GENETIC VARIATION FOR SALT AND ZINC DEFFICIENCY TOLERANCE IN *AEGILOPS TAUSCHII*

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Abstract

Salinity is a major agricultural problem limiting crop yield, particularly in arid and semi-arid regions where cereal production is common. Water and mineral nutrient uptakes in plants are affected by salt stress, and consequently, plant growth rate is reduced. In arid and semi-arid regions, zinc (Zn) deficiency is also a common constraint to crop production. Among number of solutions to these problems, selection of new genotypes with high tolerance to salt toxicity and Zn deficiency is the most sustainable and widely accepted approach. It is well known that the cultivated (modern) wheat has less genetic variation for a given trait than the wild or primitive wheats. *Aegilops tauschii* is a wild relative of wheat and the D-genome donor of wheat. In the present MSc study, salt tolerance and Zn efficiency (tolerance to Zn deficiency) of different *Aegilops tauschii* genotypes and modern wheat cultivars were investigated to identify and characterize the salt tolerant and Zn-efficient genotypes. Experiments were conducted under

greenhouse conditions by growing 116 *Aegilops tauschii* genotypes and 28 cultivated (modern) wheat cultivars at different levels of salt and Zn treatments. Genotypes were tested for severity of leaf symptoms, shoot dry weight, shoot Na, K, Ca and Zn concentrations, and ratios of K/Na and Ca/Na in shoot to determine physiological parameters associated with salt tolerance of genotypes. There was a large genetic variation in tolerance to NaCl toxicity among *Aegilops tauschii* genotypes based on the severity of leaf symptoms (leaf chlorosis and necrosis) and decreases in shoot dry matter production. This genetic variation has been evaluated and discussed in relation to the shoot concentrations of Na, K, and Ca and K/Na and Ca/Na ratios of the genotypes. The results indicated that K/Na and Ca/Na ratios are very important parameters involved in differential expression of high salt tolerance among *Aegilops tauschii* genotypes.

The *Aegilops tauschii* genotypes tested for salt tolerance was also examined for their Zn deficiency tolerance. The results obtained showed existence of a marked genetic variation in tolerance to Zn deficiency among the *Aegilops tauschii* genotypes. The selected genotypes for differential tolerance to Zn deficiency have been characterized for shoot concentrations of Zn, Na, K, Ca and P and also for dry matter production and seed concentrations of Zn. Adequate Zn supply was affective in reducing Na concentrations and increasing K/Na ratio of plants. The results of this thesis revealed new *Aegilops tauschii* genotypes with very high tolerance to both Zn deficiency and NaCl toxicity. These genotypes have been recommended for exploitation in future breeding programmes.

AEGILOPS TAUSCHII'DE TUZ STRESİ VE ÇİNKO EKSİKLİĞİNE DAYANIKLILIK İÇİN GENETİK VARYASYON

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Özet

Tahıl üretiminin yaygın olduğu kurak ve yarı kurak bölgelerde görülen tuzluluk, bitkisel verimi sınırlandıran önemli bir problemdir. Bitkinin su ve besin alımı tuz stresinden etkilenir ve bunların sonucu olarak bitkinin büyüme hızı azalır. Çinko eksikliği de kurak ve yarı kurak bölgelerde bitki üretimini yaygın olarak kısıtlamaktadır. Bu sorunları çözmek için birçok çözüm arasından, tuz toksitesi ve Zn noksanlığına dayanıklı yeni genotipleri seçmek en uzun soluklu ve yaygın olarak kabul edilen yaklaşımdır. Bilindiği gibi modern buğdaylar yabani veya ilkel buğdaylara göre belirli bir karakter göstermek için daha az genetik varyasyona sahiptir. *Aegilops tauschii* buğdayın yabani bir akrabası olup buğdaydaki D genomunun vericisidir. Bu yüksek lisans çalışmasında, tuza ve Zn eksikliğine karşı dayanıklı genotiplerin belirlenmesi için modern buğday çeşitleri ve farklı *Aegilops tauschii* genotiplerinin tuza dayanıklılığı ve çinko etkinliği incelendi. Farlı tuz ve Zn uygulamalarında yetiştirilen 116 *Aegilops tauschii* genotipi ve 28

modern buğday çeşidi kullanılarak sera koşulları altında denemeler yürütüldü. Genotiplerin tuza toleransı ile ilişkilendirilen fizyolojik özellikleri saptamak için yaprak simptom şiddetleri (yaprak sarılığı ve nekroz), yeşil aksam kuru madde ağırlığı, yeşil aksam Na, K, Ca ve Zn konsantrasyonları ile yeşil aksamdaki K/Na ve Ca/Na oranları incelendi. Yaprak simptom şiddetleri (yaprak sarılığı ve nekroz) ve yeşil aksam kuru madde ağırlığına bağlı olarak, NaCl toksitesine dayanıklılıkta *Aegilops tauschii* genotipleri arasında büyük genetik farklılıklar bulundu. Bu genetik varyasyon, genotiplerin yeşil aksam Na, K ve Ca konsantrasyonları ve yeşil aksamdaki K/Na ve Ca/Na oranlarıyla ilişkilendirilerek değerlendirilip tartışılmıştır. Seçilen *Aegilops tauschii* genotipleri arasındaki yüksek tuz dayanıklılığında farklılığın çok önemli parametreler olan K/Na ve Ca/Na oranlarıyla ilişkili olduğu gösterilmiştir.

Tuzluluk stresi için test edilmiş *Aegilops tauschii* genotipleri, Zn noksanlığına dayanıklılıklarına göre de incelendi. Elde edilen sonuçlar *Aegilops tauschii* genotipleri arasında belirgin genetik varyasyon olduğunu göstermektedir. Çinko eksikliğine dayanıklılıkta farklılığı göstermek için seçilen genotipler yeşil aksam Zn, Na, K, Ca ve P konsantrasyonları ve yeşil aksam kuru madde miktarı ile tohum Zn konsantrasyonları karakterize edildi. Yeterli Zn miktarının, Na konsantrasyonlarının azalmasında ve bitkilerin K/Na oralarının artmasında etkili olduğu gösterildi. Tez sonuçları Zn noksanlığına ve NaCl toksitesine karşı çok dayanıklı olan yeni *Aegilops tauschii* genotipleri bulunduğunu göstermektedir. Bu genotiplerin ileriki breeding programlarında değerlendirilmesi tavsiye edilmektedir.

"To my family and my spiritual-father"

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TABLE OF ABBREVIATIONS

Ca: Calcium

CIMMYT: International maize and wheat improvement center

Cl: Chlorine

DW: Dry weight

EC: Electrical conductivity

ESP: Exchangeable sodium percentage

FAO: Food and agriculture organization

HNO_3: Nitric acid

H_2O_2: Hydrogen peroxide

ICP-OES: Inductively coupled plasma optical emission spectroscopy

K: Potassium

µg: Microgram

mg: Milligram

Na: Sodium

NADPH: Nicotinamide adenine dinucleotide

ROS: Reactive oxygen species

SOD: Superoxide dismutase

Zn: Zinc

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1 INTRODUCTION

Salinization of soils is a natural phenomenon and occurs in nearly all climatic regions, from deserts to the tropical regions and even in Antarctica; and at different altitudes such as below sea level and 5000 meter high mountains. Globally, salt-affected land covers over 800 million hectares, which is over 6% of the world's total land area (FAO, 2000). Salt affected soils include saline (3.1%) and sodic (3.4%) areas (FAO, 2000). Recently saline lands have occurred through human-induced processes such as land clearing, and irrigation. Secondary salinity affects 19.5% of the current 230 million ha of irrigated land and 2.1% of the 1.5 billion ha under dryland agriculture (FAO, 2000). Irrigated land has high productivity; although only 15% of cultivated land is irrigated, one-third of the world's food is produced in irrigated land (Munns, 2005). Irrigation in semi-arid and arid regions, especially those with ineffective drainage, causes the accumulation of soluble salts in the soil water to an extent that affects plant growth (FAO, 2000). The area of arable land has increased dramatically during last two century; however a worldwide average per capita arable land was 0.38 ha in 1970 and has decreased 0.23 ha in 2000 because of a huge increase in human population (FAO, 2000). Human population was about 2.5 billion in 1950 and it is expected to be over 9 billion people a century later (World Resources Institute, 2004). The requirements of fresh water, which is already limited, have increased due to a huge rise in world population. Limited fresh water is essential for humankind, and crop plants. Global warming raise enhances yet further concern about water shortages. However, irrigation with limitless diluted sea water can solve this problem by the breeding of new crop cultivars with improved salt tolerance.

There is a negative correlation between soil productivity and salinity which inhibits plant growth. The whole plant metabolism is affected by salinity. Reduction in growth under salinity is usually related to inhibition of water uptake, ion deficiency or toxicity which may affect physiological and biochemical processes of plants (Munns and Termeat 1986**,** Greenway and

Munns 1980). Salt-affected plants display a decline in quality, inhibition in growth and reduction in crop yield.

As indicated above, all cultivated soils have certain amount of soluble salts. A saline soil contains soluble salts which are high enough to inhibit plant development. Soluble salts are divided into various types such as chlorides, sulphates and carbonates. Sodium chloride is the most soluble salt. Salt-affected soils are classified into three groups, which are saline, sodic and saline sodic soils, and characterized by electrical conductivity (EC) and exchangeable sodium percentage (ESP). Saline soils have more than 4 dS m^{-1} and less than 15% ESP (USDA, 1954).

In Turkey, 28.5 million ha is used for agricultural production and of which only 4.5 million is currently irrigated. The one-third of the irrigated area in Turkey is salt-affected (FAO, 2000). Salinization problems are associated with excessive fertilization, improper irrigation and insufficient drainage systems. Human induced soil salinity is spreading day by day and in each minute, minimal three hectares of arable land in the world is lost due to soil salinity (FAO, 2000). It is forecasted that the arable land demand in Turkey will increase from current about 2.4 persons/ha to 5 persons/ha in the near future (FAO, 2000). Therefore, water resources and saline arable lands must be managed successfully in a sustainable way.

Salinity can restrict plant development by three main ways (Marschner, 1995). These are water stress, ion toxicity (especially Cl or Na) and nutrient ion imbalance associated with a decline in K, Ca, NO or P uptake, or damage to internal transportation of these ions, whereas Cl and Na uptake increases. Crucial changes in water and ion equilibrium cause restriction of plant development and oxidative degradation of chlorophyll (including leaf chlorosis) and plasma membrane (causing lipid peroxidation), and even death of plants depending on the level of salt stress or salinity tolerance of plant.

To prevent from salt effects on plants, soil reclamation is applied to minimize soil degradation by salinization and improve current saline soils. However, it depends on the soil permeability and good quality irrigation water that is insufficient in the widespread saline soils in arid and semi-arid regions. Salinization problem is also solved by adequate drainage, but it is not sustainable (e.g., time consuming and not economically practical). Therefore, selection and breeding new plant genotypes with high salt tolerance is widely accepted approach for solution of salinity problem. These tolerant crops can be irrigated by more cost-effective brackish water that

contributes a decrease in the fresh water requirement. With rapidly consuming water resources, increasing the salt tolerance of crops has become a more important global issue.

Plants generally respond to salinity by exclusion or inclusion of ions (Greenway and Munns, 1980). Salt exclusion can be described as a mechanism that contributes to ability of plants to prevent uptake of toxic ions. In this case, plant tolerance to salinity is associated with Na exclusion (Munns, 2002). However, salt tolerance is not always related with ability to exclude toxic ions. Salt tolerant plants can also contain large quantities of salt in the shoot. In such salt-tolerant plants, toxic ions are generally accumulated in vacuoles (e.g., ion compartmentation) to maintain low concentrations of toxic ions in the cytoplasm and thus homeostatic balance at cellular level. This mechanism is described as salt inclusion of salt tolerant plants. In addition to salt exclusion and inclusion, salt tolerance can be affected from concentrations of ions and ionic relations in the substrate, duration of salt exposure, plant species, cultivar and root stock, stage of plant development, plant organ and environmental conditions (Marschner, 1995). Plants are affected from salinity in varying degrees according to stage of plant development, environmental factors, and plant species.

Salt stress influences whole plant by affecting number of metabolic pathways. Due to this complexity, a great number of parameters are used to select salt tolerant genotypes. The concentrations of Na, Cl, K and Ca in various tissues and organelles, K/Na balance for cytoplasmic homeostasis, high Ca/Na ratio and nutrient uptake, secretion and/or compartmentation into the vacuole of Na; and biosynthesis and accumulation of compatible solutes are the factors or traits commonly used to determine the level of salt sensitivity of plants. Salinity also causes phenotypic changes in plants. The rate of leaf expansion decreases as a first response to salt stress, and then chlorosis and necrosis, especially on older leaves, are observed due to salinity. The changes occurred in salt stress are used as parameters to detect salt tolerance of plants. The diagnostic parameter of salt tolerance of crops is their yield in saline versus non-saline conditions. Plants give different response to salinity in different growth stages; some plants are more sensitive to salinity during germination and some during seed formation. Plants exposed to salinity need controlling at different periods to obtain the alterations in salt response mechanisms in time. Salt tolerance has to be controlled in different growth stages at whole plant level, at cellular level (Munns *et al*., 2002; Tester and Davenport, 2003).

Selectivity between K and Na and as a consequence of this, high K/Na ratio for maintaining osmotic pressure is important in plant capacity to grow at high external Na. Osmotic adjustment is required for water uptake and prevention of ion toxicities, therefore K/Na discrimination contributes to osmotic adjustment by lowering rates of Na accumulation and raising K/Na ratio.

Wheat represent main source of the daily calorie intake both in Turkey and globally, and Turkey is one of the top ten wheat producers in the world (FAO, 2005). Salt tolerance in wheat is associated with high K/Na ratio, and bread wheats (AABBDD) with the generally higher leaf K/Na ratio is more tolerant than the durum wheats (AABB) with lower leaf K/Na ratios (Gorham, 1991; Dubcovsky *et al*., 1996). This ability in bread wheat is ascribed to the D genome. It was shown that the long arm of chromosome 4D has the *Kna1* locus which contributes K/Na discrimination by enhanced K accumulation and Na exclusion (Gorham, 1991; Dubcovsky *et al*., 1996). These results indicate that *Aegiliops tauschii* that is the donor of D genome in bread wheat may represent an important genetic source of salt stress tolerance. In the previous studies with small number of genotypes it has been shown that *Aegiliops tauschii* can be exploited to improve salt stress tolerance of cultivated wheat.

Under salt stress, plant tries to prevent water loss by closing stomata that causes reduction in CO_2 uptake (Shannon and Grieve, 1999). These events reduce photosynthesis, and the absorbed light energy is used rather for production of reactive oxygen species (ROS) instead of CO_2 fixation. Free radicals trigger oxidative stress which damage cell membrane, nucleic acids and chlorophyll. Lipid peroxidation and chlorophyll damages caused by oxidative attack of free radicals bring about leaf necrosis and chlorosis (Foyer *et al*., 1994). Plants have antioxidative defense systems against free radicals to reduce the impacts of oxidative stress and contribute to salinity tolerance (Orcutt and Nilsen, 2000).

The salinity problem is a common problem in arid and semiarid regions where Zn deficiency is also an important problem. Zinc is an essential mineral nutrient for plants. In higher plants, Zn has catalytic and structural roles in many enzymes and affects photosynthesis, RNA formation and membrane function (Brown *et al*., 1993; Römheld and Marschner, 1991). Zinc is also needed for scavenging free oxygen radicals (Marschner, 1995). One of the well-documented effects of Zn is its involvement in maintaining of the plasma membrane integrity (Welch *et al*., 1982; Cakmak and Marschner, 1988a). Due to these vital functions of Zn, crop production reduces severely in Zn deficient soils. There are a number of soil chemical and physical factors

which affect solubility of Zn in soils such as high soil pH, high $CaCO_3$, low soil organic matter and low soil moisture (Graham *et al*., 1992; Marschner, 1995). These soil factors are very typical in soils of arid and semi-arid regions. Therefore, Zn deficiency is one of the most common micronutrient deficiencies documented in semi-arid regions where salt stress is also commonly found. Zinc deficiency causes severe reductions in crop production, especially in cereal production as shown in Australia, India and Turkey (Graham *et al*., 1992; Takkar and Walker, 1993; Cakmak *et al*., 1996). It has been estimated that nearly half of cereal cultivated lands in the world suffer from low levels of Zn available to plants (Graham *et al*., 1992; Graham & Welch, 1996). As indicated above, Zn is an essential element needed for maintenance of structural and functional integrity of cell membranes. When cells are deficient in Zn, membranes show a high permeability and exudation of several compounds from roots (Welch *et al*., 1982; Cakmak and Marschner, 1988a). High membrane permeability may cause an enhanced ion uptake from soils which can be very important on soils with salinity problem. Zinc deficiency may cause enhanced uptake of toxic ions such as Na, B and Cl. The interactive effects of Zn and salt on plant growth are therefore crucially important and needs to be investigated

The aim of this study is to select salt tolerant and sensitive wild type wheat, *Aegilops tauschii*, genotypes. *Aegilops tauschii* is the donor of D genome in bread wheat. As mentioned above, better K/Na discrimination in bread wheat by enhanced K uptake and reduced Na uptake is an important trait that is affected by the genes located on D chromosome (Gorham, 1991; Dubcovsky *et al*., 1996). It is therefore important to screen number of *Aegilops tauschii* genotypes for higher salt tolerance and better K/Na discrimination. In the present thesis 116 *Aegilops tauschii* genotypes, 15 bread and 13 durum cultivars were used to study the extent of genotypic variation both for salt tolerance and Zn deficiency tolerance. Plants were grown in soil and hydroponic systems to study tolerance to salt stress in form of NaCl and the changes in concentration of Na, Ca and K. The selected salt tolerant and sensitive genotypes were also investigated for their tolerance to Zn-deficiency on a Zn deficient and salt added soil.

2 OVERVIEW

2.1 Soil Salinization

Salinity is defined as the accumulation of soluble salts in the soil water to an extent that causes a reduction in yield by preventing plant growth (Munns, 2005). Soil salinization occurs through either natural or human-induced processes. Natural salinity, also called as primary salinity, is developed during long periods by accumulating of dissolved soils in the soil or groundwater. The reasons of primary salinity are weathering of parent materials including soluble salts and deposition of oceanic salt carried by wind and rain. The intrusion of seawater into irrigation systems in coastal areas causes a decrease in quality of irrigated water and an increase in salinity. Furthermore, salinization is accelerated by climatic factors such as high evaporation in arid and semi-arid regions. Rainfall and/or underground water are insufficient in these regions; however, plants are produced by irrigating. Secondary salinization results from poor irrigation management. Hydraulic balance of the soil water is affected by improper methods of irrigation. The common reasons of secondary salinization are (i) land clearing and breeding annual crops instead of perennial crops, and (ii) irrigating by poor quality water or having poor quality drainage.

Primary salt-affected soils occur naturally and commonly not used in agricultural production in several regions. Salinization is also occurred as a result of human induced processes. Secondary salinization is increasing problem especially in arid and semi-arid lands due to intensive cultivation, fertilizer application and irrigation in these regions. In irrigated land, water is evaporated and consumed by plants, while salt is accumulated in the soil unless salts are leached from the root zone. Rainfall and management of the irrigation systems are insufficient and/or drainage systems are improper to remove salts from the soil profile in arid and semi-arid lands. On the other hand, in some conditions clearing and irrigation, in addition to rainfall,

damage hydraulic balance of the soil water and cause the accumulation of excess water. Water table is raised and soluble salts in the parent material are transported to the root zone by excess water.

Nearly 70% of the earth is covered with sea water which contains huge amount of salt. Even good quality of water in irrigation may include from 100 to 1000 g/m^3 of salt (Marschner, 1995). Irrigation water with 100 g/m^3 adds 0.1 t of salt to the soil per 1 000 m^3. Crops consume 6 000-10 000 m^3 / ha of water annually, and 0.6^{-1} t of salt accumulates in soil per each hectare (Ghassemi *et al*., 1995). Plants use the water especially by transpiration and some water is evaporated, but salts build up and cause salinity problem. Irrigation has increased dramatically during last century, correlating with an increase in human population. Consequently, the water demand has enhanced for consumption of humans and plants. These indicate that breeding of crop cultivars with improved salt tolerance is an issue of global importance to reduce the demand of plants for high quality water. Unlimited resource of seawater can be utilized for irrigation as a consequence of improving species or genotypes to salt tolerance.

Salty soil is characterized according to electrical conductivity (EC) and exchangeable sodium percentage (ESP). In saline soils, the saturation extract of salty soil has EC greater than 4dS m^{-1} (equivalent to ~40 mM NaCl l^{-1}) and ESP less than 15. The EC of saturation extract does not give the exact salt concentration at the root surface and its composition (Marschner, 1995). The concentration of neutral soluble salts except sodium salts decrease by leaching and despite having less than 4 dS m^{-1} of EC, the amount of Na is high enough to prevent root plant growth. This kind of soils is characterized as sodic soils which have greater than % 15 of ESP occupied by high Na concentration (Orcutt and Nilsen, 2000). Saline-sodic soils contain a high concentration of neutral soluble salts with an EC > 4 dS m^{-1} and the value of ESP is greater than % 15. The pH is generally less than 8.5 in both saline and saline-sodic soils; however the pH of sodic soils is high (as high as 10) (Orcutt and Nilsen, 2000).

Osmotic potential in the soil and in the root cells is important, because water is taken by plant according to gradient differences of osmotic potential between the soil and the inside of the root cells. The excessive salt in the root zone causes a decrease in soil water potential. At the low osmotic potential, plant water uptake is inhibited, resulting in physiological drought in spite of sufficient water existence (Jacoby, 1994). The excessive Na amount in sodic soils leads to degradation of soil structure and low infiltration to both water and aeration. The concentration of

Ca is important criteria in salt-affected soils since Ca uptake and transportation is affected by high Na concentrations (Marschner, 1995). Ca-containing compounds such as lime or gypsum can be applied to soils to improve soil structure, especially in sodic soils, and to restore Na toxicity symptoms in plants (Marschner, 1995; Shabala, 2006). The reason of this that Ca ions are adsorbed more strongly by negatively charged soil particles and they rather easily replace Na ions. The retention of cations is dependent upon the valance and hydrated radius of cation. Less charged cations like K, Na are bound more weakly than highly charged cations such as Al, Ca. However, the strength of adsorption is different between same charged cations and the cation with a big hydrated radius is held less tightly. Sodium ions are loosely adsorbed and ready to be leached away that leads to dispersion of sodic soil.

The most abundant salt in nature is sodium chloride (NaCl) which is the main reason of salinization. Sodium is the sixth abundant element in the earth's crust. The Na compounds account for 2.83% of the earth and 1.05 % of seawater. Sodium commonly exists as soluble forms such as sodium chloride, sodium carbonate, sodium borate, sodium nitrate and sodium sulfate. Sodium ions, in spite of their weak adsorption, build up in arid and semi-arid regions due to insufficient rainfall, poor-quality irrigation and high evaporation. The high Na concentration interrupts plant development in these areas. The other constituent of NaCl, chlorine, Cl, is the most prevalent anion in soil and seawater. It exits in the soil combined with other elements mainly Na. Chlorine is required for growth and completion of the life cycle in higher plants (Warburg and Lüttgens, 1946; Broyer *et al*., 1954; Churchill and Sze, 1984; Maschner, 1995; Harling *et al*., 1997; White, 2001). The negatively charged Cl ions are not held by negatively charged soil particles, just as same poles of magnets push each other. The Cl toxicity is more common than the Cl deficiency in nature. Chlorine is commonly found in arid and semi-arid regions (Karanlik, 2001). Chlorinity in saline regions is originated from seawater and its effectiveness in soils varies according to the distance from the sea.

2.2 Salinity and Plant Growth

2.2.1 Genetic Diversity for Salt Tolerance in Plants

The plant responses to salinity stress vary among plant species. Plants are divided into two groups according to their capacity to grow under saline conditions. Salt tolerant plant species are called halophytes, and salt sensitive plant species are called as glycophytes or nonhalophytes. Halophytic plants are naturally able to tolerate high external salinities by accumulating relatively high quantities of Na and Cl in their tissues (Orcutt and Nilsen, 2000). The halophytes have specialized cell types for adaptation to salinity such as salt glands and bladders that exclude Na and in some cases Cl (Breckle, 2002; Colmer *et al.*, 2006). Sodium is required at micronutrient level in some halophytic plants and able to replace K in some plants, even in some crop species (Subbarao, 2003; Marschner, 1995).

Most of the crops are glycophytes that can only complete their life cycle under low salt medium. Glycophytic plants have different degrees of tolerance to salinity and some has salt tolerance mechanisms to avoid salinity stress. Glycophytic crop species are characterized such as salt tolerant, moderately salt tolerant, moderately salt sensitive and salt sensitive depending on the ability to survive under saline conditions. Wheat is moderately salt-tolerant species (Mass and Hoffman, 1977).

Sodium salts, particularly NaCl, induce injury symptoms in plants. Sodium is not required for plant survival; on the other hand plants can absorb Na when excessive Na is present in soil by influencing plant growth. The high salt medium in the root zone hampers primarily plant water uptake and consequently nutrient uptake. Plants have to decline water potential in the cell to survive under salty conditions via accumulating K and/or synthesizing compatible solutes. When plant exposed to salinity stress for a long time, drought stress is observed together with carbohydrate deficit in the younger leaves. Due to high xylem transport to the older leaves, water deficit is not appeared, but Na and Cl ions build up and lead to ion toxicity in there (Marschner, 1995).

The plant stress hormone abscisic acid is synthesized under saline conditions and brings about increasing stomatal closure (Chinnusamy *et al*., 2005). This results in lowering gas

exchange and directly photosynthesis. Hence, the formation of free radicals rises and brings along breakdown of chlorophyll and membrane (Orcutt and Nilsen, 2000). Besides, ion toxicity gives rise to nutritional deficiency by interference with solute balance and nutrient uptake.

2.2.2 Salt in Plant Systems

2.2.2.1 Sodium in Plant Systems

Plants have to take nutrients from the soil to maintain their growth cycle. Arnon and Stout (1939) defined some elements as essential mineral nutrients that are required for all plants to grow and complete their life cycle. Brownell (1965) showed that Na is an essential mineral nutrient for the halophytic *Atriplex vesicaria*, however this information has still not been generalized for all plants and only some C_4 plants require Na essentially. Sodium can be classified as functional nutrient because for certain plants Na is involved in obtaining optimum biomass yield and replacing the K functions when the critical level of K is declined in the medium (Subbarao, 2003).

