.587 ns
		MS	MS	MS	MS	MS
Error	16	0.395	14.8	22	0.00324	2.891e+12

A reduction in cellular K^+ contents in leaves of wheat lines was recorded with increasing levels of salt stress, while K^+ contents were higher in the salt-tolerant lines than in the salt-sensitive lines under salt stress (**Figures 14D-F**). We found initially that K^+ contents in leaves decreased with an increase in salinity, and reductions in salt-tolerant parents Altay2000 and UZ-11CWA08 were 38.45% and 41.23%, respectively, while the magnitude of this reduction in the salt-sensitive parent Bobur was 50.91% compared to the plants under control conditions (**Figure 14D**). Lower K^+ contents were found in P1G132 (20.82) and P1G253 (32.17) (salt-sensitive), while higher K^+ contents were noted in P1G082 (36.95), P1G119 (50.59), P1G202 (36.13), and P1G264 (48.81) (salt-tolerant) (**Figure 14E**), while lines P2G027 (46.85) and P2G162 (47.72) (salt-sensitive) had lower K^+ contents compared to the tolerant lines P2G076 (49.01) and P2G243 (60.9) (**Figure 14F**). The K^+/Na^+ ratio was significantly affected by salinity. Increasing the Na^+ content led to a decrease in the K^+/Na^+ ratio in all wheat lines (**Figure 15**). However, salt-tolerant lines, including the salt-tolerant parents, showed a minimum reduction in the K^+/Na^+ ratio compared to the sensitive ones (**Figure 15**).
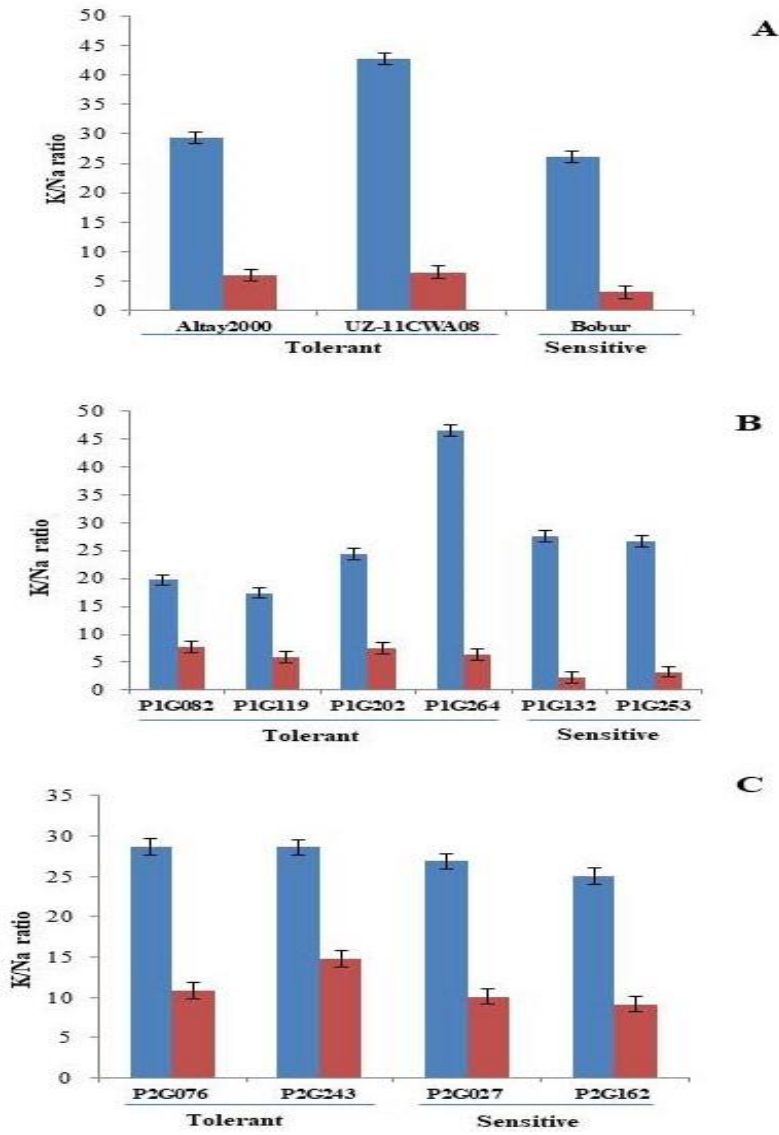
Figure 15. Effect of salt stress on the Na$^+$/K$^+$ ratio. Parents **(A)**. Contrasting F$_3$ lines of cross Bobur*Altay2000 **(B)** and of cross Bobur* UZ-11CWA08 **(C)**. non-saline (blue) and saline (red) conditions.

4.3.2. Biochemical modulations under salt stress

Biochemical analysis of leaves of different wheat lines for proline accumulation showed that proline accumulation increased significantly under saline conditions (**Figure 16**; **Table 7**). Compared to the salt-tolerant lines, the salt-sensitive lines showed a higher accumulation of proline content (**Figure 16**). In the parents, the highest percentage increase relative to that under control conditions was observed in Bobur, in which proline concentration increased by approximately 312.14%, and the lowest increase was observed in Altay2000, in which it increased by 203.24% under salt stress (**Figure 16A**).

Regarding the F_3 lines of Bobur*Altay2000, the highest and lowest percentage increases in proline accumulation relative to that under control conditions were found in P1G253 (the salt-sensitive line, 112.73%) and P1G119 (the salt-tolerant line, 4.48%), respectively (**Figure 16B**). In the F_3 lines of Bobur*UZ-11CWA08, P2G162 (the salt-sensitive line) had the highest increase in the percentage of proline accumulation relative to that under control conditions (927%), and the lowest increase observed in P2G243 (the salt-tolerant line) was 16.83% (**Figure 16C**). Interestingly, we found that the pattern of proline accumulation in the parents and both populations show the same behavior in salt-tolerant and salt-sensitive lines (**Figure 16**).
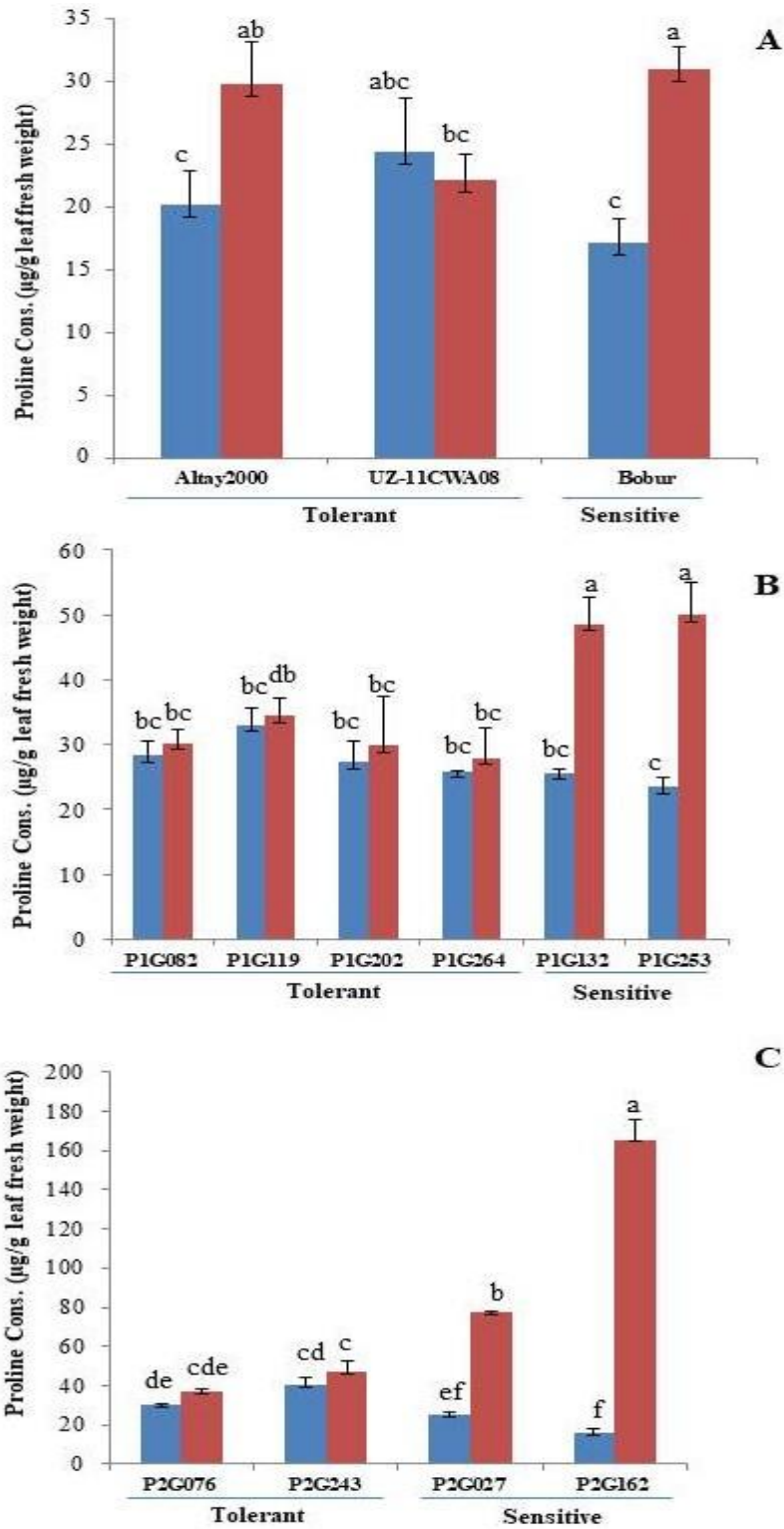
Figure 16. Effects of salinity on proline content. Parents(**A**). Contrasting F₃ lines of cross Bobur*Altay2000 (**B**) and of cross Bobur* UZ-11CWA08(**C**). non-saline (blue) and saline (red) conditions.

4.3.3. Physiological modulations under salt stress

To analyze the salt-induced physiological changes in the contrasting lines in the segregating populations and their parents, chlorophyll (Chl) content and chlorophyll fluorescence were measured in these lines after exposure to salt treatment. It has been found that the percentage reduction in total Chl content was greater at the higher salinity levels compared to the control, whereas the data obtained from the measurements show that the Chl content was consistently higher in the control treatment than in the saline treatment. The Chl content of the leaves decreased with salinity (**Figures 17A-C**). The results showed that the Chl content of salt-tolerant lines decreased slightly under salt conditions (Altay2000 (28.57%), UZ-11CWA08 (36%), P1G082 (23.52%), P1G119 (27.11%), P1G202 (22.41%), P1G264 (28.30%), P2G076 (18.75%), and P2G243 (15.09%). When compared to tolerant lines, salt-sensitive lines (Bobur (58.20%) and P1G132 (43.10%), P1G253 (47.69%) and P2G027 (48.52%), and P2G162 (55.26%) showed the greatest decrease (**Figures 17A-C**).
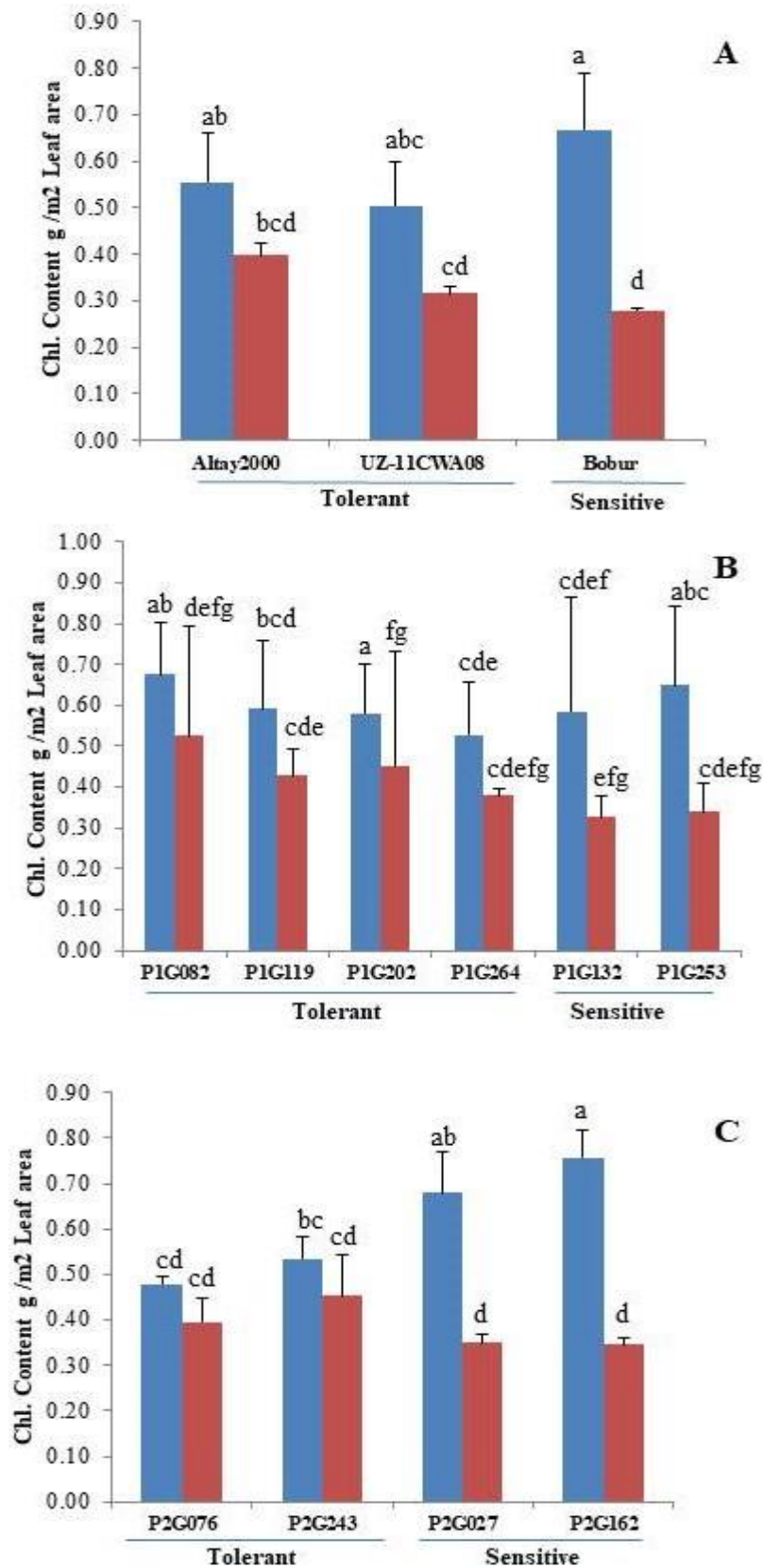
Figure 17. Effects of salinity on chlorophyll content. Parents (**A**). Contrasting F$_3$ lines of cross Bobur*Altay2000 (**B**) and of cross Bobur* UZ-11CWA08 (**C**). non-saline (blue) and saline (red) conditions.

The effects of salinity on the shape of the chlorophyll fluorescence transition curve are shown in **Figures 18-20**. Salt stress significantly inhibited fluorescence transients across all OJIP phases. The transient fluorescence curve of the lines showed a slight decrease in J and I steps compared to the control group. Calculated parameters from chlorophyll-a fluorescence are presented in **Table 8**. Under salt stress, Fv/Fm, Fo/Fm, and Fv/Fo decreased in salt-tolerant lines and increased in salt-sensitive lines (**Table S7**). The fixed area estimates decreased in all lines under salt stress, but the decrease was much greater in salt-sensitive lines than in salt-tolerant lines (**Figures 18C, 19E**, and **20C**).
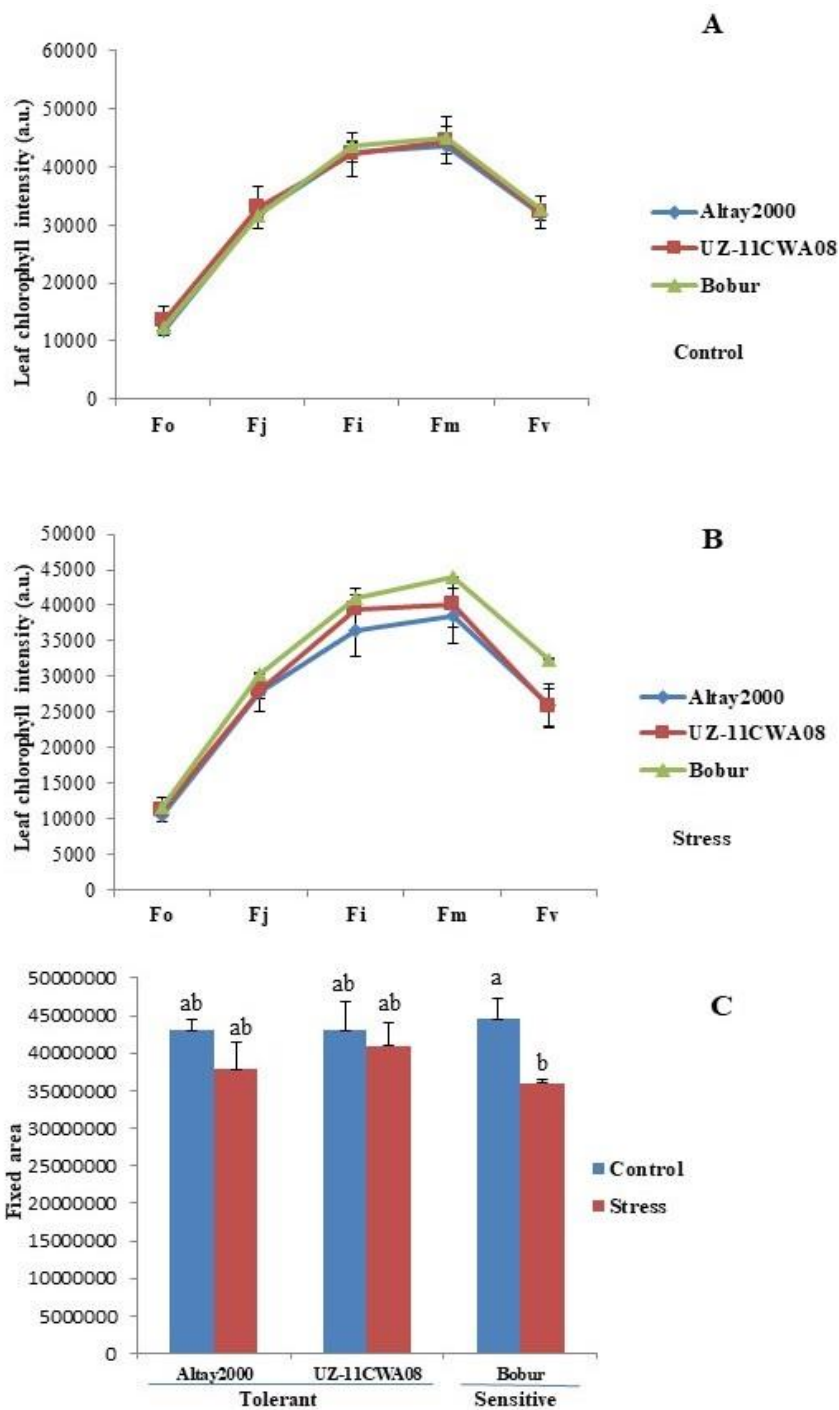
Figure 18. Effect of salt stress on the chlorophyll a fluorescence and OJIP test parameters of light-adapted leaves of two salt-tolerant (Altay2000and UZ-11CWA-8) and salt-sensitive wheat genotype (Bobur) identified in this study. **(A)** and **(B)** Chlorophyll a fluorescence kinetics curve under control and stress conditions, respectively (Fo = fluorescence intensity at 50 µs; Fj = fluorescence intensity at J-step (at 2 ms); Fi = fluorescence intensity at i-step (at 60 ms); Fm = maximal fluorescence intensity; Fv = maximal variable fluorescence). **(C)** Fix area representing the area above the chlorophyll fluorescence curve between Fo and Fm (size of the plastoquinone pool). The letters on the error bars represent what a genotype comparison means under control and salt stress conditions. Means with the same letter are not significantly different from each other.
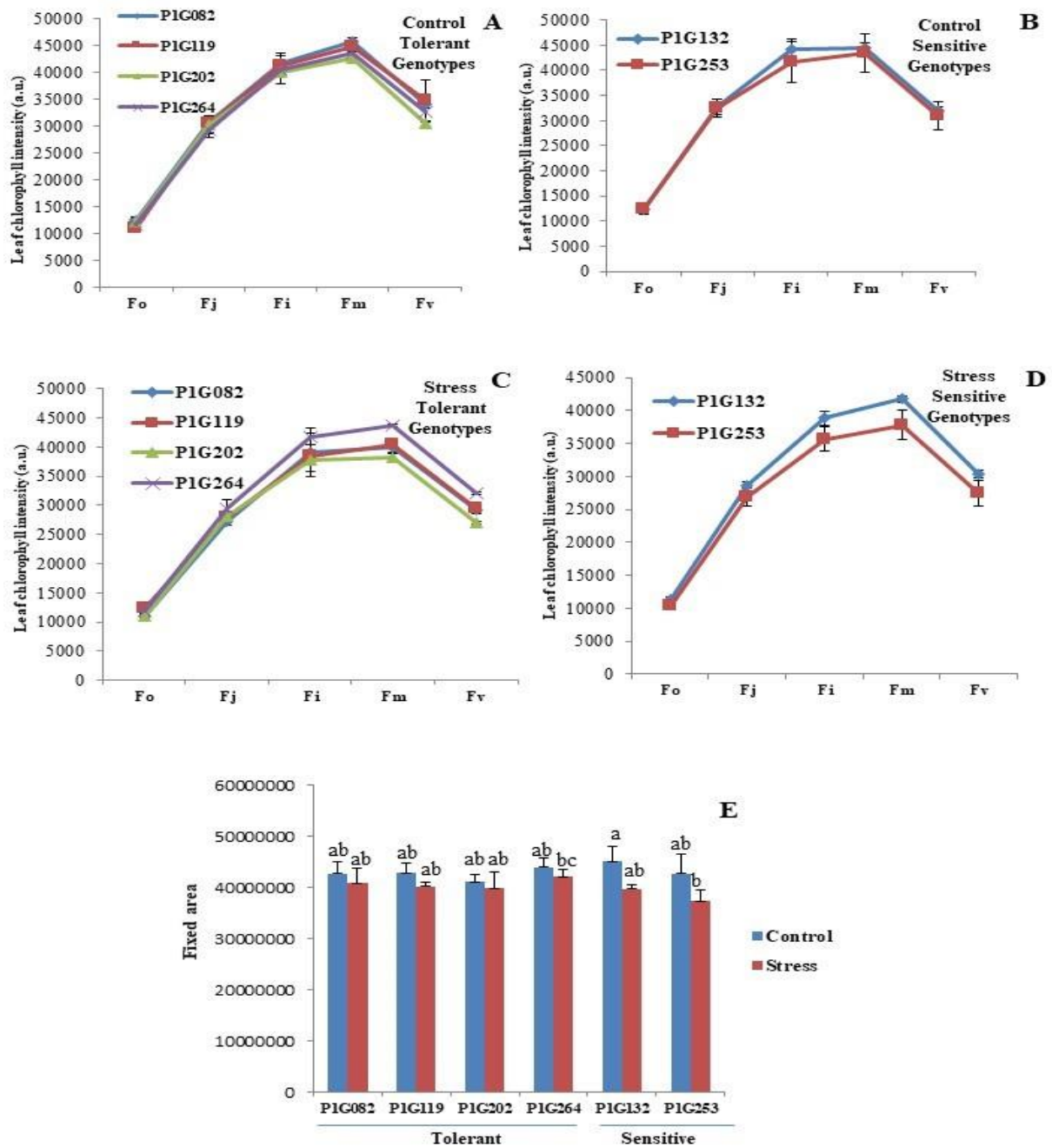
Figure 19. This study determined the effect of salt stress on the chlorophyll a fluorescence and OJIP test parameters of light-adapted leaves from contrasting F_3 lines of the cross Bobur*Altay2000. **(A)** Salt-tolerant genotypes under controlled conditions. **(B)** Salt-sensitive genotypes under control conditions, **(C)** Salt- tolerant genotypes under stress conditions. **(D)** Salt-sensitive genotypes under stress conditions Chlorophyll a has fluorescence kinetics curve (Fo = fluorescence intensity at 50 µs; Fj = fluorescence intensity at J-step (at 2 ms); Fi = fluorescence intensity at i-step (at 60 ms); Fm = maximal fluorescence intensity; Fv = maximal variable fluorescence). **(E)** Fix area representing the area above the chlorophyll fluorescence curve between Fo and Fm (size of the plastoquinone pool). The letters on the error bars represent what a genotype comparison means under control and salt stress conditions. Means with the same letter are not significantly different from each other.
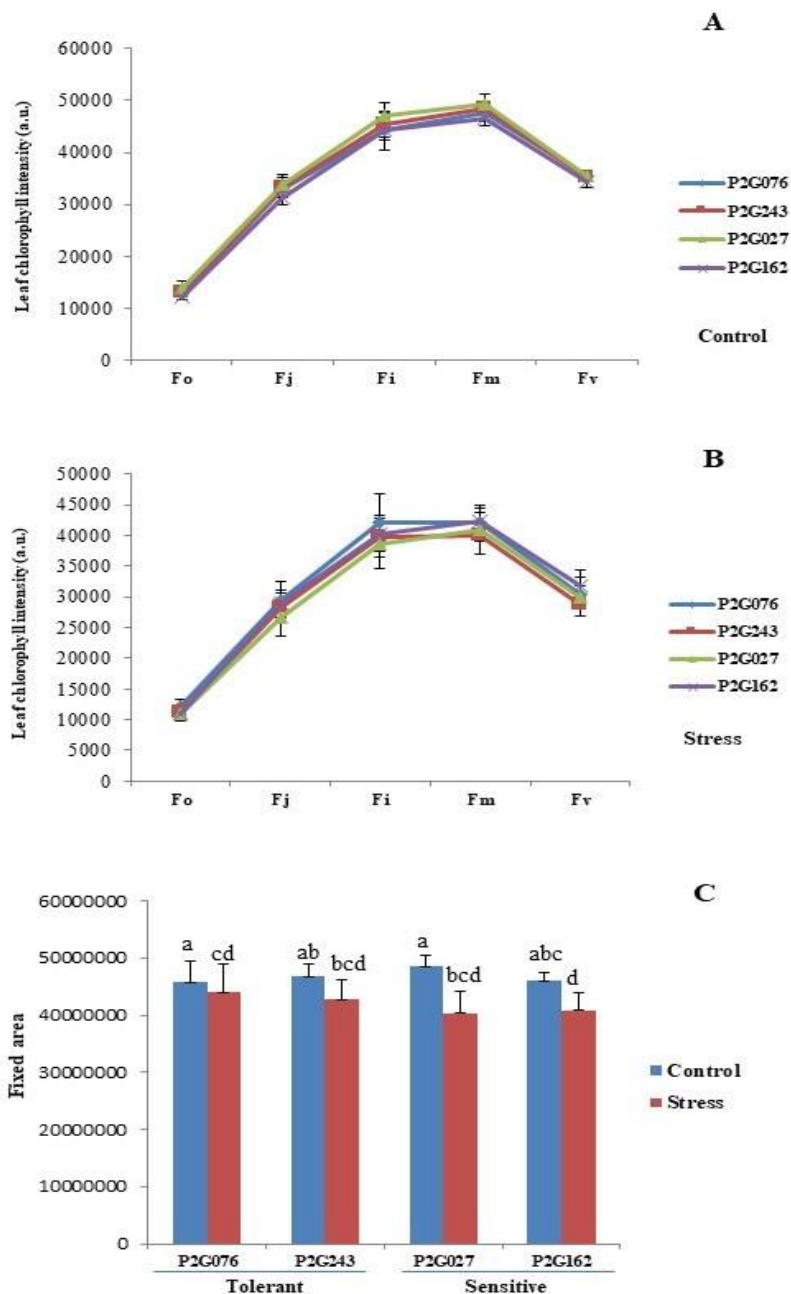
Figure 20. This study determined the effect of salt stress on the chlorophyll a fluorescence and OJIP test parameters of light-adapted leaves from contrasting F_3 lines of the cross Bobur*UZ-11CWA08. (**A**) and (**B**) Chlorophyll a fluorescence kinetics curve under control and stress conditions, respectively (Fo = fluorescence intensity at 50 µs; Fj = fluorescence intensity at J-step (at 2 ms); Fi = fluorescence intensity at i-step (at 60 ms); Fm = maximal fluorescence intensity; Fv = maximal variable fluorescence). (**C**) Fix area representing the area above the chlorophyll fluorescence curve between Fo and Fm (size of the plastoquinone pool). The letters on the error bars represent what a genotype comparison means under control and salt stress conditions. Means with the same letter are not significantly different from each other.

Table 8. Effect of salt stress on some energy fluxes of contrasting wheat genotypes.

