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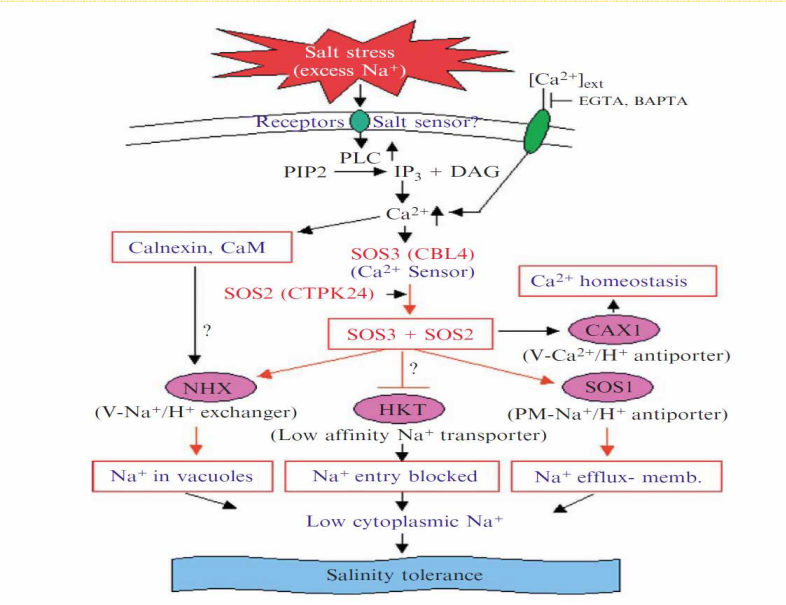
Analysis of ion content and expression of ion transporting genes in salt-stressed diploid CxE potato mapping population



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Analysis of ion content and expression of ion transporting genes in salt-stressed diploid CxE potato mapping population

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Table of contents

Acknowledgement ..... **V**
List of tables ..... **VI**
List of Figures ..... **VI**
List of appendices ..... **VII**
Summary ..... **VIII**
1. Introduction **1**
1.1 The entry of ions and physiology of salinity stress **2**
1.2 The pathway of salinity stress response **4**
1.3 Ion homeostasis for salinity tolerance **5**
1.4 Genetics of salinity tolerance **6**
2. Materials and Methods **8**
3. Results **14**
3.1 Frequency distribution of the genotypes **14**
3.2 Effect of salinity stress on AnCat content of the genotypes **16**
3.2.2 Effects of salinity on Anion content of the population **19**
3.3 Correlation analysis of the Ions **22**
3.4 Expression analysis of genes **30**
4. Discussion **33**
5. Conclusion **40**
6. Recommendation **41**
References **42**
Appendices **45**

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List of tables

Table 1. List of primers designed for amplification of candidate genes……..12

Table 2. The effect of salinity stress on cation content of leaf, stem and root…….17

Table 3. The effect of salinity stress on cation content of shoot and root……....18

Table 4. The effect of salinity stress on anion content of leaf, stem and root …… 20

Table 5. The effect of salinity stress on anion content shoot and root…… 21

Table 6. Correlation analysis between ion content of root under control ……...… 22

Table 7. Correlation analysis between ion content of stem under control……. 23

Table 8. Correlation analysis between ion content of leaf under control…… 24

Table 9. Correlation analysis between ion content of shoot under control……. 25

Table 10. Correlation analysis between ion content of root under NaCl …… 26

Table 11. Correlation analysis between ion content of stem under NaCl ……...28

Table 12. Correlation analysis between ion content of leaf under NaCl …….29

Table 13. Correlation analysis between ion content of shoot under NaCl……...30

List of Figures

Figure 1.The SOS pathway. 5

Figure 3. The Markers identified using the SOS1-3 primer pair. 31

List of appendices

Appendix 1. Formulas for ion content determination 45
Appendix 2. Correlation analysis between ion content of root and stem control 46
Appendix 3. Correlation analysis between ion content of leaf and root control 47
Appendix 4. Correlation analysis between ion content of stem and leaf control 48
Appendix 5. Correlation analysis between ion content of shoot and root control. 49
Appendix 6. Correlation analysis between ion content of root and stem salinity. 50
Appendix 7. Correlation analysis between ion content of leaf and root salinity. 51
Appendix 8. Correlation analysis between ion content of stem and leaf salinity. 52
Appendix 9. Correlation analysis between ion content of shoot and root salinity. 53
Appendix 10. Correlation analysis of root ions and growth parameters control. 54
Appendix 11. Correlation analysis of stem ions and growth parameters control. 55
Appendix 12. Correlation analysis of leaf ions and growth parameters control. 56
Appendix 13. Correlation analysis of root ions and growth parameters salinity. 57
Appendix 14. Correlation analysis of stem ions and growth parameters salinity 58
Appendix 15. Correlation analysis of leaf ions and growth parameters salinity. 59
Appendix 16. Testing uniqueness of SOS1-2 and SOS1-3 using cDNA 60
Appendix 17. Testing uniqueness of SOS1-2 and SOS1-3 using cDNA and DNA. 61
Appendix 18. List of the genotypes and lay out for identification of markers. 62

Summary

Salinity stress is a major abiotic stress effecting agricultural productivity of the world. The stress covers a large area in all climatic zones. Most of the existing crops are glycophytic; hence, improving salinity tolerance of economically significant crops is crucial. This project studied salinity-induced organ specific ionic changes and its relationship with the growth parameters. It also tried to analyze the expression of genes for ionic homeostasis in CxE diploid potato mapping population. Therefore, a total of ninety four CxE genotypes as well as parent C and parent E were grown under salinity (3 replications) and control (2 replications) condition. The harvested plant materials were pooled according to the genotype and treatment received; furthermore, each genotype was separated in to the root, stem and leaf. Eventually, ion content determination (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-}) on the organs was carried out using ion chromatography. Primers were designed, using the ESTs (expressed sequence tags) to quantify the expression of the candidate genes.

The frequency distribution of the genotypes showed a change in the position of the parents, shape of the distribution, and interval of values across treatments. The application of NaCl in to the growth medium has significantly increased the concentration of Na^+ (root, stem and leaf), K^+ (root), Mg^{2+} (stem), Ca^{2+} (stem), Cl^- (root, stem and leaf) and Na/K ratio (root, stem and leaf). However, the treatment significantly decreased the concentration of K^+ (stem and leaf), Mg^{2+} (root), and Ca^{2+} (leaf and root); whilst it did not show significant effect on Mg^{2+} (leaf). The correlation analyses showed that the interrelationship between the ions and ions with growth parameters was changing with the change in organs and growing condition. However, the experiment on the expression of genes for ion homeostasis was not successful to reveal the genetic evidence in ion transport of the CxE genotypes.

Although a statistical test was not carried out, the existing difference among the genotypes regarding their ion content might be attributed to the genetic variation with in the population. The moderately positive association observed between Na^+ and Cl^-, and similarity in the trend of changes in the root, stem and leaf after salt treatment might show the close relationship between these ions. The ionic changes and their relationship with the growth parameters might indicate that Na^+ compartmentation

and efflux were helping the genotypes more than the Na^+ exclusion strategy. However, according to the ionic changes and their relations with growth parameters; there might have been ionic toxicity. This might be due to the efficiency of the tolerance mechanism organized by the genotypes, or the treatment (120mM) was higher to be tolerated by the genotypes. The aim to get genetic evidence thorough expression analysis about the role of the ion transporters was failed. This might be attributed to poor primer design, the materials were not responding or optimum PCR reaction conditions were not met.

There is treatment dependent ion content variability among the genotypes and organs. The magnitude of salinity-induced change in ion content was dependent on the ion and the organ. Correlation between the ions showed differences across the treatment and organs. Expression analysis of genes for ion homeostasis is imperative to critically evaluate the salinity tolerance mechanisms organized by the CxE genotypes. Growing of the CxE population at different salinity levels, different environment (humidity and temperature), collecting (morphological, physiological and genetic) data on different stages of development, and optimization of the PCR reaction conditions can provide a concrete evidence to understand the mechanisms of salinity tolerance in CxE genotypes.

1. Introduction

Salinity stress is a major abiotic stress with prominent negative effects that dates back to ancient civilization. The stress continues to affect the current health, economic, social and environmental situation of the world. Rengasamy (2006) reported the existence of salinity problem in all climatic zones covering more than 100 countries. The problem occurs at different levels hampering crop production of these countries. Expansion of salinity problems is attributed to a multitude of factors: such as poor rainfall, rock weathering, wind transport, and poor irrigation (Chinnusamy and Zhu, 2003; Rengasamy, 2006). Whilst the arid and semi-arid regions of the world are prone to salinization, the majority of the irrigated land is also affected by salinity stress (Blumwald *et al*., 2004). Salinity stress restricts the use of cultivated and uncultivated land for crop production. The higher accumulation of salinity ions in soil water is deleterious to plant growth and development (reviewed in: Zhu (2001), Rengasamy (2006) and Munns and Tester (2008)). Therefore, salinity stress is greatly affecting the quality and productivity of most economically significant plant species in the world.

Due to the climate change increasing agricultural productivity through water and soil management has become problematic. Salinity also makes use of irrigation and land clearing not a practical solution to increase world crop production. Flowers (2004) indicated that the existing crops are generally non-tolerant to salinity stress. However, there is acute need for world food supply because of the alarmingly growing world population. Based on these facts developing salt stress tolerance varieties has supreme importance to boost world food production (Flowers and Flowers, 2005; Munns, 2005; Tuteja, 2007 and Sun *et al*., 2009). Thus, it is reasonable to put maximum effort to improve salinity tolerance capability of crops.

Being frequent trouble, plants evolve adaptation mechanisms to salinity stress. However, under this stress plants show differences in growth rate (Munns and Tester 2008). This reflects variability of salinity tolerance mechanisms among plants. Bartels and Sunkar (2005) reported that the variability arises from the differences in stress perception, signal transduction, gene expression programming and alteration of metabolic pathways. Blumwald *et al*., (2004) deduced that these differences have genetic basis. However, use of conventional plant breeding to exploit the existing genetic variation for crop salinity tolerance improvement was not fruitful. This is attributed to the genetic and physiological complexity of the trait (Flowers, 2004; Flowers and Flowers, 2005). On top of this, poor experimental design also contributes to the previous failures (Munns and Tester 2008; Blumwald *et al*., 2004).

The biochemistry, gene transcription, physiological and morphological functions of a plant responds to the perceived salinity stress (Zhu, 2001; Munns and Tester, 2008). Ma *et al*., (2006) and Tuteja (2007) also described the complexity of the salinity tolerance signaling pathway and the cross-talks with biotic and abiotic stresses. Hence, it is difficult to understand the molecular basis of salinity tolerance mechanisms in spite of technological advancements in molecular laboratory (Blumwald *et al*., 2004). Nevertheless, understanding the molecular backgrounds of the trait is invaluable to develop salinity tolerant varieties. To achieve this continuous genetic, physiological and biochemical dissection of the trait can set the right foundation (Chinnusamy and Zhu, 2003). In line with genome sequencing projects, the salinity tolerance mechanism dissection will contribute to crop salinity tolerance improvement programs using suitable plant breeding technologies.

1.1 The entry of ions and physiology of salinity stress

Entry of ions

Saline soil contains electrical conductivity (ECe > 4dS m^{-1}) in their saturation extract (Seeling, 2000: based on soil classification system of U.S. salinity laboratory staff, 1954). The soil water soluble salt is reported to be dominated by NaCl (Munns and Tester, 2008). Under salinity condition, ions of the soluble salt enter and are transported inside the plant together with the water. Building up of salt in the apoplast causes dehydration of the cell, in the cytoplasm it inhibits enzyme activity, and in the chloroplast it affects photosynthetic activity of plants (Munns and Tester, 2008).

The plasma membrane of the cells is impermeable to large molecules including ions. However, integral proteins embedded in the lipid bilayer of the membrane form channels and carriers. These channels and carriers control uptake of ions from the soil and transport it in the plant (Munns, 2005). Excessive accumulation of the soluble salt ions (eg, Na^+ and Cl^-) in the apoplastic pathway creates potential gradient across plasma membrane of the cell (Sun *et al*., 2009). Flowers and Flowers (2005) reported that this potential gradient allows the passive movement of ions in to the cytoplasm through the channels. Moreover, Munns (2005) described the existence of active transport ions through carriers.

Chinnusamy and Zhu, (2003) reported that the ions (Na^+ and Cl^-) in the apoplastic pathway can penetrate the hydration shells of proteins. The disruption of non-covalent bond of amino acids causes loss of protein function. This allows passage of toxic ions through plasma membrane. The similarity among ions and existence of nonselective cation channels also

facilitates the leakage of toxic ions into cytosol (Bartels and Sunkar, 2005; Sun *et al*., 2009). Plant growth and development is a result of integrated physiological processes subjected to environmental effects. Therefore, entry of the soluble salts into cytosol of the cell collapses the physiological processes of the plant.

Effects of salinity stress and the responses by plants

Initially salinity stress imposes osmotic stress that restricts the plant's ability to take up water and nutrients from the soil solution. This osmotic stress triggers diversion of some metabolic pathways (Yokoi *et al*., 2002; Parida and Das, 2005). Thus, plants respond to this stress by synthesis of compatible solutes (Zhu, 2001; Chinnusamy and Zhu, 2003; Munns, 2005). The solutes are important for regulation of osmotic homeostasis, stabilizing membrane proteins, and maintaining plant growth during saline condition. However, if the osmotic homeostasis is disrupted reactive oxygen species (ROS) are generated. As a result, enzymes for antioxidant production are activated (Zhu, 2001; Munns, 2005; Roychoudhury *et al*., 2008). If ROS production exceeds the plant's scavenging capability the ROS causes lipid peroxidation, protein oxidation, enzyme inhibition, and DNA and RNA damages (Tuteja, 2007).

High salinity levels that result in accumulation of high Na^+ and Cl^- in the cytosol causes ionic toxicity. The ionic toxicity affect cell metabolism, inhibit essential enzymes, disrupt cell division and expansion, and collapse membrane organization (Zhu, 2001; Parida and Das, 2005; Tuteja, 2007; Munns and Tester, 2008; and Roychoudhury *et al*., 2008). It also affects photosynthetic capability of the plant (Mahajan *et al*., 2008). Thus, salinity stress causes growth inhibition or even death of plants. The detoxification of ROS, re-establishing of both ionic and osmotic homeostasis as well as growth regulation are the primary mechanisms of salinity tolerance (Zhu, 2001; Chinnusamy and Zhu, 2003; Blumwald *et al*., 2004; and Munns and Tester, 2008). Thus, plants try to get rid of the salinity (Na^+) toxicity by adapting ion homeostasis using Na^+ exclusion, Na^+ compartmentation or Na^+ efflux (Bartels and Sunkar, 2005; Munns and Tester, 2008;and Sun *et al*., 2009). Compartmentation of Na^+ and Cl^- in to vacuoles has osmotic adjustment value (Yokoi *et al*., 2002). The compatible solutes produced to overcome osmotic stress may also reduce toxic effects of the ions (Munns, 2005). This shows overlapping of the response to osmotic and ionic phases of salinity tolerance. The effects of salinity stress and plant response mechanisms indicates the pivotal role of the proteins in the plasma membrane. The activity of these proteins is at least partly modified by the genetic basis of the plant. Hence, it is worth to understand the signaling cascade of salinity

stress i.e., perception of stress signal, signal transduction, and regulation of gene expression to produce proteins that may confer salinity tolerance.

1.2 The pathway of salinity stress response

Sensing the salinity stress

The external ionic and osmotic stress is at first sensed by the root cells. Then the signal transduction is partly mediated by ABA to make the shoot part alert about the existing problem (Munns and Tester, 2008). The hyperosmolarity and ion-specific signals of salt stress on apoplastic pathway, such as signals from Na^+ are sensed by membrane receptors and/or Na^+ sensitive cytosolic enzymes (Chinnusamy and Zhu, 2003). Rensink *et al*., (2005) and Tuteja (2007) described that the membrane receptors (G-protein-coupled receptors, ion channels, receptor like kinase, or histidine kinase) in turn generate secondary signaling molecules (Ca^{2+}, inositol phosphates, ROS, and ABA). These secondary signaling molecules enhance the stress signal transduction pathway (Parida and Das, 2005). The externally applied Ca^{2+} that result in the increment of cytosolic Ca^{2+} involves in ion homeostasis through activation of calmodulin (Yokoi *et al*., 2002). The key role of Ca^{2+} in the signaling web towards salinity stress is also described by Tuteja (2007). Therefore, the stress signal perceived on the membrane receptors is transduced through the secondary signaling molecules. This signal information is decoded downstream and activates the transcription factors that regulate expression of salt stress responsive genes (Mahajan *et al*., 2008). The activation of the salt responsive genes produces proteins that act in different stages of salinity tolerance pathway.

The SOS pathway

The SOS pathway plays central role in salinity tolerance of plants, and is reviewed by Bartels and Sunkar, (2005); Munns and Tester (2008) and Mahajan *et al*., (2008). The high Na^+ accumulation around the root zone and apoplastic pathway induce cytoplasmic Ca^{2+}, which is sensed by the calcineurin B-like protein (CBL4)/SOS3. SOS3 is dimerized with CBL interacting protein kinase (CIPK24)/SOS2, and create a kinase complex. The SOS2-SOS3 kinase complex is also connected with the plasma membrane by myristoyl fatty acid chain. This complex regulates activity of the plasma membrane bound (Na^+/H^+ antiporter) SOS1. Moreover, it activates the tonoplast Na^+/H^+ antiporter (NHX) and inhibits plasma membrane low affinity Na^+ transporter (HKT) (Chinnusamy and Zhu, 2003). The SOS2 may also interact with CAX1 and other calcium binding proteins (calnexin) (Tuteja, 2007). The CAX1 can also

activate NHX, which indicates the crosstalk of the SOS pathway with others to maintain cellular homeostasis (Figure 1). Plants tolerate high salinity effects by avoidance or carry out osmotic and ionic adjustments. The SOS- signaling pathway together with Ca^{2+} is pivotal regulator of Na^+ and K^+ homeostasis in salinity tolerance of plants (Yokoi *et al*., 2002).

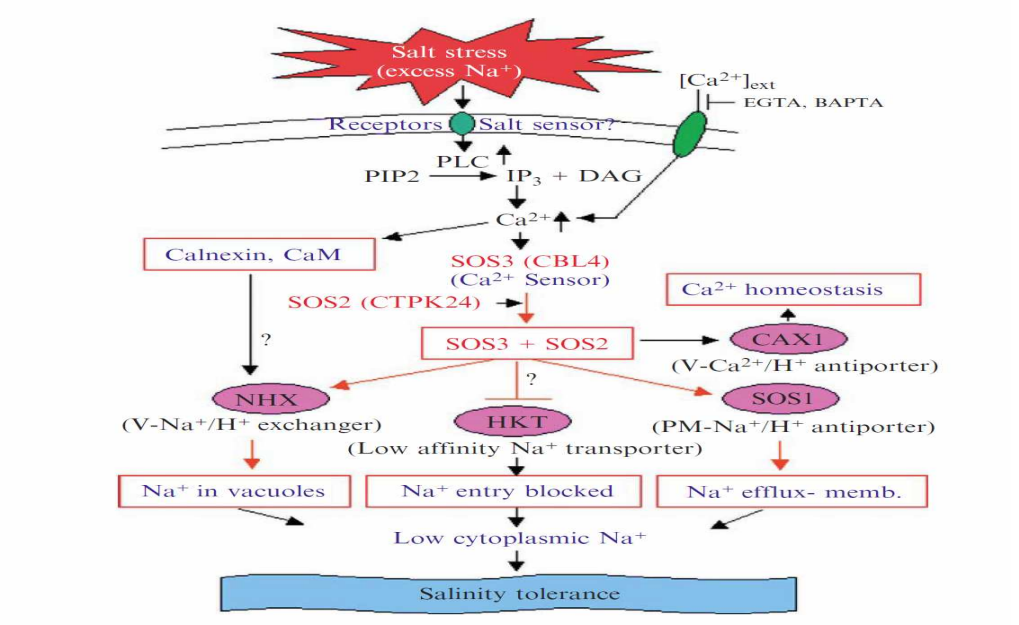

Figure 1.The SOS pathway 'A key player of salinity tolerance mechanism in plants' (Source: Tuteja, 2007).

