PHYSIOLOGICAL TRAITS UNDERLYING DIFFERENCES IN SALT TOLERANCE AMONG *GLYCINE* SPECIES

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

PHYSIOLOGICAL TRAITS UNDERLYING DIFFERENCES IN SALT TOLERANCE AMONG *GLYCINE* SPECIES

Presented by Julian Mario Lenis,

A candidate for the degree of Master of Science,

And hereby certify that in their opinion it is worthy of acceptance.

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I dedicate this thesis to:

my beloved parents,

my girlfriend, Masha,

and friends.

Without their generosity, patience, support and most of all love, the completion of this work would have been impossible.

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Chapter I

INTRODUCTION

According to Boyer (1982), the yield gap between average on-farm crop yield and the crop yield potential in optimized conditions is attributable to the following causes: (1) diseases and insects, which sporadically devastate crops at a local level, but generally do not depress average U.S. yields more than 4.1 and 2.6%, respectively, (2) weed competition, which depresses yield by 4%, and (3) inappropriate soil conditions and/or unfavorable weather, which were estimated to decrease yield potential by as much as 69%. The reduction in yield attributable to the various physiochemical factors (abiotic stresses) has been the subject of much study by plant physiologists (Raper and Kramer, 1987; Ludlow and Muchow, 1990; Nilsen and Orcutt, 1996; Araus et al., 2002). However, progress in improving plant tolerance to environmental stresses has been slow because of the complexity of the trait.

Importance and origin of salinity

Salinity is one of the major abiotic stresses that adversely affects crop productivity and quality (Chinnusamy et al., 2005). The total world area of saline and

sodic soils is 397 million and 434 million ha, respectively, totaling over 830 million ha affected by salt. This represents over 26% of the world's potentially arable land. About 20% of the current 230 million ha of irrigated land is affected by saline soils. Salinity is also a problem in dryland agriculture, affecting to varying degrees over 2% of the almost 1,500 million ha of non-irrigated land (FAO, 2008).

Although most of the salinity is natural, a significant proportion of cultivated land has become saline because of human-induced processes. Land clearing, especially in areas prone to salinization, irrigation mismanagement (either too much or too little irrigation water), use of irrigation water with high salt concentrations and over fertilization are some of the factors that contribute to the increase in salt affected area (Munns, 2005; FAO, 2008). The salinity problem is predicted to increase in the future. Blumwald and Grover (2006) predict that about 50% of the arable land will be affected by salinity by the year 2050.

According to the USDA salinity laboratory (1954), saline soil can be defined as soil having an electrical conductivity of the saturated paste extract (ECe) of 4 dS m^{-1} or more. Different tolerance thresholds and different rates of reduction in yield beyond this tolerance threshold indicate variation in mechanisms of salt tolerance among crop species (Chinnusamy et al., 2005).

Role of Na^+ and Cl^- in plants

Ions contributing to salinity include Cl^-, SO_4^{2-}, HCO_3^-, Na^+, Ca^{2+}, Mg^{2+} and rarely NO_3^- or K^+. The salts of these ions occur in highly variable concentrations and proportions (Bernstein, 1975). In saline soils, Na^+ and Cl^- are generally the dominant ions (Marschner, 1995).

The ion Na^+ is not an essential mineral element for plants although it has been shown to be beneficial for several species with a C4 metabolism (Marschner, 1995). Also, according to Kuiper (1984), plants accumulate Na^+ at the expense of K^+ and Ca^{2+}, which are essential mineral elements. Rengel (1992) considered that salt tolerance of plants was related to their ability to avoid Na^+ toxicity and maintain K^+ and Ca^{2+} levels. Soybean [*Glycine max* (L.) Merr.] is a natrophobic species, that is, no substitution of K^+ by Na^+ is possible without severely impacting growth (Marschner, 1995).

Chloride is an essential mineral element for plants. Engvild (1986) reported that more than 130 chlorinated compounds were found in higher plants, with some of them associated with antibiotic and fungicide activity. Moreover, the seeds of some legume species contain substantial amounts of chlorinated IAA (auxins) suggesting a role in growth (Hofinger and Bottger, 1979). Also, Cl^- stimulates a tonoplast H^+-ATPase, which is involved in the sequestration of Cl^- in vacuoles (Churchill and Sze, 1984). Chloride is the main inorganic anion in vacuoles and plays an important role in osmoregulation as well as in stomatal closure and opening. Furthermore, Cl^- has a stimulating effect on

asparagine synthetase, enzyme that functions in nitrogen metabolism (Rognes, 1980). Finally, the H_20 splitting system of photosystem II requires Cl^- for proper function (Marschner, 1995). In spite of the important roles Cl^- plays in plant metabolism, at high concentrations in soils, it becomes toxic to plants (Abel and Mackenzie, 1964; Bernstein, 1975; Marschner, 1995).

Effect of salinity on plants

Salinity imposes two major stresses on plants. First, salinity results in a high osmotic pressure in the soil solution (outside the plant), making it harder for the plant to extract water. This osmotic stress reduces stomatal conductance, photosynthesis and, therefore, plant growth. Second, salinity causes ion-specific stress due to the accumulation of Na^+ and Cl^- in the plant. When talking about ion-specific effects, it is important to distinguish between direct toxicities and nutritional effects. Regarding direct toxicities, Na^+ and Cl^- buildup in plant tissues promotes the formation of reactive oxygen species (ROS). ROS formation has multiple and detrimental effects such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage. Massive accumulation of Na^+ and Cl^- also results in nutrient imbalance by reduction in uptake and shoot transport as well as impaired internal distribution of mineral nutrients (Marschner, 1995; Ashraf, 2004; Yamaguchi and Blumwald, 2005; Munns, 2005; Chinnusamy et al., 2005; Tuteja, 2007; Munns, 2008; Munns et al., 2008).

In addition, Na^+ and Cl^- accumulation may impair the ability of leaves to regulate water loss under conditions of high evaporative demand (Bernstein, 1975).

Typically, plants present a 2-phase growth response to salinity. The first phase of growth response results from the effect of salt outside the plant. The cellular and metabolic processes involved are in common to drought-affected plants. At this stage, neither Na^+ nor Cl^- builds up in growing tissues at concentrations that inhibit growth. The second phase of growth response results from the ion-specific effect of salt inside the plant. Sodium and Cl^- concentrations exceed the ability of cells to compartmentalize the ions in the vacuoles. As a result, there is a rapid accumulation of salts in the cytoplasm and inhibition of enzymatic activity. In addition, salts might build up in cell walls and dehydrate the cell (Munns, 2005).

According to Munns et al. (2008), the genetic variation in growth response to the osmotic effect of salinity is likely to be small, not only within a species but also across species. On the other hand, there is evidence of genetic variability for tolerance to the ion-specific effect of salt inside the plant (Koyama et al., 2001; Lee et al., 2004; Farooq and Azam, 2006; Kao et al., 2006; Lee et al., 2006).

Phenotypic plant responses to salinity include decrease in biomass production, with shoot growth more affected than root growth; changes in chlorophyll content; leaf

necrosis; yield reduction; and, in the most severe cases, plant death (Abel and MacKenzie, 1964; Parker et al., 1983; Joly, 1989; Wang and Shannon, 1999; Essa, 2002; An et al., 2002; Katerji et al., 2003).

Environmental conditions are very important in the occurrence and severity of salinity-induced symptoms. Ehlig (1960) observed that in the spring, leaves of grapes may contain toxic levels of Na^+ and Cl^- and show no symptoms of injury. However, with the onset of hotter and dryer weather in summer, leaf scorch suddenly appeared. Therefore, salinity x environment interactions have to be considered in order to accurately assess salt tolerance of genotypes (Shannon, 1984; Li et al., 2000).

Effect of salinity on soybean

Cultivated soybean is considered to be a salt sensitive species (Läuchli, 1984). Several studies have been conducted on how salinity affects different soybean traits. Soybean plants growing under saline conditions showed severe leaf scorch. However, not all genotypes showed the same level of leaf injury, indicating the existence of genetic variability (Abel and MacKenzie, 1964; Nukaya et al., 1982; Grattan and Maas, 1985; Pantalone et al., 1997; Lee et al., 2004).

Effects of salinity on various photosynthetic parameters were also studied. Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt

stress is a general phenomenon. Chen and Yu (2007) also observed a significant decrease in chlorophyll content under high NaCl. Cicek and Cakirlar (2008) showed that chlorophyll content in soybean leaves was the result of the interaction between genotype, salinity and temperature, that is, chlorophyll content of a given genotype varied with and was dependent on salt level and temperature. Increased temperature was more consistent in decreasing chlorophyll content than increased NaCl concentration.

Joly (1989) reported a reduction in root system hydraulic conductivity when seedlings were exposed to increasing osmotic potential induced by addition of NaCl. The permeability of the root system to water was reduced by 72% as the solution osmotic potential dropped from -0.10 to -0.26 MPa. This resistance to water flow in roots resulted in decreased shoot/root ratios, showing that shoot growth of soybean seedlings exposed to high NaCl was impaired more than root growth. Also, increased salinity resulted in a reduction in dry weight accumulation and plant height (Bernstein, 1975; Läuchli and Wieneke, 1979; Marschner, 1995; Essa, 2002; Cicek and Cakirlar, 2008).

Despite reductions in plant growth from increased salinity, low salt concentrations may have a stimulating effect on growth and development in glycophytes like soybean (Meloni et al., 2001; Cicek and Cakirlar 2002, 2008). Other plant species that were not halophytes also showed a remarkable positive growth response to increasing levels of NaCl, which was attributed to Na^+ and its role in osmotic

adjustment (Flowers and Läuchli, 1983). Examples of such species are sugar and table beet, turnip, cotton, pea and wheat. However, most of the important crop species have a natrophobic behavior and low salt tolerance (Marschner, 1995).

Salt tolerance in soybean

Salt tolerance in soybean is thought to be primarily related to the ability of plants to limit accumulation of excess ions in leaves (exclusion) avoiding toxic buildups and nutrient imbalances (Abel and MacKenzie, 1964; Läuchli and Wieneke, 1979; Umezawa et al., 2002; Pathan et al., 2007). Läuchli and Wieneke (1979) reported that leaf injury in the salt sensitive cultivar Jackson growing in high salinity substrate was caused by accumulation of both Na^+ and Cl^-. Luo et al. (2005) reported that Na^+ was more toxic than Cl^- in *G. soja* seedlings, while the opposite was true for *G. max* seedlings. Significant increases in Cl^- concentration were observed in plant tissues when NaCl in the media was high. However, soybean cultivars regarded as salt tolerant had a higher capacity to reduce transport and accumulation of Cl^- into leaves and stems and keep this anion in roots, so that Cl^- was excluded from shoot and accumulated in root (Abel and MacKenzie, 1964; Wieneke and Läuchli, 1979; Läuchli and Wieneke, 1979; Maas, 1985; Essa, 2002; Luo et al., 2005).

There is conflicting information regarding accumulation of Na^+ in leaves, stems and roots of soybean plants with differential tolerance to salinity. Compared to Cl^-, a similar

response was observed for Na^+ accumulation by some researchers (Läuchli and Wieneke, 1979; Essa, 2002; Luo et al. 2005). On the other hand, Grattan and Maas (1985) did not find any difference in Na^+ concentration in leaves of salt tolerant and sensitive genotypes and concluded that soybean accessions effectively exclude Na^+ from leaves. Also, Dabuxilatu and Ikeda (2005) found trace amounts of Na^+ in leaf cells of soybean plants growing under NaCl stress and suggested that Na^+ would be retained in root cortical cells and thus not readily transported to shoot.

Many studies were carried out to investigate which plant part regulates Cl^- and Na^+ transport and accumulation. One practical approach to address this problem is grafting scions from salt tolerant plants onto roots from sensitive plants and vice versa and then, comparing ion contents in roots and scions of grafted plants exposed to varying salt concentrations. Authors agree that shoot, and not root, controls plant growth in absence of salt. However, under saline conditions, plant growth depended to a large extent on the ability of the roots to exclude Cl^- and Na^+ ions from being transported to the shoot. Therefore, shoots grafted onto includer roots (i.e., roots from sensitive accessions) contained excessive concentrations of Na^+ and Cl^- in their leaves, while shoots grafted onto excluder roots (i.e., roots from tolerant accessions) maintained low Na^+ and Cl^- concentrations. Thus, roots controlled ion accumulation in shoots (Wieneke and Läuchli, 1979; Grattan and Maas, 1985; Romero et al., 1997; Santa-Cruz et al., 2001, 2002; Estañ et al., 2005; Martinez-Rodriguez et al., 2008).

Although roots control uptake, translocation and accumulation of Na^+ and Cl^-, there could be additional mechanisms determining the final reaction of a genotype to high salt conditions. For example, White et al. (1979) determined that while roots control uptake and translocation of Zn, the ability of soybean to tolerate toxic levels of that element depends mainly on shoots and some factors other than leaf Zn levels. Similarly, Heenan and Carter (1976) demonstrated that the mechanism controlling leaf Mn toxicity in soybean resides exclusively in shoots. Moreover, the ability of shoots to tolerate toxic internal levels of Mn was more important than their ability to accumulate less Mn.

Exclusion of Na^+ and Cl^- from the transpiration stream, limited entry of these ions to the cell and their efficient compartmentalization in sub-cellular structures are important mechanisms that contribute to salt tolerance in plants (Tuteja, 2007). Studies comparing the effect of salinity on the activity of enzymes from species differing in salt tolerance showed that enzymes of saltbushes and sensitive plants were equally affected by high salt concentrations in vitro (Weimberg, 1970; Greenway and Osmond, 1972). Sequestration of toxic ions in vacuoles, and, thus, protection of cytoplasmic enzymes and organelles is thought to play an important role in salt tolerance (Bernstein, 1975). In soybean, there is evidence suggesting that ion compartmentalization in vacuoles could reduce harmful effect of toxic levels of Na^+ and Cl^- in cells. Li et al. (2006) demonstrated that two tonoplast-located soybean transporters, GmCLC1 and GmNHX1, enhanced NaCl tolerance in tobacco cells. GmCLC1 and GmNHX1 protected cells from

high salt through the sequestration of cytoplasmic Cl^- and Na^+ into vacuoles, respectively.

Salinity-induced changes in concentration of other nutrient elements such as K^+, Mg^{2+} and Ca^{2+} in different plant tissues were studied by a few authors (Läuchli and Wieneke, 1979; Nukaya et al., 1982; Essa, 2002). In most cases, results have to be analyzed on a case by case basis since they were not always in agreement.

Role of plant breeding in solving the problem of salinity

Problems with soil salinity could be solved by either altering farming practices to prevent salinization or by implementing mitigation strategies to try to remediate salinized soils (Tester and Davenport, 2003). However, the kind of changes needed in cropping systems to avoid salinization is likely to be a long and difficult process (e.g., rotation of deep-rooted herbaceous perennial pastures with annual crops). At the same time, the remediation of saline soils is usually slow and expensive, requiring large quantities of high quality water and effective soil drainage (which is not common in salt affected areas) (Munns et al., 2008). However, none of these approaches will solve the problem of growing crops on land that is already compromised (Yamaguchi and Blumwald, 2005). Thus, the development and use of crops that can tolerate high levels of salinity in soils would be a practical solution to the problem.

In order to improve salt tolerance through breeding, genetic variability for the trait is required. Several screening studies were carried out in soybean to determine tolerance of genotypes to salt (Parker et al., 1983, 1986; Yang and Blanchar, 1993; Shao et al., 1995; Xu et al., 1999). In addition to using existing genetic variability within cultivated soybeans, it is promising to introduce salt tolerance from wild relatives as has been shown in tomato (Tal, 1985) and wheat (Gorham, 1993). Salt tolerance of some wild soybeans was also evaluated (Hymowitz, 1987; Pantalone et al., 1997; Li et al., 2000; Kao et al., 2006). Results from these studies show that there is genetic variability for salt tolerance in both cultivated and wild soybean. What is more, wild *Glycine* accessions showed greater variability in response to salinity than cultivated soybean (Pantalone et al., 1997). Utilization of wild soybean relatives as potential source of genes to improve agronomic traits in cultivated soybean, including salt tolerance, has been suggested (Brown et al., 1985; Hymowitz et al., 1987; Pantalone et al., 1997; Hymowitz, 2004).

Objective of this study

Objective of this study was the identification of physiological traits underlying differences in salt tolerance among four *Glycine* species. Results of this study may lead to a deeper understanding of salt tolerance mechanisms at the whole plant level.

Chapter II

MATERIALS AND METHODS

To investigate physiological traits underlying differences for salt tolerance among four *Glycine* species, two repetitions (year 1 and 2) of the same experiment were carried out in greenhouse. Accessions evaluated in each repetition and their suggested or reported responses to salt stress are presented in Table 2.1. Temperature and humidity were recorded in every repetition of the experiment. Daily average temperature and humidity values are presented in Figures 2.1 and 2.2.

Growing of plants of different G*lycine* species

Accessions were planted in conical tubes D60L "Deepots" (Stuewe & Sons, Inc., Corvallis, OR), which were 36 cm long and 6.4 cm in diameter and contain an approximate volume of 0.983 L. Cones were filled with washed sand ("Play Sand" Short Mountain Silica, Mooresburg, TN) and placed in plastic trays with a capacity of 20 cones each (Stuewe & Sons, Inc., Corvallis, OR). A set of two trays and 40 cones was placed in a plastic container as shown in Figure 2.3 to allow submersion in nutrient solution and to subsequently increase salt to induce salt stress.

Glycine max accessions were planted directly into the sand within the cones, while *G. soja*, *G. tomentella* and *G. argyrea* accessions required seed scarification and pre-germination. Scarification was performed by treating the seeds with concentrated sulfuric acid for 15 minutes, following the procedure used in the National Plant Germplasm System (Esther Peregrine, personal communication, 2006). After treatment with acid, seeds were thoroughly rinsed with water and placed on moistened filter paper in Petri dishes at 25°C to germinate. Germinating seeds were watered as needed. Approximately 4 to 7 days after germination started, seedlings were transplanted into the sand within cones.

Following transplanting, seedlings were irrigated as needed by applying tap water to the top of the cones for a period of 7 days. After one week, trays with cones containing plants of various soybean species were immersed in a nutrient solution (NS). Nutrient solution used in the experiment was modified from Triplett et al. (1980), and the composition is presented in Table 2.2. After mixing all components of NS, pH was adjusted to 5.4 ± 0.2. Nutrient solution was always kept to a level of 6 cm from the bottom of the container through daily additions if required. Also, NS was changed every 7 days prior to NaCl treatment and every 3-4 days after initiation of NaCl treatment to avoid changes in concentration of mineral elements.

Induction of salt stress and assessing leaf scorch for tolerance

When soybean plants reached the V3 stage (Fehr and Caviness, 1977), salt treatments were imposed. About two weeks after initiation of salt treatments, individual plants were evaluated for leaf scorch. Leaf scorch is a visual assessment of plant injury (chlorosis-necrosis) from salt, scored on a 1 to 5 scale where:

1. Healthy plant (no apparent injury)
2. Slight chlorosis-necrosis (25% of leaf area with symptoms of injury)
3. Moderate chlorosis-necrosis (50% of leaf area with symptoms of injury)
4. Severe chlorosis-necrosis (75% of leaf area with symptoms of injury)
5. Complete chlorosis-necrosis (plant is withered and dead)

After rating individual plants, an average leaf scorch score was obtained for each genotype using the following formula:

Average Score = ∑ (salt rating x number of plants)/Total number of plants

Genotypes were classified based on their average score as follows:

- Tolerant: Average score ≤2.0
- Moderate: Average score between 2.1 and 3.5
- Sensitive: Average score >3.5

Measurement of chlorophyll content

After genotypes were evaluated for leaf scorch, chlorophyll content was determined on the youngest and completely developed leaf from individual plants for various salt treatments with a Minolta SPAD-502 chlorophyll-meter. The final chlorophyll content for each plant was the average of three readings.

Determination of tissue ion concentration

After leaf scorch readings, leaves (petioles included), stem and roots of individual plants were harvested separately. Roots were thoroughly rinsed with water to eliminate any sand adhered on the surface. Lengths of stems and roots were measured. All vegetative tissues were dried in an oven at 60°C for 7 days, and dry weights were determined.

Chloride (Cl^-), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentration in leaves, stems and roots were quantified both years.

Determination of Cl^- concentration

Dried tissue of various plant components were ground using a Thomas Model ED-5 laboratory Wiley mill (Thomas Scientific, Swedesboro, NJ), and 0.15 g sample was used to analyze Cl^- content.

Each ground sample (0.15 g) was placed in 30 ml of distilled H_2O on an Eberbach Corporation orbital shaker (Eberbach Corporation, Ann Arbor, MI) at 60 cycles per min for 1 hr. Standards for calibration of 25, 50, 100 and 500 mg kg^{-1} of Cl^- were made using Ricca Chemical Company's Primary Cl^- solution of 1,000 mg kg^{-1} (Arlington, TX). A standard curve was established using an ion specific electrode attached to a Fisher Scientific AR 50 dual channel pH, Ion, conductivity meter.

After standard reference curves were established, the Cl^- in solution extracted from samples of leaves, roots and stems was determined for the various soybean genotypes. The Cl^- in the solution was converted to Cl^- concentration by multiplying the mg kg^{-1} chloride in solution by volume of distilled water and dividing by weight of the plant sample.

