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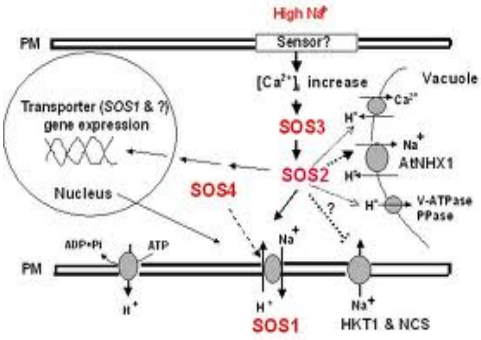
Evaluation of salt stress tolerance and ion content QTL analysis of potato grown in hydroponics and in the field



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Evaluation of salt stress tolerance and ion content QTL analysis of potato grown in hydroponics and in the field

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Contents

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Summary

Salinity stress negatively impact agricultural yield in many places in the world while affecting productivity of most important crops. Most of the existing crops are glycophytic (potato) and therefore improving tolerance for salinity is crucial. In the current study, ion concentrations in the different tissues and their relationship with agronomic data were analyzed. Additionally, QTL analysis was done for ion contents for detection of responsible regions in the genome for salinity tolerance. Therefore, 96 genotypes (including parents) from CxE diploid potato population were grown in greenhouse (hydroponics) under salinity (3 replications) and control (3 replications) conditions. Also, six tetraploid cultivars were grown in four hydroponic containers (two containers for replication 2 and two containers for replication 3) in hydroponics. In each replication one container contained control and the other salt treated plants. The harvested plant material was pooled according to the genotype and treatment received and then was separated into root, stem and leaf. Additionally, 25 tetraploid cultivars and 20 CxE genotypes were grown in the field and leaves were harvested for analysis in two different dates. Finally, ion content determination (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-}) of tissues was carried out using ion chromatography.

In CxE diploid population, the application of salt (NaCl) in to the growth media significantly increased the concentration of Cl^- and Na^+ in all tissues (except Na^+ in stem), K^+ in root and Mg^{2+} in stem. On the other hand, the concentration of Ca^{2+} (root, leaf), K^+ (leaf, stem), PO_4^{3-} in all tissues, SO_4^{2-} (leaf, stem) and K/Na ratio in root and leaf, was significantly decreased. Correlation analysis between ions and ions with growth data revealed traits, indicators, for tolerance to salinity. QTL analyses of the ion contents revealed QTLs for Na-leaf, SO_4-leaf and Mg-stem (one for each trait) under salt conditions, while two QTLs for K-leaf and one QTL for K/Na ratio were detected under control conditions.

In the six tetraploid cultivars, the application of NaCl in to the growth media significantly increased the content of Cl^- (leaf, stem), Na^+ in all tissues, Na/K ratio in all tissues, PO_4^{3-} in leaf and Mg^{2+} in stem. On the other hand, the concentration of Ca^{2+} (leaf, root), K^+ (leaf, stem) and SO_4^{2-} in leaf was significantly decreased.

Correlation analysis between ions and ions with growth data revealed traits, indicators, for tolerance to salinity. However because of the small number of plants used for the correlation analysis and the increased variation between replications, there must be a caution in the interpretation of the results.

In the tetraploid cultivars and CxE genotypes grown in test field, the addition of NaCl significantly decreased the Na^+ content in 19/06/10, while Cl^- showed no significant differences between treatments in 19/06/10. In 27/07/10 no significant differences were observed between treatments for both Na^+ and Cl^- contents so the treatment effect was not clear. Yield per plant was significantly reduced for only the 20 tetraploid cultivars. The most possible explanation is that the plants did not perceive salt stress.

In CxE diploid population grown in hydroponics, the observed differences in ion content is an indication of genetic control in the regulation of ion concentration in potato. The magnitude of salinity-induced change in ion content is dependent on ion and tissue. The ionic changes may indicate that Na^+ exclusion is more possible salinity tolerance mechanism than the Na^+ compartmentation and Na^+ efflux. However, there might have been ion toxicity. This can be attributed to low efficiency of the existing tolerance mechanism or due to high levels of the salt (120mM). Differences for ion content between the six tetraploid cultivars were not observed in contrast to the differences identified for their growth data.In the greenhouse, growing of CxE population and the six tetraploid cultivars in different salinity levels, different environment conditions (light intensity, humidity, temperature and day length), evaluation of phenotypic and physiological responses on different developmental stages (vegetative and reproductive stage) can help to obtain a more clear view of the mechanisms of salinity tolerance. QTLs can be used in Marker Assisted Selection (MAS). Generally, QTLs can be used to identify candidate genes that are important in salt tolerance, but this remain a difficult issue to cope with because the identified QTLs can contain hundreds of genes. In the field, more replications in more locations, homogeneous soil used, measurement of salt concentration in the soil, evaluation of physiological traits (like Chlorophyll Content) and analysis of ion contents in different tissues can lead to a more clear view.