1.3 Ion homeostasis for salinity tolerance

Intracellular ion homeostasis is vital for survival of living cells: it is a mechanism to keep in check the concentration of toxic ions for proper functioning of cellular machinery. Ion homeostasis is maintained by coordinated action of various pumps, ions, and Ca^+ sensors. The regulation of ion transport is essential for ion homeostasis (Zhu, 2001; Bartels and Sunkar, 2005). Thus, ion transporters are the key determinants in restoring the ionic homeostasis (Chinnusamy and Zhu, 2003).

Potassium (K^+) is one of the most essential elements required for plant growth and development. It plays crucial role in metabolism, growth, enzyme activation, and protein synthesis (Mahajan *et al*., 2008). For this reason, under normal and stress condition maintaining high K^+/Na^+ ratio is pivotal for plant survival (Chinnusamy and Zhu, 2003; Tuteja, 2007). Hence, plants try to exclude the excess entry of Na^+ in to cytosol. However, Na^+ can leak into the cytosol through K^+ selective channels (HKT), K^+ outward rectifying channels, and nonselective cation channels (Yokoi *et al*., 2002; Bartels and Sunkar, 2005; Tuteja, 2007). Therefore, plants either efflux the excess Na^+ in cytosol through plasma

membrane antiporter (SOS1) or compartmentalize it in to vacuoles through tonoplast antiporter (NHX).

Protons (H^+) are used as coupling ions for ion transport systems in the plasma membrane and tonoplast (Bartels and Sunkar, 2005; Yokoi *et al.*, 2002). Proton pumps such as P- H^+-ATPase in plasma membrane as well as the V-H^+-ATPase and H^+-PPase on the tonoplast provide energy (reviewed in Yokoi *et al.*, 2002; and Bartels and Sunkar, 2005). This energy is needed for the movement of ions across the membranes to keep ion homeostasis. The genetic determinants of salinity tolerance are the fundamentals of cellular ion homeostasis (Yokoi *et al.*, 2002; Blumwald *et al.*, 2004). The extensive genetic diversity and common molecular entities of ion homeostasis for salinity tolerance is a basic resource to dissect the trait.

1.4 Genetics of salinity tolerance

Salinity stress tolerance or susceptibility of plants is a coordinated action of many genes (Mahajan *et al.*, 2008). Salt stress inducible genes were detected by making use of molecular analysis such as investigation of transcript regulation with microarrays of a hypersensitivity assay in *Arabidopsis* (Parida and Das, 2005). Munns (2005) classified these genes for salinity tolerance into three functional groups i.e., genes for ion transport, osmotic adjustment and growth enhancers. Moreover, the product of these salt induced genes are involved in direct cellular protection (LEA proteins, antioxidants), or indirect protection (transcription factors), and in generation of regulatory molecules (ABA, salicylic acid and ethylene) (Tuteja, 2007).

Salinity stress may induce or suppress a number of genes that regulate the activity of ion transporters (Munns, 2005). The HKT gene family is involved in Na^+ exclusion, NHX1 in compartmentation of Na^+ in to vacuole, and SOS1 for Na^+ efflux (Munns and Tester, 2008). The expression of AtNHX1 gene in root hairs and guard cells of *Arabidopsis thaliana* used for PH regulation and K^+ homeostasis during salinity stress (Parida and Das, 2005). The upregulation of K^+ channels and down regulation of nonselective cation channels also occurs under salinity tolerance mechanism (Munns, 2005). SOS4 gene was identified as a gene that encodes pyridoxal (PL) kinase for biosynthesis of vitamin B6, and the SOS5 gene seems to be important for normal growth and development of plants under salinity stress (Mahajan *et al.*, 2008). Although the central role of these genes and their products in salinity stress tolerance is confirmed, detailed studies are required on the genes to unravel the complexity of the trait (Tuteja, 2007). Studies on expression analysis of the genes can potentiate the existing knowledge to choose the key genes for crop improvement programmes (Dorothea and Sunkar,

2005; Flowers and Flowers, 2005). Until now, lack of understanding about the function of candidate genes at cellular, tissue, organ and plant level has hampered their application in crop improvement (Munns, 2005). Moreover, knowledge about the crop under study is also imperative to understand its significance and basics to access the potentials.

1.5 The Potato

Potato (*Solanum tuberosum*) belongs to economically significant *Solanacea* family; is a starch-accumulating tuberous crop with a tetraploid genome that contains 48 chromosomes. According to the FAOSTAT data of 2007, the world potato production is 325.30 million tonnes (http://www.potato2008.org). This figure indicates the significant share of potato in world food production. The ploidy reduction from tetraploid to diploid strengthens the model role of potato in genetic studies (Gebhardt, 2004). Despite of its wide importance potato is very susceptible to high NaCl levels (Hmida-Sayari *et al*., 2005). Backhausen *et al*., (2005) also reviewed findings of field experiments on potato that reveals the glycophytic nature of the plant.

The significant impacts of salinity stress on food production and the means to tackle it have been described. The complexity of plant's salinity tolerance mechanisms and the difficulties to understand the trait is well documented. Ways to unravel the trait and its use for future crop improvement programmes is unanimously suggested. Moreover, the world wide importance of potato, its potentials, and the salinity effects on the crop is indicated. Therefore, the current study was carried out on diploid potato mapping population (CxE) to:

- Determine the cation and anion content in the root, leaf, stem and shoot of the CxE population. Moreover, to correlate the ionic changes caused by salinity stress with physiological and morphological components of the plant

- Analyzing the expression level of potato candidate gene families for salinity stress tolerance (SOS, HKT1, NHX). Although this objective was not successful, it was designed to get evidence for the importance of the ion transporters in reestablishing the ionic homeostasis in CxE population.

2. Materials and Methods

2.1 Experiment I

2.1.1 Genetic background of CxE population

The plant materials were obtained from the diploid potato backcross population consisting of 238 genotypes from a cross between C (USW5337.3; Hanneman RE, 1967) and E (77.2102.37; Jacobsen, 1980). Parent C is a cross between the *S. phureja* (PI225696.1) and *S. tuberosum* dihaploid (USW42); where as, parent E is derived from a cross between C and the *S. vernei-S tuberosum* backcross clone VH3-421 (Jacobsen, 1978).

2.1.2 The CxE population plant material

A total of ninety four CxE genotypes as well as parent C and parent E were used for this experiment. Plants were propagated in vitro culture, and axillary shoots were grown for two weeks on a standard MS (Murashige and Skoog) medium. After two weeks, the plantlets were transferred to the hydroponics system in green house under 18/15.6°C day/night temperature, 16 h day length and 60 /80% day/night RH (the green house condition was changing with the change in the environment). The growth medium in hydroponics had a nutrient solution (EC of 2.1 mS/cm and a pH of 5.7) of cations K^+ 7.9, Ca^{2+} 3.9, Mg^{2+} 1.6, NH_4^+ 0.6 and Na^+ 0.4 (mM); anions NO_3^- 11.0, SO_4^{2-} 2.9, PO_4^{3-} 1.94, HCO_3^- 0.4 and Cl^- 0.3 (mM); and micro nutrients Fe 24, Mn 12, B 9.8, Zn 4.4, Cu 0.7 and Mo 0.3 (µM) and Si 0.02 (mM). The plants were grown in a Randomized Complete Block Design (RBCD) with three replications of NaCl treated and two replications of control (with only standard nutrient solution) to evaluate the response of the genotypes to salinity stress. After growing for two weeks in hydroponics, the treatment (120mM of NaCl) was applied in two steps (Marcel van Culemborg, personal communication).

The Plant height, Chlorophyll content, Chlorophyll fluorescence, shoot length (cm), root length (cm), leaf area (cm²), shoot fresh weight (gm), root fresh weight (gm), shoot dry weight and root dry weight (gm); were measured and the whole plants of each genotype were harvested. The plants were harvested and pooled based on their genotype and treatment they received; then dried. These materials were used for organ specific cation and anion content (hereafter, AnCat) determination in the CxE population.

2.1.3 Ion determination

Optimization

Prior to the actual ion determination, an optimization experiment was carried out to determine: the dissolvability of the sample size, dilution factor to be used, and to recalculate the retention time of the cations and anions. Thus, a total of fifteen genotypes from the NaCl treated samples were selected because they had sufficient amount of sample for the actual analysis. Three amounts (20, 25 and 30mg) of leaf, stem and root samples of the selected genotypes under two dilution factors (100X, 1000X) were tested. A better dissolvability was observed in sample size of 25mg leaf, 25mg stem and 20mg of root. Moreover, the retention time of the cations and anions was recalculated from the output of the standards. As a result, retention time of cations Na^+ 3.67 , K^+ 5.03, Mg^{2+} 9.80 , Ca^{2+} 12..23 (min); and anions Cl^- 4.90, PO_4^{3-} 11.3 and SO_4^{2-} 13.44 (min), was stored in the MagIC Net™ software to be used for the ion content determination. All the samples were re-run, and 1000X dilution was chosen because of the consistency, reliability and less missing values of the output. (For the detailed procedure refer to sample preparation).

Sample preparation

The dried plant material of each genotype (pool of replications) was separated in to leaf, stem and root for organ specific ion content determination using Ion Chromatography (hereafter, IC). The plant material was grinded and the powder was placed in plastic containers labeled with the treatment, genotype name, and type of organ. Then a sample size of 25mg of leaf, 25mg of stem and 20mg of root of each genotype was weighed and put in a labeled glass tube.

The glass tubes containing samples were uncapped. The temperature of the ash oven (carbolite, type CWF 11/23) was set to min/max (28/575^{o}C) temperature. Using special racks, the samples were put in a known order at 28^{o}C ash oven. After starting the ash oven reached the maximum temperature of (575^{o}C) after 45min; and the ashing process continued for 5hrs. After stopping of the ash oven it took 1hr to cool down to the minimum temperature (28^{o}C). The glass tubes were relabeled. Afterwards, 1ml of 3M formic acid (hereafter, CH_2O_2) was added to the samples shaken in 99^{o}C for 15min and cooled down. Then the samples were diluted with 9ml of miliQ, followed by vortexing. Out of each sample 100µl was taken in to other labeled plastic tubes (designed for the IC), then diluted 100x using 9.9ml of miliQ and mixed using vortex (hereafter, the samples are called analytes). The analytes were ready for ion content determination using IC. The use of ion chromatography to explore ion relations at

organ and cellular levels through determination of static ion concentration is reviewed in Sun *et al*., (2009).

2.1.4 Ion content determination using IC

The IC equipment of Metrohm (www.metrohm.com); was used to determine the amount of AnCat in the leaf, stem and root of the genotypes. A single organ of the whole population from either control or salt treated condition was run at a time. Therefore, there were six runs i.e., three organs at two treatments.

The analytes of leaf, stem and root of the whole population grown under control and salinity condition were prepared immediately before each run. In addition to the 96 samples in each run blank samples size of 10ml (1000X diluted 3mM CH_2O_2), as well as anion and cation standard analytes (10ml) (in 1X dilution) were included. The blanks were prepared by taking 1ml of 3mM CH_2O_2 in too glass tubes and 9ml of miliQ was added in to it followed by shaking for 15min. The blank sample was cooled down and 100µl was taken out of it in to the plastic tubes (designed for IC) then diluted 100X.

Two types of anion and cation standards were used; the self made (hereafter, SM) and Flukas (hereafter, F®). The anion SM and cation SM were prepared in the Biochemistry Laboratory of Plant breeding, Wageningen UR in May 2009. The anion (F®), Br^- 10.05, Cl^- 10, F^- 10, NO_3^- 10, PO_4^{3-} 10, SO_4^{-2} 10.02 (mg kg^{-1}); and cation (F®), Ca^{2+} 10, Li^+ 10, Mg^{2+} 10.01, K^+ 10, Na^+ 10 (mg kg^{-1}) were obtained from Fluka®*analytical* a brand of SIGMA-ALDRICH®. Therefore, five anion SM (2, 4, 6, 8 and 10ppm), five cation SM (2, 4, 6, 8 and 10ppm), five anion F® (1, 3, 5, 7 and 9ppm), and five cation F® (1, 3, 5, 7 and 9ppm) were used for determining the sensitivity of the IC during each specific run, for all cations and anions analyzed.

The analytes including the blanks and standards were placed in the *858 professional sample processor*; by creating a working table on MagIC Net™ software. On the working table of the software the method to be used (AnCat), type of sample, sample name, position on the *sample processor*, number of samples to be injected (1) and amount of sample to be injected (20µl), and dilution factors of the analytes were filled and saved. Simultaneously, the whole devices of the IC were initialized for standardization to have an optimized isocratic eluent of 2mM Na_2CO_3 - $NaHCO_3$ and 3mM HNO_3 in the anion column (*881 compact IC pro 1*, *type* -

Metrosep A supp 4 250/4.0) and in the cation column (*881 compact IC pro 2, type-Metrosep C 4 150/4.0*) to detect the anions (Cl^-, SO_4^{2-} and PO_4^{3-}) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) respectively. Finally, the AnCat in the analyte of each genotype, in the blank and in the standards was determined using IC with an average sensitivity of Na^+(75.2%), K^+(78.70%), Mg^{2+}(80.60%), Ca^{2+}(75.22%), Cl^-(94.48%), PO_4^{3-}(103.13%), SO_4^{2-} (82.24%). The concentration (in ppm) was calculated within 19min for each, and the out put was automatically saved in the database of the MagIC Net™ software.

2.1.5 Data handling and analysis

The concentration of AnCat of each analyte was exported from MagIC Net™ software to an excel file, and summarized. Thus, the recovery (sensitivity of IC) percentage (R_i) and average recovery percentage ($\overline{R}_i$) of AnCat from SM and F® standards; and average AnCat concentration of blanks ($\bar{b}_i$) (in ppm) were calculated. Concentration of the AnCat for each genotype in mg/sample size (Y_i) and concentration of AnCat of each genotype in mg/gram of plant organ (G_i) was also calculated for the subsequent data analysis (Appendix I).

The data was organized and subjected to statistical analysis using GenStat® 12th edition. Frequency distribution was plotted to investigate the differences among genotypes, the distribution of the population for a given ion content and to locate the position of the parents on the distribution. One way and Two-way ANOVA were used to determine the significance ($P < 0.05$) differences among the organs for their AnCat content and to see if the organ differences is dependent on the treatment received. Moreover, the correlation analysis among the ions, ion content with growth parameters measured at harvest, and with growth parameters in different days was done. These analyses were used to establish possible relationship between the treatment, ion content, and growth parameters of the CxE diploid potato population.

Experiment II

2.2.1 Searching Candidate genes

Candidate genes important for salinity tolerance mechanism in potato (SOS1, SOS2, SOS3, HKT1 and NHX) were obtained from the online genetic database NCBI (http://www.ncbi.nlm.nih.gov/gene; accessed on Nov, 2009). The mRNA, complete coding sequence (CDS) of these genes were found from *Solanum lycopersicum* and *Arabidopsis*

thaliana. The FASTA format of selected CDS were blasted on TIGR under the programme BLASTN (http://blast.jcvi.org/euk-blast/plantta_blast.cgi), to find the existing ESTs (expression sequence tags) of potato which can represent the homolog gene of interest. Plant transcript assembly sequences (TA) of potato producing high-scoring segment pairs with the blasted CDS were displayed. The BLASTX and BLASTN were used to avoid the sequences (TA) that have weak protein similarities to the CDS, and further screening was done based on the percentage of sequence similarity. For further confirmation, the selected (TA) were blasted in NCBI, TBLASTX to search for its existence in the translated nucleotide database. Then the selected (TA) of the potato homolog gene was used for qRT-PCR primer design.

2.2.2 Primer design and testing

Primers were designed (Table 13), using GeneStar and manually (ClustalX 2.0.12). The length of the primer, the size of the amplicon, GC content, melting temp, annealing temp were taken in to consideration. Finally, primer pairs supposed to be unique to the genes of interest were selected. The selected primer pairs were obtained from the Biologio BV (http://www.biolegio.com/).

Table 1. List of primers designed for unique amplification of candidate genes for salinity tolerance of potato

Primer name	Sequence	Amplicon size bp[1]	Annealing temp	
			Basic Tm	Salt adjusted
HKT1 (old)-F	5' (GCCGGTTATGACGGCCACTG) 3'	275	58	53
HKT1(old)-R	5' (CTGCACGAAGCCACACTCT) 3'	275	53	48
Ef1αF*	5' (ATTGGAAACGGATATGCTCCA) 3'	101	50	45
Ef1αR*	5' (TCCTTACCTGAACGCCTGTCA) 3'	101	54	49
SOS1-1F	5' (TCTGTGTTGCGGAAGTTTTGT) 3'	111	50	45
SOS1-1R	5' (TTTTCTGTTGGAGGGATTTGT) 3'	111	49	43
SOS1-2F	5' (CAAAACTTCCGCAACACAGA) 3'	164	50	45
SOS1-2R	5' (TGCGATAATAGCGAAGACGA) 3'	164	50	45
SOS1-3F	5' (CACAGTCGGGGTTATCAAAA) 3'	224	50	45
SOS1-3R	5' (TCGCCACCAGAATCATCAC) 3'	224	51	46
HKT1-F	5' (TATATGTCCTAGCCTTGGTG) 3'	104	50	45
HKT1-R	5' (AGAATTCTGCTCCGCTACTG) 3'	104	52	47

*House keeping gene (Nicot *et al.*, 2005), [1]base pairs

2.2.3 PCR and qRT-PCR reactions

RNA from the leaf, stem and root of the genotypes was extracted and cDNA was synthesized. The PCR mix contains (2µl template, 2µl buffer (10x), 0.3µl of forward primer (10µM) and 0.3µl of reverse primer (10µM), 0.8µl dNTPs (10mM), 0.08µl Taq polymerase and 14.44µl miliQ). qRT-PCR with Biorad mix has been used for quantification of expressed genes. The concentration of DNA has been measured using spectrophotometer and diluted in to 0.05µg/µl;

and 380µl of miliQ water was added to 20µl of the diluted cDNA. Per reaction in duplo 22.5µl iQ™ SYBR green supermix (Biorad), 4.5µl of forward primer (3µM), 4.5µl of reverse primer (3µM) and 4.5µl of miliQ was added to prepare the Biorad mix. The primer master mix was prepared from 36µl per duplo and 9µl of the 20x diluted cDNA. Finally, per reaction 20µl of the mastermix was used. The cycling conditions of the qRT-PCR were; 95^{o}C for 3min, 40cycles of 95^{o}C for 15sec and 60^{o}C for 1min, and 65^{o}C for 1min.

2.2.4 Optimization trial

For this experiment the primers were designed based on the homolog gene ESTs region (TA) which is specific even to the homolog gene copies. However, for the successful reactions in the actual amplification, and expression analysis of the potato homolog genes using qRT-PCR; the primers were tested for their uniqueness, product length and optimum working PCR conditions. Hence, the selected primer pairs were tested at different PCR-program (40 cycles were used), 1.5% gel electrophoresis at 70MPV running speed. On the gel 5µl of PCR product was loaded with 2µl of the loading buffer. The gel picture was analyzed visually in reference to the 1000kb ladder to determine the size of the PCR product.

3. Results

3.1 Frequency distribution of the genotypes

The number of genotypes in an interval of the range of data value and the position of the parents (C & E) in the frequency distribution is presented. The distribution of a specific ion across treatments show a change in the range of values, shape of the frequency distribution, and exchange in the position of the parents. Moreover, the frequency distribution of the ions with in the same growing condition showed variability (Figure 2a and 2b).

Under control condition, parent 'C' has lower concentration of Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-} content, and Na/K ratio; and higher K^{+}, PO_4^{3-}, and SO_4^{2-} content than parent 'E'. Where as, under salinity condition except for Ca^{2+} parent 'C' has higher ion content than parent 'E'. In both treatments the parents are mostly laid on a range of value with higher number of genotypes. However, the parents are located in different groups of data value except for the PO_4^{3-} under salt treated condition. Most of the distributions show a unimodal nature that tends to be normal distribution although there are some skewed distributions, to the right (Fig 2a and 2b).