Determination of Na^+ concentration and other ions

About 0.25 g of dry, ground tissue from leaves, stems and roots was used to determine Na^+, Ca^{2+}, Mg^{2+} and K^+ by means of a modified wet acid dilution procedure (Mills and Jones, 1996). Samples were digested with a Hach Digesdahl™ Digestion Apparatus, 115Vac, 50/60 Hz (Hach Company, Loveland, CO) using H_2SO_4 and H_2O_2. Tissue concentrations of Na^+, Ca^{2+}, Mg^{2+} and K^+ were determined with a Perkin-Elmer™ (Wellesley, MA) atomic absorption spectrophotometer (Thomas, 1982).

Experimental design and data analysis

The experimental design was a split plot in space and time with three replications as outlined by Steel et al. (1997). Sodium chloride concentration ([NaCl]) was the main plot, and genotype (G) in a particular year (Y) was the subplot. Each G had 5 plants per replication. Four [NaCl] concentrations 0, 50, 75 and 100 mM were evaluated. Analysis of variance was conducted using PROC MIXED in SAS for all variables evaluated. Sodium chloride concentration, G and Y were considered fixed effects, while block, block x [NaCl], block([NaCl] x G]), block x Y, block([NaCl] x Y) and block([NaCl] x G x Y) were considered random effects.

In year 2, new accessions were added to the design (Table 2.1). Since they were not present in year 1, a separate analysis of variance was performed in order to

compare all eight accessions. The design was a split plot design where salt levels ([NaCl]) were main plots and the eight genotypes (G) were subplots. Analysis of variance was conducted using PROC MIXED in SAS for all variables evaluated. Sodium chloride concentration and G were considered fixed effects, while block, block x [NaCl] and block([NaCl] x G]) were considered random effects.

Bartlett's test revealed several data sets with heterogeneity of variance because variance increased with increasing levels of the variable being evaluated. Therefore, data were transformed on these variables to obtain homogeneous variance. Mean comparisons were made according to Fisher's least significance difference (LSD) at 0.05 significance level.

As previously said, new G were included in the design in year 2, so two different ANOVA were performed (i.e., pooled analysis with G present both years and individual analysis for all eight G in year 2). The following rule should be followed for correct interpretation of figures presenting comparison of means: uppercase letters on top of columns correspond to genotypes evaluated in both years; lowercase letters on top of columns correspond to genotypes only evaluated in year 2.

Regression analysis was performed using PROC REG in SAS to determine the association between variables. Regression coefficients were compared through orthogonal contrasts at 0.05 significance level.

Table 2.1. Genotype, species, response to salt stress, reference and years of evaluation to salt treatments.

Genotype	Species	Response to salt stress	Reference	Year
Fiskeby III	*G. max*	Tolerant	Carter, pers. comm.	1 and 2
M23	*G. max*	Sensitive	Shannon, pers. comm.	1 and 2
PI 468916	*G. soja*	Tolerant	Shannon, pers. comm.	1 and 2
PI 424127A	*G. soja*	Sensitive	Shannon, pers. comm.	1 and 2
PI 441008	*G. tomentella*	Tolerant	Nelson, pers. comm.	1 and 2
PI 595792	*G.argyrea*	Tolerant	Nelson, pers. comm.	1 and 2
S-100	*G.max*	Tolerant	Lee et al., 2004	2
Williams 82	*G.max*	Sensitive	Shannon, pers. comm.	2

Table 2.2. Composition of nutrient solution used in the experiment (modified from Triplett et al., 1980).

Compound	mM
$CaCl_2 . 2 H_2O$	2
$MgSO_4 . 7 H_2O$	1
K_2SO_4	0.624
$K_2HPO_4 . 3 H_2O$	1
NH_4NO_3	10
$FeSO_4 . 7 H_2O$	0.05

Compound	µM
H_3BO_3	5
$ZnSO_4 . 7 H_2O$	2
$NaMoO_4 . 2 H_2O$	0.2
$NiCl_2 . 6 H_2O$	0.22
$CuSO_4 . 5 H_2O$	0.3
$CoCl_2 . 6 H_2O$	0.02
$MnSO_4 . H_2O$	5

Figure 2.1. Daily average temperature (°C) during NaCl treatment in years 1 and 2.

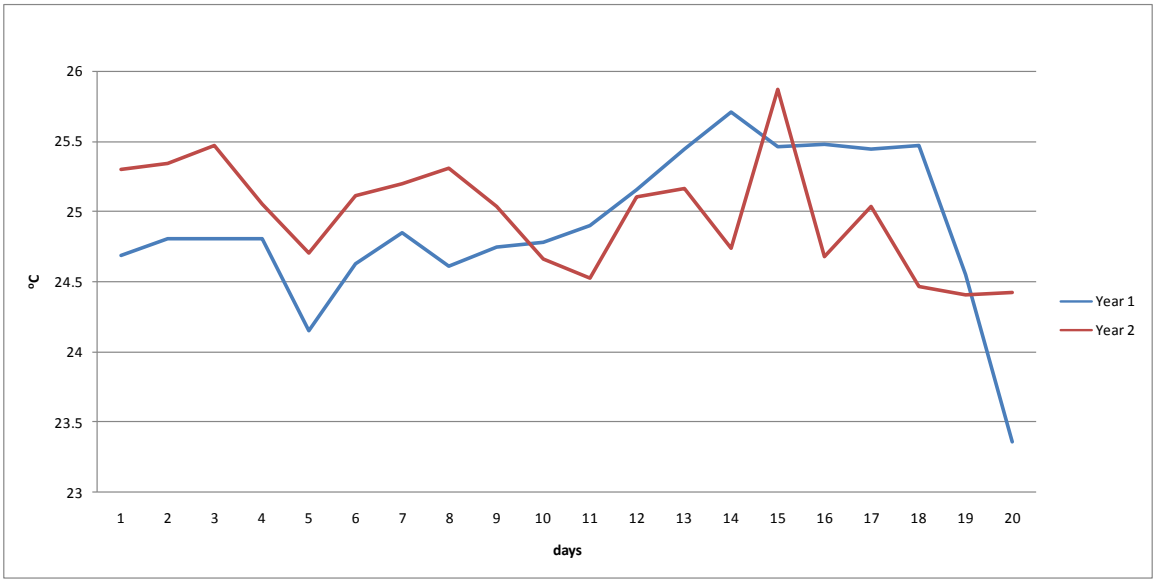


Figure 2.2. Daily average humidity (%) during NaCl treatment in years 1 and 2.

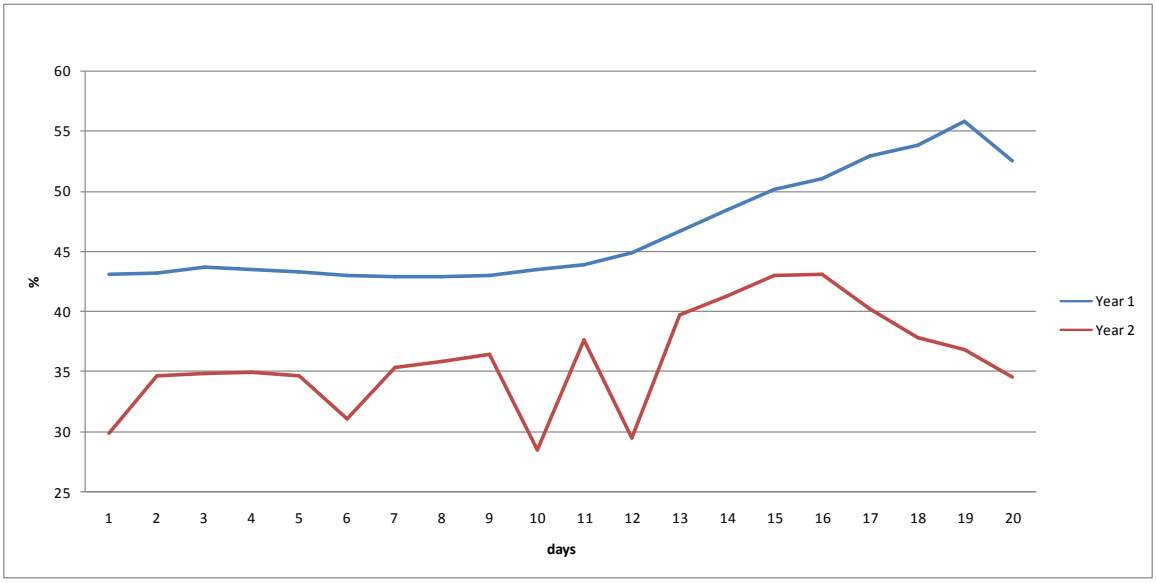


Figure 2.3. Picture of container/salt concentration (main plot) holding cones/genotypes (subplot).

Chapter III

ANALYSIS OF VARIANCE

Analysis of variance (ANOVA) was performed to test the effects of NaCl concentration ([NaCl]), year (Y) and their interactions on the levels of 22 physiological parameters of 8 soybean genotypes (G) within four *Glycine* species with different levels of salt tolerance (Table 2.1). Fisher's least significance difference (LSD) at the 0.05 significance level was used to test for significant differences among treatments. Before performing ANOVA, normality and homogeneity of variance were tested. Type of data transformation required to obtain homogeneous variance is pointed out for particular variables.

Although PI 441008 and PI 595792 were included in the experiment in both years, often enough tissue could not be collected to obtain measurable dry weights or to determine ion content during the first year. However, readings on leaf scorch and chlorophyll content were carried out on these genotypes the 2 years. Also, accessions S-100 and Williams 82 were included in the second year to have additional tolerant and sensitive *Glycine max* accessions, respectively. Consequently, a separate ANOVA was performed to compare all 8 genotypes, resulting in a split plot design with data from one year only.

For each variable, mean comparisons are presented in one of two different ways:

- If G x Y and/or [NaCl] x G x Y were significant, mean comparisons are presented for years 1 and 2 separately. Since behavior of genotypes was different in each of the repetitions, comparisons were done for individual years.
- If G x Y and [NaCl] x G x Y were not significant, pooled means across years are shown.

Mean comparisons for 2-year pooled ANOVA are denoted in uppercase letters at the top of columns, while mean comparisons for year 2 only are shown in lowercase letters on top of columns. (Figures 3.1 to 3.31).

Dry weight and length related variables were very different across genotypes, making the comparison of absolute values meaningless. To make measurements meaningful, ratios of weights and lengths for different salt rates relative to the control (0 mM NaCl) were studied. For example, if leaf dry weight of a genotype at 100 mM NaCl is shown to be 0.5, it means that at 100 mM NaCl, genotype X had half of the leaf dry weight compared to the control or 0 mM NaCl.

Leaf scorch

Square root transformation was needed to obtain homogeneous variance for leaf scorch (LS). Leaf scorch showed a highly significant G x Y interaction (Table 3.1) because LS was higher the second year than in the first, especially among accessions within annual species (Figures 3.1 and 3.2). This effect was consistent across all salt concentrations [NaCl] as shown by the non significant [NaCl] x G x Y interaction. The [NaCl] x G interaction was also highly significant. Both tolerant and sensitive genotypes had a LS=1 at 0 mM NaCl, but at increasing levels of NaCl, the sensitive accessions showed a significant higher level of leaf injury than the tolerant genotypes, and the differences increased with salt level ([NaCl]) in the solution.

In year 1, the LS scores of all tolerant genotypes responded similarly at every [NaCl] evaluated. However, in year 2, there was a clear separation among tolerant genotypes. Annual accessions (i.e., Fiskeby III, PI 468916 and S-100) showed greater injury or increased LS with increased [NaCl], while in perennial accessions (i.e., PI 441008 and PI 595792) LS remained unchanged. Sensitive accessions (i.e., M23, PI 424127A and Williams 82) started showing a moderate reaction at 50 mM NaCl, and their true susceptibility was expressed at 75 and 100 mM NaCl.

Chlorophyll content

Chlorophyll content (CC) showed homogeneous variance, so that no data transformation was necessary. The [NaCl] x G x Y interaction was significant, meaning that for a given [NaCl] the CC of genotypes were different in years 1 and 2 (Table 3.1). The significance of this 3-way interaction was due to changes in magnitude rather than in rank of accessions (Figures 3.3 and 3.4). In both years, tolerant accessions showed higher CC than sensitive genotypes, regardless the species and [NaCl]. This fact is even more noticeable when making within species comparisons. However, the differences in CC between sensitive and tolerant genotypes of the same species were smaller in year 2 than in year 1.

In both years, PI 424127A had a significant decrease in CC with [NaCl], while in M23, PI 595792 and PI 441008, CC tended to remain unchanged. Further analysis indicates that Fiskeby III and PI 468916 presented a dissimilar trend in CC in years 1 and 2. While CC increased with [NaCl] in year 1, there was a decrease in CC in year 2.

It is important to note that under NaCl stress, sensitive accessions never had a chlorophyll-meter reading higher than 25, while in tolerant genotypes the readings were sometimes over 35 (Figures 3.3 and 3.4).

Concentration of ions in leaves

Leaf Na^+ concentration

Square root transformation was needed to obtain homogeneous variance for leaf Na^+ concentration (LNac). The [NaCl] x G x Y interaction for LNac was significant (Table 3.2) because for a given [NaCl] there were changes in rank of accessions between years 1 and 2 (Table 3.2, Figures 3.5 and 3.6). In year 1, tolerant genotypes PI 468916 and Fiskeby III had significantly lower LNac than the sensitive PI 424127A and M23, whereas in year 2, the differences were smaller, and, at some [NaCl], non significant. Moreover, PI 468916 concentrated significantly lower Na^+ than M23 in year 1, but in year 2, PI 468916 tended to present similar or even higher LNac levels than M23.

LNac was higher the second year in all accessions (Figures 3.5 and 3.6). The maximum accumulation was observed in the susceptible genotype PI 424127A in both years: in year 1, LNac was about 1.5% and 2.2% in year 2, which represents almost a 50% increase in LNac.

Overall, sensitive accessions accumulated Na^+ at a greater extent than tolerant ones, especially in year 1. In year 2, sensitive accessions also concentrated more Na^+ in leaves than tolerant ones (except at 50 mM NaCl), but the differences were not always significant. In perennial accessions PI 441008 and PI 595792, which were evaluated for LNac only in the second year, Na^+ concentrations in leaves were the lowest (Figure 3.6) in comparison to *G. max* and *G. soja* accessions.

Leaf Cl^- concentration

Square root transformation was needed to obtain homogeneous variance for leaf Cl^- concentration (LClc). Since no 2- or 3-way interaction was significant for LClc, a comparison of means with 2-year pooled data is presented (Table 3.2, Figure 3.7).

Differences in LClc among genotypes were highly significant (Table 3.2); also, the increase in LClc with [NaCl] was highly significant for all genotypes in both years (Table 3.2). Overall, sensitive genotypes accumulated more Cl^- in leaves than tolerant genotypes (especially when making within-species comparisons). The non significant 2- and 3-way interactions (Table 3.2, Figure 3.7) indicate that response of genotypes was consistent across [NaCl] and years. Leaf Cl^- concentration was highest in PI 424127A (sensitive) and lowest in Fiskeby III (tolerant) among accessions evaluated the 2 years. In the one year comparison involving all eight genotypes, the tolerant lines S-100, PI 441008 and PI 595792 accumulated the same or even lower Cl^- than Fiskeby III.

There were significant differences in LClc between years 1 and 2, and these differences were consistent across [NaCl] and genotypes as shown by the non significant [NaCl] x Y, G x Y and [NaCl] x G x Y interactions (Table 3.2). Similar to LNac, LClc was higher the second year than the first year of the test. PI 424127A had the highest leaf Cl^- accumulation in both years with a maximum LClc of 7.3% and 9.7% the first and second year, respectively.

Leaf K^+ concentration

Logarithmic transformation to the base 10 was performed to obtain homogeneous variance for leaf K^+ concentration (LKc). This variable showed a highly significant G x Y interaction. Thus, the LKc of accessions in both years were different (Table 3.2, Figures 3.8 and 3.9). PI 424127A (sensitive) tended to have the highest LKc, but the difference was not always significant. On the other hand, Fiskeby III and PI 468916 tended to have the lowest LKc the first and second year, respectively, although they were not statistically different from the other genotypes (Figures 3.8 and 3.9).

In year 2, among all eight genotypes, PI 441008 along with Williams 82 tended to have the highest LKc across all NaCl concentrations although they were not consistently different from the other accessions. Conversely, tolerant lines S-100 and PI 468916 tended to show the lowest LKc (Figure 3.9).

Leaf Mg^{2+} concentration

Leaf Mg^{2+} concentration (LMgc) presented homogeneous variance, so that no transformation was required. This variable showed a significant G x Y interaction which indicates there were differences between LMgc of accessions across years. Also, the interaction [NaCl] x G was significant, meaning that at different NaCl concentrations, genotypes ranked differently for LMgc (Table 3.2, Figures 3.10 and 3.11).

Annual wild soybeans PI 424127A and PI 468916 tended to have the same pattern both years. Leaf Mg^{2+} concentration was almost identical at 0 mM NaCl, and then, at increasing [NaCl], PI 424127A had higher LMgc than PI 468916, but the difference was not significant. The *G. soja* accessions showed a significant decrease in LMgc with increases in [NaCl] in both years (Figures 3.10 and 3.11).

Regarding the *Glycine max* accessions, Fiskeby III tended to have higher LMgc than M23 in year 1, but in year 2 their Mg^{2+} content was similar. In year 2, Fiskeby III, M23 and Williams 82 had similar LMgc at each [NaCl], whereas S-100 consistently accumulated the lowest amount of Mg^{2+} in leaves. S-100 was the only cultivated soybean that exhibited a significant decrease in LMgc due to increased [NaCl] (Figures 3.10 and 3.11).

Perennial wild soybeans PI 441008 and PI 595792 had similar LMgc at all [NaCl], and there was no significant change in LMgc as [NaCl] in the solution increased (Figure 3.11).

Leaf Ca^{2+} concentration

Variance for leaf Ca^{2+} concentration (LCac) was homogeneous, so no transformation was required. LCac among genotypes were significantly different and

these differences were consistent across [NaCl] and Y as shown by the non significant 2- and 3-way interactions (Table 3.2).

In the analysis of genotypes evaluated both years, Fiskeby III, M23 and PI 424127A were similar and had the highest LCac, whereas PI 468916 exhibited significantly lower Ca^{2+} content in leaves (Figure 3.12). Also, Fiskeby III and PI 424127A tended to have higher LCac than M23 and PI 468916 at each of the NaCl treatments although the differences were not always significant.

Among genotypes for which LCac was evaluated just the second year, S-100 consistently exhibited the lowest Ca^{2+} concentration in leaves, and it was significantly lower than Fiskeby III, the other tolerant *G. max* accession, at almost every [NaCl]. Also, perennial accessions PI 441008 and PI 595792 showed similar LCac to Fiskeby III, M23 and PI 424127A. In general, LCac of genotypes showed no significant changes over increasing [NaCl] (Figure 3.12).

Concentration of ions in stems

Stem Na^+ concentration

The variable stem Na^+ concentration (SNac) required a square root transformation to obtain homogeneous variance. There was a significant [NaCl] x Y interaction, which means that the overall SNac obtained for NaCl treatments were different in years 1 and 2 (Table 3.3). However, the differences were due to changes in magnitude rather than changes in rank (data not shown).

There were significant differences among genotypes regarding SNac. Genotype differences for SNac were consistent across salt levels ([NaCl]) and years as shown by the non significant [NaCl] x G, G x Y and [NaCl] x G x Y interactions. Analysis of genotypes for which there are 2-year data showed that tolerant genotypes Fiskeby III and PI 468916 had lower SNac than sensitive M23 and PI 424127A, respectively, though the differences were not always significant at $p<0.05$. Among the 4 accessions above mentioned, Fiskeby III exhibited the lowest SNac (Figure 3.13).

Analysis of all genotypes in year 2 for SNac showed that Williams 82 and M23 (sensitive) tended to have higher SNac than Fiskeby III and S-100, but the difference was not always statistically significant. Na^+ levels in stems of S-100 and Fiskeby III were similar. Finally, perennial accessions PI 441008 and PI 595792 showed significantly lower SNac than tolerant Fiskeby III and S-100, except at 75 mM NaCl where Na^+ contents were similar (Figure 3.13).

Stem Cl^- concentration

Square root transformation was required to obtain homogeneous variance for stem Cl^- content (SClc). A significant [NaCl] x Y interaction indicates that the overall SClc obtained for various NaCl treatments were different in years 1 and 2 (Table 3.3). In year 2, the differences in SClc between [NaCl] treatments were smaller (data not shown).

Considering accessions with 2-year data, [NaCl] in the solution induced highly significant increases in SClc, and this effect was consistent for all genotypes as reflected by the non significant [NaCl] x G interaction. In addition, highly significant differences among genotypes were observed, except at 75 mM NaCl, concentration at which accessions had similar SClc. Tolerant Fiskeby III and PI 468916 tended to show lower SClc than sensitive M23 and PI 424127A, respectively, though the differences were not significant at $p<0.05$. *Glycine max* accessions tended to have lower SClc than *G. soja* genotypes, and Fiskeby III was the entry with lowest contents (Figure 3.14).

Cultivars S-100 and Williams 82, which were only evaluated in year 2, showed similar SClc as Fiskeby III and M23 at 0 and 50 mM NaCl. At 75 mM NaCl, Williams 82 was still similar to Fiskeby III and M23, but S-100 had lower levels of Cl^- in stems. Finally, at 100 mM NaCl, Williams 82 and M23 were among genotypes with the highest SClc, while Fiskeby III and S-100 showed the lowest SClc (Figure 3.14).