Introduction

Salinity is an abiotic factor cause abiotic stress and is one of the most serious, widespread environmental agricultural problems resulting in losses of yield and arable land. Generally it is considered to be a problem affecting arid and semi-arid regions of the world. However, it also affects more favourable environments. Un upward movement of soluble salts as a result from evaporation exceeding rainfall, lack of drainage and bad management of irrigation water are some of the reasons increase and lead to the problem. The increasing population in the world and therefore the need to produce more food worldwide make the salinity problem become more severe because of the more intensive cultivation of the land (Morpurgo, 1991).

The sharp increasing rates of this problem overcommit scientific community to find available strategies cope with the problem. One strategy available to cope with saline soil is to choose salt tolerant crops or select salt-tolerant cultivars within a crop. However, this strategy has its limits in the level of tolerance as well as in the number of tolerant crops or cultivars. Selecting for salinity tolerance is problematic, comes from the complexity of its polygenic nature, the various soil types, variability in climate and from the pattern and composition of the salt distribution in the soil (Greenway and Munns, 1980).

Several aspects of plant metabolism are negatively affected. Plant growth in general is reduced in glycophytes. On the other hand, plants have develop several adaptation mechanisms such as ion compartmentation, ion exclusion and osmotic adjustment (Marconi et al., 2001). Salt stress has toxic effects on plants and decrease crop productivity. In the majority of plants salt stress is cause changes in gene expression, leading to an increase synthesis of osmoprotectors and osmoregulators (Teixeira and Pereira, 2007). Accumulation of proline is function as an osmoprotector and as an osmoregulator.

Cultivated tetraploid potatoes are moderately sensitive to soil salinity with damage thresholds ranging from 15 to 30 mmol of NaCl (Maas and Hoffman, 1977), but greater stress tolerance exists in diploid wild types (Shaterian et al., 2005).

Ion Homeostasis

Ion homeostasis can be defined as the ability of a cell or an organism to maintain internal steady stage, even under environmental stress. Under salt stress are supposed to maintain a high concentration of K^+ and a low concentration of Na^+ in the cytosol (Zhu, 2003).

When plant cells are exposed to salinity (high NaCl concentrations), kinetic states of ion transport for Na^+ and Cl^- and other ions, such as K^+ and Ca^{2+} are disturbed (Binzel et al., 1988). Ionic homeostasis is created by constantly flux in and out of cells in a controlled fashion adjusted to accommodate cellular requirements (Niu et al., 1995). Thus, it is important for a plant in saline environments to keep cellular ion homeostasis for metabolic functioning and growth.

Specialized complex mechanisms common to all genotypes are created by plants that allow adaptation to saline stress conditions. Important mechanisms are those mechanisms that function to regulate ion homeostasis while mediating osmotic adjustment through the accumulation and intracellular compartmentation of ions that have very high concentrations in the external environment (Niu et al., 1995).

Ion flux across the plasma membrane and tonoplast occurs via transport proteins. This ion flux is dependent of the thermodynamic gradient ($\Delta\mu$). Electrical gradient ($\Delta\psi$) and chemical gradient are the two components that $\Delta\mu$ is consists of (Nobel, 1991). Transport of ions down the $\Delta\mu$ is passive, whereas transport against the gradient is active.

The transport proteins that mediate ion flux can be generally categorized as pumps, carriers and channels (Sussman and Harper, 1989). Pumps use metabolic energy for transport, whereas carriers couple uphill transport of one solute to the downhill movement of another, either in the same (symporter) or opposite (antiporter) direction. Channels mediate passive transport, that is in simple words movement down a free energy gradient (Niu et al., 1995).

Under typical physiological conditions, homeostatic concentration of ions in the cytosol are 100 to 200 milliM K^+, 1 to 10 milliM Na^+ and Cl^-, and 100 to 200

nanoM Ca^{2+} (Binzel et al., 1988; Bush, 1995). This is not in agreement with the current study in which homeostatic concentrations of ions in the cytosol are less than the concentrations found by Binzel et al and Bush. Unlike animal cells, plant cells do not have Na^+-ATPases or Na^+/K^+ ATPases and they rely on H^+-ATPases and H^+-pyrophosphatases to create a proton-motive force that drives the transport of all other ions and metabolites (Hasegawa et al., 2000). K^+ uptake under salt stress is maintened by upregulation of various HAK/KUP (high affinity K^+ transporter/K^+ uptake transporter) –type genes (Zhu, J.K. 2003). Uptake of Na^+ is done passively across the plasma membrane in the root-soil interface and efflux is presumed to mediated by Na^+/H^+ antiporters activities (Plett and Moller, 2010).