The amount of Na in the earth's crust is more than the amount of K, and under the saline conditions the Na content in soil further increases when compared with the K content. The monovalent cations, K and Na have similar chemical and structural properties (Table 2.2.1) (Flowers and Lauchli, 1983). The radius of hydrated K and Ns is 0.331 and 0.358 nm, respectively (Marschner, 1995). Under high saline concentrations, K transporters, even high affinity K carriers, cannot distinguish Na ions from K ions, and Na ions can enter plant cells and interfere with K uptake (Epstein, 1961; Epstein *et al*., 1963; Rains and Epstein 1965). A lot of halophytes cannot be affected by the replacement of K with Na, and they metabolically utilize Na for adaptation to saline conditions (Glenn *et al*., 1999). On the other hand, K/Na discrimination is a critical criterion in salinity tolerance of glycophytic plants (Gorham, 1991; Dubcovsky *et al*., 1996). Besides, Na ions at high external Na concentrations can enter to root cells through the non selective cation channels and passively by force of the electrochemical potential difference between soil and root cells.

Table 2.2.1 Chemical characteristic and comparison of sodium and potassium concentrations in soils, sea water, and plants (Flowers and Lauchli, 1983).

	Sodium	Potassium
Atomic number	11	19
Atomic weight	23	39.5
Concentration in lithosphere (ppm)	28.3	25.9
Soil solution (mM)	0.4-150	0.2-10
Sea water (mM)	480	10
Plant Foliage		
-Glycohytes[1]	0.2-2.0	15-50
-Halophytes[2]	25-154	10-33

[1] Grown in 5 mM K + 1 mM Na (g kg^{-1} DW)

[2] Grown in 5-8 mM K + 295-340 mM Na (g kg^{-1} DW)

Plant species are categorized as natrophiles and natrophobes based on their capacity for Na absorption by roots and Na translocations to the shoot (Shone *et al*., 1969). Natrophilic plants can absorb Na and transport it to the tops, while natrophobic plants cannot take in Na easily whereas they absorb K readily (Smith *et al*., 1980). The difference between the natrophilic and natrophobic plants depends on varieties of their ability for Na compartmentalization in their vacuole. Natrophiles are able to accumulate the excessive absorbed Na in their vacuoles to avoid the high Na concentrations in the cytosol (Subbarao, 2003).

Ion homeostasis in the cytosol is essential for metabolic activity and better water regime and uptake. Plants try to lower water potential in the cells to stimulate water uptake down osmotic potential gradient. Ions are energetically favorable to maintain osmotic potential between the soil and plant cells. However, high concentrations of some ions such as Na result in ion toxicity that affects metabolic activity. The higher plant cells have 100-200 mM K and 1-10 mM Na in their cytosol under normal conditions (Taiz and Zeiger, 2002). Metabolic enzymes are affected and protein synthesis is prevented when K/Na ratio declines. Under saline conditions, Na ions are able to substitute Ca ions that lead to increase plasma membrane permeability. As a consequence of this, the major cytoplasmic cations (e.g., Ca and K) leaks out the cells (Cramer *et al*., 1985).

The Na and/or Cl ions drift in chloroplasts and cause inhibition of photosynthesis. Either carbon metabolism or photophosphorylation may be damaged by the impaired

photosynthetic electron transport (Taiz and Zeiger, 2002). On the other hand, Na is required not for only carbon metabolism, but also for chlorophyll synthesis in some C_4 plants (Subbarao, 2003). In addition, nitrate uptake and assimilation in some C_4 plants are enhanced by Na (Ohta *et al*., 1987). Although these plants use Na as an essential mineral nutrient, their requirement is as low as micronutrient level. In addition, Na can substitute K for vacuolar function and stomatal regulation in some plants (Subbarao, 2003). Sodium cannot replace K for all functions due the specific functions of K such as cytoplasmic homeostasis, protein synthesis, but the requirement of K is declined in the presence of Na (Greenwood and Stone, 1998; Subbarao, 2003). Crop species vary widely in substitution of K by Na and in additional growth stimulation by Na that are increasing from natrophobic plants to natrophilic plants (Marschner, 1995).

2.2.2.2 Chloride in Plant Systems

Broyer and his colleagues demonstrated the Cl requirement of plants in 1954 and Cl has been classified as an essential micronutrient for higher plants. Chlorine is the most consumed essential micronutrient and found as high as macronutrients in some plants. The chlorine requirement varies among plant species, and plants contain on an average in the range of 2-20 mg Cl g^{-1} dry matter (Marschner, 1995). Chlorine exists in nature as chloride compounds and generally it is found as high as to cause toxicity in plants. Chloride is a major osmotically active solute in the vacuole and is required for osmoregulatory functions. Tonoplast proton-pumping ATPase that regulates cytosolic pH is stimulated particularly by chloride (Churchill and Sze, 1984). In addition, Cl is required for the water-splitting reaction of photosynthesis through which oxygen is produced (Warburg and Lüttgens, 1946). Chlorine may have a specific role for cell division in both leaves and roots (Harling *et al*. 1997). Besides, Cl is essential for stomatal regulation, the stabilization of membrane potential, and the regulation of electrical excitability (Marschner, 1995; White and Broadley 2001). Chloride is found in soil reserves, irrigation water, rain, and fertilizers, so Cl toxicity is more abundant than Cl deficiency in agricultural habitats. Due to this abundance, most plants generally absorb huge amount Cl and, as a result, Cl toxicity leads to burning of the leaf tips or margins, bronzing and premature yellowing of the leaves. Plant species have different response mechanisms to tolerate Cl toxicity and these mechanisms are also associated with salt tolerance.

2.3 Effects of Salinity on Plant Growth

Salinity affects features of plant metabolism and, as a consequence, growth is lowered. The intra and inter-species have different degrees of tolerance to salts in the root medium. Under salt stress, plant growth is inhibited by tree major constraints (Marschner, 1995):

(1) Restricted water uptake based on decreasing osmotic potential subjected to the excessive salt in the root medium;

(2) Ion toxicity related with the huge amount of Cl and Na uptake;

(3) Nutritional disorders by the excessive Cl and Na uptake associated with a decline in K^{+}, Ca^{2+}, NO^{3-} or P uptake, or damage internal transportation of these ions whereas Cl and Na uptake increases.

Plants give response to salinity at two-phase, that is called as a two-phase growth response to salinity (Munns, 2002). Salt stress causes quickly a decrease in the water uptake capacity of plants and the first phase of growth reduction depends on osmotic effect of the salt. Therefore, salt stress resembles water stress initially. The second phase of growth reduction takes time to develop and during the second phase, huge amounts of salt accumulate in transpiring leaves and result in a growth reduction. The second phase of growth reduction is based on the ability of the plants to tolerate the salts in the soil, so second phase response may be salt-specific.

2.3.1 Water Deficit

Salts in the root medium cause a reduction in osmotic potential and water availability. Leaves need to generate a lower water potential to maintain the osmotic potential gradient for water uptake. When water uptake is limited, root pressure-driven xylem exudation flow consequently is restricted. In saline conditions, the xylem transport of the salt stress decreases whereas ion concentration in the sap increased compared with plants in the normal conditions (Kafkali, 1991). Thus, the root and shoot growth in saline conditions are inhibited together due to limited water and mineral availability. Turgor loss in the leaf cells subjected to the decreased water uptake prevents the leaf elongation and the cell wall extensibility (Lynch *et al*., 1988), so leaf growth is usually more affected than root growth (Termaat and Munns, 1986). Root growth is inhibited under saline and Ca deficit conditions, however supplemental Ca provides an increase

root elongation in saline medium (Cramer *et al*., 1988). If salts are removed from the root zone, the salinity effects on plants can disappear and suggesting that, growth reduction by salinity depends on water stress (Marschner, 1995).

2.3.2 Ion Toxicity

In the nature, the most common salt is NaCl and as a consequence of this, Na and Cl are the most widespread ions in saline conditions. Although Cl is an essential micronutrient for all higher plants and Na is required for many halophytes (Flowers *et al*., 1977) and some C_4 species (Johnston *et al*., 1988), many crops are affected from the excessive amounts of Na and Cl. The amounts of toxic salts in saline conditions are generally much higher than the requirement of C_4 and halophytic plants. High amount of toxic ions results in ion toxicities at cellular level especially in salt sensitive plants.

Salts moved through transpiration stream are accumulated in the leaves while water evaporates and salts gradually builds up with time. Plants transpire 30-70 times more water than they use, therefore salt concentrations increase to high level enough to cause chlorosis and necrosis on the older leaves (Levitt, 1980). According to salt sensitivity, some plants are affected even at low salt medium (Sykes, 1992). Limited water uptake is not a constraint for such conditions (Greenway and Munns, 1980) and for example high chloride sensitivity in *Citrus* species depends on chloride toxicity (Maas, 1993). Chloride toxicity is more common than Na toxicity, mainly associated with the low amount of Ca in the rooting zone or poor aeration and retaining of Na in the woody roots and stems (Marschner, 1995; Tester and Davenport, 2003). On the other hand, Na toxicity is the main reason of ion-specific damage in graminaceous crops such as wheat (Kingsbury and Epstein, 1986, Tester and Davenport, 2003).

2.3.3 Nutrient Imbalance

The huge amounts of Na and Cl uptake in saline substrates influence the uptake, transport and utilization of other ions such as Ca, K in the plants. Sodium enters the plant cells trough cation channels and can interfere with Ca and/or Na transport. As a result, the nutritional balance

can be damaged by antagonism and competition of these ions between each other, and lead to K and Ca deficiencies at highly saline medium. Thus, the plant growth is reduced by depressed nutrient absorption and imbalance related to lowering Ca/Na and K/Na ratios under salinity stress. The Ca/Na ratio is a critical issue for membrane stability, water and ion transport, photosynthesis and plant nutrition.

High K/Na ratio in the cytosol is also important to avoid cellular damage due to the inhibitory effect of Na on the activity of cytosolic enzymes (Zhu, 2002). Calcium involves in enhancement of K/Na discrimination and consequently in improvement of salt tolerance (Liu and Zhu, 1997). K/Na or Ca/Na discrimination is a useful selection criterion in screening for salt tolerance (Asch *et al*., 2000; Zeng *et al*., 2003). Externally supplied Ca (Muhammed *et al*., 1987) and K (Levitt, 1980) reverse the growth inhibition and enhance plant growth under saline conditions. Besides, high Cl concentration is often accompanied by interference with NO_3 uptake. The high NH_4/NO_3 ratio causes an increase in the Na and Cl concentrations and a decrease in the Ca and K concentrations (Grattan and Grieve, 1999). In addition, the concentrations of P, Zn, Fe, B, Cu, Mo and Cu in plants demonstrate variability according to the plants species, plant developmental age, the composition and level of salinity and the concentration of these elements in the root zone (Grattan and Grieve, 1999; Hu and Schmidhalter, 2001).

2.4 Mechanisms of Adaptation to Saline Solutes

Mechanisms to minimize damage from high salinity and yield reduction under salinity stress show a large variability between major groups of plants, different varieties of a given species. Salt tolerance mechanisms occur at two level of organization: whole plant, and cellular.

2.4.1 Whole Plant Adaptation to Salinity Stress

In fact, each cell promotes the tolerance of the whole plant to high salinity and some cell types such as salt glands are specialized for whole plant adaptations. Mechanisms of salt tolerance at whole plant level are related to the level of Na uptake by roots and its distribution within the plant. Salt transport is checked at control points such as absorption from soil, loading

of xylem, unloading of xylem, loading of phloem and excreting through salt glands or bladders (Munns *et al*., 2002; Tester and Davenport, 2003). Plants can adapt to high salinity by avoidance of high Na concentrations in shoots. There are number of factors regulating Na transport to the shoot such as initial entry of Na into root epidermal, cortical and in some cases endodermal cells, Na efflux out of the root, and xylem loading. Sodium removes from the xylem in the upper part of the roots, the stem, petiole or leaf sheaths. Sodium is usually accumulated in the upper part of the root and in the different parts of the shoot such as old leaves and lower part of the shoot. In some instances, Na and Cl are retranslocated in the phloem to minimize Na accumulation in the growing tissue of the shoots. The huge amounts of Na in the shoot may be excreted through salt glands or bladders to lower Na concentrations in shoots. Stomatal closure is an important mechanism of adaptation to salinity at whole plant level (Robinson *et al*., 1997).

2.4.2 Cellular Adaptation to Salinity Stress

The ion balance is significant for regulation water uptake and energetically favorable compared with carbohydrates or amino acids. Under salinity stress, Na uptake has a significantly lower energy cost, however high cytoplasmic Na concentrations cause a decrease in K uptake and inhibit the K required functions such as protein synthesis and the activities of cytosolic enzymes. To avoid a high accumulation in the cytosol, Na is pumped into the vacuole by tonoplast Na/H antiporters that provide Na vacuolar compartmentation. Osmotic potential in the cytoplasm is regulated with K and compatible solutes (osmoprotectants) whereas Na accumulates in the vacuole**.** Elevated cytoplasmic concentrations are moderately not prohibitive for cytoplasmic reactions in the presence of osmoprotectants (Shomer-Ilan *et al*., 1991). In addition, compatible solutes stabilize membrane structure, reduce lipid peroxidation, protect mitochondrial electron transport, and diminish the amount of reactive oxygen species (Chen and Murata, 2002; Xiong *et al*., 2002; Tester and Davenport, 2003). Consequently, synthesis of osmoprotectants is important in cellular adaptation to saline medium.

Sodium can enter the root cells through non-selective cation channels, Ca transporters and even high affinity K carriers due to their antagonistic relations among different ions. Selectively absorption of K and Na in preference to Na is important for salt tolerance (Asch *et al*., 2000). K/Na discrimination is important for high cytosolic K/Na ratio that maintains cellular

metabolism. Elevated Ca/Na ratio increases membrane stability. Salinity-induced K leak and accelerated passive influx of Na through impaired membrane stability under salt stress are increased. At elevated Ca/Na ratio cytosolic K concentration raises whereas cytosolic Na concentration decline. The cytoplasmic Na can be removed via Na/H antiporters, driven by the pH gradient across the cell membrane (Blumwald, 2000). Briefly, avoiding high cytosolic Na concentration is critical point for cellular adaptation to salinity stress.

2.5 Importance of D genome in Salt Tolerance

Wheat is one of the most consumed cereal crops in the world. Global wheat consumption was 101 kg per capita (International Grains Council 1998). Wheat is grown in irrigated and rain-fed arable land where salinity stress causes yield loss (Ghassemi *et al*., 1995; Mujeeb-Kazi and Diaz de Leon, 2002). Wheat is characterized as moderately salt tolerant and its yield is declined by 50% at soil saturation extracts of 13 dS m^{-1} (Ayers and Wescot, 1976; Mass and Hoffmann, 1977). Salinity stress decrease the number of leaves per culm, the number of tillers per plant, and the number of spikelet per spike during early stages that lead to decrease seed number and consequently final seed yield decreases (Kirby, 1988; Maas and Grieve, 1990). Salt tolerance in wheat depends on regulation of Na accumulation at the root level and ionic compartmentalization (Schachtman and Munns, 1992). Sodium accumulation is related to selectively uptake of K and Na in preference to Na through plasma membrane transporters. Ionic compartmentalization is based on elevated Na uptake through tonoplast transporters.

Salt tolerance within cultivated wheat species is related to low Na concentration and high K/Na ratio in leaves (Francois *et al*., 1986; Gorham *et al*., 1987; Shah *et al*., 1987; Maas and Grieve, 1990, Munns and James, 2003; Poustini and Siosemardeh, 2004). Hexaploid (2n = 6x = 42, AABBDD) bread wheat (*Triticum aestivum*) has higher leaf K/Na ratio subjected to lower rate of Na accumulation than tetraploid (2n = 4x = 28, AABB) durum wheat (*Triticum turgidum*) (Gorham *et al*., 1987). Yield reduction of bread wheat starts at higher saline conditions compared with durum wheat, besides bread wheat yield declines at a lower rate than durum wheat with increasing salinity (Maas and Grieve, 1986). Shah *et al*. (1987) demonstrated that relatively high K/Na ratio in bread wheat is associated with its 4D chromosome. The enhanced ability of D genome to discriminate between K and Na is associated with a single locus *Kna1* on the long arm

of chromosome 4D (Dubcovsky *et al*., 1996). The K/Na discrimination character is also responsible for sodicity tolerance, but it is not useful under conditions of K deficiency (Gorham *et al*., 1997).

The diploid (2n = 2x = 14) *Aegilops tauschii* also known as *Triticum tauschii* is the D-genome donor of bread wheat (McFadden and Sears, 1946). *Aegilops tauschii* is also a good Na excluder and has a lower rate of leaf Na accumulation and higher leaf K/Na ratio (Gorham *et al*., 1991). Although the elevated K/Na ratio subjected to *Kna1* can be demonstrated at all salt concentrations, it is most apparent at low salinities. On the other hand, the trait is not unique; other mechanisms which control ion accumulation appear to be more important at higher salinities (Gorham *et al*., 1997). Schachtman *et al*. (1991) found significant differences in Na exclusion among *Aegilops tauschii* genotypes and a good correlation between Na exclusion and salinity tolerance in *Aegilops tauschii*. However, these correlations were not found for wheat cultivars (Ashraf and McNeilly 1988; Genc *et al*., 2007). The D genome contributes to also heat resistance (Ehdai and Waines, 1992) and the improvement of micronutrient efficiency (Merry *et al*., 1999).

2.6 Zinc and Plant Growth

Zinc is an essential micronutrient for higher plants. Zinc has functional and structural roles in many enzymes that involve in many vital cellular processes such as photosynthesis, cell division, protein metabolism (Marschner, 1995). Zinc is an integral component of the ribosome structure and essential for DNA replication, transcription, RNA formation, and in regulation of gene expression (Coleman, 1992; Vallee and Falchuk, 1993). In Zn-deficient plants, translation and RNA formation reduce and result in lowering of protein content and enhancement of amino acid content. Zinc participates in maintenance of structural and functional integrity of plasma membrane (Welch *et al*., 1982; Cakmak and Marschner, 1988a). Zinc plays important roles in interfering with formation of reactive O_2 species (ROS) that damage the plant defence systems (Marschner and Cakmak, 1989). Zinc inhibits generation of ROS by interfering with NADPH oxidase which enhances ROS generation (Cakmak and Marschner, 1988b; Pinton *et al*., 1994). Under Zn deficiency, generation of ROS increases by high light intensity that is associated with reduced photosynthesis in Zn deficient plants (Marschner & Cakmak, 1989; Cakmak & Engels,

1999). Plants cells have antioxidants and antioxidative defense enzymes to prevent the cells from the destructive effects of ROS. Superoxide dismutases (SODs), the most common antioxidative defense enzyme in plants, minimize generation of ROS (Fridovich 1986, Bowler *et al*. 1994), however the activity of the major SOD, CuZn-SOD reduces due to a strong photooxidative stress or conditions producing high amount of H_2O_2 (Cakmak, 2000). Thus, plasma membranes are very rapidly damaged ROS under Zn deficiency leading to enhanced membrane pathology and increased permeability.

Growth, differentiation and development of plants are inhibited by low available Zn, and thus crop yield and quality are reduced on Zn deficient soils. Zinc is physiological unavailable at extremely high and low soil pH (Harter, 1991; Marschner, 1993, 1995). Soluble Zn is very low level in calcareous soils where Zn is adsorbed to clay or $CaCO_3$ because of high pH. Zinc deficiency is one of the most common micronutrient deficiencies, especially in alkaline soils of arid and semiarid regions (Welch *et al*., 1991; Graham *et al*., 1992; Takkar & Walker, 1993; White & Zasoski, 1999; Cakmak *et al*., 1999). It is estimated that Zn deficient soils cover nearly 30% of the total arable land in the world (Sillanpää, 1982). Cereal species are generally very sensitive to Zn deficiency, especially wheat and it has been estimated that nearly half of cereal cultivated lands in the world suffer from low levels of plant available Zn (Graham *et al*., 1992; Graham & Welch, 1996; Cakmak and Braun, 1998).

Zinc efficient (tolerant) genotypes are able to grow and yield better under low Zn conditions with respect to other, Zn inefficient (sensitive) genotypes (Graham, 1984). Leaf and shoot Zn concentration are not correlated with Zn efficiency and no significant differences in Zn uptake were found between Zn efficient and Zn-inefficient genotypes (Cakmak *et al*., 1998). Among cereal species, rye and triticale are more Zn-efficient than durum and bread wheat, oat, rice, sorghum and maize (Cakmak, 1998). Durum wheat with AABB genomes is generally more sensitive to Zn deficiency than bread wheat with AABBDD genomes (Cakmak *et al*, 1997). The D genome donor of bread wheat, *Aegilops tauschii* probably contains Zn efficiency genes, so *Aegilops tauschii* could be used as a genetic resource to improve Zn efficiency in wheat (Cakmak *et al*., 1999a, b; Merry *et al*., 1999).

Under low Zn concentrations, efficient genotypes have better internal utilization of Zn, and this ability of Zn-efficient genotypes is probably an important trait to contributing better growth

under Zn deficiency (Cakmak, 1998). The activity of Zn-requiring enzymes is also an important determinant of Zn efficiency in wheat (Hacisalihoglu, 2004).

Expression of high Zn efficiency is affected from seed Zn concentrations. In comparison of genotypes for their Zn efficiency, seeds used should have more or less similar Zn concentrations (Rengel and Graham, 1995; Yilmaz *et al*., 1997).

2.7 The Interactive Effects of Zinc and Salt on Growth of Wheat

As indicated above, salinity problem is commonly associated with irrigation, especially in arid and semi-arid regions where Zn deficiency is also an important nutritional problem. Under salt stress, Zn fertilization enhances crop reduction and improves salt tolerance of plants by lowering of Na uptake in saline conditions (Alpaslan *et al*., 1998; Aktas *et al*., 2006). Zinc regulates membrane integrity and controls permeability (Welch *et al*., 1982; Cakmak and Marschner, 1988a). In Zn-deficient plants membrane integrity is impaired and membrane permeability increased, therefore Na uptake and accumulation enhanced in these plants. Under salty conditions, Zn inefficient genotypes have been found containing higher Na concentration and content than the Zn-efficient genotypes (Genc *et al*. 2005). On the other hand, Zn uptake was enhanced in saline conditions compared to native conditions (Keshavarz, 2005). Genc *et al*. (2005) demonstrated that Zn deficiency symptoms were less expressed under saline conditions compared to nonsaline conditions. It appears that improving crops with both high tolerance to salt stress and Zn deficiency is a globally important issue. Due to its high genetic potential for high Zn deficiency and salt stress tolerance, *Aegilops tauschii* can be an important genetic material to exploit it in breeding programmes (Cakmak *et al*., 1999a, b; Merry *et al*., 1999). For a successful breeding program, it is important to select highly tolerant parental lines for Zn deficiency and salt stress tolerance. In this thesis, 116 *Aegilops tauschii* genotypes have been used to study the extent of the genetic variation for salt stress and Zn deficiency tolerance. To compare the level of genetic variation in *Aegilops tauschii*, several cultivated wheat genotypes were also included in the studies.

3 MATERIAL AND METHODS

3.1 Materials

3.1.1 Plant Material and Growth Conditions

3.1.1.1 Greenhouse Conditions

3.1.1.1.1 Plant Material

A total of 116 *Aegilops tauschii* and 28 modern wheat cultivars were used in screening studies to identify the most salt-tolerant and the most salt-sensitive wheat genotypes. Of the modern wheat cultivars 15 were bread wheat (*Triticum aestivum*) and the remaining was durum wheat (*Triticum durum*).

3.1.1.1.2 Growth Conditions

Screening experiments were conducted in the greenhouse under natural light conditions. 15 seeds of each genotype were sown in plastic pots filled with 1700 g soil. The soil was obtained from Central Anatolia, so it was Zn deficient (approximately 0.1 mg extractable Zn kg^{-1}); its pH

was alkaline (around 8.00). The soil had high amount of $CaCO_3$ (14.9 %) and its texture was clay (60.6 %) and contained 0.69 % organic matter and 0.08 % salt. Before sowing of seeds, basal fertilizer treatment of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$, 100 mg P kg^{-1} soil as KH_2PO_4, 125 mg K kg^{-1} soil as KH_2PO_4, 20 mg S kg^{-1} soil as $CaSO_4.2H_2O$, and 2.5 mg F kg^{-1} soil as FeEDTA ($C_{10}H_{12}FeN_2NaO_8$) were applied into 1700 g soil and all of them was mixed thoroughly. In the studies with Zn deficiency no Zn was added into soil. For plants with adequate Zn supply, Zn was applied at a rate of 2.5 mg Zn kg^{-1} soil as $ZnSO_4.7H_2O$. In the salt treatments, at the begin of the experiment 500 mg NaCl kg^{-1} soil was added; then further rates applied, (generally when plants were 10 or 15 days old) to reach different total amounts of salt applied into soil. As described in the results section, depending on the experiments, doses of NaCl applied to soil were varied between 1000 to 5000 mg NaCl kg^{-1} soil). There were 3 replicates for each salinity level except one treatment that was in duplicate. Plants were irrigated one or two times in a day by deionized water and the pots were randomized once in a week. Plants were harvested when the differences between salinity stress and control plants were observed and only shoots were harvested and dried at 70ºC for determination of shoot dry matter production and Na, Cl, Ca and K concentration and content in shoot.

3.1.1.2 Growth Chamber Experiments

3.1.1.2.1 Plant Material

The salt-tolerant and salt-sensitive wheat genotypes selected based on pre-screening experiments in greenhouse were grown hydroponically (in nutrient solution). Two durum (Gediz, Kızıltan), two bread (Alpu-01, ES-14) wheat and nine *Aegilops tauschii* genotypes (Aegilops 20, 32, 36, 39, 95, 99, 103,108 and 115) were used in growth chamber experiments.