Stem K^+ concentration

Stem K^+ concentration (SKc) had a homogeneous variance, so that no transformation of data was required. Sodium chloride concentration in the solution had a significant effect on SKc (Table 3.3). There was a significant reduction in SKc in all genotypes, regardless the tolerance level, when [NaCl] increased from 0 to 100 mM. What is more, the decrease observed in SKc was similar across genotypes as expressed by the non significant [NaCl] x G interaction (Figure 3.15).

Also, there were significant differences in SKc among genotypes, and these differences were consistent (non significant [NaCl] x G, G x Y and [NaCl] x G x Y interactions). Tolerant accession Fiskeby III consistently had the lowest SKc although not always significantly different from M23, PI 424127A and PI 468916 which had similar SKc. In year 2, all genotypes had similar K^+ concentration in stems (Figure 3.15).

Stem Mg^{2+} concentration

Homogeneous variance was obtained for stem Mg^{2+} content (SMgc) by means of square root transformation. There were highly significant differences in SMgc among genotypes, and these differences were consistent across Y and [NaCl] as shown by the non significant 2- and 3-way interactions (Table 3.3). Two-year analyses showed that Fiskeby III consistently had the highest SMgc though the differences were not always

significant. Accessions M23, PI 424127A and PI 468916 all had similar Mg^{2+} concentration in stems (Figure 3.16).

For accessions with data for year 2 only (Figure 3.16), there were significant differences among genotypes for Mg^{2+} concentration, and even though these differences were less as [NaCl] increased, the [NaCl] x G interaction was not significant. (data not shown). Overall, increases in [NaCl] in the solution did not produce changes in SMgc of accessions as suggested by the non significant [NaCl] effect (Table 3.3).

Stem Ca^{2+} concentration

To obtain homogeneous variance, stem Ca^{2+} concentration (SCac) required a logarithm to the base 10 transformation. No effect or their interactions affected SCac. Overall, genotypes had similar Ca^{2+} levels in stems and no significant changes due to increases in [NaCl]. There was no Y effect and all interactions were not significant (Table 3.3, Figure 3.17). In *G. max* genotypes, in year 1, Fiskeby III consistently showed higher SCac than M23 (data not shown). In year 2, tolerant genotypes S-100 and Fiskeby III tended to have higher SCac than M23 and Williams 82. Although the differences were almost always non significant, the trend suggests that tolerant soybean genotypes tend to show higher SCac than sensitive ones.

Concentration of ions in roots

Root Na^+ concentration

To obtain homogeneous variance for root Na^+ concentration (RNac), a logarithm to the base 10 transformation was required. A significant [NaCl] x G x Y interaction showed that for a given [NaCl], the RNac of genotypes were different in years 1 and 2 (Table 3.4, Figures 3.18 and 3.19). Changes in rank of accessions for RNac explain the significant effect of the 3-way interaction. For example, in year 1, RNac of PI 468916 was similar to Fiskeby III at 50 mM NaCl but significantly lower at 75 and 100 mM. In year 2, a completely opposite pattern was observed. M23 was different from PI 424127A at 50 mM NaCl but similar at higher [NaCl] in year 1, whereas in year 2, these 2 accessions were different only at 100mM. Finally, another contribution to the significant 3-way interaction is the fact that although Fiskeby III did not have a significantly higher RNac than M23 in any year, the differences were greater in year 1 than in year 2, with Fiskeby III showing a trend of accumulating more Na^+ in roots than M23 at various NaCl concentrations.

In general, *G. max* accessions tended to accumulate more Na^+ in roots than *G. soja* accessions, but these differences were not always significant. Also, the perennial PI 595792 had the lowest RNac across all NaCl concentrations studied. Overall, no significant differences at $p<0.05$ were found in RNac among tolerant and sensitive genotypes.

In year 2, the level of Na^+ accumulation in roots was significantly higher than in year 1 (Table 3.4, Figures 3.18 and 3.19). Higher [NaCl] in the solution, induced a significant increase in RNac in all genotypes. A significant jump in Na^+ concentration was observed only between 0 and 50 mM NaCl but not between 50 and 75 mM or even between 50 and 100 mM.

Root Cl^- concentration

Square root transformation was required to obtain homogeneous variance for root Cl^- concentration (RClc). The increase in RClc with [NaCl] in the solution was highly significant and consistent across genotypes in both years as reflected by non significant [NaCl] x G, [NaCl] x Y and [NaCl] x G x Y interactions (Table 3.4).

There were significant and consistent differences in RClc among genotypes (Table 3.4, Figure 3.20). First, when considering genotypes for which there was 2-year data, tolerant Fiskeby III and PI 468916 tended to accumulate more Cl^- in roots than sensitive M23 and PI 424127A, respectively. This pattern was especially consistent for Fiskeby III. Second, in year 2, tolerant *G. max* genotypes S-100 and Fiskeby III had similar levels of RClc which were consistently higher than RClc in sensitive M23 and Williams 82. Third, perennial PI 595792 had a very significant increase in RClc with [NaCl], so that at 0 and 50 mM, it was among accessions with the lowest Cl^- content in roots but reached RClc similar to Fiskeby III at 100 mM NaCl. Finally, the other perennial

accession, PI 441008 showed a more moderate increase in RClc with [NaCl] and was always rather intermediate regarding Cl^- content in roots (Figure 3.20).

Root K^+ concentration

Square root transformation was performed on the variable root K^+ concentration (RKc) to obtain homogeneous variance. There were differences among genotypes in RKc as shown by the highly significant genotype effect. These differences were consistent over years and across salt concentrations as shown by the non significant [NaCl] x G, [NaCl] x Y and [NaCl] x G x Y interactions (Table 3.4, Figure 3.21).

Glycine soja accessions consistently had the lowest RKc regardless their salt tolerance level, whereas *G. tomentella* and *G. max* accessions exhibited the highest concentration of K^+ in roots. Fiskeby III always showed the highest RKc when analyzing accessions with 2-year data. In addition, Fiskeby III was consistently different for RKc from *G. soja* genotypes but not from M23 at $p<0.05$. PI 424127A tended to consistently have the lowest RKc (Figure 3.21).

Considering the eight genotypes with 1-year data, S-100, Williams 82, PI 441008 and PI 595792 were similar in RKc. The exception was PI 595792 at 0 mM NaCl, where it showed significantly lower RKc than all the other accessions.

Even though changes in [NaCl] in solution did not produce significant variations in RKc of genotypes (Table 3.4), the trend shows that there was an inverse relationship between RKc and [NaCl] (Figure 3.21). However, one accession, PI 595792, showed the opposite response in which RKc increased as salt concentration in solution increased.

Root Mg^{2+} concentration

Square root transformation was required to obtain homogeneous variance for root Mg^{2+} concentration (RMgc). There were highly significant and consistent differences in RMgc among genotypes (Table 3.4, Figure 3.22). First, considering accessions with 2-year data, Fiskeby III consistently had the highest RMgc, while the *G. soja* genotypes showed lower Mg^{2+} in roots than the *G. max* entries. Fiskeby III was always higher in RMgc than PI 468916 and PI 424127A, while M23 was different from PI 424127A but similar to PI 468916 (Figure 3.22).

Second, in year 2, PI 441008 presented the highest RMgc across all [NaCl] although the difference was not always significant at $p<0.05$. The rest of the genotypes showed rather similar levels of RMgc (Figure 3.22).

Finally, during the second year, the only accessions that showed a significant decrease in RMgc with increasing [NaCl] levels in the solution were tolerant genotypes Fiskeby III, S-100 and PI 441008.

Root Ca^{2+} concentration

The data set root Ca^{2+} concentration (RCac) was subjected to rank transformation as outlined by Conover and Iman (1981) to achieve homogeneity of variance. There was a significant [NaCl] x G x Y interaction, meaning that for a given [NaCl], the RCac of G was different in years 1 and 2 (Table 3.4).

In year 1, G were similar in RCac at 0 mM NaCl, but, in year 2, Fiskeby III was significantly higher than *G. soja* accessions. There were no further differences among G at 50, 75 and 100 mM NaCl in year 2, whereas PI 424127A at 50 mM NaCl and both *G. soja* accessions at 75 mM NaCl showed lower RCac than other genotypes in year 1 (Figures 3.23 and 3.24).

Analyzing genotypes in year 2 only, it can be seen that *G. soja* accessions showed significantly lower RCac than the others at 0 mM NaCl. At higher [NaCl], all G presented similar levels of Ca^{2+} in roots (Figure 3.24). Although there were differences in RCac among G, the differences were not consistent across [NaCl] and Y (Table 3.4, Figures 3.23 and 3.24).

Growth parameters

Leaf Dry Weight

Logarithm to the base 10 transformation was required to obtain homogeneous variance for leaf dry weight (Ldw). Since there was a highly significant G x Y interaction (Table 3.5), comparison of means is presented for years 1 and 2 separately (Figures 3.25 and 3.26). Changes in Ldw magnitude were mainly responsible for the significance of the G x Y interaction. In year 1, PI 441008 had the highest Ldw, and it was the only accession that was significantly different from sensitive genotypes PI 424127A and M23 at 50, 75 and 100 mM NaCl. Also, tolerant accessions Fiskeby III and PI 468916 tended to present higher Ldw than sensitive genotypes, but, in general, there were no differences at $p<0.05$. Sensitive accessions M23 and PI 424127A were the only ones having a significant decrease in Ldw with increased [NaCl] (Figure 3.25).

In year 2, tolerant accessions tended to show smaller variation in Ldw than sensitive ones, and, again, differences between tolerant and sensitive were not always significant. Sensitive accessions M23, Williams 82 and PI 424127A showed a significant decrease in Ldw with increasing [NaCl] in solution. On the other hand, tolerant accessions PI 595792, PI 441008, Fiskeby III and S-100 showed little change or even increased Ldw with increasing salt concentrations, especially at 50 and 75 mM NaCl. However, the observed increases were not significant at $p<0.05$ (Figure 3.26).

Stem dry weight

Logarithm to the base 10 transformation was required to obtain homogeneous variance for stem dry weight (Sdw). Since there was a significant G x Y interaction (Table 3.5), comparison of means is presented for years 1 and 2 separately (Figures 3.27 and 3.28). Considering accessions for which there are 2-year data, it is observed that in year 1, genotypes showed similar levels of Sdw at all [NaCl] but at 50mM NaCl, where PI 468916 had significantly higher Sdw than M23 and PI 424127A (Figure 3.27). In year 2, Sdw levels of Fiskeby III, PI 424127A and PI 468916 were similar at every [NaCl], while those of M23 were significantly lower (Figure 3.28). Accession M23 showed a greater decrease in Sdw than the other accessions during the second year.

In year 2, sensitive accessions M23 and Williams 82 had the lowest Sdw at every [NaCl] although they were not consistently different from some tolerant genotypes at $p<0.05$ (Figure 3.28). M23 and Williams 82 had a sustained and significant decrease in Sdw with increases in [NaCl]. All other accessions showed no significant changes in Sdw with the exception of PI 595792, whose Sdw increased significantly with increased [NaCl]. PI 441008 showed a non significant increase with [NaCl], but, at 100 mM NaCl, the Sdw was significantly reduced compared to other salt concentrations. Overall, there were significant differences in the ability of genotypes to maintain Sdw, and this feature was not altered by [NaCl] as shown by the non significant [NaCl] x G interaction (Table 3.5).

Root dry weight

Logarithm to the base 10 transformation was required to obtain homogeneous variance for root dry weight (Rdw). Overall, there were no significant main effects or interactions for Rdw (Table 3.5). However, there were some trends that are worthy to mention. Sensitive accessions PI 424127A, M23, Williams 82 and the tolerant *G. soja* line PI 468916 showed a significant decrease of about 40% in Rdw over [NaCl]. On the other hand, salt tolerant accessions Fiskeby III, S-100, PI 595792 and PI 441008 showed only small changes in Rdw, with decreases no greater than 25% in Fiskeby III and S-100. It is also interesting to note that increased NaCl seemed to induce root growth in PI 441008 (*G. tomentella*) and to a smaller extent in PI 595792 (*G. argyrea*). When considering *Glycine max* entries, it is noted that tolerant Fiskeby III and S-100 showed a smaller decrease in Rdw than the sensitive genotypes M23 and Williams 82 even though the differences were not significant at $p<0.05$ (Figure 3.29).

Stem length

Logarithm to the base 10 transformation was required to obtain homogeneous variance for stem length (Slth). A highly significant [NaCl] x G interaction was shown for Slth, meaning that genotypes presented different patterns across salt levels (Table 3.5). Changes in magnitude rather than in rank were responsible for the significant interaction. Analyzing genotypes with 2-year data, it is evident they had the same Slth

at both 0 and 50 mM NaCl. When salinity increased to 75 and 100 mM NaCl, sensitive accessions (PI 424127A and M23) had a significant decrease in Slth, while tolerant genotypes (PI 468916 and Fiskeby III) had a much smaller and not significant reduction in Slth. Different was the pattern followed by PI 595792, whose Slth was increased when NaCl was present in the nutrient solution. These five accessions showed the same behavior in year 2 (Figure 3.30).

S-100 and Williams 82 were evaluated for Slth in year 2 only. Tolerant S-100 was always among genotypes with highest Slth. Compared to other *G. max* genotypes, S-100 was consistently similar to Fiskeby III, and both accessions showed higher Slth than sensitive M23 and Williams 82. M23 and Williams 82 had the lowest Slth (Figure 3.30).

There was a significant [NaCl] effect in which increases in [NaCl] in the solution resulted in significant changes in Slth (Table 3.5). In general, the increment of NaCl in the solution from 0 to 100mM resulted in an overall reduction in Slth of about 20%.

Finally, tolerant genotype PI 441008 was not included in the evaluation of Slth due to its growth habit: rather than having a main stem like the other accessions, PI 441008 has several branches that emerge from the stem base.

Root length

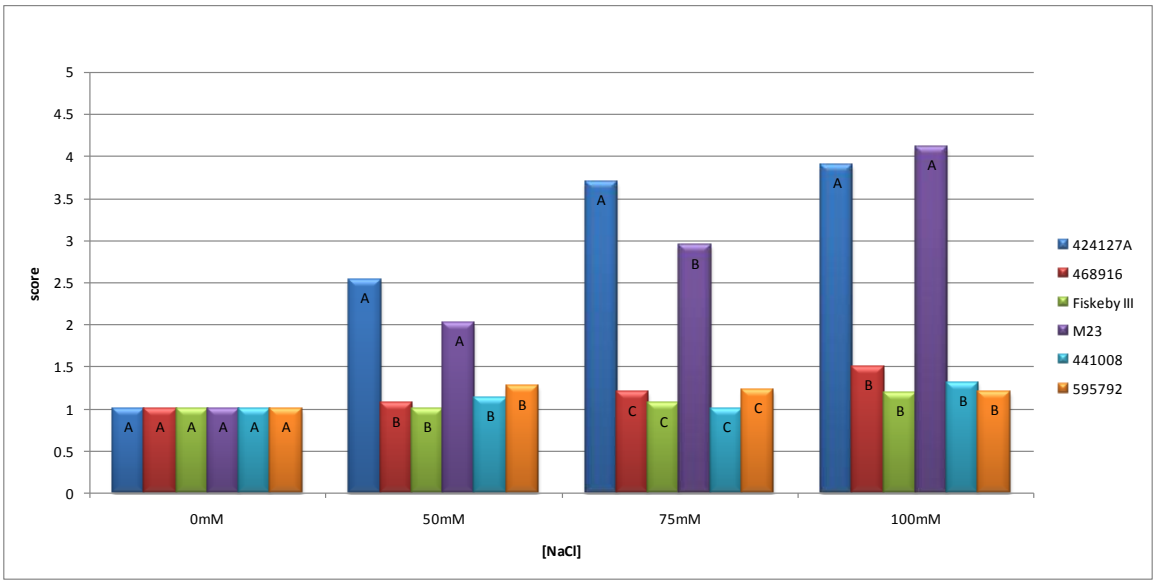
Logarithm to the base 10 transformation was required to obtain homogeneous variance for root length (Rlth). Main effects and interactions were not significant for Rlth (Table 3.5). The only observation worthy to point out is that genotypes PI 4411008, PI 595792, S-100 and Williams 82 consistently showed a slight increase in Rlth with [NaCl] although the increment was significant only for PI 595792. For the other accessions, Rlth remained unchanged (Figure 3.31).

Table 3.1. Analysis of variance, fixed effects, across 4 NaCl concentrations, 2 years and 6 genotypes of genus *Glycine* for leaf scorch and chlorophyll content.

Effect	Leaf scorch	Chlorophyll content
[NaCl]	**	ns
Genotype (G)	**	**
[NaCl] x G	**	**
Year (Y)	**	ns
[NaCl] x Y	ns	*
G x Y	**	**
[NaCl] x G x Y	ns	*

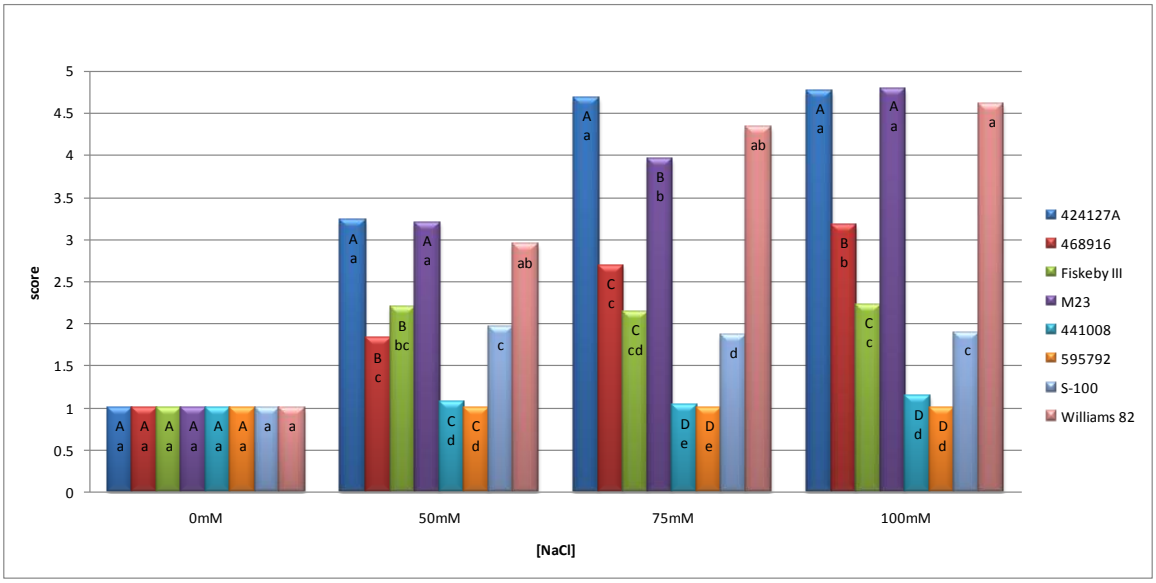
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. ns = not significant.

Figure 3.1. Leaf scorch score of 6 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



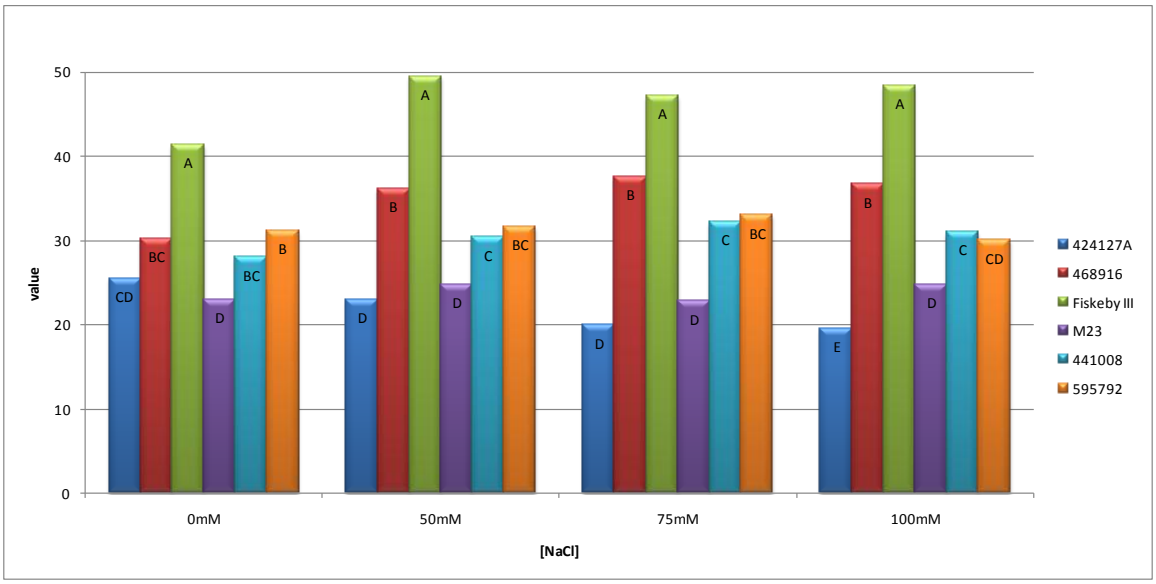
Columns with the same top letter are not significantly different at p<0.05. Genotypes with a final score below 2 are considered tolerant, between 2 and 3.5 moderate and above 3.5 sensitive.