Sensing salt stress and signaling pathways

The main effects of salinity comes from Na^+ toxicity thus, salt tolerance is approximately dependent on adaptation to Na^+ toxicity. In most species Na^+ level reach toxic concentrations before Cl^- does. Unfortunately, little is known about how Na^+ is sensed in plant cells (Zhu, 2003). A possible sensor may be the plasma membrane Na^+/H^+ antiporter SOS1 (SALT OVERLY SENSITIVE 1) (Shi et al., 2000). In Arabidopsis cells SOS1 has Na^+/H^+ exchanger activity with positive effects for Na^+ efflux (Qiu et al., 2002; Quintero et al., 2002). SOS1 has very long cytoplasmic tail and because of this it is assumed that this protein may not only transport Na^+ but also sense this ion. Therefore, SOS1 may be both a transporter and a sensor of Na^+ (Zhu, 2003).

Chemical signaling is associated by various aspects like plant growth and development and of course stress physiology and occurs through many chemicals. Calcium ions (Ca^{2+}) function as a major secondary-messanger signaling molecule and play an important role in plant growth and development under normal as well as stress conditions. The stress signal is perceived out of cell by the membrane receptors, then a signaling cascade is activated inside the cell including the generation of second messengers such as Ca^{2+} (Mahajan et al., 2008). The increase in cytosolic Ca^{2+} starts the stress signaling pathways for stress tolerance (Mahajan and Tuteja, 2005; Tuteja, 2007; Tuteja and Mahajan, 2007).

Generally perturbations of Ca^{2+} in cytosol are the results of stress signals and these stress signals are decoded by Ca^{2+} sensing proteins. Calcium sensors called CBLs and their interacting partners (CIPKs) have been discovered recently. Presumably those proteins are key networks which play an important role in plants in response to calcium and stress signaling (Mahajan et al., 2008).

Plant responses and mechanisms of salinity tolerance

In order to understand the physiological mechanisms responsible for salinity tolerance, it is necessary to know whether the growth reduction comes from osmotic effect of the salt in the soil (osmotic phase) or from the toxic effect of the salt within the plant (ionic phase). Osmotic phase starts after the salt concentration around roots increases to threshold level, and a decrease rate of shoot growth occur. Most of the plants have a threshold level of 40 milliM NaCl. The plant develops new leaves and lateral buds more slowly and fewer branches or lateral shoots are formed. One can observe that shoot growth is more sensitive than root growth, a phenomenon that also occur in drying soils with no mechanistic explanation behind this yet (Munns and Tester, 2008). On the other hand, it is logical for a plant under these conditions to maintain root structures or even increase root length in order to explore more layers and deeper in the soil find water to prevent imbalance result from evaporation and decrease of photosynthesis. Ionic phase starts when salt accumulate to toxic levels in the old leaves and they die. If the produce rate of new leaves is smaller than the rate at which they die, the photosynthetic activity of the plant will decrease and also a reduction in growth rate can be observed. Initially, osmotic stress has an immediate effect on growth and also greater effect on growth rates than the ionic stress. Ionic stress affect growth much later with less effect than osmotic stress (Munns and Tester, 2008). However, at high salinity levels or in sensitive species that is unable to control Na^{+} transport, the ionic effect dominates the osmotic effect. In current study, ionic effect dominates osmotic effect because of the high salinity levels used. Generally a plant will be able to grow in a rapid rate under salinity with an increase in tolerance in both stresses (Munns and Tester, 2008).

Three categories of salinity tolerance exist. First is tolerance to osmotic stress. Osmotic stress negatively reduces turgor and therefore leads to cell expansion which is translated to reduced growth. An increase tolerance to osmotic stress will result in greater leaf growth and stomatal conductance always refers to plants that have sufficient soil water. Therefore, this would benefit only plants when a supply of water is ensured. Second is Na^+ exclusion by roots as result plants prevent Na^+ reach toxic concentrations within leaves (Munns and Tester, 2008). Na^+ is taken up by roots and transported to the shoot via the transpiration stream. It is suggested that engineering of Na^+ exclusion from the shoot could be achieved through an alteration of plasma membrane Na^+ transport processes in the root, if these alterations were cell type specific (Moller et al. 2009). Additionally, Na^+ exclusion from leaves can be mediated by HKT transporters by removing Na^+ from the xylem sap (Horie et al. 2009). Third mechanism is tissue tolerance in which the tissue shows tolerance to accumulation of Na^+ by compartmentalization of Na^+ and Cl^- at the cellular and intracellular level in order to avoid toxic concentrations within the cytoplasm. Toxicity arrives with time, after Na^+ in leaf reach to high concentrations in the older leaves (Munns and Tester, 2008).