Energy fluxes	Genotypes	Control	Stress	Effect of salt (%)
Fm/Fo	Altay2000	3.73	3.64	2.41
	UZ-11CWA-8	3.29	3.26	0.91
	Bobur	3.68	3.81	-3.53
	P1G082	3.72	3.66	1.61
	P1G119	4.11	3.26	20.68
	P1G202	3.51	3.46	1.42
	P1G264	3.74	3.71	0.80
	P1G132	3.55	3.65	-2.82
	P1G253	3.49	3.67	-5.16
	P2G076	3.71	3.46	6.74
	P2G243	3.69	3.56	3.52
	P2G027	3.57	3.77	-5.60
	P2G162	3.79	3.88	-2.37
Fv/Fo	Altay2000	2.73	2.45	10.26
	UZ-11CWA-8	2.37	2.32	2.11
	Bobur	2.68	2.81	-4.85
	P1G082	2.76	2.68	2.90
	P1G119	3.18	2.37	25.47
	P1G202	2.51	2.45	2.39
	P1G264	2.81	2.72	3.20
	P1G132	2.56	2.66	-3.91
	P1G253	2.49	2.67	-7.23
	P2G076	2.75	2.51	8.73
	P2G243	2.68	2.57	4.10
	P2G027	2.57	2.77	-7.78
	P2G162	2.79	2.91	-4.30
Fv/Fm	Altay2000	0.73	0.67	8.22
	UZ-11CWA-8	0.72	0.71	1.39
	Bobur	0.73	0.74	-1.37
	P1G082	0.74	0.73	1.35
	P1G119	0.77	0.73	5.19
	P1G202	0.72	0.71	1.39
	P1G264	0.75	0.73	2.67
	P1G132	0.72	0.73	-1.39
	P1G253	0.71	0.73	-2.82
	P2G076	0.74	0.73	1.35
	P2G243	0.73	0.72	1.37
	P2G027	0.72	0.73	-1.39
	P2G162	0.74	0.75	-1.35

4.4 Validation of candidate genes in both segregating populations and by expression analysis

The QTLs identified in a GWAS (Oyiga *et al.,* 2018) were validated by analyzing them in contrasting lines. Firstly, it was examined whether the marker alleles of the QTL regions could distinguish these contrasting lines. For that, more than 40 SNP markers from the QTL regions were selected and tested in the parents and offspring (**Table 9**).

Table 9. SNP markers of the QTL regions in the parents and contrasting lines of both segregating populations.

SNP	Chr	Genome	Position (CM)	Alleles	Parents		Contrasting F$_3$ lines of cross Bobur*Altay2000					
							P1G082	P1G119	P1G202	P1G264	P1G132	P1G253
					Altay2000	Bobur	Tolerant	Tolerant	Tolerant	Tolerant	Sensitive	Sensitive
RAC875_c38018_278	2AL	A	110.13	T/C	T	C	C	C	T	C	C	C
Kukri_c11327_977	2AL	A	341.14	T/G	G	T	T	T	T	T	T	T
Excalibur_c20439_825	2AL	A	497.75	T/C	C	T	T	T	T	T	T	T
Excalibur_c39151_104	2AL	A	502.19	A/G	A	G	G	G	G	G	G	G
BS00066475_51	3AL	A	275.6	A/G	G	A	A	A	A	A	A	A
RAC875_c16405_84	4AS	A	147.89	T/C	C	T	T	T	T	T	T	T
Tdurum_contig33628_129	4AS	A	147.89	T/C	C	T	T	T	T	T	T	T
tplb0024k14_1812	6AS	A	115.71	T/C	C	T	T	T	T	T	T	T
BS00035083_51	7AL	A	103.7	T/C	T	C	C	C	C	C	C	C
wsnp_Ex_c43009_49439922	7AL	A	103.7	T/C	T	C	C	C	C	C	C	C
Kukri_c1831_1243	7AL	A	150.81	T/C	T	C	C	C	C	C	C	C
Ex_c2725_1442	1BS	B	201.12	A/G	G	A	G	G	G	G	G	G
BobWhite_c11044_322	1BS	B	266.71	T/C	T	C	C	C	C	C	C	C
BobWhite_c43917_288	1BS	B	269.73	A/G	G	A	G	G	G	G	G	G
RAC875_c11609_62	2BS	B	277.23	A/G	G	A	A	A	A	A	A	A
Ex_c16948_754	**2BS**	**B**	**367.4**	**A/G**	**A**	**G**	**A**	**A**	**A**	**A**	**G**	**G**
BobWhite_c5756_532	2BS	B	583.38	A/C	A	C	C	C	C	C	C	C
Kukri_c54078_114	5BL	B	257.76	T/G	T	G	G	G	G	G	G	G
Tdurum_contig25513_123	5BL	B	280.68	A/G	A	G	G	G	G	G	G	G
Tdurum_contig25513_195	5BL	B	280.68	T/C	T	C	C	C	C	C	C	C
BobWhite_c48435_165	5BL	B	280.68	T/C	C	T	T	T	T	T	T	T
RAC875_c62_1546	**1DS**	**D**	**108.87**	**A/G**	**A**	**G**	**A**	**A**	**A**	**A**	**G**	**G**
BobWhite_c5419_643	1DS	D	108.87	A/G	A	G	A	G	A	G	A	A

SNP	Chr.	Genome	Position(cM)	Alleles	Parents		Contrasting F$_3$ lines of cross Bobur* UZ-11CWA08			
					UZ-11CWA08	Bobur	P2G076 Tolerant	P2G243 Tolerant	P2G027 Sensitive	P2G162 Sensitive
CAP7_c4879_249	1AL	A	313.85	A/C	A	C	A	A	C	A
RAC875_c38018_278	2AL	A	110.13	T/C	T	C	C	C	C	T
Excalibur_c20439_825	2AL	A	497.75	T/C	C	T	C	T	T	C
Excalibur_c91176_326	2AL	A	502.77	A/G	A	G	A	G	A	G
IAAV7086	2AL	A	544.94	A/G	G	A	G	A	G	A
RFL_Contig5153_958	3AL	A	555.33	A/G	G	A	A	A	G	A
Tdurum_contig33628_129	4AS	A	147.89	T/C	C	T	C	T	C	T
Tdurum_contig33628_85	4AS	A	147.89	A/G	A	G	A	G	A	G
wsnp_Ex_c43009_49439922	7AL	A	103.7	T/C	T	C	T	T	T	T
BS00035083_51	7AL	A	103.7	T/C	T	C	T	C	T	T
D_contig25392_201	1BS	B	195.12	A/G	G	A	G	G	G	A
BobWhite_c11044_322	1BS	B	266.71	T/C	T	C	C	T	C	C
Excalibur_c65341_303	2BS	B	365.88	A/G	G	A	A	A	A	A
Ex_c16948_754	2BS	B	367.4	A/G	A	G	G	G	A	G
BobWhite_c5756_532	2BS	B	583.38	A/C	A	C	C	C	C	C
BS00032003_51	5BL	B	1.33	T/C	T	C	C	C	T	C
Tdurum_contig25513_123	5BL	B	280.68	A/G	A	G	G	G	G	G
BobWhite_c48435_165	**5BL**	**B**	**280.68**	**T/C**	**C**	**T**	**C**	**C**	**T**	**T**
Excalibur_rep_c67190_638	7BS	B	228.36	T/G	T	G	G	G	G	G
BS00087086_51	**1DS**	**D**	**108.87**	**T/C**	**T**	**C**	**T**	**T**	**C**	**C**
BS00002178_51	**1DS**	**D**	**108.87**	**A/G**	**G**	**A**	**G**	**G**	**A**	**A**

Several marker alleles of the progenies showed the same allelic pattern of classification of the contrasting lines as the phenotypic classification. In the Bobur*Altay2000 population, we found a separation by alleles between the lines on Chr. 1DS (position 108.87 cM) and Chr. 2BS (position 367.4 cM), which corresponds to the behavior of the parents. In population Bobur*UZ-11CWA08, we also found the QTL on Chr. 1DS (position 108.87 cM) and additionally, a QTL region on Chr. 5BL (position 280.68 cM) to be informative. Several other markers, however, showed a segregation pattern of their alleles that did not correspond to the phenotypic classification. Although we found most QTL regions with segregating markers, not all of them segregated in both populations. Five of the markers were polymorphic in both populations (**Table 9**).

The contrasting lines were analyzed by an ANOVA using the respective alleles as a factor. The markers on Chr. 1DS (position 108.87 cM) showed significant allelic effects for SFW, SDW, RFW, sodium content, and proline accumulation in the Bobur*Altay2000 population and for SFW, SDW, RFW, potassium content, proline accumulation, and chlorophyll content in the Bobur* UZ-11CWA08 population. For the same QTL region, an ANOVA revealed significant marker by salinity treatment interaction effects for SFW, SDW, sodium content, potassium content, proline accumulation, and Fixed-Area in the Bobur*Altay2000 population and SFW, SDW, RFW, proline accumulation, and chlorophyll content in the Bobur*UZ-11CWA08 population.

While both populations possessed these effects for the QTL region on 1DS, the same marker, which shows the same pattern of allelic segregation in the Bobur*Altay2000 on QTL 2BS (position 367.4 cM), did not show a comparable classification into the salt-sensitive or salt-tolerant group in the Bobur*UZ-11CWA08 population and did not reveal significant allelic effects for the tested traits. On the other hand, the significant QTL of Chr. 5BL (position 280.68 cM) in the Bobur*UZ-11CWA08 population could not be found in the Bobur*Altay2000 population.

Consequently, these QTLs (**Table 10**) were used to detect the informative genes and proteins involved in the response to salinity stress and the major cell regulatory mechanisms. The TGACv1 genome sequence assembly version of *Triticum aestivum* L. publicly available on JBrowse was used to detect these genes (**Table 11**; see details in **Appendix 3**).

Table 10. Colocation of SNP clusters with QTL/genes. ST_DRW Salt Tolerance Dry Root Weight.

Associated ST traits	SNP	Contrasting F₃ lines	Chr.	QTL	R² (%)	Position (bp)	Position (CM)
ST_DRW	BS00002178_51	Bobur* UZ-11CWA08	1DS	Q-1DS.1	≥13.33	33712262..33712362	108.87
ST_DRW	RAC875_c62_1546	Bobur*Altay2000	1DS	Q-1DS.2	≥13.33	32543884..32543984	108.87
ST_DRW	BS00087086_51	Bobur* UZ-11CWA08	1DS	Q-1DS.3	≥13.33	34619721..34619821	108.87
ST_DRW	Ex_c16948_754	Bobur*Altay2000	2BS	Q-2BS.1	≥12.69	699826968..699827068	367.4
ST_DRW	BobWhite_c48435_165	Bobur* UZ-11CWA08	5BL	Q-5BL.1	≥24.20	546827468..546827565	280.68

Table 11. Candidate genes for the significant marker–trait associations for salinity tolerance in the contrasting wheat genotypes. Their functions were adapted from the JBrowse (https://urgi.versailles.inra.fr/jbrowseiwgsc/gmod_jbrowse/?data=myData%2FIWGSC_RefSeq_v1.0&loc=chr3A%3A515889718..515889909&) database

Gene ID	Genes Number	Chr.	Gene Position(bp)	Gene Length(bp)	Gene Annotation
TraesCS1D02G052200	Gene_1	1DS	33469805..33474180	4,376	nucleotide binding (GO:0000166) alcohol dehydrogenase (NAD+) activity (GO:0004022) catabolic process (GO:0009056) zinc ion binding (GO:0008270) response to salt stress (GO:0009651)
TraesCS1D02G052700	Gene_2	1DS	33638501..33640761	2,261	leaf senescence (GO:0010150) abscisic acid stimulus (GO:0071215) cellular response to salicylic acid stimulus (GO:0071446) cellular response to osmotic stress (GO:0071470)
TraesCS1D02G054400	Gene_3	1DS	35788030..35788783	754	response to salt stress (GO:0009651)
TraesCS1D02G054500	Gene_4	1DS	35886292..35900512	14,221	response to salt stress (GO:0009651)
TraesCS1D02G054600	Gene_5	1DS	35926381..35927231	851	response to salt stress (GO:0009651)
TraesCS2B02G503100	Gene_6	2BS	697546485..697548362	1,878	response to toxic substance (GO:0009636) response to salt stress (GO:0009651)
TraesCS5B02G368500	Gene_7	5BL	546826331..546832103	5,773	potassium ion transmembrane transport (GO:0071805) potassium ion transmembrane transporter activity (GO:0015079)
TraesCS5B02G368800	Gene_8	5BL	547399295..547406590	7,296	sodium ion transport (GO:0006814) chloride ion homeostasis (GO:0055064) potassium ion homeostasis (GO:0055075) potassium ion transmembrane transport (GO:0071805) chloride transmembrane transport (GO:1902476) sodium:potassium:chloride symporter activity (GO:0008511) potassium:chloride symporter activity (GO:0015379)

4.4.1. *In silico* expression analysis

Using the WheatGmap web tool and based on these QTLs (**Table 10**), we found a wide range of expression for the candidate genes in different cereal tissues and at different developmental stages (**Figure 21**). Among the candidate genes, *TraesCS1D02G052200* in 1DS and *TraesCS5B02G368800* in 5BL showed the highest expression in most organs and tissues, indicating that they play important roles during development, growth, and grain filling. *TraesCS5B02G368500* was found to be semi-highest expressed in the first leaf blade, flag leaf, and leaf of 5BL. *TraesCS1D02G052700* in 1DS was highly expressed in the endosperm. Four of the significantly associated genes, *TraesCS1D02G054400*, *TraesCS1D02G054500*, and *TraesCS1D02G054600,* which were on 1DS, and *TraesCS2B02G503100* on 2BS, showed very low expression in the tissues compared to the other genes.
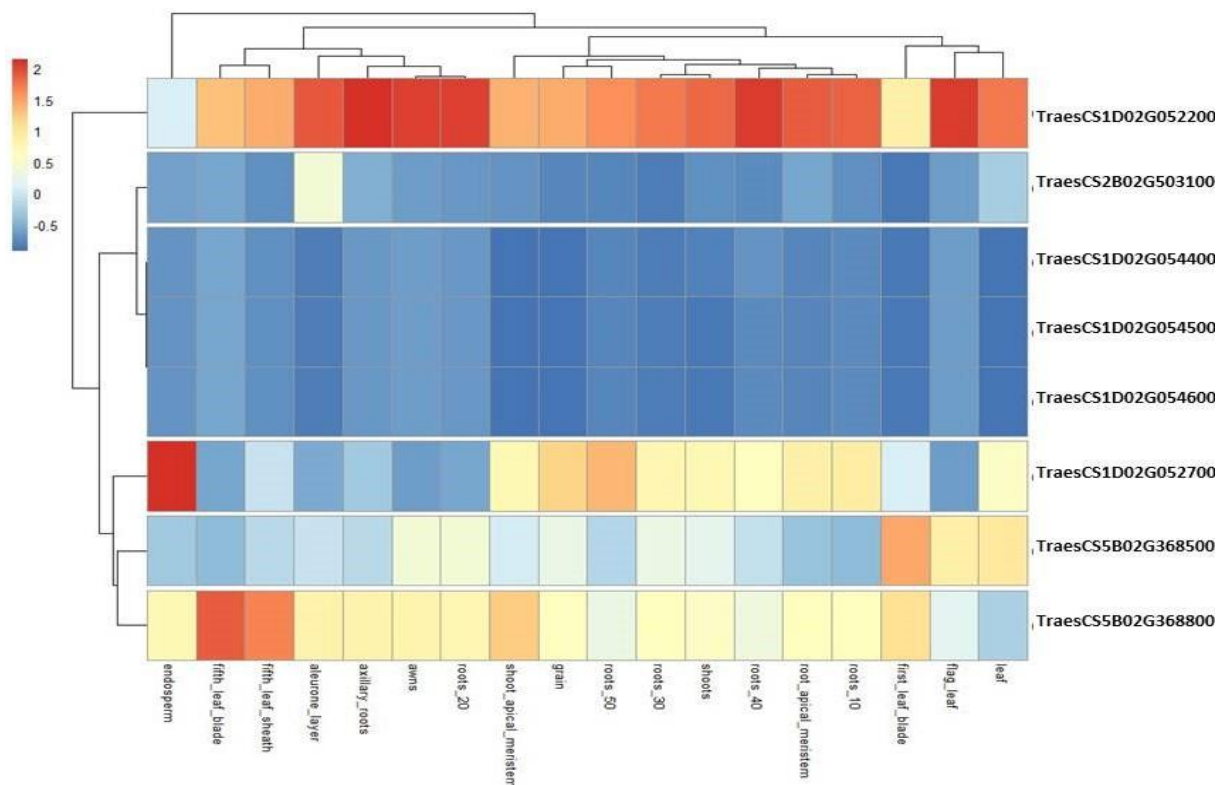
Figure 21. Expression patterns of selected candidate genes for salinity tolerance within different tissues of wheat. Expression data were obtained from the Wheat Gmap database (https://www.wheatgmap.org/expression/search/gene/) and are presented as a heatmap of transcripts per kilobase million (TPM) values.

4.4.2. Expression analysis of candidate genes

Furthermore, the candidate genes that were validated in the parents (Oyiga *et al.,* 2018) were validated in both segregating populations' contrasting lines. Quantitative Real-Time PCR (qRT-PCR) was conducted to quantify the kinetics of *ZIP7* (zinc transporter), *KefC* (glutathione-regulated potassium-efflux system protein), *AtABC8* (ABC transporter B family member 8), and *6-SFT* (sucrose: fructan-6-fructosyltransferase) expression in parents and contrasting progenies under control and salt stress conditions. **Figures (22 and 23)** show the relative expression of *ZIP7*, *KefC*, *AtABC8*, and *6-SFT* at 42 DAS (day after saline) for the contrasting parents and progenies, calculated according to the algorithm described by Livak & Schmittgen (2001). At day 42, the expression of *ZIP7*, *KeFc*, *AtABC8*, and *6-SFT* showed that they are upregulated in salt-tolerant lines, including the parents (Altay 2000, UZ-11CWA08) and lines (P1G082, P1G119, P1G202, and P1G264) and (P2G076 and P2G243) (**Figures 22 and 23**). Salt-sensitive lines, including the parent (Bobur), lines (P1G132 and P1G253), and lines (P2G027 and P2G162), on the other hand, were downregulated (**Figures 22 and 23**). *ZIP-7* differential expression was higher in both parents and progenies compared to *KefC*, *AtABC8*, and *6-SFT* expression (**Figures 22 and 23**). The contrasting lines of both populations and parents were significantly different from each other in the tested trait and showed significant interactions with the salt treatments and genes (**Table 12**).

Table 12. F-values and significance levels of the two-way ANOVA. The fold values for expression level are analyzed for the contrasting genotypes of both segregating populations and parents. F-values are shown; significance levels p: *** $p \leq 0.001$; ns not significant; SOV source of variance; DF degrees of freedom; MS mean squares.

		Parents		Contrasting lines of F_3 lines of cross Bobur*Altay2000		Contrasting lines of F_3 lines of cross Bobur* UZ-11CWA08
SOV	**DF**	**Folds values**	**DF**	**Folds values**	**DF**	**Folds values**
Genotype (G)	2	18.079***	5	14.552***	3	10.900***
Salt-Treatment (ST)	1	63.799***	1	118.088***	1	19.591***
Genes (Ge)	3	33.939***	3	2.498 ^{ns}	3	14.477***
G*ST	2	19.414***	5	17.555***	3	7.917***
G*Ge	6	5.237***	15	0.438 ^{ns}	9	0.860 ^{ns}
ST*Ge	3	27.161***	3	1.096 ^{ns}	3	13.722***
G*ST*Ge	6	8.260***	15	0.651 ^{ns}	9	0.843 ^{ns}
		MS		MS		MS
Error	48	0.51	96	0.52	64	0.722
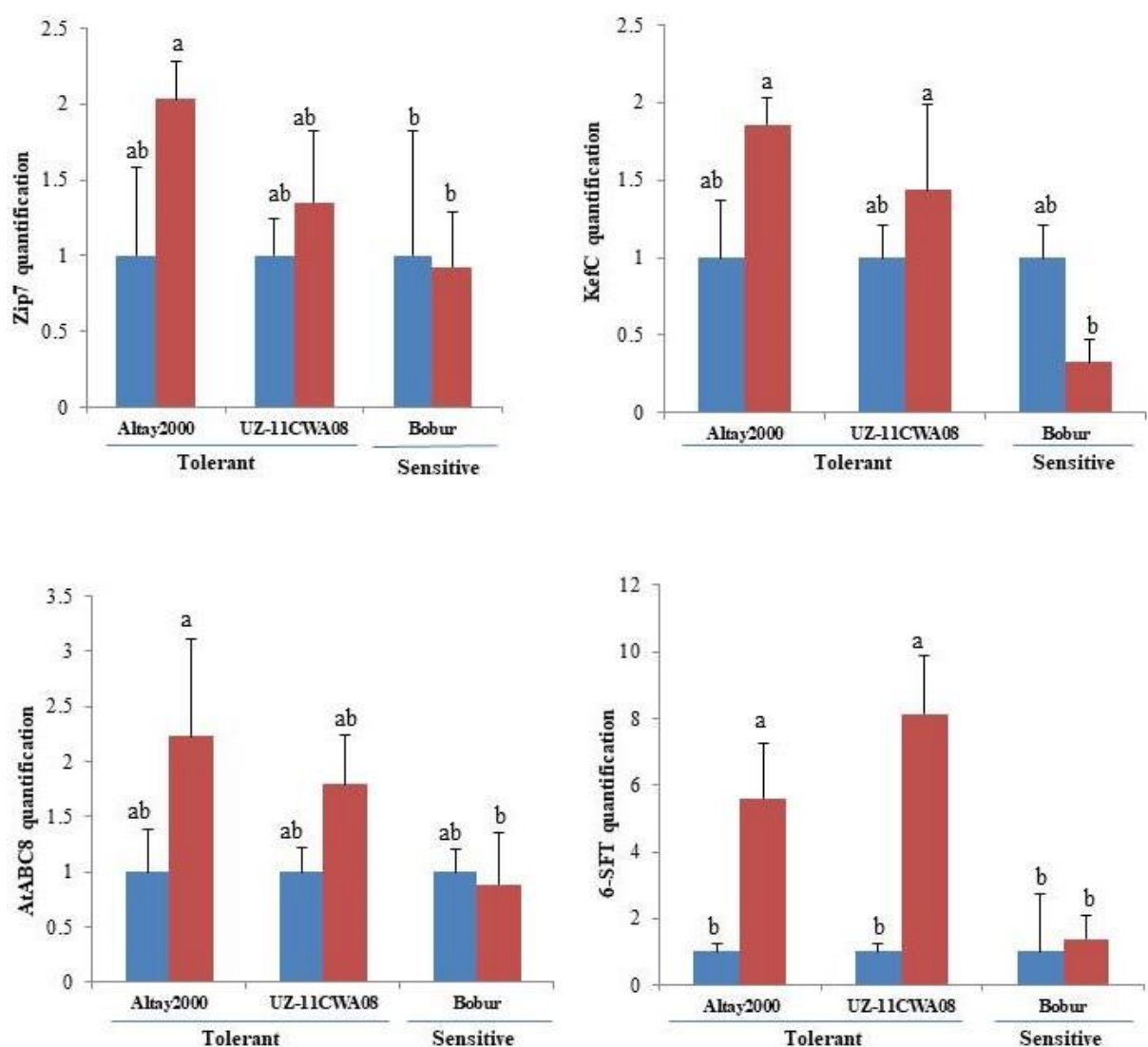
Figure 22. Expression levels of zinc transporter (*ZIP7*), glutathione-regulated potassium-efflux system protein (*KefC*), ABC transporter B family member 8 (*AtABC8*), and sucrose: fructan-6-fructosyltransferase (*6-SFT*) in leaves of two salt-tolerant plants (Altay2000 and UZ-11CWA-8) and salt-sensitive (Bobur) plant after 42 d in non-saline (blue) and saline (red) conditions were determined by the 2-ΔCT method. Efa1.1 and Efa1.2 genes were used as internal control genes. Means with the same letter are not significantly different from each other.

60
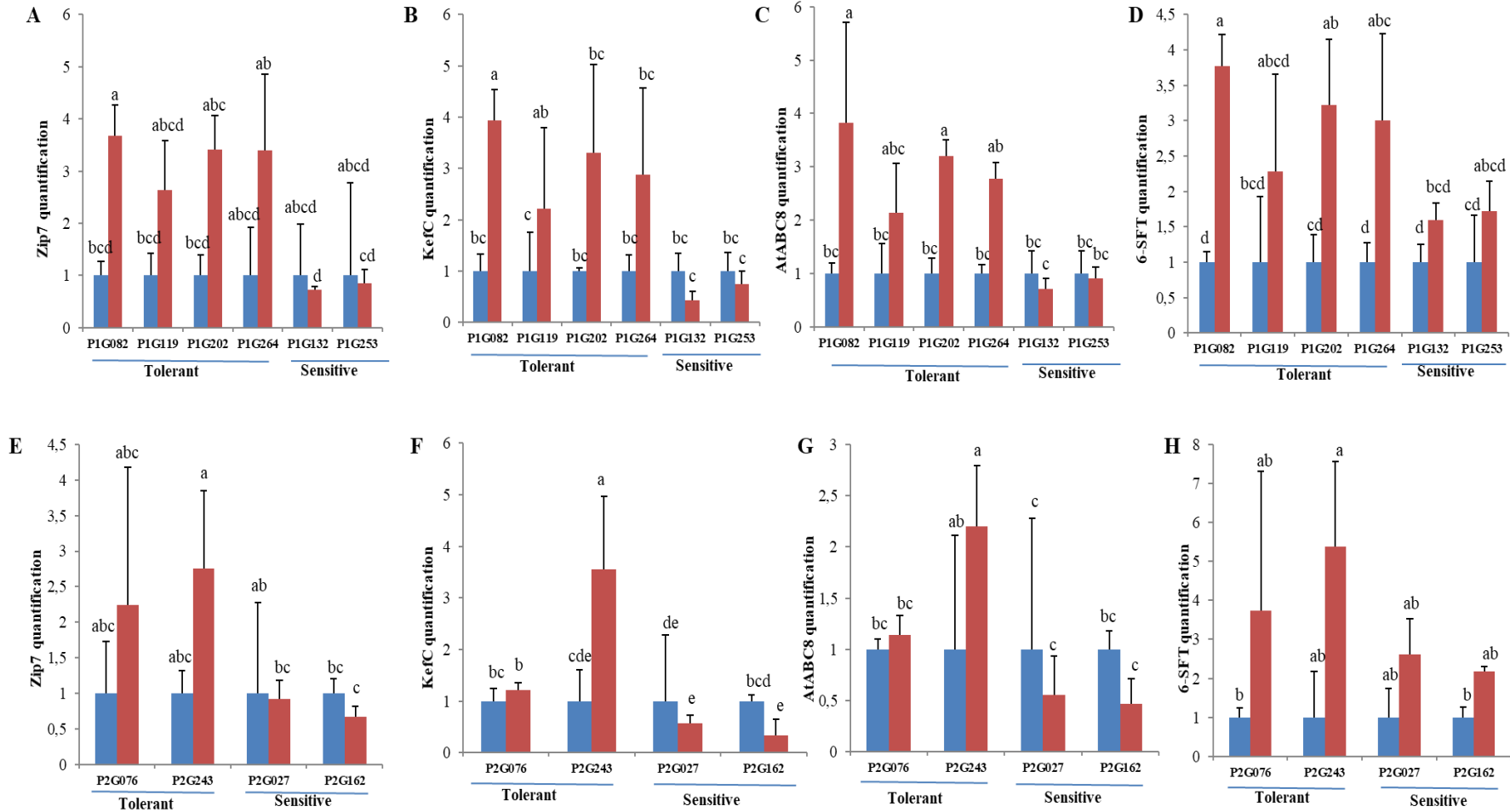
Figure 23. Expression levels of zinc transporter (*ZIP7*), glutathione-regulated potassium-efflux system protein (*KefC*), ABC transporter B family member 8 (*AtABC8*) and sucrose: fructan-6-fructosyltransferase (*6-SFT*) in leaves of (**A-D**) salt-tolerant (P1G082,P1G119,P1G202, and P1G264) and salt-sensitive (P1G132 and P1G253) of contrasting F₃ lines of cross Bobur*Altay2000; (**E-H**) salt-tolerant (P2G076 and P2G243) and salt-sensitive (P2G027 and P2G162) of contrasting F₃ lines of cross Bobur*UZ-11CWA08 after 42 d in non-saline (blue) and saline (red) conditions were determined by the 2-ΔCT method. Efa1.1 and Efa1.2 genes were used as internal control genes. Means with the same letter are not significantly different from each other.

5. Discussion

Understanding salt tolerance mechanisms and analyzing salt stress-related genes and their functions will provide a theoretical basis for understanding the stress signal network and pathways for the improvement of the target crop (Zhang *et al.,* 2018). However, plants' tolerance mechanism is a complex phenomenon that depends upon physiological and genetic responses (Afzal *et al.,* 2022). They indicated that these processes involve phenotypic evaluation as well as the identification of QTLs closely related to molecular markers. Identification of new QTLs may lead to the development of new salt-tolerant lines (Afzal *et al.,* 2022). Improving salt tolerance is a great challenge as it is a very complex trait that is under polygenic control (Said *et al.,* 2022). To improve wheat production in salinity-affected areas, access to adequate genetic diversity is critical for current and future breeding efforts. Considerable effort has been made to identify salt-tolerant wheat genotypes.