3.1.1.2.2 Growth Conditions

In nutrient solution experiments, plants were grown in growth chamber under controlled environmental conditions (light/dark regime: 16/8 h at 25/22ºC, relative humidity: 60-70 %, photon flux density: 700 μE m^{-2} s^{-1}.Seeds were germinated in perlite moistened with saturated $CaSO_4$ solution in dark for 5 days at 25ºC. Germinated seeds were planted into 2.8 L black plastic pots containing continuously aerated nutrient solution. The content of the nutrient solution was as follows: 2mM $Ca(NO_3)_2$, 1mM $MgSO_4.7H_2O$, 2 mM K_2SO_4, 0.2 mM KH_2PO_4, 10^{-6} M H_3BO_3, 10^{-6} M $MnSO_4.H_2O$, 10^{-6} M $ZnSO_4.7H_2O$, $2x10^{-7}$ M $CuSO_4.5H_2O$, $2x10^{-8}$ M $(NH_4)_6Mo_7O_{24}.4H_2O$, and 10^{-4} M FeEDTA ($C_{10}H_{12}FeN_2NaO_8$). NaCl was added into nutrient solution of one week-old modern cultivars and two week-old *Aegilops tauschii*. The concentration of NaCl added to the nutrient solution varied depending on the experiments as indicated in the legend of relevant figures and tables.

In ion uptake experiments, after following salt application, samples were taken from nutrient solution periodically during 1 day to measure ion uptake from solution. At the end of collecting samples, plants were harvested and then, shoots and roots were dried at 70°C separately to detect dry matter production, and samples taken from nutrient solution were subjected to ICP-OES (inductively coupled plasma optical emission spectroscopy) analysis for calculation of cumulative Na, K, Ca absorptions and Na, K, Ca uptake rates per g root dry weight.

3.2 Methods

3.2.1 Dry Matter Production, Salinity Tolerance Index and Zn Efficiency

Plants dried at 70°C were weighed for determination of dry matter production. The NaCl tolerance index was calculated as the ratio of shoot dry weights at different NaCl concentrations to the shoot dry weights of the control treatment (without NaCl supply) as following:

Salinity Tolerance Index = [Shoot dry matter (salt treatment) / Shoot dry matter (Control)] X 100

The Zn efficiency trait was calculated similarly, as the ratio of shoot dry matter production at Zn-deficient condition to that at Zn-sufficient condition as following:

Zinc Efficiency = [Shoot dry matter (Zn-deficient) / Shoot dry matter (Control)] X100

3.2.2 Concentration and Content

The dried root and shoot samples were ground before ICP-OES analysis. Approximately 0.2 g ground samples were digested in a microwave using 2 ml H_2O_2 and 5 ml HNO_3. After digestion, the total volume was completed up to 20 ml. For the seed samples, approximately 0.4 g seed was digested and diluted according to same procedure.

The concentration of Na, K, Ca, Zn and P were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian, Australia) at 330.237, 766.491, 370.602, 213.857 and 214.914 nm emission wavelengths, respectively. These concentrations in plant materials were checked against reference plant materials obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The Na, K, Ca, Zn and P contents (total amount per shoot) were calculated by multiplying the dry weight values of shoots with their concentration values.

For the ion uptake experiments, following NaCl applications, 15 ml samples were collected from nutrient solution in each pot at each 12-h. As time goes on, the amount of nutrient solution in each pot is reduced due to transpiration of plants and evaporation of water. Before collecting samples, volume was completed up to 2.8 L by adding water to reduce any concentration effect because of reduced water level in pots. The concentration of Na, K, Ca and Mg in the collected samples was measured by ICP-OES. The uptake results were calculated based on root dry weight unit and time as following: μmol g^{-1} root DW h^{-1}) and also calculated as cumulative absorptions based on the total absorption of the nutrients for a given time period (μmol g^{-1} root DW).

4 RESULTS

4.1 Screening for Salt Tolerance in Durum and Bread Wheat Genotypes

4.1.1 Greenhouse Experiments

4.1.1.1 Leaf Symptoms and Dry Matter Production in Modern Wheat Cultivars

The first experiment has been conducted by using selected modern wheat cultivars. As a first response to salt stress, leaf size and shoot elongation decreased in the salt-treated plants when compared the control plants (no salt treatment). Development of leaf chlorosis and necrosis on tips of the oldest leaves were typical occurred after salt treatment, and occurred particularly on salt-sensitive genotypes. There was a large genotypic variation in the appearance time and severity of the leaf symptoms.

As expected, shoot growth of all genotypes decreased under saline conditions. Reduction in shoot dry mater production differed among and within bread and durum wheat genotypes. Under salty conditions, shoot dry weight per plant varied between 230 mg (ES-14) and 366 mg (03 SE 18), with a mean value of 328 mg in bread wheat, and between 239 mg (Yelken) and 343 mg (Gediz), with a mean value of 300 mg in durum wheat (Table 4.1.1). On average, the bread wheat genotypes produced a greater amount of dry matter than the durum wheat genotypes both under saline and nonsaline conditions. The variation in decreases of the shoot dry matter by salt stress ranged between 30% (Alpu-01) and 42% (ES-14) in bread wheat and 26% (Balcalı 2000) and 38% (Kızıltan) in durum wheat (Table 4.1.1). The average decreases in shoot dry matter

production related to salt stress were not different among bread and durum wheat genotypes and these values were 36% and 32%, respectively (Table 4.1.1). The bread wheat genotypes Alpu-01, Çetinel 2000 ad 03-SE-18 and the durum wheat genotypes Balcalı 2000, Gediz and Meram showed greater NaCl tolerance and produced higher yield than the average yield under salt treated conditions (Table 4.1.1). On the other hand, the bread wheat Yakar, Ahmetağa and ES-14 and the durum wheat genotypes Yılmaz, Yelken 2000 and Kızıltan showed very high NaCl sensitivity and produced the least yield under salt stress (Table 4.1.1).

Table 4.1.1 Severity of leaf symptoms caused by NaCl treatment, shoot dry matter production and % decrease in shoot dry matter production of 15 bread and 13 durum wheat genotypes grown for 39 days with (2500 mg NaCl kg^{-1} soil) and without NaCl treatment under greenhouse conditions. Data represent means of 3 independent replications.

	Leaf	Dry matter production		
Bread Wheat Genotypes	Symptoms*	-NaCl	+NaCl	Decrease
		(mg $plant^{-1}$)		(%)
Alpu 01	5	508 ± 34	358 ± 31	30
Çetinel 2000	4.5	489 ± 20	334 ± 13	32
03 SE 18	5	545 ± 35	366 ± 11	33
Soyer 02	5	521 ± 32	348 ± 25	33
Bezostaya-1	4	550 ± 30	354 ± 32	36
BDME-10	4	505 ± 11	324 ± 23	36
Yıldız 98	3	483 ± 23	310 ± 20	36
Kırgız 95	5	560 ± 26	359 ± 13	36
Dağdaş	5	488 ± 41	312 ± 22	36
Ziyabey	4	477 ± 16	304 ± 13	36
00 KE 3	5	574 ± 21	358 ± 18	38
İzmir 85	3	573 ± 65	354 ± 24	38
Yakar	3	457 ± 26	277 ± 8	39
Ahmetağa	5	558 ± 55	335 ± 43	40
ES-14	3	397 ± 12	230 ± 8	42
Mean	4	512	328	36
Durum Wheat Genotypes				
Balcalı 2000	4	446 ± 16	331 ± 14	26
Gediz	4	472 ± 62	343 ± 6	27
Meram	3.5	455 ± 33	330 ± 13	27
Çakmak	4	408 ± 20	290 ± 44	29
Ege	4	420 ± 37	295 ± 19	30
Balcalı 85	3	436 ± 29	300 ± 27	31
Ç-1252	3.5	460 ± 3	305 ± 9	34
Şölen	3.5	461 ± 23	302 ± 11	35
Kümbet 2000	4	457 ± 7	293 ± 28	36
Tüten	3.5	492 ± 4	314 ± 53	36
Yılmaz	4.5	448 ± 23	285 ± 14	37
Yelken 2000	4	378 ± 43	239 ± 11	37
Kızıltan	3.5	431 ± 22	267 ± 8	38
Mean	4	444	300	32

*Leaf symptoms of salt stress: 1 (very severe) to 5 (very slight or no symptoms).

4.1.1.2 Shoot Concentrations of Na, K and Ca in Modern Wheat Cultivars

Significant variation in shoot Na concentrations was found among genotypes of bread and durum wheat genotypes under saline and nonsaline conditions. Durum wheat genotypes showed higher Na concentrations than bread wheat under both conditions. Under nonsaline conditions, the shoot Na concentrations in bread wheat varied from 0.012 (Kırgız 95) to 0.131 mg g^{-1} (Ahmetağa), with an average value of 0.050 mg g^{-1}, while in durum wheat the variation was between 0.35 (Balcalı 2000) and 0.82 mg g^{-1} (Yılmaz), with an average value of 0.55 mg g^{-1} (Table 4.1.2). When 2500 mg kg^{-1} NaCl was applied, Na concentrations in shoot were significantly increased in both bread and durum wheat genotypes. The most and least Na accumulated bread wheat genotypes were ES-14 (2.37 mg g^{-1}) and Ziyabey (1.19 mg g^{-1}), respectively. In the case of durum wheat genotypes, Yılmaz (15.6 mg g^{-1}) and Gediz (8.5 mg g^{-1}) had the highest and lowest shoot Na concentrations, respectively. The results indicated higher genetic capacity of durum wheats in Na uptake and accumulation when compared to bread wheats.

The shoot K concentration in bread wheat was greatly higher than the shoot concentration of K in durum wheat under both salt and control conditions indicating very high K/Na discrimination in bread wheats. On average, the shoot K concentration was 43 mg g^{-1} for bread wheat and 38 mg g^{-1} for durum wheat under nonsaline conditions. With the NaCl supply, there was a distinct reduction in K concentration, and the mean value of K concentration reduced to 35 mg g^{-1} in bread wheat and 27 mg g^{-1} in durum wheat (Table 4.1.2). In the 2500 mg kg^{-1} NaCl treatment, the genotypic variations in shoot K concentration within bread and durum wheat were low compared to the genotypic variation found for Na concentrations. Under saline conditions, the bread wheat 00-KE-3 (38.0 mg g^{-1}) and Yakar (31.6 mg g^{-1}), and the durum wheat Şölen (30.1 mg g^{-1}) and Kızıltan (23.8 mg g^{-1}) had the highest and lowest shoot K concentrations, respectively.

In most cases, when compared to nonsaline conditions, bread wheat genotypes absorbed higher Ca under saline conditions, while this absorption decreased in the case of durum wheat. The average shoot Ca concentration in bread wheat increased from 5.82 to 6.24 mg g^{-1} by applying 2500 mg kg^{-1} NaCl. However, durum wheat had generally higher shoot Ca

concentrations than bread wheat, and the average shoot Ca concentrations were 8.09 and 6.48 mg g^{-1} for durum wheat under control and saline conditions, respectively (Table 4.1.2).

Table 4.1.2 Shoot Na, K, Ca concentration of 39-day old 15 bread and 13 durum wheat genotypes grown with (2500 mg NaCl kg^{-1} soil) and without NaCl treatment under greenhouse conditions. Data represents means of 3 independent replications.

	Concentration in Shoot					
	Na		K		Ca	
Genotypes	-Na	+Na	-Na	+Na	-Na	+Na
			(mg g^{-1} DW)			
Alpu 01	0,049 ± 0,010	1,79 ± 0,21	42,4 ± 0,5	33,1 ± 1,0	5,68 ± 0,28	7,57 ± 0,05
Çetinel 2000	0,045 ± 0,010	1,95 ± 0,18	47,6 ± 2,5	35,8 ± 1,1	6,03 ± 0,10	6,29 ± 0,12
03 SE 18	0,058 ± 0,015	1,65 ± 0,07	43,6 ± 1,5	36,0 ± 1,2	6,11 ± 0,07	6,99 ± 0,41
Soyer 02	0,023 ± 0,007	1,44 ± 0,15	45,7 ± 0,9	35,5 ± 1,2	6,17 ± 0,19	6,61 ± 0,18
Bezostaya-1	0,061 ± 0,007	2,02 ± 0,07	46,2 ± 1,3	37,8 ± 0,7	5,71 ± 0,24	6,30 ± 0,09
BDME-10	0,065 ± 0,003	1,48 ± 0,11	42,9 ± 1,1	34,6 ± 0,8	5,89 ± 0,24	6,88 ± 0,20
Yıldız 98	0,020 ± 0,005	2,01 ± 0,25	45,0 ± 1,2	31,8 ± 0,8	6,16 ± 0,20	6,97 ± 0,19
Kırgız 95	0,012 ± 0,008	1,89 ± 0,08	47,0 ± 0,6	36,1 ± 1,5	5,94 ± 0,17	6,77 ± 0,14
Dağdaş	0,041 ± 0,015	1,85 ± 0,09	44,3 ± 1,8	34,4 ± 1,0	5,46 ± 0,65	5,89 ± 0,32
Ziyabey	0,051 ± 0,005	1,19 ± 0,09	36,9 ± 2,7	37,0 ± 1,1	5,79 ± 0,07	4,45 ± 0,19
00 KE 3	0,070 ± 0,012	2,14 ± 0,24	42,7 ± 1,6	38,0 ± 1,3	5,22 ± 0,06	5,63 ± 0,16
İzmir 85	0,047 ± 0,016	1,34 ± 0,04	34,8 ± 1,4	37,0 ± 1,3	5,85 ± 0,17	4,54 ± 0,05
Yakar	0,066 ± 0,009	1,76 ± 0,12	46,0 ± 2,7	31,6 ± 0,6	6,52 ± 0,24	6,88 ± 0,36
Ahmetağa	0,131 ± 0,009	1,38 ± 0,08	35,7 ± 4,9	37,4 ± 0,7	4,42 ± 0,59	4,85 ± 0,33
ES-14	0,014 ± 0,008	2,37 ± 0,03	41,8 ± 1,1	31,9 ± 1,9	6,37 ± 0,20	7,05 ± 0,21
Mean	0,050	1,75	42,9	35,2	5,82	6,24

	Concentration in Shoot					
	Na		K		Ca	
Genotypes	-Na	+Na	-Na	+Na	-Na	+Na
			(mg g^{-1} DW)			
Balcalı 2000	0,35 ± 0,02	11,0 ± 0,5	41,6 ± 0,2	23,8 ± 1,26	9,92 ± 0,52	6,84 ± 0,48
Gediz	0,36 ± 0,07	8,5 ± 0,3	35,6 ± 1,6	29,9 ± 0,87	7,58 ± 0,33	4,37 ± 0,36
Meram	0,70 ± 0,06	14,0 ± 0,9	34,0 ± 0,4	28,1 ± 1,01	7,70 ± 0,15	7,25 ± 0,12
Çakmak	0,67 ± 0,05	12,2 ± 0,7	41,4 ± 0,9	24,8 ± 0,84	7,13 ± 0,54	6,49 ± 0,09
Ege	0,38 ± 0,03	11,1 ± 0,4	37,7 ± 1,8	29,7 ± 0,70	8,43 ± 0,82	5,30 ± 0,16
Balcalı 85	0,35 ± 0,01	11,3 ± 0,4	42,7 ± 1,5	25,1 ± 0,49	7,69 ± 0,18	5,68 ± 0,19
Ç-1252	0,80 ± 0,10	12,7 ± 0,1	44,4 ± 0,6	26,0 ± 0,40	7,54 ± 0,36	7,15 ± 0,28
Şölen	0,35 ± 0,00	9,9 ± 0,3	33,1 ± 0,4	30,1 ± 1,38	8,09 ± 0,17	5,54 ± 0,06
Kümbet 2000	0,74 ± 0,06	13,8 ± 0,8	37,4 ± 0,4	29,2 ± 2,40	7,34 ± 0,28	6,77 ± 0,03
Tüten	0,35 ± 0,01	10,0 ± 0,5	34,6 ± 0,6	29,7 ± 1,42	8,50 ± 0,72	5,42 ± 0,26
Yılmaz	0,82 ± 0,02	15,6 ± 0,7	34,3 ± 0,4	28,5 ± 0,84	10,01 ± 0,10	9,33 ± 0,06
Yelken 2000	0,49 ± 0,03	13,6 ± 0,3	34,5 ± 1,2	25,9 ± 0,39	8,02 ± 0,35	7,27 ± 0,16
Kızıltan	0,73 ± 0,01	12,5 ± 0,9	37,2 ± 4,1	23,8 ± 0,94	7,18 ± 0,23	6,84 ± 0,23
Mean	0,55	12,0	37,6	27,3	8,09	6,48

The shoot concentrations of Na, K and Ca are important in development of salt tolerance in plants under saline conditions. However, there were no significant relations between salt tolerance index (expressed as the ratio of shoot dry weight in saline versus nonsaline conditions)

and the shoot Na, K and Ca concentrations within bread and durum wheat genotypes (Figure 4.1.1). However, there were some selected genotypes in which close inverse relationship exists between salt tolerance and Na concentration such as ES-14 and Gediz.

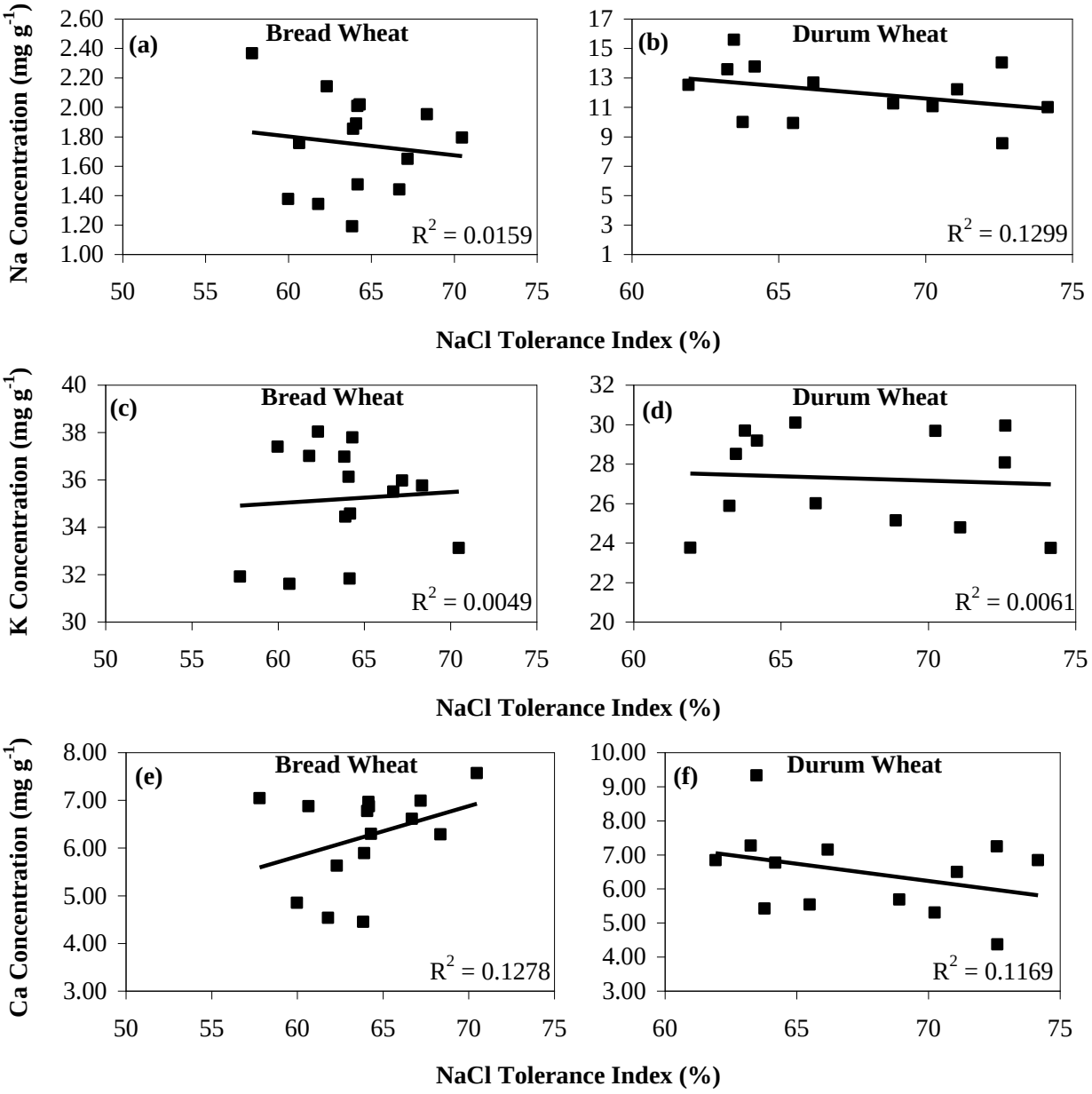

Figure 4.1.1 Correlations between NaCl tolerance index and Na, K and Ca concentration within bread and durum wheat genotypes. There are not any significant relationships in these correlations. R^2 = linear regression coefficient squared.

The K/Na and Ca/Na ratios are widely considered parameters in evaluation of genotypes for their tolerance to salt stress. These ratios were much higher in bread than durum wheat due to

higher Na absorption capacity of durum wheat. When all genotypes together considered, the K/Na and Ca/Na ratios did not however correlate with NaCl tolerance index both in bread and durum wheat genotypes (Figure 4.1.2 and 4.1.3). The K/Na ratio was correlated negatively with Na and positively with K concentrations. The correlation coefficients of K/Na ratio with shoot Na concentration were R^2 = 0.90*** for bread wheat and R^2 = 0.76*** for durum wheat; these values were much greater than the values obtained for shoot K concentration (e.g., R^2 = 0.3181* for bread wheat and R^2 = 0.4341* for durum wheat). Under salty conditions, the elevated Na concentration in shoot decreased K concentration, but there was no significant relationship between Na and K concentrations in shoot for all bread and durum wheat genotypes (Figure 4.1.2). The relationship between Ca/Na ratio and shoot Na concentration was much more significant in bread (R^2 = 0.3522*) than in durum wheat (R^2 = 0.0229). The correlation between Ca/Na ratio and shoot Na concentration tended to be negative and positive in bread and durum wheat, respectively. There was also no significant relationship between Ca/Na ratio and shoot Ca concentration within bread (R^2 = 0.1253) and durum (R^2 = 0.2765) wheat genotypes, but as expected Ca/Na ratio was correlated positively with Ca concentration. The Ca concentration correlated with Na concentrations, and this correlation was much more significant in durum (R^2 = 0.843***) than in bread wheat (R^2 = 0.2847*) (Figure 4.1.3). However, when individual genotypes considered, the genotypes with highest and lowest salt tolerance showed accordingly lower and higher Na concentrations and K/Na ratios. Such genotypes were selected for further detailed studies as described below.

According to the results of the greenhouse screening experiment, one sensitive and one tolerant bread and durum wheat genotypes were selected and these were ES-14, Alpu-01, Kızıltan, Gediz, respectively.

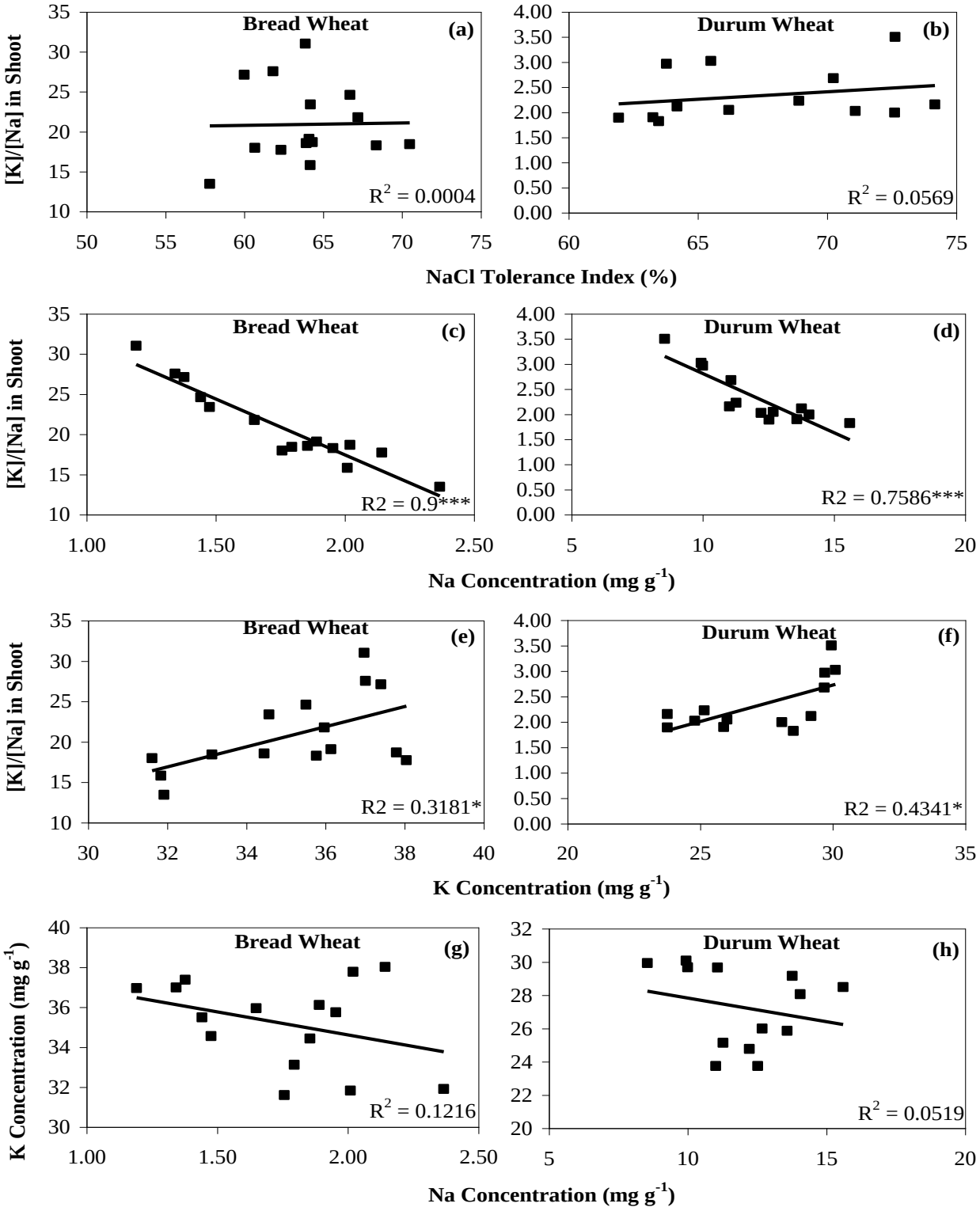

Figure 4.1.2 The relationship between the shoot K/Na ratio and NaCl tolerance index (a, b). The shoot K/Na ratio is regressed negatively on shoot Na concentration (c, d), and positively on shoot K concentration (e, f). There is no significant relationship between shoot Na and K concentration themselves (g, h). * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively, as determined using simple linear regression (solid line is the calculated linear regression line); R^2 = linear regression coefficient squared.