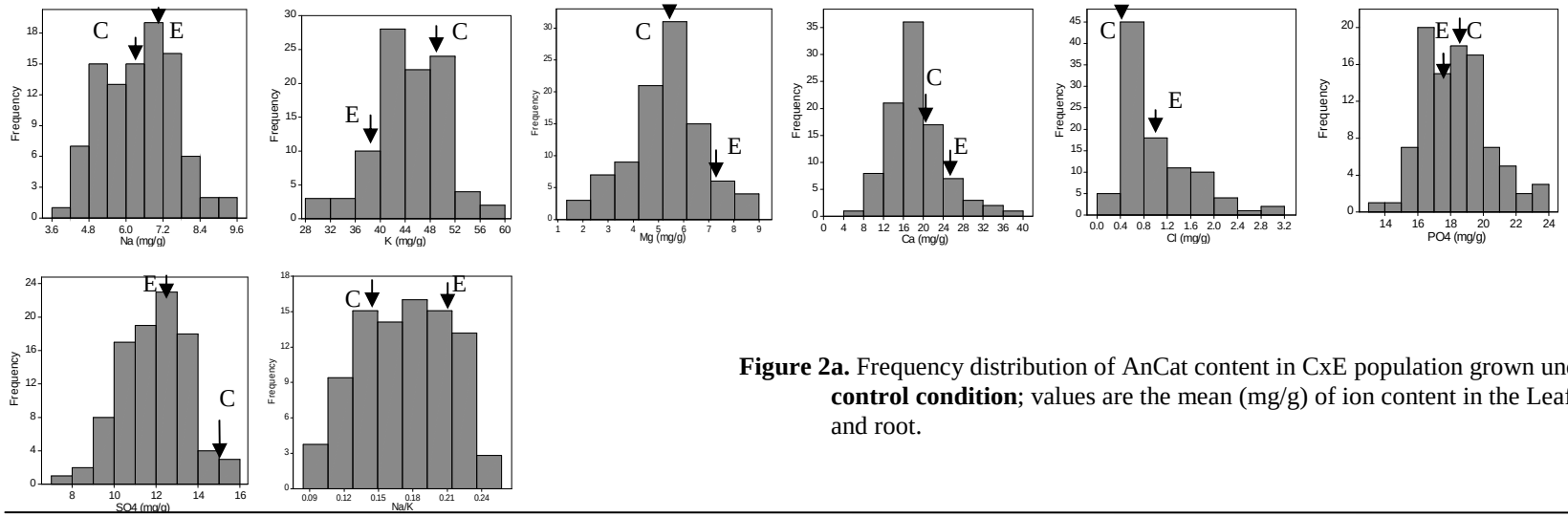

Figure 2a. Frequency distribution of AnCat content in CxE population grown under **control condition**; values are the mean (mg/g) of ion content in the Leaf, stem and root.

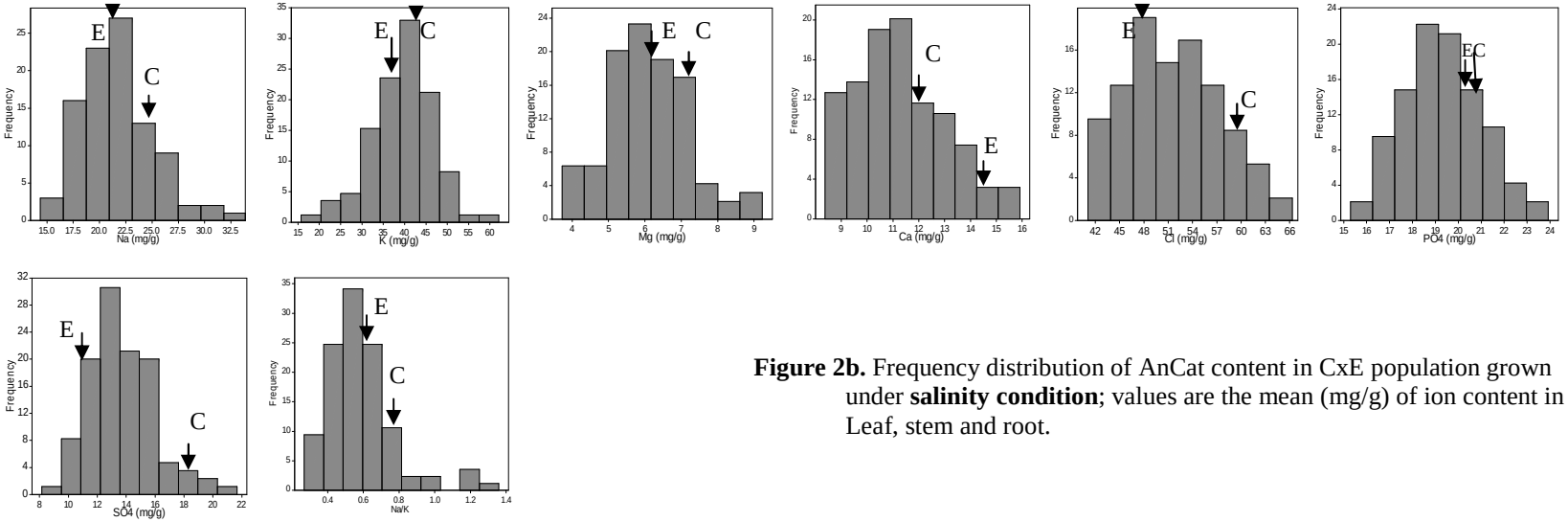

Figure 2b. Frequency distribution of AnCat content in CxE population grown under **salinity condition**; values are the mean (mg/g) of ion content in the Leaf, stem and root.

3.2 Effect of salinity stress on AnCat content of the genotypes

3.2.1 Effects of salinity on Cation content of the population

Root Stem, and Leaf

The result in table 2, shows a significant (P<0.001) interaction effect of organ and treatment on Na^+, K^+, Mg^{2+}, Ca^{2+} content, and Na/K ratio in the genotypes. There is a significant (P<0.001) treatment effect on the Na^+, K^+, Mg^{2+}, Ca^{2+} content, and Na/K ratio of the stem and root. However, the NaCl treatment effect on the leaf Mg^{2+} content was not significant (P = 0.234).

According to the result in table 2, under NaCl treated condition the genotypes have significantly (P<0.001) higher amount of Na^+ in their root, stem and leaf than under control condition. The K^+ content in leaf and stem of the genotypes is significantly (P<0.001) decreased, whilst the K^+ content in the root is significantly (P<0.001) increased due to salt treatment. The salinity stress resulted in significant (P<0.001) increment and decrement of Mg^{2+} content in stem and root respectively. Although the Ca^{2+} content in the leaf and root is significantly reduced (P<0.001), the Ca^{2+} content of the stem is significantly enhanced by the NaCl treatment. The addition of NaCl significantly (P<0.001) increased the Na/K ratio in the leaf, stem and root (Table 2).

Under control condition, there is a significant organ effect on Na^+, K^+, Mg^{2+}, Ca^{2+} content and Na/K ratio (P<0.001). Thus, significantly (P<0.001) higher Na^+, Mg^{2+}, Ca^{2+} content, and Na/K ratio is observed in the root part, whilst it contains significantly (P<0.001) lower (31.03^a) K^+ concentration. The leaf Na^+, Mg^{2+}, Ca^{2+} content, and Na/K ratio is significantly (P<0.001) higher than in the stem. However, significantly highest K^+ content (62.64^c) is observed in the stem (P<0.001) (Table 2).

Under salt treated condition, organs show significant differences (P<0.001) for their Na^+, K^+, Mg^{2+} content and Na/K ratio, and (P=0.014) for Ca^{2+} content. The root has significantly (P<0.001) higher Na^+, Mg^{2+} content and Na/K ratio but significantly lower Ca^{2+} content than leaf and stem. The stem has significantly (P<0.001) higher amount of Na^+, K^+ and Mg^{2+} than leaf. However, no significant difference is observed between the K^+ contained in stem and root; and between the Ca^{2+} contained in the leaf and stem. The leaf has significantly highest Na/K ratio than the stem (P<0.001). (Table 2).

Table 2. The effect of salinity stress on the cation content of the CxE population and the difference among the leaf, stem and root of the genotypes for their mean ion content under specific growth condition.

AnCat	Organ	Control		Salt treated		One-way	Two-way	Relative	Cv%
		Mean[1] (mg/g)	Range	Mean[1] (mg/g)	Range	P-value Tr[2]	P value tr X or[4]	in/decrease (%)	
Na^+	leaf	5.18b	2.41 - 10.54	15.36a	5.84 - 36.98	<.001		-210.167	39.3
	Stem	4.51a	1.76 - 7.95	16.87b	8.33 - 29.34	<.001	<.001	-293.426	24.9
	Root	9.6c	4.55 - 15.08	32.32c	20.38 - 48.36	<.001		-254.727	21.2
	P value Or[3]	<.001		<.001					
K^+	Leaf	40.53b	18.34 - 59.86	29.45a	16.03 - 44.42	<.001		25.1071	19.9
	Stem	62.64c	34.15 - 88.58	45.17b	2.76 - 72.51	<.001	<.001	25.8986	24.8
	Root	31.03a	11.63 - 48.25	44.53b	20.45 - 70.1	<.001		-49.5142	23
	P value Or	<.001		<.001					
Mg^{2+}	leaf	4.28b	0.004 - 8.04	4.51a	0.01 - 8.17	ns		-2404.17	31
	Stem	2.06a	0.002 - 8.99	6.55b	2.85 - 11.48	<.001	<.001	-17917.2	44.9
	Root	9.6c	0.004 - 18.63	7.23c	3.55 - 15.42	<.001		-5711.81	34.4
	P value Or	<.001		<.001					
Ca^{2+}	leaf	14.14b	8.41 - 22.22	11.88b	6.70- 17.43	<.001		14.3695	18.3
	Stem	8.48 a	0.006 - 17.64	11.53b	7.89 - 16.69	<.001	<.001	-6041.85	35.1
	Root	31.41c	6.61 - 72.96	10.43a	3.79 - 25.72	<.001		62.4052	47.2
	P value Or	<.001		0.014					
Na/K	leaf	0.13b	0.04 - 0.26	0.59 b	0.16 - 1.82	<.001		-401.654	70.1
	Stem	0.076 a	0.03 - 0.18	0.38a	0.19 - 1.29	<.001	<.001	-421.179	58.4
	Root	0.32c	0.16 - 0.54	0.77 c	0.34 - 1.79	<.001		-161.282	34.5
	P value Or	<.001		<.001					

[1]Mean value of the cations: average cation content of the 94 genotypes and their parents (C and E) in their Leaf, Stem and Root under control and salt treated condition.

[2]Treatment, [3]Organ, [4]Treatment by organ interaction, [5]non significant difference; values in the column connected by same letter are not significantly different

Table 3. The effect of salinity stress on the cation content of the CxE population and the difference among the shoot and root of the genotypes for their mean ion content under specific growth condition.

AnCat	Organ	Control		Salt treated		One-way	Two-way	Relative	Cv%
		Mean[1] (mg/g)	Range	Mean[1] (mg/g)	Range	P-value Tr[2]	P value tr X or[4]	in/decrease (%)	
Na^+	Root	9.6	4.55 - 15.08	32.32	20.38 - 48.36	<.001	<.001		21.2
	Shoot	4.86	2.7 - 6.92	16.21	9.88 - 29.47	<.001		-243.081	26.5
	P value Or[3]	<.001		<.001					
K^+	Root	31.03	11.63 - 48.25	44.53	20.45 - 70.1	<.001	<.001		23
	Shoot	51.59	30.74 - 66.83	37.31	15.75 - 53.99	<.001		25.7544	17.5
	P value Or	<.001		<.001					
Mg^{2+}	Root	9.6	0.004 - 18.63	7.23	3.55 - 15.42	<.001	<.001		34.4
	Shoot	3.23	0.05 - 8.99	5.52	2.99 - 8.19	<.001		-206.804	28.3
	P value Or	<.001		<.001					
Ca^{2+}	Root	31.41	6.61 - 72.96	10.43	3.79 - 25.72	<.001	<.001		47.2
	Shoot	11.65	4.21 - 17.83	11.67	6.78 - 16.28	ns[5]		-10.3838	21.2
	P value Or	<.001		0.031					
Na/K	Root	0.32	0.16 - 0.54	0.77	0.34 - 1.79	<.001	ns[5]		34.5
	Shoot	0.1	0.046 - 0.17	0.49	0.18 - 1.82	<.001		-426.524	62.8
	P value Or	<.001		<.001					

[1]Mean value of the cations: average cation content of the 94 genotypes and their parents (C and E) in their shoot and root under control and salt treated condition. Shoot ion content is calculated as the average of the ions contained in the leaf and stem.
[2]Treatment, [3]Organ, [4]Treatment by organ interaction, [5]non significant difference; values in the column connected by same letter are not significantly different

Root and Shoot: According to the result in table 3, except for the Na/K ratio (P=0.136) there is a significant (P<0.001) organ and treatment interaction effect on cation content of the shoot and root. Significant (P<0.001) effects of NaCl treatment on Na^+, K^+, Mg^{2+} content, and Na/K ratio of shoot is observed; whilst the Ca^{2+} content was not significantly affected (P = 0.967). As compared to the control, the shoot of genotypes grown under NaCl treated condition has significantly higher amount of Na^+, Mg^{2+} and Na/K ratio (P<0.001). However, the K^+ content is significantly reduced (P<0.001) due to salt treatment. Except K^+ under control and Ca^{2+} under salinity condition, the root has significantly higher (P<0.001) cation content than the shoot (Table 3).

3.2.2 Effects of salinity on Anion content of the population

Stem, root and leaf

The two-way ANOVA in table 4, shows the anion content in leaf, stem and root is significantly ($P < 0.001$; Cl^-, SO_4^{2-}), and ($P = 0.003$; PO_4^{3-}) affected by the organ and treatment interaction. Thus, the Cl^-, SO_4^{2-} and PO_4^{3-} content of stem and root is significantly ($P<0.001$) increased by salinity treatment. Except the PO_4^{3-} ($P=0.533$); the Cl^- and SO_4^{2-} content of leaf is significantly enhanced by salinity stress.

The amount of Cl^-, in the leaf, stem and root of the genotypes has dramatically increased due to the application of NaCl ($P<0.001$). The PO_4^{3-} content of the stem ($P<0.001$) and root (0.002) is also significantly enhanced by the NaCl treatment. Although, there is significant ($P<0.001$) reduction of SO_4^{2-} in the leaf, the NaCl treatment increased the SO_4^{2-} content of the stem and root ($P<0.001$) (Table 4).

Under both growing conditions, significantly ($P<0.001$) higher accumulation of Cl^-, SO_4^{2-} and PO_4^{3-} is observed in the root than in the leaf and stem. Under control condition the leaf Cl^-, SO_4^{2-} and PO_4^{3-} content is significantly ($P<0.001$) higher than in the stem. Where as, under salinity condition the leaf contained significantly ($P<0.001$) lower Cl^- than the stem (Table 4).

Table 4. The effect of salinity stress on the anion content of the CxE population and the difference among the leaf, stem and root of the genotypes for their mean ion content under specific growth condition.

AnCat	Organ	Control		Salt treated		One-way	Two-way	Relative	Cv%
		Mean[1] (mg/g)	Range	Mean[1] (mg/g)	Range	P-value Tr[2]	P value tr X or[4]	in/decrease (%)	
Cl^-	Leaf	0.98b	0.54 - 2.02	39.95a	23.64 - 63.48	<.001		-4294.46	29.3
	Stem	0.47a	0 - 2.4	53.178b	35.87 - 70.53	<.001	<.001	-40369.9	17.7
	Root	1.65c	0 - 7.0	61.55c	35.42 - 88.09	<.001		-6386.12	22.8
	P value Or[3]	<.001		<.001					
PO_4^{3-}	Leaf	20.08b	15.13 - 25.82	19.86b	12.18 - 25.71	ns[5]		0.297022	12.1
	Stem	13.70a	0 - 19.42	15.12a	8.13 - 22.08	<.001	0.003	-11.3219	17.6
	Root	21.26c	10.58 - 33.18	22.84c	15.96 - 28.91	0.002		-11.4008	15.9
	P value Or	<.001		<.001					
SO_4^{2-}	Leaf	10.72b	7.17 - 14.97	7.76b	4.88 - 15.82	<.001		28.0939	17.6
	Stem	5.76a	0.03 - 9.96	6.55a	2.64 - 10.01	<.001	<.001	-310.969	22.8
	Root	19.35c	9.61 - 28.46	26.85c	18.94 - 47.59	<.001		-42.1069	19.9
	P value Or	<.001		<.001					

[1]Mean value of the anions: average anion content of the 94 genotypes and their parents (C and E) in their Leaf, Stem and root under control and salt treated condition.
[2]Treatment, [3]Organ, [4]Treatment by organ interaction, [5]non significant difference; values in the column connected by same letter are not significantly different

Table 5. The effect of salinity stress on the anion content of the CxE population and the difference among the shoot and root of the genotypes for their mean ion content under specific growth condition.

AnCat	Organ	Control		Salt treated		One-way P-value Tr[2]	Two-way P value tr X or[4]	Relative in/decrease (%)	Cv%
		Mean[1] (mg/g)	Range	Mean[1] (mg/g)	Range				
Cl^-	Root	1.65	0 - 7.0	61.55	35.42 - 88.09	<.001	<.001	-6386.12	22.8
	Shoot	0.72	0.11 - 1.74	46.56	34.31 - 63.4	<.001		-7582.95	18.9
	P value Or[3]	<.001		<.001					
PO_4^{3-}	Root	21.26	10.58 - 33.18	22.84	15.96 - 28.91	0.002	ns[5]	-11.4008	15.9
	Shoot	16.94	9.08 - 20.73	17.49	13.36 - 23.38	0.065		-4.58885	11.8
	P value Or	<.001		<.001					
SO_4^{2-}	Root	19.35	9.61 - 28.46	26.85	18.94 - 47.59	<.001	<.001	-42.1069	19.9
	Shoot	8.24	4.54 - 11.47	7.15	4.68 - 12.44	<.001		10.493	16.7
	P value Or	<.001		<.001					

[1]Mean value of the cations: average cation content of the 94 genotypes and their parents (C and E) in their shoot and root under control and salt treated condition. Shoot ion content is calculated as the average of the ions contained in the leaf and stem.
[2]Treatment, [3]Organ, [4]Treatment by organ interaction, [5]non significant difference; values in the column connected by same letter are not significantly different

Root and Shoot

Table 5 shows a significant (P<0.001) organ by treatment interaction effect on Cl^- and SO_4^{2-} content; but non-significant effect on PO_4^{3-} (P=0.077) content. Except for the PO_4^{3-} content (P=0.065), the treatment showed significant (P<0.001) effect on the Cl^- and SO_4^{2-} content of the shoot. Therefore, exogenous application of NaCl significantly enhanced the concentration of Cl^- (P<0.001), but significantly (P<0.001) reduced the SO_4^{2-} content of the shoot. Under both growing conditions the concentration of Cl^-, SO_4^{2-} and PO_4^{3-} in the root is significantly (P<0.001) higher than in the shoot (Table 5).

3.3 Correlation analysis of the Ions

Correlation coefficients (r) among the AnCat content in the root, stem, leaf and shoot of the potato diploid mapping population grown under control condition is shown in table 6, 7, 8 and 9; and under salt treated condition is presented in table 10, 11, 12 and 13. In case of association between the ions in the same or different organs, ions with the growth parameters at harvest, and ions with growth parameters measured at different days; a positive correlation is expected.

3.3.1 Control condition

Root

The accumulation of Ca^{2+} showed moderately positive correlation with Na^{+} (r = 0.46) and Mg^{2+} (r = 0.57); but moderately negative correlation with K^{+} (r = -0.41) and strongly negative correlation with PO_4^{3-} (r = -0.73) (Table 6). The Ca^{2+} contained in the root has moderately positive correlation with Ca^{2+} in the stem (r = 0.25) (Appendix 2). The root Ca^{2+} also showed moderately negative correlation with chlorophyll fluorescence of lower leaves (r = -0.33) and root length (r = -0.30) (Appendix 10). The concentration of Cl^{-} in the root showed strongly positive association with Na^{+} (r = 0.74) and Na/K ratio (r = 0.51) (Table 6). Where as, the Na^{+} content of root has moderately negative correlation with PO_4^{2-} (r = -0.35) (Table 6).