In the present study, a wide range of phenotypic variability was observed for all tested traits among F_3 offspring from two crosses between salt-sensitive and salt-tolerant parents. An analysis of genetic differences was performed to verify the proposed (Oyiga *et al.,* 2018) marker-trait associations that show salt-tolerant genomic regions. Verified QTL regions and postulation of candidate genes can accelerate breeding for new high-yielding genotypes that are also salt-tolerant. The heritability of the shoot traits was higher than those of the root traits. However, before a selection of the lines concerning their shoot traits can be recommended, the correlations of the shoot traits to the target traits must be considered. Whereas TOL and SSI for SFW and SDW showed highly significant correlations with SWL in both populations, STI was highly significantly negatively correlated with SWL. Also, MP and GMP for SFW and SDW were neither highly correlated with SWL nor with each other.

5.1. Salt stress response of leaf ionic traits in salt contrasting lines

In general, the lines under salt stress showed higher Na^+ levels. Salt-sensitive lines showed the highest Na^+ concentration in the leaf compared to the salt-tolerant lines (**Figures 14A-C**). The magnitude of this reduction in the salt-sensitive lines was higher in Bobur (312.14%), P1G132 (209.52%), P1G253 (285.21%) P2G027 (110.45%), and P2G162 (131.11%) compared with the salt-tolerant lines, which were Altay2000 (204.89%), UZ-11CAW08 (285.15%), P1G082 (110.61%), P1G119 (175.71%), P1G202 (82.02%), P1G264 (270.1%), P2G076 (93.58%) and P2G243 (67.47%). No or a small increase in Na^+ under stress indicated that these genotypes were more tolerant than those that translocated high levels of Na^+ into their leaves. Higher concentrations of Na^+ impede various metabolic activities (Akram *et al.,* 2007). The different accumulations of Na^+ in the lines show that there are genetic differences in this trait and that

genotypes that accumulate only low amounts of Na^+ in their leaves react genetically differently to the increased salinity (Siddiqui *et al.,* 2017). An uptake or transport mechanism that distinguishes similar ions such as Na^+ and K^+ could be a useful selection criterion for salt tolerance in wheat and breeding for efficient nutrient uptake (Khan *et al.,* 2009).

With increased hydroponic salt concentration, all wheat lines showed reduced K^+ levels (**Figures 14D-F**). The decrease in K^+ levels is due to the presence of excess Na^+ in the growing medium, as high external Na^+ levels are known to have an antagonistic effect on K^+ uptake in the plant (Sarwar *et al.,* 2003). Salt tolerance is definitely related to K^+ content since K^+ is involved in osmotic regulation and there is competition between these two ions (Ashraf *et al.,* 2005). Salt stress drastically affects growth and decreases the process of photosynthesis due to the imbalance of the internal ionic concentration of the various cations and anions like sodium and potassium (Liu *et al.,* 2020).

The lines show genetic differences in the K^+/Na^+ ratio, with the salt-tolerant lines having significantly higher K^+/Na^+ ratios compared to the salt-sensitive lines (**Figure 15**). The data on K^+/Na^+ ratios indicate that Na^+ exclusion from leaf tissues plays a critical role in rice salt tolerance by maintaining the optimal K^+/Na^+ ratio (Kibria *et al.,* 2017).

5.2. Biochemical modulations under salt stress

It has been found that stressful environments such as drought, salinity, and high temperatures can cause damage to the plant at any stage (Bajwa *et al.,* 2018). In addition to molecular, physiological, and chemical damage, there is primarily direct damage in the photosynthesis process. This damage can impair the growth of plants at every growing stage and thus reduce the yield (Bajwa *et al.,* 2018).

Proline concentration in the leaves increased significantly under salinity (**Table 7**), with the highest increases of approximately 312% (Bobur), 112.73% (P1G253), and 927% (P2G162) in salt-sensitive lines when compared with the salt-tolerant lines (**Figure 16**). This finding is consistent with the observations of those who also observed that salt-sensitive cultivars increased proline levels under salt stress (Poustini *et al.,* 2007). Contrary to these reports, exogenous proline treatment often results in higher salt tolerance (El Moukhtari *et al.,* 2020). The present results suggest that increasing proline concentration may not be associated with salt tolerance, which is consistent with similar observations previously reported by Colmer *et al.* (1995). Proline accumulation did not seem to play a part in the improved salt tolerance of the amphiploid relative to that of wheat (Chinese Spring). The levels of proline were higher in

the leaf blades of the salt-sensitive wheat (Chinese Spring) than in those of the more salt-tolerant amphiploid (except in the oldest leaf blade) (Colmer *et al.,* 1995).

However, elevated proline levels may also confer additional regulatory or osmoprotective functions under salt stress, such as its role in the control of the activity of plasma membrane transporters involved in cell osmotic adjustment in barley roots (Cuin and Shabala, 2005). Given the fact that proline biosynthesis is a highly energy-demanding process and that only small quantities of proline are probably required for the control of plasma membrane transporters (Cuin and Shabala, 2005), the observed overproduction of proline in sensitive genotypes may not be explained by these processes but rather may reflect poor performance and greater damage in response to salt stress. Consequently, selecting higher proline levels to increase salt tolerance can be counterproductive.

5.3. Physiological modulations under salt stress

More studies are required on morphological and physiological traits to understand the complexity of salt tolerance (Mourad *et al.,* 2019; Moursi *et al.,* 2020; Mondal *et al.,* 2021; Thabet *et al.,* 2021). A reduction in the chlorophyll content was observed in all lines under salt stress. This reduction was more pronounced in the salt-sensitive lines than in the salt-tolerant ones (**Figure 17; Table 7**), which may be due to the replacement of Mg^{2+} with Na^+ in these sensitive genotypes (Demiroğlu *et al.,* 2001; Katsuhara *et al.,* 1990). Across different plants exposed to salinity stress, the reduction in chlorophyll content is an indicative response (Roychoudhury and Basu, 2008). In soybean, seedlings have a significant reduction in chlorophyll content at high NaCl levels (Chen and Yu, 2007). The reduction in chlorophyll content caused a reduction in photosynthesis (Said *et al.,* 2022). The salt-tolerant wheat genotypes revealed higher levels of chlorophyll content compared to the salt-sensitive group (Said *et al.,* 2022). Thus, the chlorophyll content would be useful in screening large numbers of genotypes (Mansour *et al.,* 2020).

The chlorophyll fluorescence transients (Fo, Fj, Fi, Fm, and Fv) in contrasting parents and both salt-tolerant and salt-sensitive lines declined (**Figures 18-20**) under saline conditions. The decrease in Fo due to salt stress indicates increased thermal dissipation (Guidi *et al.,* 2002; Bussotti *et al.,* 2011), while the decrease in Fv may be attributed to pigment losses due to the salt effect (Oyiga *et al.,* 2016). Salinity stress inhibits photosynthesis by inhibiting the photosystem II complex (PSII) on both the acceptor [QA] and donor sides (the oxygen-evolving complex OEC) and by destroying chlorophyll pigments due to toxic ion accumulation (Chen and Murata, 2011). It has been suggested to use fluorescence induction parameters to detect metabolic perturbations caused by abiotic stresses (Baker, 2008). Under salt stress, the Fv/Fm,

Fm/Fo, and Fv/Fo declined in salt-tolerant lines and increased in salt-sensitive lines (**Table 8**), suggesting different mechanisms are controlling these physiological traits in wheat, making them useful parameters for distinguishing salt-tolerant from salt-sensitive genotypes (Oyiga *et al.*, 2016). The fix area was higher in the salt-tolerant lines compared to the salt-sensitive ones (**Figures 18C, 19E, and 20C**) (**Table 7**). The result of our study was consistent with the finding of Oyiga *et al.* (2016), who found that salt stress had a negative effect on fix area in wheat genotypes.

5.4. Validation of candidate genes in both segregating populations and by expression analysis

For the development and improvement of salt-tolerant cultivars, it is important to identify the relevant genes that determine and/or influence salt tolerance in plants. This study aimed to validate putative candidate genes controlling salt tolerance in wheat by validating QTL regions and using reference sequences to identify them. In both tested biparental populations, we found a strong allele effect from members of the QTL on Chr. 1DS at 108.87cM. Because further tested QTL positions co-segregated, it is possible that a QTL on 2BS in the cross Bobur * Altay2000 or a QTL on 5BL in the cross Bobur * UZ-11CWA08 is also responsible for salt tolerance. However, we found no phenotypic response in the respective other populations of the biparental crosses with the common parent Bobur in both of these later positions. Therefore, we conclude that the responsive allele is located in the QTL region of Chr. 1DS at position 108.87 cM.

The literature findings for the putative candidate genes can be described as follows:
Using the WheatGmap web tool, the candidate genes *TraesCS1D02G052200* in 1DS and *TraesCS5B02G368800* in 5BL were found to have the highest expression in most organs and tissues of wheat, indicating that they play important roles during development, growth, and grain filling (**Figure 21**), while *TraesCS1D02G052200* encode alcohol dehydrogenase (ADH). ADH had made some variations in glycolysis and alcohol fermentation, and under flooding, stress enhanced the germination of transgenic soybeans (Tougou *et al.*, 2012). *AT1G64710* and *AT5G24760,* which are considered other ADH family genes, were reactive in the root and leaf of Arabidopsis working together during the PEG-induced water stress, which supports the conclusion that the capacity of ethanolic fermentation was improved in response to drought (Myint *et al.*, 2015). In Arabidopsis, another ADH gene that confers both biotic and abiotic stress resistance has been reported (Shi *et al.*, 2017). By regulating the ROS-related genes to maintain the ROS homeostasis in sugarcane, the sugarcane *ScADH3* gene is one of the ADH genes that appears to affect cold tolerance (Su *et al.*, 2020).

Interestingly, *TraesCS5B02G368800,* encoding cation-chloride cotransporters (CCC) was detected on chromosome 5B, and found to be highly expressed in wheat leaves (fifth leaf sheath and fifth leaf blade) (**Figure 21**). This gene is important because during osmotic and oxidative stress, CCC, such as $Na^+/K^+/2Cl^-$ cotransporters (NKCC) and K-Cl cotransporters, are activated in yeast to maintain fluid/ion homeostasis (Piechotta *et al.,* 2002). The NKCC plays a vital role in osmotic regulation and cell ionic adjustments as an integral membrane protein and functions in transporting Na^+, K^+ and $2Cl^-$ (Dith Gagnon *et al.,* 2003; Gamba 2005).

TraesCS1D02G052700 in 1DS was highly expressed in the endosperm (**Figure 21**). It encodes the sugar transporter SWEET gene, and therefore, considered sugar that will finally be exported. The sugar transporter (SWEET) gene family is a glycoprotein gene family that performs a vital role in plant growth and development, and in response to environmental stress. It can in addition regulate the transport of sugar in plants (Zhang *et al.,* 2020). They indicated that during the early phases of salt stress, a large number exhibited a rise in expression compared with controls among the SWEET genes in Kentucky bluegrass.

In osmotic stress tolerance, SWEETs may also play an important role (Chandran, 2015). For example, during senescence and osmotic stresses including cold, high salinity, and osmotic stress, *AtSWEET15*, also known as *SAG29* (senescence-associated gene 29), is strongly induced (Seo *et al.,* 2011). Comparable to *OsSWEET5* (Tang *et al.,* 2014), under normal growth conditions, constitutive overexpression of *AtSWEET15* results in enhanced leaf senescence. Compared to control plants, *AtSWEET15* overexpression lines show reduced root growth and cell viability under high salinity conditions (Chandran, 2015). In contrast, root growth decreases in *atSweet15* mutant lines is comparable to that of the control (Chandran, 2015). However, compared to the control and over-expression lines, root cells are more viable in the mutant (Seo *et al.,* 2011). So, this suggests that during osmotic stress, *AtSWEET15* may perform a role in modulating cell viability (Chandran, 2015). It has been revealed that SWEET proteins play crucial roles in plant development and stress responses, and the plant kingdom considers them one of the largest sugar transporter families (Gautam *et al.,* 2019). In tea plants, CsSWEET genes play important roles in the response to abiotic and biotic stresses and offer insights into the characteristics of SWEET genes, which could serve as the basis for further functional identification of such genes (Wang *et al.,* 2018).

TraesCS5B02G368500 in 5BL encodes a potassium transporter and shows semi-highest expression in the first leaf blade, flag leaf, and leaf (**Figure 21**). This indicated that the gene *ApKUP4* (alligator weed K^+ transporter gene), which contributes to salinity tolerance in

transgenic Arabidopsis seedlings, is essential for demonstrating plant salinity tolerance and potassium homeostasis (Song *et al.,* 2014). Also, the results of the study by Chen *et al.* (2017) demonstrate that in rice, *OsHAK1* (a high-affinity potassium transporter, that positively adjusts responses to drought stress) is considered a drought-responsive gene whose expression is related to increased dehydration tolerance through the systemic regulation of K^+ homeostasis, proline accumulation, root system architecture, plasma membrane protection, and stimulation of stress-related genes. In addition, to improve abiotic stress tolerance in cereals at the seedling and reproductive stages of plants grown under osmotic and water-limiting conditions *OsHAK1* gene overexpression does not cause any growth defect, indicating that overexpression of this ion transporter gene is a hopeful approach (Chen *et al.,* 2017). In the presence of toxic concentrations of Na^+, the high-affinity potassium transporter HKT (high-affinity K^+ transporters) gene family can selectively uptake K^+ in halophytic plants (Wang *et al.,* 2014). $SbHKT_{1;4}$ expression was upregulated more strongly in salt-tolerant sorghum accessions, correlating with a better-balanced Na^+/K^+ ratio and improved plant growth upon Na^+ stress (Wang *et al.,* 2014).

The qRT-PCR results of *ZIP7* (Milner *et al.,* 2013), *KefC* (Han *et al.,* 2015), *AtABC8* (Ma and Han 2016), and *6-SFT (*Nagaraj *et al.,* 2004) showed specific expression patterns in shoots of salt-tolerant (up-regulation) and salt-sensitive (down-regulation) lines, indicating that they are involved in ST (**Figures 22and 23**). Further analyses of the transcription of these genes in the root cells are essential, as the organ is in close contact with the solution.

6. Conclusions and perspectives

In the frame of this study, ST indices were used to classify the F_3 lines of two crosses into salt-tolerant and salt-sensitive lines with respect to their response to salt stress. The identified contrasting groups showed markedly differential physiological, biochemical, and ionic responses to salt stress. The salt-tolerant lines from the 1st population (P1G082, P1G119, P1G202, and P1G264) and 2nd population (P2G076, P2G243) of the crosses Bobur*Altay2000 and Bobur*UZ-11CWA08, respectively, showed a higher leaf K^+/Na^+ ratio, lower proline accumulation, higher chlorophyll content, and higher rates of PSII photochemical activities compared with the sensitive lines in both populations. This study provides useful information regarding the phenotypic, ionic, biochemical, and physiological variations found in the germplasm of the contrasting F_3 wheat lines. Traits like Na^+ and K^+ contents, chlorophyll content, chlorophyll-a fluorescence (ChlF), and proline accumulation that effectively differentiate the salt-tolerant and salt-sensitive groups, can be used for direct selection for salt tolerance and were all measured to validate the QTL (Q-1DS) that determine these traits under salt stress. Further, the candidate genes underlying the QTLs identified in this study need to be confirmed in follow-up studies, after which they can be used to accelerate breeding improvements in wheat salt tolerance.

References

Abd El-Mohsen, A. A., Abd El-Shafi, M. A., Gheith, E. M. S., & Suleiman, H. S. (2015). Using different statistical procedures for evaluating drought tolerance indices of bread wheat genotypes. *Advance in Agriculture and Biology, 4*(1), 19-30.

Ábrahám, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. *Plant stress tolerance,639*, 317-331.

Acuña-Galindo, M. A., Mason, R. E., Subramanian, N. K., & Hays, D. B. (2015). Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. *Crop Science*, *55*(2), 477-492.

Afzal, M., Hindawi, S. E. S., Alghamdi, S. S., Migdadi, H. H., Khan, M. A., Hasnain, M. U., ... & Sohaib, M. (2022). Potential Breeding Strategies for Improving Salt Tolerance in Crop Plants. *Journal of Plant Growth Regulation*, 1-23.

Ahmad, P., Azooz, M. M., & Prasad, M. N. V. (2013). *Salt stress in plants*. Springer

Ain, Q. U., Rasheed, A., Anwar, A., Mahmood, T., Imtiaz, M., Mahmood, T., ... & Quraishi, U. M. (2015). Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. *Frontiers in plant science*, *6*, 743.

Akhtar, J., Saqib, Z. A., Sarfraz, M., Saleem, I., & Haq, M. A. (2010). Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. *Pakistan Journal of Botany*, *42*(4), 2857-2866.

Akram, M., Malik, M. A., Ashraf, M. Y., Saleem, M. F., & Hussain, M. (2007). Competitive seedling growth and K/Na ratio in different maize (*zea mays* l.) Hybrids under salinity stress. *Pakistan Journal of Botany*, *39*(7), 2553-2563.

Allel, D., Ben-Amar, A., & Abdelly, C. (2018). Leaf photosynthesis, chlorophyll fluorescence and ion content of barley (*Hordeum vulgare*) in response to salinity. *Journal of Plant Nutrition*, *41*(4), 497-508.

Amirjani, M. R. (2010). Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. *American Journal of Plant Physiology*, *5*(6), 350-360.

Amtmann, A., & Sanders, D. (1998). Mechanisms of Na^+ uptake by plant cells. *Advances in botanical research* (pp. 75-112).

Appels, R., Nystrom-Persson, J., & Keeble-Gagnere, G. (2014). Advances in genome studies in plants and animals. *Functional & integrative genomics*, *14*(1), 1-9.

Arshi, A., Abdin, M. Z., & Iqbal, M. (2005). Ameliorative effects of CaCl2 on growth, ionic relations, and proline content of senna under salinity stress. *Journal of plant nutrition*, *28*(1), 101-125.

Arunyanark, A., Jogloy, S., Akkasaeng, C., Vorasoot, N., Kesmala, T., Nageswara Rao, R. C., ... & Patanothai, A. (2008). Chlorophyll stability is an indicator of drought tolerance in peanut. *Journal of Agronomy and Crop Science*, *194*(2), 113-125.

Ashraf, M. Y., Akhtar, K., Sarwar, G., & Ashraf, M. (2005). Role of the rooting system in salt tolerance potential of different guar accessions. *Agronomy for sustainable development*, *25*(2), 243-249.

Azizov, I. V., & Khanisheva, M. A. (2010). Pigment content and activity of chloroplasts of wheat genotypes grown under saline environment. *Proceedings of ANAS (Biological Sciences)*, *65*(5-6), 96-98.

Baheri, S. F., Javanshir, A., Kazemi, H. A., & Aharizad, S. (2003). Evaluation of different drought tolerance indices in some spring barley genotypes. *Journal of Agriculture Science*, *13*(3),95-105.

Bajwa, A. A., Farooq, M., & Nawaz, A. (2018). Seed priming with sorghum extracts and benzyl aminopurine improves the tolerance against salt stress in wheat (*Triticum aestivum* L.). *Physiology and molecular biology of plants, 24*(2), 239-249.

Baker, N. R. (2008). Chlorophyll Fluorescence: A Probe of Photosynthesis in Vivo. *Annual Review of Plant Biology*, *59*, 89–113.

Baker, N. R., & Rosenqvist, E. (2004). Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of experimental botany*, *55*(403), 1607-1621.

Balasubramanian, S., Schwartz, C., Singh, A., Warthmann, N., Kim, M. C., Maloof, J. N., ... & Weigel, D. (2009). QTL mapping in new *Arabidopsis thaliana* advanced intercross-recombinant inbred lines. *PloS ONE*, *4*(2), e4318.

Balouchi, H. R. (2010). Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation. *International Journal of Nuclear and Quantum Engineering*, *4*(1), 63-73.

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical reviews in plant sciences*, *24*(1), 23-58.

Barutcular, C., El Sabagh, A., Koc, M., & Ratnasekera, D. (2017). Relationships between grain yield and physiological traits of durum wheat varieties under drought and high temperature stress in Mediterranean conditions. *Fresenius Environmental Bulletin*, *26*(6), 4282-4291.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

Bennett, M. D., & Leitch, I. J. (1995). Nuclear DNA amounts in angiosperms. *Annals of Botany*, *76*(2), 113-176.

Betran, F. J., Beck, D., Bänziger, M., & Edmeades, G. O. (2003). Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. *Crop Science*, *43*(3), 807-817.

Bordes, J., Goudemand, E., Duchalais, L., Chevarin, L., Oury, F. X., Heumez, E., ... & Charmet, G. (2014). Genome-wide association mapping of three important traits using bread wheat elite breeding populations. *Molecular breeding*, *33*(4), 755-768.

Bose, J., Rodrigo-Moreno, A., & Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of experimental botany*, *65*(5), 1241-1257.

Bossé, Y., & Amos, C. I. (2018). A Decade of GWAS Results in Lung Cancer GWAS-Nominated Lung Cancer Loci. *Cancer Epidemiology, Biomarkers & Prevention*, *27*(4), 363-379.

Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., ... & Roux, F. (2010). Linkage and association mapping of Arabidopsis thaliana flowering time in nature. *PLoS genetics*, *6*(5), e1000940.

Bray, E. A. (2000). Response to abiotic stress. *Biochemistry and molecular biology of plants* (pp.1158-1203).

Breseghello, F., & Sorrells, M. E. (2006). Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics*, *172*(2), 1165-1177.

Bruckner, P. L., & Frohberg, R. C. (1987). Stress tolerance and adaptation in spring wheat 1. *Crop Science*, *27*(1), 31-36.

Brzyski, D., Peterson, C. B., Sobczyk, P., Candès, E. J., Bogdan, M., & Sabatti, C. (2017). Controlling the rate of GWAS false discoveries. *Genetics*, *205*(1), 61-75.

Bussotti, F., Desotgiu, R., Cascio, C., Pollastrini, M., Gravano, E., Gerosa, G., ... & Strasser, R. J. (2011). Ozone stress in woody plants assessed with chlorophyll a fluorescence. A critical reassessment of existing data. *Environmental and Experimental Botany*, *73*, 19-30.

Byrne, P. F., Bolanos, J., Edmeades, G. O., & Eaton, D. L. (1995). Gains from selection under drought versus multilocation testing in related tropical maize populations. *Crop Science*, *35*(1), 63-69.

Ceccarelli, S. (1987). Yield potential and drought tolerance of segregating populations of barley in contrasting environments. *Euphytica*, *36*(1), 265-273.

Ceccarelli, S., & Grando, S. (1991). Selection environment and environmental sensitivity in barley. *Euphytica*, *57*(2), 157-167.

Chandran, D. (2015). Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. *IUBMB life*, *67*(7), 461-471.

Chao, S., Dubcovsky, J., Dvorak, J., Luo, M. C., Baenziger, S. P., Matnyazov, R., ... & Akhunov, E. D. (2010). Population-and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). *BMC genomics*, *11*(1), 1-17.

Chen, G., Liu, C., Gao, Z., Zhang, Y., Jiang, H., Zhu, L., ... & Qian, Q. (2017). OsHAK1, a high-affinity potassium transporter, positively regulates responses to drought stress in rice. *Frontiers in plant science*, *8*, 1885.

Chen, T. H., & Murata, N. (2008). Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in plant science*, *13*(9), 499-505.

Chen, X. Q., & Yu, B. J. (2007). Ionic effects of Na^+ and Cl^- on photosynthesis in Glycine max seedlings under isoosmotic salt stress. *Journal of plant physiology and molecular biology*, *33*(4), 294-300.

Clark, A. J., Landolt, W., Bucher, J. B., & Strasser, R. J. (2000). Beech (Fagus sylvatica) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. *Environmental Pollution*, *109*(3), 501-507.

Clarke, J. M., DePauw, R. M., & Townley-Smith, T. F. (1992). Evaluation of methods for quantification of drought tolerance in wheat. *Crop Science*, *32*(3), 723-728.

Clarke, J. M., Townley-Smith, F., McCaig, T. N., & Green, D. G. (1984). Growth analysis of spring wheat cultivars of varying drought resistance 1. *Crop Science*, *24*(3), 537-541.

Colmer, T. D., Epstein, E., & Dvorak, J. (1995). Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-tolerant wheat x Lophopyrum elongatum (Host) A. Love amphiploid. *Plant physiology*, *108*(4), 1715-1724.

Cramer, G. R., Alberico, G. J., & Schmidt, C. (1994). Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Functional Plant Biology*, *21*(5), 675-692.

Cuin, T. A., & Shabala, S. (2005). Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant and Cell Physiology*, *46*(12), 1924-1933.

Cuin, T. A., Parsons, D., & Shabala, S. (2010). Wheat cultivars can be screened for NaCl salinity tolerance by measuring leaf chlorophyll content and shoot sap potassium. *Functional Plant Biology*, *37*(7), 656-664.

Dadshani, S. A. W. (2018). Genetic and physiological characterization of traits related to salinity tolerance in an advanced backcross population of wheat (Doctoral dissertation, Universitäts-und Landesbibliothek Bonn).

Darwish, M. A. H., Farhat, W. Z. E., & Elsabagh, A. (2018). Inheritance of some agronomic characters and rusts resistance in fifteen F2 wheat populations. *Cercetări Agronomice în Moldova*, *1*(173), 5-28.

De Oliveira, A. B., Alencar, N. L. M., & Gomes-Filho, E. (2013). Comparison between the water and salt stress effects on plant growth and development. *Responses of organisms to water stress* (pp.67-94).

Demiroğlu, G., Khalvati, M. A., & Avcioğlu, R. (2001). Effect of Different Salt Concentrations on the Resistance of Maize Cultivars 2. Some Physiological Characteristics and Ion Accumulation in Early Growt. *Turkish Journal of Field Crops*, *6*(2), 55-60.

Deng, W., Nickle, D. C., Learn, G. H., Maust, B., & Mullins, J. I. (2007). ViroBLAST: a stand-alone BLAST web server for flexible queries of multiple databases and user's datasets. *Bioinformatics, 23*(17), 2334-2336.

Din, J., Khan, S. U., & Ali, I. (2008). Physiological response of wheat (*Triticum aesitivum* L.) varieties as influenced by salinity stress. *Journal of Animal and Plant Sciences*, *18*(4), 125-129.

Ding, J., Li, H., Wang, Y., Zhao, R., Zhang, X., Chen, J., ... & Wu, J. (2012). Fine mapping of *Rscmv2*, a major gene for resistance to sugarcane mosaic virus in maize. *Molecular breeding*, *30*(4), 1593-1600.

Dith Gagnon, É., Forbush, B., Caron, L., & Isenring, P. (2003). Functional comparison of renal Na-K-Cl cotransporters between distant species. *American Journal of Physiology-Cell Physiology*, *284*(2), 365-370.

Dong, L., Ravelombola, W., Weng, Y., Qin, J., Zhou, W., Bhattarai, G., ... & Shi, A. (2019). Change in chlorophyll content over time well-differentiated salt-tolerant, moderately salt-tolerant, and salt-susceptible cowpea genotypes. *HortScience, 54*(9), 1477-1484.

Du, X., Xu, W., Peng, C., Li, C., Zhang, Y., & Hu, L. (2021). Identification and validation of a novel locus, Qpm-3BL, for adult plant resistance to powdery mildew in wheat using multi locus GWAS. *BMC plant biology*, *21*(1), 1-13.

Edae, E. A., Byrne, P. F., Haley, S. D., Lopes, M. S., & Reynolds, M. P. (2014). Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theoretical and applied genetics*, *127*(4), 791-807.

El Moukhtari, A., Cabassa-Hourton, C., Farissi, M., & Savouré, A. (2020). How does proline treatment promote salt stress tolerance during crop plant development?. *Frontiers in plant science*, *11*, 1127.

El-Hendawy, S. E., Hu, Y., & Schmidhalter, U. (2007). Assessing the suitability of various physiological traits to screen wheat genotypes for salt tolerance. *Journal of Integrative Plant Biology*, *49*(9), 1352-1360.

El-Hendawy, S. E., Ruan, Y., Hu, Y., & Schmidhalter, U. (2009). A comparison of screening criteria for salt tolerance in wheat under field and controlled environmental conditions. *Journal of Agronomy and Crop Science*, *195*(5), 356-367.

FAOSTAT (2017). FAO, Food and Agriculture Organization of the United Nations; http://www.fao.org/faostat/; (Last accessed 1 October 2017).

Fernandez, G. C. (1992). Effective selection criteria for assessing plant stress tolerance. In *Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress* (pp.257-270). Shanhua, Taiwan

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, *29*(5), 897-912.

Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *The New Phytologist*, *179*(4), 945–963.

Flowers, T. J., & Yeo, A. R. (1995). Breeding for salinity resistance in crop plants: where next?. *Functional Plant Biology*, *22*(6), 875-884.

Flowers, T. J., Galal, H. K., & Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology*, *37*(7), 604-612.

Flowers, T. J., Troke, P. F., & Yeo, A. R. (1977). The mechanism of salt tolerance in halophytes. *Annual review of plant physiology*, *28*(1), 89-121.

Foresman, B. J., Oliver, R. E., Jackson, E. W., Chao, S., Arruda, M. P., & Kolb, F. L. (2016). Genome-wide association mapping of barley yellow dwarf virus tolerance in spring oat (*Avena sativa* L.). *PloS one*, *11*(5), e0155376.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., ... & Dubcovsky, J. (2009). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *science*, *323*(5919), 1357-1360.

Gallagher, M. D., & Chen-Plotkin, A. S. (2018). The post-GWAS era: from association to function. *The American Journal of Human Genetics*, *102*(5), 717-730.