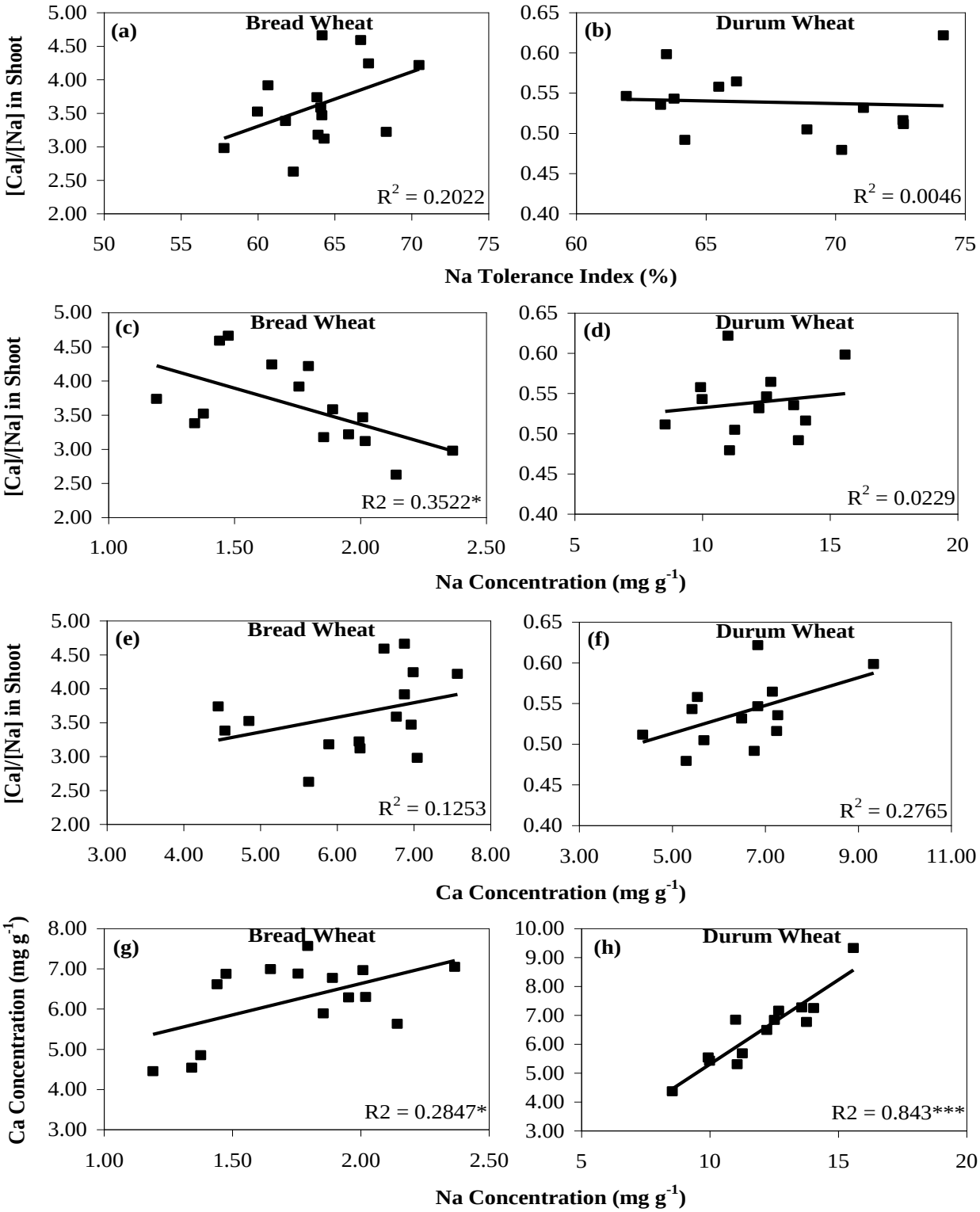

Figure 4.1.3 The relationship between the shoot Ca/Na ratio and NaCl tolerance index (a, b); shoot Na concentration (c, d), and on shoot Ca concentration (e, f). There is a positive correlation between shoot Na and K concentration themselves (g, h). * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively, as determined using simple linear regression (solid line is the calculated linear regression line); R^2 = linear regression coefficient squared.

4.1.2 Growth Chamber Experiments

In order to study the effect of salt stress on root uptake of Na, K and Ca, salt tolerant (Alpu and Gediz) and salt sensitive (ES-14 and Kızıltan) wheat cultivars were exposed to four different salinity levels (0, 25, 75 and 150 mM NaCl) in nutrient solution. After addition of salt, nutrient solution were collected during one day at 12-h intervals. The measurements indicated that there were no specific differences in the net uptake rates and also cumulative uptakes of Na, K and Ca among the selected genotypes at 0 mM NaCl treatment (control) (Figure 4.1.4).

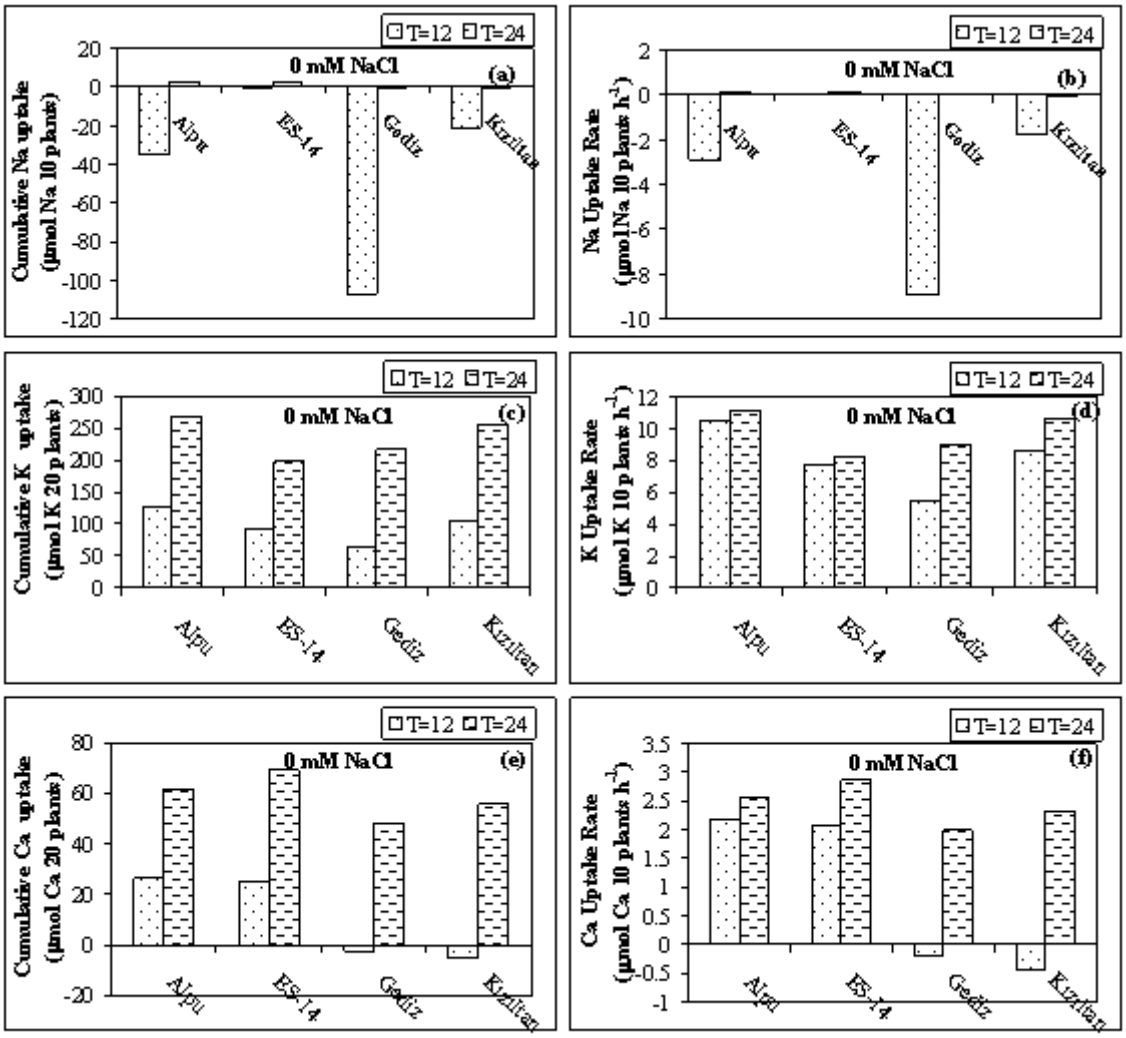

Figure 4.1.4 Influence of exposure time on concentration of Na, K and Ca in nutrient solutions of 8 days-old salt tolerant (Alpu, Gediz) and salt sensitive (ES-14, Kızıltan) wheat genotypes. Plants were grown for seven days at in nutrient solution and treated with 0 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol Na, K and Ca 20 plants (a, c, e).Uptake rates represented as µmol Na, K and Ca 20 plants per hour (b, d, f). The data represent means of four independent replications.

As expected, cumulative Na uptake progressively increased with the duration of the 25 mM NaCl treatment. The total Na uptakes were more or less similar in Alpu, ES-14 and Gediz, but the cumulative Na uptake in Kızıltan was lower compared to other cultivars after 24 h exposure time (Figure 4.1.5). The uptake rate of Na increased with exposure time in Alpu, ES-14 and Kızıltan, while there was no clear alteration in Na uptake rate of Gediz (Figure 4.1.5). Due to increases in K uptake rate with exposure time, the cumulative uptake progressively increased with the duration of NaCl treatment. However, when compared to 0 mM NaCl treatment, the cumulative K uptake

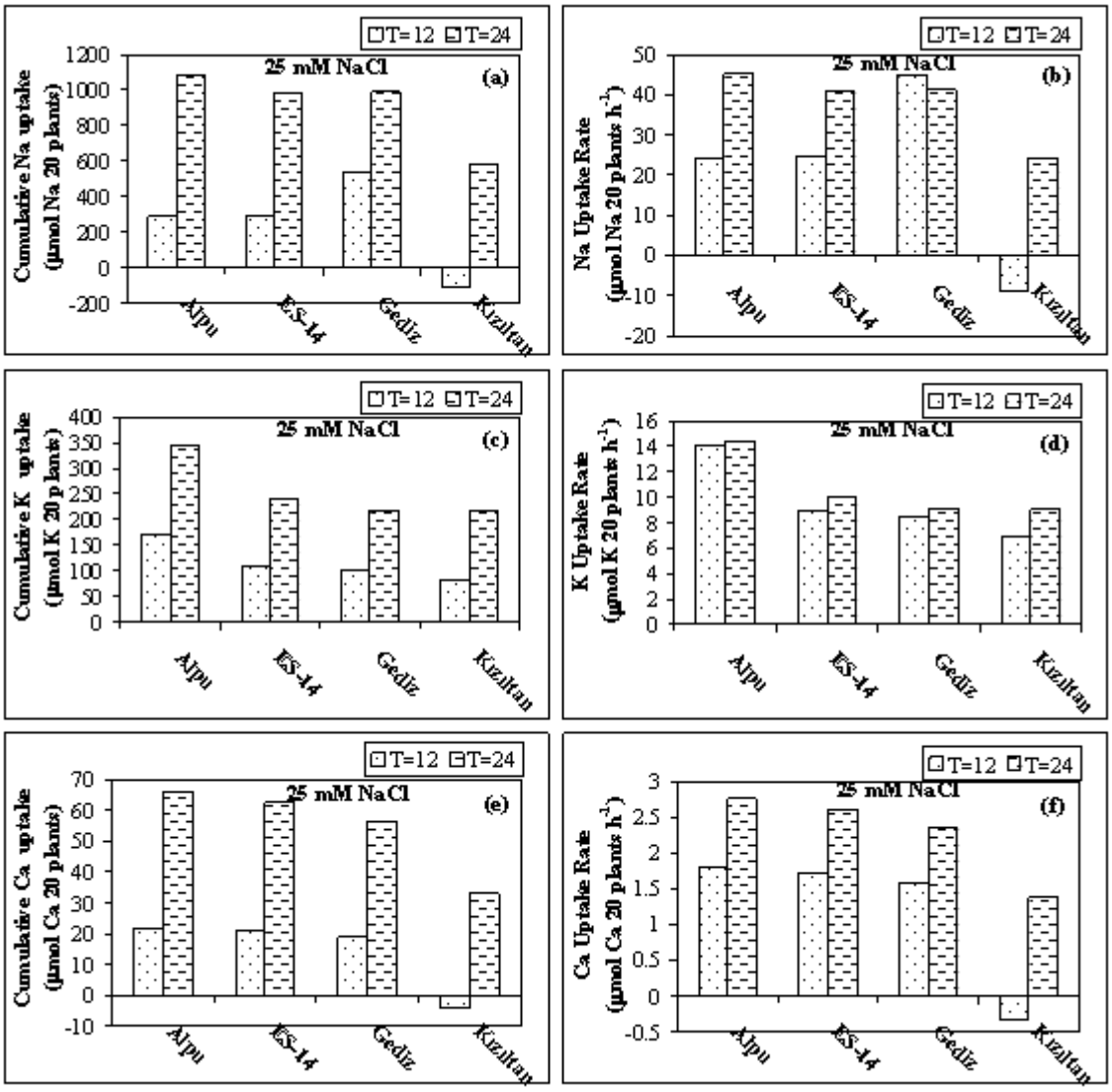

Figure 4.1.5 Influence of exposure time on concentration of Na, K and Ca in nutrient solutions of 8 days-old salt tolerant (Alpu, Gediz) and salt sensitive (ES-14, Kızıltan) wheat genotypes. Plants were grown for seven days at in nutrient solution and treated with 25 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol Na, K and Ca 20 plants (a, c, e).Uptake rates represented as µmol Na, K and Ca 20 plants per hour (b, d, f). The data represent means of four independent replications.

enhanced in salt tolerant (Alpu) and salt sensitive (ES-14) bread wheat genotypes, while it declined in salt sensitive durum wheat (Kızıltan) after 24 h exposure time. Salt tolerant durum wheat, Gediz, had more or less similar cumulative K uptake at 12 h and 24 h after 25 mM NaCl exposure. Application of 25 mM NaCl caused slight decreases in both net uptake rate and cumulative uptake of Ca in the salt sensitive genotypes, ES-14 and Kızıltan. On the other hand, the cumulative Ca uptakes and net uptake rates of Ca were increased in salt tolerant genotypes, Alpu and Gediz at 25 mM NaCl supply compared to 75 mM NaCl. Under 25 mM NaCl application, cumulative Ca uptake rate was associated by increasing Ca uptake rate with the exposure duration in all genotypes (Figure 4.1.5).

According to the results obtained at 75 mM NaCl supply, Na exclusion was observed in all genotypes at 12 h after NaCl exposure. There was a marked difference in amount of Na efflux among bread and durum wheat genotypes. Bread wheat genotypes excluded higher level of Na compared to durum wheat genotypes. Interestingly, Na uptake rates increased in salt tolerant cultivars, Gediz and especially in Alpu with regard to the measurements at 24 h after NaCl treatment. As a consequence of this, cumulative Na uptake was higher in salt tolerant genotypes than salt sensitive genotypes after 24 h. exposure time that is in agreement with the results obtained in greenhouse. The cumulative Ca uptake and net Ca uptake rates showed similarities with the results of Na uptake during the exposure time to 75 mM NaCl supply (Figure 4.1.6). The total K uptake capacity, associated to increasing of K uptake rates, in all genotypes increased with the exposure time. The cumulative K uptake was higher in Alpu compared to the others, in which total K uptake was more or less similar, at 24 h after 75 mM NaCl exposure.

The results with 150 mM NaCl treatment showed significant differences in uptake rates of Na, K and Ca between bread and durum wheat genotypes (Figure 4.1.7). Durum wheat genotypes had higher cumulative Na uptakes and net Na uptake rates compared to bread wheat. The measurement at 12 h after NaCl exposure to 150 mM NaCl demonstrated that Na exclusion occurred in salt tolerant bread wheat, Alpu, while the other genotypes showed Na uptake from the nutrient solution. However, Na uptake rate progressively increased with the exposure duration in only Alpu, while the uptake rate of Na decreased in the other genotypes. Furthermore, the total Na uptake increased after 24 h exposure time, except Gediz. Like the results obtained with 75 mM NaCl treatment

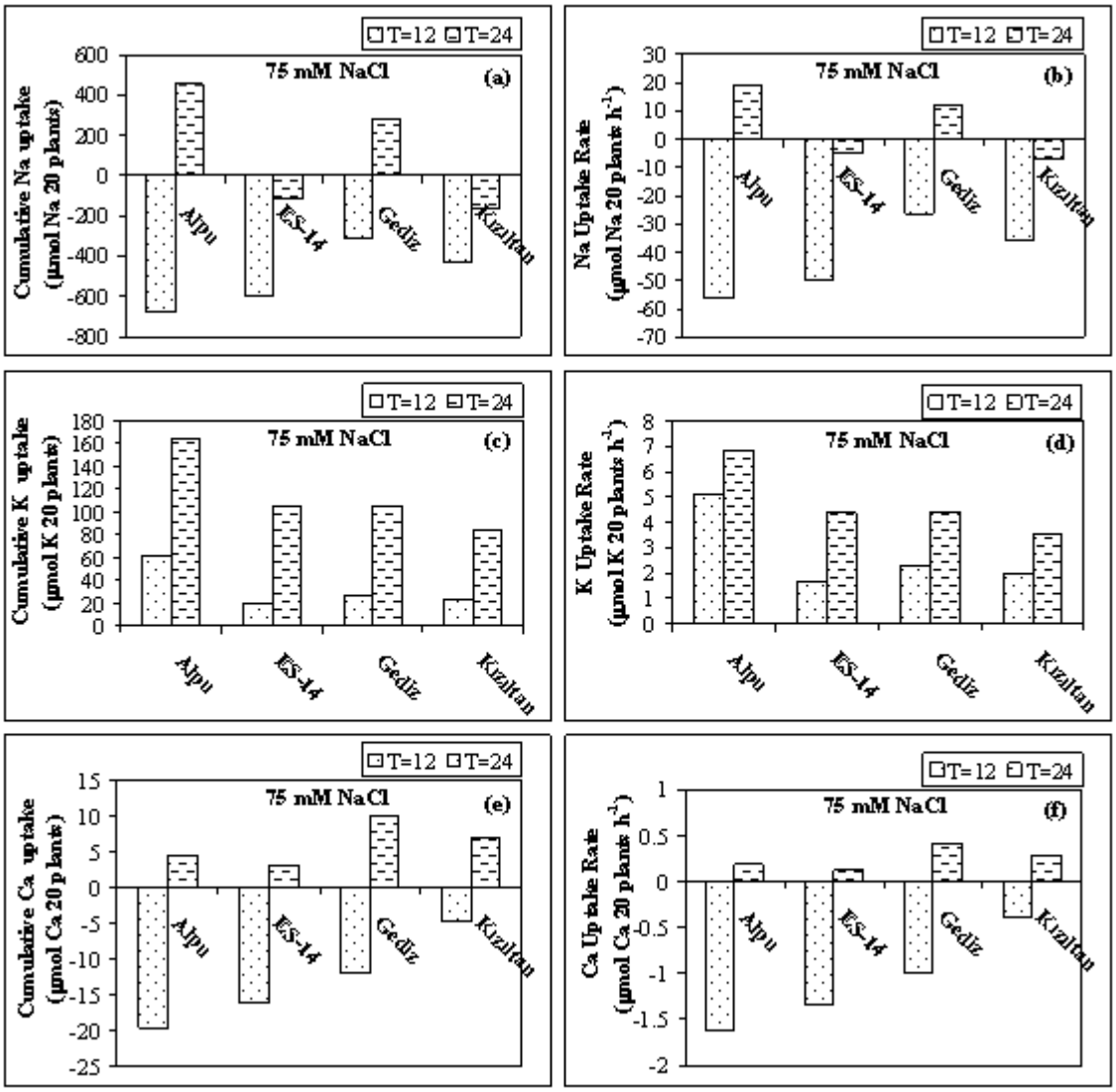

Figure 4.1.6 Influence of exposure time on concentration of Na, K and Ca in nutrient solutions of 8 days-old salt tolerant (Alpu, Gediz) and salt sensitive (ES-14, Kızıltan) wheat genotypes. Plants were grown for seven days at in nutrient solution and treated with 75 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol Na, K and Ca 20 plants (a, c, e).Uptake rates represented as µmol Na, K and Ca 20 plants per hour (b, d, f). The data represent means of four independent replications.

there were similarities between Na and Ca uptakes with respect to the results obtained at 12 h and 24 h following 150 mM NaCl exposure (Figure 4.1.7). In the case of K, there was a marked decrease in cumulative K uptake and net K uptake rate in all genotypes, except Kızıltan, at 150 mM NaCl treatment, when compared to 75 mM NaCl application. In contrast to bread wheat genotypes, the K uptake was decreased with exposure time to salt. . However, the cumulative K uptake was higher in durum wheat than in bread wheat. Salt sensitive durum wheat, Kızıltan, had higher total K uptake than the salt tolerant durum wheat, Gediz after 24 h exposure duration. In the case of

bread wheat, the salt tolerant Alpu had higher cumulative K uptake than salt sensitive ES-14. The cumulative of Na uptake and Na uptake rate at 150 mM NaCl was correlated showed similarities with the results obtained in greenhouse experiments. The cumulative and net uptake rates of Na at 150 mM NaCl showed similarities with the results obtained in greenhouse experiments. The total Na uptake capacity was higher in durum wheat than in bread wheat genotypes. Like the results obtained in greenhouse experiment, tolerant genotypes had lower Na concentration compared to the sensitive genotypes. A high genotypic variation in salinity tolerance was only observed at 150 mM NaCl leading to suggestion that salinity level could be important in ranking genotypes for their tolerance to NaCl toxicity.

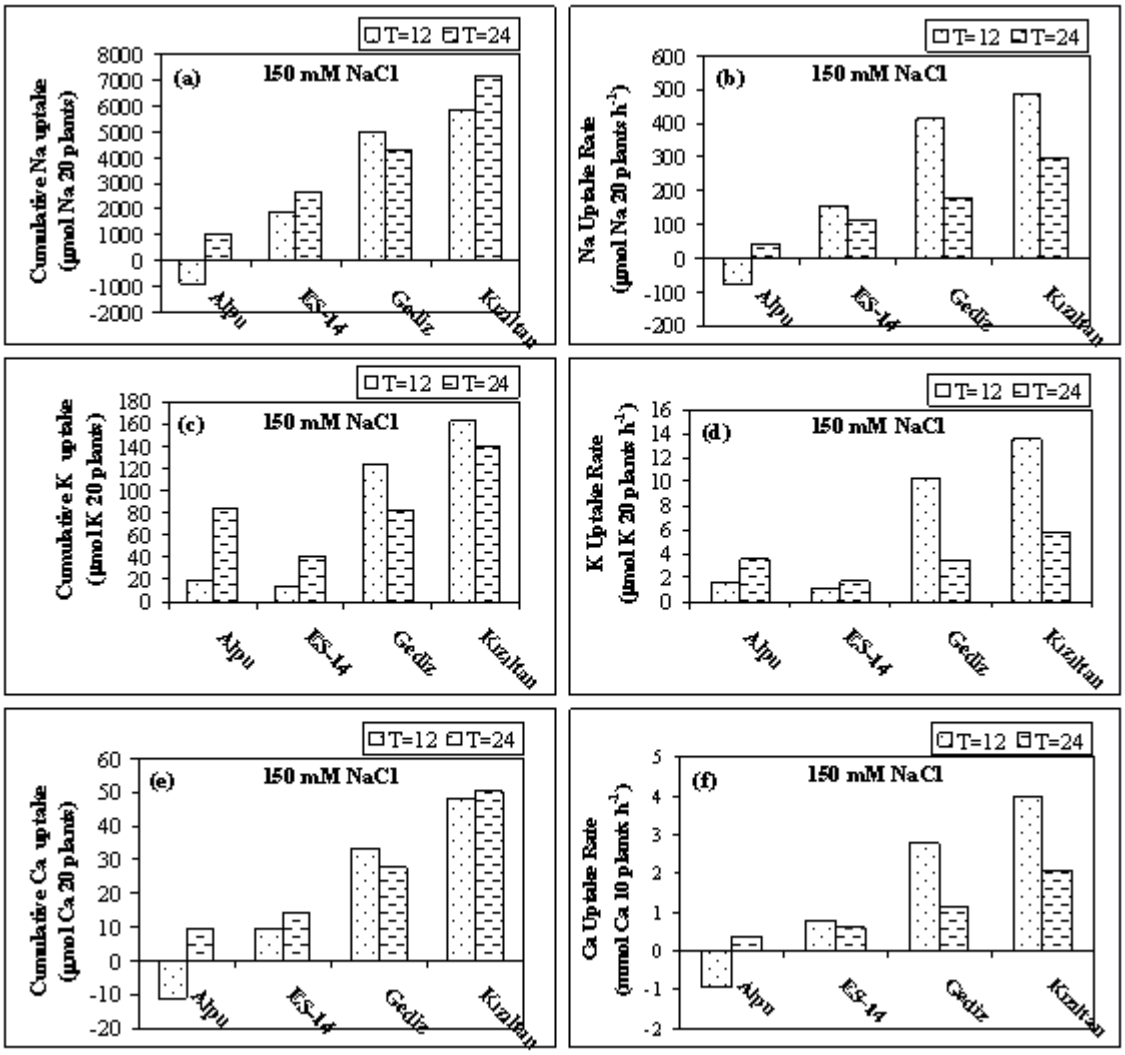

Figure 4.1.7 Influence of exposure time on concentration of Na, K and Ca in nutrient solutions of 8 days-old salt tolerant (Alpu, Gediz) and salt sensitive (ES-14, Kızıltan) wheat genotypes. Plants were grown for seven days at in nutrient solution and treated with 150 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol Na, K and Ca 20 plants (a, c, e).Uptake rates represented as µmol Na, K and Ca 20 plants per hour (b, d, f). The data represent means of four independent replications.