Ion homeostasis under salt stress

Cations (Na^+, K^+, Mg^{2+}, Ca^{2+})

When Na^+ enters cells is accumulating and become toxic to enzymes in high levels (above 100milliM). It often leads to catastrophic pathologies which affect cell survival growth and division. Additionally, membrane disorganization and osmotic imbalance which lead to growth inhibition are take place due to high concentrations of Na^+ (Mahajan et al., 2008). Cell death can be prevented by extruded or compartmentalized the excessive Na^+ in the vacuole (Hasegawa et al., 2000). The negative membrane potential across the plasma membrane of plant cell favors the passive transport of Na^+ into cells. Na^+ gets into plant cell through the high affinity K^+ transporter HKT1 (Rus et al., 2001; Mäser et al., 2002) and through non selective cation channels. The role of Na^+ efflux has to be considered, as Na^+ transported out of one cell should be a problem for neighboring cells. In Arabidopsis, Na^+ efflux is

done by Na^+/H^+ antiporter encoded by the SOS1 gene. SOS1 gene activity can only be detected in stress plants and not in unstress plants and it is an electro neutral Na^+/H^+ exchanger. Na^+ compartmentation in vacuole not only decreases Na^+ in the cytoplasm but also contributes to osmotic adjustment to maintain water uptake from saline solutions. Plastids and mitochondria may also accumulate some Na^+ thus contribute to Na^+ compartmentation (Zhu, 2003). In Arabidopsis, AtNHX family of Na^+/H^+ antiporters function in Na^+ compartmentation (Blumwald, 2000).

Potassium (K^+) acts as the major osmoticum of the cell. It controls cell expansion, plasma membrane potential and transport and ph value (Prinzenberg et al., 2010). Deficiency of potassium leads to plant growth reduction, loss of turgor, increased susceptibility to cold stress and pathogens and the development of chlorosis and necrosis (Véry and Sentenac, 2003; Ashley et al., 2006; Amtmann et al., 2008). The influx of K^+ at root comes from high and low affinity potassium carriers. Under salt stress Na^+ compete K^+ for uptake into roots. The transcript levels of several K^+ transporter genes are down- or up-regulated by salt stress, shows the different capacity of the plant to maintain K^+ in salinity. Growing plants with a shoot/root ratio > 1 will translocate most of the K^+ from root symplast to the shoot. One of the key determinants of plant salt tolerance is the maintenance of high cytosolic K^+/Na^+ ratio which was shown to be heritable in wheat but not in other species such as rice and probably involve the contribution of different genes in different species. Generally it can be concluded that K^+ uptake at root mediate via highly K^+ selective pathways whereas Na^+, at least in part, appears to move through less selective systems which sometimes are blocked by Ca^{2+}. Further identification and characterization of genes involved in K^+ translocation to the shoot and compartmentation of Na^+ in the vacuole under salt stress conditions will be of great importance to improve the knowledge about the regulation of ionic homeostasis. (Maathuis and Amtmann, 1999).

Maintenance of Mg^{2+} homeostasis in plants is vital. Mg^{2+} is essential for the function of many cellular enzymes and for the aggregation of ribosomes. It might regulate ion balance in the cell and stomatal opening. (Shaul, 2002). Mg^{2+} is the central atom of the chlorophyll molecule and fluctuations in its levels in the

chloroplast regulate the activity of key photosynthetic enzymes. A key role in Mg^{2+} homeostasis in plant cells, as in many ions, seems to play the plant vacuole. The entry of Mg^{2+} to vacuole is mediated by Mg^{2+}/H^{+} exchangers. In Arabidopsis AtMHX is the vacuolar Mg^{2+}/H^{+} exchanger determine Mg^{2+} partitioning between cytosol and vacuole of root epidermal, xylem parenchyma and meristematic cells (Shaul, 2002). However molecular details of Mg^{2+} transport are poorly understood. New genes are discovered whose products transport Mg^{2+} but little is known on their biological and physiological function (Gardner, 2003).

The significance of calcium cation comes from Ca^{2+} signal in reinstating cellular ion homeostasis (mention above in signaling pathways). SOS mutants are hypersensitive to salt. Genetic analysis confirmed that SOS1-SOS3 function in a common pathway of salt tolerance. SOS3 is a Ca^{2+} sensor and transduces the signal downstream after activating and interacting with SOS2 protein kinase. Cellular ion homeostasis is re-established after SOS3-SOS2 complex activates the Na^{+}/H^{+} antiporter activity of SOS1 (Mahajan et al., 2008).

Anions (Cl^{-}, $PO4^{-}$, $SO4^{2-}$)

Lack of information about intracellular uptake and vacuole compartmentation of Cl^{-} reported by (Binzel et al., 1988). Active uptake could be mediated by a Cl^{-}/H^{+} symporter (Niu et al., 1995). If membrane is depolarized, gradient is disturbed by Na^{+} influx across the plasma membrane, the Cl^{-} can be taken up passively through an anion channel (Skerrett and Tyerman, 1994). Nevertheless an essential adaptation for NaCl tolerance is vacuolar compartmentation of Cl^{-}. The movement from cytosol to vacuole may be achieved through channels (Niu et al., 1995).