Gamba, G. (2005). Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. *Physiological reviews*, *85*(2), 423-493.

Gaspar, T., Franck, T., Bisbis, B., Kevers, C., Jouve, L., Hausman, J. F., & Dommes, J. (2002). Concepts in plant stress physiology. Application to plant tissue cultures. *Plant growth regulation*, *37*(3), 263-285.

Gautam, T., Saripalli, G., Gahlaut, V., Kumar, A., Sharma, P. K., Balyan, H. S., & Gupta, P. K. (2019). Further studies on sugar transporter (SWEET) genes in wheat (*Triticum aestivum* L.). *Molecular Biology Reports*, *46*(2), 2327-2353.

Genc, Y., Tester, M., & McDonald, G. K. (2010). Calcium requirement of wheat in saline and non-saline conditions. *Plant and Soil*, *327*(1), 331-345.

Gilmour, A. R., Thompson, R., & Cullis, B. R. (1995). Average information REML: an efficient algorithm for variance parameter estimation in linear mixed models. *Biometrics, 51*, 1440-1450.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., ... & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *science*, *327*(5967), 812-818.

Gorlov, I. P., Moore, J. H., Peng, B., Jin, J. L., Gorlova, O. Y., & Amos, C. I. (2014). SNP characteristics predict replication success in association studies. *Human genetics*, *133*(12), 1477-1486.

Gorlova, O. Y., Xiao, X., Tsavachidis, S., Amos, C. I., & Gorlov, I. P. (2022). SNP characteristics and validation success in genome wide association studies. *Human Genetics*, *141*(2), 229-238.

Grimmer, M. K., Boyd, L. A., Clarke, S. M., & Paveley, N. D. (2015). Pyramiding of partial disease resistance genes has a predictable, but diminishing, benefit to efficacy. *Plant Pathology*, *64*(3), 748-753.

Guellim, A., Catterou, M., Chabrerie, O., Tetu, T., Hirel, B., Dubois, F., ... & Kichey, T. (2019). Identification of phenotypic and physiological markers of salt stress tolerance in durum wheat (Triticum durum Desf.) through Integrated Analyses. *Agronomy*, *9*(12), 844.

Guidi, L., Degl'Innocenti, E., & Soldatini, G. F. (2002). Assimilation of CO^2, enzyme activation and photosynthetic electron transport in bean leaves, as affected by high light and ozone. *New Phytologist*, *156*(3), 377-388.

Gupta, P. K., Balyan, H. S., & Gahlaut, V. (2017). QTL analysis for drought tolerance in wheat: present status and future possibilities. *Agronomy*, *7*(1), 5.

Han, L., Li, J. L., Wang, L., Shi, W. M., & Su, Y. H. (2015). Identification and localized expression of putative K+/H+ antiporter genes in Arabidopsis. *Acta Physiologiae Plantarum*, *37*(5), 1-14.

Haq, T. U., Gorham, J., Akhtar, J., Akhtar, N., & Steele, K. A. (2010). Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. *Functional Plant Biology*, *37*(7), 634-645.

Horn, F., Habekuß, A., & Stich, B. (2014). Genes involved in barley yellow dwarf virus resistance of maize. *Theoretical and Applied Genetics*, *127*(12), 2575-2584.

Horton, P., Ruban, A. V., & Walters, R. G. (1996). Regulation of light harvesting in green plants. *Annual review of plant biology*, *47*(1), 655-684.

Horwitz, T., Lam, K., Chen, Y., Xia, Y., & Liu, C. (2019). A decade in psychiatric GWAS research. *Molecular psychiatry*, *24*(3), 378-389.

Hossain, A. B. S., Sears, R. G., Cox, T. S., & Paulsen, G. M. (1990). Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop Science*, *30*(3), 622-627.

Hourcade, D., Bogard, M., Bonnefoy, M., Savignard, F., Mohamadi, F., Lafarge, S., ... & Cohan, J. P. (2019). Genome-wide association analysis of resistance to wheat spindle streak mosaic virus in bread wheat. *Plant Pathology*, *68*(3), 609-616.

Hu, C. A., Delauney, A. J., & Verma, D. P. (1992). A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. *Proceedings of the National Academy of Sciences*, *89*(19), 9354-9358.

Huang, B. (2000). Role of root morphological and physiological characteristics in drought resistance of plants. *Plant-Environment Interactions* (pp. 39-64). Marcel Dekker Inc., New York.

Husain, S., von Caemmerer, S., & Munns, R. (2004). Control of salt transport from roots to shoots of wheat in saline soil. *Functional Plant Biology*, *31*(11), 1115-1126.

Isla, R., Aragüés, R., & Royo, A. (1998). Validity of various physiological traits as screening criteria for salt tolerance in barley. *Field Crops Research*, *58*(2), 97-107.

Jahan, M. A. H. S., Hossain, A., Jaime, A., Da Silva, T., El Sabagh, A., Rashid, M. H., & Barutçular, C. (2019). Effect of naphthaleneacetic acid on root and plant growth and yield of ten irrigated wheat genotypes. *Pakistan Journal of Botany*, *51*(2), 451-459.

Jamil, A., Riaz, S., Ashraf, M., & Foolad, M. R. (2011). Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences*, *30*(5), 435-458.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic acids research, 36*(suppl_2), W5-W9.

Jones, R. G., & Gorham, J. (1983). Aspects of salt and drought tolerance in higher plants. *Genetic Engineering of Plants*,*26*, 355-370.

Katsuhara, M., Mimura, T., & Tazawa, M. (1990). ATP-regulated ion channels in the plasma membrane of a Characeae alga, *Nitellopsis obtusa*. *Plant physiology*, *93*(1), 343-346.

Kersey, P. J., Allen, J. E., Armean, I., Boddu, S., Bolt, B. J., Carvalho-Silva, D., ... & Staines, D. M. (2016). Ensembl Genomes 2016: more genomes, more complexity. *Nucleic acids research*, *44*(D1), D574-D580.

Khan, A. S., Asad, M. A., & Ali, Z. (2003). Assessment of genetic variability for NaCl tolerance in wheat. *Pakistan Journal of Agricultural Sciences*, *40*(1), 33-36.

Khan, M. A., Shirazi, M. U., Khan, M. A., Mujtaba, S. M., Islam, E., Mumtaz, S., ... & Ashraf, M. Y. (2009). Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*, *41*(2), 633-638.

Kiani-Pouya, A., & Rasouli, F. (2014). The potential of leaf chlorophyll content to screen bread-wheat genotypes in saline condition. *Photosynthetica*, *52*(2), 288-300.

Kibria, M. G., Hossain, M., Murata, Y., & Hoque, M. A. (2017). Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Science*, *24*(3), 155-162.

Kishor, P. K., Sangam, S., Amrutha, R. N., Laxmi, P. S., Naidu, K. R., Rao, K. S., ... & Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current science*,*88*(3), 424-438.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant methods*, *9*(1), 1-9.

Kumari, P. H., Kumar, S. A., Suravajhala, P., Jalaja, N., Giri, P. R., & Kavi Kishor, P. B. (2014). Contribution of bioinformatics to gene discovery in salt stress responses in plants. *Agricultural Bioinformatics*,109-127.

Li, H., Peng, Z., Yang, X., Wang, W., Fu, J., Wang, J., ... & Yan, J. (2013). Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. *Nature genetics*, *45*(1), 43-50.

Liang, B., Ding, H., Huang, L., Luo, H., & Zhu, X. (2020). GWAS in cancer: progress and challenges. *Molecular Genetics and Genomics*, *295*(3), 537-561.

Liu, C., Hua, J., Liu, C., Zhang, D., Hao, Z., Yong, H., ... & Li, X. (2016). Fine mapping of a quantitative trait locus conferring resistance to maize rough dwarf disease. *Theoretical and applied genetics*, *129*(12), 2333-2342.

Liu, L., Wang, B., Liu, D., Zou, C., Wu, P., Wang, Z., ... & Li, C. (2020). Transcriptomic and metabolomic analyses reveal mechanisms of adaptation to salinity in which carbon and nitrogen metabolism is altered in sugar beet roots. *BMC plant biology, 20*(1), 1-21.

Liu, S., Yang, X., Zhang, D., Bai, G., Chao, S., & Bockus, W. (2014). Genome-wide association analysis identified SNPs closely linked to a gene resistant to Soil-borne wheat mosaic virus. *Theoretical and Applied Genetics*, *127*(5), 1039-1047.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ∆∆CT method. *methods, 25*(4), 402-408.

Lu, P., Guo, L., Wang, Z., Li, B., Li, J., Li, Y., ... & Liu, Z. (2020). A rare gain of function mutation in a wheat tandem kinase confers resistance to powdery mildew. *Nature communications*, *11*(1), 1-11.

Luo, J. Y., Zhang, S., Peng, J., Zhu, X. Z., Lv, L. M., Wang, C. Y., ... & Cui, J. J. (2017). Effects of soil salinity on the expression of Bt toxin (Cry1Ac) and the control efficiency of Helicoverpa armigera in field-grown transgenic Bt cotton. *PloS one*, *12*(1), e0170379.

Lutts, S., Kinet, J. M., & Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of botany*, *78*(3), 389-398.

Lv, M., Deng, C., Li, X., Zhao, X., Li, H., Li, Z., ... & Ding, J. (2021). Identification and fine-mapping of RppCML496, a major QTL for resistance to Puccinia polysora in maize. *The Plant Genome*, *14*(1), e20062.

Ma, J. J., & Han, M. (2016). Genomewide analysis of ABCBs with a focus on ABCB1 and ABCB19 in Malus domestica. *Journal of genetics*, *95*(1), 141-149.

Maas, E. V., & Hoffman, G. J. (1977). Crop salt tolerance—current assessment. *Journal of the irrigation and drainage division*, *103*(2), 115-134.

Maischak, H., Zimmermann, M. R., Felle, H. H., Boland, W., & Mithöfer, A. (2010). Alamethicin-induced electrical long distance signaling in plants. *Plant signaling & behavior*, *5*(8), 988-990.

Mansour, E., Moustafa, E. S., Desoky, E. S. M., Ali, M. M., Yasin, M. A., Attia, A., ... & El-Hendawy, S. (2020). Multidimensional evaluation for detecting salt tolerance of bread wheat genotypes under actual saline field growing conditions. *Plants, 9*(10), 1324.

Marigorta, U. M., Rodríguez, J. A., Gibson, G., & Navarro, A. (2018). Replicability and prediction: lessons and challenges from GWAS. *Trends in Genetics*, *34*(7), 504-517.

Markwell, J., Osterman, J. C., & Mitchell, J. L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis research, 46*(3), 467-472.

Mccaig, T. N., & Clarke, J. M. (1982). Seasonal Changes in Nonstructural Carbohydrate Levels of Wheat and Oats Grown in a Semiarid Environment 1. *Crop Science*, *22*(5), 963-970.

Merelli, I., Calabria, A., Cozzi, P., Viti, F., Mosca, E., & Milanesi, L. (2013). SNPranker 2.0: a gene-centric data mining tool for diseases associated SNP prioritization in GWAS. *BMC bioinformatics*, *14*(1), 1-12.

Mian, A., Oomen, R. J., Isayenkov, S., Sentenac, H., Maathuis, F. J., & Véry, A. A. (2011). Over-expression of an Na^+ and K^+ permeable HKT transporter in barley improves salt tolerance. *The Plant Journal*, *68*(3), 468-479.

Milner, M. J., Seamon, J., Craft, E., & Kochian, L. V. (2013). Transport properties of members of the ZIP family in plants and their role in Zn and Mn homeostasis. *Journal of experimental botany*, *64*(1), 369-381.

Minolta, K. (1989). *Chlorophyll meter SPAD-502 instruction manual.* Minolta Co, Ltd, Radiometric Instruments Operations Osaka, Japan.

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. *Current science*,*80*(6) 758-763.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G. A. D., Tognetti, V. B., Vandepoele, K., ... & Van Breusegem, F. (2011). ROS signaling: the new wave?. *Trends in plant science*, *16*(6), 300-309.

Mondal, S., Sallam, A., Sehgal, D., Sukumaran, S., Farhad, M., Navaneetha Krishnan, J., ... & Biswal, A. (2021). Advances in breeding for abiotic stress tolerance in wheat. *Genomic Designing for Abiotic Stress Resistant Cereal Crops* (pp.71-103). Springer.

Mourad, A. M., Alomari, D. Z., Alqudah, A. M., Sallam, A., & Salem, K. F. (2019). Recent advances in wheat (*Triticum* spp.) breeding. *Advances in plant breeding strategies:* cereals (pp.559-593). Springer.

Moursi, Y. S., Thabet, S. G., Amro, A., Dawood, M. F., Baenziger, P. S., & Sallam, A. (2020). Detailed genetic analysis for identifying QTLs associated with drought tolerance at seed germination and seedling stages in barley. *Plants, 9*(11), 1425.

Munns, R. (1993). Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant, Cell & Environment*, *16*(1), 15-24.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, cell & environment*, *25*(2), 239-250.

Munns, R. (2005). Genes and salt tolerance: bringing them together. *New phytologist*, *167*(3), 645-663.

Munns, R., & Gilliham, M. (2015). Salinity tolerance of crops–what is the cost?. *New phytologist*, *208*(3), 668-673.

Munns, R., & James, R. A. (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and soil*, *253*(1), 201-218.

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*, 651-681.

Munns, R., James, R. A., & Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of experimental botany*, *57*(5), 1025-1043.

Munns, R., James, R. A., Xu, B., Athman, A., Conn, S. J., Jordans, C., ... & Gilliham, M. (2012). Wheat grain yield on saline soils is improved by an ancestral Na$^+$ transporter gene. *Nature biotechnology*, *30*(4), 360-364.

Muranaka, S., Shimizu, K., & Kato, M. (2002). Ionic and osmotic effects of salinity on single-leaf photosynthesis in two wheat cultivars with different drought tolerance. *Photosynthetica*, *40*(2), 201-207.

Myint, T., Ismawanto, S., Namasivayam, P., Napis, S., & Abdulla, M. P. (2015). Expression analysis of the ADH genes in Arabidopsis plants exposed to PEG-induced water stress. *World Journal of Agricultural Research*, *3*, 57-65.

Nagaraj, V. J., Altenbach, D., Galati, V., Lüscher, M., Meyer, A. D., Boller, T., & Wiemken, A. (2004). Distinct regulation of sucrose: sucrose-1-fructosyltransferase (1-SST) and sucrose: fructan-6-fructosyltransferase (6-SFT), the key enzymes of fructan synthesis in barley leaves: 1-SST as the pacemaker. *New Phytologist*, *161*(3), 735-748.

Netondo, G. W., Onyango, J. C., & Beck, E. (2004). Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop science*, *44*(3), 806-811.

Netto, A. T., Campostrini, E., de Oliveira, J. G., & Bressan-Smith, R. E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulturae*, *104*(2), 199-209.

Oyiga, B. C. (2017). Genetic variation of traits related to salt stress response in Wheat (*Triticum aestivum* L.) (Doctoral dissertation, Universitäts-und Landesbibliothek Bonn).

Oyiga, B. C., Ogbonnaya, F. C., Sharma, R. C., Baum, M., Léon, J., & Ballvora, A. (2019). Genetic and transcriptional variations in NRAMP-2 and OPAQUE1 genes are associated with salt stress response in wheat. *Theoretical and Applied Genetics*, *132*(2), 323-346.

Oyiga, B. C., Sharma, R. C., Baum, M., Ogbonnaya, F. C., Léon, J., & Ballvora, A. (2018). Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. *Plant, cell & environment*, *41*(5), 919-935.

Oyiga, B. C., Sharma, R. C., Shen, J., Baum, M., Ogbonnaya, F. C., Léon, J., & Ballvora, A. (2016). Identification and characterization of salt tolerance of wheat germplasm using a multivariable screening approach. *Journal of Agronomy and Crop Science*, *202*(6), 472-485.

Pak, V. A., Nabipour, M., & Meskarbashee, M. (2009). Effect of salt stress on chlorophyll content, fluorescence, Na^+ and K^+ ions content in rape plants (*Brassica napus* L.). *Asian Journal of Agricultural Research*, *3*(2), 28-37.

Pfrieme, A. K., Ruckwied, B., Habekuß, A., Will, T., Stahl, A., Pillen, K., & Ordon, F. (2022). Identification and validation of QTL for WDV (Wheat dwarf virus) resistance in wheat (*Triticum* spp.). *Frontiers in Plant Science*, *13*, 483.

Pham, F., Raheja, A., & Bhandari, S. (2019, May). Machine learning models for predicting lettuce health using UAV imageries. In *Autonomous Air and Ground Sensing Systems for Agricultural Optimization and Phenotyping IV* (pp. 188-201). SPIE.

Photon Systems Instruments (2016). FluorPen & PAR FluorPen. https://handheld.psi.cz/products/fluorpen-and-par-fluorpen/#download.

Piechotta, K., Lu, J., & Delpire, E. (2002). Cation chloride cotransporters interact with the stress-related kinases Ste20-related proline-alanine-rich kinase (SPAK) and oxidative stress response 1 (OSR1). *Journal of Biological Chemistry*, *277*(52), 50812-50819.

Pingault, L., Choulet, F., Alberti, A., Glover, N., Wincker, P., Feuillet, C., & Paux, E. (2015). Deep transcriptome sequencing provides new insights into the structural and functional organization of the wheat genome. *Genome biology*, *16*(1), 1-15.

Poustini, K., Siosemardeh, A., & Ranjbar, M. (2007). Proline accumulation as a response to salt stress in 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Genetic Resources and Crop Evolution*, *54*(5), 925-934.

Puram, V. R. R., Ontoy, J., Linscombe, S., & Subudhi, P. K. (2017). Genetic dissection of seedling stage salinity tolerance in rice using introgression lines of a salt tolerant landrace Nona Bokra. *Journal of Heredity*, *108*(6), 658-670.

Qadir, M., Tubeileh, A., Akhtar, J., Larbi, A., Minhas, P. S., & Khan, M. A. (2008). Productivity enhancement of salt-affected environments through crop diversification. *Land degradation & development*, *19*(4), 429-453.

Quraishi, U. M., Murat, F., Abrouk, M., Pont, C., Confolent, C., Oury, F. X., ... & Salse, J. (2011). Combined meta-genomics analyses unravel candidate genes for the grain dietary fiber content in bread wheat (*Triticum aestivum* L.). *Functional & integrative genomics*, *11*(1), 71-83.

R Core Team (2020). R: A Language and Environment for Statistical Computing. Vienna: The R Foundation.

Rajaram, S., & Van Ginkle, M. (2001). Mexico, 50 years of international wheat breeding. *The world wheat book: A history of wheat breeding.* Lavoisier Publishing, Paris, France.

Rao, S. A., & McNeilly, T. (1999). Genetic basis of variation for salt tolerance in maize (*Zea mays* L). *Euphytica*, *108*(3), 145-150.

Rashid, Z., Kaur, H., Babu, V., Singh, P. K., Harlapur, S. I., & Nair, S. K. (2021). Identification and validation of genomic regions associated with charcoal rot resistance in tropical maize by genome-wide association and linkage mapping. *Frontiers in plant science*, *12*, 726767.

Rathjen, A. J. (1994). The biological basis of genotype× environment interaction: its definition and management. In *Proceedings of the Seventh Assembly of the Wheat Breeding Society of Australia* (pp.13-17). Adelaide, Australia.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PloS one*, *8*(6), e66428.

Ren, Z. H., Gao, J. P., Li, L. G., Cai, X. L., Huang, W., Chao, D. Y., ... & Lin, H. X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature genetics*, *37*(10), 1141-1146.

Richards, R. A. (1996). Defining selection criteria to improve yield under drought. *Plant growth regulation*, *20*(2), 157-166.

Robinson, H. F., Comstock, R. E., & Harvey, P. H. (1949). Estimates of heritability and the degree of dominance in corn. *Agronomy Journal*, *41*(8), 353-359.

Rodríguez, M., Canales, E., & Borrás-Hidalgo, O. (2005). Molecular aspects of abiotic stress in plants. *Biotecnología Aplicada*, *22*(1), 1-10.

Roy, S. J., Negrão, S., & Tester, M. (2014). Salt resistant crop plants. Current opinion in Biotechnology, *26*, 115-124.

Roychoudhury, A., & Basu, S. (2008). Overexpression of an abiotic-stress inducible plant protein in the bacteria Escherichia coli. *African Journal of Biotechnology*, *7*(18), 3231–3234.

Said, A., Moursi, Y., & Sallam, A. (2022). Association mapping and candidate genes for physiological non-destructive traits: Chlorophyll content, canopy temperature, and specific leaf area under normal and saline conditions in wheat. *Frontiers in genetics, 13*.

Sanchez, D. H., Siahpoosh, M. R., Roessner, U., Udvardi, M., & Kopka, J. (2008). Plant metabolomics reveals conserved and divergent metabolic responses to salinity. *Physiologia plantarum*, *132*(2), 209-219.

Saqib, Z. A., Akhtar, J., Ul-Haq, M. A., Ahmad, I., & Bakhat, H. F. (2012). Rationality of using various physiological and yield related traits in determining salt tolerance in wheat. *African Journal of Biotechnology*, *11*(15), 3558-3568.

Saradadevi, R., Mukankusi, C., Li, L., Amongi, W., Mbiu, J. P., Raatz, B., ... & Cowling, W. A. (2021). Multivariate genomic analysis and optimal contributions selection predicts high genetic gains in cooking time, iron, zinc, and grain yield in common beans in East Africa. *The Plant Genome, 14*(3), e20156.

Sarwar, S.; Ashraf, M. Y. & Naeem, M. H. (2003) Genetic Variability of Some Primitive Bread Wheat Varieties to Salt Tolerance. *Pakistan Journal of Botany*, *35*(5), 771–777.

SAS Institute. Base SAS 9.4 Procedures Guide. Cary, NC: SAS Institute. 2015.

Schachtman, D. P., & Munns, R. (1992). Sodium accumulation in leaves of Triticum species that differ in salt tolerance. *Functional Plant Biology*, *19*(3), 331-340.

Seo, P. J., Park, J. M., Kang, S. K., Kim, S. G., & Park, C. M. (2011). An Arabidopsis senescence-associated protein SAG29 regulates cell viability under high salinity. *Planta*, *233*(1), 189-200.

Shabala, S. (2013). Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of botany*, *112*(7), 1209-1221.

Shahbaz, M., Ashraf, M., Akram, N. A., Hanif, A., Hameed, S., Joham, S., & Rehman, R. (2011). Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). *Acta Physiologiae Plantarum*, *33*(4), 1113-1122.

Shahbaz, M., Ashraf, M., Al-Qurainy, F., & Harris, P. J. (2012). Salt tolerance in selected vegetable crops. *Critical Reviews in Plant Sciences*, *31*(4), 303-320.

Shannon, M. C., Rhoades, J. D., Draper, J. H., Scardaci, S. C., & Spyres, M. D. (1998). Assessment of salt tolerance in rice cultivars in response to salinity problems in California. *Crop Science*, *38*(2), 394-398.

Sharp, R. E., Hsiao, T. C., & Silk, W. K. (1990). Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiology, 93*(4), 1337-1346.

Shi, H., Liu, W., Yao, Y., Wei, Y., & Chan, Z. (2017). Alcohol dehydrogenase 1 (ADH1) confers both abiotic and biotic stress resistance in Arabidopsis. *Plant Science*, *262*, 24-31.

Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, *5*(3), 291-317.

Siddiqui, M. N., Mostofa, M. G., Akter, M. M., Srivastava, A. K., Sayed, M. A., Hasan, M. S., & Tran, L. S. P. (2017). Impact of salt-induced toxicity on growth and yield-potential of local wheat cultivars: oxidative stress and ion toxicity are among the major determinants of salt-tolerant capacity. *Chemosphere*, *187*, 385-394.

Singh, V., Singh, A. P., Bhadoria, J., Giri, J., Singh, J., TV, V., & Sharma, P. C. (2018). Differential expression of salt-responsive genes to salinity stress in salt-tolerant and salt-sensitive rice (Oryza sativa L.) at seedling stage. *Protoplasma*, *255*(6), 1667-1681.

Soloviev, A. A., Kuklev, M. Y., & Karnaukhova, T. V. (2003). The Functional Status of Some Genotypes of Tomato Plants under Salty Conditions in Greenhouse. *Acta Horticulturae, 609*,47-50.

Sonah, H., O'Donoughue, L., Cober, E., Rajcan, I., & Belzile, F. (2015). Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. *Plant biotechnology journal*, *13*(2), 211-221.

Song, Z., Yang, S., Zhu, H., Jin, M., & Su, Y. (2014). Heterologous expression of an alligatorweed high-affinity potassium transporter gene enhances salinity tolerance in Arabidopsis thaliana. *American Journal of Botany*, *101*(5), 840-850.

Su, W., Ren, Y., Wang, D., Su, Y., Feng, J., Zhang, C., ... & Que, Y. (2020). The alcohol dehydrogenase gene family in sugarcane and its involvement in cold stress regulation. *BMC genomics*, *21*(1), 1-17.

Sukumaran, S., & Yu, J. (2014). Association mapping of genetic resources: achievements and future perspectives. *Genomics of plant genetic* resources (pp.207-235). Springer

Sukumaran, S., Dreisigacker, S., Lopes, M., Chavez, P., & Reynolds, M. P. (2015). Genome-wide association study for grain yield and related traits in an elite spring wheat population

grown in temperate irrigated environments. *Theoretical and applied genetics*, *128*(2), 353-363.

Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in plant science*, *15*(2), 89-97.

Tang, H., Krishnakumar, V., Bidwell, S., Rosen, B., Chan, A., Zhou, S., ... & Town, C. D. (2014). An improved genome release (version Mt4. 0) for the model legume Medicago truncatula. *BMC genomics*, *15*(1), 1-14.

Tavakkoli, E., Fatehi, F., Coventry, S., Rengasamy, P., & McDonald, G. K. (2011). Additive effects of Na+ and Cl–ions on barley growth under salinity stress. *Journal of Experimental Botany*, *62*(6), 2189-2203.

Tavakkoli, E., Rengasamy, P., & McDonald, G. K. (2010). High concentrations of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. *Journal of experimental botany*, *61*(15), 4449-4459.

Tavakkoli, E., Rengasamy, P., & McDonald, G. K. (2010). The response of barley to salinity stress differs between hydroponic and soil systems. *Functional Plant Biology*, *37*(7), 621-633.

Tester, M., & Davenport, R. (2003). Na^+ tolerance and Na^+ transport in higher plants. *Annals of botany*, *91*(5), 503-527.

Thabet, S. G., Moursi, Y. S., Sallam, A., Karam, M. A., & Alqudah, A. M. (2021). Genetic associations uncover candidate SNP markers and genes associated with salt tolerance during seedling developmental phase in barley. *Environmental and Experimental Botany, 188*, 104499.

Thomson, M. J., de Ocampo, M., Egdane, J., Rahman, M. A., Sajise, A. G., Adorada, D. L., ... & Ismail, A. M. (2010). Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. *Rice*, *3*(2), 148-160.

Tiwari, R. S., Picchioni, G. A., Steiner, R. L., Jones, D. C., Hughs, S. E., & Zhang, J. (2013). Genetic variation in salt tolerance at the seedling stage in an interspecific backcross inbred line population of cultivated tetraploid cotton. *Euphytica*, *194*(1), 1-11.

Tougou, M., Hashiguchi, A., Yukawa, K., Nanjo, Y., Hiraga, S., Nakamura, T., ... & Komatsu, S. (2012). Responses to flooding stress in soybean seedlings with the alcohol dehydrogenase transgene. *Plant Biotechnology*, *29*, 301–305.

U.S. Wheat Associates (2021). Wheat Letter Blog. https://www.uswheat.org/wheatletter/usw-has-published-a-world-wheat-supply-and-demand-outlook-report/.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., & Dubcovsky, J. (2006). A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, *314*(5803), 1298-1301.

Ud-Din, N., Carver, B. F., & Clutter, A. C. (1992). Genetic analysis and selection for wheat yield in drought-stressed and irrigated environments. *Euphytica*, *62*(2), 89-96.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., & Pleijel, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis research*, *91*(1), 37-46.

UNEP (2008). In Dead Water. Merging of Climate Change With Pollution, Over-Harvest, and Infestations in the World's Fishing Grounds. UNEP/GRID-Arendal, Arendal, Norway. Available online at: http://www.grida.no/_res/site/file/publications/InDeadWater_LR.pdf [Accessed on the 20 January 2009].

Verslues, P. E., Lasky, J. R., Juenger, T. E., Liu, T. W., & Kumar, M. N. (2014). Genome-wide association mapping combined with reverse genetics identifies new effectors of low water potential-induced proline accumulation in Arabidopsis. *Plant physiology*, *164*(1), 144-159.

Wang, G., Leonard, J. M., Ross, A. S., Peterson, C. J., Zemetra, R. S., Garland Campbell, K., & Riera-Lizarazu, O. (2012). Identification of genetic factors controlling kernel hardness and related traits in a recombinant inbred population derived from a soft×'extra-soft'wheat (*Triticum aestivum* L.) cross. *Theoretical and Applied genetics*, *124*(1), 207-221.

Wang, L., Yao, L., Hao, X., Li, N., Qian, W., Yue, C., ... & Wang, X. (2018). Tea plant SWEET transporters: expression profiling, sugar transport, and the involvement of CsSWEET16 in modifying cold tolerance in Arabidopsis. *Plant molecular biology*, *96*(6), 577-592.