4.2 Screening for Salt Tolerance in *Aegilops tauschii* Genotypes

4.2.1 Greenhouse Experiments

Aegilops tauschii, the D-genome donor of bread wheat was used to determine the level of salt tolerance. For this purpose, 116 *Aegilops tauschii* genotypes were grown in greenhouse with (1500 mg NaCl kg^{-1} soil) and without NaCl treatment for 26 days. There was a large variation between genotypes for salt tolerance expressed as the ratio of dry matter production at salt treatment to the dry weight without salt treatment Based on this salt tolerance index , 18 genotypes were chosen for the next experiment. Among the selected 18 genotypes, there were the 9 salt tolerant genotypes (Aegilops 95, 99, 103, 108, 115, 118, 141, 147 and 148) and the 9 salt sensitive genotypes (Aegilops 1, 20, 32, 36, 39, 40, 45, 60 and 93). These selected genotypes were grown with 0, 2500 and 5000 mg NaCl kg^{-1} soil treatment for 25 days in greenhouse. Salt tolerance index of the genotypes found in the first experiment significantly correlated with salt tolerance index found in the second experiment with 2500 mg NaCl kg^{-1} soil and 5000 mg NaCl kg^{-1} soil treatments, indicating close relationship between both experiments in terms of the suitability of the screening study. But, the close relationship found between 2 experiments with *Aegilops tauschii* was much closer at 5000 mg NaCl kg^{-1} soil than at 2500 mg NaCl kg^{-1} soil (Figure 4.2.1).

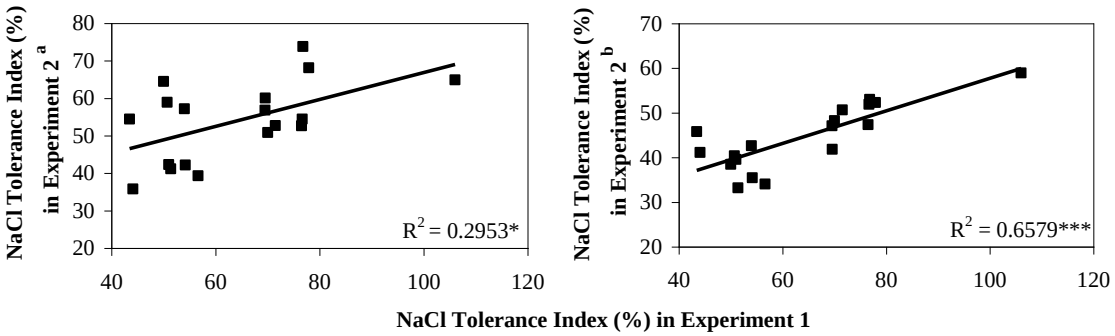

Figure 4.2.1 The relationships between NaCl tolerance index in first experiment and NaCl tolerance index obtained in second experiment. * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared. [a] under 2500 mg NaCl kg^{-1} soil supply, [b] under 5000 mg NaCl kg^{-1} soil supply

4.2.1.1 Leaf Symptoms and Dry Matter Production

The first response of *Aegilops tauschii* genotypes to salt stress was marked reduction in leaf size and shoot elongation. Thereafter, leaf chlorosis and necrosis were appeared in genotypes under salt treatment, especially in salt-sensitive genotypes. When compared to 2500 mg NaCl kg^{-1} soil, the NaCl tolerance index correlated well with severity of leaf symptoms much more significantly than the 5000 mg NaCl kg^{-1} soil treatment (Table 4.2.1). Generally, all genotypes were less affected from 2500 mg NaCl kg^{-1} soil treatment and the severity of visible symptoms were slight in 2500 mg NaCl kg^{-1} soil treated plants. Therefore, the extent of the genotypic variation under 2500 mg NaCl kg^{-1} soil treatment was small. In contrast to 2500 mg NaCl kg^{-1} soil, there was a large genotypic variation among these genotypes when exposed to 5000 mg NaCl kg^{-1} soil treatment. In the case of some (sensitive) genotypes, severe chlorosis and necrosis developed on the older leaves. Based on the severity of leaf symptoms found in the second experiment with *Aegilops tauschii* genotypes, the Aegilops genotypes 141, 108 and 147 were the most salt tolerant genotypes while the Aegilops genotypes 60, 20 and 36 were the most sensitive genotypes to salinity.

The selected 18 genotypes showed also greater genotypic differences in terms of dry matter production capacity under salt treatment, and this variation seems to be larger at 2500 mg NaCl kg^{-1} soil treatment than at 5000 mg NaCl kg^{-1} soil treatment (Table 4.2.1). Under 2500 mg NaCl kg^{-1} soil condition, the least shoot dry matter reductions were observed in the tolerant genotypes Aegilops 115, 118 and 108; and the highest reductions in dry weights were found in sensitive genotypes Aegilops 45, 20 and 40. In the case of 5000 mg NaCl kg^{-1} soil treatment Aegilops 108, 115 and 118 had the lowest reductions in shoot dry weights, while Aegilops 36, 20 and 45 showed the highest decreases in shoot dry matter reduction. Based on these results and observations, the most tolerant and the most sensitive genotypes were same in both *Aegilops* experiment, except the Aegilops genotypes 40 and 36.

There was a significant correlation between salt tolerance index and the shoot dry matter production at 2500 mg NaCl kg^{-1} soil treatment (Figure 4.2.2). In the case of 5000 mg NaCl kg^{-1} soil treatment, the correlation between NaCl tolerance trait and absolute shoot dry weight was greater than the 2500 mg NaCl kg^{-1} soil supply (Figure 4.2.2). This result indicates that the shoot dry weight under salt treatment can be used as

a selection criterion for selecting genotypes for high salt tolerance. The shoot dry matter production of the sensitive genotypes was much more affected by 5000 mg NaCl kg^{-1} soil treatment than the tolerant genotypes, except Aegilops 148.

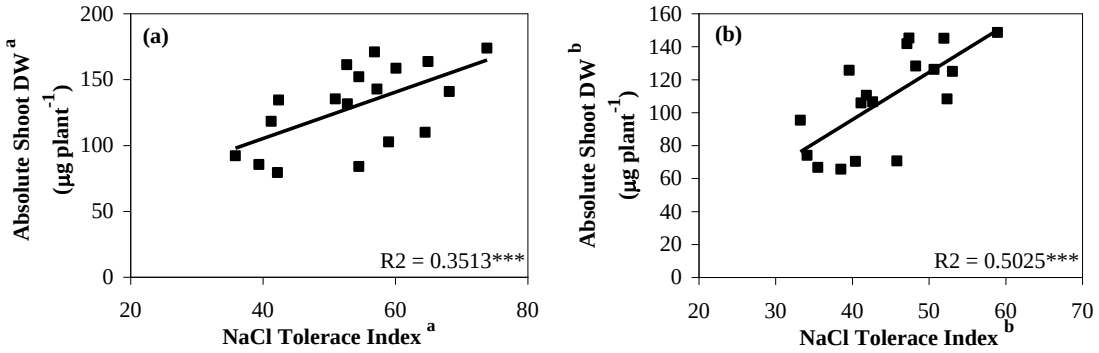

Figure 4.2.2 The relationships between NaCl tolerance index and absolute shoot dry weight under 2500 mg NaCl kg^{-1} soil condition (a) and 5000 mg NaCl kg^{-1} soil condition (b). $NaCl_{2500}$ tolerance index was calculated as: [(dry matter production at $NaCl_{2500}$/dry matter production at $NaCl_0$) X 100]; $NaCl_{5000}$ tolerance index was calculated as: [(dry matter production at $NaCl_{5000}$/dry matter production at $NaCl_0$) X 100]. *** is statistically significant at $P < 0.001$ level. R^2 = linear regression coefficient squared. [a] under 2500 mg NaCl kg^{-1} soil supply, [b] under 5000 mg NaCl kg^{-1} soil supply

Table 4.2.1 Effect of NaCl supply on leaf symptoms, shoot dry matter and NaCl tolerance index of 18 *Aegilops tauschii* genotypes grown for 25 days under greenhouse conditions. $NaCl_0$, $NaCl_{2500}$ and $NaCl_{5000}$ mean 0, 2500 and 5000 mg NaCl kg^{-1} soil treatment, respectively. $NaCl_{2500}$ tolerance index was calculated as: [(dry matter production at $NaCl_{2500}$/dry matter production at $NaCl_0$) X 100]; $NaCl_{5000}$ tolerance index was calculated as: [(dry matter production at $NaCl_{5000}$/dry matter production at $NaCl_0$) X 100]. Data represent means of 3 independent replications. All genotypes are ranked according to $NaCl_{5000}$ tolerance index.

	Leaf Symptoms**		Dry matter production			$NaCl_{2500}$	$NaCl_{5000}$
Genotypes	$NaCl_{2500}$	$NaCl_{5000}$	$NaCl_0$	$NaCl_{2500}$	$NaCl_{5000}$	Tol. Ind.	Tol. Ind.
				(µg $plant^{-1}$)		(%)	(%)
Aegilops 108*	4.5	3.5	252 ± 20	164 ± 15	149 ± 13	65	59
Aegilops 115*	4	3	236 ± 23	174 ± 2	125 ± 6	74	53
Aegilops 118*	4	3	207 ± 19	141 ± 1	108 ± 4	68	52
Aegilops 95*	4.5	3	279 ± 16	152 ± 14	145 ± 15	54	52
Aegilops 141*	4.5	4	249 ± 15	131 ± 7	126 ± 15	53	51
Aegilops 147*	4.5	3.5	266 ± 9	135 ± 16	128 ± 12	51	48
Aegilops 99*	4.5	3	306 ± 32	161 ± 19	145 ± 14	53	47
Aegilops 103*	4.5	3	301 ± 3	171 ± 9	142 ± 2	57	47
Aegilops 1	4.5	3	154 ± 14	84 ± 3	71 ± 11	54	46
Aegilops 60	4	2	249 ± 15	143 ± 8	106 ± 2	57	43
Aegilops 148*	4.5	3	264 ± 17	159 ± 9	110 ± 10	60	42
Aegilops 40	4.5	2.5	257 ± 11	92 ± 3	106 ± 3	36	41
Aegilops 32	4.5	2.5	174 ± 3	103 ± 21	70 ± 11	59	40
Aegilops 93	4.5	3	317 ± 30	134 ± 9	126 ± 12	42	40
Aegilops 39	4.5	2.5	170 ± 11	110 ± 2	66 ± 1	65	39
Aegilops 36	4	2	188 ± 8	79 ± 5	67 ± 2	42	35
Aegilops 20	4	2	217 ± 22	85 ± 12	74 ± 6	39	34
Aegilops 45	4.5	2.5	287 ± 5	118 ± 2	95 ± 1	41	33
Mean	4	3	243	130	109	54	45

*Salt tolerant genotypes based on the results obtained in experiment 1.

** Leaf symptoms of salt stress: 1 (very severe) to 5 (very slight or no symptoms).

4.2.1.2 Concentrations of Na, K, and Ca

The salt sensitive and tolerant genotypes had very similar shoot Na, K and Ca concentrations without NaCl treatment. The mean values of shoot Na, K and Ca concentrations were 0.101, 34 and 5.49 mg g^{-1} in tolerant genotypes without NaCl treatment, respectively, while in the sensitive genotypes these values were 0.098, 33 and 5.92 mg g^{-1} in the same order. The shoot Na and Ca concentrations increased, while the shoot concentration of K decreased by increasing NaCl. The shoot concentration of Na was similar among the sensitive and the tolerant genotypes, but the differences in Na concentrations between the genotypes became distinct by increasing NaCl. On average, the sensitive genotypes contained 1.7 and 1.8-fold higher Na concentrations in shoots than the tolerant genotypes at 2500 mg NaCl kg^{-1} soil treatment and 5000 mg NaCl kg^{-1} soil treatment, respectively.

When 2500 mg NaCl kg^{-1} soil was supplied, the highest and lowest shoot Na concentrations were observed in the sensitive genotype Aegilops 20 (6.22 mg g^{-1}) and tolerant genotype Aegilops 115 (1.75 mg g^{-1}), respectively (Table 4.2.2). Despite significant variation in shoot Na concentration within sensitive and tolerant genotypes, there was a weak but not significant relationship between NaCl tolerance and shoot Na concentration when all genotypes considered together (R^2 = 0.1372) (Figure 4.2.3). The tolerant and sensitive genotypes had more or less similar K and Ca concentrations at 2500 mg NaCl kg^{-1} soil (Table 4.2.3 and 4.2.4). Interestingly, the highest shoot concentrations of K and Ca were found in sensitive genotypes Aegilops 40 and 1, respectively, while tolerant genotypes Aegilops 118 and 108 had the lowest K and Ca concentrations under 2500 mg NaCl kg^{-1} soil condition. There was also a moderately significant correlation (R^2 = 0.2477*) between K concentration and NaCl tolerance index at 2500 mg NaCl kg^{-1} soil treatment (Figure 4.2.3). However, shoot Ca concentration showed no significant correlation with NaCl tolerance with 2500 mg NaCl kg^{-1} supply (R^2 = 0.0041) (Figure 4.2.3).

At 5000 mg NaCl kg^{-1} soil treatment, the shoot concentration of Na increased, and the Na concentrations of genotypes at 5000 mg NaCl kg^{-1} soil treatment generally higher than Ca concentration. Similarly also some sensitive genotypes such as Aegilops 20, 32, 36 and 39 had greater Na concentration than K concentration. On average, the shoot concentrations of Na, K and Ca were 14.9, 20.99 and 12.14 mg g^{-1},

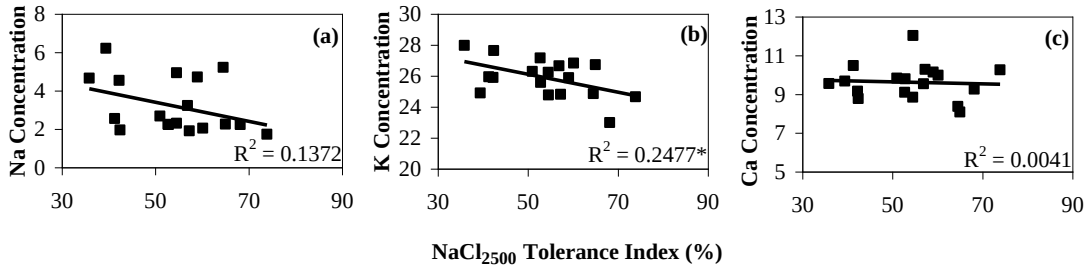

Figure 4.2.3 The NaCl tolerance index correlated with Na, K and Ca concentrations (mg g^{-1}) (a, b and c) under 2500 mg NaCl kg^{-1} condition. $NaCl_{2500}$ tolerance index was calculated as: [(dry matter production at $NaCl_{2500}$/dry matter production at $NaCl_0$) X 100. * is statistically significant at $P < 0.05$ level. R^2 = linear regression coefficient squared.

respectively. Applying NaCl at 5000 mg NaCl kg^{-1} soil resulted in increases in the shoot Na concentration. Accordingly, sensitive genotypes Aegilops 32 (28.9 mg g^{-1}) had the highest Na concentration and the lowest Na concentration was found in tolerant genotype Aegilops 115 (7.2 mg g^{-1}). On the other hand, applying NaCl at 5000 mg NaCl kg^{-1} soil decreased K concentration, mainly in the sensitive genotypes. The shoot K concentration was varied from 17.97 mg g^{-1} (Aegilops 45) to 24.53 mg g^{-1} (Aegilops 108). In the case of Ca concentration, the highest Ca concentration was found in tolerant genotype (Aegilops 95), while Aegilops 36 had the lowest shoot Ca concentration under 5000 mg NaCl g^{-1} condition. With 5000 mg NaCl kg^{-1} soil supply, there was a marked increase in Na and Ca concentrations, particularly in the sensitive genotypes. On the other hand, applying NaCl at 5000 mg NaCl kg^{-1} soil decreased K concentration, mainly in the sensitive genotypes. NaCl tolerance index at 5000 mg NaCl kg^{-1} soil treatment significantly correlated with shoot concentrations of Na, K and Ca (R^2 = 0.3496**, R^2 = 0.2822* and R^2 = 0.3466*) (Figure 4.2.4). These correlations at 5000 mg NaCl kg^{-1} soil treatment were stronger and more significant than the correlations obtained at 2500 mg NaCl kg^{-1} soil treatment. The correlation between NaCl tolerance index and shoot Na concentration was in negative direction. There was also an inverse trend between NaCl tolerance index and shoot Ca concentration. In contrast to the results obtained at 2500 mg NaCl kg^{-1} soil treatment, the tolerance index was regressed positively on shoot K concentration under 2500 mg NaCl kg^{-1} soil condition.

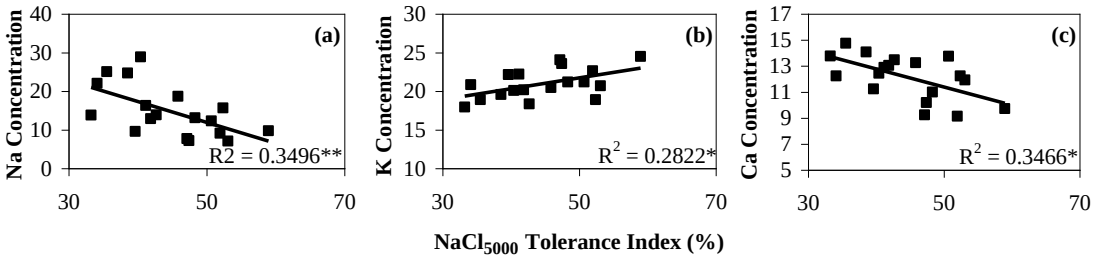

Figure 4.2.4 The NaCl tolerance index correlated with Na, K and Ca concentrations (mg g^{-1}) (a, b and c) under 5000 mg NaCl kg^{-1} condition. $NaCl_{5000}$ tolerance index was calculated as: [(dry matter production at $NaCl_{5000}$/dry matter production at $NaCl_0$) X 100. * and ** are statistically significant at $P < 0.05$ and $P < 0.01$ levels, respectively. R^2 = linear regression coefficient squared.

Table 4.2.2 Shoot Na concentration of 25-day old 18 *Aegilops tauschii* genotypes grown with (2500 mg NaCl kg^{-1} soil) and without NaCl treatment under greenhouse conditions. $NaCl_0$, $NaCl_{2500}$ and $NaCl_{5000}$ mean 0, 2500 and 5000 mg NaCl kg^{-1} soil treatment, respectively. Data represent means of 3 independent replications. All genotypes are ranked according to $NaCl_{5000}$ tolerance index.* Salt tolerant genotypes

	Na Concentration in Shoot		
Genotypes	$NaCl_0$	$NaCl_{2500}$	$NaCl_{5000}$
		(mg g^{-1} DW)	
Aegilops 108*	0.090 ± 0.014	2.27 ± 0.16	9.8 ± 0.2
Aegilops 115*	0.119 ± 0.006	1.75 ± 0.13	7.2 ± 0.3
Aegilops 118*	0.158 ± 0.026	2.24 ± 0.42	15.7 ± 3.4
Aegilops 95*	0.065 ± 0.002	2.31 ± 0.25	9.2 ± 2.6
Aegilops 141*	0.061 ± 0.002	2.24 ± 0.10	12.3 ± 0.7
Aegilops 147*	0.132 ± 0.014	2.69 ± 0.34	13.2 ± 0.6
Aegilops 99*	0.105 ± 0.020	2.26 ± 0.25	7.2 ± 0.8
Aegilops 103*	0.087 ± 0.008	3.24 ± 0.45	7.8 ± 1.0
Aegilops 1	0.110 ± 0.001	4.95 ± 0.29	18.7 ± 6.7
Aegilops 60	0.081 ± 0.002	1.92 ± 0.14	13.9 ± 0.1
Aegilops 148*	0.092 ± 0.001	2.05 ± 0.08	12.9 ± 1.1
Aegilops 40	0.120 ± 0.002	4.67 ± 0.45	16.3 ± 1.9
Aegilops 32	0.091 ± 0.014	4.73 ± 0.64	28.9 ± 1.7
Aegilops 93	0.074 ± 0.005	1.97 ± 0.14	9.6 ± 1.1
Aegilops 39	0.084 ± 0.011	5.23 ± 0.67	24.7 ± 1.7
Aegilops 36	0.111 ± 0.006	4.55 ± 0.21	25.1 ± 3.4
Aegilops 20	0.091 ± 0.010	6.22 ± 0.25	22.1 ± 2.3
Aegilops 45	0.117 ± 0.005	2.56 ± 0.37	13.8 ± 2.9
Mean	0.099	3.21	14.9

Table 4.2.3 Shoot K concentration of 25-day old 18 *Aegilops tauschii* genotypes grown with (2500 mg NaCl kg^{-1} soil) and without NaCl treatment under greenhouse conditions. $NaCl_0$, $NaCl_{2500}$ and $NaCl_{5000}$ mean 0, 2500 and 5000 mg NaCl kg^{-1} soil treatment, respectively. Data represent means of 3 independent replications. All genotypes are ranked according to $NaCl_{5000}$ tolerance index.* Salt tolerant genotypes

	K Concentration in Shoot		
Genotypes	$NaCl_0$	$NaCl_{2500}$	$NaCl_{5000}$
		(mg g^{-1} DW)	
Aegilops 108*	34.03 ± 0.74	26.74 ± 0.98	24.53 ± 0.75
Aegilops 115*	33.05 ± 0.29	24.67 ± 1.08	20.72 ± 0.86
Aegilops 118*	29.89 ± 2.34	23.01 ± 1.91	18.91 ± 0.87
Aegilops 95*	34.15 ± 0.25	26.25 ± 0.09	22.67 ± 1.67
Aegilops 141*	34.14 ± 0.06	25.59 ± 0.35	21.22 ± 0.63
Aegilops 147*	34.76 ± 0.52	26.31 ± 0.76	21.22 ± 0.97
Aegilops 99*	34.83 ± 0.30	27.18 ± 0.82	23.58 ± 1.01
Aegilops 103*	35.09 ± 0.12	26.67 ± 0.73	24.09 ± 0.81
Aegilops 1	32.12 ± 1.62	24.79 ± 0.88	20.49 ± 0.41
Aegilops 60	31.85 ± 0.35	24.83 ± 0.75	18.39 ± 0.57
Aegilops 148*	36.12 ± 0.40	26.84 ± 0.65	20.20 ± 0.64
Aegilops 40	33.98 ± 0.23	27.99 ± 0.26	22.24 ± 1.18
Aegilops 32	34.03 ± 0.19	25.89 ± 1.35	20.11 ± 1.69
Aegilops 93	33.96 ± 0.40	27.65 ± 1.16	22.17 ± 0.41
Aegilops 39	32.86 ± 1.97	24.86 ± 1.13	19.59 ± 1.37
Aegilops 36	33.26 ± 1.12	25.92 ± 1.68	18.92 ± 0.81
Aegilops 20	33.54 ± 1.02	24.92 ± 0.72	20.87 ± 1.95
Aegilops 45	32.95 ± 0.28	25.96 ± 0.90	17.97 ± 1.64
Mean	33.59	25.89	20.99

Table 4.2.4 Shoot K concentration of 25-day old 18 *Aegilops tauschii* genotypes grown with (2500 mg NaCl kg^{-1} soil) and without NaCl treatment under greenhouse conditions. $NaCl_0$, $NaCl_{2500}$ and $NaCl_{5000}$ mean 0, 2500 and 5000 mg NaCl kg^{-1} soil treatment, respectively. Data represent means of 3 independent replications. All genotypes are ranked according to $NaCl_{5000}$ tolerance index.* Salt tolerant genotypes

	Ca Concentration in Shoot		
Genotypes	$NaCl_0$	$NaCl_{2500}$	$NaCl_{5000}$
		(mg g^{-1} DW)	
Aegilops 108*	5.46 ± 0.16	8.09 ± 0.37	9.74 ± 0.35
Aegilops 115*	5.90 ± 0.09	10.26 ± 0.42	11.93 ± 0.10
Aegilops 118*	5.56 ± 0.37	9.28 ± 0.75	12.24 ± 1.67
Aegilops 95*	4.58 ± 0.25	8.86 ± 0.44	9.14 ± 0.71
Aegilops 141*	5.97 ± 0.03	9.81 ± 0.23	13.77 ± 0.33
Aegilops 147*	5.65 ± 0.21	9.84 ± 0.69	11.01 ± 0.31
Aegilops 99*	5.28 ± 0.09	9.11 ± 0.25	10.20 ± 0.40
Aegilops 103*	5.22 ± 0.36	9.56 ± 0.56	9.24 ± 0.43
Aegilops 1	6.92 ± 0.10	12.04 ± 0.24	13.26 ± 0.23
Aegilops 60	5.66 ± 0.23	10.29 ± 0.78	13.48 ± 0.82
Aegilops 148*	5.79 ± 0.21	10.00 ± 0.75	13.05 ± 0.76
Aegilops 40	5.55 ± 0.14	9.57 ± 0.29	12.90 ± 0.66
Aegilops 32	5.83 ± 0.12	10.15 ± 0.01	12.45 ± 0.50
Aegilops 93	5.28 ± 0.43	8.78 ± 0.95	11.25 ± 1.08
Aegilops 39	5.42 ± 0.23	8.38 ± 0.86	14.09 ± 1.42
Aegilops 36	5.50 ± 0.16	9.17 ± 0.73	14.75 ± 1.66
Aegilops 20	6.14 ± 0.25	9.69 ± 0.94	12.25 ± 0.26
Aegilops 45	6.99 ± 0.24	10.48 ± 0.50	13.78 ± 0.41
Mean	5.71	9.63	12.14

As expected, there were also significant correlations between K/Na, Ca/Na ratios and shoot Na concentration at both 2500 mg NaCl kg^{-1} soil (R^2 = 0.9318***, R^2 = 0.862***) and 5000 mg NaCl kg^{-1} soil treatment (R^2 = 0.7929***, R^2 = 0.8054***) (Figure 4.2.5). The correlations between K/Na, Ca/Na ratios and shoot Na concentration were also highly significant in the first experiment (R^2 = 0.5397***, R^2 = 0.4959***) (Figure 4.2.5). The K/Na and Ca/Na ratios were regressed negatively on Na concentration in all saline conditions. In general, the sensitive genotypes had higher Na concentrations associated with lower K/Na and Ca/Na ratios, when compared to tolerant genotypes. At 2500 mg NaCl kg^{-1} soil treatment, the highest K/Na and Ca/Na ratios were found in Aegilops 115, whereas the lowest ratio in Aegilops 20. When 5000 mg NaCl kg^{-1} soil was applied, the highest K/Na and Ca/Na ratios were found in Aegilops 99 and Aegilops 115, respectively. Aegilops 32 had the lowest K/Na and Ca/Na ratios under 5000 mg NaCl kg^{-1} soil condition.