Phosphate is an important structural and signaling molecule with an essential role in photosynthesis, energy conservation and carbon metabolism. Its deficiency result to reduction of growth and increase of pathogen susceptibility (Prinzenberg et al., 2010). In plants the main source of phosphorus is taken up by the roots. In higher plants proteins involved in Pi transport has been expanded the last decade by

cloning of several genes from PHT1, PHT2, PHT3 and PHT4 family, which encode proteins involved in the acquisition of Pi across the plasma membrane, chloroplast, mitochondria and Golgi respectively (Mudge et al., 2002; Versaw and Harrison, 2002; Guo et al., 2008; Cubero et al., 2009). Despite those, an important gap still remains with regard to how plants sense the availability of Pi and how the signals relay to the transcription machinery (Rouached et al., 2010). Additionally, data from literature and experimental data shows complexity between regulatory players of Pi homeostasis and other nutritional elements (Rouached et al., 2010).

In recent years the molecular identity of channels involved in transport of cations has develop rapidly. Although, the molecular identity of anion channels has lagged behind, recent studies have identified S-type (slow) and R-type (rapid) anion channels. S-type channel has been finally recognized encode members of SLAC1 family. R-type assumed that plays multiple roles but its molecular identity remains unknown (Eugene et al., 2010). Even sulphate (SO_4^{2-}) which is considered to be weakly permeated through membranes is highly permeable from R-type channel. To a lesser extent R-type channel is also selective for chloride. Also sulphate shows strong positive regulatory effect preventing the run-down of the channel activity. Thus, R-type it is suggested to function in the cell sulphate homeostasis (Eugene et al., 2010).

QTL Mapping

QTL Analysis

Over the last century, development in genetics was only associated with variation in single so-called “major genes”, although much of the natural variation observed in many crops, were due to genetic differences in many genes. QTL analysis is used to study this genetic variation, to locate the responsible genes and to explore their effects and interactions. All these are very important in agriculture and medicine (Kearsey, 1998).

The abbreviation of the word QTL is Quantitative Trait Loci which are genes responsible for quantitative traits (Geldermann, 1975). They were known as polygenes before the discovery of molecular markers. Little is known about how these genes were controlled, but for any given trait there were several such genes segregating in a Mendelian fashion and their effects were approximately additive. Many agriculturally important traits such as yield, quality and disease resistance are controlled by many genes so are known as quantitative traits or complex traits.

Principle of QTL analysis

Once a QTL is found within a plant genome is like someone find a needle in a haystack. The aim of QTL analysis is to detect an association between phenotype and the genotype of markers. Markers used to divide the mapping population into different genotypic groups. The differences between genotypic groups are based on the presence or absence of a particular marker locus (Tanksley, 1993; Young, 1996). When a marker and QTL are linked, this means they segregate and inherit together in the progeny and the mean of the group that marker is present, is significantly different from the mean of the group without the marker.

QTL detection methods

Three widely used methods for detecting QTLs are single-marker assisted analysis, simple interval mapping and composite interval mapping (Collard et al., 2005). The simplest method is single-marker analysis because it detects QTLs associated with single markers. Linear regressions, t-tests, ANOVA are some of the statistical methods used for single marker analysis. The most commonly used is linear regression because the coefficient of determination (R^2) from the marker explains the phenotypic variation comes from the QTL linked to the marker. The advantage of this method is that does not require a complete linkage map. On the other hand the further a QTL is from a marker the less likely it will be detected. The latter remain as the major disadvantage of the method (Tanksley, 1993). Problems can be minimized by using large number of segregating DNA markers covering the

entire genome. The simple interval mapping (SIM) instead of analyzing single markers, makes use of linkage maps and analyses intervals between adjacent pairs of linked markers along chromosomes simultaneously (Collard et al., 2005). The third QTL detection method which is composite interval mapping (CIM) is a combination of interval mapping with linear regression. Additionally, genetic markers are included to an adjacent pair of linked markers for interval mapping (Jansen, 1993; Zeng, 1993; Jansen and Stam, 1994; Zeng, 1994). CIM can be characterized as more precise and effective at mapping QTLs compare to the other two methods mention above (Collard et al., 2005).

LOD Significance threshold

Usually and in current study QTL analysis is carried out with interval mapping. An experimental population segregating for a quantitative trait is created. In the present study a QTL analysis was done using CxE diploid potato population and the quantitative trait was salinity. Previously, an integrated linkage map of CxE of molecular markers was calculated. A likelihood ratio statistic called LOD score was calculated on many positions on the linkage map. Subsequently, the regions shows significant values of the test statistic are supposed to contain a QTL. The experiment-wise significance level is usually 5% depend on the genome size of species being analyzed. The statistical significance in QTL analysis is a problem. It is based on complex mathematical formulae or on permutation tests. Both methods were used in this study for calculating the significance level in which values bigger than this level show an indication for that area that a QTL is exist (Van Ooijen, 1999).