Wang, M., & Xia, G. (2018). The landscape of molecular mechanisms for salt tolerance in wheat. *The crop journal*, *6*(1), 42-47.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., ... & Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*(6), 787-796.

Wang, T. T., Ren, Z. J., Liu, Z. Q., Feng, X., Guo, R. Q., Li, B. G., ... & Jing, H. C. (2014). SbHKT1; 4, a member of the high-affinity potassium transporter gene family from Sorghum bicolor, functions to maintain optimal Na$^+$/K$^+$ balance under Na$^+$ stress. *Journal of integrative plant biology*, *56*(3), 315-332.

Wang, W. X., Vinocur, B., Shoseyov, O., & Altman, A. (2000). Biotechnology of plant osmotic stress tolerance physiological and molecular considerations. *Acta horticulturae. 560*,285-292.

Wicke, B., Smeets, E., Dornburg, V., Vashev, B., Gaiser, T., Turkenburg, W., & Faaij, A. (2011). The global technical and economic potential of bioenergy from salt-affected soils. *Energy & Environmental Science*, *4*(8), 2669-2681.

Wu, D., Cai, S., Chen, M., Ye, L., Chen, Z., Zhang, H., ... & Zhang, G. (2013). Tissue metabolic responses to salt stress in wild and cultivated barley. *PLoS one*, *8*(1), e55431.

Xu, Z., & Taylor, J. A. (2009). SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic acids research*, *37*(suppl_2), W600-W605.

Yadav, S., Irfan, M., Ahmad, A., & Hayat, S. (2011). Causes of salinity and plant manifestations to salt stress: a review. *Journal of environmental biology*, *32*(5), 667- 685.

Yadawad, A., Hanchinal, R. R., Nadaf, H. L., Desai, S. A., Suma, B., & Naik, V. R. (2015). Genetic variability for yield parameters and rust resistance in F2 population of wheat (*Triticum aestivum* L.). *The Bioscan*, *10*(2), 707-710.

Yamada, K. D., Tomii, K., & Katoh, K. (2016). Application of the MAFFT sequence alignment program to large data—reexamination of the usefulness of chained guide trees. *Bioinformatics, 32*(21), 3246-3251.

Yassin, M., Fara, S. A., Hossain, A., Saneoka, H., & El Sabagh, A. (2019). Assessment of salinity tolerance bread wheat genotypes: using stress tolerance indices. *Fresenius Environmental Bulletin*, *28*(5), 4199-4217.

Yu, J., & Buckler, E. S. (2006). Genetic association mapping and genome organization of maize. *Current opinion in biotechnology*, *17*(2), 155-160.

Yu, J., Holland, J. B., McMullen, M. D., & Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics*, *178*(1), 539-551.

Zanke, C. D., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., ... & Röder, M. S. (2014). Whole genome association mapping of plant height in winter wheat (*Triticum aestivum* L.). *PloS one*, *9*(11), e113287.

Zanke, C. D., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., ... & Röder, M. S. (2015). Analysis of main effect QTL for thousand grain weight in European winter wheat (*Triticum aestivum* L.) by genome-wide association mapping. *Frontiers in plant science*, *6*, 644.

Zhang, J., Gizaw, S. A., Bossolini, E., Hegarty, J., Howell, T., Carter, A. H., ... & Dubcovsky, J. (2018). Identification and validation of QTL for grain yield and plant water status under contrasting water treatments in fall-sown spring wheats. *Theoretical and Applied Genetics*, *131*(8), 1741-1759.

Zhang, L., Dong, C., Chen, Z., Gui, L., Chen, C., Li, D., ... & Wang, J. (2021). WheatGmap: a comprehensive platform for wheat gene mapping and genomic studies. *Molecular Plant*, *14*(2), 187-190.

Zhang, M., Cao, Y., Wang, Z., Wang, Z. Q., Shi, J., Liang, X., ... & Jiang, C. (2018). A retrotransposon in an HKT1 family sodium transporter causes variation of leaf Na+ exclusion and salt tolerance in maize. *New Phytologist, 217*(3), 1161-1176.

Zhang, R., Niu, K., & Ma, H. (2020). Identification and expression analysis of the SWEET gene family from Poa pratensis under abiotic stresses. *DNA and Cell Biology*, *39*(9), 1606-1620.

Zhang, W., Chen, S., Abate, Z., Nirmala, J., Rouse, M. N., & Dubcovsky, J. (2017). Identification and characterization of Sr13, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group. *Proceedings of the national academy of sciences*, *114*(45), E9483-E9492.

Zhao, J., Ren, W., Zhi, D., Wang, L., & Xia, G. (2007). Arabidopsis DREB1A/CBF3 bestowed transgenic tall fescue increased tolerance to drought stress. *Plant Cell Reports*, *26*(9), 1521-1528.

Zhao, K., Aranzana, M. J., Kim, S., Lister, C., Shindo, C., Tang, C., ... & Nordborg, M. (2007). An Arabidopsis example of association mapping in structured samples. *PLoS genetics*, *3*(1), e4.

Zhao, K., Tung, C. W., Eizenga, G. C., Wright, M. H., Ali, M. L., Price, A. H., ... & McCouch, S. R. (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. *Nature communications*, *2*(1), 1-10.

Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and prospects of association mapping in plants. *The plant genome*, *1*(1), 5-20.

Zhu, J. K. (2001). Plant salt tolerance. *Trends in plant science*, *6*(2), 66-71.

Zhu, J. K. (2003). Regulation of ion homeostasis under salt stress. *Current opinion in plant biology*, *6*(5), 441-445.

Appendix

Appendix 1. Ranking different salt tolerance indices for 274 F$_3$ lines of cross Bobur*Altay2000.

Geno name	SWL	Ranking	STI_SFW	Ranking	STI_SDW	Ranking	SSI_SFW	Ranking	SSI_SDW	Ranking	TOL_SFW	Ranking	TOL_SDW	Ranking	ST Overall	ST %
P1G001	7.8	254	0.37	144	0.74	223	1.02	143	1.43	242	8.4	255	0.6	242	1503	78.36
P1G002	4.6	109	0.38	123	0.84	90	1.00	123	0.84	93	5.2	114	0.3	94	746	38.89
P1G003	5.1	132	0.34	179	0.76	203	1.05	170	1.04	153	5.7	142	0.5	215	1194	62.25
P1G004	5.0	125	0.36	148	0.83	94	1.03	153	0.92	119	5.3	117	0.3	100	856	44.63
P1G005	3.8	60	0.49	51	0.85	72	0.82	50	0.74	71	4.1	63	0.3	79	446	23.25
P1G006	4.0	73	0.50	45	0.92	14	0.82	47	0.45	15	4.2	65	0.2	16	275	14.34
P1G007	2.4	19	0.58	18	0.78	171	0.68	19	1.22	207	2.9	28	0.5	168	630	32.85
P1G008	5.7	176	0.43	81	0.88	51	0.93	82	0.65	55	5.9	168	0.3	59	672	35.04
P1G009	5.5	170	0.39	111	0.77	186	0.98	112	1.14	186	6.1	173	0.5	204	1142	59.54
P1G010	5.8	184	0.41	95	0.79	158	0.96	95	1.02	149	6.2	182	0.4	162	1025	53.44
P1G011	2.4	18	0.62	13	0.76	192	0.60	12	1.10	169	2.8	23	0.5	176	603	31.44
P1G012	6.7	225	0.35	166	0.75	207	1.06	175	1.39	236	7.3	228	0.6	221	1458	76.02
P1G013	6.5	215	0.38	128	0.88	47	1.01	132	0.68	60	6.8	204	0.2	52	838	43.69
P1G014	9.0	266	0.28	240	0.80	150	1.17	239	1.11	176	9.5	264	0.5	182	1517	79.09
P1G015	3.5	55	0.49	47	0.99	2	0.82	49	0.07	2	3.5	46	0.0	2	203	10.58
P1G016	5.5	165	0.41	89	0.85	78	0.96	93	0.84	91	5.8	156	0.3	89	761	39.68
P1G017	7.1	236	0.34	184	0.87	55	1.08	184	0.70	63	7.4	230	0.3	60	1012	52.76
P1G018	6.3	204	0.40	101	0.87	56	0.97	104	0.69	62	6.6	196	0.3	61	784	40.88
P1G019	4.8	115	0.43	79	0.71	248	0.93	85	1.54	251	5.5	133	0.7	249	1160	60.48
P1G020	3.2	48	0.47	60	0.81	133	0.85	60	1.01	144	3.6	50	0.4	125	620	32.33
P1G021	5.0	128	0.36	147	0.83	104	1.02	146	0.87	103	5.4	125	0.4	123	876	45.67
P1G022	4.4	99	0.45	68	0.89	28	0.88	69	0.54	28	4.7	90	0.2	36	418	21.79
P1G023	6.1	197	0.43	80	0.82	108	0.92	79	1.01	147	6.5	191	0.4	126	928	48.38
P1G024	7.3	240	0.29	230	0.80	154	1.15	229	1.03	151	7.7	236	0.4	145	1385	72.21
P1G025	5.4	162	0.38	132	0.88	54	1.01	138	0.72	68	5.7	145	0.2	51	750	39.10
P1G026	2.8	29	0.58	22	0.83	92	0.68	18	0.77	76	3.2	31	0.4	134	402	20.96
P1G027	6.0	194	0.35	163	0.79	169	1.06	172	1.18	198	6.5	192	0.5	169	1257	65.54

92

P1G028	1.2	3	0.73	5	0.84	89	0.44	4	0.88	106	1.5	3	0.3	73	283	14.75
P1G029	2.6	23	0.59	16	0.88	44	0.66	14	0.57	33	2.8	21	0.2	32	183	9.54
P1G030	3.8	64	0.47	62	0.82	117	0.88	68	0.95	127	4.2	66	0.4	127	631	32.90
P1G031	5.0	123	0.38	125	0.84	91	1.02	141	0.86	98	5.3	116	0.3	95	789	41.14
P1G032	1.8	9	0.88	2	0.98	4	0.67	16	0.57	31	2.0	8	0.2	19	89	4.64
P1G033	2.7	28	0.44	78	0.81	132	0.91	77	0.85	95	3.1	30	0.3	107	547	28.52
P1G034	6.7	222	0.24	265	0.64	264	1.23	264	1.86	266	7.5	233	0.9	261	1775	92.54
P1G035	4.0	72	0.46	67	0.88	40	0.90	70	0.61	46	4.2	68	0.2	38	401	20.91
P1G036	2.8	31	0.58	19	0.96	5	0.69	20	0.22	5	2.9	26	0.1	4	110	5.74
P1G037	4.2	85	0.36	155	0.73	232	1.04	157	1.28	219	4.8	98	0.6	224	1170	61.00
P1G038	6.0	192	0.31	212	0.78	175	1.12	211	1.11	173	6.4	188	0.5	172	1323	68.98
P1G039	5.4	159	0.30	214	0.77	181	1.12	213	1.15	189	5.9	164	0.5	167	1287	67.10
P1G040	6.6	220	0.36	153	0.82	112	1.02	145	1.06	159	7.0	214	0.4	133	1136	59.23
P1G041	3.0	38	0.55	28	0.82	119	0.74	28	1.00	142	3.4	41	0.4	121	517	26.96
P1G042	5.9	191	0.35	164	0.75	209	1.04	156	1.20	201	6.5	195	0.6	229	1345	70.13
P1G043	3.1	43	0.56	24	0.86	62	0.70	24	0.65	57	3.4	39	0.3	62	311	16.21
P1G044	6.3	207	0.29	226	0.76	199	1.15	230	1.34	227	6.8	206	0.5	183	1478	77.06
P1G045	4.4	96	0.36	149	0.81	145	1.02	140	0.94	125	4.8	97	0.4	131	883	46.04
P1G046	2.3	17	0.59	15	0.82	107	0.66	15	0.97	134	2.6	16	0.3	98	402	20.96
P1G047	3.6	59	0.36	146	0.88	48	1.03	150	0.65	52	3.9	59	0.2	43	557	29.04
P1G048	5.6	172	0.39	116	0.84	88	1.00	119	0.88	107	5.9	167	0.3	96	865	45.10
P1G049	2.0	12	0.52	33	0.78	180	0.77	32	1.09	167	2.4	14	0.4	158	596	31.07
P1G050	7.9	257	0.27	243	0.72	241	1.18	243	1.43	241	8.5	257	0.6	241	1723	89.83
P1G051	4.8	116	0.40	104	0.82	111	0.98	106	0.85	96	5.2	113	0.4	115	761	39.68
P1G052	4.6	110	0.42	84	0.82	121	0.94	86	0.88	105	5.0	108	0.4	113	727	37.90
P1G053	4.9	118	0.36	159	0.77	191	1.04	158	1.11	177	5.4	121	0.5	178	1102	57.46
P1G054	5.3	149	0.28	236	0.74	222	1.16	237	1.29	220	5.8	159	0.5	207	1430	74.56
P1G055	4.4	98	0.29	227	0.74	221	1.16	233	1.18	195	5.0	105	0.5	201	1280	66.74
P1G056	5.0	122	0.39	119	0.89	35	1.01	133	0.60	41	5.2	111	0.2	46	607	31.65
P1G057	5.3	156	0.36	152	0.79	167	1.03	149	1.06	157	5.8	155	0.5	171	1107	57.72

P1G058	4.2	89	0.48	58	0.90	23	0.85	59	0.53	25	4.4	80	0.2	21	355	18.51
P1G059	3.1	40	0.54	29	0.84	86	0.75	30	0.79	83	3.4	40	0.3	85	393	20.49
P1G060	3.9	69	0.38	129	0.82	118	1.01	129	0.87	100	4.3	72	0.4	114	731	38.11
P1G061	9.9	269	0.20	269	0.70	250	1.29	269	1.69	260	10.6	269	0.7	251	1837	95.78
P1G062	5.2	143	0.35	160	0.72	236	1.05	163	1.28	218	5.9	165	0.7	248	1333	69.50
P1G063	5.1	135	0.31	209	0.81	139	1.10	205	0.81	85	5.5	136	0.4	142	1051	54.80
P1G064	5.4	157	0.32	200	0.79	166	1.10	206	1.09	166	5.8	157	0.4	155	1207	62.93
P1G065	5.1	137	0.40	99	0.89	30	0.98	111	0.58	37	5.4	119	0.2	39	572	29.82
P1G066															0	0.00
P1G067	5.7	180	0.32	195	0.80	152	1.10	198	0.99	139	6.1	178	0.4	139	1181	61.57
P1G068	5.6	171	0.39	115	0.86	69	0.99	116	0.65	58	5.9	161	0.3	78	768	40.04
P1G069	7.0	233	0.16	270	0.68	256	1.34	270	1.38	235	7.7	235	0.7	246	1745	90.98
P1G070	5.7	179	0.29	231	0.83	103	1.16	236	0.98	136	6.0	171	0.3	106	1162	60.58
P1G071	5.2	139	0.46	66	0.87	61	0.87	65	0.65	53	5.4	127	0.3	63	574	29.93
P1G072	2.9	33	0.53	31	0.83	93	0.77	33	0.90	111	3.3	35	0.3	104	440	22.94
P1G073	7.4	243	0.30	218	0.77	189	1.14	220	1.35	228	7.9	244	0.5	199	1541	80.34
P1G074	3.6	57	0.44	73	0.93	11	0.90	71	0.41	13	3.7	52	0.1	9	286	14.91
P1G075	4.3	94	0.41	94	0.88	50	0.96	94	0.62	47	4.6	85	0.2	47	511	26.64
P1G076	4.1	79	0.34	180	0.82	115	1.06	171	0.91	114	4.4	79	0.3	91	829	43.22
P1G077	7.2	238	0.24	264	0.75	220	1.25	267	1.35	229	7.7	237	0.5	203	1658	86.44
P1G078	7.1	235	0.21	268	0.72	242	1.27	268	1.71	261	7.7	239	0.6	227	1740	90.72
P1G079	5.9	190	0.32	202	0.73	230	1.09	195	1.32	223	6.5	193	0.6	226	1459	76.07
P1G080	5.0	124	0.39	118	0.75	214	0.98	109	1.08	163	5.5	135	0.5	206	1069	55.74
P1G081	6.3	205	0.32	198	0.71	245	1.10	199	1.51	249	6.9	211	0.6	238	1545	80.55
P1G082	2.5	22	0.56	26	0.88	49	0.70	25	0.63	49	2.8	20	0.2	45	236	12.30
P1G083	3.9	68	0.39	110	0.75	213	0.97	102	1.06	161	4.4	76	0.5	173	903	47.08
P1G084	2.9	34	0.47	59	0.81	136	0.84	56	0.81	84	3.3	36	0.3	103	508	26.49
P1G085	6.3	203	0.36	151	0.77	185	1.03	148	1.14	187	6.8	205	0.5	202	1281	66.79
P1G086	3.1	42	0.51	37	0.81	144	0.79	36	0.95	129	3.5	47	0.4	138	573	29.87
P1G087	5.2	142	0.39	105	0.88	52	0.97	101	0.74	72	5.4	129	0.3	55	656	34.20

P1G088	5.2	144	0.31	213	0.73	233	1.12	215	1.27	217	5.8	154	0.6	225	1401	73.04
P1G089	4.1	83	0.34	181	0.79	161	1.07	183	0.97	131	4.5	84	0.4	143	966	50.36
P1G090	4.9	119	0.37	134	0.78	178	1.01	131	1.10	172	5.4	122	0.5	174	1030	53.70
P1G091	5.3	148	0.29	223	0.66	259	1.15	226	1.62	254	6.1	174	0.8	257	1541	80.34
P1G092	3.1	39	0.45	72	0.78	173	0.90	72	1.04	154	3.5	44	0.4	152	706	36.81
P1G093	3.9	66	0.49	48	0.85	79	0.82	48	0.71	65	4.2	67	0.3	93	466	24.30
P1G094	1.6	6	0.70	8	0.98	3	0.48	6	0.09	3	1.7	5	0.0	3	34	1.77
P1G095	5.8	183	0.26	252	0.76	193	1.20	251	1.10	170	6.3	185	0.5	210	1444	75.29
P1G096	4.1	81	0.41	91	0.87	59	0.95	92	0.64	51	4.3	75	0.2	54	503	26.23
P1G097	2.6	24	0.58	20	0.94	8	0.69	22	0.36	10	2.7	18	0.1	8	110	5.74
P1G098	5.7	177	0.29	225	0.61	268	1.13	217	1.69	259	6.6	198	0.9	265	1609	83.89
P1G099	5.5	166	0.44	76	0.90	22	0.91	76	0.55	29	5.7	149	0.2	23	541	28.21
P1G100	4.1	78	0.41	93	0.83	95	0.95	91	0.82	88	4.4	78	0.3	92	615	32.06
P1G101	4.8	113	0.38	126	0.81	138	1.00	126	1.06	155	5.2	110	0.4	135	903	47.08
P1G102	5.2	141	0.37	136	0.77	187	1.03	151	1.22	206	5.6	140	0.5	165	1126	58.71
P1G103	5.1	130	0.39	114	0.79	162	1.01	130	1.12	181	5.5	134	0.5	166	1017	53.02
P1G104	1.4	4	0.70	7	0.91	19	0.48	7	0.47	17	1.6	4	0.2	18	76	3.96
P1G105	3.3	51	0.46	65	0.89	37	0.87	64	0.54	27	3.5	48	0.2	37	329	17.15
P1G106	4.2	86	0.46	64	0.85	83	0.86	63	0.81	86	4.5	83	0.3	81	546	28.47
P1G107	1.8	8	0.66	11	0.89	38	0.55	10	0.61	44	2.0	9	0.2	34	154	8.03
P1G108	4.3	92	0.37	137	0.85	73	1.00	127	0.88	104	4.6	87	0.3	64	684	35.66
P1G109	5.8	186	0.28	233	0.68	254	1.16	232	1.63	256	6.5	194	0.7	255	1610	83.94
P1G110	4.4	97	0.39	107	0.77	183	0.98	113	1.06	160	4.9	101	0.5	177	938	48.91
P1G111	2.8	30	0.50	44	0.85	80	0.81	45	0.83	90	3.1	29	0.3	76	394	20.54
P1G112	3.2	46	0.52	34	0.85	82	0.77	34	0.72	70	3.5	42	0.3	84	392	20.44
P1G113	4.3	91	0.42	83	0.81	143	0.93	81	0.99	138	4.7	91	0.4	137	764	39.83
P1G114	2.3	16	0.71	6	0.89	29	0.47	5	0.51	21	2.5	15	0.2	50	142	7.40
P1G115	5.3	153	0.32	197	0.76	202	1.10	204	1.14	184	5.8	158	0.5	184	1282	66.84
P1G116	6.8	229	0.31	208	0.70	252	1.11	209	1.57	253	7.5	232	0.7	253	1636	85.30
P1G117	5.7	175	0.38	120	0.78	174	0.99	117	1.11	174	6.1	179	0.5	180	1119	58.34

P1G118	7.1	234	0.37	145	0.80	156	1.03	147	1.18	197	7.6	234	0.5	194	1307	68.14
P1G119	0.2	1	0.90	1	0.86	66	0.16	1	0.60	43	0.4	1	0.2	42	155	8.08
P1G120	5.1	131	0.34	177	0.72	240	1.06	181	1.32	224	5.7	144	0.6	235	1332	69.45
P1G121	7.1	237	0.34	182	0.72	234	1.06	177	1.36	232	7.8	241	0.7	250	1553	80.97
P1G122	6.5	214	0.29	228	0.76	196	1.15	228	1.16	191	7.0	215	0.5	198	1470	76.64
P1G123	3.0	36	0.44	75	0.88	41	0.91	75	0.65	56	3.2	32	0.2	29	344	17.94
P1G124	6.7	224	0.24	260	0.81	137	1.23	265	0.91	117	7.0	217	0.4	111	1331	69.40
P1G125	4.9	117	0.34	185	0.75	205	1.08	186	1.42	240	5.5	130	0.6	218	1281	66.79
P1G126	5.7	181	0.30	216	0.63	265	1.13	218	1.78	262	6.7	201	1.0	266	1609	83.89
P1G127	3.1	41	0.51	36	0.86	71	0.80	41	0.77	75	3.4	38	0.3	74	376	19.60
P1G128	5.1	136	0.33	187	0.77	188	1.08	189	1.11	175	5.6	139	0.5	185	1199	62.51
P1G129	7.8	253	0.24	258	0.76	200	1.23	263	1.37	234	8.3	253	0.5	214	1675	87.33
P1G130	8.0	259	0.25	256	0.65	263	1.22	257	1.78	263	8.9	261	0.9	260	1819	94.84
P1G131	4.8	114	0.36	154	0.74	226	1.03	152	1.33	225	5.4	118	0.6	217	1206	62.88
P1G132	8.7	264	0.26	251	0.66	260	1.22	260	1.82	265	9.6	265	0.9	262	1827	95.26
P1G133	6.5	213	0.35	161	0.75	218	1.05	167	1.41	238	7.1	221	0.6	220	1438	74.97
P1G134	4.2	87	0.42	87	0.82	124	0.93	83	0.92	120	4.6	86	0.4	122	709	36.97
P1G135	1.9	10	0.73	4	0.89	27	0.43	3	0.61	45	2.1	10	0.2	40	139	7.25
P1G136	4.1	80	0.49	52	0.86	67	0.83	52	0.72	69	4.4	77	0.3	71	468	24.40
P1G137	1.9	11	0.52	35	0.62	267	0.79	38	2.01	268	2.9	25	1.0	267	911	47.50
P1G138	4.1	82	0.35	171	0.80	147	1.06	176	0.89	108	4.5	81	0.4	132	897	46.77
P1G139															0	0.00
P1G140	3.2	47	0.45	69	0.83	106	0.90	73	0.93	123	3.5	49	0.4	109	576	30.03
P1G141	6.8	230	0.42	85	0.76	201	0.93	84	1.19	199	7.4	231	0.6	236	1266	66.01
P1G142	5.5	167	0.38	121	0.83	97	1.00	118	0.87	101	5.8	160	0.3	97	861	44.89
P1G143	6.7	221	0.37	143	0.83	105	1.01	128	0.92	121	7.0	216	0.4	124	1058	55.16
P1G144	3.3	49	0.48	57	0.88	45	0.84	57	0.58	35	3.5	43	0.2	27	313	16.32
P1G145	6.7	227	0.39	108	0.83	102	0.97	103	1.01	143	7.2	223	0.4	164	1070	55.79
P1G146	4.3	93	0.44	74	0.81	134	0.90	74	0.89	109	4.7	92	0.4	112	688	35.87
P1G147	1.6	7	0.69	9	0.89	24	0.50	8	0.57	32	1.8	7	0.2	26	113	5.89

P1G148	6.6	217	0.27	242	0.68	255	1.16	235	1.36	233	7.3	229	0.7	247	1658	86.44
P1G149	5.0	126	0.34	183	0.56	269	1.07	182	2.29	269	6.2	183	1.2	269	1481	77.22
P1G150	7.6	251	0.27	241	0.91	16	1.16	234	0.38	12	7.8	240	0.1	10	1004	52.35
P1G151	8.1	261	0.30	221	0.76	204	1.12	214	1.21	205	8.6	260	0.6	216	1581	82.43
P1G152	5.3	151	0.41	88	0.82	113	0.95	88	0.94	126	5.7	146	0.4	118	830	43.27
P1G153	1.5	5	0.67	10	0.91	20	0.54	9	0.50	19	1.7	6	0.2	17	86	4.48
P1G154	6.3	201	0.30	219	0.75	215	1.14	223	1.45	243	6.9	208	0.6	223	1532	79.87
P1G155	6.7	228	0.35	173	0.85	74	1.06	173	0.77	77	7.1	219	0.3	82	1026	53.49
P1G156	8.1	262	0.33	192	0.81	142	1.09	194	1.11	178	8.5	259	0.5	170	1397	72.84
P1G157	7.7	252	0.30	222	0.82	123	1.14	221	0.87	102	8.1	249	0.4	120	1289	67.21
P1G158	3.8	61	0.45	70	0.85	77	0.88	66	0.64	50	4.1	62	0.3	70	456	23.77
P1G159	6.1	198	0.34	178	0.66	261	1.05	169	1.48	246	7.0	212	0.8	259	1523	79.41
P1G160	5.8	187	0.35	162	0.75	216	1.04	162	1.26	213	6.4	186	0.5	212	1338	69.76
P1G161	6.5	212	0.28	237	0.76	198	1.17	240	1.34	226	7.0	213	0.5	188	1514	78.94
P1G162	3.9	67	0.49	49	0.82	116	0.81	44	0.76	73	4.2	69	0.4	119	537	28.00
P1G163	10.1	270	0.29	224	0.68	257	1.14	222	1.64	257	10.9	270	0.9	263	1763	91.92
P1G164	6.7	223	0.26	250	0.72	237	1.19	249	1.42	239	7.3	227	0.6	231	1656	86.34
P1G165	4.6	107	0.36	156	0.84	87	1.02	144	0.77	74	4.9	102	0.3	65	735	38.32
P1G166	3.1	45	0.53	30	0.89	33	0.75	29	0.59	38	3.4	37	0.2	41	253	13.19
P1G167	9.7	268	0.31	206	0.74	228	1.11	208	1.35	230	10.4	268	0.7	245	1653	86.18
P1G168	9.1	267	0.30	215	0.79	157	1.13	219	1.18	196	9.6	266	0.5	186	1506	78.52
P1G169	3.4	54	0.53	32	0.81	135	0.77	31	0.93	124	3.8	56	0.4	128	560	29.20
P1G170	7.9	258	0.31	207	0.75	219	1.10	202	1.20	203	8.5	258	0.6	240	1587	82.74
P1G171	2.1	14	0.58	17	0.89	36	0.68	17	0.57	34	2.3	12	0.2	24	154	8.03
P1G172	7.8	255	0.26	247	0.70	251	1.19	247	1.55	252	8.5	256	0.7	244	1752	91.35
P1G173	7.5	245	0.27	244	0.82	122	1.17	242	1.09	165	7.9	243	0.4	116	1377	71.79
P1G174	6.6	218	0.38	127	0.89	26	0.99	114	0.46	16	6.8	207	0.2	30	738	38.48
P1G175	2.6	26	0.48	56	0.88	43	0.85	58	0.65	54	2.8	24	0.2	28	289	15.07
P1G176	6.0	195	0.26	248	0.78	177	1.19	248	1.14	188	6.5	190	0.4	160	1406	73.31
P1G177	4.6	108	0.31	204	0.71	247	1.09	197	1.24	211	5.2	112	0.6	222	1301	67.83

