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Salinity Tolerance in Egyptian Spring Wheat Genotypes

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1 General introduction

1.1 Salinity through natural processes and human activities

From an agricultural point of view, salinity is the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth (Gorham, 1992). There are mainly two forms of soil salinity: primary and secondary salinity. Primary salinity results from the accumulation of salts in the soil or groundwater through natural processes over long period of time. Two natural processes caused primary salinity. The first is the weathering of parent materials containing soluble salts. The second is the deposition of oceanic salt carried through wind and rain. Secondary salinization results from human activities that change the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops and transpiration. The most common causes of secondary salinization are (i) land clearing and the replacement of perennial vegetation with annual crops, and (ii) irrigation schemes using salt-rich irrigation water or having insufficient drainage water.

1.2 Salinity in agriculture

Salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Estimates vary, but approximately 7% of the world's total land area is affected by salinity (Flowers et al., 1997). Most importantly, the percentage of cultivated land affected by salt is even greater. Furthermore, there is also a dangerous trend of a 10 % per year increase in the saline area throughout the world (Pannamieruma, 1984). In addition, salinity is a problem for agriculture because also only few crop species and genotypes are adapted to saline conditions. Although irrigation covers only about 15% of the cultivated land of the world, irrigated land has at least twice the productivity of rain-fed land, and may therefore produce one-third of the world's food. The reduced productivity of irrigated lands due to salinity is, therefore, a serious issue.

With the projected increase in populations of 1.5 billion people over the next two decades coupled with increased urbanization in developing countries, the world's agriculture is faced with an enormous challenge to maintain, let alone increase, our present level of food production (Owen, 2001). Ways must be found to achieve this without resorting to unsustainable farming practices and without major increases in the amount of new land under cultivation, which would further threaten forests and biodiversity. It is estimated that

productivity will need to increase by 20% in the developed countries and by 60% in the developing countries. In the light of these demographic, agricultural and ecological issues, the threat and effects of salinity become even more alarming. Reducing the spread of salinization and increasing the salt tolerance of crops and improving species or genotypes to salt tolerance, particularly the high yielding ones are, therefore, issues of global importance.

1.3 Salinity in Egypt

Egypt is an arid and semi-arid country, which covers an area of about one million square kilometers in the north-east corner of Africa and the Sinai Peninsula of south-west Asia. More than 69 million inhabitants now occupy only 4% of this area, which is mainly concentrated in the Nile Valley, the Delta and the coastal zone along the Mediterranean Sea. Thus, Egypt has one of the highest population densities in the world with an average of 1700 inhabitants per km^2. More importantly, Egypt is one of the countries that suffer severe salinity problems. For example, 33% of the cultivated land (Ghassemi et al., 1995), which comprises only 3% of total land area in Egypt, is already salinized. This salinization is mainly due to low precipitation (<25 mm annual rainfall), high temperature (during summer, temperature reaching from 35 to 45°C), high surface evaporation (1500-2400 mm/year), poor drainage system with 98% of the cultivated land under irrigated, rising water table (less than one meter below the soil surface), and irrigating with low quality water (up to salinity of 4.5 dS/m) (Amer et al., 1989). The reduction in production of soils affected by salinity is about 30% (El-Lakany et al., 1986), threatening the livelihoods of the poor farming and having a significant negative impact on the food production of Egypt as whole. Moreover, the Egyptian Government has spent large sums on reclamation, mainly on drainage projects (more than US$ 30 million annually) to solve salinity problems in irrigated area, but the annual average net income from crops grown with drainage system is more limited than for those grown without drainage system (Amer et al., 1989). Therefore, genetic improvement for salt tolerance in major crops, particularly because this approach is less expensive for poor farmers than other, has became an urgent task in dealing with salinity problems in Egyptian agriculture sector.

1.4 Effect of salinity on plant growth

Generally, Salinity can inhibit plant growth by three major ways (Greenway and Munns, 1980):

a) Water deficit arising from the more negative water potential (elevated osmotic pressure) of the soil solution;

b) Specific ion toxicity usually associated with either excessive chloride or sodium uptake; and

c) Nutrient ion imbalance when the excess of Na^+ or Cl^- leads to a diminished uptake of K^+, Ca^{2+}, NO_3^- or P, or to impaired internal distribution of one or another of these ions.

1.4.1 Effect of salinity on phenological aspects

One immediate response of plants to elevated salinity is a decrease in the rate of leaf expansion. Consequently, the total leaf area of the plant is reduced. The common decrease in leaf expansion is associated with a loss in cell turgor pressure rather than a salt-specific effect. This is supported by the evidence that Na^+ and Cl^- are always below toxic concentrations in the growing cells themselves. For example, Hu and Schmidhalter (1998) showed that wheat growing in 120 mM NaCl reacted with a 25% reduction in growth rate, Na^+ in the growing cells of leaves was at maximum only 20 mM, and Cl^- only 60 mM. However, a review by Ball (1988) found that the common decrease in leaf expansion is not related to a loss in turgor pressure and is most likely a result of a change in hormonal signaling from roots to leaves.

In the salt-sensitive genotypes, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves, resulting in death of old leaves and new leaves becoming injured and succulent (Munns and James, 2003). Consequently, the number of green and healthy leaves will ultimately decline. There is then a race against time to initiate flowers and produce seeds while there is still an adequate number of green leaves left to supply the necessary photosynthesis (Mass and Poss, 1989; Munns, 1993). Consequently, seed number and seed size are reduced.

Although salinity can induce a rapid reduction in root growth (Neumann, 1995), shoot growth decreases proportionally more than root growth, causing an increase in the root/shoot ratio. In addition, salinity significantly decreased tiller number and their appearance in wheat (Mass and Poss, 1989). Salinity significantly reduces the total dry matter yield, and the degree of reduction in total dry matter depending on genotypes and salt concentrations (Pessarakli and Huber, 1991). Salinity causes stunting of shoot.

The phenological responses to salt stress are complex. For example, Aloy (1992) found that 1000-seed weight in barley was more strongly affected by salinity than grain number per spike and spikes per plant. While in rice, spikelet and tiller number were more affected by salinity than 1000-seed weight (Zeng et al., 2002).

In addition, the response of phenological aspects to salinity changes with developmental stages of plant (Neumann, 1995). For example, many crops show a reduced tolerance to salinity during seed germination, but greater tolerance during later growth stages and vice versa in other crops. Results of salt tolerance for some crops have shown that wheat, sorghum and cowpea (Mass and Poss, 1989) were most sensitive during the vegetative and early reproductive stages, less sensitive during flowering, and least sensitive during the grain-filling stage. In contrast, sugar beet and safflower are relatively more sensitive during germination and most tolerate at late growth stage (Mass and Poss, 1989), while the tolerance of soybeans may increase or decrease during different growth periods depending on the variety. Therefore, information on the growth stage response to salinity is important in adopting suitable genetic and management strategies for saline soils. For example, if a crop is more sensitive during one stage than other, it may be possible to irrigate with saline water during the more tolerant stages of growth and use low- salinity water only during the sensitive stages of growth.

In glycophytes, growth rate is generally reduced by salinity even at low concentrations (Greenway and Munns, 1980). The reduction in growth is a consequence of several physiological responses, including water status, modification of ion balance, carbon allocation and utilization and toxic ions (Termatt and Munns, 1986; Munns, 1993), which is introduced below.

1.4.2 Effect of salinity on physiological aspects

Physiological aspects are highly sensitive to environmental factors and are, therefore, dominate in determining plant responses to stress. One approach toward understanding of physiological responses to salinity is to follow the series of events after salinity initiates. Such time studies do not prove causal relations, but they can eliminate some possibilities. For example, if leaf expansion slows before photosynthesis does, then the decrease in photosynthesis cannot cause the decrease in leaf expansion (Termaat and Munns, 1986; Munns 1993; Yeo 1998).

Basic metabolic pathways such as photosynthesis and respiration are affected by salinity. A response of respiration to salinity is primarily associated with the direct effects of salinity on enzyme function (Walker et al., 1981; Seemann and Critchly, 1985). High concentrations of salinity have often been reported to increase in respiration. This increase in respiration is greater in salt sensitive than salt tolerant species (Semikhatova et al., 1993). However, elevated salt content in tissues directly influences photosynthetic enzymes and secondarily influences gas exchange and light reactions. Originally, the results of literature cleared that salinity was inhibiting photosynthesis by stomatal and non-stomatal factors (Seemann and Critchley, 1985). In a study by Robinson et al. (1983), photosynthesis was inhibited by 65% under saline conditions. Stomatal conductance was also inhibited by a similar amount, while there was no change in chlorophyll concentrations. The reduction in photosynthesis due to non-stomatal factor may be caused by toxic ions. A negative relationship was found between photosynthesis activity and Na^+ content in leaves in a number of crop species such as rice (Yeo, 1998), and Cl^- content in woody perennials such as citrus (Waalker et al., 1981). A study with wheat (James et al., 2002) found that photosynthesis rate was reduced by a further 50% with Na^+ concentration in leaves of about 350 mM. Seemann and Critchley (1985) found that high Cl^- concentrations (250-300 mM) in the chloroplast of *Phaseolus* were correlated with the efficiency of Rubisco. Therefore, the tolerance of photosynthetic system to salinity may be associated with the capacity of the plant species to effectively compartmentalize the salts in the vacuole.

Salinity significantly reduces the total chlorophyll content and the degree of reduction in total chlorophyll depending on salt tolerance of plant species and salt concentrations. In salt-tolerant species, chlorophyll content increased, while in salt-sensitive species it was decreased (Ashraf and McNeilly, 1988). According to Velegaleti et al. (1990), the reduction in chlorophyll content was significant for salt-sensitive species, which is correlated with Cl^- accumulation.

Plant acquisition and utilization of necessary nutrients particularly K^+ and Ca^{2+} may also impair under saline conditions (e.g. ion deficiency), causing changes in ratios of K^+/Na^+ and Ca^{2+}/Na^+, thus further affecting growth and productivity of plants (Greenway and Munns, 1980; Zhu 2001). The decreases in K^+ and Ca^{2+} uptake under salinity could be due to the antagonism of Na^+ and K^+ or Ca^{2+} at sites of uptake in roots, an effect of Na^+ on the K^+ and Ca^{2+} transport into the xylem (Lynch and Läuchli, 1985) or indirect inhibition of the uptake

process in other aspects, e.g. H^+ -ATPase activity (Suhayda et al., 1990). Because Ca^{2+} is essential for maintenance of the selectivity and integrity of the cell membrane (Fageria, 1983), the deficiency of Ca^{2+} could impair both selectivity and the integrity of the membrane and thus accelerate the passive accumulation of Na^+ in plant tissue. Ca^{2+} is also needed for selective transport of ions like K^+ across membranes (Cramer et al., 1977). Therefore, some ions can serve to buffer the effect of salinity on the accumulation of other ions. For example, when excess Ca^{2+} or NH_4^+ is added to the growth medium containing high salinity, growth and nutrient accumulation can be stimulated compared to the control (Cramer, 2002).

The most observable indirect effect of salinity on plant growth is that of reduced soil water availability, because as salinity increases, soil water potential decreases. In fact, it has been difficult to separate physiological responses to low soil water potential from those in response to salinity. In general, the presence of salt in soil solution decreases the osmotic potential of soil; creating water stress and making it difficult for the plant to absorb water necessary for growth, and hence decreases leaf water potential (Munns, 1993). The decrease in leaf water potential was accompanied by a decrease in leaf osmotic potential so that leaf turgor pressure of the salinized plant was maintained (Tattini et al., 1995). Because the growth of cells is correlated with turgor pressure in the growing tissues, decreased turgor is the major cause of inhibition of plant cell expansion under saline conditions (Greenway and Munns, 1980). Reduction in turgor pressure causes reduction in stomatal conductance.

1.4.3 Effect of salinity on biochemical aspects

Several reports have shown that salt stress could generate the accumulation of toxic compounds such as reactive oxygen species (ROS) in plants, which include peroxides, superoxides and hydroxyl radicals (Burdon et al., 1996; Shen et al., 1997; Tsugane et al., 1999). These toxic molecules can then damage cellular membranes, membrane-bound structures, enzymes and DNA especially in mitochondria and chloroplasts, and can therefore severely impair plant growth and survival (Allen, 1995).

Increasing salinity is associated with a decrease in auxin, gibberellin and cytokinin levels in plant tissues, and an increase in abscisic acid (Moorby and Besford, 1983). Such changes in hormone levels are thought to be a primary process regulating the reduction in growth associated with salinity. There is little evidence that salinity directly affects the

hormone balance within the plant, and the greatest change in hormone levels caused by saline conditions results from water deficit (Blume, 1988).

Most research on the effect of salinity on enzyme activity and protein metabolism has been performed *in vitro* (Noble and Rogers, 1984). As a general rule, enzymes are inhibited by salt *in vitro* equally in glycophytes and halophytes (Greenway and Munns, 1980). The inhibition of enzyme activity by salinity is not due to osmotic influences (Blum, 1988).

1.4.4 Two-phase model of salinity effects on growth

The biphasic model for the inhibition of growth by salinity was proposed by Munns (1993). This biphasic model is very important when screening plants for salt tolerance. The first phase of growth reduction is quickly apparent, and is depended on salt outside the plant rather than salt in tissues, and growth inhibition is not due to salt specific effect. This phase is essentially due to a water deficit or osmotic stress, for which there is surprisingly little genotypic differences. The growth reduction in this phase is presumably regulated by hormonal signals coming from roots. Then there is a second phase of growth reduction, which takes time to develop, and results from internal salt injury. The rate of growth reduction in the second phase depends on the rate of leaf injury. Therefore, in some screening programs, the rate of leaf injury can be used to evaluate salt tolerance of genotypes (Munns and James, 2003).

The biphasic growth reduction model predicts that differences in growth response to salinity among genotypes of a crop should only be evident during the second phase of inhibitions, as was found for wheat, barley and maize (Fortmeier and Schubert, 1995). For example, two wheat genotypes that differed in rates of Na^+ accumulation had the same growth reduction for the first four weeks in 150 mM NaCl, and only in the fifth and sixth week were there differences in dry weight production between the two genotypes (Munns, 1993). Therefore, the failure of many short-term experiments to distinguish genotypic differences in salt tolerance is because the early response to salinity is to the osmotic effects of the salt, osmotic phase.

1.5 Mechanisms of salinity tolerance of plants

Salt tolerance refers to the ability of plants to maintain the growth under saline conditions. To achieve this, a plant must have different mechanisms to tolerate salinity. Generally, salt

tolerance is not an all-or-nothing phenomenon, and for this reason, some plants or genotypes are more salt-tolerant or sensitive than other. This results in a wide spectrum of plant responses to salinity that are defined by a wide range of adaptations at the whole plant level (Greenway and Munns, 1980; Wyn Jones and Gorham, 1983; Munns, 1993). Salt exclusion and inclusion, Na^+/K^+ discrimination and osmotic adjustment are recognized as different mechanisms for plants to tolerate salinity.

Salt exclusion means that the plants have the ability to restrict the uptake of toxic ions into the shoot (Munns, 2002). In glycophytes such as sorghum (Weimberg et al., 1982), beans (Awada et al., 1995), wheat and barley (Gorham, 1993), and corn (Alberico and Cramer, 1993), salt tolerance is associated with Na^+ exclusion. However, in some salt-sensitive genotypes, salt tolerance is not always associated with Na^+ exclusion. For example, while Na^+ exclusion was a general characteristic of a number of salt-tolerant wheat lines, a salt-sensitive line had much lower shoot Na^+ concentration than the more tolerant lines (Schachtman et al., 1989). A similar observation was noted for maize. A salt-tolerant genotype of maize transports Na^+ to leaves at twice the rate of sensitive genotype (Cramer et al., 1994). Thus, tolerance to salinity is not necessarily related with ability to exclude toxic ions.

In contrast to salt exclusion, salt inclusion is to take up large quantities of salt and to store it in the shoot, which can present problems for many physiological and biochemical events taking place in the cell. The ability of plants to maintain low cytoplasmic toxic ions is through to be one of the key determinants of salt tolerance (Yeo, 1998; Amtmann and Sanders, 1999; Ashraf, 2002). Therefore, salt tolerance of plants, whether related to exclusion or not, is associated with the ability to maintain a homeostatic ion concentration in the cytoplasm (Ungar, 1991).

Closely allied to salt exclusion and its relationship to salt tolerance is the regulation of ion selectivity, in particular the role of Na^+/K^+ discrimination, in salt tolerance (Gorham, 1993). Na^+ can be restricted K^+ uptake, and it is believed that similar mechanisms of uptake may operate for both ions (Greenway and Munns, 1980). High levels of K^+ in young expanding tissue is associated with salt tolerance in many plant species (Bhandal and Malik, 1988). It is, therefore, possible that Na^+/K^+ discrimination is associated with salt tolerance. The genetic locus that determines Na^+/K^+ discrimination in wheat (*Triticum*) has been

identified (Dvorak et al., 1994). When this locus is inserted into the genome of salt-sensitive genotypes of *Triticum*, salt tolerance is enhanced (Dvorak et al., 1994).

Additionally, osmotic adjustment is regarded as an important adaptation of plants to salinity because it helps to maintain turgor and cell volume. Plants are able to tolerate salinity by reducing the cellular osmotic potential as a consequence of a net increase in inorganic and solute accumulation (Hazegawa et al., 2000; Serraj and Sinclair, 2002). During osmotic adjustment, the cell tends to compartmentalize most of the absorbed ions in vacuoles at the same time they synthesize and accumulate compatible organic solutes in the cytoplasm in order to maintain the osmotic equilibrium between these two compartments (Serrano and Gaxiola, 1994; Hasegawa et al., 2000). Although the energetic cost of osmotic adjustment by inorganic ions is much lower than that conferred by organic molecules synthesized, this could also led to produce toxic effects because such high concentration of toxic ions may interfere with normal biochemical activities within the cell (Yeo et al., 1985). Thus, a better understanding of these mechanisms and processes would enhance our efforts to improve the salinity tolerance of crop genotypes.

1.6 Response of wheat to salinity

1.6.1 Importance of wheat in Egypt

Wheat is one of the oldest and most important of the cereal crops in Egypt. Although wheat production per unit area in Egypt has significantly increased during the past years, wheat production supplies only 40% of its annual domestic demand. The lacking ability of Egypt to produce sufficient wheat for domestic consumption are: a) the total cultivated area represents less than one quarter of the amount consumed by the population; b) Egypt had one of the highest rates of wheat consumption per capita of any country in the world (200 kg per capita, compared with a world average of less than 60 to 75 kg per capita); c) the population growth rate (2.1% annually) increases higher than the increase of wheat production; d) little efforts are made for improving salt tolerant in wheat crops, e.g. only two genotypes (Sakha 8 and Sakha 93) among Egyptian wheat genotypes are tolerate to salinity; and e) the competition among cultivated lands for wheat, forage and cotton crops. Most importantly, Egypt still is one of the largest countries that import wheat. Wheat imports in 2002/03 (July/June) are about 6.5 million tons, with a cost of about 986 million US $ annually (FAO; http://www.fao.org/). Therefore, the Egyptian Government needs to make a great effort to

increase wheat productivity. Extending wheat growing outside the Nile Valley is the first effort toward overcoming wheat problems. However, most of the area outside the Nile Valley suffers from salinity or depends on water sources that are affected by salinity, therefore, increasing salt tolerance for wheat genotypes is one of the cheap methods to spread growing wheat in these areas.

1.6.2 Response of wheat plant to salinity

Wheat is moderately tolerant to salt with threshold without yield loss at 6 dS m^{-1} and with yield 50% loss at 13 dS m^{-1} (Mass and Hoffmann, 1977).

Since the life cycle of wheat is an orderly sequence of development stage, salinity can have a significant effect on the developmental processes that occur at a particular time. The sequence of events has been separated into three distinct but continuous developmental phases (Francois and Mass, 1994). In the first phase, which encompasses the early vegetative growth stage, leaf and tiller buds are produced in the axils of the leaves and spikelet primordia is initiated. High salinity at this time reduces the number of leaves per culm, the number of tillers per plant, and the number of spikelet per spike (Mass and Grieve, 1990). The differentiation of the terminal spikelet signals completion of this phase. During the second phase, the main stem and tiller culms elongate, and the final number of florets is set (Kirby, 1988). Salinity stress during this phase may affect tiller survival and reduce the number of functional florets per spikelet. This phase ends with anthesis. Florets fertilization and grain filling occur during the third phase. Salinity during this phase affects seed number and seed size.

The effect of salinity on tiller and spikelet numbers established during the first phase has a greater influence on final seed yield than the effects exerted on yield components in the latter two phases (Kirby, 1988), indicating the probability of improving salt tolerance of wheat genotypes during early growth stages.

1.7 Strategies for breeding salt tolerant plants

Breeding for salt tolerance is a difficult and slow progress due to a combination of many factors:

1. Changes in salt tolerance with different growth stages;

2. The large number of physiological parameters that contribute to salt tolerance;
3. Lack of effective evaluation methods for salt tolerance among genotypes;
4. Low selection efficiency using multiple parameters;
5. The interactions of ionic and osmotic properties of salts in the plant.
6. Incomplete knowledge of the effects of salinity on plants; and
7. The complex interactions of salinity and environment on salt tolerance of plants.

Therefore the following aspects should be considered for improving salt tolerance in a given crop:

1. Evaluation a wide range of germplasm to assess the genetic variation and see if selection is possible from within the genotypes;
2. Identify the growth stage response when productivity is most limited by salinity, and assess whether this can be overcome by agronomic practices;
3. Assess the genotypic variation for the various traits under consideration that may have a functional role in improving salinity tolerance;
4. Choose the selection criteria;
5. Identify genetic sources for the various traits of salinity tolerance;
6. Initiate breeding programs that combine various traits from different sources into a locally adapted genotype for the ultimate development of a salt-tolerant genotype;
7. Develop screening methods that could be used to evaluate salt tolerance among genotypes and that are simple, quick and non-destructive; and
8. Choose suitable evaluation methods for helping to analyse multiple parameters simultaneously and facilitate the rankings for salt tolerance among genotypes.

1.7.1 Screening criteria

Agronomic characters such as survival, biomass accumulation and yield have been the most commonly used criteria for identifying salt tolerance among genotypes. This is largely due to their ease of measurement and because, in the end, yield (both absolute and relative) under saline conditions is usually the ultimate target. There are many problems associated with

using agronomic traits as screening criteria such as the effects of environmental conditions on the expression of these characters, differential growth and developmental patterns among genotypes, and costly and slowly with long-term growth comparisons.

Selection and breeding approaches to increase salt tolerance might be more successful, with respect to achieving maximum attainable tolerance, if selection is based directly on the relevant physiological criteria (Yeo et al., 1990; Jackson et al., 1996; Zeng et al., 2002). Consequently, salinity induces substantial differences in physiological processes at the whole plant level (Almansouri et al., 1999). These differences have been demonstrated among genotypes of several crops such as rice (Lutts et al., 1996), alfalfa and wheat (Ashraf and McNeilly, 1988). The benefit of evaluating salt tolerance for genotypes based on physiological criterion are quick, easy, non-destructive methods and also help understanding the differences of physiological mechanisms of salt tolerance among genotypes (Noble and Rogers, 1992).

The sensitivity of some crops to salinity has been attributed to the inability to keep Na^+ and Cl^- out of the transpiration stream. For many glycophytes, but not all, differences in salt tolerances between genotypes have been closely associated with reduced uptake and accumulation of Na^+ and/or Cl^- ions at the whole plant, shoot and leaf level (Francois and Mass, 1994). In such cases, genetic diversity in the trait of Na^+ and Cl^- exclusion could be useful selection traits to screen wheat genotypes for salinity tolerance.

Plant acquisition and utilization of necessary nutrients ions particularly K^+ and Ca^{2+} may be impaired under saline conditions, causing changes in ratios of K^+/Na^+ and Ca^{2+}/Na^+, thus selection among genotypes can be made for contents of K^+ and Ca^{2+} in shoots and their selectivity rather than Na^+ (Ash et al., 2000; Zhu et al., 2001).

Trait that can handle large number of genotypes is leaf injury as measured by premature loss of chlorophyll (using SPAD meter). The effectiveness of SPAD meter as a screening method has been examined in a number of studies as an index for response of chlorophyll content to stress. For instance, the close relationship between chlorophyll content and leaf injury due to salinity (Munns and James, 2003) suggested that a simple and non-destructive measure of chlorophyll content by SPAD value could be useful selection traits to screen large genotypes for salt tolerance.

Other physiological traits such as photosynthetic and water relation parameters could be useful selection traits to screen genotypes for salinity tolerance because salinity indirectly leads to water deficit and low photosynthesis for plants (James et al., 2002; Rivelli et al., 2002).

Screening and selection on the basis of individual physiological criteria cannot account for the variation in the salt tolerances among genotypes due to effects of soil properties, the osmotic effect of high ionic concentrations, competitive interference with nutrient uptake and toxic effects within the plant tissue. Thus, a combination of physiological criteria is logically a desirable objective in screening for salt tolerance of genotypes (Yeo et al., 1990; Noble and Rogers, 1992).

1.7.2 Controlled environment screening

Screening large numbers of genotypes for salinity tolerance in the field is notoriously difficult because of the variability of salinity within fields (Daniells et al., 2001) and the enormous potential for interactions with other environmental factors, ranging from soil chemical and physical properties to temperature, light flux density and seasonal fluctuations in rainfall. It would be difficult to determine the critical parameters under field conditions since any environmental change could result in dramatic change in the plant's response to salinity. Screening technique has, therefore, often been used under controlled conditions. Consequently, prediction of field performance is commonly carried out in trial plots method where the salinity of the medium can be readily adjusted to required values (Francois and Mass, 1994). Large numbers of bread and durum wheat genotypes have been screened for salt tolerance in greenhouse, the criteria being biomass production at high salinity (up to 250 mM NaCl) relative to biomass in control conditions (Kingsbury and Epstein, 1984), and a screen by Sayed (1985) of 5000 wheat lines under solution culture, based on survival of high salinity, showed considerable genetic diversity amongst tested genotypes and lines.

1.8 Objectives of the thesis

Wheat is the most important component of the Egyptian diet and only 40% of the annual domestic demands are produced in Egypt. Therefore, the wheat production in Egypt should be continuously increased in order to improve salt tolerance of Egyptian wheat genotypes. Especially, increasing salt tolerance of genotypes is much less expensive for poor farmers in

developing countries than using other management practices (Qureshi and Barrett-Lennart, 1998). Therefore, the objective of this study was to screen a large number of wheat genotypes from different regions of Egypt and to compare with salt tolerance of wheat genotypes from Australia and Germany with using the most salt tolerance genotype from India (Kharchia) as a reference in order to find out good traits or donor to improve salt tolerance for Egyptian genotypes through breeding programs.