The Potato

The potato (solanum tuberosum) belongs to Solanaceae family and it is herbaceous perennial plant. It is consist of 48 chromosomes (tetraploid). There are also diploid (24 chromosomes) and triploid (36 chromosomes) species. Solanum

tuberosum and modern varieties of this species which are tetraploid are the most widely cultivated species. Because of the complexity comes from ploidy levels of cultivated potato, diploid species are also big part of scientific research in our days.

Potato is the fourth most important food crop in the world with annual production approaching 300 million tons. More than one third of global production comes from developing countries. This is due to the fact that farmers in developing countries are provided with new technologies and special breeding lines designed for developing countries (www.cipotato.org/potato/).

One of the most significant environmental problems is increasing salinity. Potato is negatively affected by high concentration of salt (NaCl) because of the glycophytic nature of the crop. Most of the cultivated crops are glycophytes. National Land and Water Resources Audit estimates that 5.7 million hectares have a high potential for the development of dryland salinity and predicts salinity to rise to 17 million hectares by 2050.

Previously, in 2008 six tetraploid cultivars and CxE population were grown in hydroponics and some agronomic data were measured. In 2009, CxE population was again growing in hydroponics and agronomic data were measured in the greenhouse. In 2010, the CxE diploid population (grown in hydroponics in 2009) was analysed for the anion and cation concentrations, while correlation analysis and QTL analysis was also done between these ions with agronomic data from the greenhouse. In 2010 CxE population and some commercial cultivars were also grown in the field.

The current study, in 2011, is about salinity tolerance of the six tetraploid cultivars and CxE diploid population grown on hydroponics in 2008 and cultivars grown in field in 2010. The concentration of cations (Na, Mg, Ca, K) and anions (PO4, SO4, Cl) was determined as well as its correlation with agronomic data. Finally, QTL mapping of ions concentration in the CxE population and comparison of QTL analysis from last year data were also undertaken.

Comment [GL1]: These are not ions. These are elemens, and something else…

Material and Methods

Plant material

Three different types of plant material were used for this experiment:

1) The CxE diploid potato population grown in hydroponics
2) 6 tetraploid cultivars grown in hydroponics
3) 20 genotypes of CxE population (18 progenies and the two parents) and 25 tetraploid cultivars (the 5 out of 6 tetraploid cultivars grown in hydroponics and 20 more interesting tetraploid cultivars) all these grown on field.

1) The CxE population was derived from a cross between C (Hanneman Jr and Peloquin, 1967) and E (Jacobsen, 1980). Parent C is a cross between the *S. phureja* (PI225696.1) and *S. tuberosum* dihaploid (USW42) while parent E is derived from a cross between C and the *S. vernei-S tuberosum* backcross clone VH3-421 (Jacobsen, 1978). The CxE population consists of 238 genotypes. A total number of 94 genotypes as well as parent C and parent E were used for this experiment. The experiment in the greenhouse was undertaken in 2008. Plants were propagated in *in vitro* culture and axillary shoots were taken from each genotype and grown for two weeks on a standard MS (Murashige and Skoog) medium (agar solution). After two weeks period, the plantlets were transferred to hydroponic containers in greenhouse under 18/15.6^{o}C day/night temperature, 16 h day length and 60/80% day/night RH (the greenhouse conditions were affected by the changes of external 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 micronutrients Fe 24, Mn 12, B 9.8, Zn 4.4, Cu 0.7 and Mo 0.3 (µM) and Si 0.02 (mM). The CxE plants were divided in series and treatments. There were 3 replications (2, 3 and 4) and two treatments (A and B). A stands for salt and B stands for control. Totally 6 groups were created. Every group contained one replication of CxE individual. Within each of the 6 groups all plants were randomly distributed over the containers in a Randomized Complete Block Design. Each replication had 8 hydroponic containers of

treatment A and 8 hydroponic containers of treatment B. Each hydroponic container contained 12 CxE plant genotypes. The plants were left for two weeks on the standard medium. Then, the medium was refreshed: all A groups got the standard nutrient solution with extra NaCl (final concentration 120 mM). All B groups got the standard medium without extra NaCl. The 3 replications were grown for two more weeks on the hydroponics. In the meanwhile, measurements were taken (chlorophyll content and chlorophyll fluorescence). After this time replications 2 and 3 were harvested (the relevant replications for QTL mapping) and plant length and weight were measured. Then the medium was again refreshed, that time both A and B got fresh medium without extra NaCl for one more week, and then harvested in order to check the recovery rate (out of the scope of this study). Then each individual of replications 2 and 3 came together for the same treatment (pooling) and then dried.