Figure 3.2. Leaf scorch score of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



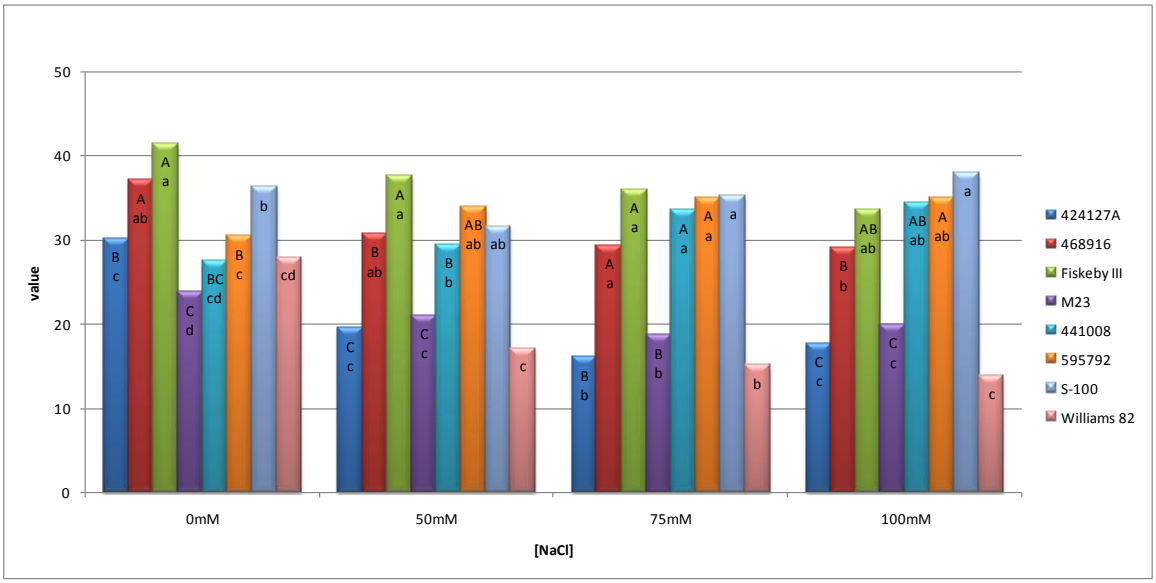
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only. Genotypes with a final score below 2 are considered tolerant, between 2 and 3.5 moderate and above 3.5 sensitive.

Figure 3.3. Chlorophyll content of 6 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



Columns with the same top letter are not significantly different at p<0.05.

Figure 3.4. Chlorophyll content of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



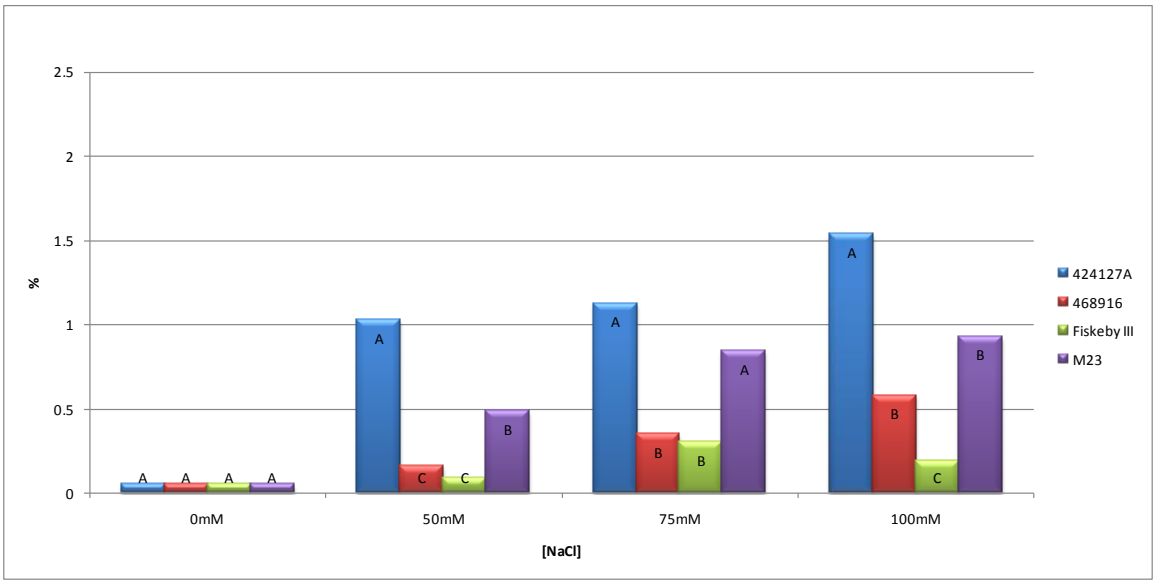
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Table 3.2. Analysis of variance, fixed effects, across 4 NaCl concentrations, 2 years and 4 genotypes of genus *Glycine* for leaf concentration of Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}.

Effect	LNac	LClc	LKc	LMgc	LCac
[NaCl]	**	**	ns	*	ns
Genotype (G)	**	**	**	**	**
[NaCl] x G	**	ns	ns	*	ns
Year (Y)	**	*	ns	ns	ns
[NaCl] x Y	**	ns	ns	ns	ns
G x Y	**	ns	**	*	ns
[NaCl] x G x Y	*	ns	ns	ns	ns

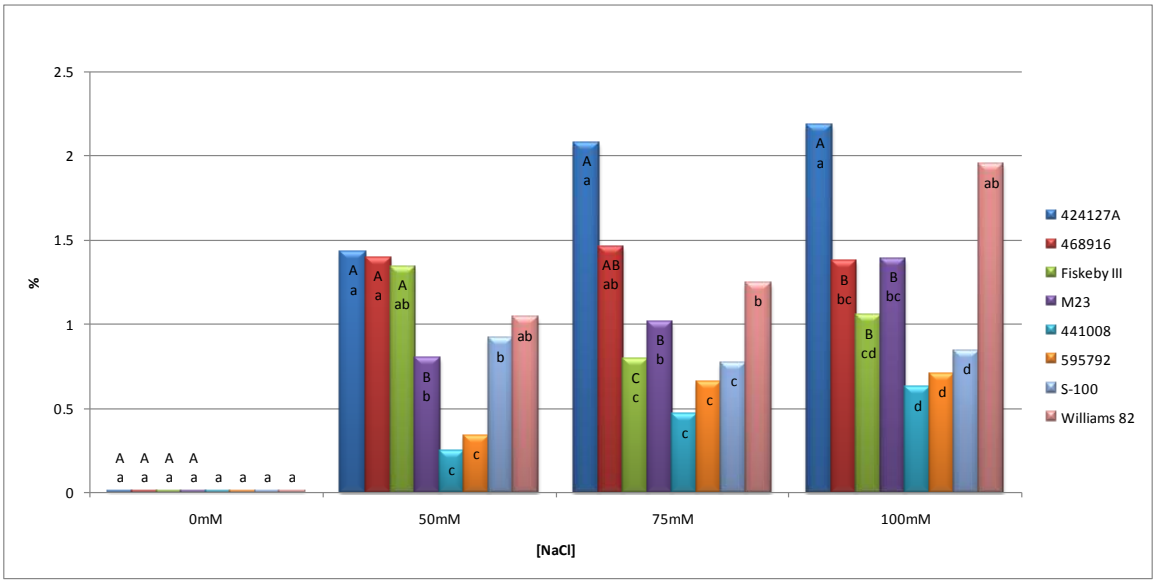
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. ns = not significant.

Figure 3.5. Leaf Na^+ concentration of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



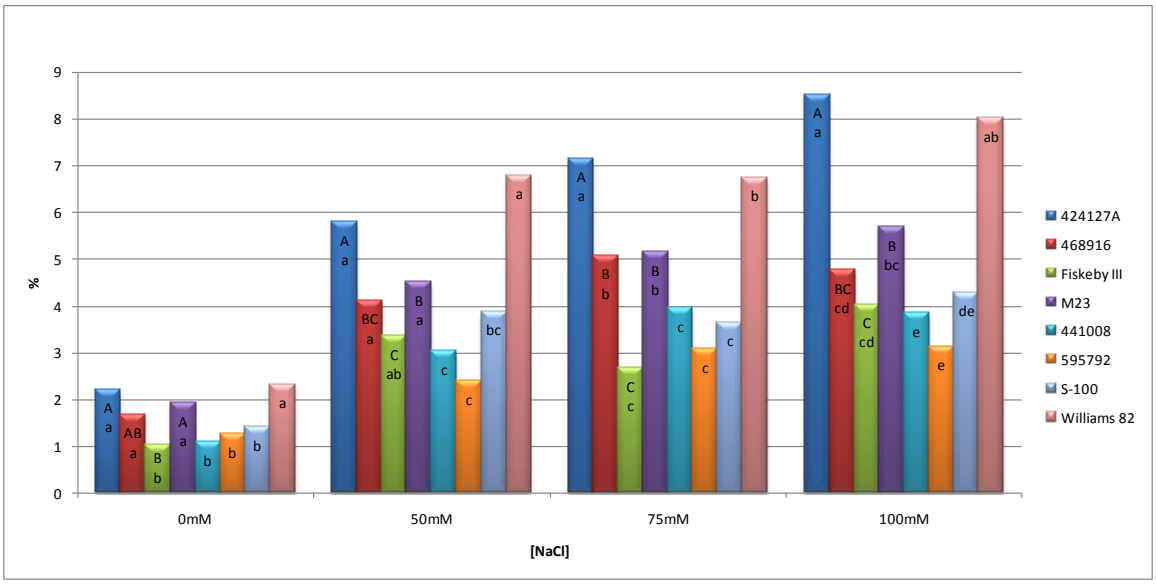
Columns with the same top letter are not significantly different at p<0.05.

Figure 3.6. Leaf Na^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



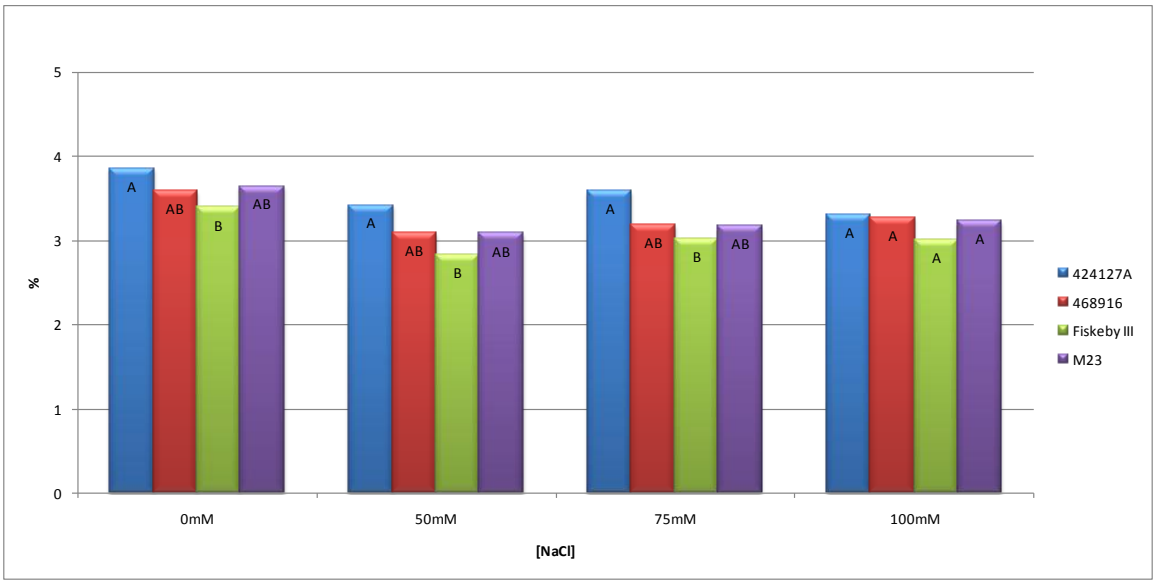
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.7. Leaf Cl^- concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



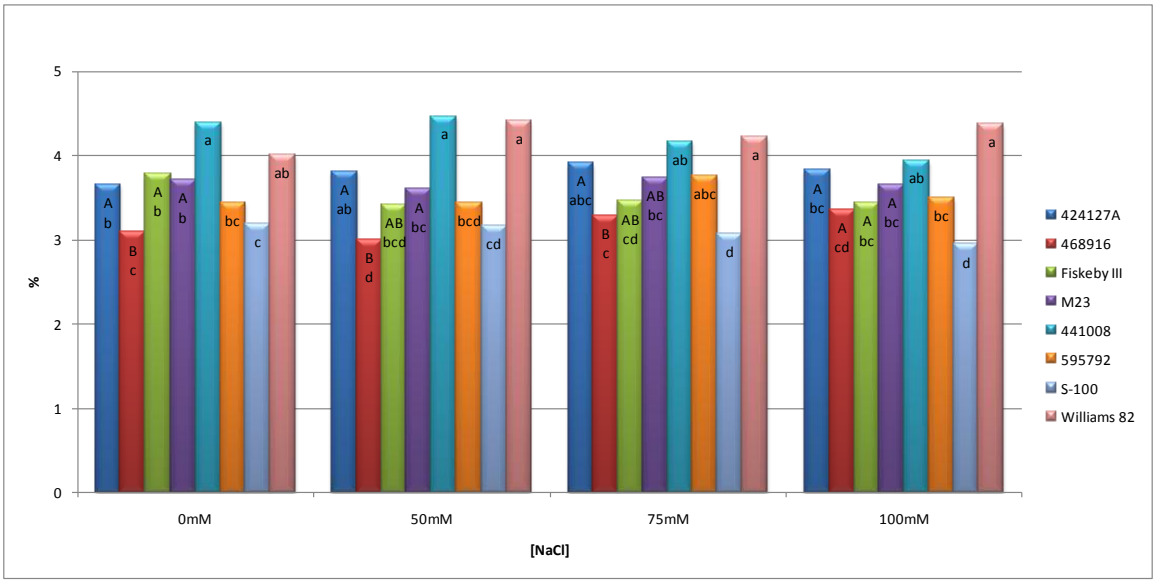
Columns with the same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.8. Leaf K^+ concentration of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



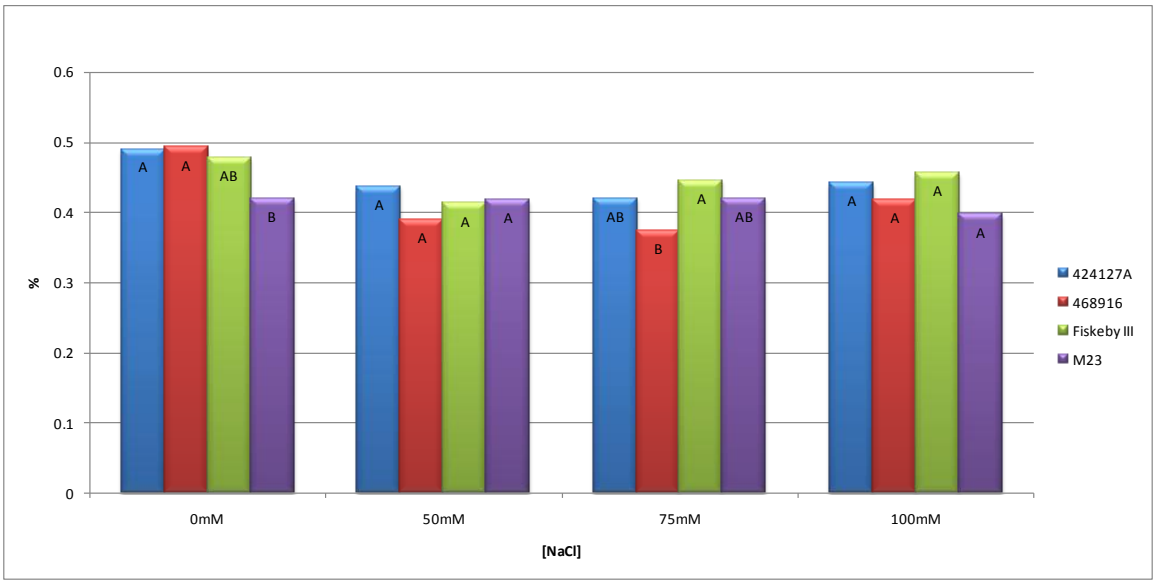
Columns with the same top letter are not significantly different at p<0.05.

Figure 3.9. Leaf K^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



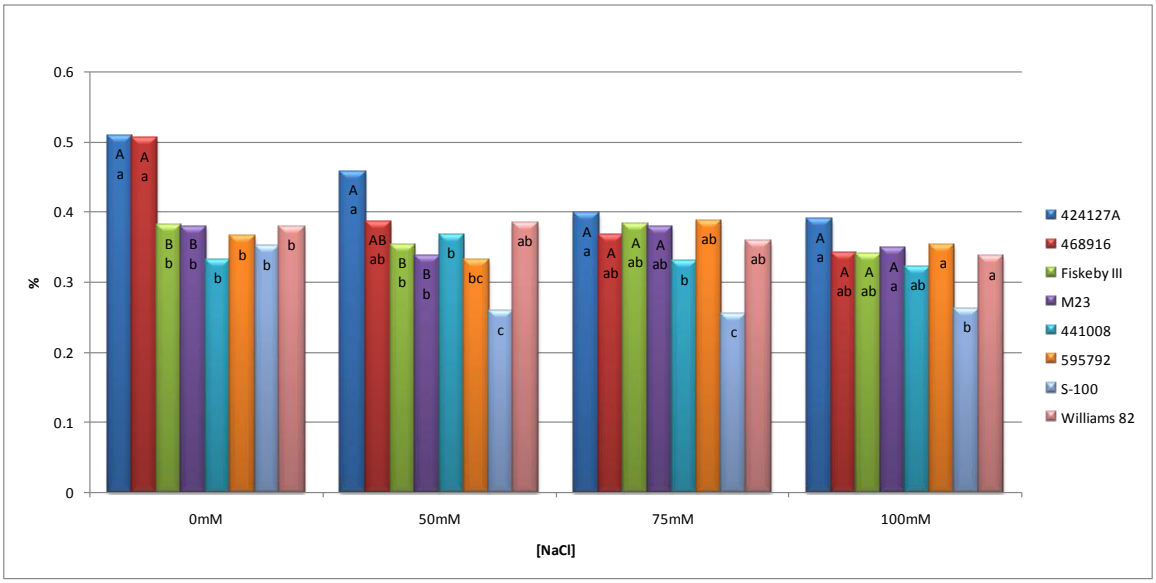
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.10. Leaf Mg^{2+} concentration of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



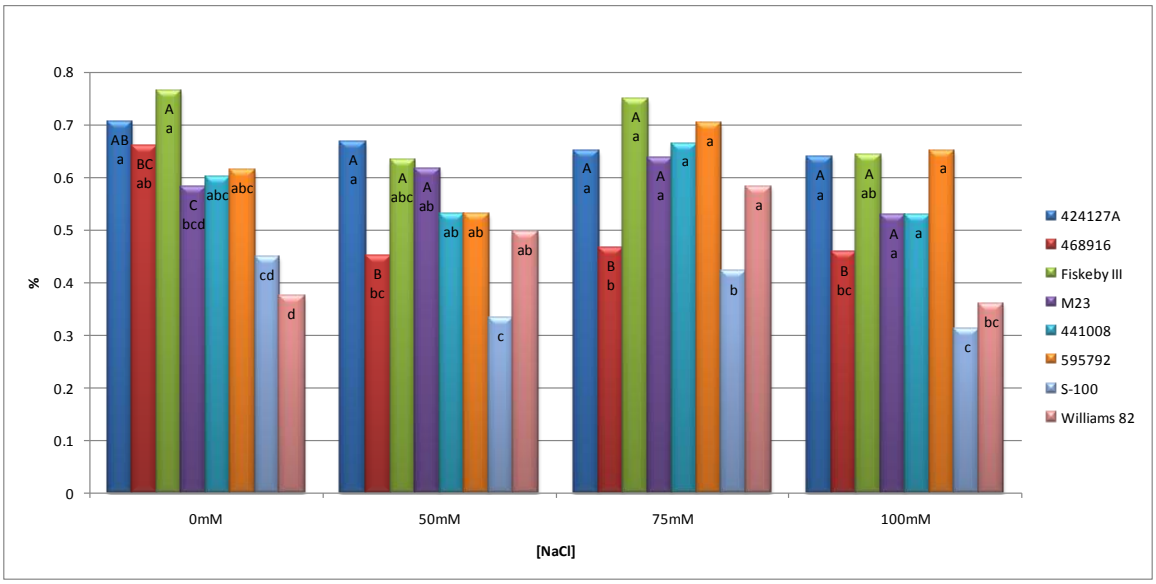
Columns with the same top letter are not significantly different at p<0.05.

Figure 3.11. Leaf Mg^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.12. Leaf Ca^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



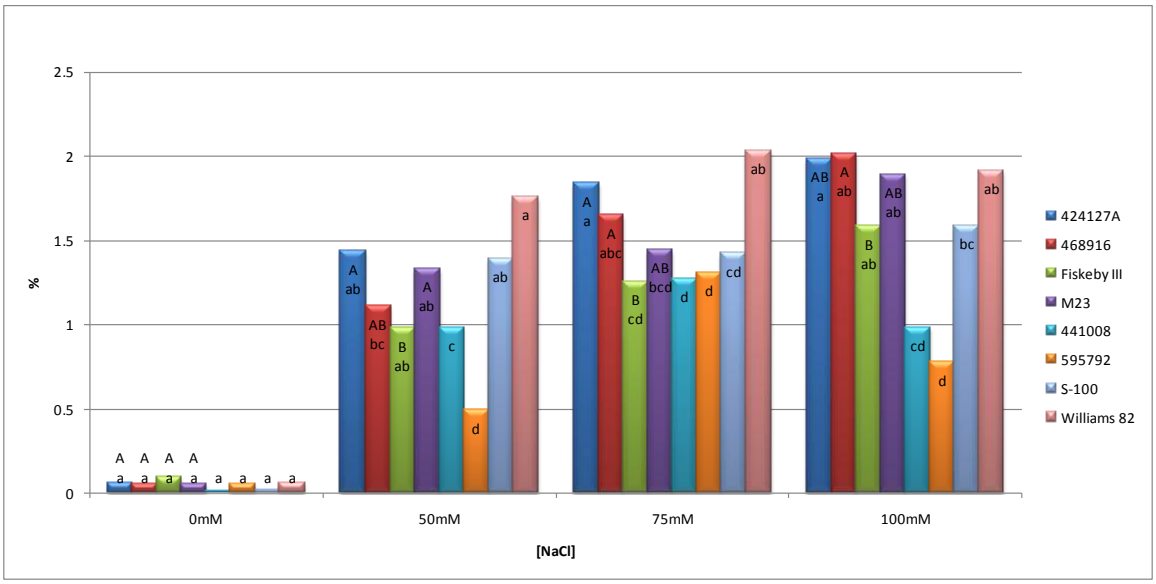
Columns with same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Table 3.3. Analysis of variance, fixed effects, across 4 NaCl concentrations, 2 years and 4 genotypes of genus *Glycine* for stem concentration of Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}.