The low success in wheat salt tolerance breeding is, at least partially, due to the lack of effective evaluation methods for salt tolerances among genotypes, low selection efficiency using overall agronomic characters and a complex phenomenon involving morphological, physiological and biochemical parameters among genotypes (Zeng et al., 2002). However, in conventional methods, salt tolerance of genotypes are usually scored and ranked based on single parameters. An appropriate statistical method will be helpful to analyze multiple parameters simultaneously to facilitate evaluation of genotypes and ranking for salt tolerance (Jollife et al., 1989; Zeng et al., 2002). In addition, salt tolerance of crops may vary with their growth stages. Therefore, the objectives of this study were to identify the relative importance of agronomic parameters associated with salt tolerance, to screen the different wheat genotypes for their salt tolerance at different growth stages, and to rank salt tolerance by using multivariate analysis for multiple agronomic parameters at different growth stages.

Because of the complex nature of salt tolerance, as well as the difficulties in maintaining long-term growth experiments, physiological traits as selection criteria are recommended for screening (Yeo et al., 1990; Noble and Rogers, 1992), which have been considered as more reliable and feasible to screen for specific traits rather than salt tolerance itself in terms of biomass or grain yield in saline soil (Munns and James, 2003). Therefore, the other objective of the study was to evaluate the association of the physiological traits of wheat as ion contents in leaves and stems, photosynthetic parameters, SPAD value and water relation parameters at different growth stages with the salt tolerance in terms of grain yield, to find out the reliable multiple physiological traits that can be used as quick, easy and economic technique as screening criteria.

The mechanisms of salt tolerance in plant have received much attention for many years, but the differences in growth response to salinity among genotypes still remain uncertain (He and Cramer, 1992). In addition, few studies have used the functional approach plant growth analysis to determine the relationship between the growth and physiological

traits (Schachtman et al., 1989). Therefore, the one objective of the study was to describe the effects of salinity on different physiological traits and how these relate to plant growth analysis.

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2 Evaluating salt tolerance of wheat genotypes at different growth stages by using multiple agronomic parameters

Abstract

Salt tolerance of wheat is known to change with growth stage. Identifying the multiple parameters associated with salt tolerance during different growth stages is important for evaluating wheat genotypes and improving their salt tolerance. Thirteen wheat genotypes from Egypt, Germany, Australia and India were grown in soil and exposed to four salinity levels (control, 50, 100 and 150 mM NaCl). Tiller number, leaf number and leaf area per plant at vegetative stage; dry weight per plant at vegetative, reproductive and maturity stages; and yield components of main spike and total grain yield at maturity were determined. The results showed that tiller number was affected more by salinity than leaf number and leaf area at the vegetative stage. Salinity decreased dry weight per plant significantly at all growth stages. Spikelet number on the main stem decreased much more with salinity than spike length, grain number and 1000-grain weight at maturity. According to cluster analysis with multiple agronomic parameters at all growth stages, the Egyptian genotypes Sakha 8 and Sakha 93 and the Indian genotype Kharchia were ranked as being the most tolerant to salinity. A change in salt tolerance with growth stages was observed for Sids 1, Gemmeza 7 and Westonia. Drysdale and Sakha 69 were ranked as being moderate tolerant. The remaining genotypes showed the lowest tolerance to salinity at all growth stages. We conclude that an increase in tiller number per plant and spikelet number per spike will improve the salt tolerance of wheat genotypes in breeding programs. Cluster analysis with multiple agronomic parameters simultaneously to evaluate the salt tolerance facilitates the rankings of salt tolerance of wheat genotypes.

2.1 Introduction

Salinity is one of the major factors reducing plant growth and productivity worldwide, and affects about 7% of the world's total land area (Flowers et al., 1997). The percentage of cultivated land affected by salt is even greater, with 23% of the cultivated land being saline and 20% of the irrigated land suffering from secondary salinization. Furthermore, there is also a dangerous trend of a 10% per year increase in the saline area throughout the world (Ponnamieruma, 1984). Egypt is one of the countries that suffer from severe salinity

problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (<25 mm annual rainfall) and irrigation with saline water (Ghassemi et al., 1995). Wheat is the most important and widely adapted food cereal in Egypt. However, Egypt supplies only 40% of its annual domestic demand for wheat (Salam, 2002). Therefore, it is necessary to increase wheat production in Egypt by raising the wheat grain yield. Obviously, the most efficient way to increase wheat yield in Egypt is to improve the salt tolerance of wheat genotypes (Epstein et al., 1980; Shannon, 1997; Pervaiz et al., 2002) because increasing the salt tolerance of wheat is much less expensive for poor farmers in developing countries than using other management practices (e.g. leaching salt from the soil surface etc.) (Qureshi and Barrett-Lennart, 1998).

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). In general, cereal plants are the most sensitive to salinity during the vegetative and early reproductive stages, and less sensitive during flowering and grain filling stages (Mass and Poss, 1989). However, a difference in the salt tolerance among genotypes may also occur at different growth stages. Zeng et al (2002) reported that various responses of different rice genotypes to salt tolerance exist at different growth stages. Similarly, Kingsbury and Epstein (1984) found that individual lines from 5000 accessions of spring wheat showed differing tolerance during their life cycle. Therefore, the salt tolerance of different wheat genotypes must be evaluated at different growth stages. Such evaluations may facilitate improvement salt tolerance of tested genotypes in breeding programs or it may prove feasible to irrigate with saline water during the more tolerant growth stages and with low salinity water only during the sensitive growth stages.

Improving salt tolerance of wheat genotypes has been inhibited by a number of factors such as the lack of effective evaluation methods for salt tolerance to screen the genotypes in breeding programs, low selection efficiency using overall agronomic parameters, and a complex phenomenon involving morphological, physiological and biochemical parameters among genotypes (Zeng et al., 2002). Compared with conventional techniques that score and rank salt tolerance genotypes based on single parameter, some success has already been realized by using multiple agronomic parameters simultaneously at different growth stages (Shannon, 1997; Zeng et al., 2002). An appropriate statistical method is needed to analyse multiple agronomic parameters simultaneously to facilitate ranking genotypes for salt tolerance (Zeng et al., 2002). Cluster analysis is commonly used multivariate statistic that has

been suggested for comparisons of genotypes means by Jolliffe et al. (1989). However, multivariate analysis in the screening of genotypes for salt tolerance has been applied only in potato (Khrais et al., 1998) and rice (Zeng et al., 2002).

The objectives of this study were to identify the relative importance of agronomic parameters associated with salt tolerance, to screen the different wheat genotypes for their salt tolerance at different growth stages and from different regions of Egypt using the most salt tolerant wheat genotypes from India as a reference, and to rank salt tolerance by using multivariate analysis of multiple agronomic parameters at different growth stages.

2.2 Materials and methods

2.2.1 Plant materials

Thirteen varieties of spring wheat (*Triticum aestivum* L.) from different countries were used in this study. Eight varieties (Sakha 8, Sakha 93, Sakha 61, Sakha 69, Giza 168, Sids 1, Sahel 1 and Gemmeza 7) were obtained from the Agricultural Research Centre in Giza, Egypt. Sakha 8 and Sakha 93 are usually cultivated in saline areas in Egypt. Of the remaining varieties, Thassos and Triso were from Germany, Westonia and Drysdale were from Australia, and Kharchia was from India.

Kharchia is the most tolerant wheat genotype, and is used as a standard for the salt tolerance test of wheat worldwide (Sharma et al., 1994; Ashraf, 2002).

2.2.2 Growth conditions

This study was carried out in a greenhouse from the middle of March to the middle of August 2002. The air temperature ranged from 23 to 28°C during the day and 15 to 18°C during the night. Relative humidity fluctuated between 45 and 85% at day/night.

Loamy soil was collected from the soil surface (0-15 cm). The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The soil consisted of 23% clay, 48% silt and 29% sand, and the organic matter content was 1.66%. The initially air-dried soil with 9% gravimetric water content was filled layer-wise in four layers in 7-l pots without a leaching possibility.

Four salt levels (control (no added NaCl), 50, 100 and 150 mM NaCl) in the soil were applied. The salinity levels of 50, 100 and 150 mM NaCl in soil solution were equivalent to an electrical conductivity of 8, 13, and 17 dS m^{-1}, respectively, which were measured at the beginning of the experiment. During the period of the experiment, the electrical conductivity at each salinity level slightly decreased due to the uptake of salt by plants. At the end of the experiment, the electrical conductivity was changed to 5.2, 10, and 14 dS m^{-1}, respectively The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinized until 10 days after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to twenty per pot.

The N, P and K were initially applied as 0.2 g NH_4NO_3 and as 0.2 g KH_2PO_4 per pot. The same amount of N, P and K was applied another three times at 20, 40 and 60 days after sowing. During the experiment, the pots were weighed daily and the water loss was replaced by adding tap water when the total amount of the water lost was around 200 g for plants to avoid suffering either drought or flooding. All treatments were replicated four times.

2.2.3 Sampling strategy

Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). Therefore, the measurements were carried out at vegetative, reproductive and grain maturity stages.

Measurements at the vegetative stage were conducted at 45 days after sowing. Three plants from each pot were harvested and separated into leaves and stems. Vegetative growth of wheat plants is characterized by the tillering and leaf appearance and growth on the tillers. Thus, the number of tillers and leaves were recorded. Leaf area was measured by using an LI-3000 Area Meter (LI-COR, Walz Co., Oregon, USA). After the fresh weight (FW) was determined, the samples were dried at 65°C for 48 hours to determine the dry weight (DW).

At 60 and 75 days after sowing, when plants were in the reproductive stage, three plants from each pot were harvested. Plants were separated into leaves, stems and spikes. FW and DW determined as above.

Grain maturity was visually estimated according to the complete loss of green colour from grumes. At grain maturity, five plants from each pot were harvested. Main spikes were

separated from the other spikes of the plants, and the spike length and spikelet number were recorded. Ears were threshed. Plant material was then dried at 65°C for 48 hours for the determination of DW. The grain number and thousand-grain weight (TGW) were also determined.

2.2.4 Ranking of genotypes for salt tolerance

Following Zeng et al. (2002), all the data were converted to salt tolerance indices before cluster analysis to allow comparisons among genotypes for salt tolerance by using multiple agronomic parameters. A salt tolerance index was defined as the observation at salinity divided by the average of the controls. Cluster group ranking numbers can be assigned to cluster groups based on cluster means, and was used to score genotypes. Cluster analysis followed the methods described by Jolliffe et al., (1989). Cluster group rankings were obtained based on Ward's minimum variance cluster analysis of the averages of the salt tolerance indices for three parameters at vegetative stage (i.e., tiller number, leaf number and leaf area per plant) and four parameters at maturity stage (i.e., spike length and spikelet number for main spike, grain number and thousand-grain weight). Cluster group rankings were obtained based on Single-Link cluster analysis of the means of the salt tolerance indices for total dry weight per plant. All procedures are described fully in the JMP User's Guide (SAS Institute, 2000). The cluster group rankings were obtained from the average of means of the multiple parameters in each cluster group. A sum was obtained by adding the number of cluster group rankings at each salt level in each genotype. The genotypes were finally ranked based on the sums such that those with the smallest and largest sums were ranked respectively as the most and least tolerant genotypes in terms of relative salt tolerance.

2.2.5 Statistical analysis of data

Data were analysed by ANOVA. According to Snedecor and Cochran (1980), LSD ($P = 0.05$) was used to compare genotypes means. Data were analysed using an ANOVA split-plot design, where salinity levels were assigned as whole plot, genotypes as sub plots and replicates as blocks.

2.3 Results

Tiller number, leaf number and leaf area at vegetative stage decreased with increasing salinity. The low salinity treatment (50 mM NaCl) reduced these parameters to a lesser

degree than moderate (100 mM NaCl) and high salinity treatments (150 mM NaCl). At 50, 100 and 150 mM NaCl, for example, tiller number was reduced by 22, 28 and 37.5%, leaf number was reduced by 6, 19 and 28% and leaf area was reduced by 8, 19 and 28%, respectively, as compared with the control treatment (Fig. 2.1).

At vegetative stage, the relative salt tolerance indices for all the measured parameters varied among genotypes (Table 2.1). The salt tolerance indices of tiller number ranged from 0.42 to 1.00 at low salinity and from 0.25 to 0.88 at high salinity among genotypes. However, the salt tolerance indices ranged from 0.86 to 1.09 for leaf number and from 0.85 to 0.99 for leaf area at low salinity and from 0.44 to 0.88 for leaf number and from 0.57 to 0.90 for leaf area at high salinity. The results at the vegetative stage showed that genotypes (Kharchia, Sakha 8 and Sakha 93) were affected the least by increasing salinity. For instance, tiller number, leaf number and leaf area at 150 mM NaCl were decreased by 25, 14 and 15% for Kharchia, 12, 18 and 14% for Sakha 8 and 16, 18 and 10% for Sakha 93, respectively, as compared with the control. However, tiller number, leaf number and leaf area per plant for genotypes (Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos, Triso and Westonia), which were the most sensitive to salinity, decreased by an average of 50, 34 and 35% at 150 mM NaCl, respectively (Fig. 2.1). To rank the salt tolerance of genotypes at the vegetative stage based on multiple parameters, genotypes were divided into four cluster groups at low and moderate salinity and five cluster groups at high salinity by using Ward's minimum variance cluster analysis (Table 2.2). At the vegetative stage, genotypes (Kharchia, Sakha 8 and Sakha 93) were ranked at the top for salt tolerance. By contrast, genotypes (Sids 1 and Sakha 61) were ranked as being the most sensitive.

To determine the variation of salt tolerance at different growth stages, the aboveground biomass per plant was measured at 45, 60 and 75 days after sowing and at final harvest. The average biomass of the 13 genotypes at 150 mM NaCl was decreased by 23, 49, 64 and 47% at 45, 60 and 75 days after sowing and at final harvest, respectively (Fig. 2.2). The averaged indices of biomass from all varieties at 50 mM NaCl ranged from 0.78 to 1.00, from 0.57 to 0.92, from 0.48 to 0.73 and from 0.59 to 0.90 at 45, 60 and 75 days after sowing and at final harvest, respectively. At high salinity, however, they ranged from 0.65 to 0.90 at day 45, from 0.40 to 0.81 at day 60, from 0.24 to 0.59 at day 75 and from 0.37 to 0.69 at final harvest (Table 2.1). The results also show a wide variation among genotypes. For instance, the biomass at 150 mM NaCl was decreased by an average of 14, 22, 46 and 31% for

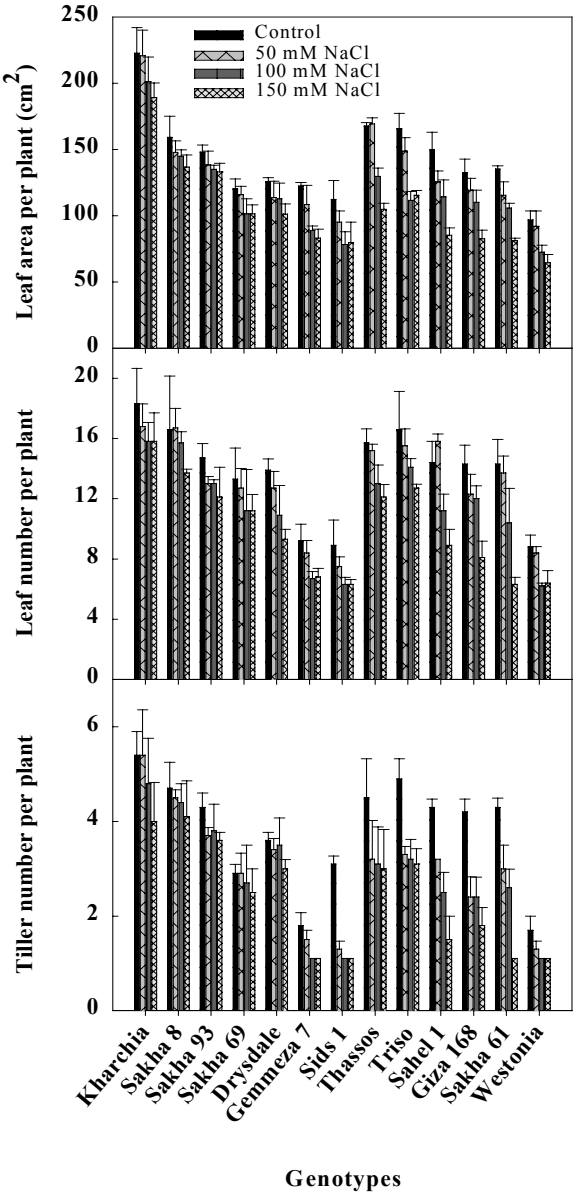

Fig. 2.1 Effect of different salinity levels on plant growth parameters (tiller number, leaf number and leaf area per plant) at day 45 for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

Table 2.1 Salt tolerance index of agronomic parameters in wheat genotypes under different salinity levels at different growth stages.

Genotypes	Salinity levels (mM NaCl)	Tiller number at day 45	Leaf number at day 45	Leaf area at day 45	Total biomass at day 45	Total biomass at day 60	Total biomass at day 75	Total biomass at final harvest	Spike length	Spikelet number	Grain number	1000-grain weight	Grain yield
	50	1.00	0.92	0.99	0.97	0.89	0.67	0.88	0.91	0.99	1.00	0.95	0.94
Kharchia	100	0.89	0.86	0.90	0.93	0.88	0.67	0.82	0.88	0.96	0.91	0.94	0.91
	150	0.75	0.86	0.85	0.90	0.81	0.59	0.69	0.86	0.90	0.87	0.94	0.83
	50	0.97	1.01	0.93	0.90	0.89	0.70	0.90	0.90	0.98	1.03	1.00	0.92
Sakha 8	100	0.93	0.95	0.91	0.88	0.81	0.60	0.79	0.84	0.86	0.89	0.99	0.82
	150	0.88	0.82	0.86	0.86	0.74	0.52	0.69	0.81	0.83	0.88	0.97	0.75
	50	0.86	0.88	0.94	0.98	0.92	0.73	0.80	0.90	0.98	1.01	0.94	0.94
Sakha 93	100	0.89	0.88	0.91	0.92	0.83	0.56	0.77	0.87	0.84	0.95	0.96	0.89
	150	0.84	0.82	0.90	0.83	0.80	0.51	0.68	0.84	0.79	0.84	0.92	0.77
	50	0.99	0.95	0.96	0.86	0.79	0.62	0.73	0.87	0.94	0.94	0.88	0.96
Sakha 69	100	0.93	0.84	0.84	0.92	0.62	0.40	0.53	0.80	0.67	0.83	0.92	0.64
	150	0.87	0.84	0.84	0.84	0.51	0.34	0.51	0.75	0.65	0.77	0.80	0.58
	50	0.94	0.91	0.90	0.86	0.73	0.66	0.78	0.89	0.93	0.93	0.99	0.84
Drysdale	100	0.98	0.78	0.90	0.92	0.63	0.45	0.71	0.77	0.70	0.85	0.97	0.61
	150	0.85	0.67	0.80	0.84	0.55	0.40	0.66	0.68	0.70	0.81	0.88	0.57
	50	0.82	0.92	0.88	1.00	0.66	0.56	0.74	0.93	1.04	1.10	0.91	0.87
Gemmeza 7	100	0.60	0.73	0.73	0.81	0.48	0.47	0.59	0.80	0.62	0.83	0.83	0.58
	150	0.60	0.74	0.68	0.76	0.43	0.38	0.55	0.71	0.64	0.84	0.69	0.50
	50	0.42	0.84	0.85	0.86	0.69	0.54	0.84	0.92	0.99	1.02	0.95	0.86
Sids 1	100	0.36	0.71	0.70	0.86	0.51	0.40	0.59	0.83	0.64	0.75	0.86	0.53
	150	0.36	0.70	0.71	0.79	0.43	0.35	0.57	0.74	0.64	0.76	0.73	0.48

Table 2.1 Continued.

Genotypes	Salinity levels (mM NaCl)	Tiller number at day 45	Leaf number at day 45	Leaf area at day 45	Total biomass at day 45	Total biomass at day 60	Total biomass at day 75	Total biomass at final harvest	Spike length	Spikelet number	Grain number	1000-grain weight	Grain yield
	50	0.71	0.97	1.01	0.85	0.65	0.50	0.72	0.91	0.91	0.98	0.92	0.74
Thassos	100	0.69	0.83	0.77	0.72	0.51	0.42	0.50	0.74	0.59	0.81	0.82	0.47
	150	0.66	0.77	0.62	0.69	0.40	0.27	0.39	0.71	0.57	0.80	0.71	0.42
	50	0.67	0.94	0.90	0.78	0.66	0.48	0.72	0.86	0.87	0.92	0.94	0.73
Triso	100	0.65	0.85	0.67	0.73	0.59	0.37	0.57	0.74	0.51	0.74	0.81	0.41
	150	0.63	0.76	0.70	0.67	0.41	0.24	0.47	0.59	0.49	0.71	0.65	0.33
	50	0.75	1.09	0.84	0.89	0.65	0.50	0.68	0.94	0.94	0.90	0.88	0.67
Sahel 1	100	0.59	0.78	0.76	0.80	0.58	0.36	0.54	0.74	0.62	0.82	0.87	0.49
	150	0.36	0.62	0.57	0.65	0.51	0.31	0.44	0.67	0.52	0.74	0.70	0.33
	50	0.57	0.86	0.90	0.85	0.62	0.57	0.70	0.85	0.89	0.98	0.87	0.75
Giza 168	100	0.56	0.84	0.83	0.86	0.49	0.37	0.60	0.70	0.57	0.79	0.86	0.51
	150	0.42	0.56	0.62	0.68	0.36	0.29	0.52	0.68	0.51	0.72	0.69	0.37
	50	0.68	0.96	0.85	0.86	0.57	0.51	0.59	0.84	0.94	0.91	0.81	0.61
Sakha 61	100	0.59	0.73	0.78	0.78	0.45	0.34	0.40	0.74	0.61	0.69	0.64	0.39
	150	0.25	0.44	0.60	0.70	0.40	0.25	0.37	0.69	0.60	0.67	0.53	0.38
	50	0.78	0.95	0.95	0.85	0.62	0.58	0.73	0.86	0.90	1.07	0.95	0.83
Westonia	100	0.67	0.70	0.75	0.80	0.49	0.40	0.42	0.78	0.59	0.78	0.91	0.38
	150	0.67	0.73	0.67	0.70	0.34	0.28	0.40	0.70	0.60	0.74	0.64	0.33

Table 2.2 Rankings of genotypes for their relative salt tolerance in terms of plant growth parameters (number of tiller, number of leaf and leaf area per plant) at day 45 in a cluster analysis (Ward's minimum variance analysis).

Genotypes	Salinity levels (mM NaCl)			Sum	Genotypes ranking	Tolerant degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	1	1	3	1	Tolerant
Sakha 93	1	1	1	3	1	Tolerant
Sakha 69	1	2	1	4	2	Tolerant
Drysdale	2	2	2	6	3	Moderate
Thassos	2	3	3	8	4	Moderate
Westonia	2	3	3	8	4	Moderate
Triso	3	3	3	9	5	Moderate
Gemmeza 7	3	4	3	10	6	Sensitive
Giza 168	3	3	5	11	7	Sensitive
Sahel 1	4	3	5	12	8	Sensitive
Sids 1	4	5	4	13	9	Sensitive
Sakha 61	4	4	5	13	9	Sensitive

genotypes (Sakha 8, Sakha 93 and Kharchia), the most salt tolerant genotypes, whereas it was decreased by an average of 29, 59, 70 and 54% for genotypes (Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos, Triso and Westonia) at 45, 60 and 75 days after sowing and at final harvest, respectively (Fig. 2.2). The biomass indices for genotypes (Kharchia, Sakha 8 and Sakha 93) were about two times greater than those for genotypes (Sakha 61, Westonia, Thassos and Triso). Based on single-link cluster analysis, genotype (Kharchia) was ranked as the most salt tolerant genotype, followed by genotypes (Sakha 8 and Sakha 93). The genotypes (Sakha 61, Sids 1, Sahel 1 and Giza 168) were ranked as the most salt sensitive genotypes (Table 2.3).

At final harvest, grain yield per plant at 150 mM NaCl was reduced by an average 22% for the most tolerant genotypes, whereas it was reduced by an average 61% for the least tolerant genotypes (Fig 2.3). On average, spike length and spikelet number on the main spike, grain number and TGW at 150 mM NaCl were reduced by 16, 16, 14 and 6%, respectively, in

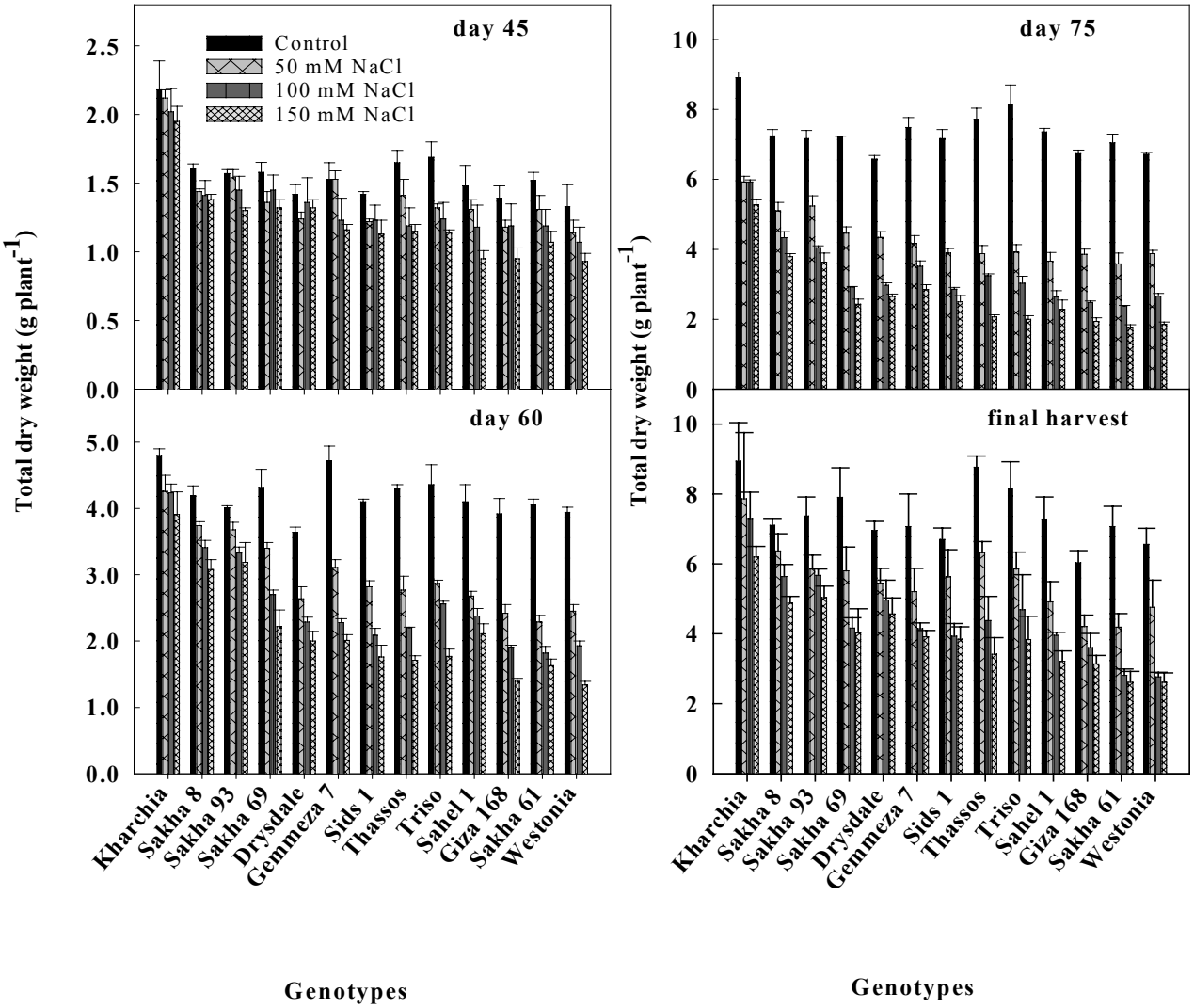

Fig. 2.2 Effect of different salinity levels on total dry weight per plant at 45, 60 and 75 days after sowing and at final harvest for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

Table 2.3 Rankings of genotypes for their relative salt tolerance in terms of total biomass per plant at different growth stages (at days 45, 60, 70 and final harvest) in a cluster analysis (Single-link cluster analysis).