97

P1G178	7.4	244	0.28	239	0.71	243	1.17	238	1.47	245	8.0	246	0.6	239	1694	88.32
P1G179	5.7	178	0.39	112	0.82	114	0.98	105	0.92	118	6.1	176	0.4	144	947	49.37
P1G180	3.3	52	0.51	41	0.75	212	0.79	37	1.06	158	3.8	57	0.5	175	732	38.16
P1G181	5.3	150	0.41	90	0.79	164	0.95	89	1.02	148	5.8	153	0.5	181	975	50.83
P1G182	7.6	249	0.23	267	0.71	244	1.24	266	1.46	244	8.2	251	0.6	232	1753	91.40
P1G183	4.0	74	0.36	158	0.74	225	1.04	159	1.23	209	4.5	82	0.5	192	1099	57.30
P1G184	5.5	168	0.35	172	0.79	160	1.06	180	1.00	140	6.0	169	0.4	147	1136	59.23
P1G185	2.1	15	0.58	21	0.86	65	0.69	21	0.78	79	2.3	13	0.3	56	270	14.08
P1G186	6.0	193	0.24	262	0.79	163	1.22	256	1.27	214	6.4	187	0.4	149	1424	74.24
P1G187	5.2	140	0.34	174	0.74	224	1.05	164	1.10	171	5.7	147	0.5	208	1228	64.03
P1G188	8.1	260	0.24	263	0.62	266	1.21	255	1.87	267	9.1	262	1.1	268	1841	95.99
P1G189	5.1	138	0.32	199	0.75	211	1.10	201	1.31	222	5.6	141	0.5	193	1305	68.04
P1G190	4.9	121	0.33	190	0.75	208	1.08	190	1.26	212	5.4	128	0.5	187	1236	64.44
P1G191	3.8	63	0.44	77	0.76	195	0.91	78	1.12	179	4.3	73	0.5	179	844	44.00
P1G192	9.0	265	0.23	266	0.71	249	1.23	262	1.50	248	9.7	267	0.7	252	1809	94.32
P1G193	3.0	37	0.56	25	0.77	190	0.71	26	1.27	215	3.6	51	0.6	219	763	39.78
P1G194	5.1	134	0.29	229	0.85	81	1.14	225	0.91	115	5.4	123	0.3	66	973	50.73
P1G195	7.8	256	0.33	191	0.82	129	1.08	191	1.02	150	8.3	252	0.4	153	1322	68.93
P1G196	5.8	182	0.38	122	0.83	100	1.00	121	0.91	116	6.1	177	0.4	110	928	48.38
P1G197	5.3	145	0.39	113	0.86	64	0.98	108	0.82	87	5.5	138	0.3	67	722	37.64
P1G198	5.4	158	0.32	193	0.83	96	1.09	193	0.85	94	5.7	152	0.3	101	987	51.46
P1G199	6.3	206	0.34	176	0.65	262	1.06	179	1.79	264	7.2	224	0.9	264	1575	82.12
P1G200	0.9	2	0.75	3	0.99	1	0.40	2	0.04	1	0.9	2	0.0	1	12	0.63
P1G201	3.9	70	0.51	38	0.95	6	0.79	35	0.25	6	4.0	61	0.1	6	222	11.57
P1G202	3.8	65	0.49	46	0.92	13	0.82	46	0.44	14	4.0	60	0.2	13	257	13.40
P1G203	5.3	146	0.32	203	0.71	246	1.09	196	1.24	210	5.9	163	0.6	233	1397	72.84
P1G204	2.0	13	0.61	14	0.94	9	0.63	13	0.32	8	2.1	11	0.1	7	75	3.91
P1G205	6.4	210	0.33	186	0.85	76	1.08	185	0.69	61	6.7	202	0.3	75	995	51.88
P1G206	5.4	160	0.37	141	0.72	238	1.04	155	1.49	247	6.0	170	0.6	228	1339	69.81
P1G207	2.7	27	0.57	23	0.89	34	0.69	23	0.58	36	2.9	27	0.2	31	201	10.48

P1G208	2.8	32	0.55	27	0.80	155	0.72	27	0.97	132	3.2	33	0.4	148	554	28.88
P1G209	5.1	129	0.38	133	0.82	120	1.00	120	0.78	78	5.4	124	0.3	99	803	41.87
P1G210	4.2	88	0.37	138	0.75	206	1.01	137	1.30	221	4.7	94	0.5	205	1089	56.78
P1G211	6.7	226	0.35	169	0.83	98	1.04	161	0.79	82	7.0	218	0.3	102	1056	55.06
P1G212	7.6	247	0.25	253	0.73	231	1.20	250	1.27	216	8.2	250	0.6	237	1684	87.80
P1G213	6.6	219	0.32	201	0.77	182	1.12	216	1.20	202	7.1	222	0.5	196	1438	74.97
P1G214	5.5	169	0.31	210	0.74	227	1.10	207	1.12	180	6.1	175	0.5	211	1379	71.90
P1G215	6.5	211	0.27	245	0.79	168	1.19	246	1.07	162	6.9	210	0.4	161	1403	73.15
P1G216	5.3	154	0.40	100	0.91	17	0.96	96	0.53	24	5.5	137	0.2	20	548	28.57
P1G217	4.2	84	0.39	109	0.78	172	1.00	125	1.15	190	4.6	88	0.4	156	924	48.18
P1G218	6.1	200	0.24	259	0.92	15	1.21	253	0.36	11	6.3	184	0.1	11	933	48.64
P1G219	2.5	21	0.49	50	0.89	31	0.83	51	0.60	40	2.7	19	0.2	22	234	12.20
P1G220	5.3	155	0.26	249	0.82	128	1.22	258	0.97	133	5.7	143	0.3	86	1152	60.06
P1G221	4.0	75	0.41	92	0.87	60	0.95	90	0.67	59	4.2	70	0.2	48	494	25.76
P1G222	4.6	105	0.36	150	0.82	109	1.03	154	1.00	141	4.9	103	0.3	90	852	44.42
P1G223	6.5	216	0.37	140	0.78	179	1.01	135	1.17	193	7.1	220	0.5	209	1292	67.36
P1G224															0	0.00
P1G225	5.5	164	0.42	86	0.89	25	0.94	87	0.52	22	5.7	150	0.2	44	578	30.14
P1G226															0	0.00
P1G227	4.5	100	0.39	106	0.86	68	0.98	107	0.79	81	4.7	93	0.3	68	623	32.48
P1G228	4.8	112	0.34	175	0.95	7	1.05	165	0.21	4	4.9	99	0.1	5	567	29.56
P1G229	2.5	20	0.65	12	0.93	10	0.56	11	0.28	7	2.7	17	0.1	12	89	4.64
P1G230	6.4	209	0.28	234	0.87	57	1.15	227	0.71	64	6.7	200	0.3	57	1048	54.64
P1G231	7.6	250	0.28	238	0.72	239	1.15	231	1.21	204	8.3	254	0.7	254	1670	87.07
P1G232	3.1	44	0.49	53	0.83	101	0.83	55	0.96	130	3.5	45	0.3	105	533	27.79
P1G233	4.6	106	0.37	135	0.82	126	1.02	139	0.89	110	5.0	107	0.4	129	852	44.42
P1G234	4.3	90	0.30	220	0.76	194	1.12	212	1.01	146	4.7	96	0.5	189	1147	59.80
P1G235	4.0	77	0.40	96	0.85	75	0.97	100	0.72	67	4.3	74	0.3	80	569	29.67
P1G236	7.3	241	0.30	217	0.80	149	1.12	210	1.19	200	7.7	238	0.4	157	1412	73.62
P1G237	5.4	161	0.40	103	0.87	58	0.96	99	0.60	39	5.7	148	0.3	69	677	35.30

P1G238	4.0	76	0.37	142	0.89	39	1.02	142	0.53	26	4.3	71	0.2	35	531	27.69
P1G239	5.6	173	0.35	165	0.74	229	1.05	166	1.35	231	6.2	180	0.6	234	1378	71.85
P1G240	7.4	242	0.32	196	0.79	165	1.10	200	1.23	208	7.9	242	0.5	195	1448	75.50
P1G241	5.0	127	0.31	211	0.84	85	1.14	224	0.82	89	5.3	115	0.3	72	923	48.12
P1G242	4.5	104	0.40	102	0.82	130	0.96	97	0.86	99	5.0	106	0.4	154	792	41.29
P1G243	2.6	25	0.49	54	0.88	42	0.83	54	0.56	30	2.8	22	0.2	33	260	13.56
P1G244	3.8	62	0.40	98	0.81	141	0.98	110	1.01	145	4.1	64	0.4	117	737	38.43
P1G245	6.4	208	0.31	205	0.85	84	1.10	203	0.92	122	6.8	203	0.3	87	1112	57.98
P1G246	3.5	56	0.50	43	0.88	53	0.81	43	0.60	42	3.7	54	0.2	49	340	17.73
P1G247	3.6	58	0.40	97	0.91	18	0.96	98	0.48	18	3.7	55	0.2	14	358	18.67
P1G248	7.6	246	0.35	167	0.79	170	1.05	168	1.10	168	8.1	248	0.5	197	1364	71.12
P1G249	4.5	103	0.39	117	0.80	146	0.99	115	1.06	156	4.9	104	0.4	146	887	46.25
P1G250	3.3	50	0.50	42	0.79	159	0.80	42	1.03	152	3.7	53	0.4	159	657	34.25
P1G251	5.9	189	0.35	168	0.86	70	1.04	160	0.79	80	6.2	181	0.3	83	931	48.54
P1G252	5.6	174	0.33	188	0.86	63	1.08	187	0.71	66	5.9	166	0.3	77	921	48.02
P1G253	8.4	263	0.27	246	0.67	258	1.17	241	1.69	258	9.2	263	0.8	258	1787	93.17
P1G254	4.0	71	0.28	232	0.50	270	1.18	244	2.66	270	5.4	120	1.4	270	1477	77.01
P1G255	6.3	202	0.33	189	0.75	210	1.08	192	1.13	183	6.9	209	0.6	230	1415	73.77
P1G256	5.3	152	0.38	131	0.82	125	1.01	134	0.90	112	5.7	151	0.4	130	935	48.75
P1G257	2.9	35	0.42	82	0.83	99	0.93	80	0.86	97	3.3	34	0.3	88	515	26.85
P1G258	5.3	147	0.48	55	0.92	12	0.83	53	0.32	9	5.5	131	0.2	15	422	22.00
P1G259	5.9	188	0.38	124	0.90	21	1.00	124	0.51	20	6.1	172	0.2	25	674	35.14
P1G260	4.5	101	0.51	40	0.81	140	0.80	40	0.91	113	4.9	100	0.4	136	670	34.93
P1G261	5.8	185	0.28	235	0.69	253	1.18	245	1.63	255	6.4	189	0.6	243	1605	83.68
P1G262	4.4	95	0.46	63	0.88	46	0.86	61	0.62	48	4.6	89	0.2	53	455	23.72
P1G263	6.1	196	0.25	255	0.75	217	1.20	252	1.51	250	6.6	197	0.5	213	1580	82.38
P1G264	5.1	133	0.36	157	0.81	131	1.06	174	0.98	135	5.5	132	0.4	108	970	50.57
P1G265	5.5	163	0.38	130	0.82	127	1.00	122	0.99	137	5.9	162	0.4	141	982	51.20
P1G266	7.6	248	0.24	261	0.80	153	1.21	254	1.09	164	8.0	247	0.4	163	1490	77.69
P1G267	6.8	231	0.25	254	0.80	151	1.22	259	0.95	128	7.3	225	0.4	151	1399	72.94

P1G268	4.5	102	0.45	71	0.89	32	0.88	67	0.52	23	4.7	95	0.3	58	448	23.36
P1G269	6.9	232	0.25	257	0.80	148	1.23	261	1.12	182	7.3	226	0.4	150	1456	75.91
P1G270	7.2	239	0.32	194	0.72	235	1.08	188	1.40	237	7.9	245	0.7	256	1594	83.11
P1G271	4.6	111	0.47	61	0.82	110	0.86	62	0.84	92	5.0	109	0.4	140	685	35.71
P1G272	6.1	199	0.37	139	0.78	176	1.01	136	1.18	194	6.6	199	0.5	191	1234	64.34
P1G273	4.9	120	0.35	170	0.77	184	1.06	178	1.14	185	5.4	126	0.5	190	1153	60.11
P1G274	3.3	53	0.51	39	0.76	197	0.80	39	1.16	192	3.9	58	0.5	200	778	40.56

Appendix 2. Ranking different salt tolerance indices for 277 F$_3$ lines of cross Bobur* UZ-11CWA08.

Geno name	SWL	Ranking	STI_SFW	Ranking	STI_SDW	Ranking	SSI_SFW	Ranking	SSI_SDW	Ranking	TOL_SFW	Ranking	TOL_SDW	Ranking	ST Overall	ST %
P2G001	6.52	250	0.29	254	0.71	246	1.33	243	1.71	242	7.11	250	0.59	240	1725	88.96
P2G002	4.49	214	0.37	219	0.84	137	1.22	217	1.01	138	4.79	213	0.31	147	1285	66.27
P2G003	0.33	5	0.88	3	0.91	61	0.23	3	0.56	59	0.49	4	0.15	50	185	9.54
P2G004	6.19	248	0.29	252	0.77	208	1.38	251	1.43	207	6.66	247	0.47	215	1628	83.96
P2G005	7.59	261	0.32	241	0.73	237	1.27	233	1.58	225	8.23	260	0.64	245	1702	87.78
P2G006	4.85	227	0.30	248	0.79	192	1.38	248	1.31	191	5.21	225	0.36	174	1505	77.62
P2G007	2.99	137	0.58	74	0.93	39	0.79	69	0.43	33	3.13	127	0.14	43	522	26.92
P2G008	2.92	130	0.53	106	0.90	68	0.89	97	0.58	63	3.11	125	0.19	68	657	33.88
P2G009	3.80	185	0.40	201	0.79	194	1.17	201	1.32	195	4.20	187	0.40	194	1357	69.98
P2G010	4.39	213	0.42	185	0.91	60	1.13	188	0.57	61	4.55	201	0.16	59	967	49.87
P2G011	4.36	211	0.39	208	0.84	130	1.15	196	0.94	126	4.65	207	0.29	136	1214	62.61
P2G012	4.59	218	0.35	235	0.75	220	1.25	225	1.51	221	5.05	222	0.46	212	1553	80.09
P2G013	2.38	90	0.54	101	0.88	92	0.88	95	0.74	87	2.60	91	0.22	84	640	33.01
P2G014	3.11	147	0.49	134	0.84	129	0.98	133	0.97	131	3.40	148	0.29	135	957	49.36
P2G015	5.34	238	0.32	242	0.80	183	1.35	245	1.26	187	5.73	236	0.40	193	1524	78.60
P2G016	4.60	219	0.39	211	0.88	88	1.19	207	0.74	91	4.82	214	0.22	90	1120	57.76
P2G017	1.87	59	0.59	66	0.87	96	0.79	68	0.79	98	2.10	61	0.23	95	543	28.00
P2G018	2.55	106	0.50	126	0.83	150	0.97	126	1.07	155	2.87	113	0.32	151	927	47.81
P2G019	3.51	166	0.45	165	0.85	120	1.04	154	0.86	108	3.77	163	0.27	115	991	51.11
P2G020	9.75	262	0.22	261	0.69	251	1.46	261	1.82	245	10.53	262	0.78	257	1799	92.78
P2G021															0	0.00
P2G022	4.90	230	0.36	228	0.77	209	1.27	232	1.47	212	5.37	229	0.47	217	1557	80.30
P2G023	5.81	245	0.36	226	0.89	76	1.26	227	0.71	78	6.06	241	0.25	105	1198	61.78
P2G024	3.58	170	0.44	167	0.80	180	1.10	174	1.23	181	3.95	169	0.38	183	1224	63.13
P2G025	4.00	198	0.39	212	0.78	199	1.26	229	1.47	214	4.42	200	0.42	199	1451	74.83
P2G026	2.93	131	0.53	112	0.87	100	0.90	101	0.78	97	3.18	133	0.25	103	777	40.07
P2G027	6.73	253	0.30	246	0.69	250	1.36	247	1.90	251	7.42	253	0.68	250	1750	90.25
P2G028	5.21	236	0.31	244	0.65	258	1.34	244	2.17	257	5.95	239	0.74	254	1732	89.32

P2G029	3.65	174	0.48	143	0.82	167	1.02	140	1.15	169	4.02	175	0.36	173	1141	58.84
P2G030	3.37	157	0.44	170	0.84	136	1.09	166	1.01	139	3.66	159	0.28	129	1056	54.46
P2G031	1.58	45	0.61	61	0.80	189	0.74	59	1.21	178	1.92	52	0.34	165	749	38.63
P2G032	2.08	75	0.54	99	0.79	196	0.89	98	1.32	196	2.45	80	0.37	181	925	47.71
P2G033	2.73	119	0.53	103	0.86	116	0.91	108	0.89	115	3.00	120	0.27	118	799	41.21
P2G034	2.16	79	0.54	98	0.83	147	0.87	92	1.03	143	2.45	81	0.29	137	777	40.07
P2G035	2.85	127	0.58	70	0.85	123	0.82	73	0.94	127	3.13	128	0.29	131	779	40.18
P2G036	2.65	116	0.50	127	0.79	193	0.97	128	1.32	193	3.03	121	0.38	187	1065	54.93
P2G037	4.08	203	0.41	197	0.74	226	1.12	185	1.54	224	4.59	206	0.51	227	1468	75.71
P2G038	3.02	138	0.47	154	0.83	148	1.04	153	1.07	154	3.32	144	0.30	142	1033	53.27
P2G039	3.35	156	0.47	149	0.73	233	1.03	148	1.69	239	3.90	168	0.55	235	1328	68.49
P2G040	4.23	208	0.39	207	0.77	207	1.18	204	1.42	205	4.69	209	0.45	210	1450	74.78
P2G041	7.18	257	0.25	258	0.72	240	1.46	260	1.77	243	7.79	257	0.61	242	1757	90.61
P2G042	7.57	260	0.29	251	0.68	253	1.40	254	2.02	256	8.35	261	0.78	256	1791	92.37
P2G043	2.38	89	0.52	117	0.83	143	0.94	119	1.02	141	2.68	96	0.30	143	848	43.73
P2G044	1.11	25	0.68	38	0.86	105	0.62	37	0.84	104	1.35	26	0.24	98	433	22.33
P2G045	3.92	190	0.40	204	0.77	212	1.19	210	1.47	213	4.35	196	0.43	202	1427	73.59
P2G046	1.38	36	0.55	90	0.75	223	0.85	83	1.50	220	1.86	51	0.48	221	924	47.65
P2G047	0.46	8	0.86	6	0.92	41	0.26	5	0.45	35	0.58	5	0.13	36	136	7.01
P2G048	2.07	74	0.53	102	0.86	114	0.91	107	0.88	113	2.30	74	0.23	92	676	34.86
P2G049	3.50	165	0.42	189	0.85	127	1.10	176	0.89	117	3.81	164	0.31	148	1086	56.01
P2G050	3.42	159	0.45	166	0.92	49	1.07	164	0.51	49	3.58	155	0.17	60	802	41.36
P2G051	4.86	228	0.33	238	0.77	206	1.31	240	1.43	206	5.30	227	0.44	205	1550	79.94
P2G052	2.45	95	0.56	85	0.89	71	0.91	104	0.74	89	2.64	94	0.19	69	607	31.30
P2G053	1.21	32	0.71	28	0.92	47	0.55	26	0.49	47	1.34	25	0.13	40	245	12.64
P2G054	3.99	196	0.42	181	0.92	51	1.13	190	0.52	52	4.15	183	0.15	48	901	46.47
P2G055	0.68	14	0.76	17	0.86	117	0.50	20	0.97	130	0.92	14	0.24	99	411	21.20
P2G056	7.40	258	0.35	233	0.88	94	1.28	234	0.76	93	7.68	256	0.28	126	1294	66.74
P2G057	3.61	172	0.41	193	0.82	166	1.11	183	1.10	161	3.95	170	0.34	166	1211	62.45
P2G058	5.08	235	0.36	230	0.74	228	1.26	228	1.62	230	5.61	235	0.53	229	1615	83.29

P2G059	4.67	222	0.41	199	0.86	119	1.18	202	0.93	123	4.95	218	0.29	133	1216	62.71
P2G060	3.93	192	0.44	174	0.77	211	1.06	161	1.34	198	4.40	199	0.47	216	1351	69.68
P2G061															0	0.00
P2G062	3.03	142	0.40	200	0.81	178	1.19	212	1.25	184	3.39	147	0.36	170	1233	63.59
P2G063	2.55	107	0.48	148	0.76	213	1.03	146	1.48	216	2.98	118	0.43	203	1151	59.36
P2G064	2.87	129	0.48	147	0.81	172	0.99	134	1.13	167	3.24	136	0.37	175	1060	54.67
P2G065	5.02	232	0.35	232	0.71	245	1.27	231	1.83	247	5.61	232	0.59	239	1658	85.51
P2G066	3.75	179	0.42	190	0.87	104	1.11	181	0.80	99	3.98	173	0.23	94	1020	52.60
P2G067	1.00	20	0.74	21	0.94	17	0.50	19	0.34	16	1.09	18	0.09	11	122	6.29
P2G068	3.29	154	0.48	140	0.79	191	1.02	143	1.29	190	3.70	160	0.41	198	1176	60.65
P2G069	3.03	140	0.51	121	0.82	158	0.94	117	1.09	159	3.36	146	0.33	158	999	51.52
P2G070	5.67	243	0.42	184	0.63	261	1.14	195	2.29	260	6.73	249	1.06	261	1653	85.25
P2G071															0	0.00
P2G072	1.88	60	0.69	35	0.90	69	0.61	35	0.64	68	2.08	60	0.20	74	401	20.68
P2G073	7.52	259	0.26	257	0.71	242	1.44	258	1.79	244	8.19	259	0.66	247	1766	91.08
P2G074	5.49	241	0.35	234	0.83	149	1.28	237	1.08	157	5.83	238	0.34	163	1419	73.18
P2G075															0	0.00
P2G076	1.13	28	0.70	32	0.93	37	0.59	32	0.46	40	1.26	22	0.12	35	226	11.66
P2G077	2.00	69	0.48	141	0.77	204	1.02	141	1.40	202	2.42	79	0.42	201	1037	53.48
P2G078	4.77	224	0.41	195	0.82	168	1.16	200	1.17	172	5.13	224	0.37	176	1359	70.09
P2G079	2.05	73	0.58	76	0.82	159	0.80	70	1.03	144	2.37	75	0.32	153	750	38.68
P2G080	3.56	167	0.42	187	0.84	132	1.10	178	0.93	124	3.84	166	0.28	121	1075	55.44
P2G081	2.03	72	0.58	71	0.95	8	0.79	67	0.29	7	2.11	62	0.08	9	296	15.27
P2G082	5.07	234	0.37	223	0.74	230	1.18	206	1.52	222	5.61	233	0.54	231	1579	81.43
P2G083	1.41	37	0.63	56	0.82	165	0.70	52	1.06	150	1.73	41	0.32	154	655	33.78
P2G084	3.80	182	0.46	160	0.73	234	1.03	149	1.61	227	4.32	194	0.53	228	1374	70.86
P2G085															0	0.00
P2G086	0.87	17	0.68	37	0.84	135	0.60	34	0.96	128	1.15	20	0.28	122	493	25.43
P2G087	0.59	13	0.86	5	0.93	31	0.27	6	0.41	28	0.70	8	0.12	31	122	6.29
P2G088	5.05	233	0.29	249	0.72	238	1.33	242	1.61	228	5.61	234	0.56	236	1660	85.61

P2G089	6.57	251	0.29	255	0.72	239	1.36	246	1.68	237	7.13	251	0.57	237	1716	88.50
P2G090	5.87	246	0.33	239	0.80	188	1.33	241	1.28	189	6.27	245	0.40	196	1544	79.63
P2G091	2.53	104	0.57	82	0.87	101	0.85	86	0.80	102	2.79	104	0.27	116	695	35.84
P2G092	4.56	216	0.36	231	0.76	214	1.28	235	1.53	223	5.01	220	0.46	211	1550	79.94
P2G093															0	0.00
P2G094	4.97	231	0.36	225	0.73	232	1.25	223	1.66	234	5.51	231	0.55	233	1609	82.98
P2G095	6.59	252	0.23	260	0.67	254	1.45	259	1.90	250	7.26	252	0.67	248	1775	91.54
P2G096	6.86	255	0.22	262	0.66	256	1.48	262	2.02	255	7.64	255	0.78	255	1800	92.83
P2G097	1.83	57	0.63	53	0.82	157	0.72	55	1.10	160	2.19	65	0.36	172	719	37.08
P2G098	4.28	210	0.37	221	0.65	259	1.24	222	2.17	258	5.11	223	0.83	259	1652	85.20
P2G099	2.94	132	0.48	144	0.88	82	1.03	147	0.73	83	3.15	130	0.21	82	800	41.26
P2G100	3.42	161	0.38	217	0.88	95	1.19	213	0.77	94	3.62	157	0.20	77	1014	52.29
P2G101	3.05	143	0.44	168	0.78	200	1.10	177	1.37	200	3.46	150	0.41	197	1235	63.69
P2G102	4.07	202	0.40	206	0.74	225	1.19	215	1.63	231	4.57	203	0.51	226	1508	77.77
P2G103															0	0.00
P2G104	6.31	249	0.31	245	0.81	173	1.38	249	1.24	182	6.68	248	0.38	182	1528	78.80
P2G105	4.12	204	0.40	205	0.76	218	1.19	211	1.49	218	4.58	205	0.47	214	1475	76.07
P2G106	2.94	133	0.38	218	0.78	197	1.29	238	1.47	211	3.32	145	0.38	188	1330	68.59
P2G107	4.53	215	0.38	216	0.84	141	1.18	203	0.98	133	4.85	216	0.32	152	1276	65.81
P2G108	4.82	226	0.34	236	0.91	58	1.30	239	0.53	54	5.00	219	0.18	66	1098	56.63
P2G109															0	0.00
P2G110															0	0.00
P2G111	2.50	103	0.47	150	0.83	152	1.00	138	1.03	146	2.80	107	0.30	140	936	48.27
P2G112	2.41	93	0.49	135	0.77	205	1.00	135	1.40	203	2.84	111	0.42	200	1082	55.80
P2G113	1.45	40	0.75	19	0.92	50	0.49	18	0.52	50	1.60	36	0.16	54	267	13.77
P2G114	3.79	181	0.42	186	0.82	163	1.13	191	1.12	164	4.12	179	0.33	159	1223	63.07
P2G115	6.81	254	0.32	243	0.71	244	1.28	236	1.70	241	7.52	254	0.71	251	1723	88.86
P2G116	2.80	124	0.53	105	0.92	44	0.92	109	0.48	45	2.94	116	0.14	44	587	30.27
P2G117	2.62	114	0.47	156	0.80	181	1.06	159	1.26	186	2.99	119	0.37	179	1094	56.42
P2G118	1.09	23	0.67	41	0.87	98	0.64	42	0.80	100	1.31	24	0.22	88	416	21.45

P2G119	4.38	212	0.43	178	0.82	161	1.13	192	1.13	166	4.76	212	0.38	185	1306	67.35
P2G120	1.30	34	0.66	44	0.83	155	0.64	44	1.06	153	1.62	37	0.32	155	622	32.08
P2G121															0	0.00
P2G122	2.78	122	0.44	173	0.82	162	1.09	172	1.13	165	3.09	124	0.31	149	1067	55.03
P2G123	3.67	175	0.44	169	0.80	182	1.05	157	1.16	170	4.07	178	0.40	195	1226	63.23
P2G124	3.69	178	0.41	198	0.75	222	1.11	182	1.45	210	4.18	185	0.49	223	1398	72.10
P2G125	7.18	256	0.30	247	0.64	260	1.40	255	2.35	261	8.10	258	0.93	260	1797	92.68
P2G126	3.57	168	0.41	196	0.68	252	1.12	184	1.88	249	4.21	189	0.65	246	1484	76.53
P2G127	5.36	239	0.34	237	0.72	241	1.26	226	1.69	240	5.97	240	0.61	243	1666	85.92
P2G128	2.87	128	0.57	80	0.80	184	0.85	85	1.26	188	3.31	142	0.44	204	1011	52.14
P2G129	3.68	177	0.50	129	0.85	121	0.98	131	0.91	121	3.97	172	0.29	138	989	51.01
P2G130	5.54	242	0.36	229	0.71	243	1.27	230	1.82	246	6.17	244	0.63	244	1678	86.54
P2G131	2.82	125	0.45	162	0.93	34	1.05	156	0.43	34	2.93	115	0.11	27	653	33.68
P2G132	0.52	12	0.81	10	0.88	85	0.36	8	0.71	76	0.72	9	0.20	75	275	14.18
P2G133															0	0.00
P2G134	3.07	145	0.46	159	0.75	221	1.02	144	1.44	208	3.52	154	0.45	208	1239	63.90
P2G135	1.01	21	0.80	13	0.90	65	0.38	13	0.60	65	1.19	21	0.18	64	262	13.51
P2G136	3.91	189	0.41	194	0.80	185	1.13	187	1.20	176	4.19	186	0.39	192	1309	67.51
P2G137	3.43	163	0.45	161	0.77	210	1.07	163	1.45	209	3.89	167	0.46	213	1286	66.32
P2G138	4.06	201	0.43	176	0.52	262	1.08	165	2.76	262	5.38	230	1.32	262	1558	80.35
P2G139	5.45	240	0.24	259	0.66	257	1.43	257	1.99	254	6.12	243	0.67	249	1759	90.72
P2G140	1.90	62	0.69	33	0.87	103	0.63	40	0.91	120	2.17	64	0.27	119	541	27.90
P2G141	2.68	117	0.56	88	0.78	198	0.83	79	1.25	185	3.13	126	0.45	209	1002	51.68
P2G142	0.92	18	0.75	18	0.97	5	0.46	17	0.18	5	0.97	16	0.05	5	84	4.33
P2G143	0.38	7	0.87	4	0.94	24	0.25	4	0.34	18	0.47	3	0.09	12	72	3.71
P2G144	3.39	158	0.49	131	0.87	99	0.95	120	0.75	92	3.63	158	0.24	100	858	44.25
P2G145	1.70	51	0.64	51	0.94	23	0.71	53	0.36	22	1.80	46	0.10	19	265	13.67
P2G146	2.60	111	0.55	96	0.88	86	0.88	96	0.73	85	2.82	109	0.22	89	672	34.66
P2G147	1.69	49	0.66	46	0.85	122	0.64	43	0.88	112	1.95	53	0.26	112	537	27.69
P2G148	4.64	221	0.38	215	0.90	70	1.22	221	0.67	71	4.84	215	0.20	76	1089	56.16