Effect	SNac	SClc	SKc	SMgc	SCac
[NaCl]	**	**	*	ns	ns
Genotype (G)	**	**	*	**	ns
[NaCl] x G	ns	ns	ns	ns	ns
Year (Y)	ns	ns	ns	ns	ns
[NaCl] x Y	**	*	ns	ns	ns
G x Y	ns	ns	ns	ns	ns
[NaCl] x G x Y	ns	ns	ns	ns	ns

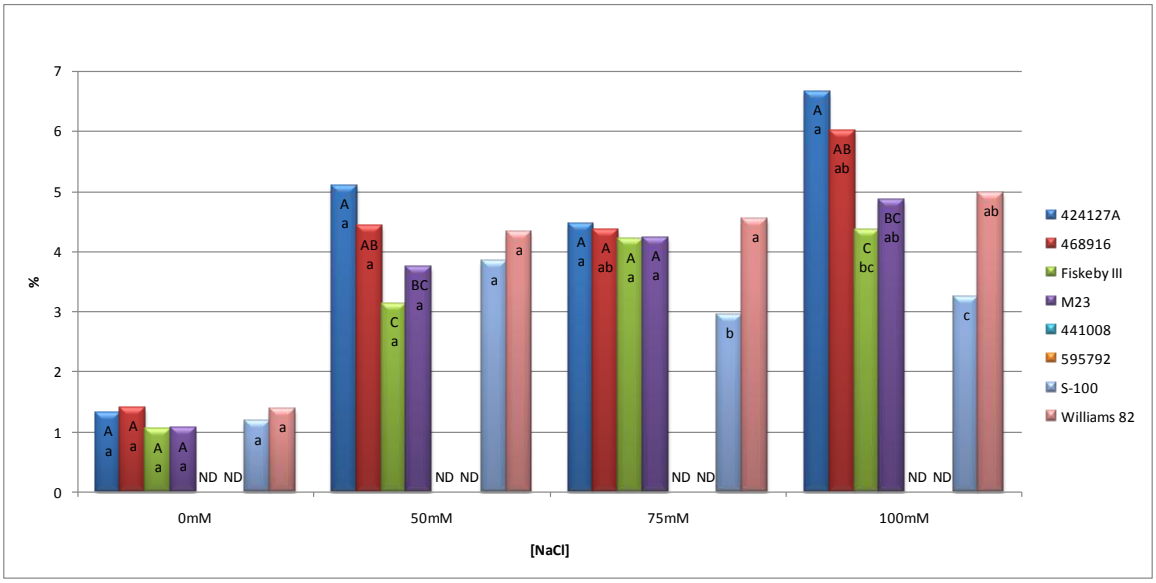
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. ns = not significant.

Figure 3.13. Stem Na^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



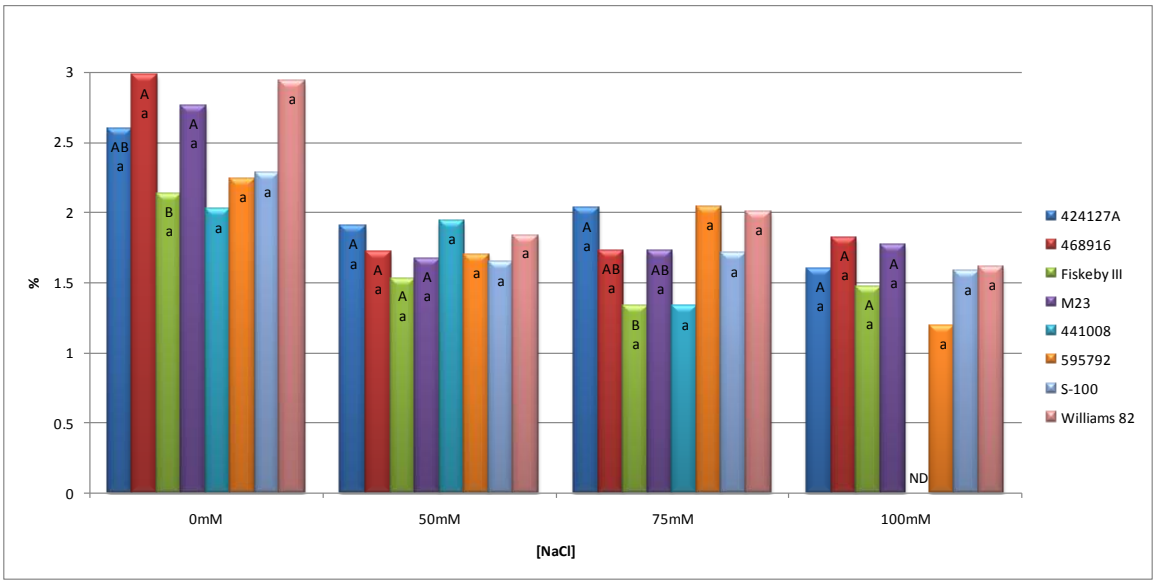
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.14. Stem Cl^- concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



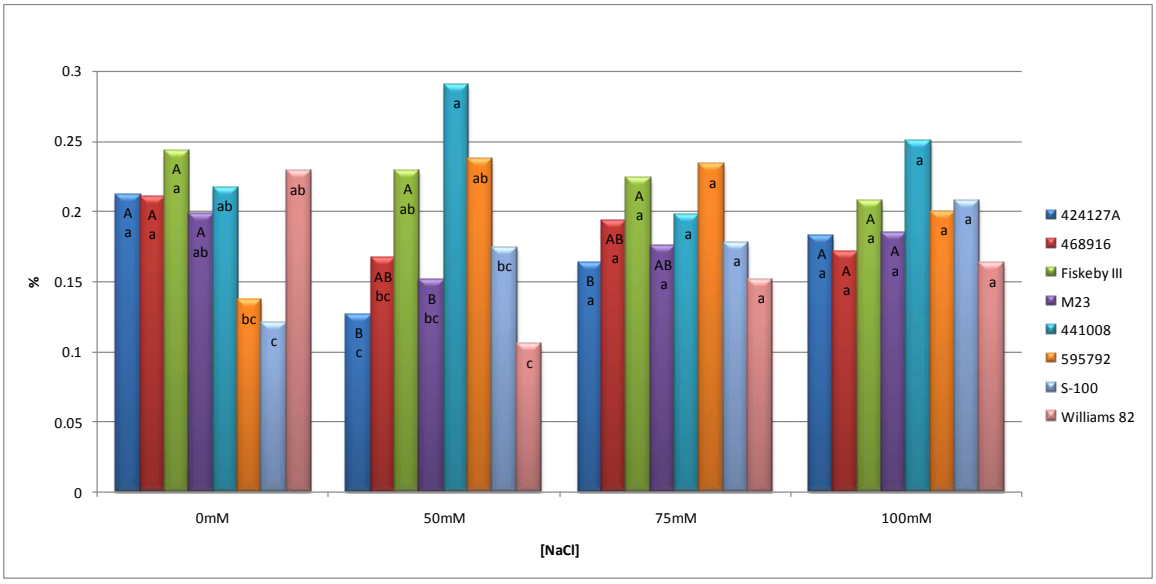
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only. ND=no data.

Figure 3.15. Stem K^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



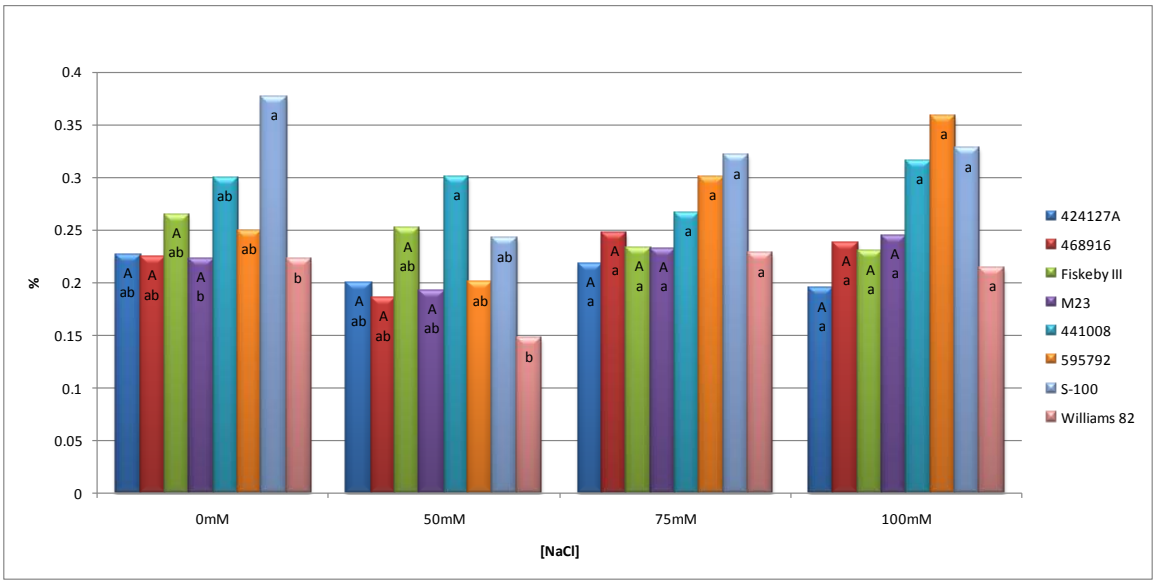
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only. ND=no data.

Figure 3.16. Stem Mg^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.17. Stem Ca^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



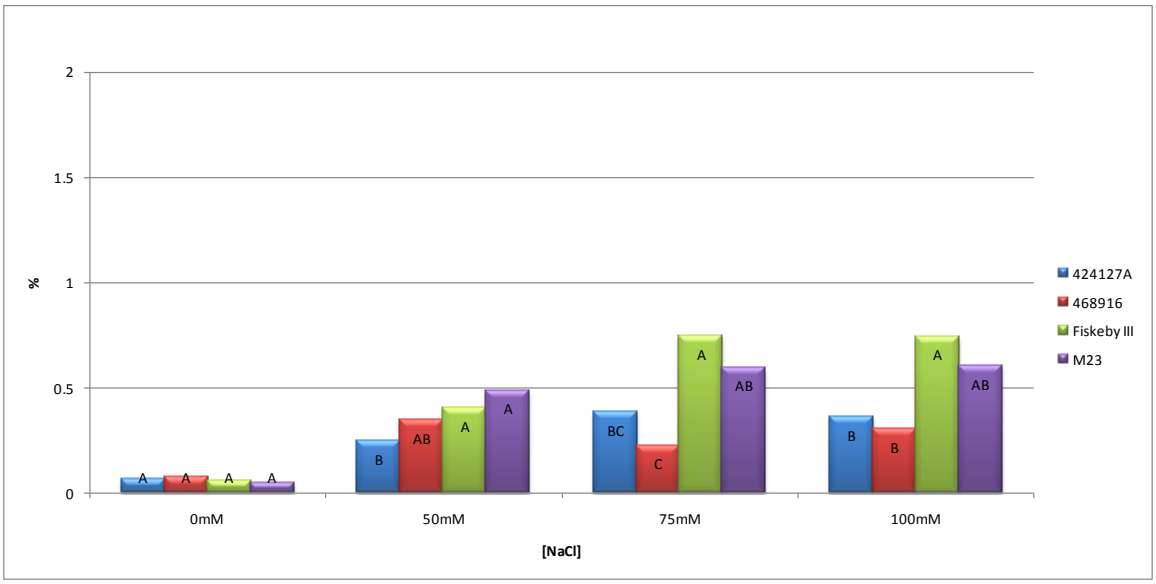
Columns with same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Table 3.4. Analysis of variance, fixed effects, across 4 NaCl concentrations, 2 years and 4 genotypes of genus *Glycine* for root concentration of Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}.

Effect	RNac	RClc	RKc	RMgc	RCac
[NaCl]	**	**	ns	ns	ns
Genotype (G)	**	**	**	**	*
[NaCl] x G	ns	ns	ns	ns	ns
Year (Y)	*	ns	*	ns	ns
[NaCl] x Y	ns	ns	ns	ns	ns
G x Y	ns	ns	ns	ns	ns
[NaCl] x G x Y	*	ns	ns	ns	*

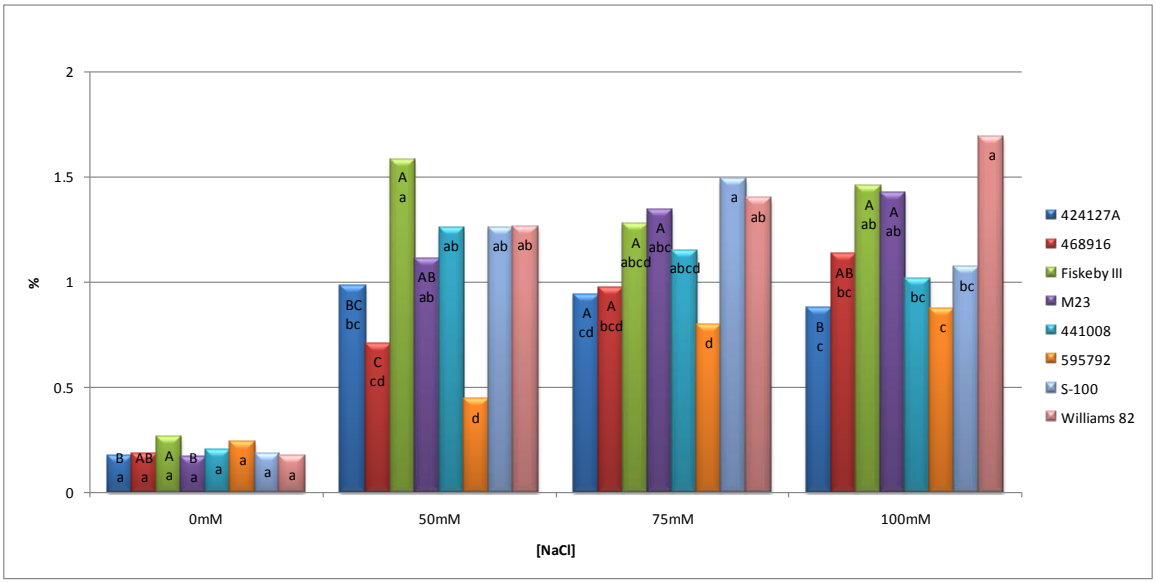
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. ns = not significant.

Figure 3.18. Root Na^+ concentration of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



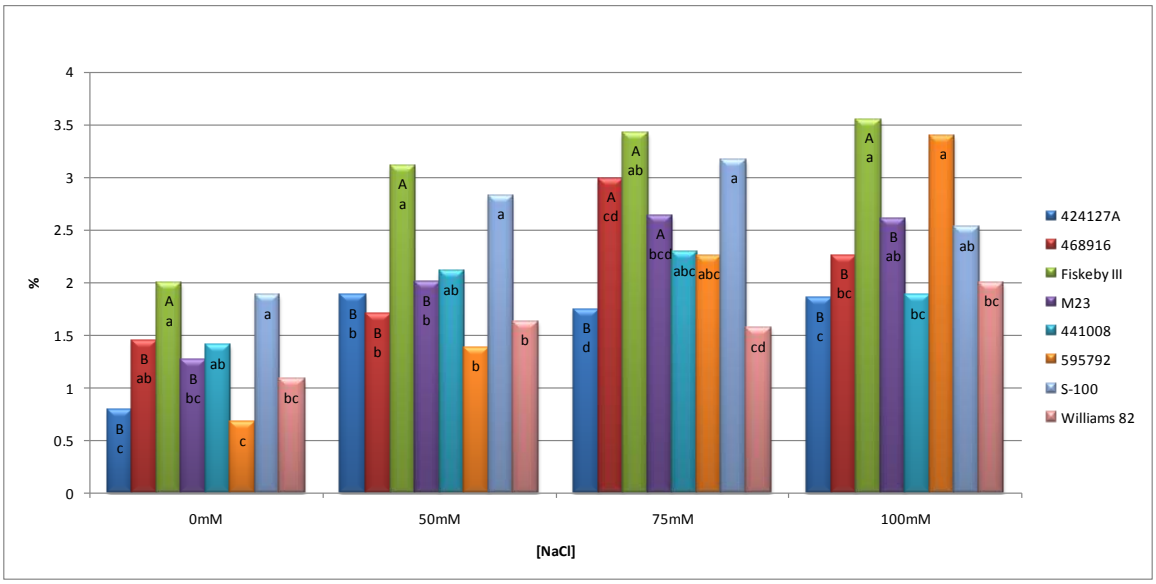
Columns with the same top letter are not significantly different at $p<0.05$.

Figure 3.19. Root Na^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



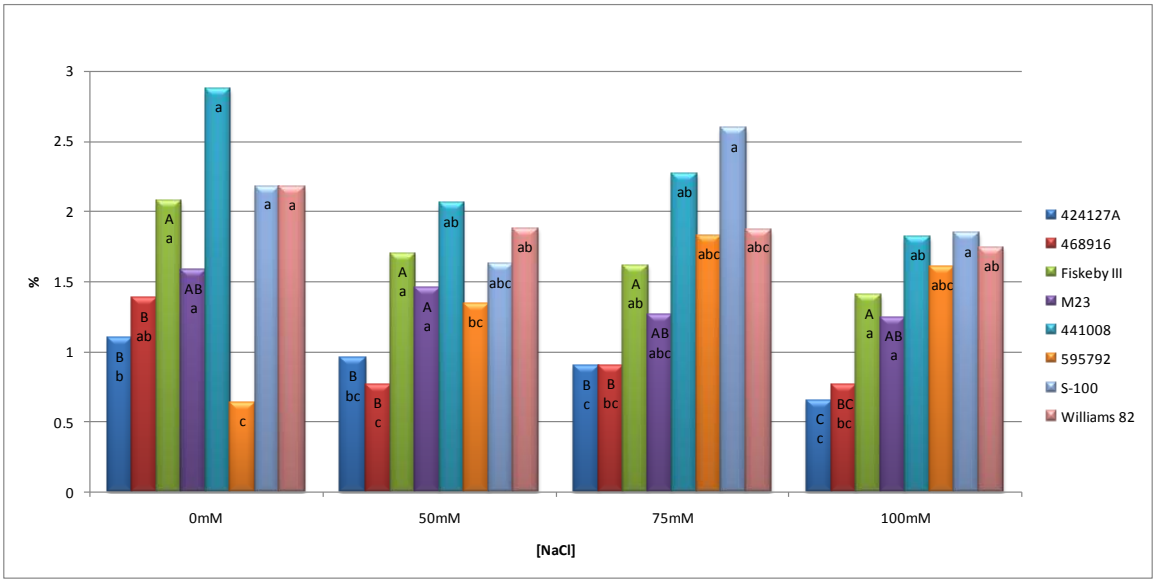
Columns with the same top letter are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.20. Root Cl^- concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



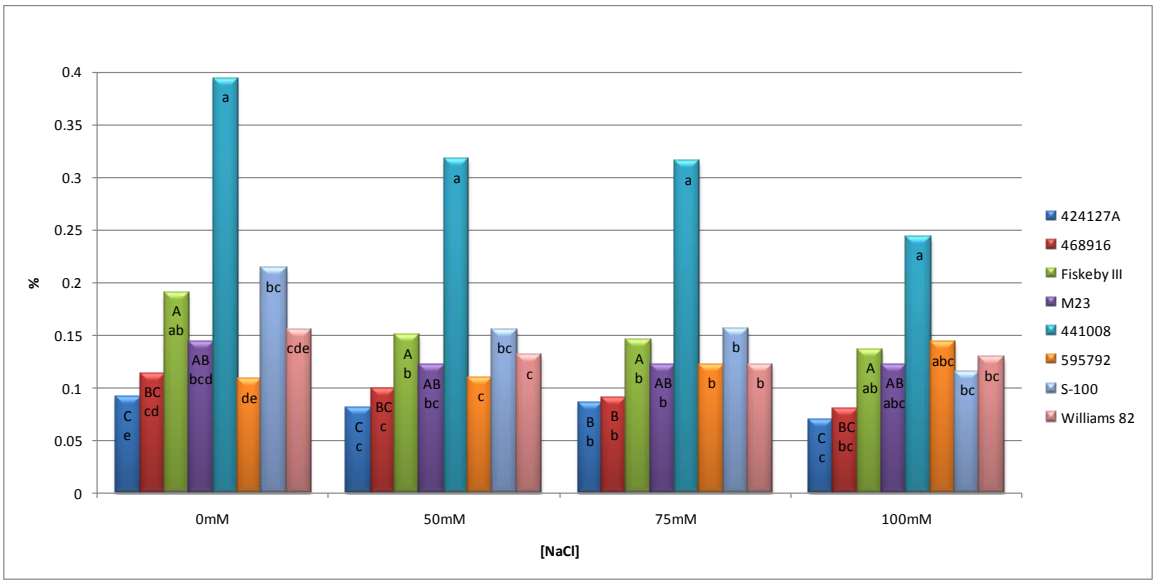
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.21. Root K^+ concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