Table 6. Correlation analysis between ion content in the root of the genotypes grown under control condition

	R[1] Ca	R Cl	R K	R Mg	R Na	R Na/K	R PO_4	R SO4
R Ca	-							
R Cl	0.17	-						
R K	**-0.41**	0.01	-					
R Mg2	**0.57**	0.00	-0.11	-				
R Na	**0.46**	**0.74**	-0.11	0.22	-			
R Na/K	**0.62**	**0.51**	**-0.66**	0.19	**0.78**	-		
R PO4	**-0.73**	-0.10	**0.62**	**-0.35**	**-0.35**	**-0.63**	-	
R SO4	0.04	0.08	**0.49**	0.09	0.20	-0.16	0.24	-

[1] Root, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

K^{+} in the root has moderately positive correlation with the SO_4^{2-} (r = 0.49) and strongly positive correlation with PO_4^{3-} (r = 0.62) (Table 6). It also showed moderately positive correlation with root fresh weight (r = 0.35) and root length (r = 0.47) (Appendix 10); moreover, with the stem length measured at day '0' and day '7'

after treatment (data not shown). The accumulation of PO_4^{3-} in the root showed moderately positive correlation with the root length (r = 0.25) (Appendix 10).

Stem

The Ca^{2+} showed moderately positive correlation with Na^{+} (r = 0.42) (Table 7). The accumulation of Ca^{2+} in the stem also has moderately positive association with the Ca^{2+} content in the leaf (r = 0.42) (Appendix 4). The Cl^{-} content of the stem showed moderately negative correlation with Mg^{2+} (r = -0.26) (Table 7). Accumulation of Cl^{-} in the stem also exhibited moderately positive association with the Cl^{-} contained in the leaf (r = 0.33) (Appendix 4).

The K^{+} contained in the stem showed moderately positive association with the concentration of PO_4^{3-} (r = 0.34) and SO_4^{2-} (r = 0.28) (Table 7); and with the stem length measured at day '0' and day '7' after treatment (data not shown).

The PO_4^{3-} in the stem has a strongly positive correlation with the SO_4^{2-} (r = 0.69) (Table 7). The stem PO_4^{3-} showed moderately positive correlation with the PO_4^{3-} in the leaf (r = 0.37) (Appendix 4). The SO_4^{2-} contained in the stem is strongly associated with the SO_4^{2-} content of the leaf (r = 0.50) (Appendix 4).

Table 7. Correlation analysis between ion content in the stem of the genotypes grown under control condition

	S Ca	S Cl	S K	S Mg	S Na	S Na/K	S PO4	S SO4
S Ca	-							
S Cl	0.15	-						
S K	-0.04	0.22	-					
S Mg	-0.05	**-0.26**	-0.05	-				
S Na	**0.42**	-0.03	0.00	0.19	-			
S Na/K	**0.35**	-0.16	**-0.57**	0.20	**0.81**	-		
S PO4	-0.03	-0.20	**0.34**	0.24	0.16	-0.05	-	
S SO4	0.18	-0.03	**0.28**	0.21	0.21	0.03	**0.69**	-

[1]Stem, for the description of the ions refer to Table 6.

Leaf

The concentration of Ca^{2+} in the leaf has strongly positive correlation with Mg^{2+} (r = 0.50), and moderately positive association with PO_4^{3-} (r = 0.33) and SO_4^{2-} (r = 0.37) (Table 8). The accumulation of Ca^{2+} in the leaf also showed moderately positive relation with the Ca^{2+} content in the root (r = 0.25) (Appendix 3). Cl^{-} content in the leaf has moderately positive correlation with the Mg^{2+} (r = 0.27) and SO_4^{2-} (r = 0.32) (Table 8). But, Cl^{-} showed moderately negative correlation with leaf area (r = -0.36),

root fresh weight (r = - 0.35), root dry weight (r = -0.28), shoot dry weight (r = -0.38), shoot fresh weight (r = - 0.31) and chlorophyll fluorescence of lower leaves (r = -0.33); whilst strongly negative correlation with the root length (r = -0.51) (Appendix 12).

The K^+ concentration in the leaf of the genotypes showed moderately positive correlation with the PO_4^{3-} (r = 0.25), but moderately negative correlation with Mg^{2+} (r = -0.26), and Na^+ (r = -0.28) (Table 8). The K^+ also has moderately positive correlation with the leaf area (r = 0.33), root dry weight (r = 0.29), shoot fresh weight (r = 0.41), shoot length (r = 0.44), and shoot dry weight (r = 0.35) of the genotypes (Appendix 12).

Leaf Mg^{2+} has moderately positive correlation with the PO_4^{3-} (r = 0.31) and SO_4^{2-} (r = 0.39) (Table 8). The Mg^{2+} in the leaf also showed moderately positive correlation with the Mg^{2+} contained in the root (r = 0.35) (Appendix 3). Whilst it showed moderately positive correlation with the chlorophyll content of the upper leaves (r = 0.32); the Mg^{2+} in the leaf has moderately negative correlation with the leaf area (r = -0.25), root length (r = -0.33) and shoot dry weight (r = -0.25) (Appendix 12).

The concentration of Na^+ in the leaf showed moderately positive correlation with the Na^+ concentration in the root (r = 0.29) (Appendix 3), and with the root length (r = 0.29) (Appendix 12). However, the leaf Na^+ has moderately negative correlation with the stem length measured at day '0' and day '7' after treatment (data not shown).

Table 8. Correlation analysis between ion content in the leaf of the genotypes grown under control condition

	L[1] Ca	L Cl	L K	L Mg	L Na	L Na/K	L PO4	L SO4
L Ca	-							
L Cl	0.12	-						
L K	0.10	0.13	-					
L Mg2	**0.50**	**0.27**	**-0.26**	-				
L Na	-0.10	-0.09	**-0.28**	-0.14	-			
L Na/K	-0.12	-0.13	**-0.69**	0.01	**0.85**	-		
L PO4	**0.33**	0.15	**0.25**	**0.31**	0.15	-0.03	-	
L SO4	**0.37**	**0.32**	-0.05	**0.39**	0.00	-0.02	**0.32**	-

[1]Leaf, for the description of the ions refer to Table 6.

The PO_4^{3-} in the leaf of the genotypes has moderately positive correlation with the SO_4^{2-} (r = 0.32) (Table 8); and with the chlorophyll content of upper leaves (r = 0.39) and stem length (r = 0.27) (Appendix 12).

Shoot

The Ca^{2+} concentration in the shoot showed moderately positive correlation with the SO_4^{2-} (r = 0.40) in the shoot (Table 9), and with the Ca^{2+} contained in the root (r = 0.29) (Appendix 5). The Na^+ in the shoot has moderately positive relation with the Na^+ contained in the root (Appendix 5).

The K^+ accumulation in the shoot showed moderately positive association with the PO_4^{3-} (r = 0.34). The Mg^{2+} in the shoot was positively correlated with the PO_4^{3-} (r = 0.29) and SO_4^{2-} (r = 0.28). Moreover, the PO_4^{3-} contained in the shoot is strongly correlated with the SO_4^{2-} concentration (Table 9).

Table 9. Correlation analysis between ion content in the shoot of the genotypes grown under control condition

	Sh[1] Ca	Sh Cl	Sh K	Sh Mg	Sh Na	Sh Na/K	Sh PO4	Sh SO4
Sh Ca	-							
Sh Cl	0.24	-						
Sh K	0.01	0.09	-					
Sh Mg2	0.18	0.04	-0.03	-				
Sh Na	0.19	-0.07	-0.07	0.18	-			
Sh Na/K	0.11	-0.08	**-0.54**	0.13	**0.82**	-		
Sh PO4	0.17	-0.12	**0.34**	**0.29**	0.17	-0.04	-	
Sh SO4	**0.40**	0.17	0.15	**0.28**	0.10	0.00	**0.50**	-

[1]Shoot, for the description of the ions refer to Table 6.

3.3.2 Salt treated condition

Root

The concentration of Ca^{2+} in the root has moderately negative correlation with the Cl^- (r = -0.39), K^+ (r = -0.48) and PO_4^{2-} (r = -0.37); but it showed strongly positive correlation with the Mg^{2+} content (r = 0.56) (Table 10). The root Ca^{2+} has also moderately negative correlation with the chlorophyll content of lower leaves (r = -0.26), chlorophyll fluorescence of lower leaves (r = -0.29), leaf area (r = -0.36), root fresh weight (r = -0.41), root length (r = -0.49), root dry weight (r = -0.37), shoot fresh weight (r = -0.37), shoot length (r = -0.48) and shoot dry weight (r = -0.38) (Appendix 13).

The Cl^- contained in the root is strongly associated with the K^+ content (r = 0.65), and moderately correlated with the Na^+ (r = 0.44) (Table 10). The Cl^- content of the root has moderately positive correlation with the leaf area (r = 0.27), root length (r = 0.32),

root dry weight (r = 0.46), shoot fresh weight (r = 0.32) and shoot dry weight (r = 0.25); and it is strongly correlated with the root fresh weight (r = 0.56) (Appendix 13).

K^+ content of the root have moderately positive association with the PO_4^{3-} content (r = 0.25) (Table 10). The root K^+ has moderately positive correlation with the leaf area (r = 0.34), root fresh weight (r = 0.48), root length (r = 0.31), root dry weight (r = 0.40), shoot fresh weight (r = 0.39), shoot length (r = 0.35) and shoot dry weight (r = 0.38) (Appendix 13).

The Mg^{2+} accumulated in the root showed moderately negative relation with the chlorophyll fluorescence of lower leaves (r = -0.34), leaf area (r = -0.31), and root length (r = -0.46) (Appendix 13).

The Na^+ in the root has moderately positive correlation with the SO_4^{2-} (r = 0.43) (Table 10). The accumulation of Na^+ in the root also showed moderately positive correlation with the Na^+ contained in the stem (r = 0.40) (Appendix 6). However, the Na^+ in the root did not show a strong correlation with the growth parameters measured at harvest (Appendix 13).

Table 10. Correlation analysis between ion content in the root of the genotypes grown under NaCl condition

	R[1] Ca	R Cl	R K	R Mg	R Na	R Na/K	R PO4	R SO4
R Ca	-							
R Cl	**-0.39**	-						
R K	**-0.48**	**0.65**	-					
R Mg	**0.56**	0.00	-0.07	-				
R Na	0.04	**0.44**	-0.21	-0.21	-			
R Na/K	**0.42**	-0.24	**-0.81**	-0.03	**0.66**	-		
R PO4	**-0.37**	0.02	**0.25**	-0.18	-0.16	**-0.29**	-	
R SO4	-0.01	0.14	0.20	-0.11	**0.43**	0.06	0.06	-

[1] Root, [Ca^{2+} (Calcium), Cl^- (Chlorine), K^+ (Potassium), Mg^{2+} (Magnesium), Na^+ (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

The Na/K ratio in the root showed moderately negative correlation with the shoot length (r = -0.33) and shoot dry weight (r = -0.26); and it has poor negative correlation with the chlorophyll content of upper leaves (r = -0.20), root fresh weight (r = -0.24), root length (r = -0.20), root dry weight (r = -0.22), and shoot fresh weight (r = -0.23) (Appendix 13).

The root PO_4^{3-} showed moderately positive correlation with the stem length (r = 0.36) (Appendix 13). The SO_4^{2-} contained in the root has moderately positive correlation with the SO_4^{2-} accumulated in the stem (r = 0.43) (Appendix 6).

Stem

The concentration of Ca^{2+} in the stem showed moderately positive correlation with the Cl^- (r = 0.48) (Table 11); and with the root dry weight (r = 0.30) (Appendix 14).

The Cl^- of stem has moderately positive correlation with the K^+ (r = 0.45) and Na^+ (r = 0.39) (Table 11). The stem Cl^- also showed moderately positive correlation with the Cl^- in the leaf (r = 0.35) (Appendix 8), and with root fresh weight (r = 0.26) and root dry weight (r = 0.27) (Appendix 14).

The K^+ accumulated in the stem showed moderately positive correlation with the K^+ in the leaf (r = 0.31) (Appendix 8). K^+ in the stem also has moderately positive correlation with the leaf area (r = 0.32), root length (r = 0.26), shoot fresh weight (r = 0.26), and shoot length (r = 0.45) (Appendix 14). The K^+ content of the stem showed moderately positive correlation with chlorophyll content of lower and upper leaves as well as with chlorophyll fluorescence at day '14' (data not shown).

The stem Mg^{2+} has moderately positive correlation with the PO_4^{3-} (r = 0.31) (Table 11); and with the Mg^{2+} in the leaf (r = 0.27) (Appendix 8). However, the Mg^{2+} in the stem has moderately negative correlation with the chlorophyll content of the lower leaves (r = -0.25) (Appendix 14).

The Na^+ content in the stem is positively correlated with the SO_4^{2-} (r = 0.48) (Table 11). Accumulation of Na^+ in the stem showed poor negative correlation with the chlorophyll content of lower leaf (r = -0.20) and stem length (r = -0.22) (Appendix 14). The stem Na^+ also has moderately negative correlation with chlorophyll content of lower leaves at day '14' (data not shown).

N/K ratio in the stem showed moderately negative correlation with the chlorophyll content of lower leaves (r = -0.28), chlorophyll fluorescence of upper leaves (r = -0.32), leaf area (r = -0.31), root fresh weight (r = -0.25), root length (r = -0.33), shoot

fresh weight (r = -0.27), shoot dry weight (r = -0.29); and strongly negative relation with the stem length (r = -0.51) (Appendix 14).

The PO_4^{3-} in the stem has moderately positive correlation with the SO_4^{2-} (r = 0.43) (Table 11). The stem PO_4^{3-} also showed moderately positive correlation with the PO_4^{3-} in the leaf (r = 0.33) (Appendix 8). The SO_4^{2-} in the stem showed moderately negative relation with the chlorophyll content of lower leaves (r = -0.33), chlorophyll content of upper leaves (r = -0.27), shoot fresh weight (r = -0.25), shoot length (r = -0.38), and shoot dry weight (r = -0.27) (Appendix 14).

Table 11. Correlation analysis between ion content in the stem of the genotypes grown under NaCl condition

	S Ca	S Cl	S K	S Mg	S Na	S Na/K	S PO4	S SO4
S Ca	-							
S Cl	**0.48**	-						
S K	0.11	**0.45**	-					
S Mg2	0.08	0.10	-0.21	-				
S Na	0.08	**0.39**	0.02	-0.12	-			
S Na/K	-0.10	-0.14	**-0.72**	0.06	**0.57**	-		
S PO4	0.05	0.07	0.15	**0.31**	0.13	0.00	-	
S SO4	0.06	0.17	0.03	0.22	**0.48**	**0.28**	**0.43**	-

[1]Stem, for the description of the ions refer to Table 10.

Leaf

The concentration of Ca^{2+} in the leaf is strongly correlated with the Cl^- (r = 0.59) and Mg^{2+} (r = 0.65); it has also moderately positive correlation with the PO_4^{3-} (r = 0.48) and K^+ (r = 0.27) (Table 12). The Ca^{2+} has moderately positive correlation with the growth parameters such as the chlorophyll fluorescence of lower leaves, leaf area, root length, shoot fresh weight, and shoot dry weight; and strongly positive correlation with the root dry weight (r = 0.53) and root fresh weight (r = 0.55), (Appendix 15).

The Cl^- has moderately positive correlation with the K^+ (r = 0.36), Mg^{2+} (r =0.33), Na^+ (r = 0.47) and PO_4^{2-} (r = 39) (Table 12). The Cl^- in the leaf showed moderately positive correlation with the root fresh weight (r = 0.38) and root dry weight (r = 0.37) (Appendix 15).

The K^+ contained in the leaf showed moderately negative correlation with Na^+ (r = -0.46) (Table 12). But, leaf K^+ has moderately positive correlation with the root fresh weight (r = 0.33), root dry weight (r = 0.31), shoot fresh weight (r = 0.40), and shoot

dry weight (r = 0.36); and strongly positive correlation with leaf area (r = 0.50) (Appendix 15). The K^+ in the leaf also showed moderately positive correlation with chlorophyll content of lower leaves at day '14' (data not shown).

The concentration of Mg^{2+} in the leaf has moderately positive correlation with the PO_4^{3-} (r = 0.43) (Table 12); and with the root fresh weight (r = 0.30), root length (r = 0.29) and root dry weight (r = 0.28) (Appendix 15).

The accumulation of Na^+ in the leaf showed moderately positive correlation with the SO_4^{2-} (r = 0.27) (Table 12). But it has moderately negative correlation with the chlorophyll content of lower leaves (r = -0.36), chlorophyll content of upper leaves (r = -0.26), leaf area (r = -0.36), root length (r = -0.27), shoot fresh weight (r = -0.34), shoot length (r = -0.42), and shoot dry weight (r = -0.35) (Appendix 15).

Table 12. Correlation analysis between ion content in the leaf of the genotypes grown under NaCl condition

	L Ca	L Cl	L K	L Mg	L Na	L Na/K	L PO4	L SO4
L Ca	-							
L Cl	**0.59**	-						
L K	**0.27**	**0.36**	-					
L Mg2	**0.65**	**0.33**	0.15	-				
L Na	0.03	**0.47**	**-0.46**	-0.06	-			
L Na/K	-0.11	0.14	**-0.75**	-0.11	**0.89**	-		
L PO4	**0.48**	**0.39**	0.15	**0.43**	0.19	0.03	-	
L SO4	0.08	0.02	-0.09	-0.07	**0.27**	**0.27**	0.15	-

[1]Leaf, for the description of the ions refer to Table 10.

Na/K ratio in the stem has moderately negative correlation with the chlorophyll content in lower leaves (r = -0.26), leaf area (r = -0.49), root fresh weight (r = -0.26), root length (r = -0.27), root dry weight (r = -0.26), shoot fresh weight (r = -0.42), shoot length (r = -0.39), and shoot dry weight (r = -0.40) (Appendix 15). The SO_4^{2-} in the leaf showed moderately positive correlation with the SO_4^{2-} contained in the root (r = 0.33) (Appendix 7).

Shoot

The Ca^{2+} in the shoot has moderately positive relation with the Mg^{2+} (r = 0.32), and PO_4^{2-} (r = 0.28); it has also strongly positive correlation with the Cl^- (r = 0.60) (Table 13). However, the shoot Ca^{2+} showed moderately negative correlation with the Ca^{2+} in the root (r = -0.28) (Appendix 9).

The Cl^- showed moderately positive correlation with the K^+ (r = 0.28) and Na^+ (r = 0.42). The K^+ and Na^+ content in the shoot of the genotypes has moderately negative correlation (r = -0.35). The Mg^{2+} concentration in the shoot is positively correlated with the PO_4^{2-} (r = 0.35); and the shoot Na^+ has moderately positive correlation with SO_4^{2-} (r = 0.31) (Table 13). The SO_4^{2-} in the shoot has moderately positive correlation with the SO_4^{2-} in the root (r = 0.40) (Appendix 9).

Table 13. Correlation analysis between ion content in the shoot of the genotypes grown under NaCl condition.

	Sh Ca	Sh Cl	Sh K	Sh Mg	Sh Na	Sh Na/K	Sh PO4	Sh SO4
Sh Ca	-							
Sh Cl	**0.60**	-						
Sh K	0.22	**0.28**	-					
Sh Mg	**0.32**	0.19	-0.12	-				
Sh Na	0.02	**0.42**	**-0.35**	0.00	-			
Sh Na/K	-0.15	0.10	**-0.70**	0.06	**0.84**	-		
Sh PO4	**0.28**	0.21	0.09	**0.35**	0.19	0.09	-	
Sh SO4	0.09	0.13	-0.02	0.18	**0.41**	**0.31**	0.24	-

[1]Shoot, for the description of the ions refer to Table 10.

3.4 Expression analysis of genes

At the beginning the cDNA of three genotypes (200, 250 and 777) were used to test the functioning of the primer pairs (SOS1-1, SOS1-2, SOS1-3, HKT1-new and Ef1α). Although two PCR programs were used no PCR products were observed (data not shown). Using new PCR reagents the primer pairs (SOS1-1, SOS1-2, SOS1-3, HKT1-new, Ef1α) were tested using cDNA of genotype 200, 250 and 777, and the parents (C and E). Two PCR programs that differ in the duration of annealing temperature (1min and 30sec) were used; and the PCR program with 30sec gave a result to make further optimization on SOS1-1, SOS1-2 and SOS1-3 and Ef1α. Hence, the DNA and cDNA of the genotype-200, parent C, and parent E was used under two PCR programs (annealing temperature, 50^{o}C and 53^{o}C) both having the duration of 30 sec. Primer pairs SOS1-2 and SOS1-3 gave a band of expected size under both PCR programs while the other primer pairs failed. As a result, the PCR cycle with 94^{o}C (30sec), 53^{o}C (30sec) and 72^{o}C (45sec) was selected because it showed stronger band intensity than 50^{o}C (30sec).