Genotypes	Salinity levels (mM NaCl)			Sum	Genotypes ranking	Tolerant degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	2	2	5	2	Tolerant
Sakha 93	1	2	2	5	2	Tolerant
Sakha 69	2	3	3	8	3	Moderate
Drysdale	2	3	3	8	3	Moderate
Thassos	3	4	3	10	4	Moderate
Westonia	3	4	4	11	5	Moderate
Triso	4	4	4	12	6	Sensitive
Gemmeza 7	3	4	5	12	6	Sensitive
Giza 168	3	5	5	13	7	Sensitive
Sahel 1	3	5	5	13	7	Sensitive
Sids 1	3	5	5	13	7	Sensitive
Sakha 61	4	5	5	14	8	Sensitive

the three most tolerant genotypes (Kharchia, Sakha 8 and Sakha 93), by 31, 43, 25 and 33%, respectively, in the least tolerant genotypes (Fig. 2.3).

The salt tolerance indices of yield components of the main spike (i.e. spike length, spikelet number, grain number and thousand grain weight (TGW)) were decreased with increasing salinity (Table 2.1). The variation of the indices among genotypes increased from low to high salinity. For instance, salt tolerance indices for spikelet number and TGW ranged from 0.89 to 1.04 and from 0.81 to 1.00 at low salinity among genotypes, respectively, whereas the indices ranged from 0.49 to 0.90 for spikelet number and from 0.53 to 0.97 for TGW at high salinity (Table 2.1). The salt tolerance indices at high salinity ranged from 0.59 to 0.86 for spike length and from 0.67 to 0.88 for total grain number among genotypes. According to the cluster analysis, the genotypes were divided into four cluster groups at low salinity and five cluster groups at moderate and high salinity (Table 2.4). The results show

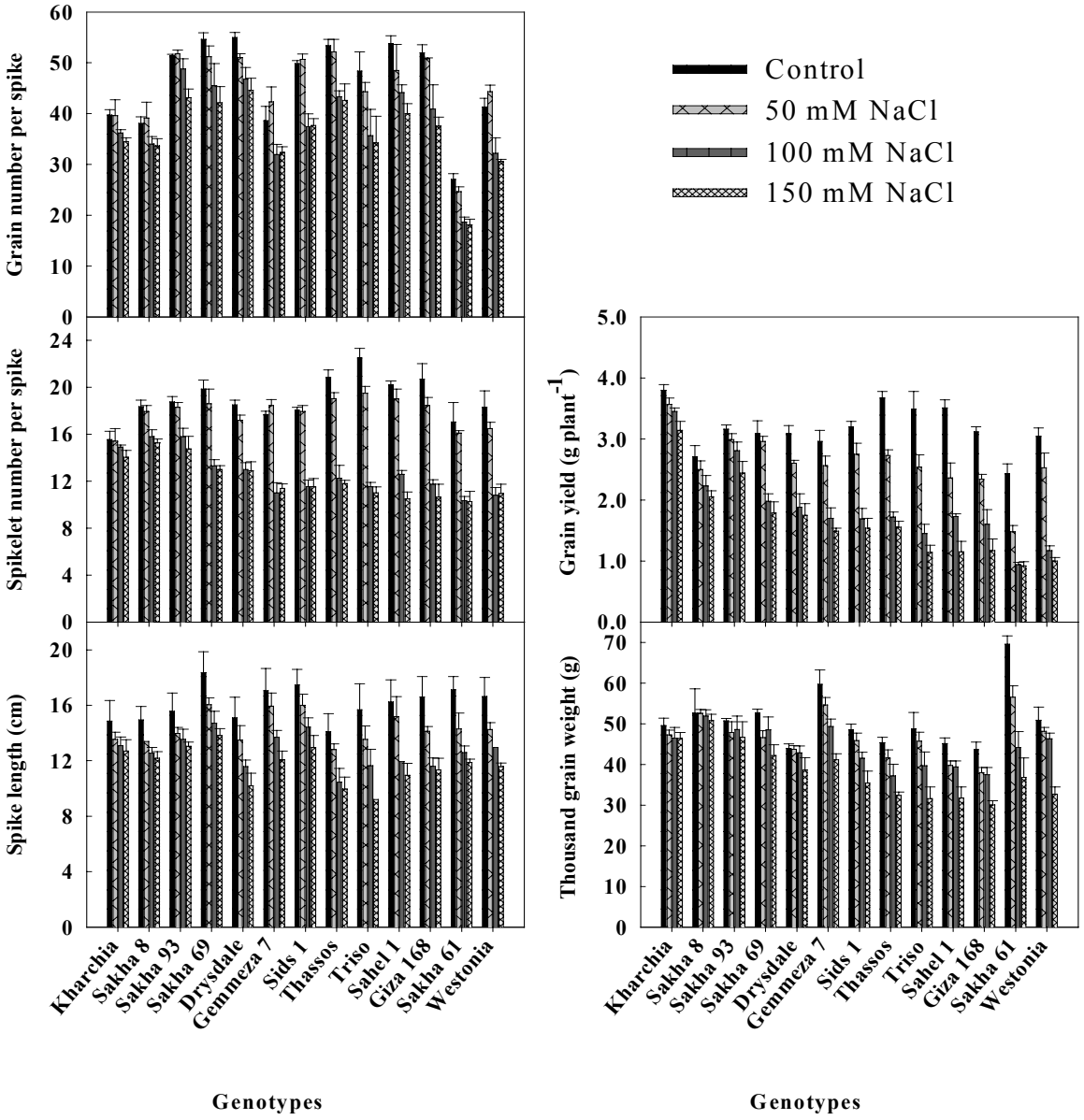

Fig. 2.3 Effect of different salinity levels on yield of main spike (spike length, spikelet number, grain number and thousand grain yield) and total grain yield per plant for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

Table 2.4 Rankings of genotypes for their relative salt tolerance in terms of yield components of main spike (i.e. spike length, spikelet and grain numbers and 1000-Grain weight) in a cluster analysis (Ward's minimum variance analysis).

Genotypes	Salinity levels (mM NaCl)			Sum	Genotypes ranking	Tolerant degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	1	1	3	1	Tolerant
Sakha 93	1	1	1	3	1	Tolerant
Drysdale	2	2	2	6	2	Tolerant
Sakha 69	2	2	2	6	2	Tolerant
Sids 1	1	3	3	7	3	Moderate
Gemmeza 7	1	3	3	7	3	Moderate
Thassos	2	3	4	9	4	Moderate
Sahel 1	3	4	4	11	5	Moderate
Giza 168	3	5	4	12	6	Sensitive
Triso	3	4	5	12	6	Sensitive
Westonia	3	4	5	12	6	Sensitive
Sakha 61	3	5	5	13	7	Sensitive

that genotypes (Kharchia, Sakha 8 and Sakha 93) were ranked as the most tolerant genotypes, whereas genotypes (Giza 168, Triso, Westonia and Sakha 61) were ranked as the least tolerant among all genotypes. The genotypes (Sakha 69 and Drysdale) were intermediate between the most and least tolerant genotypes. The salt tolerance indices of grain yield per plant for genotypes (Kharchia, Sakha 8 and Sakha 93) were at least two times greater than for genotypes (Sids 1, Giza 168, Sahel 1, Thassos, Triso and Sakha 61), the most salt sensitive genotypes, at high salinity (Table 2.1). Based on simultaneous analysis of the means of salt tolerance indices in grain yield per plant using single-linked cluster analysis, the genotypes were divided into four cluster groups at low salinity and five cluster groups at moderate and high salinity. The genotypes (Kharchia, Sakha 8 and Sakha 93) were ranked as the most tolerant genotypes and genotypes (Giza 168, Thassos, Triso, Sahel 1, Westonia and Sakha 61) as the least tolerant among all genotypes (Table 2.5).

Table 2.5 Rankings of genotypes for their relative salt tolerance in terms of grain yield per plant in a cluster analysis (Single –link cluster analysis).

Genotypes	Salinity levels (mM NaCl)			Sum	Genotypes ranking	Tolerant degree
	50	100	150			
Kharchia	1	1	1	3	1	Tolerant
Sakha 8	1	2	2	5	2	Tolerant
Sakha 93	1	2	2	5	2	Tolerant
Sakha 69	1	3	3	7	3	Moderate
Drysdale	1	3	3	7	3	Moderate
Gemmeza 7	2	4	4	10	4	Moderate
Sids 1	2	4	4	10	4	Moderate
Giza 168	3	5	5	13	6	Sensitive
Thassos	3	5	5	13	6	Sensitive
Triso	3	5	5	13	6	Sensitive
Sahel 1	4	5	5	14	7	Sensitive
Westonia	4	5	5	14	7	Sensitive
Sakha 61	4	5	5	14	7	Sensitive

2.4 Discussion

Salt tolerance among wheat genotypes was evaluated in this study using a cluster analysis. As pointed out by Khrais et al. (1998) and Zeng et al. (2002), the advantages of using a multivariate analysis in the evaluation of salt tolerance are that it allows: a) a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking; b) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels, especially when the salt tolerance indices are averaged across salt levels; and c) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels. Because there is variation of salt tolerance among the agronomical parameters and also among the different growth stages for wheat plants, the sensitive parameters, which can be single or multiple parameters, must be identified at different growth stages before using the cluster analysis.

Improving the grain yield of wheat is always the main target in plant breeding. Therefore, the evaluation of final grain yield and growth parameters determining grain yield

is a critical aspect of breeding programs. The final yields of wheat are determined by the number of spikes per plant and yield components such as spikelet number, grain number and grain weight. The number of spikes is highly correlated with the number of tillers. The effect of salinity on tiller number and spikelet number, which both initiate during early growth stages, has a greater influence on final grain yield than on yield components in the later stages (Mass et al., 1983; Mass and Poss, 1989). Among wheat genotypes, however, the salt tolerance also changes at different growth stages (Kingsbury and Epstein, 1984; Ashraf and Waheed, 1993; Zeng et al., 2002).

Vegetative growth of wheat plants is characterized by the tillering and leaf appearance and growth on the tillers. At the vegetative growth stage, therefore, the three agronomic parameters (i.e., tiller number, leaf number and leaf area per plant) were used to evaluate genotypes for salt tolerance. Generally, the values of the three agronomic parameters decreased with increasing salinity (Fig. 2.1). However, salt sensitive genotypes showed a greater reduction in tiller number (e.g. by about 41%) than tolerant ones (e.g. by about 11%). This may indicate that tiller number and their behaviour under salinity can be used as simple and non-destructive measurement to evaluate wheat genotypes in breeding programs. Nicolas et al. (1994) found that salt stress during tiller emergence can inhibit their formation and can cause their abortion at later stages. When salinity levels are greater than 7.5 dS m^{-1} or 50 mM NaCl, most of the secondary tillers of moderately tolerant genotypes were eliminated, and the number of primary tillers for salt sensitive wheat genotypes was greatly reduced (Eugene et al., 1994). Paradkis (1940) found that high-tillering varieties of wheat had greater grain yield on poor soil than low-tillering ones, whereas low-tillering varieties on rich soil produced as much as or more than the high-tillering ones. Therefore, increasing the salinity tolerance in wheat may require an increase in the capacity of tillering (Islam and Sedgley, 1981).

The various yield components showed different responses to salinity. The TGW was least sensitive to salinity, whereas spikelet number was the most sensitive yield component, which is in agreement with observation in rice (Zeng and Shannon, 2000). Although final yield is directly determined after anthesis, the grain yield can be described in terms of components that are determined sequentially in the course of phenological development (Evans et al., 1975). Grain number is determined during the period of spike emergence to anthesis and grain weight is determined between anthesis and maturity (the least sensitive stage in wheat) (Kirby, 1988; Mass and Grieve, 1990; Frank et al., 1997). Because spikelets

initiate at the vegetative stage, the negative effect of salinity on spikelet number indicates that the number of spikelets per spike together with number of tillers per plant are sensitive parameters at the vegetative stage. This suggests that evaluation for salt tolerance among genotypes can be based on the genetic diversity in tiller and spikelet numbers. Another advantage is that the tiller number, together with spikelet number can again be used as a simple and non-destructive measurement to evaluate large number of wheat genotypes in breeding programs; especially, because the two parameters can be determined at early growth stages.

When the developmental pattern of genotypes is so different between growth stages, assessment of the actual salt tolerance of the genotypes may be determined by comparisons of their biomass production over a long growth period (Leland et al*.,* 1994; Munns et al., 2000), which therefore serve as another criterion to evaluate the salt tolerance. The results in this study indicate that the ranking among genotypes for salt tolerance based on the DW per plant at different growth stages was close to that based on agronomic parameters at the vegetative stage (Tables 2.2 and 2.3). This indicates that the reduction in DW was closely related to those in tiller and leaf number and leaf area (Hu et al., 1997). The reduction in total biomass in the sensitive genotypes was probably due to the extra energy utilization for osmotic accumulation, which is much more ATP consuming for osmotic adjustment (Wyn Jones and Gorham, 1993).

A similar salt tolerance at different growth stages was observed in genotypes (Kharchia, Sakha 8 and Sakha 93). The characteristics of these genotypes are more tillers, higher leaf number and greater leaf area compared with other genotypes, less effect of salinity on final grain yield and the yield components of the main spike, and the salt tolerance of these genotypes remained almost unchanged at the different growth stages. Therefore, these characters of salt tolerant genotypes should be introduced in a cross breeding programs as an elite salt tolerance germplasm to incorporate different desirable agronomic traits. In addition, although the Indian wheat genotype (Kharchia) is the most salt tolerant one, the Egyptian genotypes (Sakha 8 and Sakha 93) have the same salt tolerant characters. Compared to genotypes (Sakha 8 and Sakha 93), however, Kharchia shows higher yield under non-saline conditions. Working with appropriate breeding programs, which aim to increase the yield in genotypes (Sakha 8 and Sakha 93), may be more meaningful than working with Kharchia. A change in salt tolerance with growth stages was observed for genotypes (Sids 1,

Gemmeza 7 and Westonia). Sids 1 and Gemmeza 7 were ranked as having intermediate salt tolerance based on the parameters of yield components of the main spike and final grain yield (Tables 2.4 and 2.5), which may also be resulted from a slight decrease in the values of EC with growing time due to the salt uptake of plant, whereas they were ranked as having poor salt tolerance based on the parameters at the vegetative stage and total DW (Tables 2.2 and 2.3). The opposite trend was observed in genotype (Westonia). Therefore, the results suggest that it might be possible to improve the salt tolerance of genotypes (Sids 1 and Gemmeza 7) for salt tolerance by increasing tillering ability and/or by irrigating more frequently to alleviate soil salt stress at early growth stages. The genotypes (Giza 168, Triso, Thassos, Sahel 1 and Sakha 61) were more sensitive at all growth stages. Furthermore, genotypes (Drysdale and Sakha 69) were more sensitive at moderate and high salinity levels and, to become more tolerant at low salinity levels, it is suggested that maintaining the salinity at low levels is an important strategy for improving the growth of these two genotypes. Because the Australian genotype (Drysdale) is also drought-tolerant, Sakha 69 may possibly be used for drought tolerant genotypes in Egypt because both showed similar characters in this study.

In conclusion, because genotypes (Kharchia, Sakha 8 and Sakha 93) were identified as the most salt tolerant genotypes in the cluster analysis, they can be utilized through appropriate selection and breeding programs for further improvement in salt tolerance of Egyptian wheat genotypes. Because genotypes (Sids 1 and Gemmeza 7) were more sensitive to salinity at early growth stages and more tolerant at the later stages, their salt tolerance can be improved by developing strategies for agronomic management according to the different growth stages, indicating that the degree of salt tolerance of wheat genotypes to salinity must be evaluated according to different growth stages. When a large number of genotypes have to be evaluated in salt tolerance breeding by using multiple agronomic parameters, cluster analysis can be used to facilitate the ranking of the genotypes for salt tolerance.

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3 Validity of various physiological traits as screening criteria for salt tolerance in wheat genotypes

Abstract

Success of improving the salt tolerance of genotypes requires effective and reliable screening traits in breeding programs. Since no single process can account for the variation of plant response to salinity, combined physiological traits could be reliable and feasible as screening criteria for salt tolerance. Thirteen wheat genotypes from Egypt, Germany, Australia and India were grown in soil with four salinity levels (control, 50, 100 and 150 mM NaCl) in a greenhouse. The physiological traits (ion contents in leaves and stems (Na^+, Cl^-, K^+, Ca^{2+}), the ratios of K^+/Na^+ and Ca^+/Na^+ in the leaves and stem, net photosynthesis rate, stomatal conductance, respiration rate, transpiration rate, SPAD value, leaf water relations) were measured at different growth stages in order to identify the validity of them in genotypic variation and their association with the salt tolerance in terms of grain yield. The interaction between salinity and genotypes was highly significant for most parameters. Grain yield and the tested physiological traits were scored based on the salt tolerance indices at 150 mM NaCl and then ranked. The physiological traits except for Na^+ and Cl^- in stems and leaf transpiration rate at 150 mM NaCl showed a significant genotypic variation, indicating the traits that have a significant genotypic variation may be possibly used as screening criteria. However, this study also showed that some traits in genotype Westonia were not associated with its salt tolerance in terms of grain yields. Thus, combined physiological traits such as Na^+ and Cl^- excluder traits along with ionic selectivity or photosynthetic and water relation parameters should be considered in screening salt tolerance of wheat genotypes rather than only single specific physiological traits. According to the analysis of the linear regression of the scores of the physiological traits against those of grain yield, the slopes for the traits of K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+, Na^+ and Cl^- in leaves, photosynthesis rate, stomatal conductance, and SPAD value were greatest. From a practical point of view, however, the traits of net photosynthesis rate, stomatal conductance and SPAD value should be considered to use as screening criteria. Since the Indian genotype, Kharchia, is more tolerant to salinity than Egyptian ones, the results here may suggest that Kharchia is a good donor to increase the salt tolerance for Egyptian wheat genotypes in breeding programs.

3.1 Introduction

35-50% of the world's population in about 80 countries is in semiarid area where salinization is a major problem. To solve the world's food problem, therefore, an increase in the food production in semiarid regions is particularly important. The salinity problem is most extreme in semiarid regions because precipitation is infrequent there, soils have some natural salinity, irrigation is required, and evaporation is relatively high. A typical country in semiarid areas is Egypt. Approximately one million of 3.2 million ha cultivated land in Egypt is already salinized (Ghassemi et al., 1995). Egypt has low and fluctuating precipitation (5-200 mm/year), high surface evaporation (1500-2400 mm/year) and high temperature (Attia, 1997). In addition, the secondary salinization in fields has been also occurring due to irrigation with brackish water or with saline groundwater along with poor drainage systems in most areas (El-Saidi, 2002). Both leaching salt from soil surface and genetic improvement of salinity tolerance in current genotypes (Kingsbury and Epstein, 1984; Shannon, 1997) have been proposed as the most effective strategies to solve the salinity problems. Although leaching salt from soil surfaces can ameliorate salt stress, it is not feasible on a large scale in semiarid regions due to the lack of good quality water resources, low soil permeability, and high cost of amendments (Qureshi et al., 1990). Therefore, the improvement of current genotypes to be more salt tolerance is an alternative option to solve salinity problems in order to meet a continuing increases in the food demand in those regions (Zeng et al., 2002). Unfortunately, improving salt tolerance of genotypes is often inhibited by the lack of effective evaluation methods for salt tolerance among genotypes (Shannon, 1997; Zahid et al., 2002). Therefore, it is very important to develop an effective evaluation approach for screening salt tolerant genotypes, which should be reliable, quick, easy, practical and economic. Therefore, our objectives were to test the validity of different physiological traits as screening methods, which should be reliable and practical for evaluating genotypic variation in salt tolerance.

Screening large numbers of genotypes for salinity tolerance in the field is difficult due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctuations in rainfall (Srivastava and Jana, 1984; Yeo et al., 1990; Munns and James, 2003). Significant genetic variation for salt tolerance might exist, but the confounding presence of drought stress makes it difficult to identify genotypes with salt tolerance. Screening techniques have, therefore, often been used under controlled environments. Because of the complex nature of salt tolerance, as well as the difficulties in maintaining long-term growth experiments,

physiological traits as selection criteria are recommended for screening (Yeo et al., 1990; Noble and Rogers, 1992), which have been considered as more reliable and feasible to screen for specific traits rather than salt tolerance itself in terms of biomass or yield in saline soil (Munns and James, 2003). Salinity directly causes ion toxicity and imbalance and indirectly leads to water deficit and low photosynthesis for plants. Thus, physiological traits used for screening germplasm for salinity tolerance have included Na^+ and Cl^- exclusion (Rogers and Noble, 1992; Garcia et al., 2002), and K^+/Na^+ or Ca^{2+}/Na^+ discrimination (Asch et al., 2000; Zeng et al., 2003). Specific traits such as Na^+ exclusion can be subject to less environmental influence than growth rates (Munns and James, 2003). The work in the literature has shown that the effect of salinity on photosynthesis rate (A), stomatal conductance (g_S) and transpiration rate (E) (Sergey et al., 1998; James et al., 2002), leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and turgor pressure (T_p) (Guerrier, 1996; Rivelli et al., 2002) are also associated with the plant salt tolerance. The benefit of evaluating salt tolerance based on physiological traits has recently been proposed due to the availability of new devices like SPAD meter for specific traits determination that are more quick and easy to use in the field measurements. Screening for specific physiological traits can reduce the time needed to grow plants under salinity and can eliminate the need to grow plants under controlled conditions (Munns et al., 1995; Munns and James, 2003). Thus, it can be a quick, easy and economic technique. However, using specific physiological traits or single trait in breeding programs has not until now been as good as expected (Jackson et al., 1996) because no single process can account for the variation in the plant response to salinity due to effects of soil properties, the osmotic effect of high ionic concentrations, competitive interference with nutrient uptake and toxic effects within the plant tissue. Thus, a combination of physiological traits is logically a desirable objective in screening for salt tolerance of genotypes. To have effective and reliable techniques for using physiological traits, however, it is still not clear how many physiological traits should be used and how those physiological traits could optimally be combined.

Furthermore, physiological characters such as ion accumulation and water relations and photosynthesis change with the growth stages; ion accumulation varies in the different organs of plants. In order to find out what time and which organs can be chosen for using the physiological traits of ion effects to screen genotypic variation, therefore, the physiological traits at different growth stages and in different plant organs should be evaluated as well.

The objectives of this study were to evaluate the association of the physiological traits of wheat such as ion contents in leaves and stems, photosynthetic parameters, SPAD value and water relation parameters at different growth stages with the salt tolerance in terms of grain yield, to find out the reliable multiple physiological traits that can be used as quick, easy and economic technique as screening criteria, and to obtain good donor genotypes of salt tolerance for improving Egyptian wheat genotypes in breeding programs in comparison with the most salt tolerant genotypes from India and Australia.

3.2 Materials and methods

3.2.1 Plant materials

Thirteen varieties of spring wheat (*Triticum aestivum* L.) from different countries were used in this study. Eight varieties (Sakha 8, Sakha 93, Sakha 61, Sakha 69, Giza 168, Sids 1, Sahel 1 and Gemmeza 7) were obtained from Agricultural Research Centre, Giza, Egypt. Sakha 8 and Sakha 93 are usually cultivated in saline areas in Egypt. Thassos and Triso were from Germany, Westonia and Drysdale were from Australia, and Kharchia was from India.

Kharchia is the most tolerant wheat genotype, and is used as a standard for the salt tolerance test of wheat worldwide (Sharma et al., 1994; Ashraf, 2002).

3.2.2 Growth conditions

This study was carried out in a greenhouse from the middle of March to the middle of August 2002. The air temperature ranged from 23 to 28°C during the day and 15 to 18°C during the night. Relative humidity fluctuated between 45 and 85% at day/night.

Loamy soil was collected from the soil surface (0-15 cm). The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The soil consisted of 23% clay, 48% silt and 29% sand, and the organic matter content was 1.66%. The initially air-dried soil with 9% gravimetric water content was filled layer-wise in four layers in 7-l pots without a leaching possibility.

Four salt levels (control (no added NaCl), 50, 100 and 150 mM NaCl) in the soil were applied. The salinity levels of 50, 100 and 150 mM NaCl in soil solution were equivalent to an electrical conductivity of 8, 13, and 17 dS m^{-1}, respectively, which were measured at the beginning of the experiment. During the period of the experiment, the electrical conductivity

at each salinity level slightly decreased due to the uptake of salt by plants. At the end of the experiment, the electrical conductivity was changed to 5.2, 10, and 14 dS m^{-1}, respectively The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinized until 10 days after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to twenty per pot.