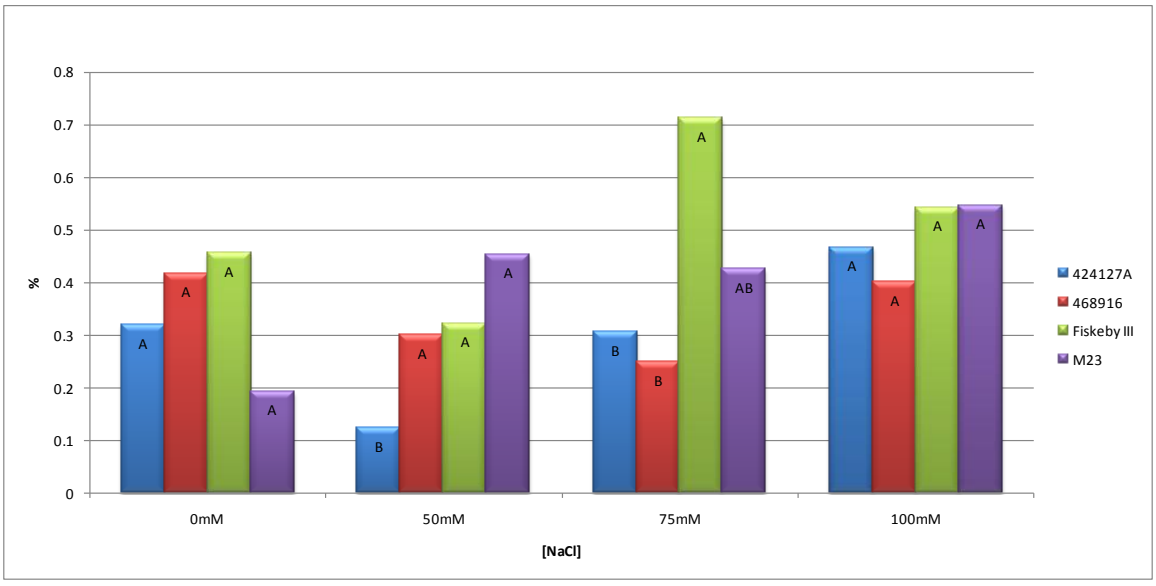
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.22. Root Mg^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



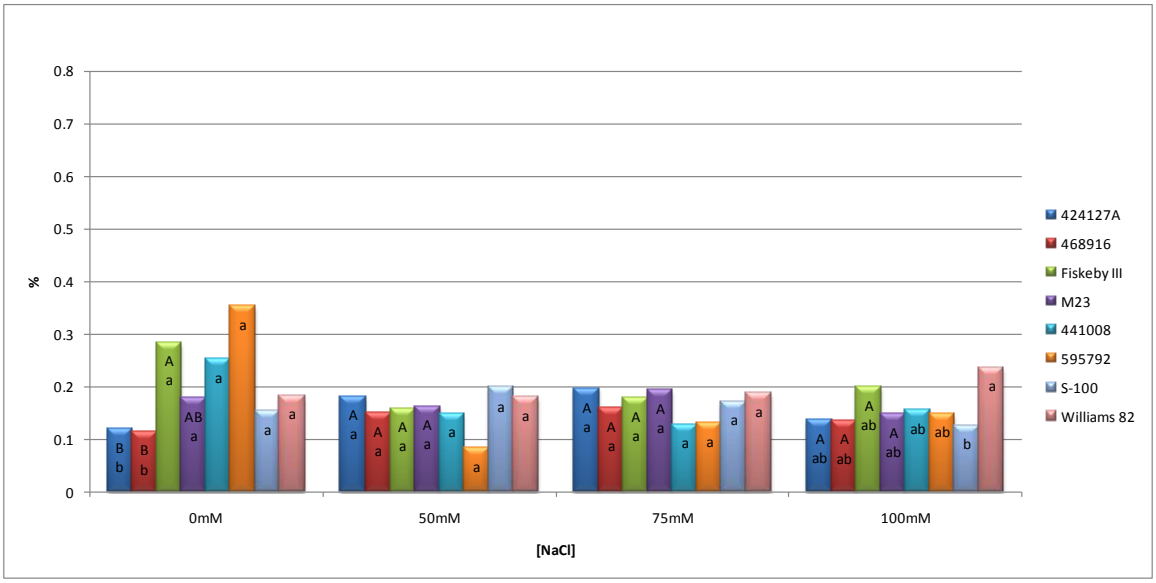
Columns with the same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.23. Root Ca^{2+} concentration of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



Columns with same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.24. Root Ca^{2+} concentration of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



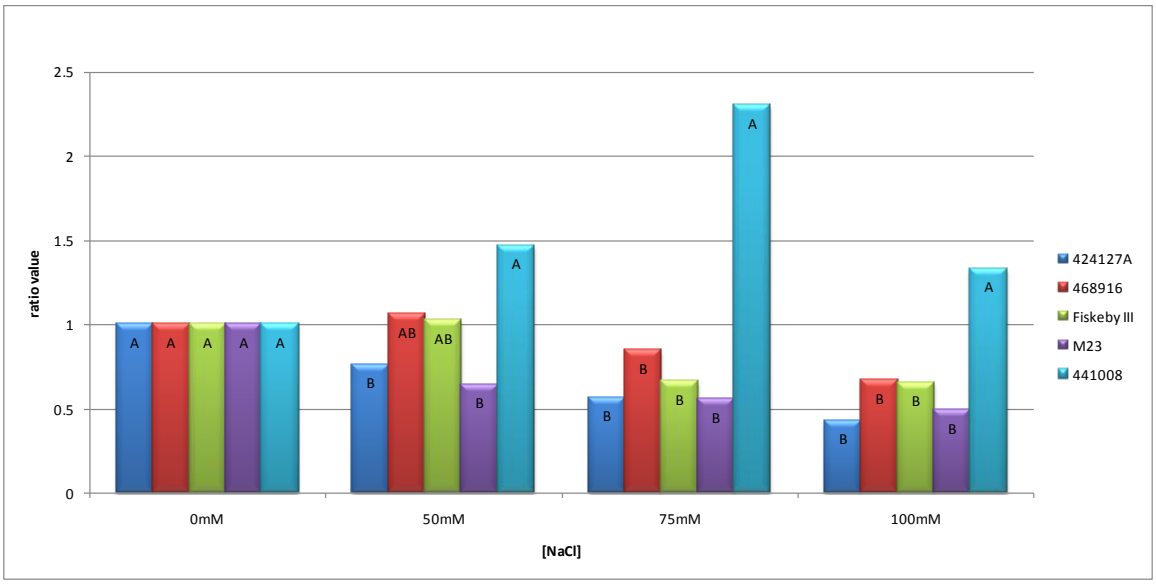
Columns with same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Table 3.5. Analysis of variance, fixed effects, across 4 NaCl concentrations, 2 years and genotypes of genus *Glycine* for leaf, stem and root dry weight and stem and root length.

Effect	Ldw	Sdw	Rdw	Slth	Rlth
[NaCl]	*	ns	ns	*	ns
Genotype (G)	**	**	ns	**	ns
[NaCl] x G	ns	ns	ns	**	ns
Year (Y)	ns	ns	ns	ns	ns
[NaCl] x Y	ns	ns	ns	ns	ns
G x Y	**	*	ns	ns	ns
[NaCl] x G x Y	ns	ns	ns	ns	ns

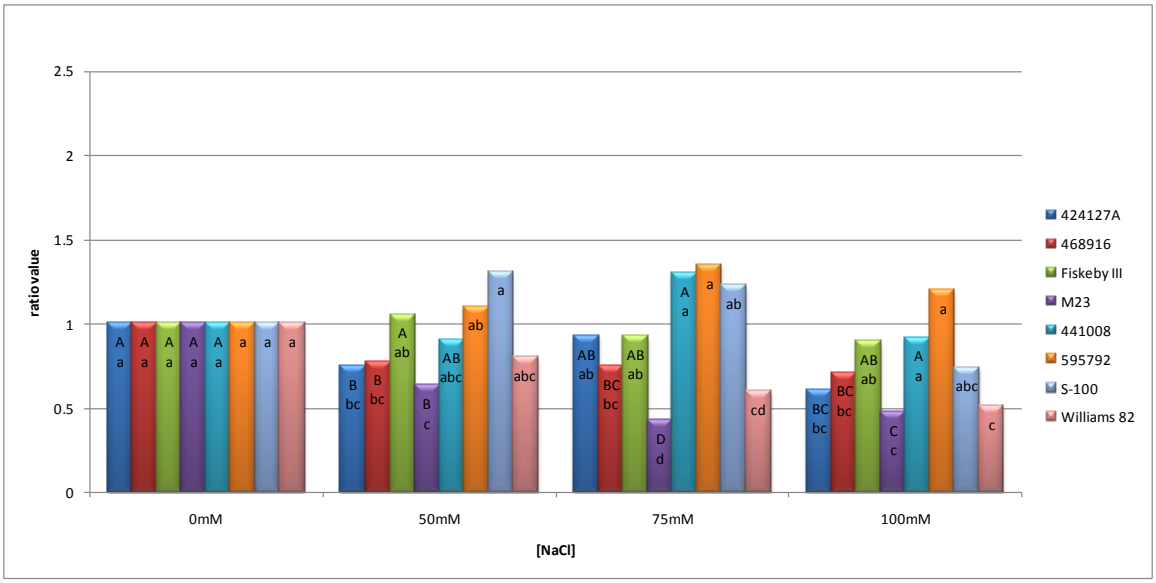
*, ** Significant at the 0.05 and 0.01 probability levels, respectively. ns = not significant.

Figure 3.25. Leaf dry weight ratio of 5 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



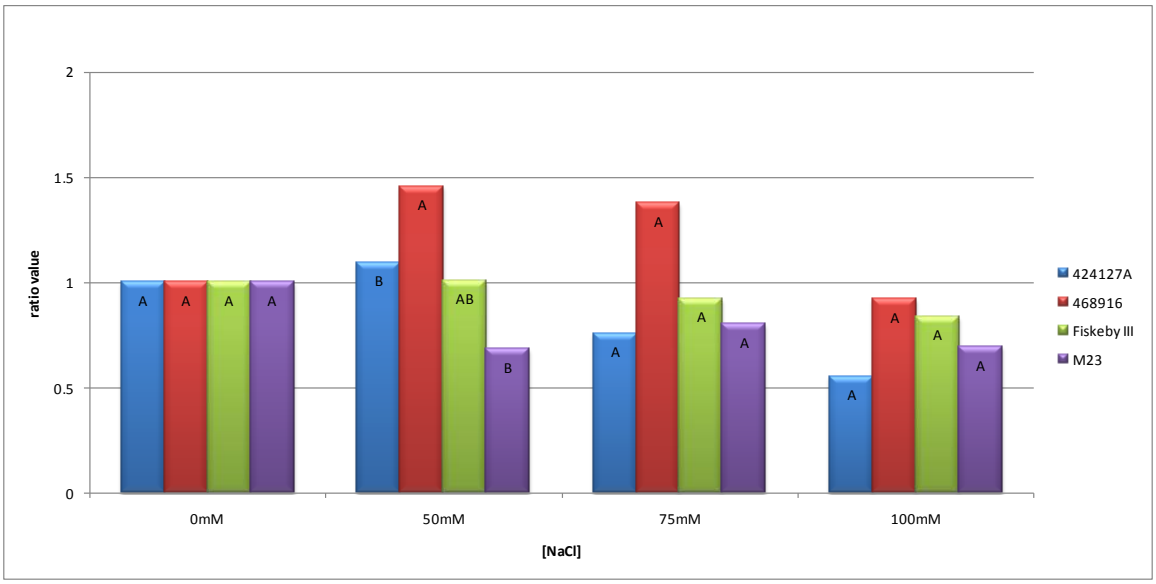
Columns with the same top letter are not significantly different at p<0.05.

Figure 3.26. Leaf dry weight ratio of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



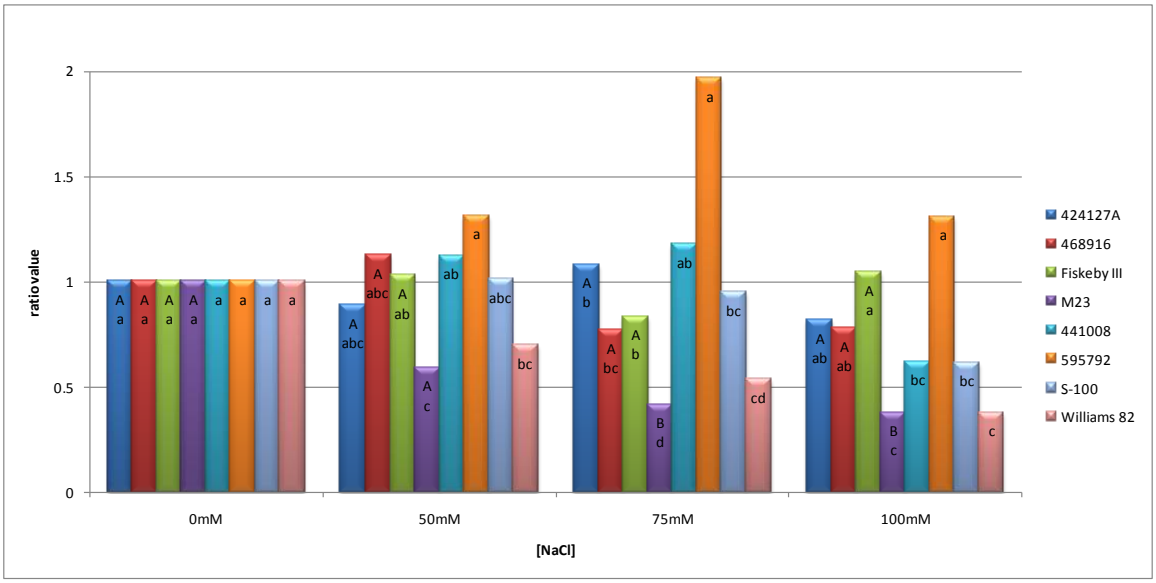
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.27. Stem dry weight ratio of 4 genotypes of genus *Glycine* at 4 NaCl concentrations in year 1.



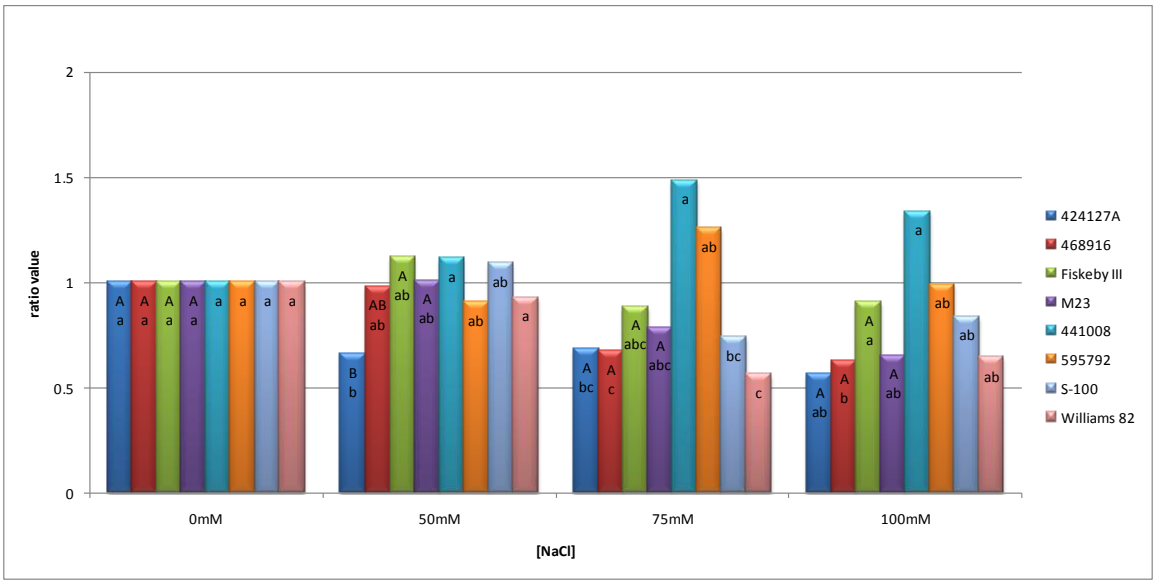
Columns with the same top letter are not significantly different at p<0.05.

Figure 3.28. Stem dry weight ratio of 8 genotypes of genus *Glycine* at 4 NaCl concentrations in year 2.



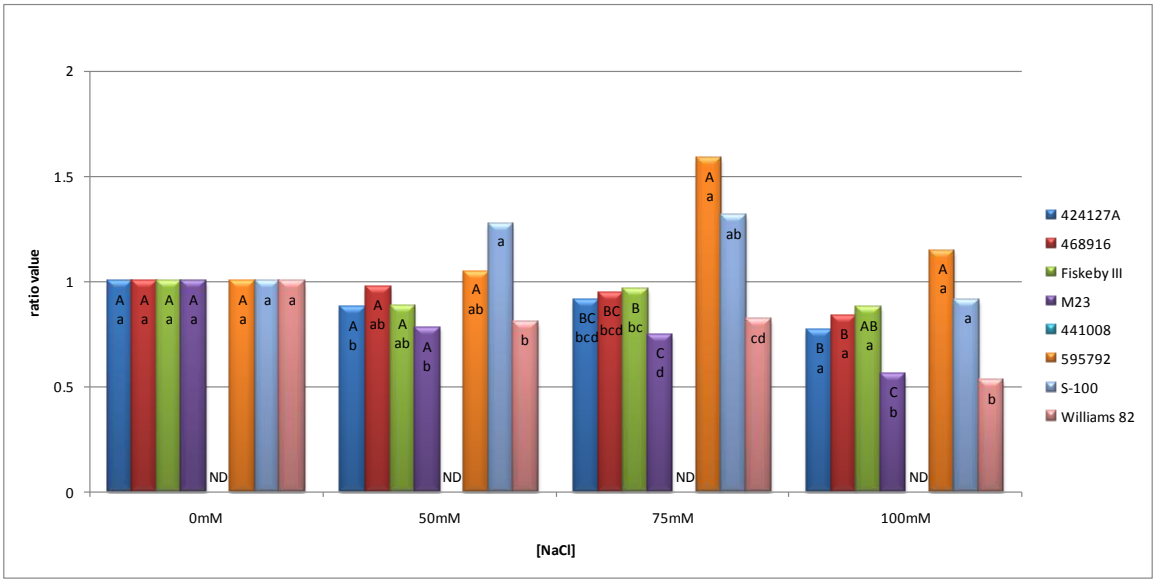
Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.29. Root dry weight ratio of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



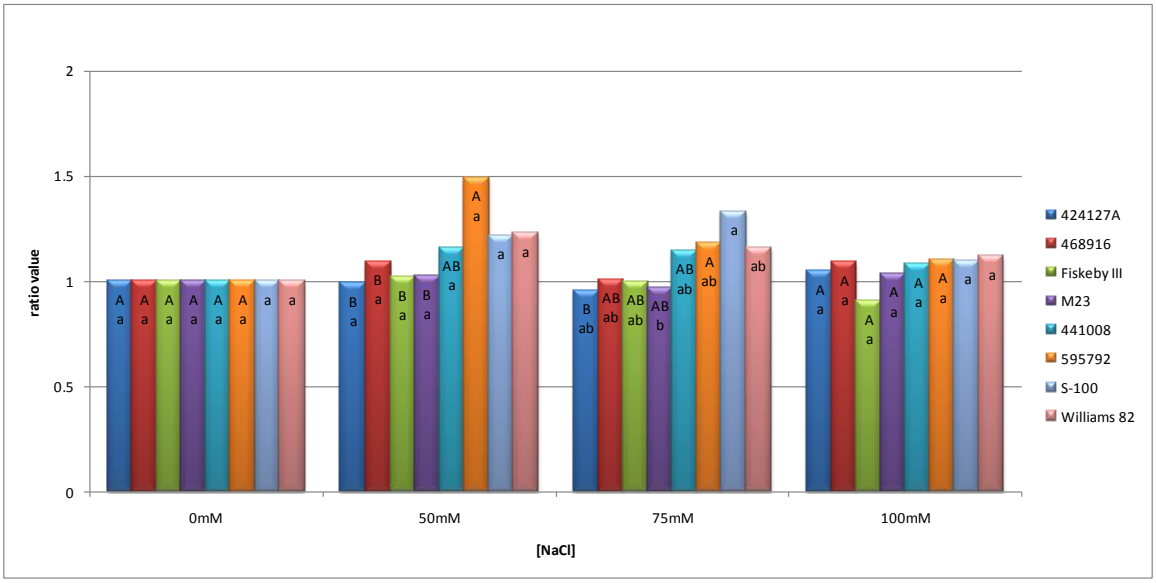
Columns with the same top letter and case type are not significantly different at $p<0.05$. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Figure 3.30. Stem length ratio of 7 genotypes of genus *Glycine* at 4 NaCl concentrations.



Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only. ND=no data.

Figure 3.31. Root length ratio of 8 genotypes of genus *Glycine* at 4 NaCl concentrations.