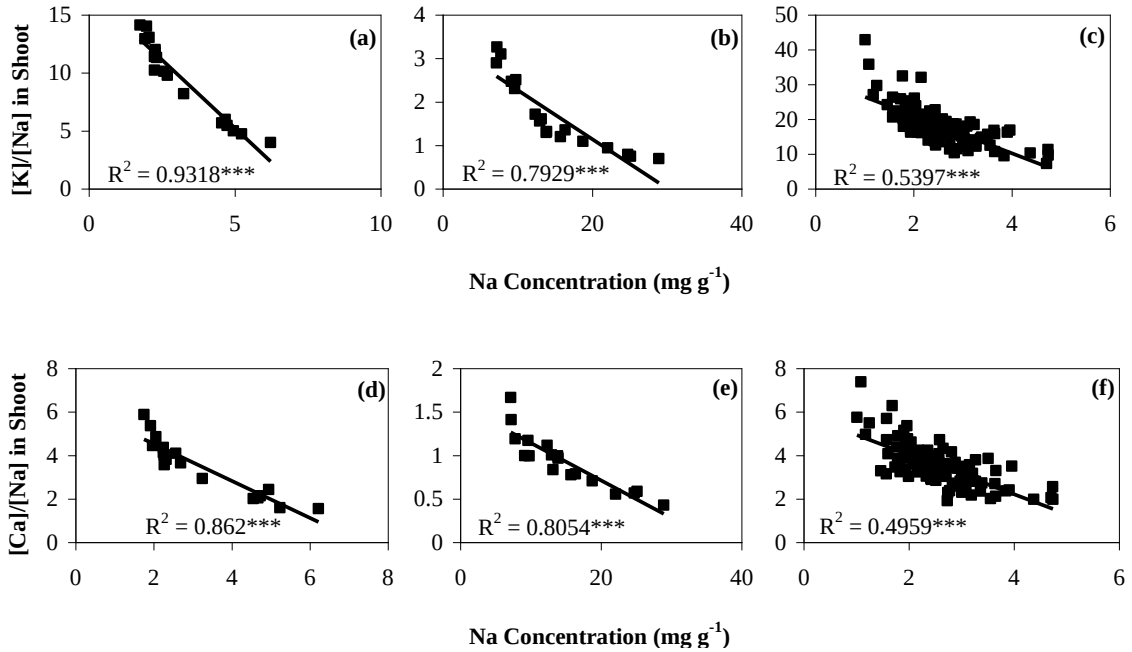

Figure 4.2.5 The relationships between the shoot K/Na ratio and shoot Na concentrations under 2500 mg NaCl kg^{-1} condition (a), 5000 mg NaCl kg^{-1} condition (b) and in the first experiment (c). The relationships between the shoot Ca/Na ratio and shoot Na concentrations under 2500 mg NaCl kg^{-1} condition (d), 5000 mg NaCl kg^{-1} condition (e) and in the first experiment (f). The shoot K/Na and Ca/Na ratios are regressed negatively on shoot Na concentration. *** is statistically significant at $P < 0.001$ level. R^2 = linear regression coefficient squared.

The relationship between K/Na ratio and shoot K concentration was much more significant at 5000 mg NaCl kg^{-1} soil (R^2 = 0.558***) than at 2500 mg NaCl kg^{-1} soil treatment (R^2 = 0.0275). In the case of first experiment, the K/Na ratio showed a very significant correlation with shoot K concentration (R^2 = 0.1002***) (Figure 4.2.6). The

relationship between Ca/Na ratio and shoot Ca concentration was not significant at both 2500 and 5000 mg NaCl kg^{-1} soil, but there was moderately significant relation in the first experiment (R^2 = 0.0493*) (Figure 4.2.7).

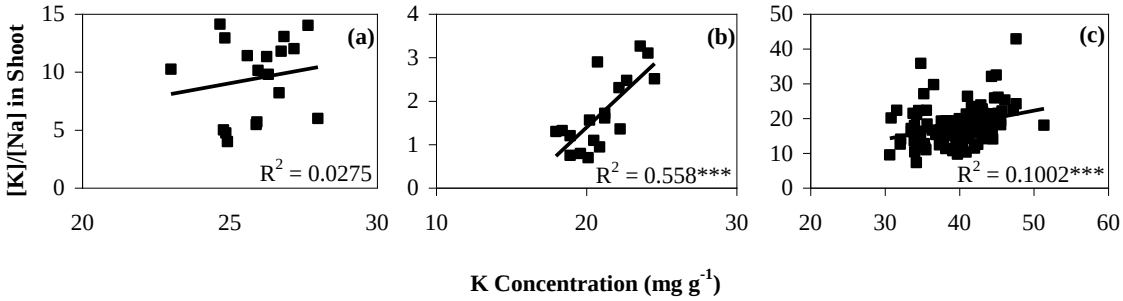

Figure 4.2.6 The relationships between the shoot K/Na ratio and shoot K concentrations under 2500 mg NaCl kg^{-1} condition (a), 5000 mg NaCl kg^{-1} condition (b) and in the first experiment (c). The shoot K/Na ratio is regressed positively on shoot K concentration. * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared.

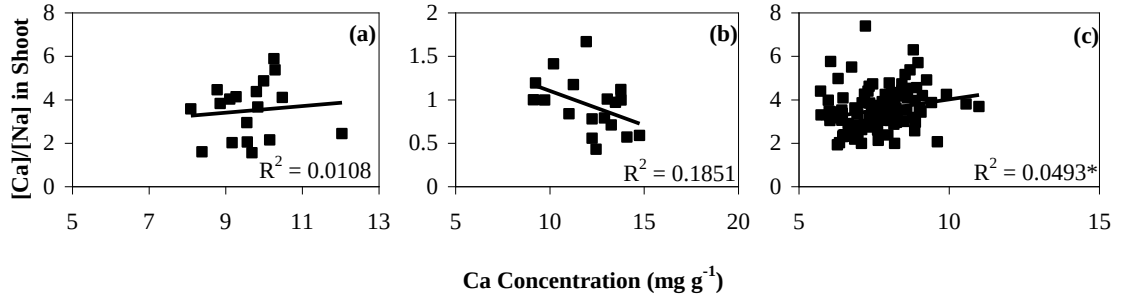

Figure 4.2.7 The relationships between the shoot Ca/Na ratio and shoot Ca concentrations under 2500 mg NaCl kg^{-1} condition (a), 5000 mg NaCl kg^{-1} condition (b) and in the first experiment (c). * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared

There was an inverse trend between shoot K and Na concentrations. The K concentration correlated with Na concentration at 5000 mg NaCl kg^{-1} soil (R^2 = 0.2973*) more significantly than at 2500 mg NaCl kg^{-1} soil treatment (R^2 = 0.0134). In the case of the first experiment, there was, however, no significant correlation (R^2 = 0.0017). Shoot Ca concentration was correlated positively with Na concentration. The correlation between shoot Na and Ca concentrations was much more significant at 5000 mg NaCl kg^{-1} soil supply (R^2 = 0.3982**) than in the first experiment (R^2 = 0.0568*) (Figure 4.2.8). On the other hand, the relationship between Na and Ca concentration at 2500 mg NaCl kg^{-1} soil supply was very poor (R^2 = 0.018). The shoot K concentration showed a very significant correlation with Ca concentration at 5000 mg NaCl kg^{-1} soil treatment (R^2 = 0.6743***) and in the first experiment (R^2 = 0.3526***) in contrast to 2500 mg NaCl kg^{-1} soil treatment (R^2 = 0.0824) (Figure 4.2.8).

The results obtained at 5000 mg NaCl kg^{-1} soil supply were similar to the first experiment results supporting the idea that Na concentration is an important selection criteria under salinity stress. Based on their Na uptake capacity at 5000 mg NaCl kg^{-1} soil treatment, 9 genotypes were selected to use in the further experiments related to physiological effects of salinity stress among these genotypes. Five of these selected genotypes (Aegilops 95, 99, 103,108 and 115) were salt tolerant and they had lower Na concentration at 5000 mg NaCl kg^{-1} soil supply. The other 4 genotypes (Aegilops 20, 32, 36 and 39) were sensitive to salinity and had higher Na concentration at 5000 mg NaCl kg^{-1} soil supply.

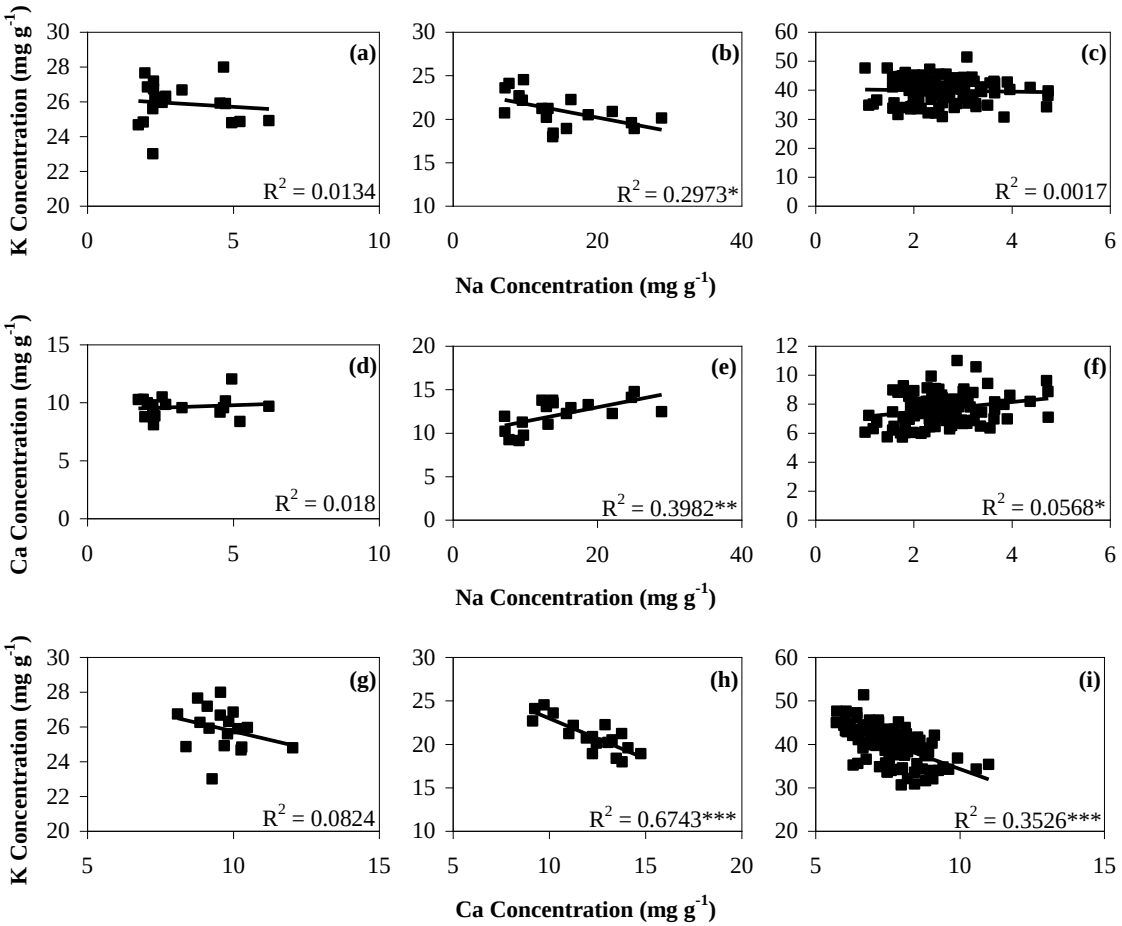

Figure 4.2.8 The relationships between the shoot K and Na concentrations under 2500 mg NaCl kg^{-1} condition (a), 5000 mg NaCl kg^{-1} condition (b) and in the first experiment (c). The relationships between the shoot Ca and Na concentrations under 2500 mg NaCl kg^{-1} condition (d), 5000 mg NaCl kg^{-1} condition (e) and in the first experiment (f). The relationships between the shoot K and Ca concentrations under 2500 mg NaCl kg^{-1} condition (g), 5000 mg NaCl kg^{-1} condition (h) and in the first experiment (i). * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared.

4.2.2 Growth Chamber Experiments

In order to study the effect of NaCl application on uptake rate of Na, K and Ca, nutrient solution experiment has been conducted in growth chamber by using selected salt tolerant and salt sensitive *Aegilops tauschii* genotypes. Plants grown in nutrient solution were exposed to 75 mM NaCl, and the uptake solution was sampled at 0 h, 12 h and 24 h after NaCl exposure. The salinity stress influenced similarly the cumulative Na uptake and net Na uptake rate. Some genotypes showed Na efflux (Na exclusion) when exposed to NaCl stress. Sodium exclusion was observed mainly in salt tolerant genotypes, Aegilops 95, 99, 108 and 115, and in the salt sensitive genotype, Aegilops 36, at 12 h after exposure duration (Figure 4.2.9).

The results of Na uptake at 12 h after exposure time showed that salt tolerant genotype, Aegilops 103 and salt sensitive genotypes, Aegilops 20, 32 and 39, absorbed Na in contrast to the other genotypes in which Na excluded. However, the results obtained at 24 h, showed that Aegilops 95, 99 and 108 absorbed Na, while Na exclusion was found in Aegilops 103, 20 and 32. Aegilops 115 and 36 continued to show Na efflux at 24 h after NaCl exposure duration. The absorption of Na during 24 h was observed only in one genotype that was the salt tolerant Aegilops 39.

The total Ca uptake and net Ca uptake rates were similarly affected from NaCl supply. Such similarities were also detected in 75 mM and 150 mM NaCl exposure to modern cultivars (Figure 4.2.10). In the case of K, root uptake continued increasingly during 24 h after NaCl exposure in all genotypes, except Aegilops 36 that absorbed considerably low K from the nutrient solution (Figure 4.2.11). The sensitivity to salinity of Aegilops 36 seems to be associated with the low level of K/Na ratio, due to its low K uptake capacity. Aegilops 99 and 108, that showed the highest efflux rate in Na and Ca at 12 h after exposure duration, removed also K from solution at 12 h.

The cumulative K uptake was higher in Aegilops 95,103, 108 and 39 at 24 h after NaCl exposure. In the case of Aegilops 108, cumulative uptake of K and Na was greater when compared to other genotypes at 12 h after the NaCl treatment. Na exclusion and K uptake in Aegilops 103 progressively increased with the duration of the NaCl treatment. The high salinity tolerance of Aegilops 103 is particularly associated with the capacity of this genotype to exclude Na from roots.

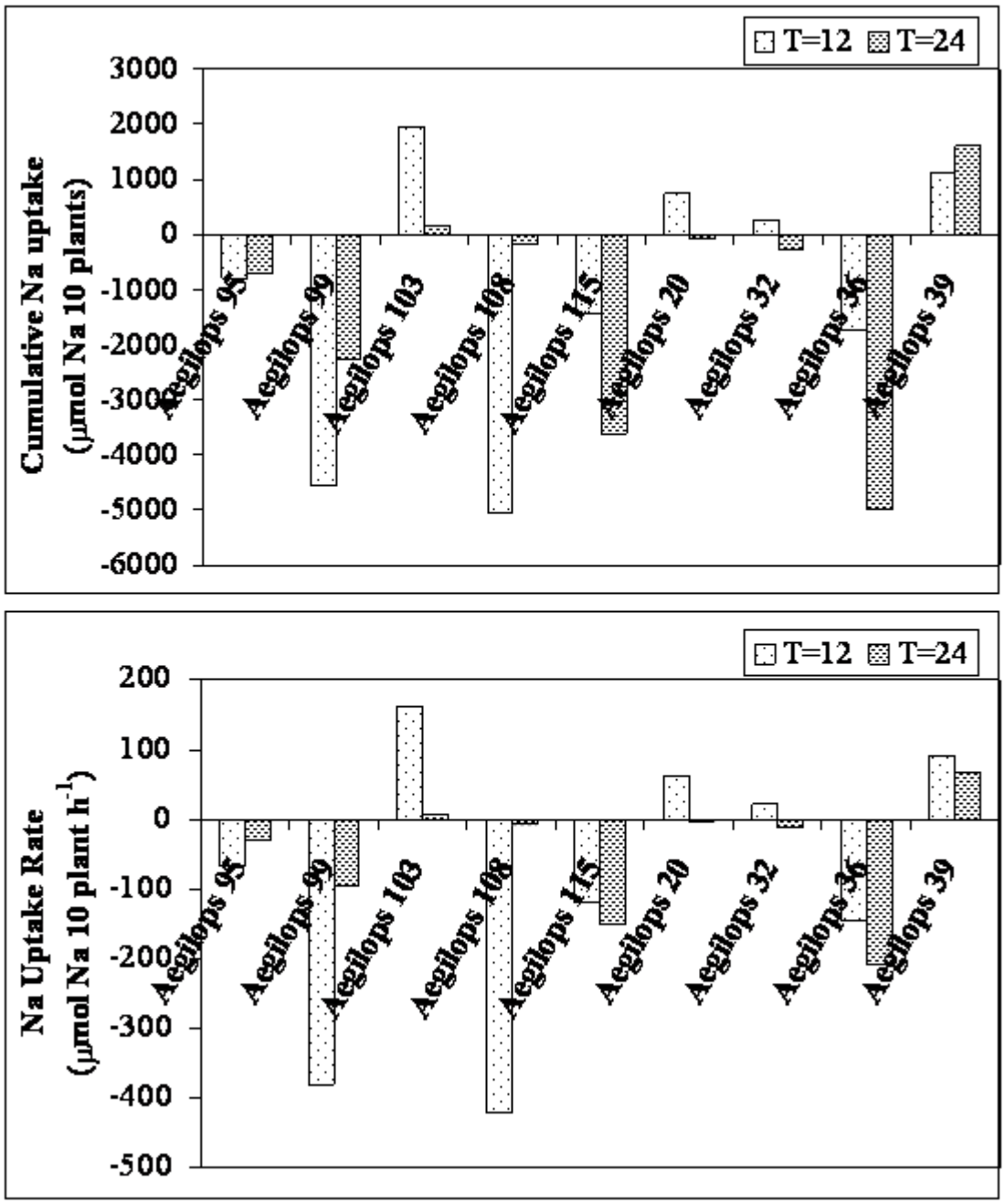

Figure 4.2.9 Influence of exposure time on concentration of Na in nutrient solutions of 15days-old salt tolerant (Aegilops 95, 99, 103, 108 and 115) and salt sensitive (Aegilops 20, 32, 36 and 39) wheat genotypes. Plants were grown for fourteen days at in nutrient solution and treated with 75 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as μmol Na 10 plants. Uptake rates represented as μmol Na 10 plants per hour. The data represent means of four independent replications.

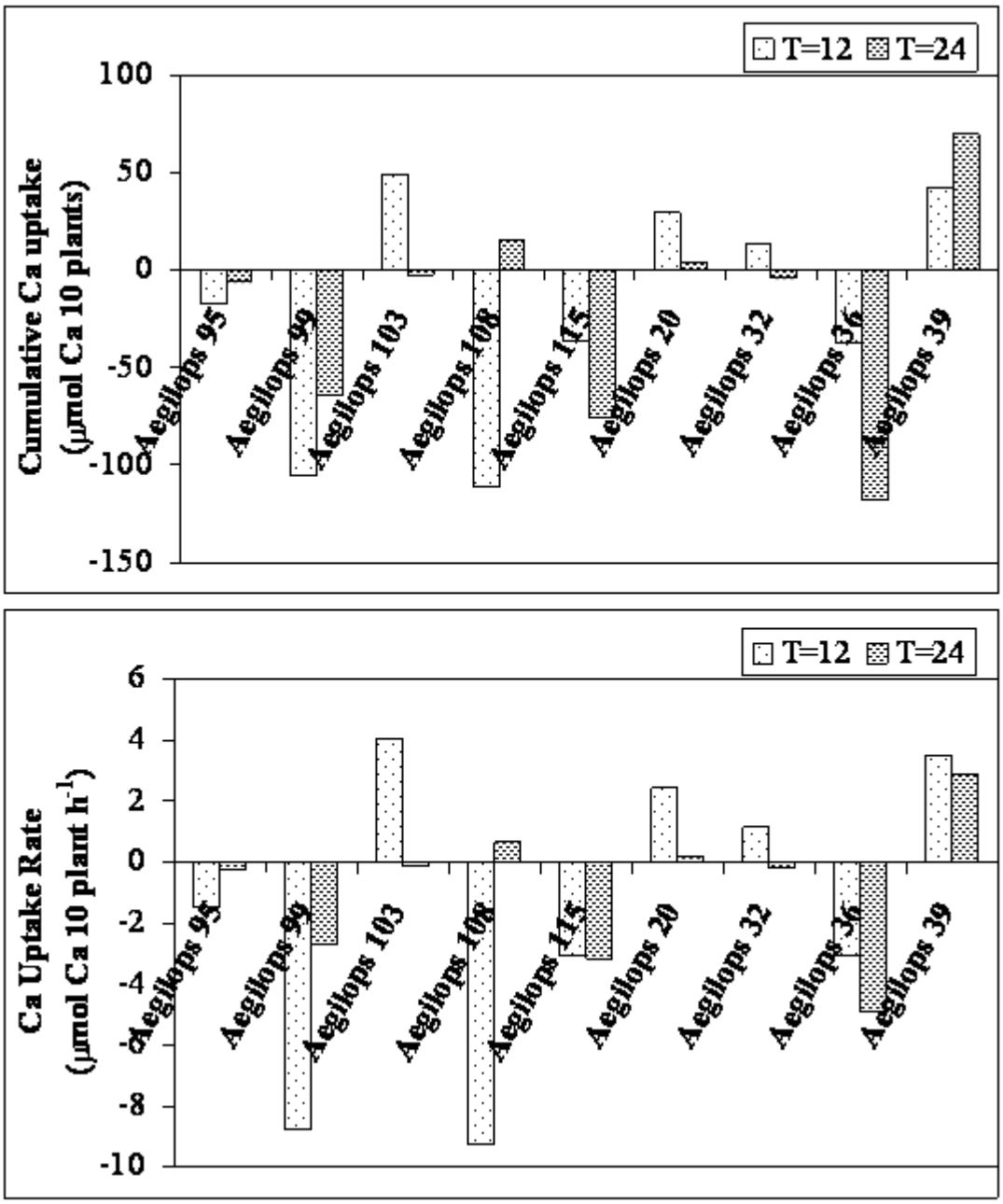

Figure 4.2.10 Influence of exposure time on concentration of Ca in nutrient solutions of 15days-old salt tolerant (Aegilops 95, 99, 103, 108 and 115) and salt sensitive (Aegilops 20, 32, 36 and 39) wheat genotypes. Plants were grown for fourteen days at in nutrient solution and treated with 75 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol Ca 10 plants. Uptake rates represented as µmol Ca 10 plants per hour. The data represent means of four independent replications.

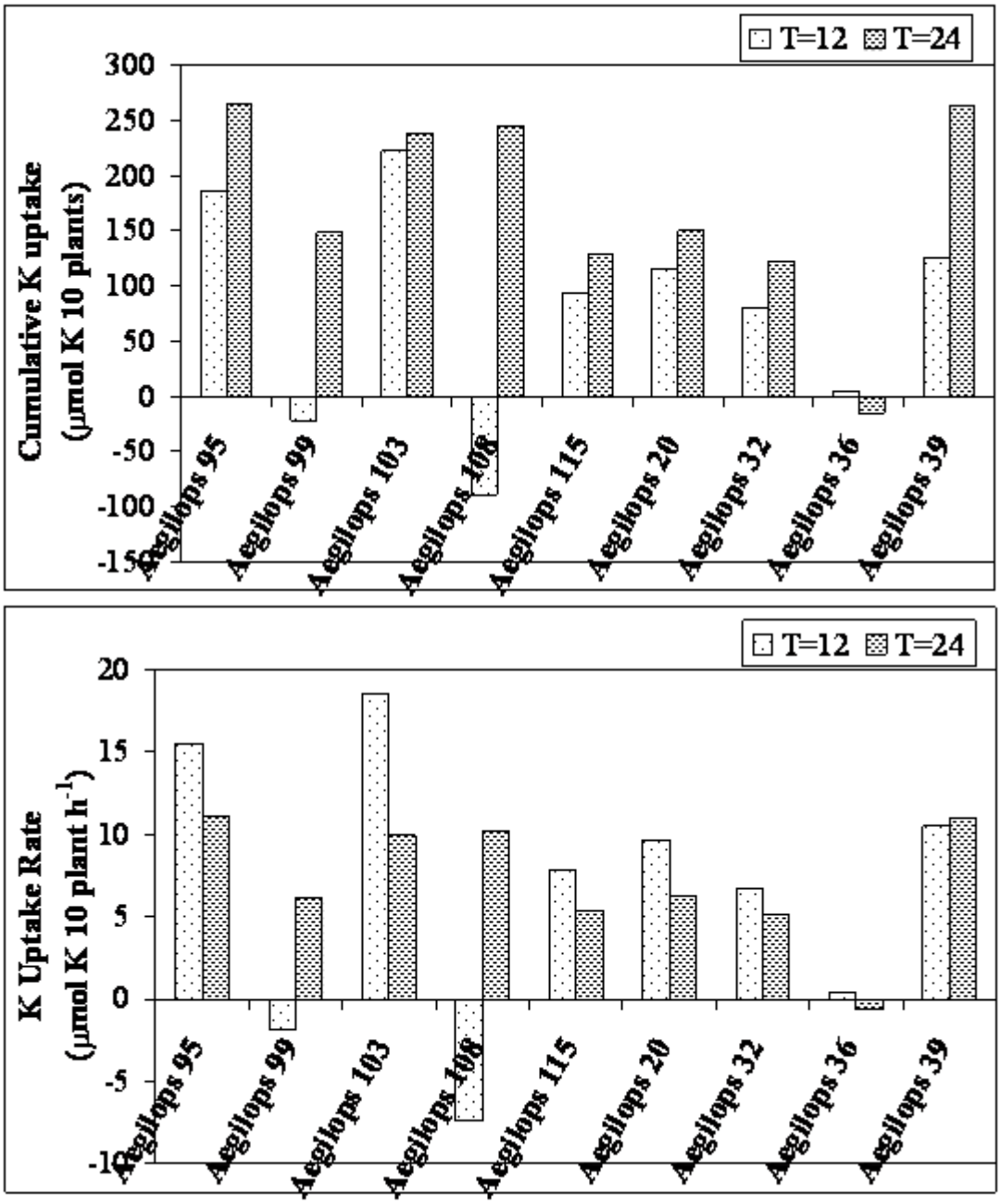

Figure 4.2.11 Influence of exposure time on concentration of K in nutrient solutions of 15days-old salt tolerant (Aegilops 95, 99, 103, 108 and 115) and salt sensitive (Aegilops 20, 32, 36 and 39) wheat genotypes. Plants were grown for fourteen days at in nutrient solution and treated with 75 mM NaCl for 24 hours before harvest. Cumulative uptake rate represented as µmol K 10 plants. Uptake rates represented as µmol K 10 plants per hour. The data represent means of four independent replications.