The N, P and K were initially applied as 0.2 g NH_4NO_3 and as 0.2 g KH_2PO_4 per pot. The same amount of N, P and K was applied another three times at 20, 40 and 60 days after sowing. During the experiment, the pots were weighed daily and the water loss was replaced by adding tap water when the total amount of the water lost was around 200 g for plants to avoid suffering either drought or flooding. All treatments were replicated four times.

3.2.3 Analysis of ion concentrations

Oven dried samples of leaves and stems of plant at 45 days after sowing and at final harvest was ground into fine powder by passing through a 0.5-mm diameter sieve. The concentration of mineral elements in leaves and stems were measured as follows:

For the determination of Na^+, K^+ and Ca^{2+} contents, 300 mg ground dry material of stems or leaves was digested by adding 3 ml concentrated HNO_3 (65%) and 2 ml H_2O_2 (30%) for 30 min at 2600 kpa (80 psi) in a MDS-2100 microwave oven (CEM Corp., Matthews, NC). After digestion, each sample was then brought up to 50 ml final volume with distilled-deionized water. The concentration of Na^+ was determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia). The K^+ and Ca^{2+} contents were determined with a flame photometer (ELEX 6361, Eppendorf, Netheler-Hinz GmbH., Germany).

For Cl^-, 100 mg of ground samples were extracted with 100 ml distilled water and were shaken for one hour and then filtered. Chloride was determined using an ion chromatography analyzer (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA).

3.2.4 Photosynthetic parameter measurements

Photosynthesis rate (A), stomatal conductance (g_s), respiration rate (R) and transpiration rate (E) were determined on the second fully expanded youngest leaf at 45 and 60 days after

sowing. Measurements were made with a LI-COR 6400 portable gas exchange system (Analytical Development Company, England). Because the leaf did not fill the leaf chamber, the leaf area was determined independently and photosynthetic parameters were estimated with a re-computation program (LI-COR, Lincoln, NE). Measurements were conducted in a growth chamber during the light period. Plants were transferred into a growth chamber with air temperature of 25°C, photosynthetic photon flux density of 1150 μmol m^{-2} s^{-1} and CO_2 was set at 400 μmol mol^{-1}, one day before the measurements of photosynthesis were performed.

3.2.5 Leaf chlorophyll measurement

Leaf chlorophyll content was determined using a hand-held SPAD 502 meter (Minolta, Osaka, Japan). Average SPAD chlorophyll readings were calculated from five measurements from the leaf tip to the leaf base. The measurement was made at 45 and 60 days after sowing.

3.2.6 Water relation measurements

Leaf water potential (Ψ) and osmotic potential (Ψ_π) from the middle of the second youngest fully developed leaf blade were measured for two times at 45 and 60 days after sowing. Ψ was measured with a pressure bomb (PMS Instrument Co., model 1002, Corvalis Co., Oregon, USA) according to the technique followed by Scholander et al., (1965). Immediately after Ψ was determined, the same leaf material was frozen in dry ice. The leaf samples were thawed at room temperature placed in a syringe, the leaf sap was expressed under pressure and then Ψ_π was determined with a vapour pressure osmometer (Wescor 5100C, Wescor Inc., Logan, USA). Turgor pressure (T_p) was estimated as the difference between Ψ_π and Ψ.

3.2.7 Ranking and scoring of genotypes for salt tolerance

In order to allow comparisons among genotypes, a salt-tolerant genotype, Kharchia, was chosen as a reference, i.e., a standard against which all the other genotypes were compared. Thus, the measurements of the plants from the other genotypes in each salinity treatment were divided by the means of the reference in the same salinity level to convert to relative values, i.e., the salt tolerance indices. The indices were then used to score and rank the genotypes. Genotypes were classified into five classes according to the formula: Number of classes = $1 + 3.3 \log_{10} n$, where n is the number of tested genotypes (Josef, 1985). The class

intervals of indices were defined as the difference between high and low salt indices divided by the number of class. Scores were assigned to the class intervals from the highest to the lowest in grain yield, K^+ and Ca^{2+}contents in leaves and stems, the ratios of K^+/Na^+ and Ca^{2+}/Na^+ in leaves and stems, *A*, g_S, SPAD value and T_p or from the lowest to the highest in Na^+, Cl^-, *E*, *R*, Ψ, and Ψ_π.

3.2.8 Statistical analysis of data

A factorial experimental design with 13 genotypes and four salinity levels was arranged in a completely randomized design with 4 replications. Data were analyzed through ANOVA tests, using COSTAT Version 3.03 (software, Berkeley, CA 94701). Relationships between the scores of grain yield and the scores of different physiological parameters were analysed by simple linear regression by using JMP user's Guide (SAS institute, 2000).

3.3 Results and discussion

Highly significant differences among salinity levels and genotypes ($P < 0.001$ or $P < 0.01$) in grain yield and the physiological parameters, except Ca^{2+}/Na^+ ratio in leaves at final harvest, among genotypes were observed in this study (Tables 3.1-3.3). The interaction between salinity and genotypes was also highly significant ($P < 0.001$ or $P < 0.01$) for most parameters, except for the Ca^{2+}/Na^+ in leaves and stems at final harvest, indicating there are differential responses of genotypes to salinity from low to high levels (Tables 3.1-3.3). Thus, the relationships between the physiological traits and salt tolerance (i.e. grain yield) at 150 mM NaCl are presented and discussed here.

In order to evaluate and select reliable physiological parameters to use for screening criteria for salt tolerance of wheat genotypes, therefore, an objective measure based on the grain yield was included in Tables 3.4-3.7. Grain yield of genotypes were scored based on the salt tolerance indices at 150 mM NaCl (Tables 3.4-3.7). The smallest number of score was ranked at the top, representing the most salt tolerant genotype, and the largest number of score was ranked at the bottom. The salt tolerance of genotypes decreases with increasing number of scores. Since Kharchia was used as the standard, i.e., as salt tolerance reference, the number of scores for Kharchia was one for grain yield (Tables 3.4-3.7). Among the Egyptian wheat genotypes (Sakha 8 and Sakha 93) were the most salt tolerant genotypes compared with others. The genotypes (Giza 168 and Sakha 61) were the most salt sensitive

Table 3.1 Mean squares and F-tests of main effects of salinity and genotypes and their interactions for grain yield and ion contents in leaves at 45 days after sowing and at final harvest.

Source•	df	Grain yield	Ion content in leaves at day 45						Ion content in leaves at final harvest					
			Na^+ x10^4	Cl^- x10^4	K^+ x10^4	Ca^{2+} x10^4	K^+/Na^+ x10^2	Ca^{2+}/Na^+	Na^+ x10^4	Cl^- x10^4	K^+ x10^4	Ca^{2+} x10^4	K^+/Na^+ x10^2	Ca^{2+}/Na^+
Salinity (S)	3	25.9^{***}	301.4^{***}	1237^{***}	136.6^{***}	31.6^{***}	15.3^{***}	146.7^{***}	7925.6^{***}	6691^{***}	202.8^{***}	88.2^{***}	21.4^{***}	723.1^{***}
Genotype (G)	12	4.02^{***}	21.5^{***}	44.8^{***}	33.2^{***}	2.7^{***}	0.29^{***}	1.76^{***}	112.6^{***}	314.6^{***}	22.12^{***}	13.4^{***}	0.03^{*}	0.56^{NS}
S x G	36	0.38^{***}	4.11^{***}	6.1^{***}	2.1^{***}	0.19^{***}	0.06^{***}	0.36^{***}	32.5^{***}	51.6^{***}	2.7^{***}	0.91^{***}	0.04^{***}	0.67^{NS}

• The main effect of salt were tested using the first order interaction, replicate x salt, as the error term. The main effect of genotype and the interaction between salt and genotype were tested using the highest order interaction, replicate x salt x genotypes, as the error term.

^{NS} , **,*** Not significant, significant at 0.05 and significant at 0.001probability level, respectively, in *F*-test.

Table 3.2 Mean squares and F-tests of main effects of salinity and genotypes and their interactions for grain yield and ion contents in stems at 45 days after sowing and at final harvest.

Source[•]	df	Grain yield	Ion content in stems at day 45						Ion content in stems at final harvest					
			Na^+ x10^4	Cl^- x10^4	K^+ x10^4	Ca^{2+} x10^4	K^+/Na^+ x10^2	Ca^{2+}/Na^+	Na^+ x10^4	Cl^- x10^4	K^+ x10^4	Ca^{2+} x10^4	K^+/Na^+ x10^2	Ca^{2+}/Na^+
Salinity (S)	3	25.9^{***}	321.5^{***}	624^{***}	135.3^{***}	4.54^{***}	21.7^{***}	27.13^{***}	6018.6^{***}	1655^{***}	182.2^{***}	10.5^{***}	60.7^{***}	94.4^{***}
Genotype (G)	12	4.02^{***}	15.5^{***}	11.6^{***}	22.1^{***}	2.15^{***}	0.09^{***}	0.61^{***}	100.58^{***}	9.0^{***}	11.7^{***}	1.21^{***}	0.03^{***}	0.07^{**}
S x G	36	0.38^{***}	3.62^{***}	1.8^{***}	2.7^{***}	0.09^{***}	0.04^{**}	0.07^{***}	23.24^{***}	1.5^{***}	2.54^{***}	0.13^{***}	0.04^{***}	0.03^{NS}

[•] The main effect of salt were tested using the first order interaction, replicate x salt, as the error term. The main effect of genotype and the interaction between salt and genotype were tested using the highest order interaction, replicate x salt x genotypes, as the error term.

^{NS} , **,*** Not significant, significant at 0.01 and significant at 0.001probability level, respectively, in *F*-test.

Table 3.3 Mean squares and F-tests of main effects of salinity and genotypes and their interactions for net photosynthesis rate (*A*), stomatal conductance (g_S), respiration rate (*R*), transpiration rate (*E*), chlorophyll content (SPAD value), leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and turgor pressure (T_p) at 45 and 60 days after sowing.

Source•	df	Photosynthetic and water relation parameters at day45								Photosynthetic and water relation parameters at day 60							
		A x10^2	g_S x10^4	*R* x10^2	*E* x10^2	SPAD x10^2	Ψ	Ψ_π	T_p	*A* x10^2	g_S x10^4	*R* x10^2	*E* x10^2	SPAD x10^2	Ψ	Ψ_π	T_p
Salinity (S)	3	9.7^{***}	40^{***}	3.2^{***}	1.2^{***}	0.41^{***}	6.7^{***}	12.3^{***}	0.8^{***}	18.2^{***}	54.1^{***}	6.2^{***}	3.6^{***}	8.6^{***}	9.4^{***}	17.8^{***}	2.2^{***}
Genotype (G)	12	1.6^{***}	3.1^{***}	0.81^{***}	0.3^{***}	2.5^{***}	0.9^{***}	0.14^{***}	0.4^{***}	2.4^{***}	3.88^{***}	1.3^{***}	0.43^{***}	6.8^{***}	1.1^{***}	0.7^{***}	0.2^{***}
S x G	36	0.2^{***}	0.7^{***}	0.1^{***}	0.01^{***}	0.21^{***}	0.11^{***}	0.03^{***}	0.09^{***}	0.4^{***}	0.49^{***}	0.17^{***}	0.01^{***}	0.5^{***}	0.12^{***}	0.09^{***}	0.01^{**}

• The main effect of salt were tested using the first order interaction, replicate x **salt, as the error term. The main effect of genotype and the interaction between salt and genotype were tested using the highest order interaction, replicate** x **salt** x **genotypes, as the error term.**

**,*** Significant at 0.01 and significant at 0.001probability level, respectively, in *F*-test.

genotypes. The genotypes (Sakha 8 and Sakha 93) were ranked as number 2 compared with number 1 for genotype (Kharchia), suggesting there is a potential to improve the salt tolerance of Egyptian wheat genotypes by using Kharchia as donor. German genotypes, (Thassos and Triso), and Australia genotypes (Drysdale and Westonia) also showed a genotypic difference in salt tolerance. Drysdale from Australia were moderately tolerant to salinity, and Triso from Germany and Westonia from Australian were most sensitive to salinity according to their scores on grain yield.

In order to find out the association of physiological traits with the plant tolerance objective (grain yield), ion contents in leaves and stems at different harvests, and leaf photosynthesis and water relations measured at different sampling times at 150 mM NaCl were also scored according to the salt tolerance indices, and the results are presented in Tables 3.4-3.7. The relationships between the scores of physiological traits and grain yield were further analyzed using linear regression (Tables 3.8-3.10). If the regression coefficient is significant, the slope of the equation may reflect the degree of genotypic variation. The slope with a higher value may indicate a greater variation among genotypes than those with smaller values. In general, the scores on ion contents in leaves, leaf net photosynthesis rate (A), stomatal conductance (g_S), chlorophyll content (SPAD value), leaf water potential (Ψ), and leaf turgor pressure (T_p), regardless of measuring time and leaf osmotic potential (Ψ_π) at day 60 were significantly correlated with the scores on grain yield, indicating that any of these parameters may be used as screening criteria for the salt tolerance of wheat genotypes (Tables 3.8-3.10). However, Na^+ and Cl^- in stems and leaf transpiration rate (E), regardless of measuring time, were not significantly correlated with grain yield, suggesting that the investigated organs can be important factors limiting the evaluation of salt tolerance. The correlations between the numbers of scores on grain yield and on physiological traits among the genotypes indicate genotypic variation and the possible use of physiological traits as screening criteria of salt tolerance. For some genotypes, however, their physiological traits could not be well associated with salt tolerance (Tables 3.4-3.7). For instance, genotype (Westonia) was classified as most sensitive to salinity according to its scores on grain yield, but Na^+ accumulation in plant was scored as the number 1 in leaves and stems at both sampling times. The scores on Cl^- accumulation in plants were ranked between number 1 and 4. Thus, the physiological traits should be further evaluated for their effectiveness and reliability in order to determine whether

Table 3.4 Scores among wheat genotypes for their relative salt tolerance at 150 mM NaCl on ion contents in leaves at day 45 and final harvest and on grain yield.

Genotypes	Ion contents in leaves at day 45						Ion contents in leaves at final harvest						Grain yield
	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	
Kharchia	1	1	1	1	1	1	1	1	1	1	1	1	**1**
Sakha 8	1	1	1	1	1	1	1	1	1	2	1	1	**2**
Sakha 93	1	1	1	1	1	1	1	1	1	2	1	1	**2**
Sakha 69	2	3	3	3	4	4	2	2	2	3	3	3	**3**
Drysdale	2	3	2	3	4	4	2	4	2	2	3	3	**3**
Gemmeza 7	3	5	4	5	5	5	4	3	5	4	5	4	**4**
Sids 1	3	5	5	5	4	5	4	4	4	4	5	4	**4**
Thassos	4	5	5	5	5	5	5	4	5	4	5	5	**4**
Triso	5	5	5	5	5	5	5	5	5	5	5	5	**5**
Sahel 1	3	5	4	5	5	5	4	4	4	5	5	5	**5**
Giza 168	5	5	5	5	5	5	5	5	5	5	5	5	**5**
Sakha 61	5	5	5	5	5	5	5	4	5	5	5	5	**5**
Westonia	1	3	4	4	3	4	1	4	5	5	4	4	**5**

Table 3.5 Scores among wheat genotypes for their relative salt tolerance at 150 mM NaCl on ion contents in stems at day 45 and final harvest and on grain yield.

Genotypes	Ion contents in stems at day 45						Ion contents in stems at final harvest						Grain yield
	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	Na^+	Cl^-	K^+	Ca^{2+}	K^+/Na^+ ratio	Ca^{2+}/Na^+ ratio	
Kharchia	1	1	1	1	1	1	1	1	1	1	1	1	**1**
Sakha 8	1	1	1	1	1	1	1	1	1	1	1	1	**2**
Sakha 93	1	1	1	1	1	1	1	1	1	1	1	1	**2**
Sakha 69	1	2	4	4	3	4	1	1	2	4	2	3	**3**
Drysdale	1	2	4	4	3	4	1	1	2	4	2	4	**3**
Gemmeza 7	3	2	5	5	5	5	4	1	4	4	5	5	**4**
Sids 1	3	2	4	5	4	5	3	1	4	4	4	4	**4**
Thassos	3	5	4	5	5	5	3	5	4	5	4	5	**4**
Triso	5	5	4	5	5	5	5	5	5	5	5	5	**5**
Sahel 1	5	2	4	5	5	5	5	1	4	5	5	5	**5**
Giza 168	5	2	5	5	5	5	5	1	4	5	5	5	**5**
Sakha 61	3	2	5	5	5	5	4	1	5	5	5	5	**5**
Westonia	1	1	4	5	2	4	1	1	5	5	2	4	**5**

Table 3.6 Scores among wheat genotypes for their relative salt tolerance at 150 mM NaCl on net photosynthesis rate (*A*), stomatal conductance (g_S), respiration rate (*R*), transpiration rate (*E*), and chlorophyll content (SPAD value) at 45 and 60 days after sowing and on grain yield.

Genotypes	Photosynthesis parameters and SPAD value at day 45					Photosynthesis parameters and SPAD value at day 60					Grain yield
	A	g_S	*R*	*E*	SPAD	*A*	g_S	*R*	*E*	SPAD	
Kharchia	1	1	1	3	1	1	1	1	3	1	**1**
Sakha 8	1	1	1	3	1	2	2	1	3	2	**2**
Sakha 93	1	1	1	3	1	2	2	1	3	2	**2**
Sakha 69	1	1	1	4	1	3	3	2	3	3	**3**
Drysdale	1	1	2	1	1	3	3	2	1	3	**3**
Gemmeza 7	3	3	4	3	2	5	4	5	3	4	**4**
Sids 1	2	3	2	4	3	4	4	3	3	3	**4**
Thassos	3	2	3	5	5	5	5	5	5	5	**4**
Sahel 1	3	3	3	1	2	5	5	4	1	4	**5**
Triso	4	5	5	5	5	5	5	5	5	5	**5**
Giza 168	5	5	5	4	5	5	5	5	3	5	**5**
Sakha 61	5	5	5	4	5	5	5	5	4	5	**5**
Westonia	2	2	2	2	3	5	4	5	3	5	**5**

Table 3.7 Scores among wheat genotypes for their relative salt tolerance at 150 mM NaCl on leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and leaf turgor pressure (T_p) at 45 and 60 days after sowing and on grain yield.

Genotypes	Water relation parameters at day 45			Water relation parameters at day 60			Grain yield
	Ψ	Ψ_π	T_P	Ψ	Ψ_π	T_P	
Kharchia	1	1	1	1	1	2	**1**
Sakha 8	1	1	1	1	1	1	**2**
Sakha 93	1	1	2	1	1	1	**2**
Sakha 69	3	1	3	3	3	2	**3**
Drysdale	3	2	3	2	3	1	**3**
Gemmeza 7	5	3	5	4	5	3	**4**
Sids 1	4	3	3	4	3	3	**4**
Thassos	5	5	4	5	5	4	**4**
Sahel 1	3	2	3	4	4	2	**5**
Triso	5	5	5	5	5	4	**5**
Giza 168	5	3	5	5	5	3	**5**
Sakha 61	5	3	5	5	5	4	**5**
Westonia	5	1	5	5	4	5	**5**

only single specific physiological trait or multi-traits will be needed as screening criteria for salt tolerance in wheat genotypes.

3.3.1 Traits of Na^+ and Cl^- exclusion

Traits used for screening germplasm have included Na^+ exclusion (Yeo and Flowers, 1986) and Cl^- exclusion (Rogers and Nobel, 1992). It is more reliable and feasible to screen for specific traits rather than salt tolerance itself because traits such as Na^+ exclusion can be subject to less environmental influences than growth rates (Munns and James, 2003). In this study, salt tolerance for most salt tolerant genotypes (Kharchia, Sakha 8 and Sakha 93) were associated with the characters of Na^+ and Cl^- exclusion in leaves, which is in agreement with the work in the literature (Kingsbury and Epstein, 1984; Schachtman and Munns, 1992; Dvorak et al., 1994; Chhipa and Lal, 1995; Asch et al., 2000; Zhu et al., 2001; Munns and James, 2003). Several mechanisms may control leaf Na^+ accumulation in salt tolerant genotypes. The net uptake of Na^+ and Cl^- may be controlled by the roots, by the net loading of Na^+ and Cl^- in the xylem, and/or the removal of Na^+ and Cl^- by the leaf sheath. Since the scores on Na^+ and Cl^- in stems for most of the salt tolerant genotypes were low, i.e., the stems did not store more Na^+ and Cl^- compared with leaves, the mechanism of control of net Na^+ and Cl^- accumulation in leaves may be only due to the higher selectivity of the roots and/or to low net loading of Na^+ and Cl^- in the xylem. Interestingly, the salt tolerance of the salt-sensitive genotype (Westonia) was not associated with its scores on Na^+ accumulation in leaves and stems, i.e., Westonia has also the character of Na^+ exclusion in plants (Tables 3.4 and 3.5). This may suggest that when specific traits of Na^+ or Cl^- exclusion in leaves are used for screening criteria of salt tolerance of wheat genotypes, other physiological traits should be included, which may be able to show a better association with salt tolerance of Westonia as well.

Because Na^+ and Cl^- accumulation in stems regardless of measuring time was not significantly correlated with the grain yield, Na^+ and Cl^- accumulation in leaves will be recommended as screening criteria (Table 3.8). Genotypic variation of Na^+ or Cl^- exclusion in leaves was greater at final harvest than at 45 days after sowing. From practical and economic point of view, traits of Na^+ and Cl^- exclusion at early stages should be used since genotypic variation of Na^+ and Cl^- exclusion in leaves in both measuring times were significant.

3.3.2 Trait of ion selectivity

In general, genotypic variation in Na^+ exclusion may result in K^+/Na^+ and Ca^{2+}/Na^+ discrimination. The net ion concentration in plant is the result of ion uptake through selective and non-selective channels in the plant cell membrane and subsequent loading in xylem. In plants, a large number of K^+ selective membrane channels and non-selective cation channels permeable to both K^+ and Na^+ have been identified in different plant species (Amtmann and Sanders, 1999). The results in Tables 3.4 and 3.5 show that the scores of genotypes on K^+ and Ca^{2+} contents in leaves and stems and ratios of K^+/Na^+ and Ca^{2+}/Na^+ in leaves and stems also were largely associated with their scores on grain yield at both harvest. The results in Tables 3.4 and 3.5 demonstrate a significant genotypic difference in K^+ and Ca^{2+} contents in plants and in the discrimination of K^+/Na^+ or Ca^{2+}/Na^+, which is consistent with earlier reports in wheat (Dvorak et al., 1994; Chhipa and Lal, 1995) and rice (Asch et al., 2000; Zhu et al., 2001). The slopes from the linear regression of the scores on K^+ and Ca^{2+} in leaves at both sampling times against the scores on grain yield were greater than those for Na^+ in leaves, indicating a greater genetic difference in ion selectivity of K^+ and Ca^{2+} over Na^+ at 150 mM NaCl in wheat genotypes (Table 3.8). Similarly, Cramer et al. (1994) also found that for maize genotypes, the concentration of K^+ and Ca^{2+} and their ratios over Na^+ were also more related with salt tolerance in two hybrids than traits of Na^+ exclusion. Because of the higher selectivity of Ca^{2+} and K^+ in the most tolerant genotypes, higher Ca^{2+} and/or K^+ over Na^+ in leaves appears to protect the plant from the effects of toxic ions (Rengel, 1992). In fact, it is possible that a high K^+/Na^+ or Ca^{2+}/Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ (Cuin et al., 2003; Mark and Romola, 2003). Thus, the ratios of K^+/Na^+ and Ca^{2+}/Na^+ are important factors to be considered as selection criteria. Although there was no association of Na^+ accumulation in leaves for the salt sensitive genotype (Westonia) its selectivity of Ca^{2+} over Na^+ was strongly associated with its salt tolerance. In contrast with Na^+ and Cl^- in stems, genotypic variation in K^+, Ca^{2+}, K^+/Na^+ and Ca^{2+}/Na^+ was also significant (Table 3.8). Although, genotypic variation in K^+, Ca^{2+}, K^+/Na^+ and Ca^{2+}/Na^+ in leaves and stems in both measuring times is significant, the traits of selectivity of K^+ and Ca^{2+} over Na^+ in leaves at early stages should be used since it is much easier to sample leaves and only significant genotypic variation in leaves for Na^+ and Cl^- exclusion was found.

Table 3.8 Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on ion contents in leaves and stems (Y) at day 45 and final harvest at 150 mM NaCl.

Sampling time	Organs	Parameters	Regression equations	Slope	r^2
Day 45	**Leaves**	**Ca^{2+}/Na^+**	Y = - 0.38 + 1.05 X	1.05	0.78^{***}
Day 45	**Leaves**	**Ca^{2+}**	Y = - 0.28 + 1.03 X	1.03	0.84^{***}
Day 45	**Leaves**	**K^+**	Y = - 0.37 + 0.94 X	0.94	0.84^{***}
Day 45	**Leaves**	**K^+/Na^+**	Y = - 0.24 + 0.94 X	0.94	0.71^{***}
Day 45	**Leaves**	**Cl^-**	Y = - 0.17 + 0.94 X	0.94	0.75^{***}
Day 45	**Leaves**	**Na^+**	Y = 0.67 + 0.23 X	0.23	0.42^{**}
Day 45	**Stems**	**Ca^{2+}/Na^+**	Y = - 0.35 + 1.05 X	1.05	0.78^{***}
Day 45	**Stems**	**K^+**	Y = 0.21 + 0.95 X	0.95	0.50^{***}
Day 45	**Stems**	**Ca^{2+}**	Y = - 0.44 + 0.95 X	0.95	0.96^{***}
Day 45	**Stems**	**K^+/Na^+**	Y = 0.06 + 0.71 X	0.71	0.65^{***}
Day 45	**Stems**	**Na^+**	Y = 0.72 + 0.21 X	0.21	0.14^{NS}
Day 45	**Stems**	**Cl^-**	Y = 0.75 + 0.18 X	0.18	0.18^{NS}
Harvest	**Leaves**	**Ca^{2+}**	Y = - 0.22 + 1.10 X	1.10	0.83^{***}
Harvest	**Leaves**	**K^+/Na^+**	Y = - 0.34 + 1.03 X	1.03	0.84^{***}
Harvest	**Leaves**	**Ca^{2+}/Na^+**	Y = - 0.34 + 1.00 X	1.00	0.89^{***}
Harvest	**Leaves**	**Na^+**	Y = 0.69 + 0.95 X	0.95	0.65^{***}
Harvest	**Leaves**	**K^+**	Y = - 0.69 + 0.94 X	0.94	0.78^{***}
Harvest	**Leaves**	**Cl^-**	Y = - 0.12 + 0.87 X	0.87	0.66^{***}
Harvest	**Stems**	**Ca^{2+}**	Y = - 0.03 + 1.07 X	1.07	0.96^{***}
Harvest	**Stems**	**Ca^{2+}/Na^+**	Y = - 0.33 + 1.03 X	1.03	0.82^{***}
Harvest	**Stems**	**K^+**	Y = 0.47 + 0.89 X	0.89	0.45^{***}
Harvest	**Stems**	**K^+/Na^+**	Y = 0.12 + 0.67 X	0.67	0.69^{***}
Harvest	**Stems**	**Na^+**	Y = 0.90 + 0.21 X	0.21	0.12^{NS}
Harvest	**Stems**	**Cl^-**	Y = 1.14 + 0.01 X	0.01	0.03^{NS}

^{NS} , **,*** Not significant, significant at 0.01 and significant at 0.001probability level, respectively, in *F*-test.