To test the primer pairs SOS1-2 and SOS1-3, six genotypes with highest Na^+ content and six genotypes that have lowest Na^+ content were selected. The cDNA of these genotypes from the plants under salinity and control condition was tested using the

previously selected PCR cycle i.e., 53°C (30sec). Although further test on the HKT1 new and old primer pairs were also carried out at this stage, it could not show a product. Hence, the SOS1-2 and SOS1-3 primer pairs were further tested using the other PCR program of 50°C (30sec). After a repeated test, the use of the PCR cycle with 53°C (30sec) was reaffirmed to be used for further test of these primer pairs.

After repeated test, the SOS1-2, SOS1-3 and Ef1α using 94°C (30sec), 53°C (30sec) and 72°C (45sec); stronger bands were obtained (Appendix 16). Further testing of these primers was carried out using cDNA of six genotypes from each growing condition; and DNA of another twelve genotypes including parent C and parent E. These genotypes were selected based on their relative higher or lower Na/K ratio. This trial gave stronger bands and was repeated to check its reproducibility. As a result, the primer pair SOS1-2 has shown a product in both of the parents (C and E) DNA, but the SOS1-3 has given a product for only parent 'C' DNA (Appendix 17). Thus, SOS1-3 has been used to find markers in the CxE population (Figure 3).

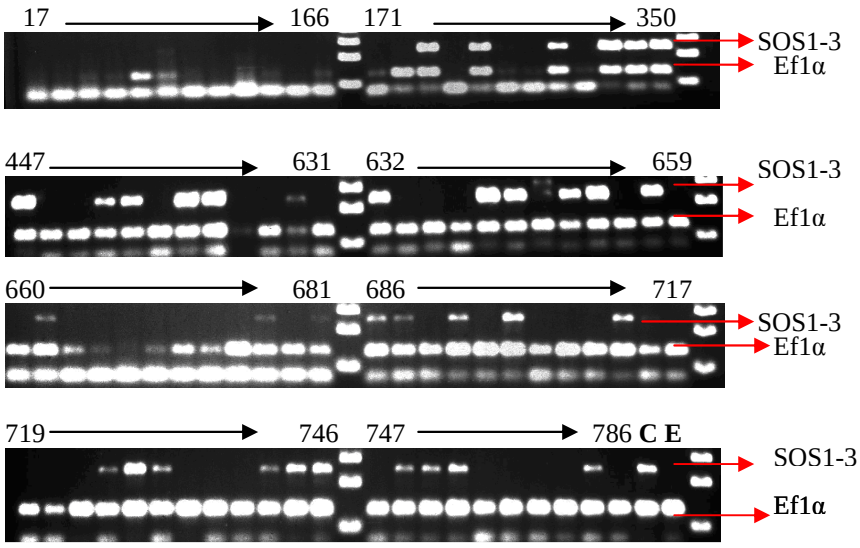

Figure 3. The Markers identified in genotypes of the CxE population using the SOS1-3 primer pair. For the genotype number of each lane refer to Appendix 18.

SOS1-2 primer was chosen to carry out expression analysis of SOS1 gene; and was further tested to optimize the PCR program to be used for the final qRT-PCR. Thus, using 94°C (30sec), 55°C (30sec) and 72 °C (45sec) PCR; the same result as the previous 53°C (30sec) was obtained but with stronger band intensity (data not shown).

Before, the use of the primer SOS1-2, another optimization was recommended using the qRT PCR. Therefore, four genotypes (196, 607, 667 and 723) have been selected and the test was carried out on SOS1-2 and Ef1α using the cDNA, RNA and DNA of the genotypes. However, there was strong primer dimmer as detected from the double peak of melting temperatures. When the qRT-PCR products were run in gel-electrophoresis the primer dimmers gave as strong band as the products. Moreover, there was a product from the RNA of the genotypes using both the SOS1-2 and Ef1α (data not shown). To carry out further test, cDNA of the four genotypes has been used at different primer concentrations (10, 5, 3, 2, 1 and 0.5μM). The result for these primer combinations was also showed strong primer dimmer (data not shown).

4. Discussion

The frequency distribution of CxE population showed the variability among the genotypes regarding their ion contents (Figure 2a and Figure 2b). Since a single measurement was done for each sample, statistical test of genotypic effect on ion content was not carried out. However, the samples were pooled (3 plants of NaCl treatment and 2 plants of the control) for ion content determination. Thus, there may be higher probability to rule out the environmental effect on differences among the genotypes. As a result, the existing difference among the genotypes regarding their ion content might be attributed to the genetic variation with in the population. Munns and Tester, (2008) also reviewed that there is a genetic variation for ion content with in many plant species. Similarly, the existence of genetic variation is reported with in tomato population (Lapushner *et al*., 1973), sweet potato genotypes (George *et al*., 2002), alphalpha cultivars (Silva *et al*., 2005) and rice (Roychoudhury *et al*., 2008). This genetic variation is due to the differences of the genes for controlling salt uptake, transport and osmotic regulation (Munns, 2005). According to the result in Figure 2a and 2b; the variability among the genotypes shows difference across the treatment. This variability difference can be observed from the changes in the range of values, shape of frequency distribution, and from the position of the parents across treatments. This can be explained by the environmental sensitivity of plant salinity tolerance (Koyama *et al*., 2001; Bartels and Sunkar, 2005; Munns and Tester, 2008).

The result of this experiment indicated that, there is a significant treatment and organ interaction effect on most of the ions contained in the root, stem and leaf of the genotypes. Thus, the concentration of AnCat in different organs of the plant showed variability. Moreover, the ion content of the genotypes in their root, stem and leaf was influenced by the application of NaCl into the growth medium (Table 2 and 4). The effects of NaCl application into the growth medium on the ion content, plant growth and their relations is reported in different plants (Martin and Koebner, 1995; Khan *et al*., 1999; Khan *et al*., 2001; Karimi *et al*., 2005; Tarakcioglu and Inal, 2002).

In this experiment the addition of NaCl has significantly increased the Na^+ (Table 2) and Cl^- (Table 4) content in the root, stem and leaf. Experimental evidences also indicated that there is increment of both Na^+ and Cl^- due to NaCl treatment (Gadallah,

1999; Ferreira *et al*., 2001; Khan *et al*., 2001; Parida and Das, 2005; Roychoudhury *et al*., 2008; Karimi *et al*., 2005). This increment of Na^+ and Cl^- is due to higher availability of the Na^+ and Cl^- on the growth medium, that create large electrochemical gradient for passive entry of these ions in to the plants (Sun *et al*., 2009; Blumwald *et al*., 2004; and Flowers and Flowers 2005). In the current experiment, Na^+ and Cl^- under salinity condition showed moderately positive relationship in the root, stem and leaf of the genotypes (Table 10, 11 and 12). Similarly, Martin and Koebner (1995) described the synergistic interaction between Na^+ and Cl^- to cause salt toxicity on wheat. Both of these ions can also disrupt functioning of ion channels to facilitate their entry in to plant cells (Chinnusamy and Zhu, 2003; Bartels and Sunkar, 2005; Sun *et al*., 2009). Under both control and salinity condition of this experiment there is significantly higher accumulation of Cl^- in the root than leaf and stem. The leaf has higher and lower Cl^- content than the stem under control and salinity condition respectively (Table 4). This result followed the same trend for the Na^+ content in these organs under both growing conditions (table 2). Hence, the similarity of this trend might also strengthen the evidence for the existence of association in the accumulation of Na^+ and Cl^- ions in different parts of the plant.

In our experiment, despite of the significant increment of Cl^- under salinity condition in the root (6386%), stem (40367%) and leaf (4294.5 %) (Table 4); it did not show negative correlation with the growth parameters measured at harvest (Appendix 13, 14 and 15). However, under salinity stress the negative effects of Cl^- on plant physiological and morphological components were reported (Khan *et al*., 2001; Martin and Koebner, 1995; Roychoudhury *et al*., 2008). Under salinity condition, the Cl^- has moderately positive correlation with the K^+ in the root, stem and leaf (Table 10, 11 and 12). This correlation with K^+ might have masked the negative effects of Cl^- on the growth parameters under salinity condition. Alternatively, there is a concentration level of Cl^- beyond which it will become toxic (Munns and Tester, 2008). The Cl^- enters in to cytosol through the Cl^-/H^+ symporters (Sun *et al*., 2009). So that, the genes that encode the protein channels for Cl^- entry might have been downregulated in the CxE population. Thus, larger proportion of Cl^- is held on the apoplast where it is less toxic (Blumwald *et al*., 2004; Munns, 2005). However, under control condition the Cl^- in the leaf showed moderately negative correlation with

some of the growth parameters (Appendix 12). This might have been due to lack of Cl^- to the extent it can help the plant's physiological processes.

Although under control condition the root has higher Na^+ content followed by the leaf; under salt treated condition, the root has significantly higher Na^+ followed by the stem (Table 2). This result is not in agreement with Ferreira *et al*., (2001) who reported the highest Na^+ content in the leaves than in the root of Guava (*Psidium guajava* L.) grown under salinity stress. However, the result for the salinity condition of this experiment is in agreement with Munns and Tester (2008), who described the tendency of Na^+ to be held in the woody roots and stems. Under salinity condition of this experiment, the lower accumulation of Na^+ in the leaf but higher accumulation in the root may be due to the export of Na^+ from the leaf back to the root through the phloem (Munns, 2005; Munns and Tester, 2008).

Higher accumulation of Na^+ in the leaf reflected the detrimental effects of salinity stress on aromatic rice (Roychoudhury, 2008). However, there is relatively lower accumulation of Na^+ in the leaf under salinity condition of the current experiment (Table 2). This might indicate the existence of adaptation mechanism by the CxE genotypes to the salinity stress through limiting Na^+ concentration in the leaf. But, the leaf Na^+ content under salinity condition showed moderately negative correlation with the chlorophyll content of lower leaves, chlorophyll content of upper leaves, leaf area, root length, shoot fresh weight, shoot length, and shoot dry weight (Appendix 15). This is similar to the description of Munns and Tester (2008), that is smaller proportion of Na^+ that reach the leaf can show ionic toxicity. The degeneration of chlorophyll, inhibition of shoot and root length in rice due to higher foliar Na^+ is also reported by Roychoudhury *et al*., (2008). Therefore, in this experiment the increment of Na^+ in the leaf due to the NaCl treatment might have been to the extent it can show up the impacts of ionic toxicity. The higher accumulation of Na^+ in plants is detrimental to physiological and morphological characteristics (Chinnusamy and Zhu, 2003; Bartels and Sunkar, 2005; Munns, 2005; Tuteja, 2007; and Munns and Tester, 2008). Under salinity condition the Na^+ content in the root and stem showed poor correlation with the growth parameters measured at harvest (Appendix 13 and 14). This might indicate the increasing of negative correlation between Na^+ and the growth parameters from the root to the leaf (Appendix 13, 14 and 15).

The NaCl treatment increased the Na^+ and K^+ in the root (Table 2). The Na^+ in the root did not have negative correlation with the growth parameters (Appendix 13). Inhibition of Na^+ influx, Na^+ compartmentation via tonoplast Na^+/H^+ antiporters, and extrusion of Na^+ through plasma membrane Na^+/H^+ antiporters are very important mechanisms for salinity tolerance (Sun *et al*., 2009; Apse and Blumwald, 2002). Exclusion of excess Na^+ entry by roots is the first line of defense deployed by plants (Roychoudhury *et al*., 2008), if this failed the Na^+ concentration in the leaf will grow to a toxic level (Munns, 2005). Therefore, in this experiment the significant increment of Na^+ after NaCl treatment in root, and showing negative correlation with the growth parameters when reaching the leaf of the genotypes might indicate the poor efficiency of Na^+ exclusion. However, the increasing of K^+ together with the Na^+ in the root might be due to capability of the genotypes to keep the Na^+ in the root below the toxic level. This may be achieved through compartmentalizing of Na^+ in to vacuole and efflux of Na^+ out of the cytosol in to the apoplast. The K^+ concentration can increase up to non toxic level to keep the electrochemical gradient across the tonoplast (Munns and Tester, 2008).

In this experiment there is significant reduction of K^+ in leaf (25%) and stem (26%) (Table 2). Similarly, the reduction of K^+ content after NaCl treatment is reported in different plants (Ferreira *et al*., 2001; Tarakcioglu and Inal, 2002; Parida and Das; 2005). Although the increasing of Na^+ in the stem under salinity condition might not be a reason for the K^+ reduction in the stem ($r = 0.02$) (Table 11); the leaf Na^+ showed negative correlation ($r = -0.46$) with the K^+ contained in the leaf (Table 12). There is entry of Na^+ through K^+ channels and nonselective cation channels (Bartels and Sunkar, 2005; Sun *et al*., 2009). Therefore, the interference of the Na^+ on the K^+ acquisition pathway of the CxE genotypes in this experiment might be well exhibited at the leaf. The reduced K^+ in the stem and leaf under salinity condition has positive relationship with some of the growth parameters (Appendix 14 and 15). Hence, this reduction might have been to the extent that can affect some of the growth parameters.

The K^+ contained in the root under salt treated condition has moderately positive correlation with the leaf area, root fresh weight, root length, root dry weight, shoot fresh weight, shoot length and shoot dry weight (Appendix 13). The importance of K^+ for plant metabolism and growth has been reported by Mahajan *et al*., (2008); that is

why, plants under salinity stress maintains higher K^+ and lower Na^+ concentrations (Parida and Das, 2005). In this experiment the Na/K ratio in the root, stem and leaf has significantly increased (Table 2); and showed moderately negative correlation with most of the growth parameters under salinity condition (Appendix 13, 14 and 15). Similar results were reported on *Vicia faba* (Gadallah, 1999) and lettuce (*Lactuca sativa* L.) (Tarakcioglu and Inal, 2002). Therefore, in spite of the salinity tolerance mechanisms used by the CxE population these ionic changes and their relation with the growth parameters might be indicating the existence of salinity stress. This might be attributed to poor salinity tolerance strategy of the CxE genotypes or the treatment (120mM of NaCl) might have been too intense for the genotypes.

According to the result in table 2, due to salt treatment significant reduction of Ca^{2+} content in the leaf (14.3695%) and root (62.4052%) of the genotypes is observed; while the Ca^{2+} in stem is significantly increased (6041.9%). However, the application of NaCl reduced the Ca^{2+} content in stem and leaf whilst it was stable in the root (Ferreira *et al*., 2001). Increasing of NaCl and $CaCl_2$ has enhanced the Ca^{2+} content of *Vicia faba* (Gadallah, 1999). Application of NaCl also increases the cytosolic Ca^{2+} (Munns and Tester, 2008; Bartels and Sunkar, 2005). In this experiment, the reduced Ca^{2+} in the root and leaf is accompanied by the negative correlation between Na^+ with K^+ (r = -0.21, root and r = -0.46, leaf) (Table 10 and 12). But the increased of Ca^{2+} in the stem is where there is poorest correlation between the Na^+ and K^+ (r = 0.02) (Table 11). Moreover, increased Ca^{2+} in the stem showed moderately positive correlation with the root dry weight (r = 0.30) and leaf area (r = 0.22) (Appendix 14). Accordingly, the significant importance of Ca^{2+} in the SOS pathway for ion homeostasis is described by several authors (Yokoi *et al*., 2002; Bartels and Sunkar, 2005; Tuteja, 2007; Mahajan *et al*., 2008; and Munns and Tester, 2008). Sun *et al*., (2009) also reported the importance of Ca^{2+} in reducing the K^+ efflux due to severe salinity.

In this experiment the application of NaCl increased the Mg^{2+} content in the stem (17917%), and reduced in the root (5711.8%); but the Mg^{2+} (2404%) in the leaf was stable after the treatment (Table 2). However, the result of Ferreira *et al*., (2001) showed that the Mg^{2+} levels in stems and roots were not affected by salinity but

decreased in the leaves of Guava (*Psidium guajava* L.). Application of NaCl has reduced the Mg^{2+} contents of *Kochia prostrate* (Karimi *et al*., 2005).

Under both control and salinity conditions the root has significantly higher SO_4^{2-} and PO_4^{3-} concentration followed by the leaf (Table 4). Although NaCl treatment has significantly increased PO_4^{3-} concentration in the stem and root; the PO_4^{3-} concentration in the leaf was not significantly changed (Table 4). The result of Tarakcioglu and Inal, (2002) showed increased PO_4^{3-} concentration in lettuce (*Lactuca sativa* L.) due to salinity stress. Under salinity condition PO_4^{3-} in the root showed moderately positive correlation with the shoot length (Appendix 13). Schachtman *et al*., (1998) also described the importance of PO_4^{3-} in promoting plant growth and development. The increase of PO_4^{3-} in the root and stem after salinity treatment might indicate the use of PO_4^{3-} for the activity of Na^+/H^+ antiporters; since, the increased Na^+ in these organs failed to show negative correlation with growth parameters measured at harvest.

In this experiment the NaCl treatment has significantly increased the concentration of SO_4^{2-} in the root and leaf, but significantly reduced the stem SO_4^{2-} content (Table 4). Under salinity condition the stem SO_4^{2-} showed moderately negative relation with the chlorophyll content of lower and upper leaves, shoot fresh weight, shoot length, and shoot dry weight (Appendix 14). Maruyama-Nakashita *et al*., (2004) described that SO_4^{2-} is required for the synthesis of sulfur-containing amino acids in plants; thus, lack of SO_4^{2-} can limit plant growth and development. In this experiment, under salinity condition the SO_4^{2-} and Na^+ showed moderately positive relationship in the root ($r = 0.43$), stem ($r = 0.48$) and leaf ($r = 0.25$) (Table 10, 11 and 12). Under salinity condition there is highest SO_4^{2-} concentration in the root (Table 4), where the Na^+ did not show negative correlation with growth parameters. Where as, in the leaf the SO_4^{2-} and Na^+ showed weak correlation than in the root. These correlations might be indicating the importance of SO_4^{2-} in maintaining functioning of the ionic channels for ion homeostasis in CxE population.

The differentially expressed genes of a plant in response to the NaCl treatment are the genes encoding proteins for ion homeostasis maintenance, metabolism, regulation of other genes, or for signal transduction (Sahu and Shaw, 2009). The genetic studies on potato in response to stressful conditions have led to the identification of a large

number of stress regulated expression of genes (Leone *et al*., 1999). However, the current experiment did not bring a result to be used as a genetic evidence about the function of the ion transporters in salinity tolerance of CxE diploid potato mapping population. This might be due to the poor primer design or the different PCR conditions used for optimization were not ideal for the primer pairs to amplify the gene of interest. Even the primers with the optimized PCR condition (SOS1-2 and Ef1α primer pairs) failed to work at the qRT-PCR because they were giving strong bands due to the primer dimmer. After qRT-PCR, the electrophoresis gel showed a product for the RNA which indicates the possibility of contamination of the RNA with DNA. Moreover, there was larger product size when DNA was amplified using the SOS1-2 primer; where as, the SOS1-3 enabled to give a product only on DNA of parent C (Appendix 17). The larger size obtained using SOS1-2 may be due to the existence of intron in the amplified region; and SOS1-3 might have been allele specific which might be due to the sequence differences between parent C and E caused by SNP, deletions or insertions in the specific gene amplified.

5. Conclusion

There is treatment dependent variability among the genotypes of CxE population in terms of their ion content. The application of NaCl in to the growth medium caused significant changes of different magnitude in different ions and organs. The root, stem and leaf of the genotypes have different ion content under specific growing condition.

The ionic changes due to salinity tolerance altered the interrelationship among the ions. The correlation value between same ions of different organs is dependent on the type of ion and the organ. Moreover, the ions contained in different organs have different correlation value with a specific growth parameter. The application of NaCl in to the growth medium increased Na^+ and Cl^- content, and Na/K ratio in the organs of genotypes. The salinity stress reduced the K^+ content in the leaf and stem of the genotypes. Expression analysis of genes for ion homeostasis is imperative to critically evaluate the genetics of salinity tolerance mechanisms organized by the CxE genotypes.

6. Recommendation

- Doing salinity tolerance experiment on the CxE population at different salinity stress levels could be more informative through investigating the trends of ion content, growth parameters and gene expression at the different levels of stress.