4.3 Screening for Zinc Deficiency in Salt Stress Tolerant *Aegilops Tauschii* Genotypes

Previously, *Aegilops tauschii* has been shown as an important genetic resource to improve Zn efficiency in wheat (Cakmak *et al*., 1999a, b; Merry *et al*., 1999). The *Aegilops tauschii* genotypes we selected are different from the genotypes used by Cakmak *et al*., (1999a, b) and Merry *et al*., (1999). Based on the results of the first greenhouse screening experiment with *Aegilops tauschii*, 42 genotypes were selected to study the genetic variation for Zn deficiency tolerance, defined as the ratio of shoot dry weight at low Zn supply to the dry weight at high Zn supply. Among these genotypes used for Zn deficiency tolerance studies, 23 selected genotypes were salt tolerant and other 19 genotypes were salt sensitive based on the NaCl tolerance index. Plants were grown for 42 days under Zn deficiency (0 mg Zn kg^{-1} soil) and sufficient Zn supply (2 mg Zn kg^{-1} soil) on a Zn deficient soil from Central Anatolia (Eskisehir location).

There was a substantial genetic variation in tolerance to Zn deficiency among the *Aegilops tauschii* genotypes tested. This variation was about 5-fold among the genotypes (Table 4.3.1). Under adequate Zn supply, there was no significant difference in shoot growth between Zn efficient (tolerant) and inefficient (sensitive) genotypes (Table 4.3.1). However, Zn efficient genotypes had higher shoot dry weight compared to Zn-inefficient genotypes under Zn deficiency.

Dry matter production was decreased under Zn deficiency, and the magnitude of these decreases in the shoot dry matter by Zn deficiency varied from 11% (Aegilops 90) to 82% (Aegilops 20), with an average value of 34% (Table 4.3.1). In addition to dry matter reduction, Zn deficiency resulted in development leaf chlorosis and necrosis particularly on older leaves of the Zn deficiency sensitive genotypes. The selected genotypes showed marked differences in the severity of visual symptoms. Higher tolerance to Zn deficiency significantly positively correlated with the less severity of leaf symptoms caused by Zn deficiency (R^2 = 0.5571***) (Figure 4.3.1). Zinc inefficient genotypes showed severe deficiency symptoms, while Zn deficiency symptoms developed slightly in Zn efficient genotypes. According to these results, the 5 most Zn-efficient genotypes (Aegilops 90, 72, 125, 62 and 99) and the 5 most Zn-inefficient genotypes (Aegilops 40, 36, 18, 32 and 20) were selected to repeat this

experiment. Zinc efficiency ratio also highly correlated with the shoot dry weight of these genotypes under Zn deficiency ($R^2 = 0.6246$***) (Figure 4.3.1).

Table 4.3.1 The effect of Zn supply (+Zn = 2 mg kg^{-1} soil) on leaf symptoms of Zn deficiency, shoot dry weight, and Zn efficiency ratio of the 23 most NaCl tolerant and the 19 most NaCl sensitive genotypes selected among 116 *Aegilops tauschii* genotypes grown with (1500 mg NaCl kg^{-1} soil) and without NaCl supply under greenhouse conditions. Selection of the 42 genotypes was based on NaCl tolerance index. Data represent means of 3 independent replications.

Genotypes	Leaf Symptoms**	Dry matter production		Zn efficiency ratio***
		-Zn	+Zn	
		(mg $plant^{-1}$)		(%)
Aegilops 90*	3.5	603 ± 48	676 ± 30	89
Aegilops 72*	4	662 ± 85	743 ± 38	89
Aegilops 125*	3.5	532 ± 9	601 ± 18	89
Aegilops 62*	4.5	619 ± 84	716 ± 24	87
Aegilops 99*	4	773 ± 61	933 ± 58	83
Aegilops 42*	3	571 ± 96	706 ± 64	81
Aegilops 45	2	704 ± 94	878 ± 58	80
Aegilops 126	3.5	856 ± 102	1077 ± 161	80
Aegilops 138	4	610 ± 66	793 ± 110	77
Aegilops 112*	2.5	443 ± 12	579 ± 23	77
Aegilops 134	3.5	640 ± 42	839 ± 15	76
Aegilops 56	3	557 ± 71	732 ± 48	76
Aegilops 69	2.5	598 ± 90	812 ± 43	74
Aegilops 127	3	450 ± 39	614 ± 29	73
Aegilops 147*	3	498 ± 24	680 ± 66	73
Aegilops 95*	3.5	592 ± 37	813 ± 83	73
Aegilops 129*	3	683 ± 37	947 ± 55	72
Aegilops 93	4	697 ± 48	969 ± 165	72
Aegilops 141*	3	522 ± 13	728 ± 99	72
Aegilops 44	2	589 ± 52	824 ± 63	71
Aegilops 116	3	646 ± 60	914 ± 26	71
Aegilops 103*	2	595 ± 4	854 ± 96	70
Aegilops 148*	3	613 ± 53	894 ± 155	69
Aegilops 73*	3.5	502 ± 19	737 ± 118	68
Aegilops 133*	3	571 ± 62	842 ± 129	68
Aegilops 142*	2	509 ± 20	753 ± 20	68
Aegilops 104*	2	597 ± 29	913 ± 145	65
Aegilops 84*	2.5	670 ± 42	1033 ± 121	65
Aegilops 106*	3.5	677 ± 21	1060 ± 111	64
Aegilops 60	4.5	783 ± 41	1271 ± 118	62
Aegilops 118*	1.5	481 ± 24	800 ± 148	60
Aegilops 115*	2.5	575 ± 56	980 ± 185	59
Aegilops 5	1	406 ± 39	710 ± 137	57
Aegilops 21	1	543 ± 9	970 ± 113	56
Aegilops 83	1	404 ± 4	768 ± 102	53
Aegilops 23	2	395 ± 115	793 ± 146	50
Aegilops 30*	1	393 ± 69	848 ± 148	46
Aegilops 40	1	409 ± 50	892 ± 129	46
Aegilops 36	1	331 ± 29	727 ± 6	46
Aegilops 18*	1	159 ± 3	605 ± 38	26
Aegilops 32	1	156 ± 31	844 ± 140	18
Aegilops 20	1	147 ± 10	833 ± 176	18
Mean	3	542	826	66

*Salt tolerant genotypes based on the results obtained in experiment 1.

**Leaf symptoms of salt stress: 1 (very severe) to 5 (very slight or no symptoms).

***Zn efficiency = (dry weight at –Zn/dry weight at +Zn) X 100.

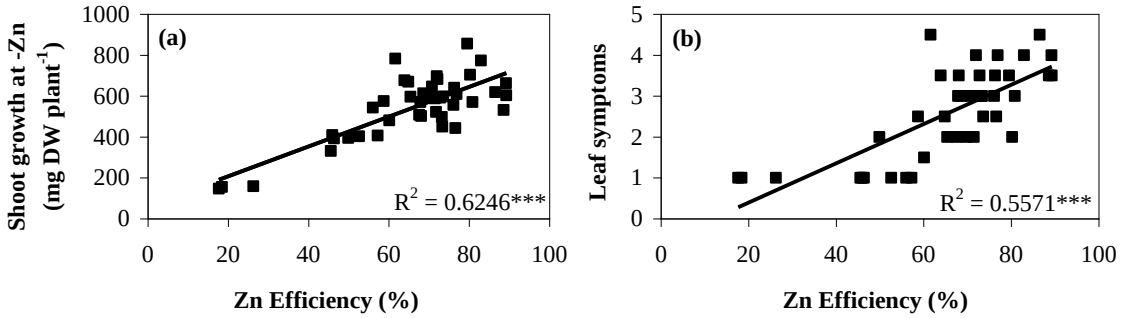

Figure 4.3.1 Relationships between Zn efficiency, shoot dry weight under Zn-deficient condition (a) and Zn deficiency symptoms on leaves (b). *** is statistically significant at $P < 0.001$ level. R^2 = linear regression coefficient squared.

4.3.1 Leaf Symptoms and Dry Matter Production in Selected Genotypes under Varied Zn Supply

From the greenhouse experiment described in Table 4.3.1, 10 *Aegilops tauschii* genotypes have been selected to study further the relationship between Zn deficiency tolerance and salt tolerance. Zinc deficiency symptoms of the selected 10 genotypes, such as chlorosis and necrosis on the older leaves, were observed in plants grown without Zn supply. The visual symptoms in the second Zn-experiment were, however, not severe as much as in the first Zn-experiment. This reason could depend on differences in the length of growth time between these experiments. Plants in the first experiment were grown for 42 days, while plants in the second experiment were harvested at 25-day old due to more severe stress with NaCl treatment. Nevertheless, these symptoms highly correlated with the results obtained in the first results (R^2 = 0.7692***) (Figure 4.3.2). The severity of leaf symptoms also correlated with the Zn efficiency ratio in the second experiment (R^2 = 0.7211**) (Figure 4.3.2).

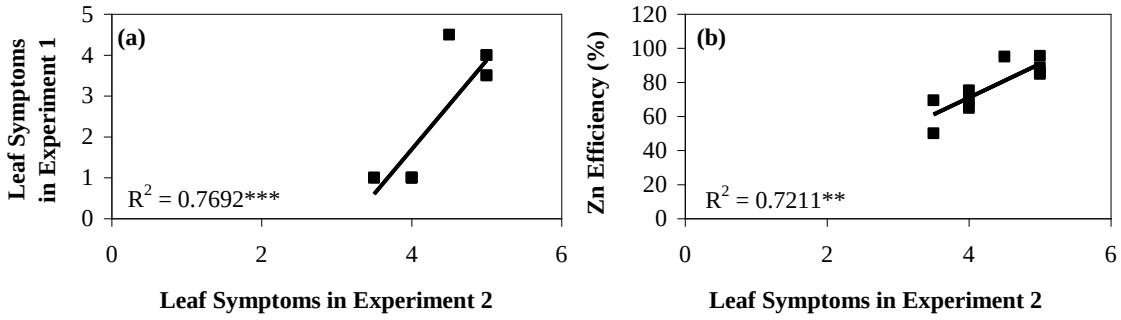

Figure 4.3.2 Relationships between leaf symptoms in experiment 2, leaf symptoms in experiment 1 (a) and Zn efficiency in experiment 2 (b). ** and *** are statistically significant at $P < 0.01$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared.

Dry matter production was more or less similar in all genotypes under sufficient Zn supply, but dry matter production, particularly in the sensitive genotypes reduced with an average value of 22% under Zn deficiency (Table 4.3.2). The relationship between Zn efficiency ratio and absolute shoot growth was very significant under Zn deficiency (R^2 = 0.6331**) (Figure 4.3.3). Zinc efficient genotypes produced higher yield under Zn deficiency, when compared to Zn-inefficient genotypes. Shoot dry weight of the Zn-efficient genotypes was, on average, 1.7-fold higher compared to the Zn-inefficient genotypes.

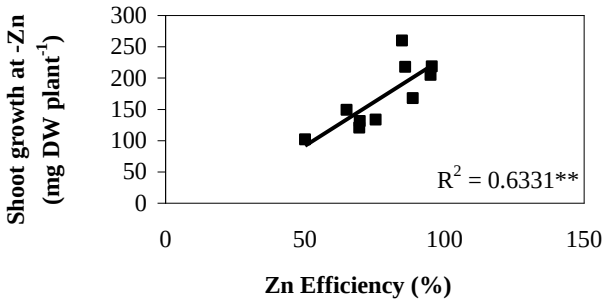

Figure 4.3.3 Relationships between Zn efficiency, shoot dry weights under Zn-deficient conditions. *** is statistically significant at $P < 0.001$ level. R^2 = linear regression coefficient squared.

Table 4.3.2 The effect of Zn supply (+Zn = 2 mg kg^{-1} soil) on leaf symptoms of Zn deficiency, shoot dry weight, and Zn efficiency ratio of the 23 most NaCl tolerant and the 19 most NaCl sensitive genotypes selected among 116 *Aegilops tauschii* genotypes grown with (1500 mg NaCl kg^{-1} soil) and without NaCl supply under greenhouse conditions. Selection of the 42 genotypes was based on NaCl tolerance index.

Genotypes	Leaf Symptoms**	Dry matter production		Zn efficiency ratio***
		-Zn	+Zn	
		(mg $plant^{-1}$)		(%)
Aegilops 125*	5	218 ± 13	223 ± 19	95
Aegilops 62*	4.5	205 ± 10	226 ± 27	95
Aegilops 90*	5	168 ± 18	190 ± 14	89
Aegilops 72*	5	218 ± 31	254 ± 18	86
Aegilops 99*	5	260 ± 30	306 ± 32	85
Aegilops 18	4	133 ± 60	174 ± 17	75
Aegilops 36	4	131 ± 12	188 ± 8	70
Aegilops 32	3.5	121 ± 24	174 ± 3	69
Aegilops 40	4	149 ± 52	226 ± 55	65
Aegilops 20	3.5	102 ± 24	208 ± 23	50
Mean	4	170	217	78

*Zn-efficient genotypes based on the Zn efficiency trait obtained in experiment 1.
**Leaf symptoms of salt stress: 1 (very severe) to 5 (very slight or no symptoms).
***Zn efficiency = (dry weight at –Zn/dry weight at +Zn) X 100.

4.3.2 Seed Size, Seed-Zn Concentration and Zn Content

Seeds of these selected genotypes were analyzed to investigate the relationship between seed Zn concentration and tolerance to Zn deficiency. The seed weights of the genotypes tested differed greatly. Generally, Zn efficient genotypes were bigger in seed size than the Zn-inefficient genotypes, however the variation in seed mass was only 1.7-fold within Zn efficient and Zn-inefficient genotypes. The highest and lowest Zn efficiency ratios were found in Aegilops 125 and Aegilops 20, respectively, while the seed weights of these genotypes were very similar (110 in Aegilops 125 and 100 μg in Aegilops 20). There was a weak but not significant correlation between seed size and Zn efficiency ratio (Figure 4.3.4). Furthermore, there was no significant correlation between Zn efficiency ratio and seed Zn concentration in these genotypes (Figure 4.3.4) indicating that higher Zn efficiency is not directly related to higher seed Zn concentration. . In contrast to Zn concentration and seed weight, there was moderately significant correlation between Zn content (total amount of Zn per seed) and the Zn efficiency trait (R^2 = 0.4572*) (Figure 4.3.4). On average, Zn-efficient genotypes had 470 ng Zn $seed^{-1}$ whereas 299 ng Zn $seed^{-1}$ was found in the Zn-inefficient genotypes.

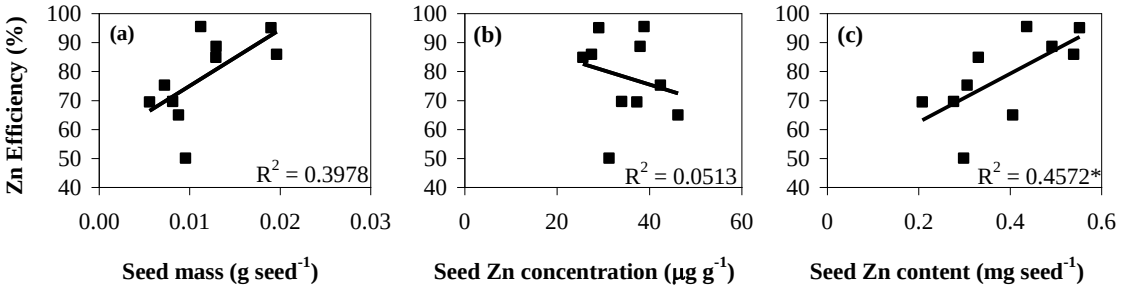

Figure 4.3.4 Correlation between Zn efficiency and seed mass (a); seed Zn concentration (b); and seed Zn content (c). * is statistically significant at $P < 0.05$ level. R^2 = linear regression coefficient squared.

4.3.3 Zn Concentration and Content in Shoot

There was no distinct variation in Zn concentration between Zn efficient and Zn inefficient genotypes under both Zn deficiency and adequate Zn supply. Under -Zn conditions, the variation in Zn concentration ranged between 8.11 (Aegilops 20) and 10.06 mg kg^{-1} DW (Aegilops 90), with an average value of 9.03 mg kg^{-1} DW (Table 4.3.3). In the case of sufficient Zn supply, the shoot concentration of Zn varied from

28.71 (Aegilops 36) to 34.45 mg kg^{-1} DW (Aegilops 99), with a mean value of 31.45 mg kg^{-1} DW (Table 4.3.3). Despite the existence of the extreme Zn concentrations found in Zn efficient (Aegilops 90 and 99) and Zn-inefficient (Aegilops 20 and 36) genotypes, there was moderately significant relation between Zn efficiency and Zn concentration (R^2 = 0.4805*) (Figure 4.3.5). However, shoot Zn content showed more significant correlation with Zn efficiency ratio (R^2 = 0.7012**) (Figure 4.3.5). When compared to the Zn-inefficient genotypes, total amount of Zn per shoot was higher in Zn-efficient genotypes. Zinc-inefficient genotype Aegilops 20 had the lowest Zn content, while Zn efficient Aegilops 99 had the highest content in Zn deficient soils (Table 4.3.3).

Table 4.3.3 Shoot Zn concentration and content of 25-day old 10 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without (0 mg Zn kg^{-1} soil) Zn supplied under greenhouse conditions.

	Zn Concentration in Shoot (mg kg^{-1} DW)		Zn Content in Shoot (µg $plant^{-1}$)	
Genotypes	-Zn	+Zn	-Zn	+Zn
Aegilops 125*	9.74 ± 0.64	32.26 ± 2.77	2.12 ± 0.08	7.55 ± 0.66
Aegilops 62*	9.40 ± 1.33	29.56 ± 1.85	2.05 ± 0.14	7.28 ± 0.68
Aegilops 90*	10.06 ± 0.35	31.36 ± 1.12	1.56 ± 0.09	5.97 ± 0.63
Aegilops 72*	9.05 ± 1.10	31.95 ± 1.28	1.72 ± 0.10	8.12 ± 0.66
Aegilops 99*	9.10 ± 0.54	34.45 ± 4.35	2.37 ± 0.40	9.34 ± 0.44
Aegilops 18	8.27 ± 0.49	30.35 ± 5.47	0.81 ± 0.11	5.24 ± 0.81
Aegilops 36	9.60 ± 1.95	28.71 ± 1.14	1.13 ± 0.09	5.39 ± 0.38
Aegilops 32	8.12 ± 0.32	31.06 ± 0.54	0.86 ± 0.11	5.41 ± 0.12
Aegilops 40	8.88 ± 0.69	34.18 ± 2.16	1.06 ± 0.28	8.67 ± 1.10
Aegilops 20	8.11 ± 0.12	30.61 ± 2.17	0.71 ± 0.07	5.74 ± 0.06
Mean	9.03	31.45	1.44	6.87

*Zn-efficient genotypes based on the Zn efficiency trait obtained in experiment 1.

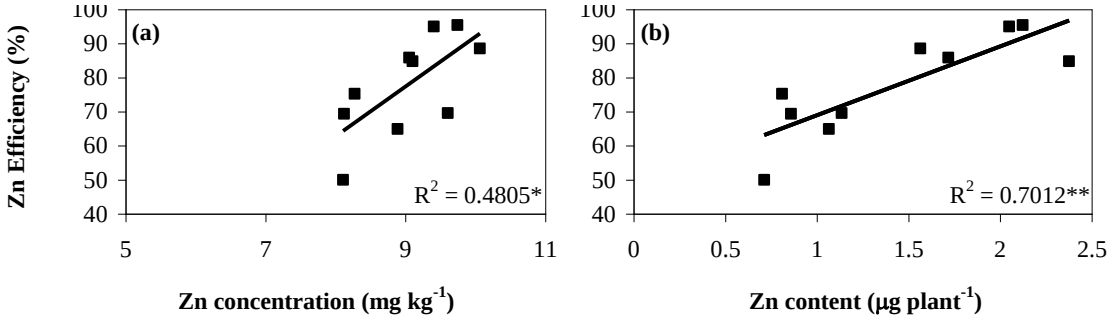

Figure 4.3.5 Relationships between Zn efficiency, Zn concentration (a) and Zn content (b) in shoot of 5 Zn-efficient and 5 Zn-inefficient *Aegilops tauschii* genotypes. * and ** are statistically significant at $P < 0.05$ and $P < 0.01$ levels, respectively. R^2 = linear regression coefficient squared.

4.4 Effect of Salinity Stress on Growth of Wheat under Zn Deficiency

The relationship between Zn deficiency and salinity stress was studied in the selected 15 *Aegilops tauschii* genotypes. Among the selected genotypes, there were 5 Zn-efficient genotypes (Aegilops 90, 72, 125, 62 and 99), 5 Zn-inefficient genotypes (Aegilops 40, 36, 18, 32 and 20), 5 salt-tolerant genotypes (Aegilops 95, 99, 103, 108 and 115) and 4 salt-susceptible genotypes (Aegilops 20, 32, 36 and 39). All these genotypes were grown for 25 days at 2 levels of Zn (0 and 2 mg Zn kg^{-1} soil) and 2 levels of salinity (0 and 2500 mg NaCl kg^{-1} soil). The dry matter production results were shown in Table 4.4.1.

Table 4.4.1 The effects of Zn deficiency, 2500 mg NaCl kg^{-1} soil treatment with (2 mg Zn kg^{-1} soil) and without Zn supply on leaf symptoms, shoot dry weight, and the tolerance traits of the 15 *Aegilops tauschii* genotypes grown for 25 days under greenhouse conditions. [1], Zn deficient salty (2500 mg NaCl kg^{-1} soil) condition; [2], Zn deficient (without salt supply) condition; [3], saline (2500 mg NaCl kg^{-1} soil) condition with sufficient Zn (2 mg kg^{-1} soil) supply. The results are given in order of [1, 2, 3]. Data represent means of 3 independent replications.

	Leaf Symptoms[1,2,3]			Dry matter production				Tolerance Index[1,2,3]		
				-NaCl		+NaCl				
				-Zn	+Zn	-Zn	+Zn			
				(mg $plant^{-1}$)				(%)		
Aegilops 108	4.5	4.5	4.5	225 ± 12	252 ± 20	156 ± 1	171 ± 8	89	62	68
Aegilops 72	5	4.5	4.5	235 ± 17	254 ± 18	153 ± 0	133 ± 18	92	60	53
Aegilops 90	5	4	4.5	168 ± 18	190 ± 14	113 ± 8	141 ± 26	88	59	74
Aegilops 115	5	4	4	217 ± 8	252 ± 33	148 ± 12	174 ± 2	86	59	69
Aegilops 125	5	4.5	4.5	218 ± 13	223 ± 19	129 ± 18	151 ± 20	98	58	68
Aegilops 62	4.5	4.5	4.5	205 ± 10	242 ± 4	137 ± 14	146 ± 12	85	57	60
Aegilops 99	5	4.5	4.5	260 ± 30	306 ± 32	170 ± 13	161 ± 19	85	55	53
Aegilops 103	5	4.5	4.5	218 ± 8	301 ± 3	164 ± 6	171 ± 9	73	55	57
Aegilops 95	5	4.5	4.5	225 ± 13	279 ± 16	144 ± 7	152 ± 14	81	52	54
Aegilops 32	3.5	3.5	4.5	108 ± 13	174 ± 3	80 ± 16	103 ± 21	62	46	59
Aegilops 36	4	3.5	4	131 ± 12	188 ± 8	84 ± 13	79 ± 5	70	45	42
Aegilops 18	4	4	4.5	99 ± 6	174 ± 17	68 ± 8	102 ± 13	57	39	59
Aegilops 40	4	4	4.5	177 ± 30	257 ± 11	99 ± 10	92 ± 3	69	39	36
Aegilops 39	3.5	3.5	4.5	89 ± 8	166 ± 11	59 ± 10	110 ± 2	54	35	66
Aegilops 20	3.5	3	4	88 ± 7	208 ± 23	67 ± 3	85 ± 12	42	32	41
Mean	4	4	4	178	231	118	132	75	50	57

Zinc deficiency and/or salinity stress caused leaf symptoms and reduction in plant growth. As expected, these effects were observed more severely when plants were exposed to 2500 mg NaCl kg^{-1} supply together with Zn deficiency. On average, dry matter production decreased only 25% under Zn deficiency and %43 under salinity stress whereas 50% under combination of salt stress with Zn deficiency. Based on these results, salt stress reduced plant growth much more severely than Zn deficiency.