3.3.3 Traits of photosynthetic parameters and SPAD value

Salinity causes not only ion toxicity and imbalance, but it also indirectly leads to low photosynthesis in plants. At low or moderate soil salinity, decreased growth is primarily associated with a reduction in photosynthetic area rather than a reduction in photosynthesis per unit leaf area (Munns, 1993). At high salinity, however, leaf photosynthesis can be reduced by lowered stomatal conductance as a result of water imbalance (Brugnoli and Lauteri, 1991) or by non-stomatal factors may be caused by toxic ions. Evidence in support of this comes from strong negative correlations between ions and photosynthetic activity, where Na^+ and Cl^- has been implicated primarily in crop species such as rice (Yeo et al., 1985) and wheat (Rawson, 1986), and Cl^- in woody perennials such as citrus (Walker et al., 1993) and grapevine (Downton, 1977; Walker et al., 1981). Because photosynthesis, stomatal conductance and chlorophyll content in leaves can be measured by a non-destructive, rapid and easy technique using a porometer and SPAD meter, these physiological traits may be important to be used as screening criteria if they would be closely associated with salt tolerance of genotypes at a given level of salinity. In this study, significant genotypic variation in net photosynthesis rate, stomatal conductance and SPAD value were observed for both sampling times (Table 3.6). However, the genotypic variation was greater at 60 days after sowing than at 45 days. From the economic point of view, the earlier sampling time is better. However, our study suggests that the measurements of photosynthesis, stomatal conductance and SPAD value were more reliable at 60 days after sowing than at 45 days (Table 3.9). Practically, evaluating the genotypes for salt tolerance should be directly made in the field by measuring photosynthesis by porometer and chlorophyll by SPAD meter. Compared with using a porometer to measure the photosynthesis rate and stomatal conductance, the SPAD meter is much more handy and practical for large scale screening when there is a large number of test genotypes to be evaluated through breeding programs. The effectiveness of SPAD meter as a screening method has been examined in a number of studies as an index for response of chlorophyll content to stress. For instance, this technique was used for screening groundnut genotypes for tolerance to iron-deficiency chlorosis (Samdur et al., 2000) and it is also used to estimate tissue tolerance for high Na^+ accumulation (Munns and James, 2003). In both studies, a closer relationship between SPAD value and tolerance to iron-deficiency chlorosis and high Na^+ accumulation were observed. The previous studies also showed that the SPAD value was linearly correlated with maximum

net photosynthesis rate in soybean (Ma et al., 1995), in rice (Laza et al., 1996), and in wheat (Gutierrez-Rodriguez et al., 2000).

Although the transpiration rate is important for controlling the accumulation of salt ions in shoots (Walker et al., 1990; Storey 1995; Moya et al., 1999), the scores among genotypes on leaf transpiration rate were not similar to those on grain yield and the correlation between them was not significant at both sampling times (Tables 3.6 and 3.9). It seems that the transpiration rate as screening criteria may be more important for drought stress than for salt stress, since two genotypes (Drysdale and Sahel 1) with drought tolerance character were ranked as number 1 according to their scores on the transpiration rate compared with other genotypes (Table 3.6). Because one of the stresses caused by salinity is osmotic stress or water deficit, the trait of leaf transpiration in the salt tolerance of genotypes should be improved in order to further increase their salt tolerance.

3.3.4 Traits of leaf water relations

Under saline conditions, low osmotic potentials of the soil solution induce water deficit in plant tissue. As a consequence, the turgor in plants may decrease, resulting from a faster decrease in water potential than in osmotic potential. Salt tolerance of wheat genotypes may also vary with their leaf water relations. Leaf water potential was significantly correlated with grain yield at both sampling times, but it was greater at 45 days after sowing than at 60 days (Table 3.10). Munns (1993) proposed that water deficit in plant occurs before plants suffer from ionic effects (ion toxicity and ion imbalance). Genotypic variation in Na^+ and Cl^- exclusion in leaves was greater in later stages than early stages. Thus, greater genotypic variation in leaf water relations in early stages than in later stages indicates that potentially using leaf water potential as screening criterion may provide the possibility of having a rapid and economic technique. However, there are also some disadvantages, e.g. leaf water potential is sensitive to environmental conditions such as light intensity. Surprisingly, the scores of genotype (Westonia) on leaf water potential were closely associated with those of its grain yield. Thus, successful strategies to establish screening criteria must include physiological traits of leaf water potential along with Na^+ and Cl^- exclusion and/or leaf photosynthesis. Data in Tables 3.7 and 3.10 also show that leaf turgor pressure at 45 days after sowing was similar to leaf water potential at 45 days. However, osmotic potential at 45 days was not suitable to be used as criterion since there was no correlation between osmotic potential and grain yield.

Table 3.9 Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on net photosynthesis rate (*A*), stomatal conductance (g_S), respiration rate (*R*), transpiration rate (*E*), and chlorophyll content (SPAD value) (Y) at 45 and 60 days after sowing at 150 mM NaCl.

Sampling time	Parameters	Regression equations	Slope	r^2
Day 45	**R**	Y = 0.55 + 0.50 X	0.50	0.46^{**}
Day 45	**A**	Y = 0.46 + 0.45 X	0.45	0.47^{**}
Day 45	**g_S**	Y = 0.33 + 0.45 X	0.45	0.46^{**}
Day 45	**SPAD**	Y = 0.35 + 0.45 X	0.45	0.47^{**}
Day 45	**E**	Y = 2.2 + 0.15 X	0.15	0.002^{NS}
Day 60	**A**	Y = - 0.22 + 1.04 X	1.04	0.98^{***}
Day 60	**g_S**	Y = - 0.02 + 0.97 X	0.97	0.97^{***}
Day 60	**SPAD**	Y = - 0.22 + 0.92 X	0.92	0.95^{***}
Day 60	**R**	Y = - 0.19 + 0.91 X	0.91	0.83^{***}
Day 60	**E**	Y = 1.66 + 0.13 X	0.13	0.001^{NS}

^{NS} , **,*** Not significant, significant at 0.01 and significant at 0.001probability level, respectively, in *F*-test.

In conclusion, the tested physiological traits except for the traits of Na^+ and Cl^- in stems and leaf transpiration rate show a significant genotypic variation, indicating the traits, which have a significant genotypic variation, may be possibly used as screening criteria. However, this study also found that some traits in the genotype (Westonia) were not associated with its salt tolerance in terms of grain yields. Thus, the combined physiological traits should be considered in screening salt tolerance of wheat genotype rather than only single specific physiological trait. According to the analysis of linear regression of the scores of the physiological traits against those of grain yield, the slopes for the traits of K^+, Ca^{2+}, K^+/Na^+, Ca/Na^+, Na^+ and Cl^- in leaves, photosynthesis rate, stomatal conductance, and SPAD value are greatest. From a practical point of view, however, the traits of net photosynthesis rate, stomatal conductance and SPAD value should be considered as screening criteria. Since Kharchia is more tolerant to salinity than Egyptian salt tolerant genotypes, the results here may suggest that Kharchia is a good donor to increase the salt tolerance of Egyptian wheat genotypes in breeding programs.

Table 3.10 Equations of linear regression, slopes and regression coefficients between the scores on grain yield (X) and the scores on leaf water potential (Ψ), leaf osmotic potential (Ψ_π) and leaf turgor pressure (T_p) (Y) at 45 and 60 days after sowing at 150 mM NaCl.

Sampling time	Parameters	Regression equations	Slope	r^2
Day 45	**Ψ**	Y = - 0.26 + 0.97 X	0.97	0.88^{***}
Day 45	**T_p**	Y = - 0.18 + 0.86 X	0.86	0.77^{***}
Day 45	**Ψ_π**	Y = 0.74 + 0.21 X	0.21	0.15^{NS}
Day 60	**Ψ**	Y = - 0.21 + 0.92 X	0.92	0.77^{***}
Day 60	**Ψ_π**	Y = - 0.22 + 0.94 X	0.94	0.88^{***}
Day 60	**T_p**	Y = 0.25 + 0.21 X	0.21	0.24^{NS}

^{NS} , **,*** Not significant, significant at 0.01 and significant at 0.001probability level, respectively, in *F*-test.

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4 Growth, ion contents, gas exchange, and water relations of wheat genotypes differing in salt tolerance

Abstract

Although the mechanisms of salt tolerance in plants have received much attention for many years, the differences in salt tolerance among genotypes still remain uncertain. To investigate factors determining salt tolerance differences among wheat genotypes and their relation to salt stress, thirteen genotypes from Egypt, Germany, Australia and India that differ in degree of salt tolerance were grown in soil with four salinity levels (control, 50, 100 and 150 mM NaCl) in a greenhouse. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) at the period between 45 and 60 days after sowing, ion contents in leaves and stems at day 45 and final harvest, photosynthetic parameters, chlorophyll content (SPAD value), and leaf water relations at days 45 and 60 were determined. Salinity significantly reduced RGR and NAR, while LAR was not affected. The reduction in RGR was related to a reduction in NAR in all genotypes, but not with LAR, except in salt-sensitive genotypes (Sakha 61, Giza 168, Thassos, Triso and Westonia) and both moderate-tolerant genotypes (Sakha 69 and Drysdale). This indicates that the growth of salt-tolerant genotypes (Sakha 8, Sakha 93 and Kharchia) was affected by salinity primarily due to a decline in photosynthetic capacity rather than a reduction in leaf area; however, both NAR and LAR are important factors in determining RGR of moderate-tolerant and salt-sensitive genotypes. Na^+ content in leaves did not always predict salt tolerance differences among genotypes. Na^+ and Cl^- contents in leaves and stems at day 45 were significantly correlated with RGR in moderate-tolerant genotypes except Sakha 69 and with salt-sensitive genotypes except Westonia, but not with salt-tolerant genotypes. Both K^+ and Ca^{2+} contents in leaves and stems were significantly associated with salt tolerance differences among genotypes. And both ions contents in leaves and stems at day 45 were significantly correlated with RGR in moderate-tolerant and salt-sensitive genotypes, but not with salt-tolerant genotypes. Reduction in *A* for salt-tolerant genotypes was due to g_S, while in moderate-tolerant and salt-sensitive genotypes it was due to a combination of stomatal and non-stomatal factors. Leaf Ψ and Ψ_π were significantly decreased by salinity and were always less negative in salt-tolerant genotypes than moderate-tolerant and salt-sensitive genotypes, especially at day 60. Leaf T_P was increased by salinity and was more closely related to salt tolerance differences among

genotypes than osmotic adjustment. It was concluded that salt tolerance of genotypes (Sakha 8, Sakha 93 and Kharchia) was found to be associated with Na^+ and Cl^- exclusion, to maintain high K^+ and Ca^{2+} contents in leaves and stems, less reduction in A and g_S, less negative value for leaf Ψ and leaf Ψ_π and maintenance of positive leaf turgor pressure which result in a better growth under salinity.

4.1 Introduction

Salinity limits the plant production in nearly 40% of agricultural lands worldwide (Gorham, 1992). Therefore, there is a need for salt-tolerant genotypes to add saline lands into proper cultivation to meet the needs of the earth's increasing population, which will rise to 8.5 billion by the year 2025 (Ghassemi et al., 1995). Achieving this by plant breeding requires understanding the physiological mechanisms of salt tolerance among genotypes so that the traits of salt tolerance can be introduced in the genotypes of interest through genetic engineering and molecular markers.

Relative growth rate has been considered to make more appropriate comparisons of growth among species or genotypes under salinity than absolute growth rate (Cramer et al., 1994). The RGR gives a relative basis on which to compare growth rates of plants, since it takes into account both the initial and ending plant weights over a specified time period (Hunt, 1990). The RGR is a function of the net assimilation rate (NAR), which is an index of the photosynthetic-assimilatory capacity of the plant per unit leaf area, and the leaf area ratio (LAR), which is an index of the leafiness of the plant (Hunt, 1990). However, the relative contribution of components RGR to growth reduction is still conflicting. For example, some reports have shown that salinity affects LAR, but not NAR (Curtis and Läuchli, 1986). In contrast, other reports have found that NAR, but not LAR was affected by salinity (Cramer et al., 1990). This difference may suggest that on the whole plant level, growth components makes it possible to clarify whether genotypic variation in salt tolerance can be attributed to morphological changes or photosynthetic response (Ishikawa et al., 1991). Most importantly, few studies have employed plant growth as an approach to determine the physiological basis of the response of whole plants to salinity (Schachtman et al., 1989). Therefore, the first step in this study was to analyse the effects of salinity on growth components of RGR and how this was related to genotypic variation in salt tolerance among genotypes.

Salinity inhibits plant growth mainly by water deficits, ion toxicity and ion imbalance. (Greenway and Munns, 1980). Thus, the differences in these aspects among genotypes may reflect their salt tolerance. For example, one important aspect of salt tolerance is the ability of a plant to exclude Na^+ and/or Cl^- from the shoots. In wheat, genotypic variation in salt tolerance has been found to associate with low rates of Na^+ transport and high selectivity for K^+ over Na^+ (Schachtman and Munns, 1992), whereas there is little genotypic variation in rates of Cl^- transport (Gorham et al., 1990). In contrast, a negative correlation between salt tolerance and toxic ion exclusion has also been found in alfalfa (Ashraf et al., 1986), maize (Cramer et al., 1994), and lentil (Ashraf and Waheed, 1993). In addition, in some crops such as cotton, rice and *Triticum tauschii*, Na^+ exclusion did not always predict the salt tolerance of all genotypes (Yeo and Flowers, 1983; Leidi and Saiz, 1997). Therefore, it is necessary to identify whether genotypes differing in salt tolerance use ion exclusion to tolerate salinity.

Since photosynthesis is a major factor in the determination of growth, the sensitivity of photosynthesis to salinity in different genotypes is of interest (Heuer and Plaut, 1989). A close association was found between growth and photosynthesis rate in six *Brassica* species differing in salt tolerances (Ashraf, 2001). Similarly, in wheat (James et al., 2002) found that genotypic variations in dry matter production are likely to be accounted for by differences in photosynthesis rate. In contrast, other studies found no or little association between growth and photosynthesis rate, e.g. in *Hibiscus cannabinus* (Curtis and Läuchli, 1986), *Trifolium repens* (Rogers and Noble, 1992), and *Triticum aestivum* (Hawkines and Lewis, 1993). Additionally, reduction in photosynthesis rate by salinity could be due to lower stomatal conductance (g_S) (Seemann and Critchley, 1985). Therefore, genotypic differences in g_S under salinity are also of interest. For instant, Rivelli et al. (2002) observed that g_S of a low Na^+ durum landrace were reduced to a greater extent than that of a high Na^+ durum landrace when plants were grown in a short-term experiment at 150 mM NaCl. In view of the above mentioned, it was hypothesized that genotypic differences in salt tolerance may be associated with differing responses with respect to their photosynthetic parameters.

The presence of salt in soil solution decreases the osmotic potential of soil; creating water stress and making it difficult for the plant to absorb water necessary for growth, and hence decreased leaf water potential (Munns, 1993). The decrease in leaf water potential was accompanied by a decrease in leaf osmotic potential so that leaf turgor pressure of the

salinized plant was maintained (Tattini et al., 1995). Even more striking are the findings of Rivelli et al. (2002), who found that there were little differences between genotypes that differed in Na^+ exclusion in the effect of salinity on water relations. In contrast, the leaf water potential and leaf osmotic potential were always less negative in salt-tolerant genotypes of sorghum than the salt-sensitive one (Serraj and Sinclair, 2002). Generally, plants are able to tolerate salinity by reducing leaf osmotic potential by synthesis of organic solutes or by compartmentation of inorganic ions, in a process called osmotic adjustment (Hazegawa et al., 2000). The osmotic adjustment that occurs in leaves contributes to maintain water uptake and cell turgor, which are essential for plants to tolerate salinity. Therefore, genotypic difference in salt tolerances may reflect differing response with respect to their leaf water relations.

The main objective of this study was to describe the effect of salinity on ion contents, leaf photosynthetic parameters, chlorophyll content and leaf water relations of wheat genotypes, and how they reflected salt tolerance differences among genotypes, with particular reference to RGR and its components.

4.2 Materials and methods

4.2.1 Plant materials

Thirteen varieties of spring wheat (*Triticum aestivum* L.) from different countries were used in this study. Eight varieties (Sakha 8, Sakha 93, Sakha 61, Sakha 69, Giza 168, Sids 1, Sahel 1 and Gemmeza 7) were obtained from the Agricultural Research Centre in Giza, Egypt. Sakha 8 and Sakha 93 are usually cultivated in saline areas in Egypt. Of the remaining varieties, Thassos and Triso were from Germany, Westonia and Drysdale were from Australia, and Kharchia was from India.

Kharchia is the most tolerant wheat genotype, and is used as a standard for the salt tolerance test of wheat worldwide (Ashraf, 2002).

4.2.2 Growth conditions

This study was carried out in a greenhouse from the middle of March to the middle of August 2002. The air temperature ranged from 23 to 28°C during the day and 15 to 18°C during the night. Relative humidity fluctuated between 45 and 85% at day/night.

Loamy soil was collected from the soil surface (0-15 cm). The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The soil consisted of 23% clay, 48% silt and 29% sand, and the organic matter content was 1.66%. The initially air-dried soil with 9% gravimetric water content was filled layer-wise in four layers in 7-l pots without a leaching possibility.

Four salt levels (control (no added NaCl), 50, 100 and 150 mM NaCl) in the soil were applied. The salinity levels of 50, 100 and 150 mM NaCl in soil solution were equivalent to an electrical conductivity of 8, 13, and 17 dS m^{-1}, respectively, which were measured at the beginning of the experiment. During the period of the experiment, the electrical conductivity at each salinity level slightly decreased due to the uptake of salt by plants. At the end of the experiment, the electrical conductivity was changed to 5.2, 10, and 14 dS m^{-1}, respectively The final water content (25% on dry soil basis) was achieved by adding tap water or salt solution to each layer. To avoid an osmotic shock for seedling emergence, however, the topmost soil layer was not salinized until 10 days after sowing. Twenty-five seeds were sown in each pot. One week after sowing, the seedlings were thinned to twenty per pot.

The N, P and K were initially applied as 0.2 g NH_4NO_3 and as 0.2 g KH_2PO_4 per pot. The same amount of N, P and K was applied another three times at 20, 40 and 60 days after sowing. During the experiment, the pots were weighed daily and the water loss was replaced by adding tap water when total amount of the water lost was around 200 g for plants to avoid suffering either drought or flooding. All treatments were replicated four times.

4.2.3 Growth analysis

Three plants at 45 and 60 days after sowing were randomly sampled from each pot. Plants were harvested and separated into leaves and stems. Leaf area was measured by using a LI-3000 Area Meter (LI-COR, Walz Co., Oregon, USA). After leaf area was determined, the samples were dried at 65°C for 48 hours and then dry weight was determined. RGR (g g^{-1} day^{-1}), NAR (g m^{-2} day^{-1}) and LAR (m^2 g^{-1}) were derived from the following equations (Hunt, 1990):

$$RGR = \frac{1}{W} \times \frac{\partial W}{\partial T} \qquad [1]$$

$$NAR = \frac{1}{L_A} \times \frac{\partial W}{\partial T} \quad [2]$$

$$LAR = \frac{L_A}{W} \quad [3]$$

where W, T and L_A represent plant dry weight (g), time (day) and leaf area (m^2), respectively.

4.2.4 Analysis of ion concentrations

Oven dried samples of leaves and stems of plant at 45 days after sowing were ground into fine powder by passing through a 0.5-mm diameter sieve. The concentration of mineral elements in leaves and stems were measured as follows:

For the determination of Na^+, K^+ and Ca^{2+} contents, 300 mg of ground dry material of stems or leaves was digested by adding 3 ml concentrated HNO_3 (65%) and 2 ml H_2O_2 (30%) for 30 min at 2600 kpa (80 psi) in a MDS-2100 microwave oven (CEM Corp., Matthews, NC). After digestion, each sample was then brought up to 50 ml final volume with distilled-deionized water. The concentration of Na^+ was determined with an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Mulgrave Victoria, Australia). The K^+ and Ca^{2+} contents were determined with a flame photometer (ELEX 6361, Eppendorf, Netheler-Hinz GmbH., Germany).

For Cl^-, 100 mg of ground samples were extracted with 100 ml distilled water and were shaken for one hour and then filtered. Chloride was determined using an ion chromatography analyser (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA).

4.2.5 Photosynthetic parameter measurements

Photosynthesis rate (*A*), stomatal conductance (g_s), and respiration rate (*R*) were determined on the second fully expanded youngest leaf at 45 and 60 days after sowing. Measurements were made with a LI-COR 6400 portable gas exchange system (Analytical Development Company, England). Because the leaf did not fill the leaf chamber, the leaf area was determined independently and photosynthetic parameters were estimated with a re-computation program (LI-COR, Lincoln, NE). Measurements were conducted in a growth chamber during the light period. Plants were transferred into a growth chamber with air

temperature of 25°C, photosynthetic photon flux density of 1150 μmol m^{-2} s^{-1} and CO_2 was set at 400 μmol mol^{-1} for one day before the measurements of photosynthesis were performed.

4.2.6 Leaf chlorophyll measurement

Leaf chlorophyll content was determined using a hand-held SPAD 502 meter (Minolta, Osaka, Japan). Average SPAD chlorophyll readings were calculated from five measurements from the leaf tip to the leaf base. The measurement was made at 45 and 60 days after sowing.

4.2.7 Water relation measurements

Leaf water potential (Ψ) and osmotic potential (Ψ_π) from the middle of the second youngest fully developed leaf blade were measured for two times at 45 and 60 days after sowing. Ψ was measured with a pressure bomb (PMS Instrument Co., model 1002, Corvalis Co., Oregon, USA) according to the technique followed by Scholander et al., (1965). Immediately after Ψ was determined, the same leaf material was frozen in dry ice. The leaf samples were thawed at room temperature placed in a syringe, the leaf sap was expressed under pressure and then Ψ_π was determined with a vapour pressure osmometer (Wescor 5100C, Wescor Inc., Logan, USA). Turgor pressure (T_p) was estimated as the difference between Ψ_π and Ψ.

4.2.8 Experimental design and statistical analysis

A factorial experimental design with 13 genotypes and four salinity levels was arranged in a completely randomized design with 4 replications. Data were analyzed using an analysis of variance split plot design, where salinity treatments were assigned as whole plot, genotypes as sub plots and replicates as blocks. Statistical analysis and correlations between variables were done using COSTAT Version 3.03 (software, Berkeley, CA 94701).

4.3 Results

4.3.1 Growth response of genotypes to salinity

Relative growth rate (RGR) decreased significantly with salinity (Fig. 4.1). RGR was reduced by 19.1, 36.8 and 44%, respectively, for the levels of 50, 100 and 150 mM NaCl compared with the control. The response of NAR to salinity showed a similar trend. However, no significant effect of salinity on LAR was observed. At 50, 100 and 150 mM NaCl, for example, NAR was decreased by 19.6, 34.8 and 40.6%, while LAR was decreased by only

1.4, 5.4 and 8.1%, respectively, compared with the control. Although RGR and NAR were decreased significantly by salinity, genotypic differences for both variables were observed. RGR and NAR for salt-tolerant genotypes were decreased much less than for moderate-tolerant and salt-sensitive genotypes at all salinity treatments (Fig. 4.1). RGR and NAR of salt-tolerant genotypes (Kharchia, Sakha 8 and Sakha 93) were decreased by an average of 6.8 and 8.6% at low salinity, 9.4 and 9.9% at moderate salinity and 8.1 and 6% at high salinity, respectively, compared with the control. RGR and NAR of salt-sensitive genotypes (Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7 and Westonia) decreased significantly with salinity (Fig. 4.1). They were decreased by an average of 29.8 and 30% at low salinity, 50.7 and 49% at moderate salinity and 58.6 and 57.2% at high salinity, respectively, compared with the control. RGR and NAR were significantly lower in salt-sensitive genotypes (Thassos and Triso) at control and they were decreased at 150 mM NaCl by an average of 49.3 and 36.7%, respectively, compared with the control. RGR of moderate-tolerant genotypes (Sakha 69 and Drysdale) was not affected significantly by low salinity, while it was reduced by an average of 34.5 and 33.3% at 100 and 150 mM NaCl, respectively, compared with the control (Fig. 4.1). LAR showed a different pattern among genotypes. LAR for salt-sensitive genotypes (Sids 1, Sahel 1 and Gemmeza 7) was slight increased by salinity, while it was decreased by an average of 11.2 and 15.8% at 100 and 150 mM NaCl, respectively, compared with the control for the other salt-sensitive genotypes (Sakha 61, Giza 168, Thassos, Triso and Westonia). LAR for moderate-tolerant genotypes was also decreased by salinity such as the mentioned salt-sensitive genotypes. LAR for salt-tolerant genotypes was not affected significantly by salinity (Fig. 4.1).