2) The same as CxE population was done for the six tetraploid cultivars with the only difference that each group contained one hydroponic container and each hydroponic container contained two replications of each cultivar and those replications were pooled together after harvest and then dried. The experiment was undertaken in 2008.

3) The last material came from a field trial. The plants were grown in a coastal area in 2010. The underground water was contaminated by sea water. Some of the tetraploid cultivars were replicated two times while some others were replicated three times and these were pooled before dried. Only the leaves were harvested at two different dates (19/06/10 and 27/10/10) and dried.

Ion determination

Optimization

Prior to the ion determination an optimization experiment was carried out to determine the dissolvability of the sample amount, time in the ashing oven, dilution factor to be used and to calculate the retention times of cations and anions. From

the same experiment done in the past in our lab, 1000X dilution factor was chosen because of the reliability and less missing values of the output. The time in ashing oven was determined at a maximum temperature of 575^{o}C. Additionally, calculations for the same experiment last year showed the retention time of anions and cations and these retention times were stored in the MagIC Net^{TM} software. The retentions times were recalculated from the output of the standards and were Na^{+} 3.67, K^{+} 5.03, Mg^{2+} 9.80, Ca^{2+} 12.23 (min); for cations and Cl^{-} 4.90, PO_4^{3-} 11.3, SO_4^{2-} 13.44 (min) for anions. The sample amount chosen for tetraploid cultivars grown in hydroponics was 25mg for leaves and stems and 20mg for roots. The same amount was used for CxE population but poor dissolvability of the roots and stem control were observed, so we decided to make our own optimization for the appropriate amount of sample used for CxE population. Thus, 12 genotypes (6 genotypes for control root and 6 for salt root) were randomly selected and three amounts (18mg, 15mg and 12mg) were tested, each amount in 4 genotypes (2 genotypes for control root and 2 for control salt) under 1000X dilution factor. The results showed good dissolvability at 15mg and therefore in CxE population the sample amounts used for leaves, stems and roots were 25mg, 25mg and 15mg respectively. A second run was done for stems control with the same results (poor dissolvability) and after a third run with the same problem existed at that time for 15mg sample amount we decided to use stem control results for comparisons within the population but not in QTL analysis because of the unreliable nature of the results. Moreover, the sample amount from genotypes and tetraploid cultivars come from field was 25mg for leaves (only leaves were used for analysis).

Sample preparation

The dried plant material of each genotype (after pooling) was separated into leaf, stem and root, then grinded and the powder was placed in plastic containers labeled with the treatment, genotype name, type of organ and date (date was written only for field samples). The appropriate sample amount was weighed and put in a labeled glass tubes. The next step was the input of the samples in the ash

oven (glass tubes were uncapped), for a minimum of 5 hours at a maximum temperature of 575^{o}C. Special racks were used for the ash oven.

In the laboratory the first important thing to do was to calibrate the pipettes and dispensers before use. After cooling down the ashed samples, 1ml of 3M formic acid was added to the samples and the capped tubes were shaken for 15min at 99^{o}C and then were allowed to cool down. Once the ash was dissolved, 9ml of milliQ water (10X diluted) was added and then the solutions were mixed properly. 100µl from this sample was put into 9.9ml milliQ water and then mixed properly (the sample was now 1000X diluted). On the other hand, if the dissolvability was poor 9ml of milliQ were added and put in the shaker for 30min., and when the ash was dissolved 100µl from this sample was put in 9.9ml milliQ water and mixed properly (1000X diluted). For this last transfer special plastic tubes designed for IC (ion chromatography) were used in both cases. The last step before putting the samples in IC was to prepare the standards for the calibration and the blanks. The stock solutions available were 10mg/Kg=10ppm. Specifically, the concentrations were 10.0 mg/Kg±0.2% of the following anions F, Cl, Br, NO_3, SO_4, PO_4 and 10 mg/Kg±0.2% of the following cations Li, Na, K, Mg, Ca. Primary materials for standards were certified by EMPA/BAM. Anion standards were produced with certified high purity materials (NaF, NaCl, NaBr, $NaNO_3$, Na_2HPO4, Na_2SO_4) and ultrapure water (18 MOhm, 0.22 µm filtered). Cation standards were produced with high purity materials (Li_2CO_3, NaCl, KCl, MgO, $CaCO_3$), ultrapure water (18 MOhm, 0.22 µm filtered) and hydrochloric acid. Standard 1-5 were the cations standards and 6-10 were the anions standards. Standard 1 and 6 contained 0.5ppm cations and anions respectively, and were made by diluted 0.5ml cation stock solution and 0.5ml anion stock solution respectively with 9.5ml milliQ water and then mixed properly. In the same way was done for standard 2 and 7 that contained 1ppm stock solution and diluted with 9ml milliQ, standard 3 and 8 contained 2ppm stock solution diluted with 8ml milliQ etc. Standard 4 and 9 contained 4ppm stock solution and standard 5 and 10 contained 6ppm stock solution. Those concentrations for standards were designed according to the IC (ion chromatography) software in order to be recognized by the software. The

blanks (2 for each run) were prepared by adding 1ml 3M formic acid to 9ml milliQ and then 100µl of this was taken and put in 9.9ml milliQ and finally mixed properly.