P2G149	1.53	42	0.72	27	0.93	30	0.56	28	0.42	30	1.64	38	0.12	30	225	11.60
P2G150	1.18	30	0.70	30	0.81	171	0.58	30	1.17	173	1.53	33	0.35	168	635	32.75
P2G151	3.24	153	0.48	142	0.95	11	1.03	151	0.33	13	3.32	143	0.08	10	623	32.13
P2G152	3.98	195	0.51	119	0.92	42	0.95	122	0.46	41	4.14	181	0.15	52	752	38.78
P2G153	3.13	149	0.50	125	0.94	20	0.98	129	0.34	19	3.24	137	0.11	24	603	31.10
P2G154	2.27	85	0.70	31	0.95	15	0.59	31	0.33	15	2.38	77	0.11	26	280	14.44
P2G155	3.05	144	0.57	79	0.89	77	0.83	78	0.70	74	3.27	139	0.22	87	678	34.97
P2G156	3.75	180	0.37	222	0.73	235	1.22	219	1.68	238	4.24	193	0.49	225	1512	77.98
P2G157	3.23	152	0.46	157	0.85	125	1.05	158	0.94	125	3.50	152	0.26	114	983	50.70
P2G158	3.58	171	0.49	137	0.87	97	1.01	139	0.73	84	3.83	165	0.25	106	899	46.36
P2G159	2.50	101	0.55	92	0.92	43	0.88	93	0.47	43	2.63	93	0.13	41	506	26.10
P2G160	2.16	80	0.50	128	0.88	87	0.98	130	0.74	88	2.37	76	0.21	83	672	34.66
P2G161	1.06	22	0.73	23	0.83	153	0.52	23	1.07	156	1.40	29	0.34	164	570	29.40
P2G162	6.00	247	0.29	253	0.74	227	1.39	252	1.61	229	6.53	246	0.53	230	1684	86.85
P2G163	2.15	78	0.55	93	0.81	176	0.88	94	1.21	177	2.49	85	0.33	161	864	44.56
P2G164	0.98	19	0.77	16	0.93	38	0.45	16	0.46	37	1.11	19	0.13	38	183	9.44
P2G165	1.12	26	0.68	36	0.76	216	0.61	36	1.48	217	1.59	35	0.47	219	785	40.48
P2G166	1.72	53	0.62	59	0.70	248	0.75	63	1.95	252	2.30	72	0.59	238	985	50.80
P2G167	2.77	121	0.52	113	0.70	247	0.94	118	1.88	248	3.50	153	0.73	253	1253	64.62
P2G168	2.61	112	0.56	87	0.97	3	0.86	89	0.18	3	2.66	95	0.05	4	393	20.27
P2G169	2.57	108	0.53	107	0.83	151	0.92	111	1.08	158	2.90	114	0.33	162	911	46.98
P2G170	1.94	63	0.55	97	0.84	128	0.90	102	1.00	137	2.22	67	0.28	127	721	37.18
P2G171	2.63	115	0.52	116	0.94	21	0.91	106	0.34	17	2.74	101	0.11	23	499	25.73
P2G172	2.32	86	0.45	163	0.90	63	1.09	167	0.61	67	2.47	82	0.16	55	683	35.22
P2G173	2.19	82	0.49	132	0.73	236	0.98	132	1.68	236	2.74	102	0.55	234	1154	59.52
P2G174	1.89	61	0.61	62	0.94	19	0.76	64	0.36	23	1.99	55	0.10	18	302	15.58
P2G175	2.58	110	0.51	123	0.86	115	0.94	116	0.84	103	2.84	110	0.26	110	787	40.59
P2G176	2.46	97	0.55	91	0.93	33	0.85	84	0.42	29	2.57	89	0.12	28	451	23.26
P2G177	1.66	48	0.64	50	0.89	75	0.69	49	0.66	69	1.85	49	0.20	73	413	21.30
P2G178	0.84	16	0.81	8	0.93	36	0.37	9	0.47	42	0.97	15	0.13	37	163	8.41

P2G179	1.72	52	0.69	34	0.93	28	0.60	33	0.42	31	1.85	48	0.13	39	265	13.67
P2G180	4.21	207	0.43	177	0.76	219	1.18	205	1.63	232	4.69	210	0.48	220	1470	75.81
P2G181	1.57	44	0.72	24	0.95	12	0.54	24	0.31	10	1.67	39	0.10	13	166	8.56
P2G182	4.89	229	0.36	227	0.78	203	1.25	224	1.41	204	5.36	228	0.47	218	1533	79.06
P2G183	4.70	223	0.39	214	0.92	52	1.22	218	0.53	53	4.85	217	0.16	53	1030	53.12
P2G184	0.05	1	0.93	1	0.86	110	0.13	1	0.87	111	0.30	1	0.25	109	334	17.23
P2G185	3.68	176	0.39	210	0.76	215	1.19	209	1.47	215	4.12	180	0.45	207	1412	72.82
P2G186	1.44	39	0.67	43	0.84	138	0.63	39	0.97	129	1.71	40	0.28	123	551	28.42
P2G187	1.57	43	0.66	45	0.86	118	0.66	46	0.91	119	1.86	50	0.29	132	553	28.52
P2G188	2.17	81	0.53	111	0.79	195	0.93	114	1.33	197	2.54	87	0.37	178	963	49.66
P2G189	4.19	205	0.32	240	0.80	190	1.39	253	1.39	201	4.57	202	0.38	189	1480	76.33
P2G190	2.15	77	0.59	67	0.94	18	0.81	72	0.35	20	2.25	69	0.10	20	343	17.69
P2G191	2.49	99	0.53	109	0.88	84	0.92	113	0.72	79	2.69	98	0.21	80	662	34.14
P2G192	2.02	71	0.58	73	0.86	107	0.82	74	0.85	105	2.27	70	0.25	104	604	31.15
P2G193	1.97	67	0.57	81	0.84	140	0.84	80	1.01	140	2.27	71	0.29	139	718	37.03
P2G194	0.30	4	0.83	7	0.80	187	0.33	7	1.24	183	0.68	7	0.38	190	585	30.17
P2G195	2.98	135	0.41	191	0.88	79	1.14	193	0.73	82	3.20	134	0.22	85	899	46.36
P2G196	1.97	66	0.63	55	0.81	169	0.73	57	1.18	174	2.30	73	0.33	160	754	38.89
P2G197	2.62	113	0.58	72	0.84	142	0.83	76	1.06	152	2.96	117	0.35	167	839	43.27
P2G198	3.44	164	0.47	153	0.70	249	1.06	160	1.97	253	4.15	182	0.71	252	1413	72.87
P2G199	2.35	87	0.58	75	0.97	2	0.84	81	0.16	2	2.39	78	0.04	2	327	16.86
P2G200	0.06	2	0.78	15	0.67	255	0.45	15	2.26	259	0.88	13	0.82	258	817	42.14
P2G201	3.20	151	0.47	152	0.87	102	1.10	175	0.89	116	3.45	149	0.25	107	952	49.10
P2G202	1.13	27	0.66	47	0.84	133	0.67	47	0.98	134	1.40	30	0.27	120	538	27.75
P2G203	1.17	29	0.72	26	0.84	139	0.56	27	1.03	142	1.47	31	0.31	146	540	27.85
P2G204	0.50	11	0.80	14	0.85	126	0.40	14	0.99	135	0.75	10	0.26	111	421	21.71
P2G205	0.48	9	0.81	12	0.74	231	0.38	11	1.67	235	0.97	17	0.49	224	739	38.11
P2G206	3.42	162	0.53	104	0.74	224	0.92	110	1.59	226	3.97	171	0.55	232	1229	63.38
P2G207	0.24	3	0.90	2	0.90	66	0.21	2	0.67	70	0.42	2	0.18	67	212	10.93
P2G208															0	0.00

P2G209	3.92	191	0.52	115	0.88	91	0.91	105	0.72	81	4.16	184	0.24	101	868	44.77
P2G210	2.95	134	0.56	86	0.86	112	0.87	90	0.86	109	3.23	135	0.28	128	794	40.95
P2G211	5.29	237	0.42	182	0.78	201	1.10	173	1.31	192	5.73	237	0.45	206	1428	73.65
P2G212	2.98	136	0.51	120	0.91	59	0.95	121	0.56	60	3.16	132	0.18	62	690	35.59
P2G213	2.74	120	0.55	95	0.93	35	0.90	100	0.46	38	2.86	112	0.12	33	533	27.49
P2G214	1.73	54	0.64	49	0.94	16	0.70	51	0.35	21	1.82	47	0.10	15	253	13.05
P2G215	2.50	102	0.59	69	0.88	80	0.80	71	0.71	77	2.70	99	0.21	81	579	29.86
P2G216	1.95	64	0.68	39	0.95	13	0.62	38	0.33	14	2.04	59	0.10	16	243	12.53
P2G217	2.45	96	0.52	118	0.83	145	0.96	123	1.06	151	2.75	103	0.30	141	877	45.23
P2G218															0	0.00
P2G219	2.15	76	0.58	77	0.95	7	0.83	75	0.30	9	2.23	68	0.08	8	320	16.50
P2G220	1.47	41	0.67	42	0.95	14	0.64	45	0.32	11	1.57	34	0.10	21	208	10.73
P2G221	1.69	50	0.59	68	0.97	6	0.78	65	0.19	6	1.74	42	0.05	6	243	12.53
P2G222	5.76	244	0.28	256	0.82	160	1.40	256	1.11	162	6.10	242	0.35	169	1489	76.79
P2G223	0.76	15	0.81	11	0.95	10	0.38	12	0.33	12	0.84	12	0.08	7	79	4.07
P2G224	3.80	184	0.49	136	0.86	108	1.00	136	0.85	106	4.05	177	0.25	108	955	49.25
P2G225	3.57	169	0.46	158	0.91	62	1.06	162	0.59	64	3.75	161	0.18	61	837	43.17
P2G226	3.95	194	0.37	220	0.84	131	1.22	220	0.98	132	4.23	192	0.28	125	1214	62.61
P2G227	1.20	31	0.72	25	0.94	22	0.55	25	0.38	25	1.30	23	0.10	17	168	8.66
P2G228	0.36	6	0.81	9	0.81	170	0.37	10	1.19	175	0.68	6	0.33	157	533	27.49
P2G229	1.97	68	0.53	110	0.86	106	0.92	112	0.86	107	2.21	66	0.23	96	665	34.30
P2G230	1.75	55	0.63	54	0.98	1	0.69	50	0.11	1	1.78	44	0.03	1	206	10.62
P2G231	2.80	123	0.43	179	0.86	109	1.12	186	0.86	110	3.04	122	0.24	102	931	48.01
P2G232	1.59	46	0.68	40	0.91	56	0.64	41	0.56	57	1.75	43	0.16	58	341	17.59
P2G233	3.80	183	0.39	213	0.90	64	1.16	198	0.57	62	3.98	174	0.18	63	957	49.36
P2G234	1.10	24	0.73	22	0.86	113	0.51	22	0.90	118	1.36	27	0.26	113	439	22.64
P2G235	1.36	35	0.66	48	0.93	40	0.67	48	0.45	36	1.49	32	0.13	42	281	14.49
P2G236	3.31	155	0.49	138	0.90	67	1.00	137	0.60	66	3.49	151	0.18	65	779	40.18
P2G237	2.85	126	0.57	78	0.89	78	0.85	82	0.72	80	3.08	123	0.23	93	660	34.04
P2G238	4.63	220	0.41	192	0.74	229	1.14	194	1.64	233	5.24	226	0.60	241	1535	79.16

P2G239	2.40	92	0.56	84	0.92	53	0.85	87	0.52	51	2.55	88	0.15	49	504	25.99
P2G240	3.13	150	0.47	151	0.91	55	1.03	150	0.54	55	3.28	140	0.15	51	752	38.78
P2G241	3.02	139	0.47	155	0.93	29	1.10	179	0.46	39	3.13	129	0.11	25	695	35.84
P2G242	3.42	160	0.50	130	0.88	83	1.04	155	0.80	101	3.61	156	0.19	71	856	44.15
P2G243	1.97	65	0.61	64	0.97	4	0.74	60	0.18	4	2.02	57	0.05	3	257	13.25
P2G244	1.64	47	0.61	63	0.81	177	0.74	61	1.15	168	2.00	56	0.37	177	749	38.63
P2G245	1.43	38	0.63	57	0.81	174	0.75	62	1.23	180	1.79	45	0.36	171	727	37.49
P2G246	3.12	148	0.48	139	0.92	48	0.97	127	0.47	44	3.26	138	0.14	45	689	35.53
P2G247															0	0.00
P2G248	3.81	186	0.48	146	0.88	81	1.02	145	0.74	90	4.03	176	0.22	86	910	46.93
P2G249	2.49	100	0.62	58	0.84	134	0.74	58	1.00	136	2.79	106	0.30	144	736	37.96
P2G250	2.54	105	0.50	124	0.92	46	0.96	124	0.49	46	2.69	97	0.15	47	589	30.38
P2G251	2.01	70	0.61	60	0.94	25	0.73	56	0.36	24	2.13	63	0.12	32	330	17.02
P2G252	3.03	141	0.43	175	0.93	27	1.10	180	0.40	26	3.15	131	0.12	34	714	36.82
P2G253	2.39	91	0.56	89	0.89	72	0.87	91	0.69	73	2.59	90	0.20	78	584	30.12
P2G254	2.57	109	0.54	100	0.88	90	0.90	99	0.77	95	2.81	108	0.24	97	698	36.00
P2G255	4.25	209	0.40	203	0.76	217	1.19	208	1.49	219	4.73	211	0.48	222	1489	76.79
P2G256	2.43	94	0.49	133	0.88	89	0.97	125	0.74	86	2.62	92	0.19	72	691	35.64
P2G257	4.06	200	0.39	209	0.82	164	1.16	199	1.11	163	4.38	197	0.32	156	1288	66.43
P2G258	4.81	225	0.29	250	0.88	93	1.38	250	0.77	96	5.04	221	0.23	91	1226	63.23
P2G259															0	0.00
P2G260	2.46	98	0.53	108	0.86	111	0.94	115	0.88	114	2.73	100	0.27	117	763	39.35
P2G261	2.36	88	0.55	94	0.93	32	0.86	88	0.41	27	2.48	83	0.12	29	441	22.74
P2G262	4.00	197	0.42	188	0.80	186	1.16	197	1.32	194	4.39	198	0.39	191	1351	69.68
P2G263	3.85	188	0.45	164	0.81	175	1.09	168	1.23	179	4.22	191	0.37	180	1245	64.21
P2G264	1.83	58	0.63	52	0.89	73	0.71	54	0.69	72	2.02	58	0.19	70	437	22.54
P2G265	4.01	199	0.44	171	0.83	144	1.09	171	1.03	145	4.33	195	0.32	150	1175	60.60
P2G266	4.59	217	0.40	202	0.95	9	1.19	214	0.30	8	4.68	208	0.10	14	872	44.97
P2G267	4.20	206	0.42	180	0.80	179	1.09	169	1.16	171	4.58	204	0.38	184	1293	66.68
P2G268	3.09	146	0.48	145	0.89	74	1.04	152	0.70	75	3.30	141	0.21	79	812	41.88

P2G269	3.94	193	0.42	183	0.85	124	1.13	189	0.92	122	4.21	190	0.28	124	1125	58.02
P2G270	3.61	173	0.44	172	0.91	54	1.09	170	0.55	56	3.77	162	0.16	57	844	43.53
P2G271	2.22	84	0.56	83	0.83	156	0.83	77	1.04	149	2.52	86	0.31	145	780	40.23
P2G272	2.69	118	0.51	122	0.94	26	1.02	142	0.43	32	2.79	105	0.10	22	567	29.24
P2G273	1.23	33	0.71	29	0.91	57	0.56	29	0.56	58	1.39	28	0.16	56	290	14.96
P2G274	2.20	83	0.52	114	0.83	154	0.91	103	1.03	147	2.48	84	0.29	130	815	42.03
P2G275	3.82	187	0.37	224	0.78	202	1.21	216	1.36	199	4.20	188	0.38	186	1402	72.31
P2G276	1.81	56	0.60	65	0.92	45	0.79	66	0.50	48	1.95	54	0.14	46	380	19.60
P2G277	0.48	10	0.74	20	0.83	146	0.51	21	1.04	148	0.77	11	0.29	134	490	25.27

.

Appendix 3. Overall ST Ranking for 274 F_3 cross Bobur*Altay2000 lines.

Genoname	Overall ST Ranking	Status
P1G200	0.625651721	Tolerant
P1G094	1.772679875	Tolerant
P1G204	3.910323253	Tolerant
P1G104	3.962460897	Tolerant
P1G153	4.483837331	Tolerant
P1G032	4.640250261	Tolerant
P1G229	4.640250261	Tolerant
P1G036	5.735140772	Tolerant
P1G097	5.735140772	Tolerant
P1G147	5.891553702	Tolerant
P1G135	7.24713243	Tolerant
P1G264	7.40354536	Tolerant
P1G107	8.02919708	Tolerant
P1G171	8.02919708	Tolerant
P1G119	8.081334724	Tolerant
P1G029	9.541188738	Tolerant
P1G207	10.47966632	Tolerant
P1G015	10.58394161	Tolerant
P1G201	11.57455683	Tolerant
P1G219	12.20020855	Tolerant
P1G082	12.30448384	Tolerant
P1G166	13.19082377	Tolerant
P1G202	13.39937435	Tolerant
P1G243	13.55578728	Tolerant
P1G185	14.07716371	Tolerant
P1G006	14.33785193	Tolerant
P1G028	14.75495308	Tolerant
P1G074	14.91136601	Tolerant
P1G175	15.06777894	Tolerant
P1G043	16.21480709	Tolerant
P1G144	16.31908238	Tolerant
P1G105	17.15328467	Tolerant
P1G246	17.72679875	Tolerant
P1G123	17.93534932	Tolerant
P1G058	18.5088634	Tolerant
P1G247	18.66527633	Tolerant
P1G127	19.60375391	Tolerant
P1G112	20.4379562	Tolerant
P1G059	20.49009385	Tolerant
P1G111	20.54223149	Tolerant
P1G035	20.90719499	Tolerant
P1G026	20.95933264	Tolerant
P1G046	20.95933264	Tolerant

P1G022	21.79353493	Tolerant
P1G258	22.00208551	Tolerant
P1G072	22.94056309	Tolerant
P1G005	23.25338895	Tolerant
P1G268	23.35766423	Tolerant
P1G262	23.72262774	Tolerant
P1G158	23.77476538	Tolerant
P1G093	24.29614181	Tolerant
P1G136	24.4004171	Tolerant
P1G221	25.75599583	Moderate Tolerant
P1G096	26.22523462	Moderate Tolerant
P1G084	26.48592284	Moderate Tolerant
P1G075	26.64233577	Moderate Tolerant
P1G257	26.85088634	Moderate Tolerant
P1G041	26.95516163	Moderate Tolerant
P1G238	27.68508863	Moderate Tolerant
P1G232	27.78936392	Moderate Tolerant
P1G162	27.99791449	Moderate Tolerant
P1G099	28.20646507	Moderate Tolerant
P1G106	28.46715328	Moderate Tolerant
P1G033	28.51929093	Moderate Tolerant
P1G216	28.57142857	Moderate Tolerant
P1G208	28.88425443	Moderate Tolerant
P1G047	29.04066736	Moderate Tolerant
P1G169	29.19708029	Moderate Tolerant
P1G228	29.5620438	Moderate Tolerant
P1G235	29.66631908	Moderate Tolerant
P1G065	29.82273201	Moderate Tolerant
P1G086	29.87486966	Moderate Tolerant
P1G071	29.9270073	Moderate Tolerant
P1G140	30.03128259	Moderate Tolerant
P1G225	30.13555787	Moderate Tolerant
P1G049	31.07403545	Moderate Tolerant
P1G011	31.43899896	Moderate Tolerant
P1G056	31.64754953	Moderate Tolerant
P1G100	32.06465068	Moderate Tolerant
P1G020	32.32533889	Moderate Tolerant
P1G227	32.48175182	Moderate Tolerant
P1G007	32.84671533	Moderate Tolerant
P1G030	32.89885297	Moderate Tolerant
P1G087	34.20229406	Moderate Tolerant
P1G250	34.2544317	Moderate Tolerant
P1G260	34.93222106	Moderate Tolerant
P1G008	35.03649635	Moderate Tolerant
P1G259	35.14077164	Moderate Tolerant

P1G237	35.29718457	Moderate Tolerant
P1G108	35.66214807	Moderate Tolerant
P1G271	35.71428571	Moderate Tolerant
P1G146	35.87069864	Moderate Tolerant
P1G092	36.80917623	Moderate Tolerant
P1G134	36.96558916	Moderate Tolerant
P1G197	37.64337852	Moderate Tolerant
P1G052	37.90406674	Moderate Tolerant
P1G060	38.11261731	Moderate Tolerant
P1G180	38.16475495	Moderate Tolerant
P1G165	38.32116788	Moderate Tolerant
P1G244	38.42544317	Moderate Tolerant
P1G174	38.47758081	Moderate Tolerant
P1G002	38.89468196	Moderate Tolerant
P1G025	39.10323253	Moderate Tolerant
P1G016	39.67674661	Moderate Tolerant
P1G051	39.67674661	Moderate Tolerant
P1G193	39.7810219	Moderate Tolerant
P1G113	39.83315954	Moderate Tolerant
P1G068	40.04171011	Moderate Tolerant
P1G274	40.56308655	Moderate Tolerant
P1G018	40.87591241	Moderate Tolerant
P1G031	41.13660063	Moderate Tolerant
P1G242	41.29301356	Moderate Tolerant
P1G209	41.86652763	Moderate Tolerant
P1G076	43.22210636	Moderate Tolerant
P1G152	43.274244	Moderate Tolerant
P1G013	43.69134515	Moderate Tolerant
P1G191	44.00417101	Moderate Tolerant
P1G222	44.42127216	Moderate Tolerant
P1G233	44.42127216	Moderate Tolerant
P1G004	44.62982273	Moderate Tolerant
P1G142	44.89051095	Moderate Tolerant
P1G048	45.09906152	Moderate Tolerant
P1G021	45.6725756	Moderate Tolerant
P1G045	46.0375391	Moderate Tolerant
P1G249	46.24608968	Moderate Tolerant
P1G138	46.76746611	Moderate Tolerant
P1G083	47.08029197	Moderate Tolerant
P1G101	47.08029197	Moderate Tolerant
P1G137	47.49739312	Moderate Tolerant
P1G252	48.01876955	Moderate Tolerant
P1G241	48.12304484	Moderate Tolerant
P1G217	48.17518248	Moderate Tolerant
P1G023	48.38373306	Moderate Tolerant

P1G196	48.38373306	Moderate Tolerant
P1G251	48.54014599	Moderate Tolerant
P1G218	48.64442127	Moderate Tolerant
P1G256	48.74869656	Moderate Sensitive
P1G110	48.90510949	Moderate Sensitive
P1G179	49.37434828	Moderate Sensitive
P1G089	50.3649635	Moderate Sensitive
P1G114	50.57351408	Moderate Sensitive
P1G194	50.72992701	Moderate Sensitive
P1G181	50.83420229	Moderate Sensitive
P1G265	51.1991658	Moderate Sensitive
P1G198	51.45985401	Moderate Sensitive
P1G205	51.87695516	Moderate Sensitive
P1G150	52.34619395	Moderate Sensitive
P1G017	52.7632951	Moderate Sensitive
P1G103	53.02398332	Moderate Sensitive
P1G010	53.44108446	Moderate Sensitive
P1G155	53.49322211	Moderate Sensitive
P1G090	53.70177268	Moderate Sensitive
P1G230	54.64025026	Moderate Sensitive
P1G063	54.79666319	Moderate Sensitive
P1G211	55.05735141	Moderate Sensitive
P1G143	55.16162669	Moderate Sensitive
P1G080	55.73514077	Moderate Sensitive
P1G145	55.78727842	Moderate Sensitive
P1G210	56.77789364	Moderate Sensitive
P1G183	57.29927007	Moderate Sensitive
P1G053	57.455683	Moderate Sensitive
P1G057	57.71637122	Moderate Sensitive
P1G245	57.97705944	Moderate Sensitive
P1G117	58.34202294	Moderate Sensitive
P1G102	58.70698644	Moderate Sensitive
P1G040	59.22836288	Moderate Sensitive
P1G184	59.22836288	Moderate Sensitive
P1G009	59.54118874	Moderate Sensitive
P1G234	59.80187696	Moderate Sensitive
P1G220	60.06256517	Moderate Sensitive
P1G273	60.11470282	Moderate Sensitive
P1G019	60.47966632	Moderate Sensitive
P1G070	60.58394161	Moderate Sensitive
P1G037	61.00104275	Moderate Sensitive
P1G067	61.57455683	Moderate Sensitive
P1G003	62.25234619	Moderate Sensitive
P1G128	62.51303441	Moderate Sensitive
P1G131	62.87799791	Moderate Sensitive

P1G064	62.93013556	Moderate Sensitive
P1G187	64.02502607	Moderate Sensitive
P1G272	64.33785193	Moderate Sensitive
P1G190	64.44212722	Moderate Sensitive
P1G027	65.53701773	Moderate Sensitive
P1G141	66.00625652	Moderate Sensitive
P1G055	66.73618352	Moderate Sensitive
P1G085	66.78832117	Moderate Sensitive
P1G125	66.78832117	Moderate Sensitive
P1G115	66.84045881	Moderate Sensitive
P1G039	67.10114703	Moderate Sensitive
P1G157	67.20542231	Moderate Sensitive
P1G223	67.36183525	Moderate Sensitive
P1G177	67.83107404	Moderate Sensitive
P1G189	68.03962461	Moderate Sensitive
P1G118	68.1438999	Moderate Sensitive
P1G195	68.92596455	Moderate Sensitive
P1G038	68.97810219	Moderate Sensitive
P1G124	69.39520334	Moderate Sensitive
P1G120	69.44734098	Moderate Sensitive
P1G062	69.49947862	Moderate Sensitive
P1G160	69.76016684	Moderate Sensitive
P1G206	69.81230448	Moderate Sensitive
P1G042	70.12513034	Moderate Sensitive
P1G248	71.11574557	Moderate Sensitive
P1G173	71.79353493	Moderate Sensitive
P1G239	71.84567258	Moderate Sensitive
P1G214	71.89781022	Moderate Sensitive
P1G024	72.21063608	Moderate Sensitive
P1G156	72.8362878	Moderate Sensitive
P1G203	72.8362878	Moderate Sensitive
P1G267	72.94056309	Moderate Sensitive
P1G088	73.04483837	Moderate Sensitive
P1G215	73.14911366	Moderate Sensitive
P1G176	73.30552659	Moderate Sensitive
P1G236	73.61835245	Moderate Sensitive
P1G255	73.77476538	Moderate Sensitive
P1G186	74.24400417	Moderate Sensitive
P1G054	74.55683003	Moderate Sensitive
P1G133	74.97393118	Moderate Sensitive
P1G213	74.97393118	Moderate Sensitive
P1G095	75.28675704	Sensitive
P1G240	75.49530761	Sensitive
P1G269	75.91240876	Sensitive
P1G012	76.01668405	Sensitive

P1G079	76.06882169	Sensitive
P1G122	76.64233577	Sensitive
P1G254	77.00729927	Sensitive
P1G044	77.05943691	Sensitive
P1G149	77.21584984	Sensitive
P1G266	77.68508863	Sensitive
P1G001	78.362878	Sensitive
P1G168	78.51929093	Sensitive
P1G161	78.93639208	Sensitive
P1G014	79.09280501	Sensitive
P1G159	79.40563087	Sensitive
P1G154	79.87486966	Sensitive
P1G073	80.34410845	Sensitive
P1G091	80.34410845	Sensitive
P1G081	80.55265902	Sensitive
P1G121	80.96976017	Sensitive
P1G199	82.11678832	Sensitive
P1G263	82.37747654	Sensitive
P1G151	82.42961418	Sensitive
P1G170	82.74244004	Sensitive
P1G270	83.10740355	Sensitive
P1G261	83.68091762	Sensitive
P1G098	83.8894682	Sensitive
P1G126	83.8894682	Sensitive
P1G109	83.94160584	Sensitive
P1G116	85.29718457	Sensitive
P1G167	86.1835245	Sensitive
P1G164	86.33993743	Sensitive
P1G077	86.44421272	Sensitive
P1G148	86.44421272	Sensitive
P1G231	87.06986444	Sensitive
P1G129	87.33055266	Sensitive
P1G212	87.79979145	Sensitive
P1G178	88.32116788	Sensitive
P1G050	89.83315954	Sensitive
P1G078	90.71949948	Sensitive
P1G069	90.9801877	Sensitive
P1G172	91.3451512	Sensitive
P1G182	91.39728884	Sensitive
P1G163	91.91866528	Sensitive
P1G034	92.544317	Sensitive
P1G253	93.16996872	Sensitive
P1G192	94.31699687	Sensitive
P1G130	94.83837331	Sensitive
P1G132	95.25547445	Sensitive

| P1G061 | 95.77685089 | Sensitive |
| P1G188 | 95.98540146 | Sensitive |

Appendix 4. Overall ST Ranking for 277 F$_3$ cross Bobur* UZ-11CWA08 lines.