4.3.2 Ion contents

Salinity increased Na^+ and Cl^- contents in leaves and stems at day 45 and final harvest. For example, the contents of Na^+ in leaves and stems were increased by about 2.2, 4.2 and 6.8-fold at day 45, and 8, 17.8 and 39.1-fold at final harvest at 50, 100 and 150 mM NaCl, respectively. The contents of Cl^- in leaves and stems were also increased by about 8.9, 12.2 and 13.7-fold at day 45, and 17.1, 20.5 and 28.3-fold at final harvest, respectively, compared with the control (Fig. 4.2). Genotypes differed significantly in the ability to exclude Na^+ and Cl^- from the leaves and stems (Fig. 4.2). At day 45, the salt-tolerant genotypes (Sakha 8, Sakha 93 and Kharchia) had low Na^+ content in leaves at all salinity treatments, followed by

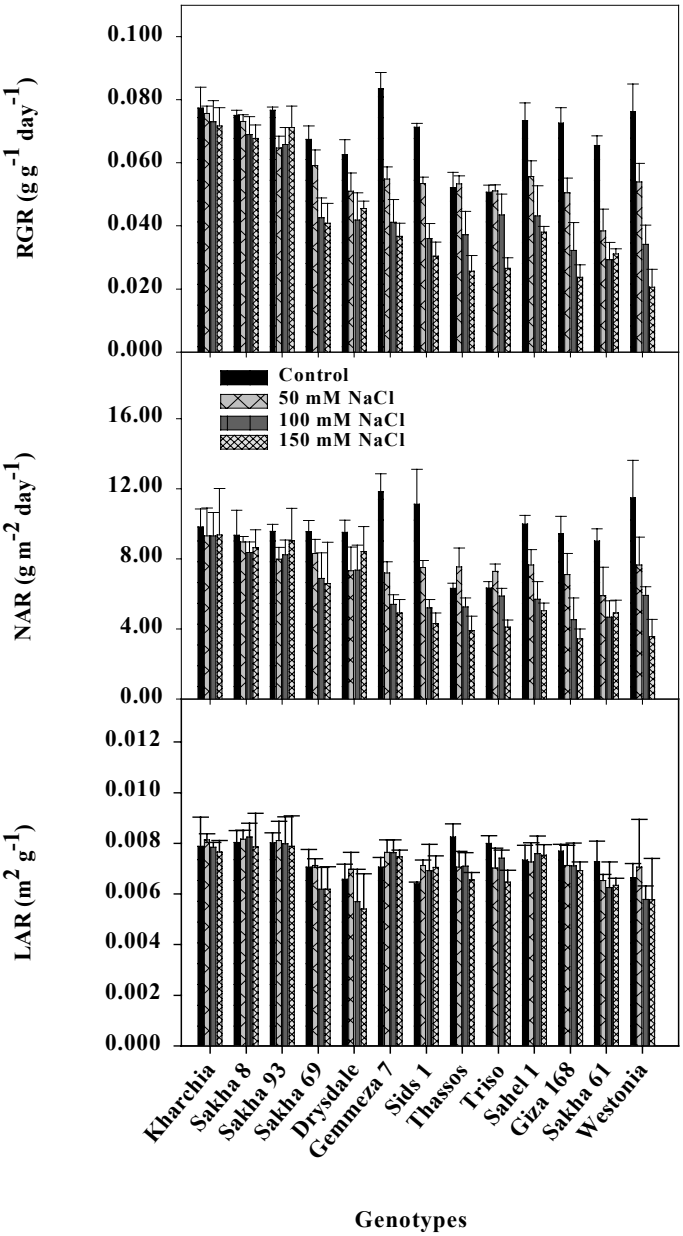

Fig. 4.1 Effect of different salinity levels on relative growth rate (RGR), net assimilation rate (NAR) and leaf area ratio (LAR) for different wheat genotypes following 45 days after sowing. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

moderate-tolerant genotypes (Sakha 69 and Drysdale) and salt-sensitive genotype (Westonia). The same pattern was found at final harvest except for Kharchia, which had lower Na^+ content in leaves at 50 and 100 mM NaCl than the other genotypes (Fig. 4.2). Low Na^+ content in stem was found in all salt-tolerant genotypes and the salt-sensitive genotype (Westonia) at both measurements as well as in both moderate-tolerant genotypes at final harvest (Fig. 4.2). The high Na^+ contents in leaves and stems at both measurements and all salinity treatments were observed in salt-sensitive genotypes (Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos and Triso). Only two genotypes (Thassos and Triso) had high Cl^- content in stems at both measurements. The low Cl^- content in leaves at all salinity treatments was found in salt-tolerant genotypes at day 45, and Kharchia at final harvest (Fig. 4.2). The high Cl^- content in leaves at all salinity treatments was found in all salt-sensitive genotypes and moderate-tolerant genotype (Drysdale) at both measurements.

Salinity reduced K^+ and very markedly Ca^{2+} contents in leaves and stems. Furthermore, the decrease in K^+ content in leaves was greater than in stems, while the decrease in Ca^{2+} content in leaves and stems was similar (Fig. 4.3). At 100 and 150 mM NaCl, for example, the contents of K^+ and Ca^{2+} in leaves were decreased by approximately 30 and 50% at day 45, and 39.6 and 49.1% at final harvest, respectively. The contents of K^+ and Ca^{2+} in stems were decreased by approximately 27.3 and 45% at day 45, and 27.4 and 54.3% at final harvest, respectively. There were significant differences in K^+ and Ca^{2+} contents in leaves and stems among genotypes (Fig. 4.3). At 100 and 150 mM NaCl, the salt-tolerant genotypes had higher K^+ content in leaves by about 18.6 and 36.4% at day 45 and 14.9 and 42.2% at final harvest, and higher Ca^{2+} content in leaves by about 32.2 and 49% at day 45 and 19.2 and 55% at final harvest than that of moderate-tolerant and salt-sensitive genotypes, respectively. The differences in K^+ content in stems among genotypes were little at day 45, while at final harvest the salt-tolerant genotypes had greater K^+ content in stems by about 8 and 21.2% at all salinity treatment than that of moderate-tolerant and salt-sensitive genotypes, respectively (Fig. 4.3). The Ca^{2+} contents in stems of salt-tolerant genotypes was greater by about 48 and 64% at day 45, and 36 and 55% at final harvest than that of moderate-tolerant and salt-sensitive genotypes, respectively (Fig. 4.3)

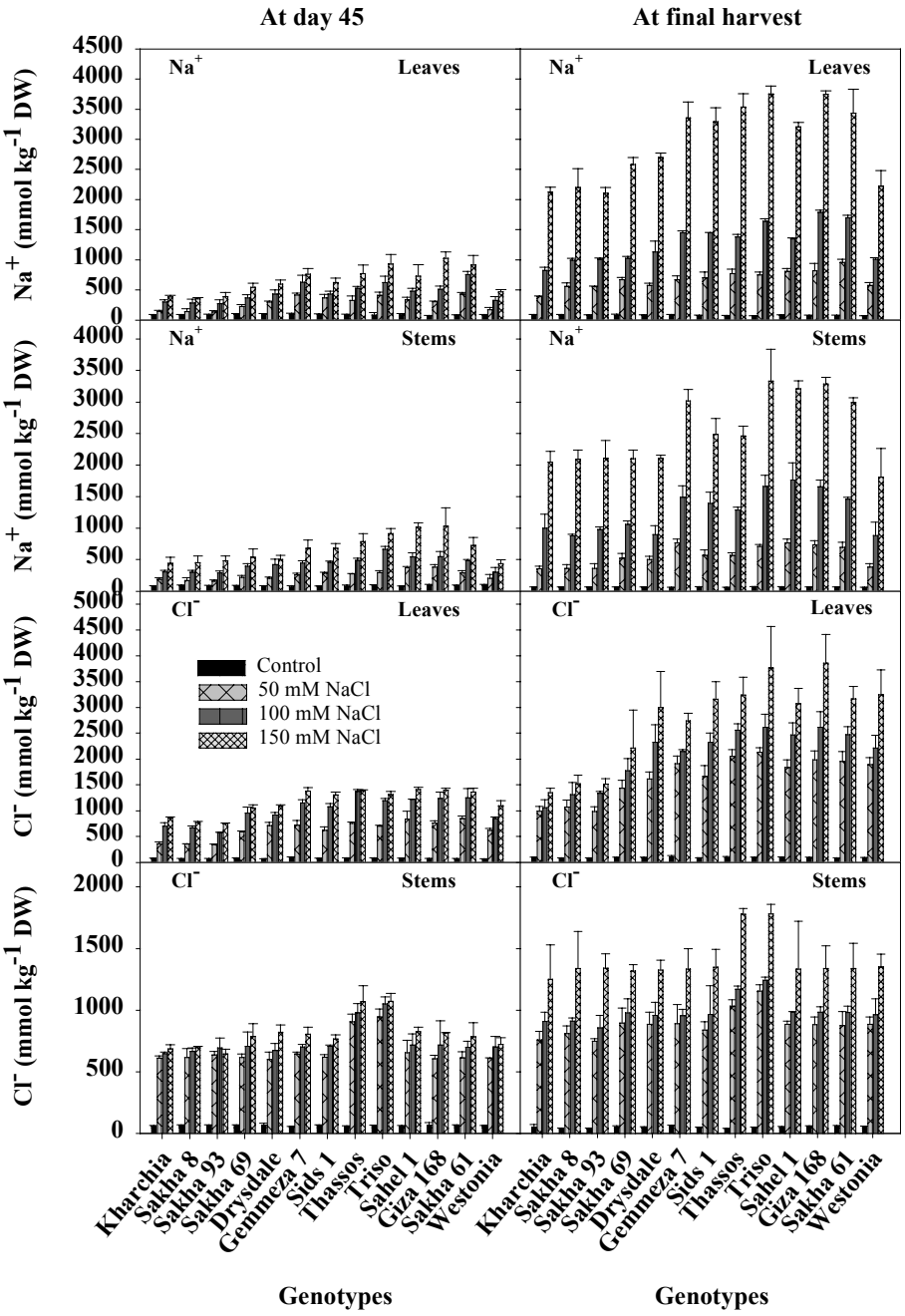

Fig. 4.2 Effect of different salinity levels on sodium and chloride contents in leaves and stems at days 45 and final harvest for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

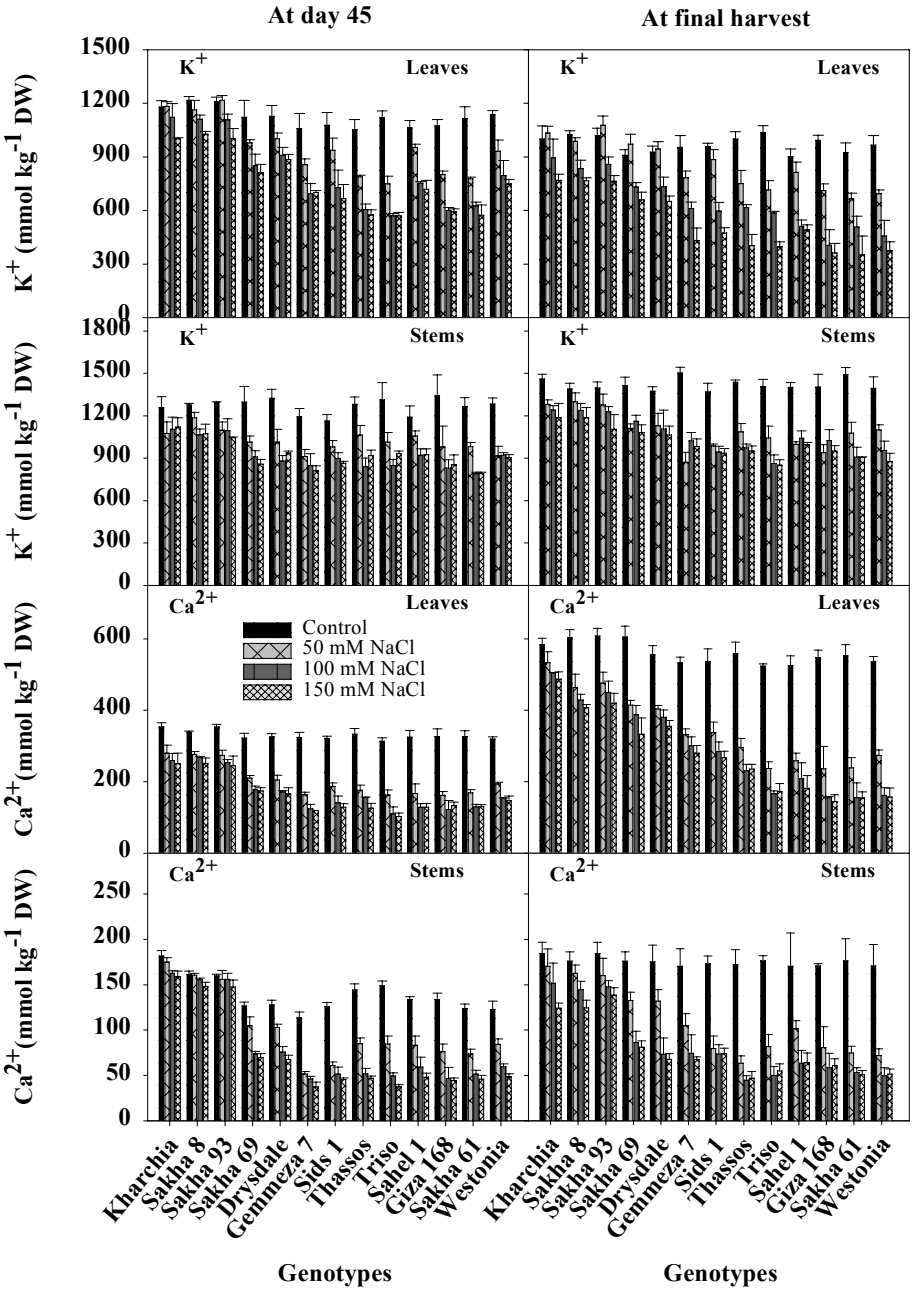

Fig. 4.3 Effect of different salinity levels on potassium and calcium contents in leaves and stems at days 45 and final harvest for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

4.3.3 Photosynthetic parameters

Photosynthesis rate (A) and stomatal conductance (g_S) were decreased significantly by salinity at days 45 and 60 (Fig. 4.4). At 50, 100 and 150 mM NaCl, for example, A was reduced by 14.8, 22.7 and 27.9% at day 45, and by 18.9, 36.8 and 39.6% at day 60. However, g_S was reduced by 31.3, 43.2 and 48.5% at day 45, and 28.9, 52.5 and 55.7% at day 60, respectively, compared with the control. Conversely, respiration rate (R) was increased by salinity. At both measurements, R was increased by approximately 1.8, 2.5 and 1.4 times at 50, 100 and 150 mM NaCl, respectively, compared with the control (Fig. 4.4). Photosynthesis rate and stomatal conductance differed significantly among genotypes at all salinity treatments (Fig. 4.4). In salt-tolerant genotypes, A was decreased much less than g_S. At 100 and 150 mM NaCl, for example, A was decreased by an average of 6.9 and 14.3% at day 45, and 12.6 and 15.5% at day 60, while g_S was decreased by an average of 27.1 and 33.3% at day 45, and 30.2 and 35.8% at day 60, respectively, as compared with the control (Fig. 4.4). In salt-sensitive genotypes (Sakha 61, Giza 168, Sids 1, Sahel 1, Gemmeza 7, Thassos and Triso), A and g_S were decreased significantly at all salinity treatments. At 50, 100 and 150 mM NaCl, for example, A was decreased by an average of 28, 34.2 and 39% at day 45, and 27.5, 48.8 and 51.9% at day 60. Whereas, g_S was decreased by an average of 44.9, 56.5 and 60.7% at day 45, and 34.8, 64 and 67.2% at day 60, respectively, compared with the control (Fig. 4.4). Moreover, it is worth noting that the A and g_S for moderate-tolerant genotypes and salt-sensitive genotypes (Westonia) were less affected by salinity as well as salt-tolerant genotypes at day 45, but this decrease was greater at day 60. Respiration rate was increased by much less in salt-tolerant, moderate-tolerant and salt-sensitive genotypes (Sids 1 and Westonia) by salinity than other genotypes at day 45. Whereas, at day 60, R for salt-sensitive genotypes was higher about 2.3 and 2.5 times than that of salt-tolerant and 1.4 and 1.8 times than that of moderate-tolerant genotypes at low and both moderate and high salinity, respectively.

4.3.4 Chlorophyll content (SPAD value)

Chlorophyll content was reduced slightly with salinity. At moderate and high salinity, for example, it was reduced by only about 3.5% at day 45 and by about 17% at day 60, compared to control (Fig. 4.4). Chlorophyll content of salt-tolerant genotypes (Sakha 8, Sakha 93 and Kharchia) was slightly increased with salinity at days 45 and 60. The opposite case was found

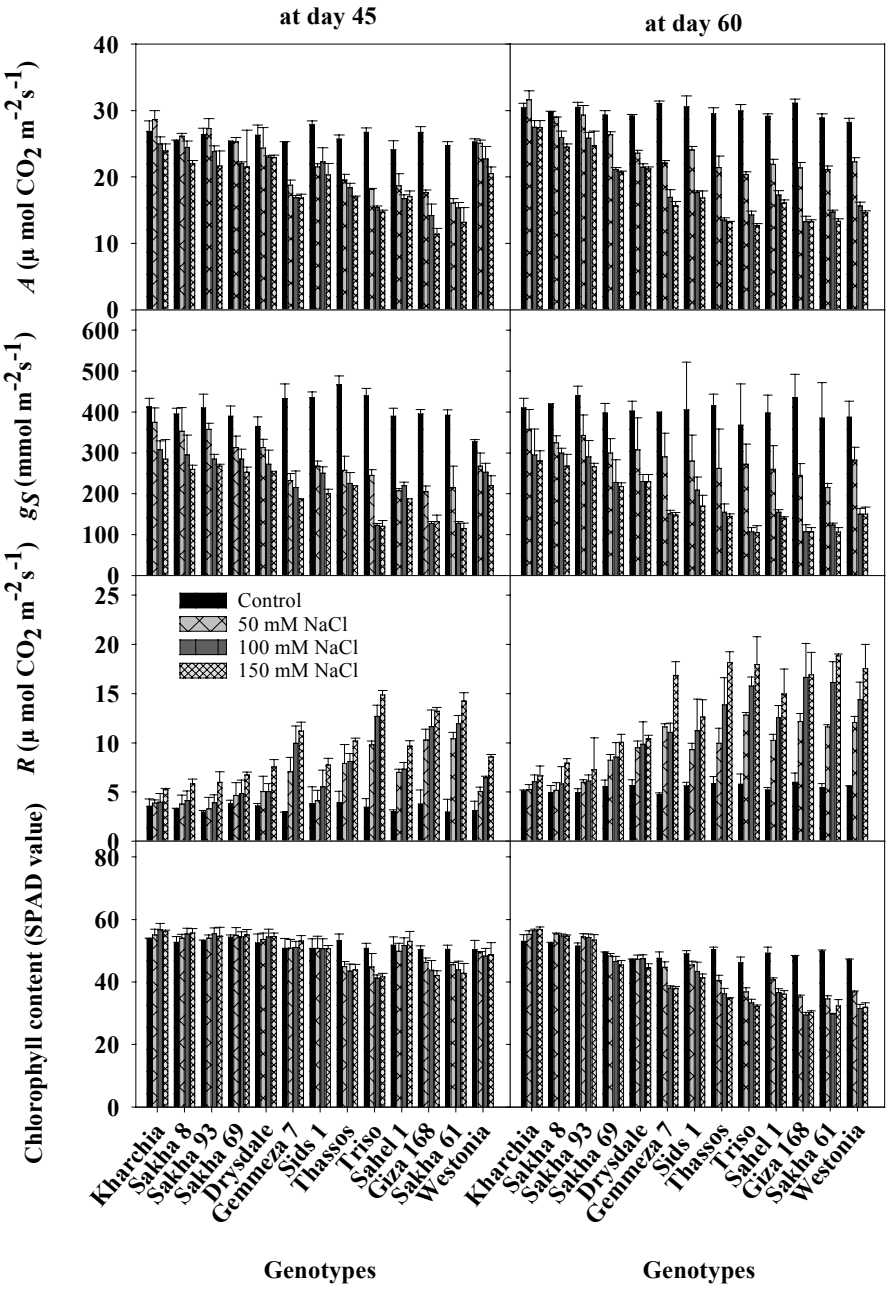

Fig. 4.4 Effect of different salinity levels on photosynthesis parameters and chlorophyll content (SPAD value) at 45 and 60 days after sowing for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

in salt-sensitive genotypes, e.g., chlorophyll content was decreased by about 6 and 8% at day 45 and by about 19 and 28% at day 60 at low and both moderate and high salinity levels, respectively, as compared with the control. Chlorophyll content of moderate-tolerant genotypes (Sakha 69 and Drysdale) showed a changing pattern with regarding measurements. It was slightly increased with increasing salinity at day 45, while at day 60 it was decreased by about 7% at 150 mM NaCl except Drysdale, which increased slightly at 50 and 100 mM NaCl, compared to control (Fig. 4.4).

4.3.5 Water relations

Salinity significantly affected leaf water potential (Ψ) and osmotic potential (Ψ_π) at days 45 and 60 (Fig. 4.5). Leaf Ψ was decreased by –0.45, –0.71 and –0.81 MPa at day 45, and by –0.56, –0.84 and –0.95 MPa at day 60. Leaf Ψ_π was decreased by –0.62, –0.92 and –1.05 MPa at day 45, and –0.57, –1.03 and –1.22 MPa at day 60. While, Leaf turgor pressure (T_P) was increased by 0.20, 0.24 and 0.28 MPa at day 45, and by 0.02, 0.19 and 0.27 MPa at day 60, when salinity increased from control to 50, 100 and 150 mM NaCl, respectively (Fig. 4.5). There were initial differences in leaf water potential (Ψ) among genotypes at days 45 and 60. The salt-tolerant genotypes had significantly less negative value for leaf Ψ at both measurements. The opposite case was found for moderate-tolerant and salt-sensitive genotypes (Fig. 4.5). It is noted that the differences in leaf Ψ_π among genotypes showed up clearly only at low salinity at day 45, while there was only little difference at moderate and high salinity. At 50, 100 and 150 mM NaCl, for example, leaf Ψ_π were decreased by an average of –0.33, –0.85 and –1.03 MPa for salt-tolerant, –0.74, –1.04 and –1.09 MPa for moderate-tolerant and –0.70, –1.00 and –1.10 MPa for salt-sensitive genotypes, respectively, compared with the control. At day 60, the differences in leaf Ψ_π among genotypes were clear at all salinity treatments. At day 45, the salt-tolerant genotypes showed the highest value for T_p at all salinity treatments, while the salt-sensitive genotypes (Sakha 61, Giza 168, Gemmeza 7, Thassos, Triso and Westonia) showed the lowest value. At day 60, the salt-tolerant and moderate-tolerant genotypes showed the highest value for T_P, especially at moderate and high salinity. However, the salt-sensitive genotype (Sakha 61, Triso and Westonia) had the lowest value for T_P at all salinity treatment (Fig. 4.5).

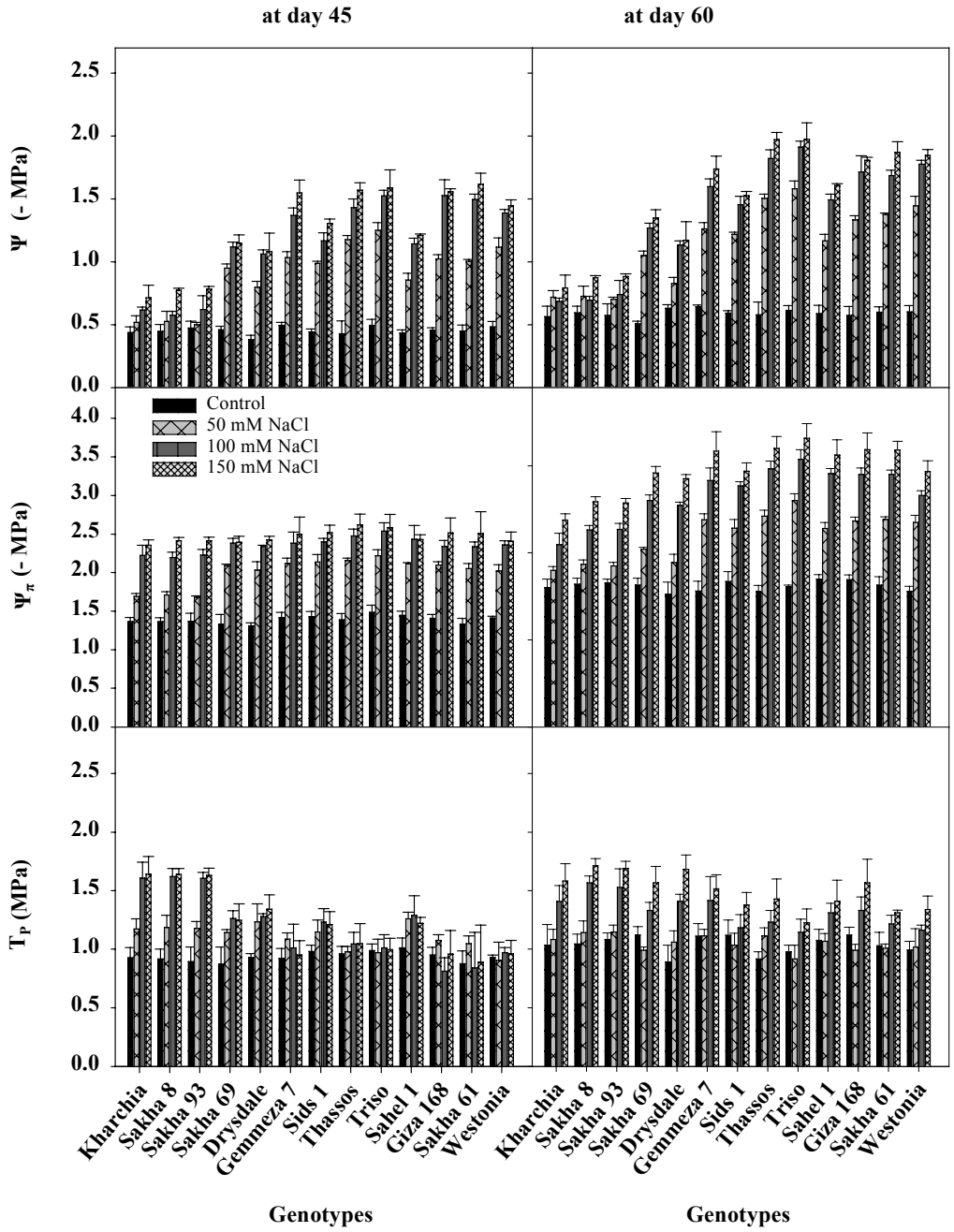

Fig. 4.5 Effect of different salinity levels on water relation parameters at 45 and 60 days after sowing for different wheat genotypes. Error bars represent standard deviations. Error bars fit within the plot symbol if not shown.