- Salinity tolerance in plants is intermingling with other stress tolerance mechanisms. Hence, it is very important to grow the CxE genotypes under salinity condition at different environmental components such as the temperature and humidity.

- Morphological, physiological and genetic data collected at different stages of plant development may provide concrete evidence about the response of the CxE genotypes to salinity stress.

- For the expression analysis of the genes in the CxE genotypes, it would have been important to measure the concentration of the existing cDNA so as confirming the quality of the material. Moreover, development of other primer pairs (based on the cDNA and DNA sequences) and carrying out optimization of PCR and qRT-PCR conditions could help to get reliable genetic information.

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Appendices

Appendix 1: Formulas used for ion content determination (mg/g).

i = specific cation or anion

j = Genotypes

$R_i = \left(r_i / p_i \right) * 100$; R_i (recovery of each cations and anions), r_i (concentration in ppm obtained from IC), p_i (the ppm of AnCat in the standards).

$\overline{R}_i = \sum \left(R_i / N_i \right)$; $\overline{R}_i$ (average recovery percentage of AnCat), N_i (the number of standards used).

$Y_i = ((x_i - \bar{b}_i) / \overline{R}_i * 100) / d_i$; Y_i (concentration of AnCat, in mg/sample size used) x_i (concentration of AnCat in ppm, determined by the IC), $\bar{b}_i$ (average of AnCat from the blank analytes in ppm obtained from IC), d_i (dilution factor used i.e., 1000X).

$G_i = \left(Y_i / M_j \right) * 1000$; G_i (Amount of AnCAt in mg /gram of plant organ), M_j (the (amount of sample ashed for each genotype), 1000 (conversion factor of milligram to gram).

Appendix 2. Correlation analysis between ion content in the root and stem of the genotypes grown under control condition.

Ions	R[1] Ca	R Cl	R K	R Mg	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4	S[2] Ca	S Cl	S K	S Mg2	S Na	S Na/Ca	S Na/Cl	S Na/K	S Na/Mg	S PO4	S SO4
R Ca	-																					
R Cl	0.17	-																				
R K	**-0.41**	0.01	-																			
R Mg	**0.57**	0.00	-0.11	-																		
R Na	**0.46**	**0.74**	-0.11	0.22	-																	
R Na/Ca	**-0.69**	0.19	**0.30**	**-0.40**	0.05	-																
R Na/Cl	0.03	**-0.82**	0.02	0.22	**-0.35**	**-0.26**	-															
R Na/K	**0.62**	**0.51**	**-0.66**	0.19	**0.78**	-0.15	**-0.26**	-														
R Na/Mg	0.12	0.19	**-0.29**	**-0.60**	0.14	-0.08	**-0.26**	**0.38**	-													
R PO_4	**-0.73**	-0.10	**0.62**	**-0.35**	**-0.35**	**0.62**	-0.08	**-0.63**	-0.19	-												
R SO_4	0.04	0.08	**0.49**	0.09	0.20	0.03	0.02	-0.16	-0.13	0.24	-											
S Ca	**0.25**	-0.13	-0.23	0.01	-0.01	-0.24	0.14	0.13	0.12	**-0.26**	0.04	-										
S Cl	**0.28**	-0.06	-0.24	**0.29**	0.07	**-0.26**	0.21	0.20	-0.02	-0.18	0.02	0.15	-									
S K	0.17	0.07	0.09	0.07	0.19	-0.13	0.08	0.10	0.18	0.00	0.02	-0.04	0.22	-								
S Mg	-0.04	-0.06	-0.01	-0.07	-0.15	-0.13	-0.11	-0.13	-0.10	0.00	0.03	-0.05	**-0.26**	-0.05	-							
S Na	0.15	0.06	-0.22	-0.06	0.16	-0.09	-0.10	**0.25**	0.15	-0.15	0.06	**0.42**	-0.03	0.00	0.19	-						
S Na/Ca	0.06	-0.04	0.01	0.13	0.10	-0.04	0.07	0.06	-0.05	-0.11	0.05	**-0.46**	-0.15	-0.01	0.16	-0.05	-					
S Na/Cl	**-0.29**	0.01	0.12	**-0.27**	-0.04	**0.31**	-0.13	-0.11	0.02	**0.25**	0.07	-0.16	**-0.72**	-0.17	0.04	0.12	0.06	-				
S Na/K	0.01	0.03	-0.22	-0.09	0.02	0.00	-0.15	0.13	0.03	-0.12	0.05	**0.35**	-0.16	**-0.57**	0.20	**0.81**	-0.04	0.18	-			
S Na/Mg	-0.04	0.18	0.06	0.07	0.16	0.12	-0.11	0.06	-0.07	0.02	-0.03	0.16	-0.05	-0.07	**-0.41**	0.08	-0.08	0.11	0.09	-		
S PO_4	0.08	0.10	0.13	0.06	0.05	0.02	-0.08	-0.04	0.01	0.08	0.04	-0.03	-0.20	**0.34**	0.24	0.16	-0.01	0.18	-0.05	-0.06	-	
S SO_4	**0.30**	0.07	-0.09	0.20	0.14	-0.19	0.03	0.15	0.03	**-0.27**	0.15	0.18	-0.03	**0.28**	0.21	0.21	-0.08	0.01	0.03	-0.08	**0.69**	-

[1]Root, [2]Stem, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ions are calculated in mg/g of organ of the genotypes.

Appendix 3. Correlation analysis between ion content in the leaf and root of the genotypes grown under control condition.

Ions	L[1] Ca	L Cl	L K	L Mg2	L Na	L Na/Ca	L Na/Cl	L Na/K	L Na/Mg	L PO4	L SO4	R[2] Ca	R Cl	R K	R Mg2	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4
L Ca	-																					
L Cl	0.12	-																				
L K	0.10	0.13	-																			
L Mg2	**0.50**	**0.27**	**-0.26**	-																		
L Na	-0.10	-0.09	**-0.28**	-0.14	-																	
L Na/Ca	**-0.57**	-0.12	**-0.29**	**-0.33**	**0.85**	-																
L Na/Cl	-0.13	**-0.67**	**-0.30**	-0.22	**0.73**	**0.65**	-															
L Na/K	-0.12	-0.13	**-0.69**	0.01	**0.85**	**0.75**	**0.67**	-														
L Na/Mg	-0.21	0.19	0.19	**-0.68**	0.07	0.16	-0.07	-0.04	-													
L PO_4	**0.33**	0.15	0.25	**0.31**	0.15	-0.03	0.07	-0.03	-0.07	-												
L SO_4	**0.37**	**0.32**	-0.05	**0.39**	0.00	-0.17	-0.21	-0.02	-0.01	**0.32**	-											
R Ca	**0.25**	**0.41**	-0.10	**0.50**	0.04	-0.06	-0.20	0.06	-0.22	0.17	**0.37**	-										
R Cl	-0.19	-0.06	0.10	-0.19	0.19	0.21	0.22	0.06	0.00	0.15	-0.07	0.17	-									
R K	0.05	**-0.52**	-0.05	-0.13	0.12	0.05	**0.42**	0.09	-0.11	0.04	0.05	**-0.41**	0.01	-								
R Mg2	0.22	0.13	-0.09	**0.35**	-0.02	-0.08	-0.04	0.04	-0.12	0.14	0.16	**0.57**	0.00	-0.11	-							
R Na	-0.08	-0.04	-0.13	0.06	**0.29**	**0.27**	0.25	0.25	-0.15	0.17	0.07	**0.46**	**0.74**	-0.11	0.22	-						
R Na/Ca	**-0.33**	**-0.37**	-0.08	**-0.43**	0.12	0.23	**0.36**	0.11	0.13	-0.09	**-0.31**	**-0.69**	0.19	**0.30**	**-0.40**	0.05	-					
R Na/Cl	**0.37**	-0.02	-0.13	**0.35**	-0.17	**-0.27**	-0.15	-0.02	-0.16	-0.06	0.16	0.03	**-0.82**	0.02	0.22	**-0.35**	**-0.26**	-				
R Na/K	-0.05	**0.34**	-0.09	0.19	0.12	0.14	-0.10	0.11	-0.07	0.12	0.09	**0.62**	**0.51**	**-0.66**	0.19	**0.78**	-0.15	**-0.26**	-			
R Na/Mg	-0.01	0.20	0.02	0.04	-0.11	-0.09	-0.19	-0.11	-0.05	-0.01	0.04	0.12	0.19	-0.29	**-0.60**	0.14	-0.08	**-0.26**	**0.38**	-		
R PO_4	-0.22	**-0.28**	0.01	**-0.30**	-0.03	0.05	0.17	-0.03	0.17	-0.01	**-0.31**	**-0.73**	-0.10	**0.62**	**-0.35**	**-0.35**	**0.62**	-0.08	**-0.63**	-0.19	-	
R SO_4	-0.10	-0.24	**-0.29**	-0.10	0.17	0.18	0.23	**0.26**	-0.07	-0.21	0.08	0.04	0.08	**0.49**	0.09	0.20	0.03	0.02	-0.16	-0.13	0.24	-

[1]Leaf, [2] Root, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

Appendix 4. Correlation analysis between ion content in the stem and leaf of the genotypes grown under control condition.

Ions	S[1] Ca	S Cl	S K	S Mg2	S Na	S Na/Ca	S Na/Cl	S Na/K	S Na/Mg	S PO4	S SO4	L[2] Ca	L Cl	L K	L Mg2	L Na	L Na/Ca	L Na/Cl	L Na/K	L Na/Mg	L PO4	L SO4
S Ca	-																					
S Cl	0.15	-																				
S K	-0.04	0.22	-																			
S Mg2	-0.05	**-0.26**	-0.05	-																		
S Na	**0.42**	-0.03	0.00	0.19	-																	
S Na/Ca	**-0.46**	-0.15	-0.01	0.16	-0.05	-																
S Na/Cl	-0.16	**-0.72**	-0.17	0.04	0.12	0.06	-															
S Na/K	**0.35**	-0.16	**-0.57**	0.20	**0.81**	-0.04	0.18	-														
S Na/Mg	0.16	-0.05	-0.07	**-0.41**	0.08	-0.08	0.11	0.09	-													
S PO4	-0.03	-0.20	**0.34**	0.24	0.16	-0.01	0.18	-0.05	-0.06	-												
S SO4	0.18	-0.03	**0.28**	0.21	0.21	-0.08	0.01	0.03	-0.08	**0.69**	-											
L Ca	**0.42**	0.25	-0.04	0.25	0.03	-0.20	-0.23	0.05	-0.03	0.15	**0.41**	-										
L Cl	0.10	**0.33**	-0.20	**0.27**	-0.11	-0.16	**-0.28**	0.03	-0.01	0.01	0.12	0.12	-									
L K	0.12	0.03	0.14	0.08	0.06	-0.01	0.02	-0.02	-0.07	**0.29**	0.12	0.10	0.13	-								
L Mg2	-0.08	-0.02	0.11	-0.22	0.20	0.24	0.08	0.07	0.17	-0.03	-0.05	**0.50**	**0.27**	**-0.26**	-							
L Na	0.04	0.09	0.00	0.03	0.22	0.16	0.04	0.14	0.04	0.12	0.12	-0.10	-0.09	**-0.28**	-0.14	-						
L Na/Ca	-0.21	-0.16	-0.01	-0.09	0.23	**0.35**	**0.32**	0.16	-0.04	-0.07	-0.21	**-0.57**	-0.12	**-0.29**	**-0.33**	**0.85**	-					
L Na/Cl	-0.21	**-0.27**	0.06	-0.19	0.08	0.23	0.22	0.03	-0.07	-0.15	-0.18	-0.13	**-0.67**	**-0.30**	-0.22	**0.73**	**0.65**	-				
L Na/K	-0.03	0.04	-0.07	-0.03	0.13	0.12	0.02	0.10	0.05	-0.08	0.02	-0.12	-0.13	**-0.69**	0.01	**0.85**	**0.75**	**0.67**	-			
L Na/Mg	0.05	-0.17	-0.07	0.12	0.08	0.03	**0.27**	0.10	-0.08	0.15	0.08	-0.21	0.19	0.19	**-0.68**	0.07	0.16	-0.07	-0.04	-		
L PO4	0.20	-0.24	-0.08	0.12	0.02	-0.08	0.15	0.01	-0.15	**0.37**	0.20	**0.33**	0.15	0.25	**0.31**	0.15	-0.03	0.07	-0.03	-0.07	-	
L SO4	0.20	0.03	-0.02	0.14	-0.01	-0.08	-0.05	-0.02	-0.08	0.25	**0.50**	**0.37**	**0.32**	-0.05	**0.39**	0.00	-0.17	-0.21	-0.02	-0.01	**0.32**	-

[1]Stem, [2] Leaf, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

Appendix 5. Correlation analysis between ion content in the shoot and root of the genotypes grown under control condition.

Ions	Sh[1] Ca	Sh Cl	Sh K	Sh Mg2	Sh Na	Sh Na/Ca	Sh Na/Cl	Sh Na/K	Sh Na/Mg	Sh PO4	Sh SO4	R[2] Ca	R Cl	R K	R Mg2	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4
Sh Ca	-																					
Sh Cl	0.24	-																				
Sh K	0.01	0.09	-																			
Sh Mg2	0.18	0.04	-0.03	-																		
Sh Na	0.19	-0.07	-0.07	0.18	-																	
Sh Na/Ca	**-0.74**	**-0.29**	-0.12	0.02	**0.39**	-																
Sh Na/Cl	-0.24	**-0.81**	-0.15	-0.04	**0.49**	**0.56**	-															
Sh Na/K	0.11	-0.08	**-0.54**	0.13	**0.82**	**0.41**	**0.46**	-														
Sh Na/Mg	-0.02	-0.10	-0.06	**-0.74**	0.37	0.17	**0.31**	**0.31**	-													
Sh PO4	0.17	-0.12	**0.34**	**0.29**	0.17	-0.03	0.15	-0.04	-0.15	-												
Sh SO4	**0.40**	0.17	0.15	**0.28**	0.10	-0.23	-0.11	0.00	-0.16	**0.50**	-											
R Ca	**0.29**	-0.17	-0.16	0.09	-0.04	**-0.31**	0.25	0.08	0.09	**-0.28**	-0.01	-										
R Cl	**0.42**	-0.07	**-0.45**	**0.26**	0.02	**-0.38**	0.13	**0.32**	0.10	**-0.28**	-0.12	0.17	-									
R K	0.08	0.11	0.05	0.01	0.08	-0.14	-0.01	0.03	0.15	0.00	-0.14	**-0.41**	0.01	-								
R Mg2	**0.28**	-0.17	-0.09	0.16	-0.10	**-0.39**	0.12	0.00	-0.06	-0.19	-0.04	**0.57**	0.00	-0.11	-							
R Na	0.11	0.17	-0.05	-0.05	**0.30**	0.03	-0.18	0.22	0.01	-0.11	0.16	0.46	**0.74**	-0.11	0.22	-						
R Na/Ca	-0.11	0.13	0.08	-0.04	0.22	0.23	-0.16	0.10	-0.08	0.09	0.10	**-0.69**	0.19	**0.30**	**-0.40**	0.05	-					
R Na/Cl	**-0.27**	0.20	**0.33**	-0.19	0.16	**0.40**	-0.23	-0.10	-0.08	0.20	0.15	0.03	**-0.82**	0.02	0.22	**-0.35**	**-0.26**	-				
R Na/K	0.06	0.06	-0.02	-0.01	0.22	0.09	-0.09	0.15	-0.08	-0.08	0.24	**0.62**	**0.51**	**-0.66**	0.19	**0.78**	-0.15	**-0.26**	-			
R Na/Mg	-0.23	0.23	0.00	-0.18	0.25	**0.46**	-0.21	0.17	0.03	0.14	0.02	0.12	0.19	-0.29	**-0.60**	0.14	-0.08	**-0.26**	**0.38**	-		
R PO4	0.14	0.15	0.11	0.11	0.13	-0.04	-0.09	0.04	0.00	0.04	-0.09	**-0.73**	-0.10	**0.62**	**-0.35**	**-0.35**	**0.62**	-0.08	**-0.63**	-0.19	-	
R SO4	**0.39**	-0.01	-0.02	0.20	0.12	**-0.30**	0.12	0.13	0.04	**-0.33**	0.13	0.04	0.08	**0.49**	0.09	0.20	0.03	0.02	-0.16	-0.13	0.24	-

[1]Shoot, [2] Root, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes. The shoot ion content is calculated as the mean of the ions in the leaf and stem.

Appendix 6. Correlation analysis between ion content in the root and stem of the genotypes grown under salinity condition.

Ions	R[1] Ca	R Cl	R K	R Mg2	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4	S[2] Ca	S Cl	S K	S Mg2	S Na	S Na/Ca	S Na/Cl	S Na/K	S Na/Mg	S PO4	S SO4
R Ca	-																					
R Cl	**-0.39**	-																				
R K	**-0.48**	**0.65**	-																			
R Mg2	**0.56**	0.00	-0.07	-																		
R Na	0.04	**0.44**	-0.21	-0.21	-																	
R Na/Ca	**-0.78**	**0.50**	**0.33**	**-0.54**	**0.33**	-																
R Na/Cl	**0.42**	**-0.45**	**-0.77**	-0.19	**0.59**	-0.13	-															
R Na/K	**0.42**	-0.24	**-0.81**	-0.03	**0.66**	-0.09	**0.88**	-														
R Na/Mg	**-0.32**	0.23	-0.08	**-0.81**	**0.70**	**0.55**	**0.47**	**0.39**	-													
R PO4	**-0.37**	0.02	**0.25**	-0.18	-0.16	0.23	-0.18	**-0.29**	0.00	-												
R SO4	-0.01	0.14	0.20	-0.11	**0.43**	0.16	**0.32**	0.06	**0.32**	0.06	-											
S Ca	-0.09	0.11	-0.12	-0.05	0.23	0.07	0.12	0.17	0.11	-0.04	0.16	-										
S Cl	-0.14	0.20	-0.05	-0.21	**0.30**	0.21	0.08	0.12	**0.30**	-0.05	-0.03	**0.48**	-									
S K	**-0.29**	0.07	0.08	**-0.35**	0.17	0.24	0.08	0.02	**0.31**	0.17	-0.04	0.11	**0.45**	-								
S Mg2	-0.10	0.03	0.09	0.12	-0.20	0.09	-0.24	-0.22	-0.16	0.25	-0.08	0.08	0.10	-0.21	-							
S Na	**0.32**	-0.07	**-0.33**	-0.03	**0.40**	-0.13	**0.43**	**0.40**	0.25	-0.14	0.17	0.08	**0.39**	0.02	-0.12	-						
S Na/Ca	**0.34**	-0.17	-0.21	0.02	0.16	-0.17	**0.29**	0.22	0.11	-0.09	0.04	**-0.58**	0.00	-0.06	-0.13	**0.74**	-					
S Na/Cl	**0.45**	-0.23	**-0.31**	0.15	0.20	**-0.30**	**0.40**	**0.34**	0.02	-0.12	0.21	-0.22	**-0.31**	**-0.31**	-0.18	**0.75**	**0.75**	-				
S Na/K	**0.52**	-0.13	**-0.27**	**0.36**	0.09	**-0.32**	0.21	0.23	-0.15	-0.23	0.11	-0.10	-0.14	**-0.72**	0.06	**0.57**	**0.55**	0.72	-			
S Na/Mg	0.23	-0.04	-0.23	-0.12	**0.38**	-0.14	**0.41**	**0.40**	**0.28**	**-0.29**	0.14	0.01	0.12	0.23	**-0.82**	**0.55**	**0.41**	**0.46**	0.15	-		
S PO4	0.14	-0.03	-0.08	-0.05	0.07	-0.05	0.11	0.09	0.10	0.15	-0.11	0.05	0.07	0.15	**0.31**	0.13	0.11	0.11	0.00	-0.15	-	
S SO4	**0.31**	-0.16	-0.18	-0.10	**0.25**	-0.14	**0.39**	0.24	0.23	-0.03	**0.34**	0.06	0.17	0.03	0.22	**0.48**	**0.38**	**0.40**	**0.28**	0.09	**0.43**	-

[1]Root, [2]Stem, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ions are calculated in mg/g of organ of the genotypes.

Appendix 7. Correlation analysis between ion content in the leaf and root of the genotypes grown under salinity condition.