However, the leaf symptoms under salt stress together with Zn deficiency highly correlated with Zn deficiency symptoms (R^2 = 0.6819***), when compared to the symptoms under only salinity condition (R^2 = 0.0491). It was important to notice that the ability of the genotypes to grow under salt stress on Zn deficient soil correlated much more significantly with Zn efficiency ratio (R^2 = 0.8717***) rather than the salt tolerance trait (R^2 = 0.3115*) (Figure 4.4.1). There was a strong relationship between absolute shoot growth under Zn deficiency and Zn efficiency ratio (R^2 = 0.743***) (Figure 4.4.2). In the case of Zn deficient salty condition, the tolerance ratio was highly related to the absolute shoot production (R^2 = 0.7314***) (Figure 4.4.2). On the other hand, the relationship between the absolute shoot growth and salinity tolerance index was moderately significant (R^2 = 0.3802*) (Figure 4.4.2). These results indicated that plants exposed to both salinity stress and Zn deficiency affected more from Zn deficiency rather than salinity stress.

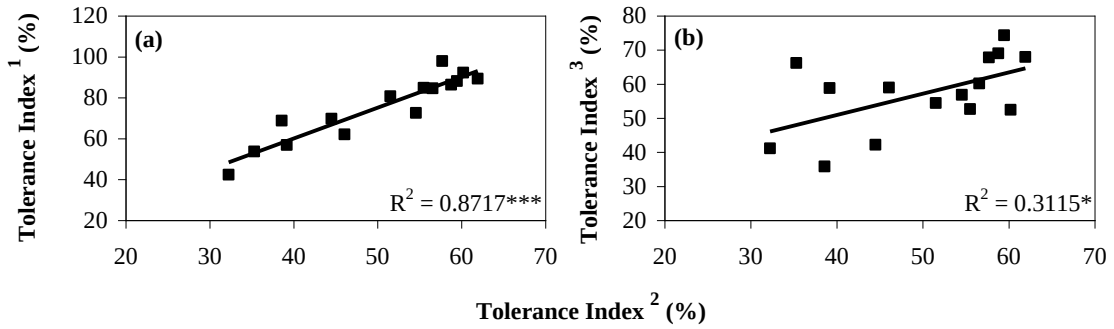

Figure 4.4.1 Relationships between tolerance index in Zn deficient salty (2500 mg NaCl kg^{-1} soil) condition and tolerance index in Zn deficient (without salt supply) condition (a); and tolerance index in saline (2500 mg NaCl kg^{-1} soil) condition with sufficient Zn (2 mg kg^{-1} soil) supply (b). [1], Zn deficient salty (2500 mg NaCl kg^{-1} soil) condition; [2], Zn deficient (without salt supply) condition; [3], saline (2500 mg NaCl kg^{-1} soil) condition with sufficient Zn (2 mg kg^{-1} soil) supply. * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared.

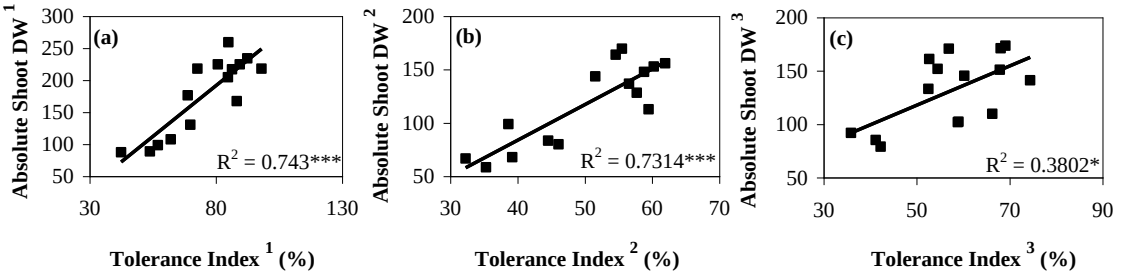

Figure 4.4.2 Relationships between absolute shoot dry weights and tolerance traits within tolerance index in Zn deficient (without salt supply) condition (a), saline (2500 mg NaCl kg^{-1} soil) condition with sufficient Zn (2 mg kg^{-1} soil) supply (b), and Zn deficient salty (2500 mg NaCl kg^{-1} soil) condition (c). [1], Zn deficient salty (2500 mg NaCl kg^{-1} soil) condition; [2], Zn deficient (without salt supply) condition; [3], saline (2500 mg NaCl kg^{-1} soil) condition with sufficient Zn (2 mg kg^{-1} soil) supply. * and *** are statistically significant at $P < 0.05$ and $P < 0.001$ levels, respectively. R^2 = linear regression coefficient squared.

As expected, applying NaCl at 2500 mg kg^{-1} caused a marked increase in Na concentration of plants and this increase was higher under 0 mg Zn kg^{-1} soil supply than 2 mg Zn kg^{-1} soil application (Table 4.4.2). Nevertheless, there were some exceptions such as Aegilops 40, 62, 95, 103 and 108 that had higher shoot Na concentration, when salt applied with adequate Zn supply. On average, Na concentration, under saline conditions, increased from 3.55 mg g^{-1} to 4.66 mg g^{-1} with 2 mg Zn kg^{-1} soil supply. Zinc concentration of shoots was enhanced by NaCl supply in all genotypes under Zn deficiency. However this increase in shoot Zn concentration by NaCl supply under Zn deficiency was not sufficient to compensate Zn deficiency. The shoot concentration of Zn in the plants treated with NaCl under Zn deficiency was as average 10 mg kg^{-1} DW that is close or less than the widely considered critical Zn deficiency concentration in literature (e.g., 12-15 mg kg^{-1} DW) (Table 4.4.3). Shoot K concentration declined with the NaCl supply, while increases in Zn applications did not result in any consistent effect on shoot concentration of K. The mean values of shoot K concentrations were 33.39, 31.66, 25.85 and 26.07 mg g^{-1} under control, Zn deficient, Zn deficient saline and only saline conditions, respectively (Table 4.4.4). The shoot concentrations of Ca, in most of these genotypes, slightly increased under Zn deficiency. In the case of salinity stress, there was a huge raise in Ca uptake, particularly under insufficient Zn supply. Shoot Ca concentrations, on average, were 5.59, 5.90, 10.34 and 9.15 mg g^{-1} under control, Zn deficient, Zn deficient saline and only saline conditions, respectively (Table 4.4.5). Due to the inhibiting effect of Zn on Na uptake, K/Na and Ca/Na ratios increased along with increased Zn supply, except Aegilops 40, 62, 95, 103 and 108 that had higher Na absorption capacities under Zn sufficient saline conditions (Table 4.4.6). Under Zn deficiency, the P uptake increased in all genotypes. There was no marked increase in P concentrations of plants with the NaCl supply. The mean values of shoot P concentrations were 3.38, 3.98, 3.75 and 3.28 mg g^{-1} under control, Zn deficient, Zn deficient saline and only saline conditions, respectively (Table 4.4.7).

Table 4.4.2 Shoot Na concentration of 25-day old 15 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without Zn supply under saline (2500 mg NaCl kg^{-1} soil) and nonsaline treatments. Data represent means of 3 independent replications.

	Na Concentrations in Shoot			
	-NaCl		+NaCl	
	-Zn	+Zn	-Zn	+Zn
	(mg g^{-1} DW)			
Aegilops 108	0.116 ± 0.004	0.098 ± 0.004	1.99 ± 0.05	2.27 ± 0.16
Aegilops 72	0.088 ± 0.009	0.071 ± 0.013	2.67 ± 0.02	1.98 ± 0.05
Aegilops 90	0.109 ± 0.018	0.099 ± 0.002	3.40 ± 0.39	2.49 ± 0.19
Aegilops 115	0.083 ± 0.008	0.107 ± 0.021	2.16 ± 0.22	1.75 ± 0.13
Aegilops 125	0.117 ± 0.022	0.086 ± 0.004	4.12 ± 0.40	3.09 ± 0.14
Aegilops 62	0.115 ± 0.010	0.071 ± 0.007	2.79 ± 0.10	3.35 ± 0.15
Aegilops 99	0.099 ± 0.010	0.089 ± 0.031	2.10 ± 0.26	1.90 ± 0.26
Aegilops 103	0.098 ± 0.008	0.087 ± 0.008	1.95 ± 0.43	3.24 ± 0.45
Aegilops 95	0.093 ± 0.016	0.065 ± 0.002	1.89 ± 0.35	2.31 ± 0.25
Aegilops 32	0.138 ± 0.012	0.091 ± 0.014	5.95 ± 0.66	4.73 ± 0.64
Aegilops 36	0.089 ± 0.010	0.111 ± 0.006	6.77 ± 0.61	4.55 ± 0.21
Aegilops 18	0.124 ± 0.010	0.080 ± 0.000	8.53 ± 1.98	5.44 ± 0.32
Aegilops 40	0.087 ± 0.026	0.110 ± 0.017	4.02 ± 0.16	4.67 ± 0.45
Aegilops 39	0.151 ± 0.009	0.090 ± 0.003	10.99 ± 0.81	5.23 ± 0.67
Aegilops 20	0.132 ± 0.005	0.091 ± 0.010	10.57 ± 4.08	6.22 ± 0.25
Mean	0.109	0.090	4.66	3.55

Table 4.4.3 Shoot Zn concentration of 25-day old 15 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without Zn supply under saline (2500 mg NaCl kg^{-1} soil) and nonsaline treatments. Data represent means of 3 independent replications.

	Zn Concentrations in Shoot			
	-NaCl		+NaCl	
	-Zn	+Zn	-Zn	+Zn
	(mg kg^{-1} DW)			
Aegilops 108	8.06 ± 0.15	31.18 ± 2.07	9.52 ± 0.32	28.90 ± 1.97
Aegilops 72	8.47 ± 0.66	31.95 ± 1.28	10.78 ± 0.58	29.04 ± 2.00
Aegilops 90	10.06 ± 0.35	31.36 ± 1.12	11.44 ± 1.20	32.07 ± 1.63
Aegilops 115	7.55 ± 0.15	30.88 ± 3.74	10.76 ± 0.84	32.42 ± 1.28
Aegilops 125	9.74 ± 0.64	32.26 ± 2.77	11.54 ± 0.09	29.48 ± 1.79
Aegilops 62	8.70 ± 0.75	29.56 ± 1.85	10.57 ± 0.69	26.39 ± 0.99
Aegilops 99	9.10 ± 0.54	32.04 ± 1.82	10.22 ± 0.21	30.02 ± 0.82
Aegilops 103	7.49 ± 0.06	30.85 ± 1.61	9.47 ± 0.47	31.00 ± 2.80
Aegilops 95	7.54 ± 0.25	34.03 ± 0.81	10.76 ± 0.95	31.73 ± 2.99
Aegilops 32	8.12 ± 0.32	31.06 ± 0.54	10.68 ± 0.37	29.43 ± 1.24
Aegilops 36	8.48 ± 0.26	28.71 ± 1.14	10.31 ± 0.31	28.13 ± 2.84
Aegilops 18	8.27 ± 0.49	27.53 ± 3.46	10.13 ± 0.07	31.68 ± 2.09
Aegilops 40	8.88 ± 0.69	34.18 ± 2.16	9.88 ± 0.14	31.13 ± 2.62
Aegilops 39	9.09 ± 0.70	25.26 ± 2.29	10.21 ± 0.54	31.02 ± 1.07
Aegilops 20	8.11 ± 0.12	30.61 ± 2.17	9.94 ± 0.50	26.14 ± 1.15
Mean	8.51	30.76	10.41	29.91

Table 4.4.4 Shoot K concentration of 25-day old 15 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without Zn supply under saline (2500 mg NaCl kg^{-1} soil) and nonsaline treatments. Data represent means of 3 independent replications.

	K Concentrations in Shoot			
	-NaCl		+NaCl	
	-Zn	+Zn	-Zn	+Zn
	(mg g^{-1} DW)			
Aegilops 108	31.90 ± 0.52	34.03 ± 0.74	25.89 ± 2.64	26.74 ± 0.98
Aegilops 72	31.88 ± 0.47	32.47 ± 0.46	26.13 ± 0.51	26.52 ± 0.64
Aegilops 90	31.82 ± 1.93	32.18 ± 0.56	24.93 ± 1.25	26.08 ± 1.05
Aegilops 115	31.38 ± 0.45	33.05 ± 0.29	25.18 ± 0.89	24.67 ± 1.08
Aegilops 125	30.52 ± 1.51	31.54 ± 0.31	24.42 ± 0.73	24.55 ± 0.83
Aegilops 62	32.50 ± 2.68	34.58 ± 1.64	27.24 ± 0.63	27.29 ± 0.35
Aegilops 99	34.11 ± 0.30	34.83 ± 0.30	27.91 ± 0.98	27.18 ± 0.82
Aegilops 103	32.67 ± 0.39	35.09 ± 0.12	26.27 ± 1.10	26.67 ± 0.73
Aegilops 95	32.39 ± 0.61	34.15 ± 0.25	26.34 ± 0.45	26.25 ± 0.09
Aegilops 32	31.01 ± 0.84	34.03 ± 0.19	25.71 ± 0.33	25.89 ± 1.35
Aegilops 36	31.85 ± 0.59	33.26 ± 1.12	26.90 ± 0.72	25.92 ± 1.68
Aegilops 18	30.05 ± 2.98	31.31 ± 3.76	25.05 ± 0.75	25.52 ± 1.89
Aegilops 40	32.37 ± 1.05	33.98 ± 0.23	28.08 ± 1.29	27.99 ± 0.26
Aegilops 39	30.63 ± 0.67	32.86 ± 1.97	23.35 ± 2.09	24.86 ± 1.13
Aegilops 20	29.85 ± 0.98	33.54 ± 1.02	24.28 ± 0.27	24.92 ± 0.72
Mean	31.66	33.39	25.85	26.07

Table 4.4.5 Shoot Ca concentration of 25-day old 15 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without Zn supply under saline (2500 mg NaCl kg^{-1} soil) and nonsaline treatments. Data represent means of 3 independent replications.

	Ca Concentrations in Shoot			
	-NaCl		+NaCl	
	-Zn	+Zn	-Zn	+Zn
	(mg g^{-1} DW)			
Aegilops 108	5.27 ± 0.30	5.46 ± 0.16	9.55 ± 1.77	8.09 ± 0.37
Aegilops 72	6.39 ± 0.04	5.76 ± 0.31	10.82 ± 0.62	9.18 ± 0.98
Aegilops 90	5.21 ± 0.22	4.96 ± 0.13	9.18 ± 0.46	8.16 ± 1.08
Aegilops 115	6.29 ± 0.14	5.90 ± 0.09	10.92 ± 0.32	10.26 ± 0.42
Aegilops 125	7.69 ± 0.23	6.81 ± 0.17	12.24 ± 0.77	11.49 ± 0.94
Aegilops 62	6.34 ± 0.84	5.35 ± 0.48	9.52 ± 0.16	8.37 ± 0.11
Aegilops 99	5.91 ± 0.05	5.28 ± 0.09	9.44 ± 0.40	9.11 ± 0.25
Aegilops 103	5.61 ± 0.23	5.22 ± 0.36	9.31 ± 0.48	8.98 ± 1.08
Aegilops 95	5.63 ± 0.26	4.58 ± 0.25	8.91 ± 0.15	8.86 ± 0.44
Aegilops 32	5.50 ± 0.08	5.83 ± 0.12	10.78 ± 1.10	9.14 ± 1.75
Aegilops 36	5.55 ± 0.03	5.50 ± 0.16	11.06 ± 2.23	9.17 ± 0.73
Aegilops 18	6.07 ± 0.22	6.16 ± 0.37	10.78 ± 0.89	8.87 ± 0.79
Aegilops 40	5.77 ± 0.44	5.55 ± 0.14	10.25 ± 0.24	9.57 ± 0.29
Aegilops 39	5.38 ± 0.07	5.42 ± 0.23	11.22 ± 1.36	8.38 ± 0.86
Aegilops 20	5.91 ± 0.29	6.14 ± 0.25	11.07 ± 1.13	9.69 ± 0.94
Mean	5.90	5.59	10.34	9.15

Table 4.4.6 Shoot K/Na and Ca/Na ratios with (2 mg kg^{-1}) and without Zn supply under under saline (2500 mg NaCl kg^{-1} soil).Data represent means of 3 independent replications.

	K/Na Ratio in Shoot		Ca/Na Ratio in Shoot	
	+NaCl			
	-Zn	+Zn	-Zn	+Zn
Aegilops 108	13	12	4.81	3.57
Aegilops 72	10	13	4.05	4.63
Aegilops 90	7	10	2.70	3.28
Aegilops 115	12	14	5.06	5.88
Aegilops 125	6	8	2.97	3.72
Aegilops 62	10	8	3.41	2.50
Aegilops 99	13	14	4.50	4.79
Aegilops 103	13	8	4.78	2.77
Aegilops 95	14	11	4.71	3.83
Aegilops 32	4	5	1.81	1.93
Aegilops 36	4	6	1.63	2.02
Aegilops 18	3	5	1.26	1.63
Aegilops 40	7	6	2.55	2.05
Aegilops 39	2	5	1.02	1.60
Aegilops 20	2	4	1.05	1.56
Mean	8	9	3	3

Table 4.4.7 Shoot P concentration of 25-day old 15 *Aegilops tauschii* genotypes grown with (2 mg Zn kg^{-1} soil) and without Zn supply under saline (2500 mg NaCl kg^{-1} soil) and nonsaline treatments. Data represent means of 3 independent replications.

	P Concentrations in Shoot			
	-NaCl		+NaCl	
	-Zn	+Zn	-Zn	+Zn
	(mg g^{-1} DW)			
Aegilops 108	3.24 ± 0.17	3.11 ± 0.23	3.38 ± 0.02	2.96 ± 0.16
Aegilops 72	3.67 ± 0.12	3.03 ± 0.24	3.81 ± 0.56	3.10 ± 0.53
Aegilops 90	5.09 ± 0.53	3.95 ± 0.09	4.61 ± 0.86	3.23 ± 0.47
Aegilops 115	4.02 ± 0.68	3.11 ± 0.20	3.79 ± 0.34	3.38 ± 0.40
Aegilops 125	4.09 ± 0.57	3.30 ± 0.43	4.19 ± 0.51	3.64 ± 0.18
Aegilops 62	4.02 ± 0.55	3.18 ± 0.17	3.98 ± 0.66	3.09 ± 0.16
Aegilops 99	3.83 ± 0.31	3.22 ± 0.28	3.79 ± 0.43	3.17 ± 0.33
Aegilops 103	3.30 ± 0.46	2.83 ± 0.09	3.64 ± 0.53	2.72 ± 0.26
Aegilops 95	3.32 ± 0.13	3.08 ± 0.09	3.91 ± 0.16	2.90 ± 0.12
Aegilops 32	4.32 ± 0.58	3.40 ± 0.20	3.69 ± 0.70	3.75 ± 0.38
Aegilops 36	4.17 ± 0.56	3.73 ± 0.27	4.28 ± 0.86	3.45 ± 0.08
Aegilops 18	4.16 ± 0.46	4.02 ± 0.35	3.03 ± 0.73	3.47 ± 0.24
Aegilops 40	4.04 ± 0.26	3.41 ± 0.05	3.82 ± 0.52	3.53 ± 0.36
Aegilops 39	4.44 ± 0.11	3.95 ± 0.47	2.98 ± 0.92	3.70 ± 0.08
Aegilops 20	3.96 ± 0.30	3.42 ± 0.25	3.34 ± 0.52	3.04 ± 0.35
Mean	3.98	3.38	3.75	3.28

5 CONCLUSION

The results of the study demonstrated existence of a large variation in tolerance to NaCl tolerance. The NaCl tolerance level was associated with the differences in shoot Na concentration. Selected salt tolerant genotypes had lower Na concentration compared to salt sensitive genotypes used in this work. In the literature, Gorham *et al*. (1987) showed as first that NaCl tolerance is associated with low Na uptake. The differences in the NaCl tolerance between bread and durum wheat was related to higher Na uptake capacity in durum wheat than in bread wheat. As expected, there were marked differences in Na concentrations among bread and durum wheat genotypes. Shoot Na concentration was approximately 7-fold higher in durum wheat than in bread wheat under saline conditions in greenhouse experiment. In contrast to huge variation among bread and durum wheat genotypes, the decreases in shoot dry matter between them showed no significant variability, and even in durum wheat, this reduction, on average, was lower compared to bread wheat. In addition, there was considerably variation in Na concentration within salt tolerant and salt sensitive wheat cultivars. The salt sensitive genotypes had greater Na concentration compared to salt tolerant genotypes. The significant relationship between Na concentration and NaCl tolerance was demonstrated in *Aegilops tauschii* (Schachtman *et al*., 1991). In this study, significant differences in Na concentration related to the salinity tolerance were also found within *Aegilops tauschii*.

The results obtained in growth chamber experiments showed similarities with the results of greenhouse experiments. The salt tolerant Alpu and Gediz cuyltivars had lower cumulative Na uptake compared to salt-sensitive ES-14 and Kızıltan cultivars. In addition, in well agreement with their higher sensitivity to salt stress the durum wheat cultivars showed greater cumulative Na uptake than bread wheat cultivars. In the case of *Aegilops tauschii*, the cumulative Na uptake was also higher in salt sensitive genotypes, except Aegilops 36. It was also important to notice that the salt tolerant

genotypes exhibited greater Na efflux, when compared to sensitive genotypes, except Aegilops 103 and 36.

The K/Na ratio, that is a significant parameter involved in salinity tolerance, was significantly correlated with shoot Na and K concentrations and these correlations were in negative and positive directions, respectively (Garcia *et al*., 1997). The Ca/Na ratio that is also important parameter for salt stress tolerance was negatively correlated with shoot Na concentration, but this ratio was not significantly correlated with shoot Ca concentration. The shoot Ca concentration increased with NaCl supply in greenhouse experiment, except durum wheat genotypes. Such close relationship was also found in spinach (Wilson *et al*., 2000), red orach (Wilson *et al*., 2000), rice (Zeng *et al*., 2003) and wheat (Khoshgoftarmanesh *et al*., 2006). In contrast to results of greenhouse experiments, the Ca concentration decreased with NaCl supply in growth chamber experiment.

This work showed also a significant genotypic variation in tolerance to Zn deficiency among 42 *Aegilops tauschii* that were selected based on their salinity tolerance trait. Tolerance to Zn deficiency was associated with relative shoot growth that is a most reliable parameter. Absolute shoot dry matter yield under Zn deficiency also was widely used to determine the tolerance to Zn deficiency (Torun *et al*., 2000; Hacisalihoglu *et al*., 2004). Under Zn deficiency, all Zn-efficient genotypes produced higher yield compared to Zn-inefficient genotypes. Zinc efficiency is associated with the severity of leaf symptoms (Cakmak *et al*., 1998). The severity of leaf symptoms in *Aegilops tauschii* genotypes were increasing with low levels of Zn efficiency (relative growth). There was no significant variation in Zn concentration in shoot and seed within Zn-efficient and Zn-inefficient genotypes (Torun *et al*., 2000; Hacisalihoglu *et al*., 2004). The total amount of Zn per shoot was higher in Zn-efficient genotypes than in Zn-inefficient genotypes.

In this study, the interactive effects of salt stress and Zn nutrition on growth of the selected *Aegilops tauschii* genotypes were investigated. Shoot Na concentration generally reduced with Zn supply to the saline conditions. This result can be interpreted at level of Zn effects on structural integrity of cell membranes. Previously, it has been shown that Zn deficiency increases permeability of cell membranes (Welch *et al*., 1982; Cakmak and Marschner 1988a) which may cause an increased passive uptake of Na. Like under saline conditions, genotypic variation in shoot Na concentration was also observed under salt stress together with Zn deficiency. Salt tolerant genotypes had

lower Na concentrations compared to the salt sensitive genotypes. Therefore, shoot Na concentration may also be used as a screening parameter for assessing genotypic variation in tolerance to salinity stress under Zn deficiency. Under Zn deficiency, Zn concentration in shoot slightly increased with NaCl application (Keshavarz *et al*., 2005). However, the increase was not sufficiently high to correct Zn deficiency.

Absolute shoot growth under Zn deficiency is a reliable parameter for Zn deficiency tolerance, and this parameter under salt treatment was highly correlated with tolerance to the stress conditions. The correlation between Zn efficiency and shoot growth in Zn inadequate conditions was also very specific under Zn deficiency. But, there was no significant correlation between NaCl index and absolute shoot growth in saline conditions supplemented with adequate Zn. Genc *et al*. (2005) indicated that the severity of Zn deficiency declined with salinity supply, but the severity of leaf symptoms increased with the addition of NaCl to Zn deficient soil. Therefore, the severity of Zn deficiency symptoms highly correlated with the severity of leaf symptoms under Zn deficiency with salt supply.

In conclusion, the results of the present thesis demonstrate existence of a substantial genetic variation in tolerance salt stress and Zn deficiency stress among the *Aegilops tauschii* genotypes. New *Aegilops tauschii* genotypes were identified having both high Zn deficiency tolerance and high NaCl tolerance. These stress factors are very common in semi-arid conditions where wheat is commonly cultivated such as in Central Anatolia. The selected *Aegilops tauschii* genotypes are highly promising in improving cultivated (modern) wheat for high Zn efficiency and high NaCl tolerance and therefore such *Aegilops tauschii* genotypes should be exploited in breeding programmes in future.

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APPENDIX

Chemicals

All chemicals and standart solutions were supplied by Merck (Germany), SIGMA (USA), Fluka (Switzerland), Applichem (Germany) and Riedel de Häen (Germany).

Equipment

Centrifuge:	Kendro Lab. Prod., Heraeus Multifuge 3 S-R, GERMANY
Distilled water:	Millipore, Elix-S, FRANCE Millipore, MilliQ, Academic, FRANCE
Inductively coupled plasma-optical emission spectroscopy (ICP-OES):	Varian, Vista-Pro ccd, AUSTRALIA
Magnetic stirrer:	IKA®-WERKE, GERMANY VELP Scientifica, Microstirrer, ITALY
Microliter Pipette:	Gilson, Pipetman, FRANCE Eppendorf, GERMANY