4.4 Discussion

To understand the mechanisms of salt tolerance among genotypes, the thirteen genotypes were classified into three groups, salt-tolerant, moderate- tolerant and salt-sensitive genotypes. This classification was made based on the rankings of these genotypes in terms of grain yield and agronomic parameters from the results presented in Chapter 2. The Genotypes (Sakha 8, Sakha 93 and Kharchia) were classified as salt-tolerant genotypes. Sakha 69 and Drysdale were identified as moderate-tolerant. The other genotypes, Sakha 61, Giza 168, Sahel 1, Sids 1, Gemmeza 7, Thassos, Triso and Westonia were classified as salt sensitive-genotypes.

At the whole-plant level, RGR explains whether genotypic differences in salt tolerance can be attributed to photosynthetic response (NAR) or morphological changes (LAR) (Hunt, 1990; Ishikawa et al., 1991). The results presented here demonstrate that the decrease in RGR of salt-tolerant genotypes (Sakha 8, Sakha 93 and Kharchia) is related to NAR, but not with LAR (Fig. 4.1). This indicates that the growth of these genotypes was affected by salinity primarily by a decline in photosynthesis rate or imbalance between photosynthesis and respiration rates of the whole plant rather than a reduction in leaf area. These results are in agreement with the reports by Cramer et al. (1994). Similar trend was found in salt-sensitive genotypes (Sids 1, Sahel 1 and Gemmeza 7). However, the reduction in RGR of other salt-sensitive genotypes (Sakha 61, Giza 168, Thassos, Triso and Westonia) and moderate-tolerant genotypes (Sakha 69 and Drysdale) was associated with NAR and LAR (Fig. 4.1). This indicates that leaf expansion and photosynthesis rate is the growth-limiting factor in theses genotypes (Morales et al., 1997).

Exclusion of harmful ions (Na^+ and Cl^-) from the shoots has been found to associate with genotypic variation in salt tolerance (Greenway and Munns, 1980). For the genotypes that cannot exclude toxic ions from the shoots, salt build up to toxic levels in the leaves, is the major cause of growth reduction (Munns, 1993). The results of the present study found that the salt-tolerant genotypes had the lowest Na^+ and Cl^- contents in leaves and stems at day 45 and final harvest (Fig. 4.2). This indicates that these genotypes had the ability to exclude harmful ions from the shoots, which contributed to their salt tolerance (Schachtman and Munns, 1992). The salt-sensitive genotype (Westonia) had also low Na^+ contents in leaves and stems at both measurements (Fig. 4.2). This indicates that Na^+ exclusion did not always predict salt tolerance of all wheat genotypes. Similarly, in rice (Yeo and Flowers, 1983), maize (Cramer et al., 1994), and cotton (Leidi and Saiz, 1997), salt tolerance of some

genotypes does not correlate with the content of leaf Na^+. The salt-sensitive genotypes had the highest content of Na^+ in leaves and stems and content of Cl^- in leaves (Fig. 4.2). Furthermore both Na^+ and Cl^- contents in leaves and stems at day 45 was significantly correlated with RGR in salt-sensitive genotypes, while in salt-tolerant genotypes and salt-moderate (Sakha 69) it did not (Table 4.1). This can be interpreted that the reduction of salt tolerance in the salt-sensitive genotypes can be attributed, at least in part, to high accumulation of harmful ions in shoots. It also noteworthy that RGR of salt-sensitive genotype (Westonia) was significantly correlated with Cl^- content in leaves at day 45, but not with Na^+ content (Table 4.1). This indicates that the Cl^- rather than Na^+ seems to be more closely related with salt tolerance in this genotype. These results agree with the findings in salt-sensitive bean genotypes (Montero et al., 1998) and *Trifolium repens* (Rogers and Noble, 1992).

Changes in ion contents and their relations appeared to be associated with salt tolerance differences among genotypes. In this study, the contents of K^+ and Ca^{2+} in leaves and stems of salt-tolerant genotypes were slightly decreased with increasing salinity (Fig. 4.3). K^+ and Ca^{2+} contents together with Na^+ content for salt-tolerant genotypes indicate that these genotypes had the ability to discriminate K^+ and Ca^{2+} against Na^+. Therefore, maintenance of higher contents of K^+ and Ca^{2+} in salt-tolerant genotypes may have been one of the factors for their superiority of salt tolerance as compared to other genotypes. In contrast, K^+ content in leaves and Ca^{2+} contents in leaves and stems of salt-sensitive genotypes were decreased significantly with salinity. At high salinity level, for example, K^+ and Ca^{2+} contents in leaves were much less by about 36.4 and 49% at day 45, and 42.2 and 55% at final harvest than in the salt-tolerant genotypes. And Ca^{2+} content in stems was much less by about 64% at day 45 and 55% at final harvest (Fig. 4.3). In moderate-tolerant genotypes, Ca^{2+} contents in leaves and stems were more affected by salinity than K^+ (Fig. 4.3). Furthermore, the relationship between K^+ and Ca^{2+} contents in leaves and stems at day 45 and RGR in salt-sensitive and moderate-tolerant genotypes was highly significant, however not in salt-tolerant genotypes except in Sakha 8 and Sakha 93 for K^+ content in stems (Table 4.1). This indicates that both ions play an important role for differing salt tolerances among genotypes because essential physiological processes will be affected by reduction in the uptake of K^+ and Ca^{2+}. For example, at the cellular and whole plant level, K^+ is involved in the maintenance of tissue rigidity, leaf stomatal movement, turgor maintenance and osmoregulation, and is one of the most prominent inorganic solutes in charge balance, protein synthesis and homeostasis

Table 4.1 Correlation coefficients between relative growth rate (RGR) following 45 days after sowing and ion content in leaves and stems at days 45 of different wheat genotypes. Correlation analysis was performed using the replicates of each treatment with data combined across salt levels.

Genotypes	RGR				RGR			
	Ion contents in leaves at day 45				Ion contents in stems at day 45			
	Na^+	Cl^-	K^+	Ca^{2+}	Na^+	Cl^-	K^+	Ca^{2+}
Kharchia	-0.06^{NS}	-0.13^{NS}	0.23^{NS}	0.01^{NS}	-0.04^{NS}	-0.13^{NS}	0.04^{NS}	0.15^{NS}
Sakha 8	-0.46^{NS}	-0.41^{NS}	0.43^{NS}	0.47^{NS}	-0.45^{NS}	-0.47^{NS}	0.62^{*}	0.47^{NS}
Sakha 93	-0.08^{NS}	-0.40^{NS}	0.04^{NS}	0.47^{NS}	-0.11^{NS}	-0.47^{NS}	0.55^{*}	0.15^{NS}
Sakha 69	-0.46^{NS}	-0.49^{NS}	0.71^{**}	0.74^{***}	-0.46^{NS}	-0.74^{NS}	0.71^{**}	0.79^{***}
Drysdale	-0.67^{**}	-0.73^{**}	0.68^{**}	0.75^{***}	-0.74^{***}	-0.72^{**}	0.75^{***}	0.78^{***}
Gemmeza 7	-0.88^{***}	-0.96^{***}	0.95^{***}	0.96^{***}	-0.85^{***}	-0.93^{***}	0.89^{***}	0.93^{***}
Sids 1	-0.83^{***}	-0.90^{***}	0.89^{***}	0.89^{***}	-0.87^{***}	-0.85^{***}	0.85^{***}	0.86^{***}
Thassos	-0.83^{***}	-0.75^{***}	0.74^{***}	0.62^{*}	-0.86^{***}	-0.60^{*}	0.69^{**}	0.70^{**}
Triso	-0.77^{***}	-0.69^{**}	0.60^{*}	0.56^{*}	-0.81^{***}	-0.50^{*}	0.46^{NS}	0.69^{**}
Sahel 1	-0.88^{***}	-0.91^{***}	0.87^{***}	0.88^{***}	-0.85^{***}	-0.87^{***}	0.80^{***}	0.93^{***}
Giza 168	-0.84^{***}	-0.91^{***}	0.91^{***}	0.84^{***}	-0.78^{***}	-0.82^{***}	0.79^{***}	0.91^{***}
Sakha 61	-0.83^{***}	-0.92^{***}	0.93^{***}	0.96^{***}	-0.81^{***}	-0.92^{***}	0.94^{***}	0.94^{***}
Westonia	-0.49^{NS}	-0.92^{***}	0.91^{***}	0.89^{***}	-0.47^{NS}	-0.44^{NS}	0.78^{***}	0.92^{***}

^{NS,*,**,***} Not significant, significant at 0.05, significant at 0.01 and significant at 0.001 probability level, respectively, df = 14 (error).

(Chow et al. 1990). Ca^{2+} is important in cell membrane biology during salt stress, for example, in the preservation of membrane integrity (Rengel, 1992), influencing K^+/Na^+ selectivity (Cramer, 2002), and is also used as a second messenger in many signal transudation pathways within the cell (Knight, 2000). In this context, it is interesting to speculate that maintenance of lower K^+ and Ca^{2+} content in salt-sensitive genotypes and Ca^{2+} content in moderate-tolerant genotypes may have been involved in the reduction of their salt tolerance. This result is evidenced from K^+ deficiency affected salt tolerance of maize

(Botella et al., 1997), and Ca^{2+} enhanced salt tolerance in barley (Huang and Redman, 1995), sorghum (Bernstein et al., 1993) and *Brassica* species (He and Cramer, 1992)

As mentioned before, RGR was associated with NAR of all genotypes. The NAR, which represents a balance between photosynthesis and respiration rates of the whole plant, decreases due to a reduction in *A* or an increase in *R* (Cramer et al., 1990). It is clear, therefore, that the balance between both parameters may play an important role in the differences in salt tolerance among genotypes under salinity. *A* and *R* of salt-tolerant genotypes were slightly affected by increasing salinity (Fig. 4.4), which caused slight decreases in RGR as well. In contrast, *A* of salt-sensitive genotypes was much less by about 1.4 times at low salinity and about 1.6 times at moderate and high salinity than in salt-tolerant genotypes (Fig. 4.4). *R* was also much higher by about 2.1 times at low salinity and about 2.3 times at moderate and high salinity than in salt-tolerant genotypes. These results led to reduce NAR of salt-sensitive genotypes by about 30, 49 and 57.2% at 50, 100 and 150 mM NaCl, respectively, compared with the control. This result indicates that both *A* and *R* plays an important role in salt tolerance differences among genotypes. Decreases in *A* and increase in *R* may slow down or stop growth (Tattini et al., 1995). In study of *Xanthium*, Schwarz and Gale (1981) found that 80% of the reduced carbon assimilation could be accounted for by a reduction in *A* and 20-25% was the result of increased *R*. The percentage that contributes to reduce carbon assimilation would vary with the species and their salt tolerance. Additionally, Semikhatova et al. (1993) found that increased *R* is mainly attributed to additional energy cost for the salt economy of the cell, i.e. pumping out ions from the cytoplasm into the vacuole. Therefore, increases in *R* in salt-sensitive genotypes may be related to accumulation of harmful ions in cytoplasm which could reduce the efficiency of RuBP carboxylase and other enzyme that are related to photosynthetic capacity (Seemann and Critchley, 1985).

Reduction in *A* by salinity could be due to either stomatal or non-stomatal factors (Heuer and Plaut, 1989). The data presented here show that g_S was significantly decreased by salinity for all genotypes. At moderate and high salinity, for example, g_S was reduced by about 31, 36 and 60% for salt-tolerant, moderate-tolerant and salt-sensitive genotypes, respectively, with regarding measurements (Fig. 4.4). Furthermore, *A* was significantly correlated with g_S in all genotypes (Table 4.2). It is also noteworthy that a reduction in g_S for salt-tolerant genotypes was reduced to a greater extent than *A*. This indicates that a reduction in *A* for these genotypes mainly is due to a reduction in g_S. Similarly, Robinson et al., (1983)

found that g_S of salt-tolerant spinach genotypes was decreased by 350 mM NaCl, but little significant decrease in *A* was observed. The reduction in *A* of moderate-tolerant and salt-sensitive genotypes was associated with a combination of stomatal and non-stomatal factors. This result is supported by the significant correlation between *A* and g_S and ion contents of Na^+ and Cl^- in leaves at day 45 in moderate-tolerant and salt-sensitive genotypes (Table 4.2). This indicates that both stomatal and non-stomatal factors affect *A* in moderate-tolerant and salt-sensitive genotypes (Heuer and Plaut, 1989).

The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content (Delfine et al., 1999). The results of chlorophyll content (SPAD value) showed a varying pattern among genotypes. In salt-tolerant genotypes, chlorophyll content increased with salinity and was not correlated with *A* regardless of measurements (Fig. 4.4 and Table 4.2). However, in salt-sensitive genotypes (Sakha 61, Giza 168, Thassos and Triso), chlorophyll content was decreased by an average of 15% at day 45 and 33% at day 60 at moderate and high salinity and was significantly correlated with *A* regardless of the measurements (Table 4.2). In moderate-tolerant genotypes and salt-sensitive genotypes (Gemmeza 7, Sids 1 and Sahel 1), chlorophyll content was decreased by salinity and was significantly correlated with *A* at day 60, but at day 45 it did not (Fig. 4.4 and Table 4.2). This indicates that the responses of chlorophyll content to salt stress depended on salt tolerance differences among wheat genotypes. Similarly, in alfalfa (Winicov and Seeman, 1991), sunflower (Ashraf, 1999) and cowpea (Murillo-Amador et al., 2002), therefore responses of chlorophyll content to salinity depended on salinity level and the degree of salt tolerance of genotypes. In cowpea, for example, Murillo-Amador et al. (2002) found that chlorophyll content of salt-tolerant genotypes was increased under salinity, whereas in salt-sensitive genotypes it showed a different pattern.

It is known that the excess of salinity in the growth medium causes reduction in leaf water potential (Ψ) which combined with a decrease in leaf osmotic potential (Ψ_π) causes that leaf turgor (T_P) of the salinized plant is maintained (Tattini et al., 1995). Our results showed that the decrease in leaf Ψ_π exceeded always that of leaf Ψ. Therefore, leaf T_P was enhanced by salinity in all genotypes (Fig. 4.5). One of the most interesting findings, osmotic adjustment (the difference in leaf Ψ_π between the salt and control) of salt-sensitive and moderate-tolerant genotypes was greater than that of salt-tolerant genotypes, especially at low and moderate salinity (Fig. 4.5). This suggests that the achievement of osmotic adjustment

Table 4.2 Correlation coefficients between photosynthesis rate (*A*) at days 45 and 60 and Na^+ and Cl^- contents in leaves at day 45, stomatal conductance (g_S) and chlorophyll content (SPAD value) at days 45 and 60. Correlation analysis was performed using the replicates of each treatment with data combined across salt levels.

Genotypes	*A* at day 45				*A* at day 60	
	Na^+ at day 45	Cl^- at day 45	g_S at day 45	Chl. at day 45	g_S at day 60	Chl. at day 60
Kharchia	-0.43 ^{NS}	-0.42 ^{NS}	0.69**	-0.33 ^{NS}	0.63**	-0.38 ^{NS}
Sakha 8	-0.40^{NS}	-0.49 ^{NS}	0.72***	-0.46 ^{NS}	0.81***	-0.39 ^{NS}
Sakha 93	-0.48 ^{NS}	-0.47 ^{NS}	0.72***	-0.36 ^{NS}	0.81***	-0.14 ^{NS}
Sakha 69	-0.44 ^{NS}	-0.50*	0.80***	-0.04 ^{NS}	0.89***	0.83 ***
Drysdale	-0.63**	-0.68**	0.66**	-0.37 ^{NS}	0.87***	0.31 ^{NS}
Gemmeza 7	-0.93***	-0.93***	0.96***	-0.19 ^{NS}	0.95***	0.90 ***
Sids 1	-0.90***	-0.81***	0.90***	0.13 ^{NS}	0.82***	0.86 ***
Thassos	-0.87***	-0.94***	0.97***	0.89***	0.91***	0.95***
Triso	-0.88***	-0.97***	0.98***	0.87***	0.89***	0.97***
Sahel 1	-0.77***	-0.93***	0.89***	-0.07 ^{NS}	0.93***	0.97 ***
Giza 168	-0.88***	-0.98***	0.96***	0.84***	0.98***	0.98***
Sakha 61	-0.88***	-0.94***	0.93***	0.84***	0.95***	0.92***
Westonia	-0.79***	-0.76***	0.64**	0.06 ^{NS}	0.96***	0.95 ***

^{NS,*,**,***} Not significant, significant at 0.05, significant at 0.01 and significant at 0.001 probability level, respectively, df = 14 (error).

would, thus, not imply an increase in salt tolerance (Heuer and Plaut, 1989). A similar result was observed for rice genotypes reported by Lutts et al. (1996). Whereas, the ability of genotypes to maintain higher turgor pressure plays an important role in their salt tolerance (Heuer and Plaut, 1989), because leaf T_P is an important factor for the regulation of the leaf function and metabolic process at the cell level (Lacerda et al., 2003). This result is clear in salt-tolerant genotypes (Fig. 4.5). In addition, osmotic adjustment was achieved by the uptake of inorganic ions like Na^+, Cl^- and K^+ (Hazegawa et al., 2000). This suggests that the turgor pressure of most salt-sensitive genotypes was achieved probably by accumulating of Na^+ and

Cl^-. Most importantly, although Na^+ and Cl^- has an important to avoid loss of turgor pressure in salt-sensitive genotypes that accumulate higher contents of both ions under salinity, this could also led to produce toxic effects on leaf function because some anabolic enzymes are sensitive to both ions (Yeo et al., 1985). However, Leigh and Story (1993) proposed that a plant's capacity to include toxic ions can be a beneficial trait only when the absorption of toxic ions is accompanied by the ability to regulate internal toxic ions. The minor contribution of Na^+ and Cl^- and the major contribution of K^+ to maintenance of leaf turgor pressure in salt-tolerant genotypes is likely a factor to confer salt tolerance to these genotypes (Serraj and Sinclair, 2002).

In conclusion, RGR and NAR were inhibited significantly by salinity and associated with salt tolerance differences among genotypes. LAR was not affected by salinity nor it was affected in salt-sensitive genotypes (Sakha 61, Giza 168, Thassos, Triso and Westonia) and moderate-tolerant genotypes (Sakha 69 and Drysdale). Na^+ contents in leaves and stems did not accurately predict salt tolerance of all genotypes. The differences in salt tolerance among genotypes were significantly associated with K^+ and Ca^{2+} contents in leaves and stems and the balance between *A* and *R*. The reduction in *A* for salt-tolerant genotypes was correlated with stomatal factor, while in moderate-tolerant and salt-sensitive genotypes it was correlated with stomatal and non-stomatal factors. The differences in salt tolerance among genotypes were more associated with leaf T_P than osmotic adjustment.

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5 General discussion

Improving salt tolerance of genotypes is often inhibited by the lack of effective evaluation methods for salt tolerance among genotypes (Zeng et al., 2003). Therefore, it is very important to develop an effective evaluation approach for screening salt tolerant genotypes, which should be reliable, quick, easy, practical and economic. Although direct screening based on grain yield takes more time, is laborious and expensive, the evaluation of salt tolerance of genotypes based on final grain yield is necessary before any recommendation can be made with regard to the authenticity of reliable different traits as screening criteria or recommendation of selected genotypes for using a good donor to increase the salt tolerance for wheat genotypes in breeding programs. The agronomic and physiological traits may be important not only to be used as quick and easy screening criteria if they would be closely associated with grain yield of genotypes (Noble and Rogers, 1992; Munns and James, 2003), but also to improve the salt tolerance that needs a better understanding of salt tolerance mechanisms of wheat genotypes. In this study, therefore, genotypic differences for salt tolerance were identified among genotypes on the grain yield and agronomic parameters at different growth stages; ranking of grain yield was used as a reference to determine validity of physiological traits as screening criteria for salt tolerance of wheat genotypes; and to understand salt tolerance mechanisms among wheat genotypes by analyzing variation of physiological mechanisms.

5.1 Screening salt tolerance of genotypes based on grain yield

The results in Chapter 2 showed that wide genotypic differences were observed among genotypes in the total grain yield per plant. For example, the salt tolerance indices of grain yield per plant for Kharchia, Sakha 8 and Sakha 93 were at least two times greater than for Sids 1, Giza 168, Sahel 1, Thassos, Triso and Sakha 61 (Table 2.1). Based on simultaneous analysis of the means of salt tolerance in grain yield per plant using single-linked cluster analysis, Kharchia was ranked as the most salt-tolerance genotype at different salinity levels, followed by the Egyptian wheat genotypes (Sakha 8 and Sakha 93). In addition, Kharchia shows higher grain yield, especially under non-saline conditions, than Egyptian genotypes (Sakha 8 and Sakha 93) (Fig. 2.3). This indicates that working with appropriate breeding programs, which aim to increase the yield in Sakha 8 and Sakha 93, may be more meaningful than working with Kharchia. The Egyptian genotypes (Sahel 1, Giza 168 and Sakha 61),

German genotypes (Thassos and Triso) and Australian genotype (Westonia) were ranked as the most salt-sensitive (Table 2.5). Furthermore, the Egyptian genotypes (Sakha 69, Gemmeza 7 and Sids 1) and Australian genotypes (Drysdale) were more tolerant at low salinity level and became more sensitive at moderate and high salinity levels, suggesting that maintaining the salinity at low levels is an important strategy for improving the growth of these genotypes (Mass and Poss, 1989).

5.2 Screening salt tolerance of genotypes based on agronomic parameters

Because evaluating salt tolerance among genotypes based on grain yield needs a long period for the experiment, the work in the literature suggests that evaluating salt tolerance of genotypes on the basis of agronomic parameters, especially for parameters that initiate at early growth stage and significantly correlate with grain yield, can be used as more quick and feasible traits to screen large number of genotypes rather than grain yield. Therefore, it is necessary to identify salinity-sensitive agronomic parameters that initiate at early growth stage. Tiller and spikelet numbers of wheat initiate during vegetative stages (as most sensitive stage in wheat) (Mass and Grieve, 1990; Frank et al., 1997), and are sensitive parameters to salinity in other crops such as rice (Zeng et al., 2002). The results presented in Chapter 2 showed that tiller number per plant and spikelet number per spike were significantly reduced by salinity (Figs 2.1 and 2.3), and wide genotypic differences observed for both yield components (Table 2.1), indicate that evaluation for salt tolerance among genotypes could be based on genetic diversity in tiller and spikelet numbers. The advantage of the utilization of both parameters in the evaluation for salt tolerance is that both parameters can represent the evaluation of genotypic differences for salt tolerances in terms of total grain yield. Thus, both parameters can be used as more quick and feasible traits to evaluate large number of wheat genotypes in breeding programs rather than grain yield.

The advantage of the utilization of tiller and spikelet numbers in the evaluation for salt tolerance is that both parameters can be determined at early growth stage. Furthermore, the salt tolerance of wheat was more sensitive at early growth stage than later growth stage (Mass and Poss, 1989). Therefore, screening salt tolerance of genotypes at early growth stage based on agronomic parameters can shorten the period for experiments to screen salt tolerance of wheat genotypes. However, it is only true if the changes in salt tolerance exhibit the same pattern in all genotypes at all growth stages (Allen et al., 1985; Ashraf and Waheed, 1993). The results in Chapter 2 showed that the salt tolerance of the most salt-tolerant (Sakha

8, Sakha 93 and Kharchia) and the most salt-sensitive (Sahel 1, Giza 168, Sakha 61, Thassos and Triso) genotypes was similar at different growth stages. However, salt tolerance of genotypes (Gemmeza 7, Sids 1 and Westonia) at early growth stage does not correlate with that at later growth stage (Chapter 2). Therefore, screening salt tolerance of these genotypes by agronomic parameters has not as effective at the early growth stage.

In conventional methods, genotypes are usually scored and ranked on single parameters. Because the ranking among genotypes for relative salt tolerance may vary with different agronomic parameters and salinity levels, the methods for evaluating salt tolerance by agronomic parameters should be improved. Cluster analysis was used in this study to help the analysis of multiple agronomic parameters simultaneously and facilitate the scores and rankings for salt tolerance among genotypes. The result of Chapter 2 showed that by simply adding the numbers in cluster group rankings at different salinity levels, salt tolerance among genotypes could be estimated more conveniently and accurately. This advantage will increase the efficiency of agronomic parameters as screening technique and will be more obvious when a large number of genotypes need to be evaluated for salt tolerance breeding.

5.3 Physiological processes of salt tolerance in wheat genotypes

A salt tolerance of genotypes is usually the result of a combination of different physiological mechanisms. In order to develop practicable strategies for selecting salt tolerance of wheat genotypes by physiological traits, it is necessary to have better understanding of the physiological mechanisms of salt tolerance genotypes. Dracup (1991) reported that the efficiency of physiological traits as selection criteria depended on genetic diversity in physiological mechanisms of salt tolerance.

The mechanisms for salt tolerance often depended on the morphological and physiological complexity of the organized plant rather than on tolerance at the cellular level (Dracup, 1991). At the whole-plant level, RGR explains whether genotypic differences in growth can be attributed to photosynthetic response, net assimilation rate (NAR), or morphological changes, leaf area ratio (LAR), (Hunt, 1990; Ishikawa et al., 1991). The results in Chapter 4 showed that the reduction in RGR of salt-tolerant genotypes (Sakha 8, Sakha 93 and Kharchia) is related to NAR, but not with LAR (Fig 4.1). Similar trend was found in salt-sensitive genotypes (Sids 1, Sahel 1 and Gemmeza 7). However, the reduction in RGR of other salt-sensitive genotypes (Sakha 61, Giza 168, Thassos, Triso and Westonia) and moderate-

tolerant genotypes (Sakha 69 and Drysdale) was related to NAR and LAR. This indicates that the growth of salt-tolerant genotypes was affected by salinity primarily due to a decline in photosynthesis rate rather than a reduction in leaf area. However, leaf area and photosynthesis rate are the growth limited factors in moderate-tolerant and salt-sensitive genotypes (Jeannette et al., 2003). Thus, it seems that the RGR had the potential to provide the information needed for comparing parallel morphological and physiological variation among genotypes.

The RGR of all tested genotypes was related to NAR. Net assimilation rate represents the combined physiological processes of photosynthesis and respiration. The results in Chapter 4 showed that photosynthesis rate (*A*) and respiration rate (*R*) for salt-tolerant genotypes were slightly affected by salinity. However, *A* of salt-sensitive genotypes was decreased significantly by salinity, e.g. at moderate and high salinity it was decreased by about 35 and 50% at days 45 and 60, respectively, compared with the control. Respiration rate of salt-sensitive genotypes was much higher by about 2.1- 2.3 times than that of salt-tolerant genotypes (Fig. 4.4). These differences among genotypes in *A* and *R* led to also a large difference in NAR, indicating that both *A* and *R* play an important role in salt tolerance and growth differences among genotypes. The decrease in *A* and increase in *R* may slow down or stop growth (Tattini et al., 1995). Similarly, Schwarz and Gale (1981) reported that 80% of the reduced carbon assimilation could be accounted for a reduction in *A*, and 20-25% for increased *R*.