Columns with the same top letter and case type are not significantly different at p<0.05. Uppercase letters correspond to 2-year pooled ANOVA; lowercase letters correspond to year 2 ANOVA only.

Chapter IV

REGRESSION ANALYSES

To investigate the degree of association between some of the physiological traits studied, regression analyses were performed using PROC REG in SAS. Regression coefficients (b) were compared through orthogonal contrasts at the 0.05 significance level.

In year 2, new accessions were added to the design (Table 2.1). Although PI 441008 (*G. tomentella*) and PI 595792 (*G. argyrea*) were included in the experiment in both years, often enough tissue could not be collected to obtain measurable dry weights or to determine ion content during the first year. Consequently, there are regressions performed with 2-year data as well as regressions carried out with data resulting from year 2 only. Regressions with year 2 data were performed to compare all 8 genotypes.

Leaf scorch on [NaCl] in nutrient solution

To investigate the rate of leaf injury induced by increasing concentrations of NaCl in solution, the regression of leaf scorch score over increasing salt concentrations was performed. There was a highly significant association between the increase of NaCl

in solution and leaf scorch in all genotypes. Exceptions were the tolerant perennial accessions PI 441008 and PI 595792, which did not show any change in leaf appearance as [NaCl] in solution increased (Table 4.1).

In the regressions with composite data, sensitive PI 424127A and M23 showed the highest rates of leaf scorch, while tolerant Fiskeby III and PI 468916 showed significantly lower rates of leaf damage. Rates of leaf scorch were 86 and 75% lower in Fiskeby III and PI 468916 than in M23 and PI 424127A, respectively (Table 4.1).

Regressions performed with data from year 2 show sensitive accessions PI 424127A, M23 and Williams 82 with leaf scorch rates ranging from 0.038 to 0.040 and belonging to the same statistical group. Tolerant genotypes PI 468916, Fiskeby III and S-100 had b values ranging from 0.009 to 0.021, and they were significantly different from sensitive accessions. Among tolerant genotypes, PI 468916 showed the highest leaf injury rate, and it was significantly different from those of Fiskeby III and S-100 (Table 4.1).

Leaf Na^+ concentration on [NaCl] in nutrient solution

The regression of leaf Na^+ concentration over various salt concentrations was performed to investigate the increase of Na^+ in leaves per unit of NaCl increase in solution. For all genotypes there was a highly significant association between the

increase of NaCl in the solution and the Na^+ levels in leaves. Also, there were significant differences in the rate of Na^+ accumulation (b value=regression coefficient) among genotypes (Table 4.2).

In the 2-year regression analysis, Fiskeby III showed the lowest rate of Na^+ accumulation (2.36), while PI 424127A had the highest (8.71). Within-same-species comparisons show tolerant Fiskeby III and PI 468916 accumulating significantly less Na^+ than sensitive M23 and PI 424127A, respectively.

In the regression analysis for year 2, sensitive Williams 82 and M23 accumulated Na^+ at a higher rate than tolerant Fiskeby III and S-100, and the same was observed between PI 424127A and PI 468916. In addition, S-100 and the perennial PI 441008 and PI 595792 showed similar accumulation rates to Fiskeby III but significantly lower than those of the other accessions.

Leaf Cl^- concentration on [NaCl] in nutrient solution

The regression of leaf Cl^- concentration on [NaCl] in solution gives the rate of Cl^- accumulation in leaves with increasing levels of NaCl in the solution. All genotypes showed a highly significant association between the increase of NaCl in the solution and Cl^- levels in leaves. Also, there were significant differences in the rate of Cl^- accumulation (b value=regression coefficient) among genotypes (Table 4.3).

In the 2-year regression analysis, Fiskeby III showed the lowest rate of Cl^- accumulation (7.03) while PI 424127A the highest (18.46). Within-same-species comparisons show tolerant Fiskeby III and PI 468916 accumulating significantly less Cl^- than sensitive M23 and PI 424127A, respectively (Table 4.3).

In year 2, sensitive PI 424127A accumulated significantly more Cl^- than tolerant PI 468916; the same pattern was observed when comparing sensitive Williams 82 and tolerant S-100. Moreover, Fiskeby III showed a lower rate of Cl^- accumulation than Williams 82, but the difference was not significant. In addition, Fiskeby III and M23 presented similar Cl^- accumulation rates. Perennial PI 441008 and PI 595792 were among accessions with the lowest accumulation levels (Table 4.3).

Leaf scorch on Leaf Na^+ concentration

The present regression was carried out to quantitatively determine the increase in leaf injury per unit increase of Na^+ in leaves. All genotypes with 2-year data showed a highly significant association between the increase of Na^+ levels in leaves and leaf injury (leaf scorch). Also, there were significant differences in the rate of scorching (b value=regression coefficient) among genotypes (Table 4.4). M23 was the accession that showed the highest rate of leaf injury, and it was significantly different from all the other accessions. Within-species comparisons show that tolerant Fiskeby III and PI 468916 were significantly different from sensitive M23 and PI 424127A, respectively.

In year 2, perennial accessions PI 441008 and PI 595792 showed no significant association between increases of Na^+ in leaves and leaf scorch. On the other hand, all annual genotypes presented significant regressions. Again, sensitive cultivar M23 showed the highest Na^+-induced leaf injury rate, and it was significantly different from the rest. The other sensitive *G. max* accession, Williams 82, showed the second highest rate of Na^+-induced injury, and it was significantly different from tolerant S-100 but not from Fiskeby III at the $p<0.05$ significance level. Regarding *G. soja* accessions, sensitive PI 424127A presented a significantly higher rate of injury than tolerant PI 468916 (Table 4.4).

Leaf scorch on Leaf Cl^- concentration

To investigate the rate of leaf injury per unit increase of Cl^- in leaves, the present regression was performed. All genotypes with 2-year data showed a highly significant association between the increase of Cl^- levels in leaves and leaf injury (leaf scorch). Also, there were significant differences in the rate of Cl^--induced scorching (b value=regression coefficient) among genotypes (Table 4.5). As previously observed for Na^+-induced leaf scorch, M23 was the accession that showed the highest rate of leaf injury resulting from Cl^- present in leaves. Furthermore, within species comparisons show that tolerant Fiskeby III and PI 468916 were significantly different from their sensitive counterparts (Table 4.5).

Regressions from year 2 data show that tolerant perennial accessions PI 441008 and PI 595792 presented no association between the increase of Cl^- in leaves and leaf scorch. Regressions were highly significant for all other genotypes. M23 exhibited the highest rate of leaf injury due to Cl^- in leaves, and it was significantly different from all other accessions except sensitive Williams 82. Within species comparisons show tolerant Fiskeby III and S-100 (*G. max*) and PI 468916 (*G. soja*) with significantly lower leaf injury rate than sensitive M23 and Williams 82 and PI 424127A, respectively (Table 4.5).

Leaf dry weight on [NaCl] in nutrient solution

The regression of leaf dry weight over the four salt concentrations was performed to investigate the effect of increasing levels of NaCl in solution on leaf dry weight. In the analysis with data over both years, only M23, PI 424127A and Fiskeby III showed a significant and negative association. Less than 10% of the variation in leaf dry weight was explained by increasing levels of NaCl in Fiskeby III and PI 424127A. The only accession that showed an important R^2 value was M23. The rate of decrease in dry weight shown by M23 was significantly greater than the observed in Fiskeby III and PI 424127A (Table 4.6).

In the second year, only the sensitive accessions M23, Williams 82 and PI 424127A showed significant associations between [NaCl] in solution and leaf dry weight.

Williams 82 and M23 showed the greatest rates of decrease in leaf dry weight per unit increase of NaCl in solution, and they were similar at the 0.05 significance level. On the contrary, as it was previously observed in the composite analysis, PI 424127A showed a rate of leaf dry weight decrease significantly lower than that of M23. Also, PI 424127A showed a low R^2 value (0.12), meaning that although the association is significant, only 12% of variation in dry weight of leaves was explained by changes in NaCl in solution (Table 4.6).

COMPARATIVE ANALYSES

In this section, a comparative analysis of Na^+ and Cl^- accumulation rates in both salt sensitive and tolerant genotypes is presented. Also, observed differences regarding Na^+ and Cl^- toxicity in both salt tolerant and sensitive accessions are shown. These analyses were carried out using regression coefficients (b values) resulting from 2-year data analysis of accessions Fiskeby III, M23, PI 468916 and PI 424127A, which were previously presented in the above section "Regression Analyses" of this chapter (Tables 4.2 to 4.5).

Leaf Cl^- and Na^+ accumulation rates

The observed rates of Cl^- accumulation in sensitive M23 and PI 424127A were 1.6 and 2.2 times higher than in tolerant Fiskeby III and PI 468916, respectively. Similarly, Na^+ accumulation occurred at 2.2 and 2.4 higher rates in M23 and PI 424127A, respectively than in their tolerant counterparts. Regardless of the salt tolerance level and species considered, Cl^- consistently accumulated at a rate at least 2.1 times higher than Na^+ (Tables 4.7 and 4.8).

Cl^-- and Na^+-induced leaf scorch

Regardless the species, sensitive accessions showed higher rates of leaf injury per unit of Cl^- and Na^+ accumulated in leaves than tolerant ones. In general, both Cl^- and Na^+ were two times more toxic in sensitive genotypes than in tolerant ones; the exception was observed in *G. soja* accessions: Na^+ was 50% more toxic in sensitive PI 424127A than in tolerant PI 468916 (Tables 4.9 and 4.10).

When analyzing leaf injury rate per unit increase of Cl^- or Na^+ in leaves, it is evident that, regardless of species and salt tolerance level, Na^+ has a greater toxic effect than Cl^-. In both M23 and Fiskeby III, the damage potential of Na^+ was 3.1 greater than that of Cl^-. In addition, the toxic effect of Na^+ was 2.2 and 3.1 greater than that of Cl^- in PI 424127A and PI 468916, respectively. In PI 424127A, the toxicity of Na^+ relative to that of Cl^- was the lowest among the four accessions analyzed (Tables 4.9 and 4.10).

Table 4.1. Regression analysis of leaf scorch on [NaCl] (mM) in nutrient solution for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf scorch-[NaCl] in nutrient solution					
	Composite			Year 2		
	intercept	b	R^2	intercept	b	R^2
Fiskeby III	0.98	0.005c	0.13**	1.05	0.013c	0.54**
M23	0.94	0.035a	0.78**	1.09	0.038a	0.89**
PI 468916	0.95	0.009b	0.23**	0.96	0.021b	0.68**
PI 424127A	1.06	0.036a	0.79**	1.15	0.040a	0.89**
PI 441008	-	-	ns	-	-	ns
PI 595792	-	-	ns	-	-	ns
S-100	-	-	-	1.14	0.009c	0.19**
Williams 82	-	-	-	1.07	0.038a	0.85**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at p<0.05.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

Table 4.2. Regression analysis of leaf Na^+ concentration (mmol kg^{-1}) on [NaCl] (mM) in nutrient solution for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf Na^+ concentration-[NaCl] in nutrient solution					
	Composite			Year 2		
	intercept	b	R^2	intercept	b	R^2
Fiskeby III	18.90	2.36c	0.22**	32.78	4.63cd	0.56**
M23	15.71	5.14b	0.66**	13.76	5.90c	0.84**
PI 468916	21.94	3.70bc	0.36**	45.96	6.70bc	0.73**
PI 424127A	32.91	8.71a	0.67**	42.66	9.97a	0.74**
PI 441008	-	-	-	-7.76	2.89d	0.58**
PI 595792	-	-	-	3.97	3.22d	0.73**
S-100	-	-	-	70.15	3.54d	0.26**
Williams 82	-	-	-	2.58	8.04ab	0.68**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at p<0.05.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

Table 4.3. Regression analysis of leaf Cl^- concentration (mmol kg^{-1}) on [NaCl] (mM) in nutrient solution for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf Cl^- concentration-[NaCl] in nutrient solution					
	Composite			Year 2		
	intercept	b	R^2	intercept	b	R^2
Fiskeby III	278.33	7.03c	0.28**	426.43	12.36bc	0.65**
M23	628.73	11.25b	0.45**	862.87	12.22bcd	0.64**
PI 468916	532.95	8.52bc	0.29**	797.54	12.34bcd	0.51**
PI 424127A	659.68	18.46a	0.66**	954.76	18.48a	0.74**
PI 441008	-	-	-	347.55	8.75cd	0.72**
PI 595792	-	-	-	392.50	5.49d	0.65**
S-100	-	-	-	453.76	8.03cd	0.36**
Williams 82	-	-	-	729.47	15.97ab	0.70**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at p<0.05.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

Table 4.4. Regression analysis of leaf scorch on leaf Na^+ concentration (mmol kg^{-1}) for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf scorch-Leaf Na^+ concentration					
	Composite			Year 2		
	intercept	b†	R^2	intercept	b†	R^2
Fiskeby III	0.92	2.66bc	0.82**	0.99	2.75bcd	0.88**
M23	1.19	5.57a	0.80**	1.14	6.04a	0.93**
PI 468916	0.95	2.30c	0.75**	0.97	2.34d	0.79**
PI 424127A	1.42	3.35b	0.75**	1.44	3.26bc	0.79**
PI 441008	-	-	-	-	-	ns
PI 595792	-	-	-	-	-	ns
S-100	-	-	-	0.99	2.60cd	0.71**
Williams 82	-	-	-	1.60	3.56b	0.71**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at p<0.05.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

† Multiply the reported numbers in this column by 10^{-3} to obtain the actual numbers.

Table 4.5. Regression analysis of leaf scorch on leaf Cl^- concentration (mmol kg^{-1}) for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf scorch-Leaf Cl^- concentration					
	Composite			Year 2		
	intercept	b†	R^2	intercept	b†	R^2
Fiskeby III	0.75	0.87b	0.62**	0.75	0.90d	0.58**
M23	0.63	1.79a	0.59**	-0.09	2.14a	0.66**
PI 468916	0.70	0.74b	0.52**	0.48	0.94d	0.61**
PI 424127A	0.61	1.51a	0.70**	0.15	1.64bc	0.69**
PI 441008	-	-	-	-	-	ns
PI 595792	-	-	-	-	-	ns
S-100	-	-	-	0.62	1.20cd	0.54**
Williams 82	-	-	-	0.25	1.83ab	0.70**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at $p<0.05$.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

† Multiply the reported numbers in this column by 10^{-3} to obtain the actual numbers.

Table 4.6. Regression analysis of leaf dry weight (g) on [NaCl] (mM) in nutrient solution for genotypes of genus *Glycine*. Two-year data composite analysis and year 2 data analysis.

Genotype	Leaf dry weight-[NaCl]					
	Composite			Year 2		
	intercept	b	R^2	intercept	b	R^2
Fiskeby III	1.83	-0.005b	0.08**	-	-	ns
M23	1.58	-0.010a	0.46**	1.73	-0.012a	0.60**
PI 468916	-	-	ns	-	-	ns
PI 424127A	1.02	-0.004b	0.09**	1.46	-0.006b	0.12*
PI 441008	-	-	ns	-	-	ns
PI 595792	-	-	-	-	-	ns
S-100	-	-	-	-	-	ns
Williams 82	-	-	-	2.42	-0.014a	0.36**

b=regression coefficient; R^2=coefficient of determination; b with the same letter are not significantly different at $p<0.05$.

*, ** Significant associations at the 0.05 and 0.01 probability levels, respectively. ns=not significant.

Table 4.7. Cl^- and Na^+ accumulation rates (mmol kg^{-1} per mM unit increase of NaCl in NS) in leaves of *Glycine max* genotypes M23 (salt sensitive) and Fiskeby III (salt tolerant) and their comparisons.

	M23 (S)	Fiskeby III (T)	S/T
Cl^-	11.25	7.03	1.6
Na^+	5.14	2.36	2.2
Cl^-/Na^+	2.2	3.0	

(S)=sensitive; (T)=tolerant.

Table 4.8. Cl^- and Na^+ accumulation rates (mmol kg^{-1} per mM unit increase of NaCl in NS) in leaves of *Glycine soja* genotypes PI 424127A (salt sensitive) and PI 468916 (salt tolerant) and their comparisons.

	PI 424127A (S)	PI 468916 (T)	S/T
Cl^-	18.46	8.52	2.2
Na^+	8.71	3.70	2.4
Cl^-/Na^+	2.1	2.3	

(S)=sensitive; (T)=tolerant.

Table 4.9. Cl^-- and Na^+-induced leaf scorch rates (leaf scorch score per mmol kg^{-1} unit increase of Cl^- and Na^+ in leaves) of *Glycine max* genotypes M23 (salt sensitive) and Fiskeby III (salt tolerant) and their comparisons.

	M23 (S)	Fiskeby III (T)	S/T
Cl^-	1.79	0.87	2.1
Na^+	5.57	2.66	2.1
Na^+/Cl^-	3.1	3.1	

(S)=sensitive; (T)=tolerant.

Table 4.10. Cl^-- and Na^+-induced leaf scorch rates (leaf scorch score per mmol kg^{-1} unit increase of Cl^- and Na^+ in leaves) of *Glycine soja* genotypes PI 424127A (salt sensitive) and 468916 (salt tolerant) and their comparisons.

	PI 424127A (S)	PI 468916 (T)	S/T
Cl^-	1.51	0.74	2.0
Na^+	3.35	2.30	1.5
Na^+/Cl^-	2.2	3.1	

(S)=sensitive; (T)=tolerant.

Chapter V

DISCUSSION

Leaf scorch and Na^+ and Cl^- concentration in leaves

Genotypes of four *Glycine* species were compared for salinity-induced leaf scorch (marginal and tip burn and other necrotic leaf symptoms) at four NaCl levels (0, 50, 75 and 100 mM) in a greenhouse experiment. Level of injury varied among genotypes. Sensitive accessions were moderately to severely damaged due to exposure of roots to high [NaCl] in the nutrient solution, while tolerant genotypes had slight to no injury. The harmful effect of salinity on leaf health was previously reported for several crops including soybean (Bernstein and Hayward, 1958; Abel and MacKenzie, 1964; Grattan and Maas, 1985; Pantalone et al., 1997; Lee et al., 2004).

Among *G. max* genotypes, Fiskeby III and S-100 were similar and showed the lowest levels of leaf scorch. S-100 was previously reported as a salt tolerant variety (Lee et al., 2004). Genotype PI 468916 (*G. soja*), showed tolerance based on leaf scorch, but to a lesser degree than Fiskeby III and S-100. Perennial genotypes PI 441008 (*G. tomentella*) and PI 595792 (*G. argyrea*) showed high salt tolerance, with no injury to

leaves even at the highest salt concentration. Previous studies have suggested the utilization of wild relatives as a potential source of genes to improve agronomic traits in crop species, including salt tolerance (Brown et al., 1984; Hymowitz et al., 1987; Pantalone et al., 1997; Hymowitz, 2004; Humphreys and Humphreys, 2005).

This is the first report presenting the reaction of these genotypes to high [NaCl] concentrations. The exception is the soybean cultivar S-100, which was previously reported as salt tolerant by Lee et al. (2004).

Experimental conditions were important in determining the severity of salinity-induced leaf scorch. Sensitive and some of the tolerant genotypes showed higher leaf scorch the second year than in the first. This could be directly related to higher temperatures and lower humidity, and consequently increased transpiration rates at the time plants were undergoing salt stress (Figures 2.1 and 2.2). Similar observations regarding the increase in severity of symptoms with hotter and dryer weather were reported by Ehlig (1960) and confirmed by Bernstein (1975). Salinity level x environment interactions were considered to be significant in assessing salt reaction (Shannon, 1984; Li et al., 2000; Cicek and Cakirlar, 2008). Thus, environmental conditions can modify the salinity tolerance of genotypes (Li et al., 2000), which explains the fact that some of the tolerant accessions had higher leaf scorch during the second year.

Changes in leaf scorch were significantly associated with NaCl in the solution. However, increases in leaf injury were better explained by the variation in the contents of Na^+ and Cl^- in leaves. Tolerant accessions accumulated Cl^- in leaves at lower rates than sensitive accessions, which is in agreement with previous studies (Bernstein, 1975; Wieneke and Läuchli, 1979). In addition, this study shows that also Na^+ was transported to leaves at lower rates in tolerant than in sensitive genotypes.

Salt tolerance in soybean, especially at high NaCl concentrations, is thought to be primarily related to the ability of the plants to limit the accumulation of excess ions in leaves and, thus, avoid toxic buildups and nutrient imbalances (Läuchli and Wieneke, 1979; Umezawa et al., 2002; Pathan et al., 2007). Soybean is considered to be a natrophobic species, that is, no substitution of K^+ for Na^+ is possible without severely impacting growth (Marschner, 1995). In these experiments, all genotypes regardless the salt tolerance level had a significant increase in LNac with increased [NaCl] in nutrient solution. Sensitive genotypes, however, accumulated significantly more Na^+ in leaves than tolerant genotypes. Similar observations were reported by Läuchli and Wieneke (1979), Essa (2002) and Luo et al. (2005) and are in disagreement with Grattan and Maas (1985) and Dabuxilatu and Ikeda (2005). Also, in tolerant accessions, rate of Na^+ accumulation in leaves was at least 2.2 times less than in sensitive genotypes. The distribution of ions in soybean varieties differing in salt tolerance suggested that Na^+ exclusion from leaf tissues appears to play an important role in salt tolerance of cultivar

Lee (Läuchli and Wieneke, 1979). The correlation between Na^+ exclusion from leaves and salt tolerance was also previously observed in other crops (Jacoby and Ratner, 1974; Martinez-Rodriguez et al., 2008).