Ions	L[1] Ca	L Cl	L K	L Mg2	L Na	L Na/Ca	L Na/Cl	L Na/K	L Na/Mg	L PO4	L SO4	R[2] Ca	R Cl	R K	R Mg2	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4
L Ca	-																					
L Cl	**0.59**	-																				
L K	**0.27**	**0.36**	-																			
L Mg2	**0.65**	**0.33**	0.15	-																		
L Na	0.03	**0.47**	**-0.46**	-0.06	-																	
L Na/Ca	**-0.49**	0.09	**-0.47**	**-0.37**	**0.81**	-																
L Na/Cl	**-0.36**	-0.18	**-0.75**	**-0.28**	**0.77**	**0.81**	-															
L Na/K	-0.11	0.14	**-0.75**	-0.11	**0.89**	**0.77**	**0.86**	-														
L Na/Mg	-0.04	-0.12	-0.17	**-0.49**	0.05	0.04	0.15	0.11	-													
L PO4	**0.48**	**0.39**	0.15	**0.43**	0.19	-0.07	-0.04	0.03	-0.17	-												
L SO4	0.08	0.02	-0.09	-0.07	**0.27**	0.13	**0.29**	**0.27**	0.16	0.15	-											
R Ca	**-0.32**	-0.03	-0.21	-0.20	**0.30**	**0.45**	**0.33**	**0.30**	-0.11	-0.03	-0.18	-										
R Cl	0.25	0.19	**0.34**	0.11	-0.20	**-0.31**	**-0.37**	**-0.28**	-0.10	0.13	-0.02	**-0.39**	-									
R K	0.15	-0.06	0.23	0.09	**-0.27**	**-0.32**	**-0.29**	**-0.26**	-0.15	0.16	0.08	**-0.48**	**0.65**	-								
R Mg2	**-0.33**	-0.18	-0.19	-0.11	0.09	**0.31**	0.20	0.16	-0.16	-0.12	**-0.25**	**0.56**	0.00	-0.07	-							
R Na	0.11	0.24	**0.35**	-0.05	-0.06	-0.11	-0.22	-0.22	-0.05	0.02	0.05	0.04	**0.44**	-0.21	-0.21	-						
R Na/Ca	**0.32**	0.21	**0.33**	0.13	-0.18	**-0.32**	**-0.33**	**-0.28**	0.12	0.09	0.24	**-0.78**	**0.50**	**0.33**	**-0.54**	**0.33**	-					
R Na/Cl	-0.11	0.08	0.07	-0.16	0.12	0.17	0.09	0.03	0.03	-0.08	0.08	**0.42**	**-0.45**	**-0.77**	-0.19	**0.59**	-0.13	-				
R Na/K	-0.03	0.22	0.13	-0.08	0.13	0.13	0.01	0.02	0.07	-0.06	-0.06	**0.42**	-0.24	**-0.81**	-0.03	**0.66**	-0.09	**0.88**	-			
R Na/Mg	**0.30**	**0.29**	**0.31**	0.05	-0.06	-0.23	-0.24	-0.19	0.10	0.10	0.22	**-0.32**	0.23	-0.08	**-0.81**	**0.70**	**0.55**	**0.47**	**0.39**	-		
R PO4	0.14	-0.04	-0.03	0.21	0.01	-0.05	0.05	0.04	-0.07	0.10	0.18	**-0.37**	0.02	**0.25**	-0.18	-0.16	0.23	-0.18	**-0.29**	0.00	-	
R SO4	-0.04	-0.12	0.17	-0.22	-0.19	-0.15	-0.14	-0.20	-0.08	-0.07	**0.33**	-0.01	0.14	0.20	-0.11	**0.43**	0.16	**0.32**	0.06	**0.32**	0.06	-

[1]Leaf, [2] Root, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

Appendix 8. Correlation analysis between ion content in the stem and leaf of the genotypes grown under salinity condition.

Ions	S[1] Ca	S Cl	S K	S Mg2	S Na	S Na/Ca	S Na/Cl	S Na/K	S Na/Mg	S PO4	S SO4	L[2] Ca	L Cl	L K	L Mg2	L Na	L Na/Ca	L Na/Cl	L Na/K	L Na/Mg	L PO4	L SO4
S Ca	-																					
S Cl	**0.48**	-																				
S K	0.11	**0.45**	-																			
S Mg2	0.08	0.10	-0.21	-																		
S Na	0.08	**0.39**	0.02	-0.12	-																	
S Na/Ca	**-0.58**	0.00	-0.06	-0.13	**0.74**	-																
S Na/Cl	-0.22	**-0.31**	**-0.31**	-0.18	**0.75**	**0.75**	-															
S Na/K	-0.10	-0.14	**-0.72**	0.06	**0.57**	**0.55**	**0.72**	-														
S Na/Mg	0.01	0.12	0.23	**-0.82**	**0.55**	**0.41**	**0.46**	0.15	-													
S PO4	0.05	0.07	0.15	**0.31**	0.13	0.11	0.11	0.00	-0.15	-												
S SO4	0.06	0.17	0.03	0.22	**0.48**	**0.38**	**0.40**	**0.28**	0.09	**0.43**	-											
L Ca	0.24	0.12	0.05	-0.04	-0.04	-0.17	-0.09	-0.06	0.00	-0.10	0.17	-										
L Cl	**0.34**	**0.35**	0.05	0.05	0.16	-0.07	-0.06	0.07	-0.11	0.02	0.17	**0.59**	-									
L K	0.13	-0.07	**0.31**	0.06	**-0.27**	**-0.30**	-0.23	**-0.31**	-0.11	0.04	0.12	**0.27**	**0.36**	-								
L Mg2	0.10	0.05	-0.20	**0.27**	0.17	0.08	0.16	0.21	-0.01	0.00	**0.35**	**0.65**	**0.33**	0.15	-							
L Na	0.00	0.03	-0.09	-0.10	0.21	0.20	0.19	0.20	-0.06	-0.15	0.08	0.03	**0.47**	**-0.46**	-0.06	-						
L Na/Ca	-0.19	-0.05	-0.12	-0.07	0.22	**0.32**	0.24	0.23	-0.06	-0.06	-0.04	**-0.49**	0.09	**-0.47**	**-0.37**	**0.81**	-					
L Na/Cl	-0.25	-0.23	-0.13	-0.15	0.08	**0.26**	0.23	0.15	0.02	-0.17	-0.03	**-0.36**	-0.18	**-0.75**	**-0.28**	**0.77**	**0.81**	-				
L Na/K	-0.18	0.09	**-0.28**	-0.17	**0.42**	**0.53**	**0.35**	**0.42**	0.03	-0.13	-0.06	-0.11	0.14	**-0.75**	-0.11	**0.89**	**0.77**	**0.86**	-			
L Na/Mg	-0.02	-0.01	0.13	-0.23	-0.08	-0.03	-0.09	-0.10	-0.05	-0.03	-0.24	-0.04	-0.12	-0.17	**-0.49**	0.05	0.04	0.15	0.11	-		
L PO4	0.19	0.05	-0.13	0.24	0.23	0.12	0.23	0.22	0.00	**0.33**	0.23	**0.48**	**0.39**	0.15	**0.43**	0.19	-0.07	-0.04	0.03	-0.17	-	
L SO4	-0.06	0.01	-0.21	-0.14	**0.29**	**0.28**	**0.30**	**0.29**	0.01	-0.09	**0.40**	0.08	0.02	-0.09	-0.07	**0.27**	0.13	**0.29**	**0.27**	0.16	0.15	-

[1]Stem, [2] Leaf, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

Appendix 9. Correlation analysis between ion content in the shoot and root of the genotypes grown under salinity condition.

Ions	Sh[1] Ca	Sh Cl	Sh K	Sh Mg2	Sh Na	Sh Na/Ca	Sh Na/Cl	Sh Na/K	Sh Na/Mg	Sh PO4	Sh SO4	R[2] Ca	R Cl	R K	R Mg2	R Na	R Na/Ca	R Na/Cl	R Na/K	R Na/Mg	R PO4	R SO4
Sh Ca	-																					
Sh Cl	**0.60**	-																				
Sh K	0.22	**0.28**	-																			
Sh Mg2	**0.32**	0.19	-0.12	-																		
Sh Na	0.02	**0.42**	**-0.35**	0.00	-																	
Sh Na/Ca	**-0.55**	0.01	**-0.41**	-0.18	**0.80**	-																
Sh Na/Cl	**-0.39**	**-0.26**	**-0.57**	-0.13	**0.76**	**0.84**	-															
Sh Na/K	-0.15	0.10	**-0.70**	0.06	**0.84**	**0.78**	**0.82**	-														
Sh Na/Mg	-0.21	0.10	-0.14	**-0.74**	**0.63**	**0.65**	**0.60**	**0.50**	-													
Sh PO4	**0.28**	0.21	0.09	**0.35**	0.19	0.01	0.06	0.09	-0.13	-												
Sh SO4	0.09	0.13	-0.02	0.18	**0.41**	**0.28**	**0.37**	**0.31**	0.12	0.24	-											
R Ca	**-0.28**	0.23	0.04	**-0.27**	0.20	**0.26**	-0.01	0.07	**0.28**	0.08	0.06	-										
R Cl	-0.10	0.24	-0.06	-0.24	**0.32**	**0.26**	0.10	0.21	**0.35**	-0.05	-0.09	**-0.39**	-									
R K	**-0.31**	0.22	0.17	**-0.35**	**0.30**	**0.34**	0.10	0.08	**0.38**	0.11	0.05	**-0.48**	**0.65**	-								
R Mg2	-0.18	0.08	0.11	0.03	-0.17	0.14	**-0.26**	-0.20	-0.09	**0.29**	-0.18	**0.56**	0.00	-0.07	-							
R Na	**0.39**	-0.19	**-0.37**	0.05	0.14	-0.21	**0.30**	**0.29**	0.07	-0.06	-0.07	0.04	**0.44**	-0.21	-0.21	-						
R Na/Ca	**0.50**	**-0.30**	**-0.33**	**0.26**	0.00	**-0.33**	**0.26**	0.20	-0.13	-0.08	-0.08	**-0.78**	**0.50**	**0.33**	**-0.54**	**0.33**	-					
R Na/Cl	**0.48**	**-0.37**	**-0.36**	0.23	-0.07	**-0.39**	**0.26**	0.17	-0.17	-0.02	0.01	**0.42**	**-0.45**	**-0.77**	-0.19	**0.59**	-0.13	-				
R Na/K	**0.41**	**-0.27**	**-0.30**	0.24	-0.15	**-0.33**	0.09	0.09	-0.21	-0.04	-0.13	**0.42**	-0.24	**-0.81**	-0.03	**0.66**	-0.09	**0.88**	-			
R Na/Mg	**0.37**	-0.20	**-0.35**	0.02	0.22	-0.23	**0.40**	**0.36**	0.11	**-0.26**	0.07	**-0.32**	0.23	-0.08	**-0.81**	**0.70**	**0.55**	**0.47**	**0.39**	-		
R PO4	0.06	0.07	0.06	-0.11	0.05	0.03	0.01	0.01	0.12	0.15	-0.11	**-0.37**	0.02	**0.25**	-0.18	-0.16	0.23	-0.18	**-0.29**	0.00	-	
R SO4	0.06	-0.10	-0.05	-0.21	0.17	0.08	**0.27**	0.10	**0.27**	0.10	**0.40**	-0.01	0.14	0.20	-0.11	**0.43**	0.16	**0.32**	0.06	**0.32**	0.06	-

[1]Shoot, [2] Root, [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes. The shoot ion content is calculated as the mean of the ions in the leaf and stem.

Appendix 10. Correlation analysis between ions contained in the root and the growth parameters of the genotypes grown under control condition.

	CC LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm UL[5]	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.71**	-													
CC-UL	**0.48**	**0.26**	-												
Fv/Fm-UL	0.05	-0.11	-0.09	-											
Fv/Fm-LL	**0.29**	**-0.46**	-0.13	0.13	-										
LA_cm	0.19	**-0.28**	-0.04	-0.20	0.21	-									
Rfw	0.19	-0.18	0.07	-0.01	0.15	**0.78**	-								
RL	0.16	-0.24	-0.03	-0.07	**0.43**	**0.62**	**0.64**	-							
Rdw	0.18	-0.16	0.08	-0.11	0.12	**0.80**	**0.94**	**0.57**	-						
Rfw/Sfw	-0.02	0.01	-0.07	0.22	-0.08	-0.09	**0.36**	0.09	**0.26**	-					
Rdw/Sdw	-0.23	**0.37**	0.07	0.02	**-0.46**	**-0.33**	0.01	**-0.43**	0.06	**0.49**	-				
Sfw	0.21	-0.14	0.15	**-0.25**	0.18	**0.92**	**0.71**	**0.61**	**0.74**	**-0.26**	**-0.36**	-			
SL	**0.32**	-0.21	0.23	-0.22	0.19	**0.57**	**0.31**	**0.42**	**0.36**	**-0.37**	**-0.43**	0.70	-		
Sdw	0.23	-0.21	0.08	-0.22	0.25	**0.94**	**0.75**	**0.71**	**0.77**	-0.08	**-0.45**	**0.94**	**0.64**	-	
RL/SL	-0.01	-0.17	-0.19	0.10	**0.31**	0.21	**0.40**	**0.69**	**0.33**	**0.35**	-0.14	0.11	**-0.31**	**0.25**	-
R[0] Ca	-0.08	0.13	0.09	0.01	**-0.33**	-0.10	-0.02	**-0.30**	-0.01	0.09	**0.31**	-0.09	-0.09	-0.16	-0.23
R Cl	-0.10	0.03	-0.11	-0.17	-0.14	0.14	0.14	0.12	0.19	-0.02	-0.01	0.14	-0.03	0.13	0.12
R K	0.07	-0.12	-0.07	0.06	0.08	0.22	**0.35**	**0.47**	0.23	0.13	-0.12	0.22	0.05	0.24	**0.41**
R Mg	0.01	0.11	0.15	-0.06	-0.16	-0.08	0.01	-0.15	0.01	0.14	**0.35**	-0.05	0.00	-0.10	-0.16
R Na	-0.10	0.06	-0.05	-0.21	-0.23	0.03	0.09	0.03	0.11	0.12	0.05	0.02	-0.07	0.04	0.09
R Na/Ca	-0.14	-0.05	**-0.27**	-0.15	0.19	-0.04	-0.10	0.23	-0.09	-0.13	**-0.26**	-0.05	0.01	0.04	0.23
R Na/Cl	**0.27**	-0.11	0.23	0.13	0.06	-0.07	0.00	-0.05	-0.07	0.16	0.04	-0.09	0.04	-0.04	-0.03
R Na/K	-0.12	0.15	0.05	-0.14	**-0.27**	-0.12	-0.15	**-0.27**	-0.08	-0.02	0.09	-0.12	-0.07	-0.13	-0.20
R Na/Mg	-0.14	0.06	-0.09	0.11	-0.24	-0.09	-0.14	-0.20	-0.15	-0.15	-0.18	-0.10	-0.13	-0.13	-0.11
R PO4	0.10	-0.11	-0.05	0.03	0.22	-0.08	-0.13	**0.25**	-0.21	-0.16	**-0.28**	-0.03	0.07	0.00	0.21
R SO4	-0.16	0.10	-0.11	0.08	-0.11	0.05	0.16	**0.25**	0.10	0.13	0.08	0.05	-0.24	0.04	**0.46**

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

Appendix 11. Correlation analysis between ions contained in the stem and the growth parameters of the genotypes grown under control condition.

	CC LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm UL[5]	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.71**	-													
CC-UL	**0.48**	**0.26**	-												
Fv/Fm-UL	0.05	-0.11	-0.09	-											
Fv/Fm-LL	**0.29**	**-0.46**	-0.13	0.13	-										
LA_cm	0.19	**-0.28**	-0.04	-0.20	0.21	-									
Rfw	0.19	-0.18	0.07	-0.01	0.15	**0.78**	-								
RL	0.16	-0.24	-0.03	-0.07	**0.43**	**0.62**	**0.64**	-							
Rdw	0.18	-0.16	0.08	-0.11	0.12	**0.80**	**0.94**	**0.57**	-						
Rfw/Sfw	-0.02	0.01	-0.07	0.22	-0.08	-0.09	**0.36**	0.09	**0.26**	-					
Rdw/Sdw	-0.23	**0.37**	0.07	0.02	**-0.46**	**-0.33**	0.01	**-0.43**	0.06	**0.49**	-				
Sfw	0.21	-0.14	0.15	**-0.25**	0.18	**0.92**	**0.71**	**0.61**	**0.74**	**-0.26**	**-0.36**	-			
SL	**0.32**	-0.21	0.23	-0.22	0.19	**0.57**	**0.31**	**0.42**	**0.36**	**-0.37**	**-0.43**	**0.70**	-		
Sdw	0.23	-0.21	0.08	-0.22	0.25	**0.94**	**0.75**	**0.71**	**0.77**	-0.08	**-0.45**	**0.94**	**0.64**	-	
RL/SL	-0.01	-0.17	-0.19	0.10	**0.31**	0.21	**0.40**	**0.69**	**0.33**	**0.35**	-0.14	0.11	**-0.31**	**0.25**	-
S Ca	-0.22	**0.32**	0.13	0.03	-0.04	0.02	0.05	-0.08	0.05	0.14	0.17	0.03	-0.10	0.02	0.01
S Cl	0.06	-0.05	-0.02	0.06	-0.18	-0.10	-0.10	-0.19	-0.11	0.11	**0.28**	-0.16	0.00	-0.17	-0.21
S K	0.13	-0.10	0.10	0.07	-0.13	0.10	0.03	0.13	-0.02	-0.14	**-0.26**	0.11	0.20	0.10	-0.06
S Mg	0.08	-0.21	-0.13	0.21	0.24	0.01	0.03	0.03	0.00	0.12	-0.05	-0.04	-0.13	-0.06	0.20
S Na	-0.13	0.01	-0.11	0.00	0.12	0.01	-0.06	0.09	-0.08	0.07	-0.25	-0.05	0.06	0.01	0.03
S Na/Ca	-0.03	-0.05	-0.08	-0.15	-0.05	-0.05	-0.02	0.12	-0.03	0.06	0.00	-0.07	0.01	-0.06	0.08
S Na/Cl	-0.10	0.08	0.00	-0.12	0.21	-0.02	-0.08	0.08	-0.05	-0.20	**-0.27**	0.03	-0.08	0.05	0.14
S Na/K	-0.18	0.05	-0.17	0.00	0.14	-0.07	-0.08	0.00	-0.07	0.14	-0.04	-0.12	-0.07	-0.08	0.06
S Na/Mg	0.04	0.10	0.20	**-0.28**	-0.08	0.19	0.18	0.13	0.17	0.02	0.02	0.15	0.06	0.17	0.08
S PO4	0.17	-0.24	0.04	0.01	0.12	-0.10	-0.05	0.10	-0.03	-0.17	-0.23	-0.08	0.06	-0.11	0.14
S SO4	0.12	-0.11	0.11	0.06	-0.01	-0.13	-0.09	-0.07	0.00	-0.18	0.01	-0.11	-0.03	-0.20	0.04

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

Appendix 12. Correlation analysis between ions contained in the leaf and the growth parameters of the genotypes grown under control condition.