Reduction in *A* by salinity could be due to stomatal and/or non-stomatal factors (Rawson et al., 1988; Belkhodja et al., 1994). The stomatal conductance of all genotypes was significantly affected by salinity. It was reduced at moderate and high salinity by about 31, 36 and 60% for salt-tolerant, moderate-tolerant and salt-sensitive genotypes, respectively, compared with the control (Fig. 4.4). Furthermore, photosynthesis rate of salt-tolerant genotypes was significantly correlated with stomatal conductance, and it was reduced much less than stomatal conductance (Fig. 4.4 and Table 4.2). This indicates that stomatal conductance is the major limitation to photosynthesis rate of salt-tolerant genotypes. The significant reduction in photosynthesis rate for moderate-tolerant and salt-sensitive genotypes was due to a combination of stomatal and non-stomatal factors. The non-stomatal limitations were associated with toxic ions effect and reduction in chlorophyll content (Table 4.2). The significant correlation of photosynthesis rate with stomatal conductance in all genotypes

indicated that screening for stomatal conductance may be the most effective way of selecting wheat genotypes. Similarly, Munns and James (2003) reported that stomatal conductance could provide a positive screen for salinity tolerance, and be better than a negative screen such as leaf injury. The photosynthesis rate of salt-tolerant genotypes did not correlate with toxic ions content in leaves (Table 4.2). This would indicate that different mechanisms in the ion specific effects might be observed among tested genotypes, which are introduced below.

The results of ion contents showed that the salt-tolerant genotypes had the lowest Na^+ and Cl^- contents in leaves and stems at day 45 and final harvest (Fig. 4.2). Interestingly, the salt-sensitive genotype (Westonia) had the ability to exclude Na^+ from the shoots such as salt-tolerant genotypes (Fig. 4.2). This indicates that this criterion (salt ions exclusion) did not always predict salt tolerance differences among wheat genotypes. A similar result was found in rice (Yeo and Flowers, 1986) and maize (Cramer et al., 1994). The other salt-sensitive genotypes had the highest contents of Na^+ in leaves and stems and Cl^- content in leaves (Fig. 4.2). Moreover, the contents of K^+ and Ca^{2+} in leaves and stems of salt-tolerant genotypes were decreased slightly with increasing salinity. In contrast, K^+ content in leaves of salt-sensitive genotypes and Ca^{2+} contents in leaves and stems of moderate-tolerant and salt-sensitive genotypes were decreased significantly with salinity (Fig 4.3). Therefore, the increase in salt ions and/or decrease in essential ions in moderate-tolerant and salt-sensitive genotypes may affect efficiency of RuBP carboxylase and other enzymes that are related to the photosynthetic process (Seemann and Critchley, 1985). Therefore, *A* and NAR of these genotypes were decreased significantly under salinity and RGR was highly correlated with NAR in these genotypes. Although, the reduction in *A* of salt-tolerant genotypes did not relate with ion effects (Table 4.2), the NAR of these genotypes was also related to RGR (Fig 4.1). This indicates that the reduction in NAR of these genotypes may be related to feedback inhibition of carbon metabolism. Munns and Termaat (1986) postulated that inhibition of photosynthesis could be a consequence of feedback inhibition of carbon metabolism rather than ion excess directly affecting photosynthetic metabolism. In addition, the massive accumulation of soluble carbohydrates for osmotic adjustment diverts most of the energy otherwise available for growth and in turn both carbohydrate accumulation, and reduced sink strength may negatively affect net assimilation rate by feedback mechanisms (Bohnert et al., 1995).

In general, plants are able to tolerate salinity by reducing leaf osmotic potential; by synthesis of organic solutes; or by compartmentation of inorganic ions, in a process called osmotic adjustment (Hazegawa et al., 2000). The decrease in leaf water potential was accompanied by a decrease in leaf osmotic potential so that leaf turgor pressure of the salinized plant was maintained (Tattini et al., 1995). The inorganic ions, especially Na^+, Cl^- and K^+, were generally the major components of osmotic adjustment. Most importantly, although the energetic cost of osmotic adjustment by inorganic ions is much lower than that conferred by organic solutes synthesized, this could also lead to produce toxic effects because such high concentration of toxic ions may interfere with normal biochemical activates within the cell (Yeo et al., 1985). The results in Chapter 4 showed that although the osmotic adjustment of moderate-tolerant and salt-sensitive genotypes was higher than that of salt-tolerant genotype, this osmotic adjustment did not avoid the toxic effects of salt ions on the reduction of growth in these genotypes. This indicates that the achievement of osmotic adjustment was not an important mechanism conferring salt tolerance to wheat genotypes, probably because osmotic adjustment in salt-sensitive genotypes was achieved by toxic ions, which interfere with normal biochemical activation within the cell. The salt-tolerant genotypes have the higher value for leaf turgor pressure than moderate-tolerant and salt-sensitive genotypes (Fig 4.5). Therefore, the ability of genotypes to maintain higher turgor pressure plays an important role in their salt tolerance (Heuer and Plaut, 1989), because leaf turgor pressure is an important factor for the regulation of the leaf function and metabolic process at the cell level to maintain higher growth under salinity. This indicates that leaf turgor pressure is more related with salt tolerance of genotypes than osmotic adjustment. Similarly, Rivelli et al (2002) reported that osmotic adjustment occurred in all salt-stressed wheat genotypes, with one of the most salt-sensitive genotypes having the greatest osmotic adjustment.

As mentioned above, wide genotypic differences were observed among genotypes for salt tolerance mechanisms, indicating that physiological traits could be used as selection criteria of salt tolerance. However, the reliability of using physiological traits should be evaluated by their association with the plant salt tolerance (grain yield). In this study, the reliability of different physiological traits as selection criteria was judged by comparing the scores of different physiological traits at different harvest at 150 mM NaCl with their scores on grain yield (Tables 3.4-3.7); and by analysis of the relationships between the scores of physiological traits and grain yield by using linear regression (Tables 3.8-3.10). If the scores

of physiological traits are consistent and correlated with their scores on grain yield, the physiological traits could be reliable and feasible as screening criteria for salt tolerance (Zeng et al., 2003). The results in Chapter 3 showed that the scores on ion contents in leaves, leaf photosynthesis rate, stomatal conductance, chlorophyll content (SPAD value), and leaf water potential regardless of measuring time and leaf turgor pressure at day 45, and leaf osmotic potential at day 60 were significantly correlated with the scores on grain yield, indicating that any of these traits may be used as screening criteria for the salt tolerance of wheat genotypes. However, Na^+ and Cl^- contents in stems and leaf transpiration rate regardless of measuring time were not significantly correlated with the scores on grain yield. Furthermore, the scores on photosynthesis rate, stomatal conductance, respiration rate, SPAD value, leaf turgor pressure, and leaf osmotic potential were more consistent with their scores on grain yield at day 60 than at day 45. This indicates that some aspects should be considered when the physiological traits could be used as selection criteria for the salt tolerance of wheat genotypes, which will be introduced in the following section.

5.4 Physiological traits used as quick and easy criteria for selecting salt tolerant genotypes

Because screening salt tolerance of genotypes based on agronomic parameters have many inherent disadvantages such as differential growth and developmental patterns between genotypes, and logistical and time constraints with long-term growth comparisons, especially for a large number of genotypes tested for salt tolerance breeding, some researchers have suggested that the evaluation of salt tolerance among genotypes might be more successful if evaluation is based directly on the relevant physiological traits (Yeo et al., 1990; Jackson et al., 1996; Zeng et al., 2002). The advantage of the utilization of physiological traits in the evaluation for salt tolerance among genotypes are the physiological criteria are able to supply more objective information than agronomic parameters when screening for component traits of complex characters (Yeo, 1994); screening for specific physiological traits can reduce the time needed to grow plants under salinity; and can eliminate the need to grow plants under controlled conditions (Munns and James, 2003) Furthermore, the benefit of evaluating salt tolerance based on physiological traits has recently been proposed due to the availability of new devices like SPAD meter for specific traits determination that are more quick and easy to use in field measurements. However, selection based on physiological criteria is fraught with uncertainties and unknowns.

To effectively use the physiological traits as quick and reliable criteria for screening salt tolerance of genotypes, the following aspects should be considered:

i) Time when the physiological traits are used as an effective screening criterion. In our study, we found that significant genotypic variation in net photosynthesis rate, stomatal conductance and SPAD values were observed at 45 and 60 days after sowing. However, the genotypic variation was greater at 60 days after sowing than at 45 days (Table 3.9). In contrast, genotypic differences in leaf water potential were greater at 45 days after sowing than at 60 days (Table 3.10). The other trait such as leaf turgor pressure was significantly correlated with grain yield at day 45, but not at day 60. The opposite observation was found in leaf osmotic potential (Table 3.10), which may be due to the early response to salinity is to the osmotic effects of the salt outside the roots (water deficit). However, the salt-specific effect, ion toxicity and ion imbalance, takes more times to show up (Munns, 1993). The osmotic stress of the salt outside the roots reduces the amount of water absorption, which affects on leaf water relations (Munns, 2002). However, accumulation of the toxic ions inside the plant or reduction in the uptake of essential ions effects on the enzymes and mechanisms process that are related with photosynthesis machinery and chlorophyll content (Huang and Redman, 1995; He and Cramer, 1992; Cramer, 2002). Therefore, leaf water relations (leaf water potential and leaf turgor pressure) can be used as economic criteria because it is one of the most important indicators for evaluation of salt tolerance among genotypes at early growth stage. Although, the measurement of leaf water relation in our study is effective as screening criterion at early growth stage, this method is impractical for screening large number of genotypes (typically ca. 30 samples per hour using a pressure chamber) (Jongdee et al., 2002). Therefore, evaluating the genotypes for salt tolerance by photosynthetic parameters and SPAD values is much practical for large scale screening when there are large number of test genotypes to be evaluated through breeding programs.

ii) The organs of plant in using ion contents as physiological traits. Some researchers have found the lack of correlation between the scores on absolute ion concentrations in the shoots and the scores on grain yield (Yeo and Flowers, 1986; Zeng et al., 2003). In that study, shoot ion contents were measured based on the total above ground biomass. This approach may not identify some salt tolerance components such as ion distribution in organs of plant. Therefore, analysis of ion contents in different organ of plants is necessary when ion contents are used as screening criteria. The results in Chapter 3 showed that the scores among

genotypes based on Na^+ and Cl^- contents in leaves at day 45 and final harvest were significantly associated with the scores on grain yield. However, the scores among genotypes based on both ion contents in stems regardless of measuring times were inconsistent and not significantly correlated with their scores on grain yield (Tables 3.4 and 3.8). This suggests that Na^+ and Cl^- accumulation in leaves is more reliable as screening criteria for evaluation of salt tolerance among wheat genotype than their contents in stems, which is in agreement with the work in literature (Asch et al., 2000; Munns and James, 2003).

iii) The number of plant samples. This is very important when the numbers of plants are limited, especially in breeding programs. The analysis of ion contents requires destructive plant harvesting and subsequent tissue analysis. Therefore, the criterion of ion contents cannot be used as screening criteria in case of limiting plant number (Noble and Rogers, 1992). In this case, the other physiological criteria can be used as rapid and quick screening technique without requiring plant harvest such as measuring of photosynthetic parameters by using porometer and total chlorophyll contents by using SPAD meter. The results in Chapter 3 showed that the scores on photosynthesis rate, stomatal conductance and SPAD value at 60 days after sowing were consistent with their rankings in terms of grain yield (Table 3.6). The slopes from the linear regression of the scores on the three parameters against the scores on grain yield were highly significant at days 45 and 60 (Table 3.9). Most importantly, the results of SPAD value are consistent with their photosynthesis rate and stomatal conductance. Therefore, our results suggest that a simple and non-destructive measure of chlorophyll content (measured using a SPAD meter) would be an adequate screening method for evaluating large number of genotypes in breeding programs without requiring plant harvest, especially this method is much more handy and practical for large scale screening. The SPAD value found in other studies is closely correlated with tolerance to iron-deficiency chlorosis (Samdur et al., 2000), high Na^+ accumulation in leaves (Munns and James, 2003) and with maximum net photosynthesis rate (Laza et al., 1996).

Most importantly, although measuring of ion contents are not feasible as a screen criterion because analysis of ion contents takes more time and needed many plants at harvest, the physiological criteria should include these criteria (ion exclusion or selectivity), because many physiological mechanisms and the strategies for mitigating salinity problems in crop production through management practices depended on better understanding of physiological mechanisms of ion effects. Therefore, we can suggest that measuring of ion contents can be

valuable to investigate a small number of genotypes that have been obtained by quicker selection methods (Cramer et al., 1994; Zhu et al., 2001).

iv) Combined physiological traits are more reliable and feasible as screening criteria for salt tolerance rather than only single specific physiological traits. The results in Chapter 3 showed that some physiological traits such as Na^+ exclusion and transpiration rate failed to evaluate salt tolerance for all tested genotypes. For example, the scores of Na^+ content in leaves and stems for salt-sensitive genotype (Westonia) was inconsistent with their rankings in terms of grain yield (Tables 3.4 and 3.5). The scores among genotypes on leaf transpiration rate were not similar to those on grain yield and the correlation between them was not significant at days 45 and 60 (Tables 3.6 and 3.9). Therefore, the results suggest that a combination of physiological traits is logically a desirable objective in screening for salt tolerance of genotypes. Jackson et al (1996) reported that using specific physiological traits or single traits in breeding programs has not until now been as good as expected because no single process can account for the variation in the plants response to salinity due to effects of soil properties, the osmotic effect of high ionic concentrations, competitive interference with nutrient uptake and toxic effects within the plant tissue and the effects of other environmental factors such as temperature and light.

5.5 Conclusion

From this study, we conclude that screening salt tolerance of genotypes based on agronomic parameters is more reliable when genotypes are ranked for salt tolerance based on the means of multiple parameters. Screening salt tolerance based on salt-sensitive agronomic parameters can be made at early growth stage for genotypes when their salt tolerance does not change with growth stages. Physiological parameters, not all, are more quick, easy, economic and feasible for large scale screening when there are large number of tested genotypes to be evaluated through breeding programs. The time of measuring physiological traits must be considered for using those traits as selection criteria. Combined physiological traits should be considered in screening salt tolerance of wheat genotypes rather than only single specific physiological trait. Better understanding of physiological processes of salt tolerance may help breeding programs because the physiological traits could be more effective for screening salt tolerance than others. Although screening salt tolerance based on ion analysis takes more time and needs more plant harvest, the physiological criteria should include these criteria (ion exclusion or selectivity). Therefore, we suggest that these criteria could be used to investigate

a small number of genotypes selections that have been obtained by quicker selection methods. The growth of salt-tolerant genotypes was affected by salinity primarily due to decline in photosynthetic capacity that is correlated to the lower stomatal conductance. The decline in photosynthetic capacity and leaf area is the growth-limited factor in moderate-tolerant and salt-sensitive genotypes. The decline in photosynthesis capacity in these genotypes is related to ion effects, lower stomatal conductance, increase in the respiration rate and/or reduction in chlorophyll content. Although this study provides important approach and information about how to evaluate salt tolerance among wheat genotypes, the experiments were conducted in the controlled environment. Therefore, there is a need to further study under the field conditions.

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6 Summary

In Egypt, most extension regions growing wheat plants suffer from severe salinity problems. However, there is still no salt tolerant wheat genotype available in Egypt to overcome salinity problems. The low success in developing wheat salt tolerance is, at least partially, due to low selection efficiency using multiple agronomic parameters and lack of effective evaluation methods for salt tolerance among genotypes in the screening process. Therefore, this study aimed: (i) to identify useful agronomic parameters for evaluation of salt tolerance; (ii) to develop effective approaches to evaluating genotypes by multiple agronomic parameters for salt tolerance at different growth stages; and (iii) to find out reliable multiple physiological traits that can be used as quick, easy and economic screening criteria for salt tolerance among wheat genotypes. Thirteen wheat genotypes from Egypt, Germany, Australia and India were grown in soil with four salinity levels (control, 50, 100 and 150 mM NaCl) in a greenhouse. The parameters of plant growth and yield, yield components of the main spike, ion content in leaves and stems, photosynthetic and water relation parameters, and SPAD value were determined.

The present study demonstrated that the number of leaves and tillers per plant, and spikelet per main spike were reduced significantly by salinity, and also showed wide genotypic differences that can be used for evaluating plant salt tolerance, especially at early growth stages. However, grain number per spike and thousand-grain weight were less sensitive to salinity. Few genotypic differences were identified among genotypes on these two parameters. Although the genotypic differences in the number of leaves and tillers varied with the levels of salinity and the growth stages, using multiple agronomic parameters at different growth stages makes it possible to rank plant salt tolerance. According to cluster analysis with multiple agronomic parameters at different growth stages, the Egyptian wheat genotypes (Sakha 8 and Sakha 93) and the Indian genotype (Kharchia) were ranked as most tolerant to salinity. A change in salt tolerance with growth stages was observed for genotypes (Sids 1, Gemmeza 7 and Westonia). Drysdale and Sakha 69 were ranked as moderate-tolerant genotypes. Sakha 61, Giza 168, Sahel 1, Thassos and Triso were ranked as the salt-sensitive genotypes at all growth stages. This information is very important not only for breeders interested in improving salt tolerance of Egyptian wheat genotypes, but also for farmers who grow wheat in areas where the soil and ground water has a high salinity. We confirm that cluster group ranking of genotypes based on multiple agronomic parameters can be applied in salt tolerance breeding to evaluate salt tolerance and may have great advantage over conventional methods.

Screening techniques developed under controlled environments are difficult to apply to screening large numbers of genotypes for salinity tolerance in the field due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctuations in rainfall. Because of the complex nature of salt tolerance, as well as the difficulties in maintaining long-term growth experiments, physiological traits as selection criteria are recommended for screening. In order to have reliable and feasible physiological traits, however, there is a need to have a better understanding of the physiological processes of salt tolerance. The study here showed that salinity directly causes ion toxicity and nutrient imbalance and indirectly leads to water deficit and low photosynthesis for plants, especially at high level of salinity. The use of physiological traits as selection criteria in salt tolerance breeding requires the genetic variation among genotypes. The physiological traits (ion contents in leaves and stems (Na^+, Cl^-, K^+, Ca^{2+}), the ratios of K^+/Na^+ and Ca^{2+}/Na^+ in the leaves and stem, net photosynthesis rate, stomatal conductance, respiration rate, transpiration rate, SPAD value, leaf water relations), except for Na^+ and Cl^- in stems and leaf transpiration rate at 150 mM NaCl, showed a significant genotypic variation, indicating the traits that have a significant genotypic variation may be possibly used as screening criteria. However, this study also showed that some traits in genotype Westonia were not associated with its salt tolerance in terms of grain yield. Thus, combined physiological traits such as Na^+ and Cl^- excluder traits along with ionic selectivity or photosynthesis rate should be considered in screening salt tolerance of wheat genotypes rather than only single specific physiological traits. According to the analysis of the linear regression of the physiological traits against the grain yield, the slopes for the traits of K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+, Na^+ and Cl^- in leaves, photosynthesis rate, stomatal conductance, and SPAD value were greatest. From a practical point of view, however, the traits of net photosynthesis rate, stomatal conductance and SPAD value should be considered as screening criteria.

In conclusion, the study on the evaluation of salt tolerance of Egyptian wheat genotypes compared with the standard salt tolerance genotype of wheat Kharchia based on physiological and agronomic traits is not only important for Egyptian farmers to develop different strategies to increase wheat salt tolerance by agricultural practices, but it also suggests that breeders should consider to include the physiological and agronomic traits such as more tillers, high leaf number, great leaf area, low Na^+ and Cl^- content, high selectivity of K^+ and Ca^{2+} over Na^+, high photosynthesis rate, high stomatal conductivity, and less respiration rate in breeding programs for increasing the salt tolerance of Egyptian wheat genotypes.

7 Zusammenfassung

In den meisten neu kultivierten Gebieten Ägyptens treten starke Versalzungsprobleme im Weizenanbau auf. Bisherige Weizensorten weisen im allgemeinen eine nicht genügende Salztoleranz auf. Als Folge der niedrigen Selektionseffizienz bei der Verwendung multipler agronomischer Parameter und des Fehlens effizienter Evaluierungsmethoden bei der Auslese ist die Entwicklung neuer salztoleranter Sorten sehr schwierig. Ziel dieser Arbeit war es, (i) nützliche agronomische Parameter für die Evaluierung der Salztoleranz zu identifizieren, (ii) effiziente Vorgehensweisen in der Evaluierung der Salztoleranz von Genotypen in verschiedenen Entwicklungsstadien basierend auf multiplen agronomischen Parametern zu entwickeln, und (iii) verlässliche multiple physiologische Eigenschaften herauszufinden, die als schnelle, einfache und ökonomische Kriterien der Salztoleranz eingesetzt werden können. 13 Genotypen aus Ägypten, Indien, Australien und Deutschland wurden bei vier Salzniveaus (Kontrolle, 50, 100 und 150 mM NaCl) in Boden in einem Gewächshaus angebaut. Das Wachstum der Pflanzen und der Ertrag, sowie die Ertragskomponenten der Hauptähre, der Ionengehalt der Blätter und der Stängel, die Photosyntheserate und SPAD-Werte wurden bestimmt.

Diese Arbeit zeigt, dass die Zahl der Blätter und der Triebe pro Pflanze, sowie die Anzahl Ährchen pro Hauptähre signifikant durch Salzstress reduziert werden. Es fanden sich breite genotypische Unterschiede, die für die Evaluierung der Salztoleranz speziell in frühen Entwicklungsstadien genutzt werden können. Die Anzahl Körner pro Ähre sowie das Tausendkorngewicht reagierten weniger empfindlich auf Salzstress. Obschon die genotypischen Unterschiede in der Zahl der Blätter und in der Triebzahl in Abhängigkeit der Salzkonzentration und der Entwicklungsstadien variierten, ergab sich die Möglichkeit mittels multiplen agronomischen Parametern Rangierungen der Salztoleranz aufzustellen. Basierend auf einer Clusteranalyse mittels multiplen agronomischen Parametern wurden die ägyptischen Genotypen Sakha 8 und Sakha 93 sowie die indische Referenzsorte Kharchia als salztolerant bewertet. Eine entwicklungsbedingte Änderung in der Salztoleranz ergab sich bei Sids 1, Gemezza 7 und Westonia. Drysdale und Sakha 69 wurden als mitteltolerant bewertet. Als am meisten salzempfindlich erwiesen sich Sakha 61, Giza 168, Sahel 1, Thassos und Triso. Diese Informationen sind nicht nur für Züchter zur Verbesserung ägyptischer Weizengenotypen von Interesse, sondern auch für Landwirte die Weizen in salinen Gebieten anbauen. Mit dieser Arbeit wird bestätigt, dass, basierend auf multiplen agronomischen Parametern, Clustergruppierungen in der Bewertung von Genotypen gegenüber Salztoleranz vorgenommen werden können und diese große Vorteile gegenüber konventionellen Methoden aufweisen.

Auslesetechniken, die unter kontrollierten Bedingungen entwickelt werden, können unter Feldbedingungen aufgrund der Heterogenität der chemischen und physikalischen Bodeneigenschaften und der saisonalen Fluktuation der Niederschläge bei einer großen Anzahl von Genotypen nur erschwert in der Auslese auf Salztoleranz eingesetzt werden. Aufgrund der komplexen Natur der Salztoleranz, wie auch der Schwierigkeit vergleichbare Wachstumsbedingungen langfristig aufrechtzuerhalten, werden physiologische Eigenschaften als Selektionskriterien in der Auslese empfohlen. Um verlässliche und brauchbare physiologische Eigenschaften abzuklären, sind bessere Kenntnisse der physiologischen Prozesse der Salztoleranz unerlässlich. In dieser Arbeit wurde gezeigt, dass Salinität direkt Ionentoxizität und Nährstoffungleichgewichte verursacht und indirekt, speziell bei hoher Salinität, zu Wasserdefizit und niedriger Photosynthese führt. Um physiologische Kriterien in der Züchtung auf Salztoleranz zu nutzen, ist eine genotypische Variabilität erforderlich. Eine signifikante genotypische Variabilität und damit ein möglicher Einsatz als Auslesekriterien wurde bei den physiologischen Eigenschaften Ionengehalt in Blättern und Stängeln (Na^+, Cl^-, K^+, Ca^{2+}), K^+/Na^+- und Ca^{2+}/Na^+-Verhältnis in den Blättern und im Stängel, Nettophotosyntheserate, stomatäre Leitfähigkeit, Respirationsrate, Transpirationsrate, SPAD-Wert und Blattwasserstatus festgestellt. Eine Ausnahme bildeten Na^+ und Cl^- im Stängel und die Blatttranspiration bei 150 mM NaCl. Beim Genotyp Westonia standen einige Eigenschaften jedoch nicht in Beziehung zum Ertrag. Kombinierte physiologische Eigenschaften wie das Ausschlussvermögen bei Na^+ und Cl^- zusammen mit der Ionenselektivität oder der Photosyntheserate sind daher gegenüber einzelnen physiologischen Merkmalen vorzuziehen. Lineare Regressionen zwischen physiologischen Eigenschaften und dem Kornertrag ergaben die größten Steigungen bei den Merkmalen K^+, Ca^{2+}, K^+/Na^+, Ca^{2+}/Na^+, Na^+ und Cl^- in den Blättern, Photosyntheserate, stomatäre Leitfähigkeit und SPAD-Wert. Aus praktischen Überlegungen sind die Eigenschaften Nettophotosyntheserate, stomatäre Leitfähigkeit und SPAD-Wert vorteilhaft.

Die Ergebnisse dieser Arbeit zeigen, dass die Evaluierung der Salztoleranz ägyptischer Weizensorten basierend auf physiologischen und agronomischen Eigenschaften einerseits von Bedeutung für Landwirte ist, um verschiedene Strategien zur Verbesserung der Salztoleranz durch Bewirtschaftungsmaßnahmen zu entwickeln. Andererseits ergibt sich aus dieser Arbeit, dass Züchter zur Verbesserung der Salztoleranz ägyptischer Weizensorten physiologische und agronomische Eigenschaften berücksichtigen sollten wie eine höhere Trieb- und Blattzahl, eine große Blattfläche, niedrige Na^+und Cl^- Gehalte, eine hohe K^+/Ca^{2+} Selektivität gegenüber Na^+, eine hohe Photosyntheserate und stomatäre Leitfähigkeit sowie eine niedrige Respirationsrate

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