Chloride is an essential mineral element for plants and plays multiple roles in plant metabolism (Marschner, 1995). However, at high concentrations in soils, Cl^- becomes toxic to plants (Abel and Mackenzie, 1964; Bernstein, 1975; Marschner, 1995). All soybean genotypes, regardless the salt tolerance level, had a significant increase in LClc with increased [NaCl] in nutrient solution. Sensitive genotypes, however, accumulated significantly more Cl^- in leaves than tolerant genotypes. Similar observations were reported by Abel and MacKenzie (1964), Läuchli and Wieneke (1979), Grattan and Maas (1985), Essa (2002) and Luo et al. (2005). In this study, tolerant accessions showed rates of Cl^- accumulation in leaves at least 1.6 times lower than sensitive genotypes. The association between salt tolerance of plants and their ability to effectively exclude Cl^- from leaves was previously reported by several researchers (Abel and MacKenzie, 1964; Grattan and Mass, 1985; Wieneke and Läuchli, 1979).

This study also suggests that differences between tolerant and sensitive genotypes is not only related to the level of toxic ions transported and accumulated in leaves, a process primarily controlled by roots (Grattan and Maas, 1985; Wieneke and Läuchli, 1979; Martinez-Rodriguez et al., 2008), but also on the ability of particular genotypes to withstand these ions without developing injury, a process that is regulated by shoots. In these experiments, it was observed that leaf scorch injury in sensitive

genotypes was about twice as high as in tolerant ones per unit of Na^+ or Cl^- increase in leaves, showing a differential sensitivity of accessions to the accumulated ions. Shoot-regulated tolerance to toxic elements is a mechanism that was previously observed for Zn and Mn (Heenan and Carter, 1976; White et al., 1979). One of the mechanisms allowing tolerant genotypes to resist toxic ions in leaves could be enhanced intracellular compartmentalization. One piece of evidence supporting this idea comes from Li et al. (2006), who demonstrated that two tonoplast-located soybean transporters, GmCLC1 and GmNHX1, enhanced NaCl tolerance in tobacco cells. GmCLC1 and GmNHX1 had a protection effect against NaCl through the sequestration of cytoplasmic Cl^- and Na^+ into vacuoles, respectively.

Comparisons between Na^+- and Cl^--induced leaf scorch indicated that Na^+ in leaves was 2 to 3 times more harmful than Cl^- regardless of species and tolerance level to salinity. Also, the proportion of the variability in leaf scorch explained by LNac was much more important than that explained by LClc. Previous studies comparing relative toxicity of ionic components of NaCl are in partial agreement with these results in that Na^+ was more toxic than Cl^- in *G. soja* accessions, but the opposite situation was true in *G. max* genotypes (Luo et al., 2005). Disagreement between these studies could be due to differences in genotypes and experimental conditions.

K^+, Mg^{2+} and Ca^{2+} concentration in leaves

Leaf K^+ concentration (LKc) of genotypes showed no significant changes across [NaCl], so K^+ was not replaced by Na^+. In a study by Läuchli and Wieneke (1979), salinity increased K^+ content of soybean leaves in one experiment, but there was no variation in LKc in a second experiment. On the other hand, Essa (2002) observed that increased salinity resulted in a decrease in LKc of soybean genotypes. Differences found in the above studies regarding the response of K^+ concentration in leaves would indicate that LKc responds to factors other than just salinity. Sensitive *G. max* and *G. soja* genotypes tended to have higher LKc than their tolerant counterparts, but the differences were not significant; these results are in agreement with Grattan and Maas (1985) but disagree with Essa (2002). Tolerant line PI 441008 was among the highest in LKc, feature that was previously observed in *G. tomentella* species by Kao et al. (2006).

Leaf Mg^{2+} concentration (LMgc) remained unchanged across [NaCl], except in G. soja accessions, which had a significant decrease. The different response observed in *G. soja* accessions could be a result of the increase in Na^+ at the expense of Mg^{2+}. Magnesium competes with other cations for absorption sites (Bernstein and Hayward, 1958). No pattern was found between tolerance to salinity and LMgc since both sensitive and tolerant genotypes showed similar levels of Mg^{2+} in leaves at all [NaCl]. In the study by Essa (2002), there was a significant decrease in LMgc as salinity levels increased in the most sensitive accession. In the tolerant and moderately tolerant genotypes, Mg^{2+} decreased with increasing salinities, but, at the highest salinity level,

there was an increase, and LMgc was similar to the control plants. Nukaya et al. (1982) observed that increasing levels of NaCl resulted in significant increases in Mg^{2+} contents in leaves. However, LMgc decreased at the highest salt concentration to levels similar to the control.

Lynch and Läuchli (1985) suggested that high Na^{+} in substrate inhibit Ca^{2+} uptake and transport and may induce Ca^{2+} deficiency. On the other hand, Bernstein and Pearson (1956) as well as Lessani and Marschner (1978) found appreciable reduction in growth of plants when growing in saline substrate with no decrease in Ca^{2+} content. In this study, leaf Ca^{2+} concentration (LCac) of genotypes showed no significant changes across [NaCl], which indicates that Ca^{2+} was not replaced by Na^{+}. These results are in agreement with those presented by Nukaya et al. (1982) and Kao et al. (2006) but in disagreement with Essa (2002). The only differences observed in the present work were those due to the distinct leaf Ca^{2+} content of genotypes. Salt tolerance of genotypes was not correlated to LCac.

Na^{+}, Cl^{-}, K^{+}, Mg^{2+} and Ca^{2+} concentration in stems

Stem Na^{+} concentration (SNac) and Cl^{-} concentration (SClc) followed the same pattern as LNac and LClc, respectively, that is, there was a significant increase in SNac and SClc with increasing levels of NaCl in all genotypes. Tolerant accessions had a lower stem accumulation of Na^{+} and Cl^{-} than sensitive genotypes. However, the difference in

Na^+ and Cl^- concentration between tolerant and sensitive genotypes was more pronounced in leaves. Similar results were also observed by Luo et al. (2005), Läuchli and Wieneke (1979) and Abel and MacKenzie (1964).

Stem K^+ concentration (SKc), unlike LKc, showed significant decreases as NaCl in the growth media increased, which is in agreement with the results reported by Läuchli and Wieneke (1979). This phenomenon was observed in all genotypes, regardless species and tolerance level to salinity, and it indicates that plants were better able to maintain LKc than SKc. Since this pattern has occurred widely among species, SKc appears not to be a factor determining salt tolerance/sensitivity of genotypes.

As previously observed in leaves, Mg^{2+} concentration in stems (SMgc) was not significantly affected by increasing salinity levels. Genotypes showed significant differences in their SMgc, with tolerant genotypes showing higher Mg^{2+} concentrations than sensitive ones. No previous work has been reported on variation of SMgc as affected by increasing salt levels.

Stem Ca^{2+} concentration (SCac) showed no variation with increasing levels of salinity. Also, SCac was very similar among all genotypes. However, a trend was observed showing tolerant genotypes with higher SCac than sensitive ones, especially in *G. max* accessions. No previous work could be found showing behavior of SCac with increasing salt levels.

Na^+, Cl^-, K^+, Mg^{2+} and Ca^{2+} concentration in roots

Increases in NaCl in the nutrient solution resulted in significant increases in root Na^+ concentration (RNac) in all genotypes. In *G. max*, tolerant genotypes tended to show higher RNac than sensitive ones, but differences were not significant. Similar observations were reported by Läuchli and Wieneke (1979). Also, Dabuxilatu and Ikeda (2005) suggested that higher tolerance to Na^+ in soybean would be related to the retention of this ion in vacuoles of root cortical cells, making transport of Na^+ to leaves more difficult. *G. soja* accessions showed the same pattern as *G. max* although it was less clear. Tolerant *G. argyrea* line PI 595792 had the lowest RNac, while *G. tomentella* accession PI 441008 had intermediate RNac.

All genotypes showed a significant increase in root Cl^- concentration (RClc) as salinity increased and differences were observed among genotypes for RClc. In general, tolerant accessions accumulated more Cl^- in roots than sensitive ones. These results are in agreement with studies carried out by Wieneke and Läuchli (1979), Läuchli and Wieneke (1979) and Luo et al. (2005), but disagree with those presented by Abel and MacKenzie (1964), who did not find differences in RClc of sensitive and tolerant genotypes. Perennial accessions showed different patterns in RClc accumulation. PI 441008 (*G. tomentella*) had intermediate RClc (between that of tolerant and sensitive *G. max* accessions), while PI 595792 (*G. argyrea*) showed low Cl^- accumulation at the lowest NaCl levels, and the highest RClc at 100 mM NaCl among all accessions studied. The behavior of PI 595792 regarding changes in RClc associated to increasing levels of

salinity may be explained by reports showing genotypes with a semihalophytic trait, which behave as "includers" or "excluders" depending on the [NaCl] in the media (Martinez-Rodriguez et al., 2008; Perez-Alfocea et al., 1993, 1996; Tester and Davenport, 2003).

Increasing [NaCl] did not significantly affect root K^+ concentration (RKc). However, all genotypes except PI 595792, tended to decrease in RKc as salt concentration increased. Läuchli and Wieneke (1979) reported that RKc was lowered by NaCl in one experiment but remained unchanged in a second experiment, so our results are in partial agreement. PI 595792 showed a significant increase in RKc as salinity increased, and these results could be related to the suggested semihalophytic feature of this accession. The *G. soja* genotypes had the lowest RKc regardless their tolerance level, while PI 441008 (*G. tomentella*) and tolerant *G. max* genotypes had the highest RKc. RKc in PI 595792 was intermediate.

Behavior of root Mg^{2+} concentration (RMgc) was very similar to that of RKc. Increasing [NaCl] did not significantly affect RMgc of accessions. However, all genotypes showed a decreasing trend of RMgc as salinity increased. The exception was PI 595792, accession that had a non significant increasing trend. As previously observed in RKc, *G. soja* genotypes had the lowest RMgc, regardless their tolerance level, while PI 441008 (*G. tomentella*) and tolerant *G. max* genotypes had the highest RMgc.

Although differences were not significant, there was a trend showing tolerant *G. max* accessions with higher RKc and RMgc than sensitive ones. It is possible that the higher RKc and RMgc observed in tolerant genotypes helps them to maintain a better ionic balance as RNac increases with increased salinity.

Overall, salinity produced no consistent effect on root Ca^{2+} concentration (RCac) of genotypes. No differences in RCac were observed between tolerant and sensitive genotypes. PI 595792 (*G. argyrea*) was the only accession showing a significant decrease in RCac with increasing salt levels. It is possible that in this particular accession, the uptake of Na^{+} and especially K^{+} and Mg^{2+} increases at the expense of Ca^{2+}. Bernstein and Hayward (1958) concluded that ion toxicity due to Na^{+} and Ca^{2+} is different in glycophytes and halophytes: while Na^{+} is more toxic than Ca^{2+} in glycophytes, the opposite is observed in halophytes. Preferential absorption of Na^{+}, K^{+} and Mg^{2+} over Ca^{2+} could be a mechanism that PI 595792 has to prevent toxicity, which is another feature of its likely semihalophytic reaction to increased salinity.

Chlorophyll content

Chlorophyll content was significantly higher in salt tolerant genotypes than in sensitive ones, and the difference was especially evident with an increase in NaCl concentration. Several researchers have studied the effects of salinity on various

photosynthetic parameters. No report, however, has shown such large differences in chlorophyll content between salt tolerant and sensitive accessions.

Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon. Chen and Yu (2007) also observed a significant decrease in chlorophyll content at high NaCl. However, a recent report (Cicek and Cakirlar, 2008) has shown that the content of chlorophyll in soybean leaves was a function of the interaction between genotype, salinity and temperature, meaning that for a given genotype, chlorophyll content increases or decreases depending on salt level and temperature. In the same study, decreases in chlorophyll content were more related to increased temperature rather than to increased salinity. In our experiments, variations in chlorophyll content among various genotypes was the result of the [NaCl] x G x Y 3-way interaction, which is in agreement with Cicek and Cakirlar (2008).

In general, plant exposure to increasing levels of salt resulted in decreased chlorophyll content of sensitive accessions and increased chlorophyll content of tolerant accessions. In sensitive lines PI 424127A and Williams 82 the decrease in chlorophyll in leaves was significant, while in M23, the decreasing trend was not significant. Tolerant genotypes PI 441008, PI 595792 and S-100 showed an increase in chlorophyll content with higher [NaCl], but this increase was not always significant. In Fiskeby III and PI 468916, chlorophyll had a significant increase in year 1 but a significant decrease in year 2. The difference in leaf chlorophyll in Fiskeby III and PI 468916 in years 1 and 2 could

be associated with a higher sensitivity of these accessions to salinity under higher temperature as pointed out by Cicek and Cakirlar (2008).

Growth parameters

All annual accessions showed a negative correlation between leaf dry weight (Ldw) and [NaCl]; however, the decrease in leaf biomass was significant only in sensitive genotypes (PI 424127A, M23 and Williams 82). Tolerant genotypes Fiskeby III and S-100 had a slight increase in Ldw at 50 mM NaCl. The stimulating effect of low salt concentrations on growth and development of glycophytes is a phenomenon that was observed in other studies (Meloni et al., 2001; Cicek and Cakirlar 2002, 2008). Leaf dry weight of perennial genotypes PI 441008 and PI 595792 showed non-significant increases as salinity increased, which suggests a beneficial or neutral effect of NaCl on growth of these accessions. The high tolerance to salinity of some wild perennial soybeans was previously investigated (Hymowitz et al., 1987; Pantalone et al., 1997).

Annual *Glycine* species showed a decreasing trend in stem dry weight (Sdw) as [NaCl] increased, but it was significant only in sensitive M23 and Williams 82 genotypes. Sdw of PI 468916 appeared to be enhanced by some NaCl in the solution. The other annual accessions maintained Sdw close to control values, especially tolerant ones. As previously observed in leaves, Sdw in PI 441008 and PI 595792 tended to increase with exposure to higher levels of NaCl.

Root dry weight (Rdw) was not significantly affected by increasing salt levels. However, two patterns were observed. In annual species, regardless the tolerance level, there was a decreasing trend in Rdw as salinity increased, while Rdw in perennial species had an increasing trend suggesting again some NaCl-induced growth. Among annual genotypes, tolerant cultivars Fiskeby III and S-100 showed the smallest reductions in Rdw in response to higher salinity.

Salinity induced significant decreases in stem length (Slth) in sensitive accessions. On the other hand, in annual tolerant accessions, Slth remained unchanged although a non significant increase in Slth of S-100 suggests some growth stimulation. Finally, Slth in perennial tolerant genotype PI 595792 was significantly stimulated by increased NaCl concentrations.

In general, root length (Rlth) of genotypes was not significantly affected by increased salinity. The exception was genotype PI 595792, which had a significant increase in Rlth at 50 mM NaCl. Although no statistically significant, tolerant genotypes S-100 and PI 441008 tended to show an increase in Rlth as salinity increased.

The arresting effect of high NaCl concentrations on growth parameters of salt sensitive plants have been studied in many species and by many researchers. In our experiments, shoot growth was much more affected by salinity than root growth. Our results are in agreement with previous findings (Bernstein, 1975; Läuchli and Wieneke, 1979; Joly, 1989; Marschner, 1995; Essa, 2002; Cicek and Cakirlar, 2008).

Chapter VI

SUMMARY AND CONCLUDING REMARKS

Salinity is one of the major abiotic stresses that adversely impacts crop productivity and quality (Chinnusamy et al., 2005), affecting both irrigated and non-irrigated lands (FAO, 2008).

Although most of the salinity is natural, a significant proportion of cultivated land has become saline because of human-induced processes. If soil salinization keeps increasing at the actual rates, about 50% of the arable land will be affected by salinity by the year 2050 (Blumwald and Grover, 2006). The kind of changes needed in cropping systems to avoid and/or solve salinization is likely to be a long, expensive and difficult process (Munns et al., 2008). Development and utilization of salt tolerant crops and varieties is one of the most cost effective strategies for coping with soil salinity (Essa, 2002).

A better understanding of the physiological traits underlying differences in salt tolerance among cultivars of a crop species and among related species would be useful in the development of salt tolerant genotypes (Lauchli and Wieneke, 1979; Pantalone et al., 1997).

In these experiments, leaf scorch was a variable that effectively identified soybean plant response to salinity. Leaf scorch is a trait that can be easily and quickly determined by exposing roots of soybean plants in V3-V4 stage (Fehr and Caviness, 1977) to a salt solution. To accurately assess response of accessions to NaCl, it is important to monitor changes in temperature and humidity which can influence the response of genotypes. For example, Fiskeby III (maturity group 00) is an early maturing genotype adapted to the conditions of northern U.S. and southern Canada with tolerance to salt. If evaluations for salt tolerance of Fiskeby III are carried out under higher than optimum temperatures, leaf scorch would be much higher than at the temperatures to which Fiskeby III is adapted and, thus, the effect of other factors (e.g., heat stress) would be confounded with salt damage.

It has been consistently shown that tolerant genotypes have the ability to maintain Na^+ and Cl^- levels in shoots (i.e., stems and especially leaves) at significantly lower levels than sensitive genotypes. This is achieved by an exclusion mechanism controlled by the roots that limits transport of Na^+ and Cl^- to shoots. Sensitive genotypes accumulate damaging amounts of Na^+ and Cl^- in shoots through an inclusion mechanism which allows movement of these ions to the upper parts of the plant.

The tolerant reaction to salinity not only depends on the amount of Na^+ and Cl^- accumulated in leaves, but also on the capacity of the leaf tissue to tolerate phytotoxic levels of these ions. In this study, sensitive accessions not only accumulated more Na^+ and Cl^- in leaf tissues but also developed more leaf injury per unit of Na^+ and/or Cl^- in

the tissue. The higher sensitivity of susceptible genotypes leaf tissue could be the consequence of insufficient compartmentalization of these ions in vacuoles and greater sensitivity of cytoplasmic organelles to salt stress.

In these experiments, Na^+ was more toxic than Cl^- regardless of soybean species and tolerance level to salinity. In addition, Na^+ concentration in leaves explained a higher proportion of variation in leaf scorch than Cl^- concentration in leaves. Differences with previous studies could be attributed to several factors such as different phenological stage during exposure to NaCl stress, nutrient solution composition and genotypes.

Leaf concentrations of K^+, Mg^{2+} and Ca^{2+} did not vary significantly due to increases in NaCl concentration. This indicates that there was no substitution of any of those cations for Na^+. Previous studies show similar results, but there are also disagreements. The only species that showed a significant decrease in leaf Mg^{2+} concentration with NaCl was *G. soja* regardless of tolerance level to salinity.

In stems, Mg^{2+} and Ca^{2+} remained unaffected by increasing NaCl concentrations, and tolerant genotypes tended to have higher concentrations of these nutrient elements than sensitive ones. In our experiments and other studies, K^+ level in stems significantly decreased when NaCl concentrations increased in both sensitive and tolerant genotypes. It is possible that, when facing a situation of reduced K^+ uptake due

to competition with Na^+ (root K^+ concentration tended to decrease too, but the reduction was not significant) plants mobilize K^+ from stems into leaves to keep foliar K^+ levels adequate for vital functions.

In roots, Ca^{2+} remained unchanged, and all accessions tended to show similar levels. Both K^+ and Mg^{2+} tended to decrease as salinity increased, but this trend was not significant. Tolerant accessions tended to show higher levels of K^+ and Mg^{2+} in roots than sensitive ones. It is possible that the higher K^+ and Mg^{2+} concentrations in roots of tolerant genotypes help them maintain a better ion balance as Na^+ levels increase with increased salinity. K^+ and Mg^{2+} could be also acting in electrical balance as counterions of Cl^-. Tolerant PI 5959792 had completely different patterns in K^+, Mg^{2+} and Ca^{2+} concentration in roots compared to the other accessions, with significant increases in K^+ and Mg^{2+} and a decrease in Ca^{2+}. This could represent a mechanism of salt tolerance different from the other accessions. Significant reductions in Ca^{2+} to favor increases in the other cations could indicate a halophytic trait response to salinity since Ca^{2+} is toxic in halophytes (Bernstein and Hayward, 1958).

Chlorophyll content of genotypes was dependent on the interaction among genotypes, NaCl concentration and temperature. Despite the differential response of genotypes to various NaCl treatments under various environmental factors, tolerant genotypes had always higher chlorophyll content than sensitive ones in our experiments. It would be very interesting to correlate chlorophyll content and tolerance/sensitivity to salinity of a large number of genotypes. If significant and high

correlations between chlorophyll content and salt tolerance are found, this could represent the ideal screening method for breeders since chlorophyll meter readings are easy, reliable, cheap and quick to perform.

Salinity had a negative effect on dry matter accumulation although tolerant accessions were less affected than sensitive ones. Our experiments confirmed previous results indicating that shoot growth is more sensitive to salinity than root growth.

Overall, good levels of salt tolerance were found in *G. max* and *G. soja* species. Outstanding levels of tolerance to salinity were observed in *G. argyrea* and *G. tomentella* species, which showed no leaf injury and displayed growth stimulation at the NaCl concentrations evaluated.

Genetic improvement of soybeans for salt tolerance is technically feasible since there is intra and interspecies genetic variability. Incorporation of salt tolerant genes into elite material can be done with traditional hybridizations in crosses between *G. max* accessions or between *G. max* and *G. soja* accessions. Since crosses between perennial *Glycine* species and annual *Glycine* species do not yield fertile F_1 progeny (Hymowitz, 2004), molecular breeding techniques to introduce salt tolerant genes coming from perennial species would need to be employed.

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