Genoname	Overall ST Ranking	Status
P2G143	3.713254255	Tolerant
P2G223	4.074265085	Tolerant
P2G142	4.332129964	Tolerant
P2G067	6.291903043	Tolerant
P2G087	6.291903043	Tolerant
P2G047	7.013924703	Tolerant
P2G178	8.406395049	Tolerant
P2G181	8.561113976	Tolerant
P2G227	8.664259928	Tolerant
P2G164	9.437854564	Tolerant
P2G003	9.541000516	Tolerant
P2G230	10.62403301	Tolerant
P2G220	10.72717896	Tolerant
P2G207	10.93347086	Tolerant
P2G149	11.60391955	Tolerant
P2G076	11.65549252	Tolerant
P2G216	12.53223311	Tolerant
P2G221	12.53223311	Tolerant
P2G053	12.63537906	Tolerant
P2G214	13.04796287	Tolerant
P2G243	13.25425477	Tolerant
P2G135	13.51211965	Tolerant
P2G145	13.66683858	Tolerant
P2G179	13.66683858	Tolerant
P2G113	13.76998453	Tolerant
P2G132	14.18256833	Tolerant
P2G154	14.44043321	Tolerant
P2G235	14.49200619	Tolerant
P2G273	14.95616297	Tolerant
P2G081	15.26560083	Tolerant
P2G174	15.57503868	Tolerant
P2G219	16.50335224	Tolerant
P2G199	16.86436307	Tolerant
P2G251	17.019082	Tolerant
P2G184	17.2253739	Tolerant
P2G232	17.58638473	Tolerant
P2G190	17.68953069	Tolerant
P2G276	19.59773079	Tolerant
P2G168	20.26817947	Tolerant
P2G072	20.68076328	Tolerant

P2G055	21.19649304	Tolerant
P2G177	21.29963899	Tolerant
P2G118	21.45435792	Tolerant
P2G204	21.7122228	Tolerant
P2G044	22.3310985	Tolerant
P2G264	22.53739041	Tolerant
P2G234	22.64053636	Tolerant
P2G261	22.74368231	Tolerant
P2G176	23.25941207	Tolerant
P2G277	25.27075812	Moderate Tolerant
P2G086	25.42547705	Moderate Tolerant
P2G171	25.7349149	Moderate Tolerant
P2G239	25.99277978	Moderate Tolerant
P2G159	26.09592573	Moderate Tolerant
P2G007	26.92109335	Moderate Tolerant
P2G213	27.48839608	Moderate Tolerant
P2G228	27.48839608	Moderate Tolerant
P2G147	27.69468798	Moderate Tolerant
P2G202	27.74626096	Moderate Tolerant
P2G203	27.84940691	Moderate Tolerant
P2G140	27.90097989	Moderate Tolerant
P2G017	28.00412584	Moderate Tolerant
P2G186	28.41670964	Moderate Tolerant
P2G187	28.5198556	Moderate Tolerant
P2G272	29.24187726	Moderate Tolerant
P2G161	29.39659618	Moderate Tolerant
P2G215	29.86075297	Moderate Tolerant
P2G253	30.11861784	Moderate Tolerant
P2G194	30.17019082	Moderate Tolerant
P2G116	30.27333677	Moderate Tolerant
P2G250	30.37648272	Moderate Tolerant
P2G153	31.09850438	Moderate Tolerant
P2G192	31.15007736	Moderate Tolerant
P2G052	31.30479629	Moderate Tolerant
P2G120	32.07839092	Moderate Tolerant
P2G151	32.1299639	Moderate Tolerant
P2G150	32.74883961	Moderate Tolerant
P2G013	33.00670449	Moderate Tolerant
P2G131	33.67715317	Moderate Tolerant
P2G083	33.78029912	Moderate Tolerant
P2G008	33.88344507	Moderate Tolerant
P2G237	34.038164	Moderate Tolerant
P2G191	34.14130995	Moderate Tolerant
P2G229	34.29602888	Moderate Tolerant
P2G146	34.65703971	Moderate Tolerant

P2G160	34.65703971	Moderate Tolerant
P2G048	34.86333161	Moderate Tolerant
P2G155	34.96647757	Moderate Tolerant
P2G172	35.22434244	Moderate Tolerant
P2G246	35.5337803	Moderate Tolerant
P2G212	35.58535327	Moderate Tolerant
P2G256	35.63692625	Moderate Tolerant
P2G091	35.84321815	Moderate Tolerant
P2G241	35.84321815	Moderate Tolerant
P2G254	35.99793708	Moderate Tolerant
P2G252	36.82310469	Moderate Tolerant
P2G193	37.0293966	Moderate Tolerant
P2G097	37.08096957	Moderate Tolerant
P2G170	37.18411552	Moderate Tolerant
P2G245	37.49355338	Moderate Tolerant
P2G249	37.95771016	Moderate Tolerant
P2G205	38.11242909	Moderate Tolerant
P2G031	38.62815884	Moderate Tolerant
P2G244	38.62815884	Moderate Tolerant
P2G079	38.67973182	Moderate Tolerant
P2G152	38.78287777	Moderate Tolerant
P2G240	38.78287777	Moderate Tolerant
P2G196	38.88602372	Moderate Tolerant
P2G260	39.35018051	Moderate Tolerant
P2G026	40.07220217	Moderate Tolerant
P2G034	40.07220217	Moderate Tolerant
P2G035	40.17534812	Moderate Tolerant
P2G236	40.17534812	Moderate Tolerant
P2G271	40.22692109	Moderate Tolerant
P2G165	40.48478597	Moderate Tolerant
P2G175	40.58793192	Moderate Tolerant
P2G210	40.94894275	Moderate Tolerant
P2G033	41.20680763	Moderate Tolerant
P2G099	41.25838061	Moderate Tolerant
P2G050	41.36152656	Moderate Tolerant
P2G268	41.87725632	Moderate Tolerant
P2G274	42.03197524	Moderate Tolerant
P2G200	42.1351212	Moderate Tolerant
P2G225	43.16658071	Moderate Tolerant
P2G197	43.26972666	Moderate Tolerant
P2G270	43.52759154	Moderate Tolerant
P2G043	43.73388345	Moderate Tolerant
P2G242	44.14646725	Moderate Tolerant
P2G144	44.2496132	Moderate Tolerant
P2G163	44.55905106	Moderate Tolerant

P2G209	44.76534296	Moderate Tolerant
P2G266	44.97163486	Moderate Tolerant
P2G217	45.22949974	Moderate Tolerant
P2G158	46.36410521	Moderate Sensitive
P2G195	46.36410521	Moderate Sensitive
P2G054	46.46725116	Moderate Sensitive
P2G248	46.93140794	Moderate Sensitive
P2G169	46.98298092	Moderate Sensitive
P2G046	47.6534296	Moderate Sensitive
P2G032	47.70500258	Moderate Sensitive
P2G018	47.80814853	Moderate Sensitive
P2G231	48.01444043	Moderate Sensitive
P2G111	48.27230531	Moderate Sensitive
P2G201	49.09747292	Moderate Sensitive
P2G224	49.25219185	Moderate Sensitive
P2G014	49.3553378	Moderate Sensitive
P2G233	49.3553378	Moderate Sensitive
P2G188	49.66477566	Moderate Sensitive
P2G010	49.87106756	Moderate Sensitive
P2G157	50.69623517	Moderate Sensitive
P2G166	50.79938112	Moderate Sensitive
P2G129	51.00567303	Moderate Sensitive
P2G019	51.10881898	Moderate Sensitive
P2G069	51.52140278	Moderate Sensitive
P2G141	51.67612171	Moderate Sensitive
P2G128	52.14027849	Moderate Sensitive
P2G100	52.29499742	Moderate Sensitive
P2G066	52.60443528	Moderate Sensitive
P2G183	53.12016503	Moderate Sensitive
P2G038	53.27488396	Moderate Sensitive
P2G077	53.48117586	Moderate Sensitive
P2G030	54.4610624	Moderate Sensitive
P2G064	54.66735431	Moderate Sensitive
P2G036	54.92521919	Moderate Sensitive
P2G122	55.02836514	Moderate Sensitive
P2G080	55.44094894	Moderate Sensitive
P2G112	55.80195977	Moderate Sensitive
P2G049	56.00825168	Moderate Sensitive
P2G148	56.1629706	Moderate Sensitive
P2G117	56.42083548	Moderate Sensitive
P2G108	56.62712739	Moderate Sensitive
P2G016	57.76173285	Moderate Sensitive
P2G269	58.01959773	Moderate Sensitive
P2G029	58.84476534	Moderate Sensitive
P2G063	59.3604951	Moderate Sensitive

P2G173	59.51521403	Moderate Sensitive
P2G265	60.59824652	Moderate Sensitive
P2G068	60.64981949	Moderate Sensitive
P2G023	61.78442496	Moderate Sensitive
P2G057	62.45487365	Moderate Sensitive
P2G011	62.60959257	Moderate Sensitive
P2G226	62.60959257	Moderate Sensitive
P2G059	62.71273853	Moderate Sensitive
P2G114	63.07374936	Moderate Sensitive
P2G024	63.12532233	Moderate Sensitive
P2G123	63.22846828	Moderate Sensitive
P2G258	63.22846828	Moderate Sensitive
P2G206	63.38318721	Moderate Sensitive
P2G062	63.58947911	Moderate Sensitive
P2G101	63.69262506	Moderate Sensitive
P2G134	63.89891697	Moderate Sensitive
P2G263	64.20835482	Moderate Sensitive
P2G167	64.62093863	Moderate Sensitive
P2G107	65.80711707	Moderate Sensitive
P2G002	66.27127385	Moderate Sensitive
P2G137	66.32284683	Moderate Sensitive
P2G257	66.42599278	Moderate Sensitive
P2G267	66.68385766	Moderate Sensitive
P2G056	66.73543063	Moderate Sensitive
P2G119	67.35430634	Moderate Sensitive
P2G136	67.50902527	Moderate Sensitive
P2G039	68.48891181	Moderate Sensitive
P2G106	68.59205776	Moderate Sensitive
P2G060	69.67509025	Moderate Sensitive
P2G262	69.67509025	Moderate Sensitive
P2G009	69.98452811	Moderate Sensitive
P2G078	70.08767406	Moderate Sensitive
P2G084	70.8612687	Moderate Sensitive
P2G124	72.09902011	Moderate Sensitive
P2G275	72.30531202	Moderate Sensitive
P2G185	72.82104177	Moderate Sensitive
P2G198	72.87261475	Moderate Sensitive
P2G074	73.1820526	Moderate Sensitive
P2G045	73.59463641	Moderate Sensitive
P2G211	73.64620939	Moderate Sensitive
P2G040	74.78081485	Moderate Sensitive
P2G025	74.83238783	Moderate Sensitive
P2G037	75.70912842	Sensitive
P2G180	75.81227437	Sensitive
P2G105	76.07013925	Sensitive

P2G189	76.32800413	Sensitive
P2G126	76.53429603	Sensitive
P2G222	76.79216091	Sensitive
P2G255	76.79216091	Sensitive
P2G006	77.61732852	Sensitive
P2G102	77.77204745	Sensitive
P2G156	77.97833935	Sensitive
P2G015	78.59721506	Sensitive
P2G104	78.80350696	Sensitive
P2G182	79.06137184	Sensitive
P2G238	79.16451779	Sensitive
P2G090	79.62867457	Sensitive
P2G051	79.93811243	Sensitive
P2G092	79.93811243	Sensitive
P2G012	80.09283136	Sensitive
P2G022	80.29912326	Sensitive
P2G138	80.35069624	Sensitive
P2G082	81.43372873	Sensitive
P2G094	82.980918	Sensitive
P2G058	83.29035585	Sensitive
P2G004	83.96080454	Sensitive
P2G098	85.19855596	Sensitive
P2G070	85.25012893	Sensitive
P2G065	85.50799381	Sensitive
P2G088	85.61113976	Sensitive
P2G127	85.92057762	Sensitive
P2G130	86.53945333	Sensitive
P2G162	86.84889118	Sensitive
P2G005	87.77720474	Sensitive
P2G089	88.49922641	Sensitive
P2G115	88.86023724	Sensitive
P2G001	88.96338319	Sensitive
P2G028	89.32439402	Sensitive
P2G027	90.25270758	Sensitive
P2G041	90.61371841	Sensitive
P2G139	90.71686436	Sensitive
P2G073	91.07787519	Sensitive
P2G095	91.54203198	Sensitive
P2G042	92.36719959	Sensitive
P2G125	92.67663744	Sensitive
P2G020	92.77978339	Sensitive
P2G096	92.83135637	Sensitive

Appendix 3. Colocation of SNP clusters with QTL/genes and the candidate genes for the significant marker–trait associations for salinity tolerance in the contrasting wheat genotypes. ST_DRW Salt Tolerance Dry Root Weight

Associated ST traits	SNP	Contrasting F$_3$ lines	Chr.	QTL	R^2 (%)	Position (bp)	Position (CM)
ST_DRW	BS00002178_51	Bobur* UZ-11CWA08	1DS	Q-1DS.1	≥13.33	33712262..33712362	108.87
ST_DRW	RAC875_c62_1546	Bobur*Altay2000	1DS	Q-1DS.2	≥13.33	32543884..32543984	108.87
ST_DRW	BS00087086_51	Bobur* UZ-11CWA08	1DS	Q-1DS.3	≥13.33	34619721..34619821	108.87
ST_DRW	Ex_c16948_754	Bobur*Altay2000	2BS	Q-2BS.1	≥12.69	699826968..699827068	367.4
ST_DRW	BobWhite_c48435_165	Bobur* UZ-11CWA08	5BL	Q-5BL.1	≥24.20	546827468..546827565	280.68

BS00002178_51 (Chr.1DS) 33712262..33712362 bp

Gene ID	Chr.	Gene Position(bp)	Gene Length(bp)	Gene Annotation
TraesCS1D02G053000	1DS	33711972..33712886	915	**transport** (GO:0006810) **cellular process** (GO:0009987) **signaling** (GO:0023052)
TraesCS1D02G052900	1DS	33705560..33706322	763	**transport** (GO:0006810) **cation channel activity** (GO:0005261) **metal ion transmembrane transporter activity** (GO:0046873)
TraesCS1D02G052200	1DS	33469805..33474180	4,376	**nucleotide binding** (GO:0000166) **alcohol dehydrogenase (NAD+) activity** (GO:0004022) **zinc ion binding** (GO:0008270) **response to salt stress** (GO:0009651)
TraesCS1D02G052300	1DS	33552707..33553135	429	**zinc ion binding** (GO:0008270)
TraesCS1D02G052700	1DS	33638501..33640761	2,261	**leaf senescence** (GO:0010150) **abscisic acid stimulus** (GO:0071215) **cellular response to salicylic acid stimulus** (GO:0071446) **cellular response to osmotic stress** (GO:0071470)

RAC875_c62_1546 (Chr.1DS) 32543884..32543984 bp

Gene ID	Chr.	Gene Position (bp)	Gene Length (bp)	Gene Annotation
TraesCS1D02G050500	1DS	30664423..30665861	1,439	**ATP binding** (GO:0005524)
TraesCS1D02G051000	1DS	31060344..31061952	1,609	**ATP binding** (GO:0005524)
TraesCS1D02G051600	1DS	32423812..32424632	821	**ATP binding** (GO:0005524)
TraesCS1D02G051700	1DS	32479445..32482026	2,582	**zinc ion binding** (GO:0008270)
TraesCS1D02G051900	1DS	32543984..32546795	2,812	**zinc ion binding** (GO:0008270)
TraesCS1D02G052000	1DS	32737866..32740293	2,428	**ATP binding** (GO:0005524)

RAC875_c62_1546 (Chr.1DS) 32543884..32543984 bp

Gene ID	Chr.	Gene Position (bp)	Gene Length (bp)	Gene Annotation
TraesCS1D02G052200	1DS	33469805..33474180	4,376	**zinc ion binding** (GO:0008270) **catabolic process** (GO:0009056) **response to salt stress** (GO:0009651)
TraesCS1D02G052300	1DS	33552707..33553135	429	**zinc ion binding** (GO:0008270)
TraesCS1D02G052700	1DS	33638501..33640761	2,261	**leaf senescence** (GO:0010150) **cellular response to osmotic stress** (GO:0071470)
TraesCS1D02G052900	1DS	33705560..33706322	763	**cation channel activity** (GO:0005261) **metal ion transmembrane transporter activity** (GO:0046873)
TraesCS1D02G053100	1DS	33801952..33805289	3,338	**hydrolase activity** (GO:0016787)
TraesCS1D02G053500	1DS	34636225..34639650	3,426	**ATP binding** (GO:0005524)
TraesCS1D02G053700	1DS	34943205..34944312	1,108	**calcium ion binding** (GO:0005509)
TraesCS1D02G053800	1DS	34944703..34946404	1,702	**hydrolase activity** (GO:0016787)
TraesCS1D02G054100	1DS	35639168..35645648	6,481	**response to stress** (GO:0006950) **hydrolase activity, acting on ester bonds** (GO:0016788)
TraesCS1D02G054200	1DS	35649432..35654365	4,934	**response to stress** (GO:0006950) **hydrolase activity, acting on ester bonds** (GO:0016788)
TraesCS1D02G054300	1DS	35786547..35787507	961	**response to stress** (GO:0006950)
TraesCS1D02G054400	1DS	35788030..35788783	754	**response to salt stress** (GO:0009651)
TraesCS1D02G054500	1DS	35886292..35900512	14,221	**response to salt stress** (GO:0009651)
TraesCS1D02G054600	1DS	35926381..35927231	851	**response to salt stress** (GO:0009651)
TraesCS1D02G054800	1DS	36315758..36316553	796	**cellular response to stress** (GO:0033554)
TraesCS1D02G054900	1DS	36322597..36323744	1,148	**ATP binding** (GO:0005524)

BS00087086_51 (Chr.1DS) 34619721..34619821 bp

Gene ID	Chr.	Gene Position (bp)	Gene Length (bp)	Gene Annotation
TraesCS1D02G052200	1DS	33469805..33474180	4,376	**zinc ion binding** (GO:0008270) **catabolic process** (GO:0009056) **response to salt stress** (GO:0009651)
TraesCS1D02G052300	1DS	33552707..33553135	429	**zinc ion binding** (GO:0008270)
TraesCS1D02G052700	1DS	33638501..33640761	2,261	**leaf senescence** (GO:0010150) **cellular response to osmotic stress** (GO:0071470)
TraesCS1D02G052900	1DS	33705560..33706322	763	**cation channel activity** (GO:0005261) **metal ion transmembrane transporter activity** (GO:0046873)
TraesCS1D02G053100	1DS	33801952..33805289	3,338	**hydrolase activity** (GO:0016787)
TraesCS1D02G053500	1DS	34636225..34639650	3,426	**ATP binding** (GO:0005524)
TraesCS1D02G053700	1DS	34943205..34944312	1,108	**calcium ion binding** (GO:0005509)
TraesCS1D02G053800	1DS	34944703..34946404	1,702	**hydrolase activity** (GO:0016787)
TraesCS1D02G054100	1DS	35639168..35645648	6,481	**response to stress** (GO:0006950) **hydrolase activity, acting on ester bonds** (GO:0016788)
TraesCS1D02G054200	1DS	35649432..35654365	4,934	**response to stress** (GO:0006950) **hydrolase activity, acting on ester bonds** (GO:0016788)
TraesCS1D02G054300	1DS	35786547..35787507	961	**response to stress** (GO:0006950)
TraesCS1D02G054400	1DS	35788030..35788783	754	**response to salt stress** (GO:0009651)
TraesCS1D02G054500	1DS	35886292..35900512	14,221	**response to salt stress** (GO:0009651)
TraesCS1D02G054600	1DS	35926381..35927231	851	**response to salt stress** (GO:0009651)
TraesCS1D02G054800	1DS	36315758..36316553	796	**cellular response to stress** (GO:0033554)
TraesCS1D02G054900	1DS	36322597..36323744	1,148	**ATP binding** (GO:0005524)

Ex_c16948_754 (Chr.2BS) 699826968..699827068 bp

Gene ID	Chr.	Gene Position(bp)	Gene Length (bp)	Gene Annotation
TraesCS2B02G504400	2BS	698948580..698951170	2,591	**voltage-gated ion channel activity (GO:0005244)** **calcium channel activity** (GO:0005262)
TraesCS2B02G504500	2BS	698951855..698954968	3,114	**protein kinase activity** (GO:0004672) **metal ion binding** (GO:0046872)
TraesCS2B02G504600	2BS	698963317..698969654	6,338	**calcium ion transport** (GO:0006816) **ATP binding** (GO:0005524) **zinc ion binding** (GO:0008270)
TraesCS2B02G504700	2BS	699039998..699049444	9,447	**response to stress** (GO:0006950) **positive regulation of programmed cell death** (GO:0043068)
TraesCS2B02G504900	2BS	699106129..699110782	4,654	**protein phosphorylation** (GO:0006468)
TraesCS2B02G505000	2BS	699173218..699176344	3,127	**response to metal ion** (GO:0010038) **enzyme binding** (GO:0019899) **copper ion binding** (GO:0005507)
TraesCS2B02G505100	2BS	699177857..699179469	1,613	**response to stress** (GO:0006950)
TraesCS2B02G505300	2BS	699223169..699225929	2,761	**response to stress** (GO:0006950) **lipid catabolic process** (GO:0016042)
TraesCS2B02G505400	2BS	699281542..699286967	5,426	**ATP binding** (GO:0005524)
TraesCS2B02G505500	2BS	699827467..699830441	2,975	**zinc ion binding** (GO:0008270)
TraesCS2B02G506000	2BS	701359791..701360810	1,020	**ATP binding** (GO:0005524)
TraesCS2B02G506200	2BS	701490662..701490934	273	**response to stress** (GO:0006950) **metal ion transport** (GO:0030001) **potassium channel activity** (GO:0005267)

Ex_c16948_754 **(Chr.2BS) 699826968..699827068 bp**

Gene ID	Chr.	Gene Position (bp)	Gene Length (bp)	Gene Annotation
TraesCS2B02G506400	2BS	702724421..702727614	3,194	**zinc ion binding** (GO:0008270)
TraesCS2B02G507100	2BS	703972469..703976356	3,888	**phosphorylation** (GO:0016310)
TraesCS2B02G507500	2BS	704129187..704130949	1,763	**oxidoreductase activity, acting on CH-OH group of donors** (GO:0016614)
TraesCS2B02G507700	2BS	704291232..704293317	2,086	**oxidoreductase activity, acting on CH-OH group of donors** (GO:0016614)
TraesCS2B02G507800	2BS	704295292..704297547	2,256	**oxidoreductase activity, acting on CH-OH group of donors** (GO:0016614)
TraesCS2B02G508000	2BS	704412130..704414285	2,156	**oxidoreductase activity, acting on CH-OH group of donors** (GO:0016614)
TraesCS2B02G508100	2BS	704425924..704429773	3,850	**oxidoreductase activity, acting on CH-OH group of donors** (GO:0016614)
TraesCS2B02G501700	2BS	697141935..697142588	654	**response to stress** (GO:0006950)
TraesCS2B02G501800	2BS	697156916..697157553	638	**response to stress** (GO:0006950)
TraesCS2B02G502100	2BS	697294756..697295142	387	**response to stress** (GO:0006950)
TraesCS2B02G502300	2BS	697340985..697341371	387	**response to stress** (GO:0006950)
TraesCS2B02G502600	2BS	697378406..697378986	581	**response to stress** (GO:0006950)
TraesCS2B02G502800	2BS	697398239..697398802	564	**response to stress** (GO:0006950)
TraesCS2B02G503000	2BS	697440940..697444611	3,672	**catabolic process** (GO:0009056)
TraesCS2B02G503100	2BS	697546485..697548362	1,878	**response to toxic substance** (GO:0009636) **response to salt stress** (GO:0009651)
TraesCS2B02G503200	2BS	697571625..697587793	16,169	**zinc ion binding** (GO:0008270)
TraesCS2B02G503300	2BS	697665455..697666303	849	**response to stress** (GO:0006950)
TraesCS2B02G503400	2BS	697707263..697714188	6,926	**ATP binding** (GO:0005524)
TraesCS2B02G503500	2BS	697767267..697768957	1,691	**protein kinase binding** (GO:0019901)
TraesCS2B02G503600	2BS	697976012..697980451	4,440	**protein kinase binding** (GO:0019901)
TraesCS2B02G503700	2BS	697986239..697996332	10,094	**ATP binding** (GO:0005524)
TraesCS2B02G503800	2BS	698002411..698004562	2,152	**response to oxidative stress** (GO:0006979) **cellular oxidant detoxification** (GO:0098869)

				ATP binding (GO:0005524) **hydrolase activity** (GO:0016787)
TraesCS2B02G504000	2BS	698212664..698217597	4,934	**zinc ion binding** (GO:0008270)
TraesCS2B02G504100	2BS	698294968..698296747	1,780	**protein kinase activity** (GO:0004672)
TraesCS2B02G504300	2BS	698303626..698306747	3,122	**protein kinase activity** (GO:0004672)

BobWhite_c48435_165 (Chr.5BL) 546827468..546827565 bp

Gene ID	Chr.	Gene Position(bp)	Gene Length(bp)	Gene Annotation
TraesCS5B02G368500	5BL	546826331..546832103	5,773	**potassium ion transmembrane transport** (GO:0071805) **potassium ion transmembrane transporter activity** (GO:0015079)
TraesCS5B02G367200	5BL	545817415..545819994	2,580	**protein kinase activity** (GO:0004672) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G367300	5BL	545922998..545924246	1,249	**protein kinase activity** (GO:0004672) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G367500	5BL	546072781..546075278	2,498	**protein serine/threonine kinase activity** (GO:0004674) **calmodulin binding** (GO:0005516) **ATP binding** (GO:0005524)
TraesCS5B02G367600	5BL	546093997..546097128	3,132	**protein serine/threonine kinase activity** (GO:0004674) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G367700	5BL	546115790..546124256	8,467	**protein serine/threonine kinase activity** (GO:0004674) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G367800	5BL	546144826..546145902	1,077	**protein serine/threonine kinase activity** (GO:0004674) **ATP binding** (GO:0005524)
TraesCS5B02G367900	5BL	546317678..546326440	8,763	**protein kinase activity** (GO:0004672) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524) **response to stress** (GO:0006950)
TraesCS5B02G368000	5BL	546486031..546489464	3,434	**protein serine/threonine kinase activity** (GO:0004674) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)

BobWhite_c48435_165 (Chr.5BL) 546827468..546827565 bp

Gene ID	Chr.	Gene Position(bp)	Gene Length(bp)	Gene Annotation
TraesCS5B02G368137	5BL	546533965..546538744 bp	4,780	**protein kinase activity** (GO:0004672) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G368200	5BL	546564774..546569512 bp	4,739	**protein serine/threonine kinase activity** (GO:0004674) **calcium ion binding** (GO:0005509) **ATP binding** (GO:0005524)
TraesCS5B02G368300	5BL	546703873..546705896 bp	2,024	**leaf senescence** (GO:0010150)
TraesCS5B02G368400	5BL	546808606..546813282 bp	4,677	**nucleotide binding** (GO:0000166) **mRNA splicing, via spliceosome** (GO:0000398)
TraesCS5B02G368600	5BL	546845714..546868426 bp	22,713	**zinc ion binding** (GO:0008270)
TraesCS5B02G368700	5BL	546867095..546868036 bp	942	**zinc ion binding** (GO:0008270)
TraesCS5B02G368800	5BL	547399295..547406590 bp	7,296	**sodium ion transport** (GO:0006814) **chloride ion homeostasis** (GO:0055064) **potassium ion homeostasis** (GO:0055075) **potassium ion transmembrane transport** (GO:0071805) **chloride transmembrane transport** (GO:1902476) **sodium:potassium:chloride symporter activity** (GO:0008511) **potassium:chloride symporter activity** (GO:0015379)
TraesCS5B02G368900	5BL	547652774..547657481 bp	4,708	**ATP binding** (GO:0005524) **response to hydrogen peroxide** (GO:0042542)
TraesCS5B02G369100	5BL	548107622..548108983 bp	1,362	**zinc ion binding** (GO:0008270)
TraesCS5B02G369300	5BL	548340275..548352701 bp	12,427	**hydrolase activity** (GO:0016787)
TraesCS5B02G369500	5BL	548478746..548479336 bp	591	**response to stress** (GO:0006950) **zinc ion binding** (GO:0008270)
TraesCS5B02G369600	5BL	548565536..548565799 bp	264	**response to stress** (GO:0006950) **signal transduction** (GO:0007165) **zinc ion binding** (GO:0008270) **hydrolase activity** (GO:0016787)