	CC[1] LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm[5] UL	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.71**	-													
CC-UL	**0.48**	**0.26**	-												
Fv/Fm-UL	0.05	-0.11	-0.09	-											
Fv/Fm-LL	**0.29**	**-0.46**	-0.13	0.13	-										
LA	0.19	**-0.28**	-0.04	-0.20	0.21	-									
Rfw	0.19	-0.18	0.07	-0.01	0.15	**0.78**	-								
RL	0.16	-0.24	-0.03	-0.07	**0.43**	**0.62**	**0.64**	-							
Rdw	0.18	-0.16	0.08	-0.11	0.12	**0.80**	**0.94**	**0.57**	-						
Rfw/Sfw	-0.02	0.01	-0.07	0.22	-0.08	-0.09	**0.36**	0.09	**0.26**	-					
Rdw/Sdw	-0.23	**0.37**	0.07	0.02	**-0.46**	**-0.33**	0.01	**-0.43**	0.06	**0.49**	-				
Sfw	0.21	-0.14	0.15	**-0.25**	0.18	**0.92**	**0.71**	**0.61**	**0.74**	**-0.26**	**-0.36**	-			
SL	**0.32**	-0.21	0.23	-0.22	0.19	**0.57**	**0.31**	**0.42**	**0.36**	**-0.37**	**-0.43**	**0.70**	-		
Sdw	0.23	-0.21	0.08	-0.22	0.25	**0.94**	**0.75**	**0.71**	**0.77**	-0.08	**-0.45**	**0.94**	**0.64**	-	
RL/SL	-0.01	-0.17	-0.19	0.10	**0.31**	0.21	**0.40**	**0.69**	**0.33**	**0.35**	-0.14	0.11	**-0.31**	**0.25**	-
L Ca	0.03	0.04	0.13	0.05	-0.11	0.00	0.12	-0.13	**0.05**	0.15	0.20	0.00	-0.07	-0.05	-0.04
L Cl	-0.14	**0.35**	0.23	0.10	**-0.33**	**-0.36**	**-0.35**	**-0.51**	**-0.28**	-0.04	**0.38**	**-0.31**	-0.14	**-0.38**	**-0.47**
L K	0.23	-0.16	0.12	-0.06	0.10	**0.33**	0.20	0.07	**0.29**	-0.18	-0.16	**0.41**	**0.44**	**0.35**	-0.23
L Mg	0.09	0.15	**0.32**	0.08	-0.24	**-0.25**	-0.20	**-0.33**	-0.24	0.02	0.21	-0.21	-0.14	**-0.25**	-0.21
L Na	-0.10	0.00	-0.11	-0.25	-0.01	0.15	0.19	**0.29**	0.12	0.13	-0.01	0.14	0.04	0.16	0.18
L Na/Ca	-0.07	-0.02	-0.11	-0.23	0.02	0.09	0.06	0.24	0.05	0.01	-0.10	0.09	0.04	0.12	0.14
L Na/Cl	0.02	-0.22	-0.22	**-0.28**	0.12	**0.29**	**0.33**	**0.45**	0.23	0.12	-0.17	0.23	0.08	**0.27**	**0.36**
L Na/K	-0.15	0.09	-0.08	-0.15	-0.02	-0.02	0.07	0.18	-0.01	0.20	0.07	-0.06	-0.16	-0.01	0.23
L Na/Mg	-0.11	0.02	-0.11	-0.08	0.11	0.08	0.05	0.13	0.07	-0.07	-0.08	0.07	0.08	0.06	0.08
L PO4	0.19	0.08	**0.39**	-0.18	-0.11	-0.03	0.06	-0.04	0.07	-0.06	0.08	0.09	**0.27**	-0.02	-0.25
L SO4	-0.05	0.19	0.20	0.11	**-0.26**	-0.03	0.15	0.01	0.17	0.11	**0.30**	-0.01	-0.08	-0.09	0.06

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

Appendix 13. Correlation analysis between ions contained in the root and the growth parameters of the genotypes grown under salinity condition.

	CC LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm UL[5]	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.58**	-													
CC-UL	**0.60**	**0.30**	-												
Fv/Fm-UL	**0.27**	-0.05	0.25	-											
Fv/Fm-LL	**0.36**	**-0.32**	0.09	**0.48**	-										
LA	-0.02	-0.03	-0.04	0.14	**0.31**	-									
Rfw	0.07	0.04	0.12	0.19	**0.28**	**0.71**	-								
RL	0.10	0.00	0.11	0.21	**0.40**	**0.57**	**0.60**	-							
Rdw	0.04	0.09	0.14	0.18	**0.26**	**0.73**	**0.97**	**0.55**	-						
Rfw/Sfw	0.05	-0.01	0.03	0.10	0.15	-0.08	**0.54**	0.22	**0.46**	-					
Rdw/Sdw	-0.12	0.12	-0.03	-0.03	-0.07	-0.19	**0.32**	-0.09	**0.33**	0.74	-				
Sfw	0.08	0.04	0.15	0.13	**0.26**	**0.92**	**0.80**	**0.58**	**0.81**	-0.04	-0.14	-			
SL	**0.38**	-0.13	**0.32**	0.24	**0.30**	**0.47**	**0.41**	**0.37**	**0.39**	-0.01	**-0.28**	**0.55**	-		
Sdw	0.08	0.00	0.11	0.11	**0.26**	**0.92**	**0.73**	**0.57**	**0.75**	-0.09	**-0.28**	**0.97**	**0.58**	-	
RL/SL	-0.13	0.11	-0.06	0.08	0.23	**0.25**	**0.33**	**0.76**	**0.30**	0.24	0.07	0.22	**-0.28**	0.19	-
R Ca	**-0.26**	0.04	-0.24	-0.14	**-0.29**	**-0.36**	**-0.41**	**-0.49**	**-0.37**	-0.21	0.06	**-0.37**	**-0.48**	**-0.38**	-0.17
R Cl	0.09	0.02	0.11	0.08	0.22	**0.27**	**0.56**	**0.32**	**0.46**	0.51	0.28	**0.32**	0.18	**0.25**	0.22
R K	0.09	0.06	0.14	0.11	0.23	**0.34**	**0.48**	**0.31**	**0.40**	**0.31**	0.05	**0.39**	**0.35**	**0.38**	0.09
R Mg	-0.11	0.13	0.02	-0.07	**-0.34**	**-0.31**	-0.19	**-0.46**	-0.19	-0.04	0.08	-0.22	-0.16	-0.24	**-0.35**
R Na	-0.07	-0.07	-0.17	0.06	0.11	0.16	0.14	0.11	0.09	0.14	0.17	0.06	-0.16	-0.01	0.23
R Na/Ca	0.17	-0.10	0.07	0.10	**0.34**	0.22	**0.29**	**0.39**	0.24	0.21	0.08	0.21	0.20	0.16	**0.25**
R Na/Cl	-0.15	-0.10	**-0.26**	0.00	-0.10	-0.10	**-0.36**	-0.19	**-0.32**	**-0.30**	-0.07	-0.24	**-0.34**	-0.25	0.01
R Na/K	-0.08	-0.13	-0.20	-0.02	-0.11	-0.15	-0.24	-0.20	-0.22	-0.13	0.06	-0.23	**-0.33**	**-0.26**	0.01
R Na/Mg	0.00	-0.11	-0.14	-0.01	**0.28**	**0.27**	0.18	**0.36**	0.15	0.12	0.06	0.15	-0.02	0.12	**0.36**
R PO4	0.06	0.00	0.06	0.22	0.12	0.12	0.13	0.15	0.11	0.01	-0.18	0.15	**0.36**	0.19	-0.09
R SO4	-0.09	-0.02	-0.14	0.15	0.12	0.22	0.10	0.02	0.08	-0.01	-0.06	0.14	-0.04	0.09	0.03

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

Appendix 14. Correlation analysis between ions contained in the stem and the growth parameters of the genotypes grown under salinity condition.

	CC LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm UL[5]	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.58**	-													
CC-UL	**0.60**	**0.30**	-												
Fv/Fm-UL	**0.27**	-0.05	0.25	-											
Fv/Fm-LL	**0.36**	**-0.32**	0.09	**0.48**	-										
LA	-0.02	-0.03	-0.04	0.14	**0.31**	-									
Rfw	0.07	0.04	0.12	0.19	**0.28**	**0.71**	-								
RL	0.10	0.00	0.11	0.21	**0.40**	**0.57**	**0.60**	-							
Rdw	0.04	0.09	0.14	0.18	**0.26**	**0.73**	**0.97**	**0.55**	-						
Rfw/Sfw	0.05	-0.01	0.03	0.10	0.15	-0.08	**0.54**	0.22	**0.46**	-					
Rdw/Sdw	-0.12	0.12	-0.03	-0.03	-0.07	-0.19	**0.32**	-0.09	**0.33**	**0.74**	-				
Sfw	0.08	0.04	0.15	0.13	**0.26**	**0.92**	**0.80**	**0.58**	**0.81**	-0.04	-0.14	-			
SL	**0.38**	-0.13	**0.32**	0.24	**0.30**	**0.47**	**0.41**	**0.37**	**0.39**	-0.01	**-0.28**	**0.55**	-		
Sdw	0.08	0.00	0.11	0.11	**0.26**	**0.92**	**0.73**	**0.57**	**0.75**	-0.09	**-0.28**	**0.97**	**0.58**	-	
RL/SL	-0.13	0.11	-0.06	0.08	0.23	**0.25**	**0.33**	**0.76**	**0.30**	0.24	0.07	0.22	**-0.28**	0.19	-
S Ca	-0.17	0.14	-0.05	0.18	0.02	0.22	0.25	0.05	**0.30**	0.11	0.15	0.17	-0.04	0.12	0.09
S Cl	-0.05	0.01	-0.04	0.12	0.23	0.21	**0.26**	0.20	**0.27**	0.19	**0.25**	0.19	0.01	0.08	**0.26**
S K	0.11	-0.18	-0.05	0.21	0.14	**0.32**	0.12	**0.26**	0.08	-0.13	-0.23	**0.26**	**0.45**	0.24	-0.01
S Mg	**-0.25**	**0.31**	0.00	-0.19	-0.17	-0.02	0.10	-0.03	0.13	0.16	0.17	0.03	-0.07	0.02	0.04
S Na	-0.20	0.07	-0.15	-0.11	0.05	0.00	-0.12	-0.04	-0.11	-0.19	0.04	0.01	-0.22	-0.05	0.13
S Na/Ca	-0.07	-0.03	-0.10	-0.25	0.03	-0.16	**-0.30**	-0.11	**-0.32**	**-0.25**	-0.07	-0.13	-0.16	-0.14	0.00
S Na/Cl	-0.20	0.07	-0.15	-0.22	-0.12	-0.17	**-0.35**	-0.23	**-0.33**	**-0.35**	-0.14	-0.15	**-0.26**	-0.14	-0.08
S Na/K	**-0.28**	0.14	-0.18	**-0.32**	-0.11	**-0.31**	**-0.25**	**-0.33**	-0.22	-0.05	0.20	**-0.27**	**-0.51**	**-0.29**	-0.02
S Na/Mg	0.11	-0.21	-0.07	0.10	0.12	-0.01	-0.13	-0.03	-0.15	-0.15	-0.04	-0.06	-0.03	-0.09	-0.02
S PO4	-0.21	0.15	-0.09	-0.07	-0.09	-0.22	-0.16	-0.07	-0.17	0.05	0.15	-0.20	-0.16	-0.22	0.05
S SO4	**-0.33**	0.13	**-0.27**	-0.10	-0.02	-0.18	-0.21	-0.17	-0.21	0.02	0.15	**-0.25**	**-0.38**	**-0.27**	0.10

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

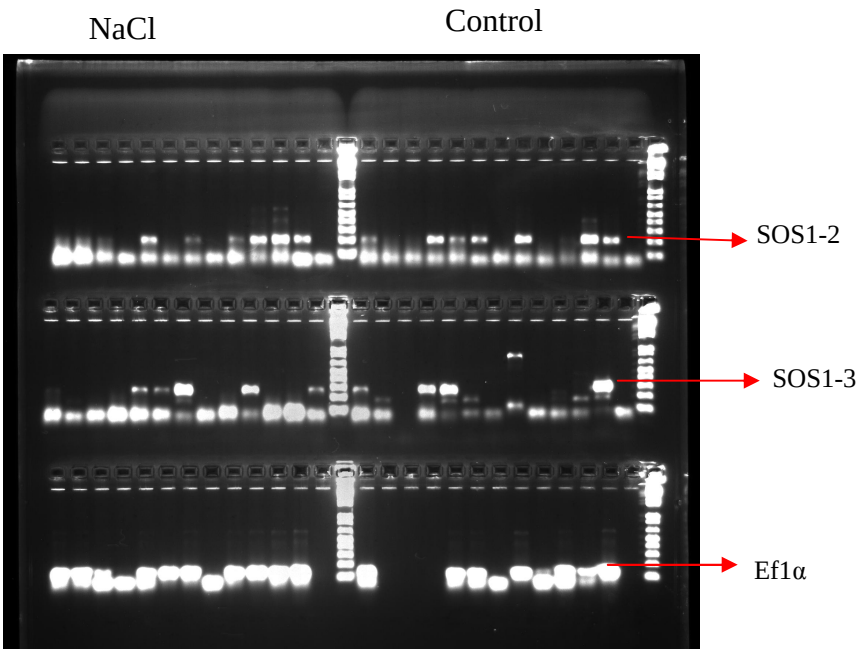
Appendix 15. Correlation analysis between ions contained in the leaf and the growth parameters of the genotypes grown under salinity condition.

	CC LL[2]	CC UL/LL[3]	CC UL[4]	Fv/Fm UL[5]	Fv/Fm LL	LA[6]	Rfw[7]	RL[8]	Rdw[9]	Rfw/ Sfw[10]	Rdw/ Sdw[11]	Sfw[12]	SL[13]	Sdw[14]	RL/ SL[15]
CC-LL	-														
CC-UL/LL	**-0.58**	-													
CC-UL	**0.60**	**0.30**	-												
Fv/Fm-UL	**0.27**	-0.05	0.25	-											
Fv/Fm-LL	**0.36**	**-0.32**	0.09	**0.48**	-										
LA	-0.02	-0.03	-0.04	0.14	**0.31**	-									
Rfw	0.07	0.04	0.12	0.19	**0.28**	**0.71**	-								
RL	0.10	0.00	0.11	0.21	**0.40**	**0.57**	**0.60**	-							
Rdw	0.04	0.09	0.14	0.18	**0.26**	**0.73**	**0.97**	**0.55**	-						
Rfw/Sfw	0.05	-0.01	0.03	0.10	0.15	-0.08	**0.54**	0.22	**0.46**	-					
Rdw/Sdw	-0.12	0.12	-0.03	-0.03	-0.07	-0.19	**0.32**	-0.09	**0.33**	**0.74**	-				
Sfw	0.08	0.04	0.15	0.13	**0.26**	**0.92**	**0.80**	**0.58**	**0.81**	-0.04	-0.14	-			
SL	**0.38**	-0.13	**0.32**	0.24	**0.30**	**0.47**	**0.41**	**0.37**	**0.39**	-0.01	**-0.28**	**0.55**	-		
Sdw	0.08	0.00	0.11	0.11	**0.26**	**0.92**	**0.73**	**0.57**	**0.75**	-0.09	**-0.28**	**0.97**	**0.58**	-	
RL/SL	-0.13	0.11	-0.06	0.08	0.23	**0.25**	**0.33**	**0.76**	**0.30**	0.24	0.07	0.22	**-0.28**	0.19	-
L Ca	0.16	-0.13	0.06	0.22	**0.34**	**0.33**	**0.55**	**0.40**	**0.53**	**0.43**	**0.27**	**0.36**	0.19	**0.30**	**0.30**
L Cl	-0.13	-0.07	-0.22	0.04	0.23	0.10	**0.38**	0.09	**0.37**	**0.53**	**0.53**	0.05	-0.15	-0.01	0.19
L K	0.02	-0.10	-0.07	0.18	0.13	**0.50**	**0.33**	0.20	**0.31**	0.00	0.01	**0.40**	0.24	**0.36**	0.01
L Mg	0.18	-0.08	0.13	0.23	0.19	0.18	**0.30**	**0.29**	**0.28**	0.25	0.05	0.21	0.24	0.23	0.14
L Na	**-0.36**	0.16	**-0.26**	-0.20	-0.04	**-0.36**	-0.14	**-0.27**	-0.14	0.22	**0.33**	**-0.34**	**-0.42**	**-0.35**	0.04
L Na/Ca	**-0.42**	0.17	**-0.32**	**-0.35**	**-0.26**	**-0.49**	**-0.42**	**-0.50**	**-0.42**	-0.06	0.19	**-0.49**	**-0.48**	**-0.47**	-0.21
L Na/Cl	**-0.33**	**0.25**	-0.13	-0.21	-0.20	**-0.47**	**-0.43**	**-0.37**	**-0.41**	-0.15	-0.01	**-0.42**	**-0.35**	**-0.39**	-0.11
L Na/K	**-0.26**	0.16	-0.15	-0.19	-0.07	**-0.49**	**-0.26**	**-0.27**	**-0.26**	0.15	0.18	**-0.42**	**-0.39**	**-0.40**	0.04
L Na/Mg	0.02	0.02	0.05	**-0.27**	0.05	-0.07	-0.03	0.07	0.02	-0.01	0.06	-0.03	-0.01	-0.02	0.07
L PO4	0.02	0.12	0.15	0.14	0.01	-0.05	0.19	0.05	0.17	**0.34**	**0.38**	0.01	0.08	-0.08	-0.02
L SO4	-0.19	**0.33**	0.08	0.09	-0.02	-0.07	0.02	-0.03	0.04	0.09	0.07	-0.02	-0.05	-0.04	0.05

[0]Root [Ca^{2+} (Calcium), Cl^{-} (Chlorine), K^{+} (Potassium), Mg^{2+} (Magnesium), Na^{+} (Sodium), PO_4^{2-} (Phosphate) and SO_4^{2-} (Sulphate)]; the ion content is calculated in mg/g of organ of the genotypes.

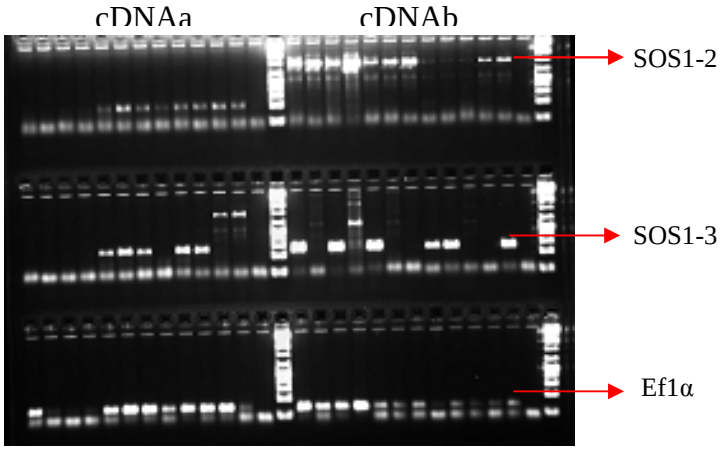
[1]Chlorophy content,[2]Lower leaf, [3] Upper to lower leaf ratio, [4]Upper leaf , [5]Chlorophy fluorescence, [6]Leaf area, [7]Root fresh weight, [8]Root length, [9]Root dry weight, [10] Root to shoot fresh weight ratio, [11]Root to shoot dry weight ratio, [12]Shoot fresh weight, [13]Stem length, [14]Stem dry weight, and [15] root to stem length ratio

Appendix 16. Testing the uniqueness of SOS1-2 and SOS1-3 primer combination using cDNA of genotypes.



Testing of the SOS1-2 and SOS1-3 using PCR cycles [94^{o}C (30sec), 53^{o}C (30sec) and 72 ^{o}C (45sec)]. Under each growing condition (NaCl and control) and primer combination the first six lanes were genotypes having relatively high Na^{+} content and the next six lanes were the genotypes have low Na^{+} content.

Appendix 17. Testing the uniqueness of SOS1-2 and SOS1-3 primer combination using cDNA and DNA of genotypes.



Testing of the SOS1-2 and SOS1-3 using PCR cycles [94°C (30sec), 53°C (30sec) and 72 °C (45sec)]. The first part (left) has six genotypes that have cDNA obtained from the salt treated (a) and control (b) condition. In the second part (right) there are ten genotypes genotypes, as well as the Parent C and Parent E which are in the first and second lanes. The lane before each marker is control.

Appendix 18: List of the genotypes used in the experiment and lay out of for the identification of markers in the CxE population using the SOS1-3 primer combination.

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**		**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
Genotype	17	27	69	72	82	84	110	141	145	155	159	166	**M**	171	196	200	202	218	222	232	233	250	268	276	350	**M**
No.	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**		**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	
Genotype	447	602	603	604	607	608	609	615	624	628	630	631	**M**	632	633	634	636	640	642	648	651	653	656	658	659	**M**
No.	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**		**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	
Genotype	660	663	664	666	667	668	669	673	674	675	680	681	**M**	686	688	689	695	698	701	702	709	712	714	715	717	**M**
No.	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**	**81**	**82**	**83**	**84**		**85**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**	
Genotype	719	721	723	724	726	728	732	733	736	738	740	746	**M**	747	752	753	757	761	765	769	777	782	786	C	E	**M**

NB: ‘M’ is position of the marker on gel-electrophoresis (picture).