Ion Chromatography (IC)

The sample preparation was done immediately before each run of the IC. The samples (hereafter analytes) were loaded onto machine for the ion content determination. According to our type of plant material and division was made in different tissues, 9 runs in total were done.

The IC equipment of Metrohm was used to determine the amount of ion concentrations in the leaves, stems and roots of the genotypes. Cation measurement was done on a Metrohm 881 Compact IC pro (2.881.0010) using a Metrosep C4 150, 150/4.0 mm column equipped with a Metrosep C4/4 Guard column. Anion measurement was done on a Metrohm 881 Compact IC pro (2.881.0020) using a Metrosep A Supp 4, 250/4.0 mm column equipped with a Metrosep A Supp 4/6 Guard column. Both IC systems were equipped with a Metrohm 858 Professional Sample Processor and a Metrohm 800 Dosino.

Before the IC system started, a check was made for every buffer, rinsing fluid that were completely filled and the waste bottles were empty (sample holder and big can beside apparatus). The analytes including blanks and standards were loaded in the 858 professional sample processor by creating a working table on workplace in Magic Net. On the working table some details referred to each sample were filled in. The *method* used was ANCAT (ANionCATion), the name of the sample was filled in on *ident,* the selected correct type in the *sample type,* the *position,* number of *injections* that was 1, *volume* that was 20µl*, sample amount* was 1 and *info 1* left empty.

Each run was started with an injection of a blank which was always an inappropriate one. This means that the first injection could not recognize retention times and peaks of the ions. After this the system expected to be ok and then the second injection was again an injection of blank to check and confirmed if the system was ok. After these two injections, the standards containing ions of interest were

analyzed to check if all the peaks were recognized and detected correctly. If not, the retention times were adjusted before all the samples are analyzed. The blanks and standards were analyzed at the beginning, at the middle and at the end of the run. Standards were measured multiple times aiming to have more accurate calibration curve. The run was ended with a blank. Before the start of each run the system was left to equilibrate. Each injection consumed 2ml of sample (10ml solution in each tube) and in combination with the fact that the needle was not going on the lowest point of the tube we could do 4 injections of each analyte. Every analyte was finished after 18 minutes and within one minute machine was consumed approximately 1ml of anion buffer and 0.9ml of cation buffer. Therefore, before of the end of each run the buffers were refilled at least one time.

When each run ended, a check was done to see if every peak was recognized and if the measured concentration (in ppm) was in accordance with the prepared concentration. The shift in retention times for peaks were adjusted (in order for the software to recognize the compound) and also the calibration curves were adjusted by turning off the incorrect standards used for the curve. Then if everything was ok the concentrations of the ions were exported from Magic Net software to an Excel file.

Data analysis

The data were subjected to statistical analysis using Genstat 13th edition. One and two-way analysis of variance (ANOVA) were used to determine the significant differences among the different tissues and treatments for their ion contents in CxE and tetraploid cultivars grown in hydroponics, while significance differences between dates and treatments for ion contents were determine for field genotypes and cultivars. Moreover, correlation analysis was done for the six tetraploid cultivars and CxE diploid population grown in hydroponics with agronomic data collected at harvest of the plants and also with some growth parameters measured in different days of growth. These analyses were done to find possible relationships between the

treatments, tissues, ion contents and growth parameters of CxE population, tetraploid cultivars in hydroponics and cultivars grown in field.

MapQTL for mapping the ions in CxE population

MapQTL is software designed for the mapping of quantitative trait loci (QTL) in experimental populations of diploid species. It was designed by Kyazma B.V., Wageningen, Netherlands (Van Ooijen, 2009). By QTL analysis regions of the genome that are responsible for phenotypic variation for a quantitative trait of interest and the associated genotypic effects can be estimated by the program. MapQTL is a MS-Windows^{R} program easy to use and extremely fast.

The first step was to prepare the data files needed to be input in a relatively simple plain text format. The three files were the *locus genotype file* (also called *loc-file*), the *map file* and the *quantitative data file* (also called *qua-file*). The *loc-file* had the genotype codes for the loci of a single segregating population. The *map file* had the map positions of all loci and the *qua-file* had the data of the quantitative traits of interest of all individuals. The traits used was the ions concentrations of CxE population of both treatments and all tissues except ions concentrations of control stem that excluded from QTL analysis because of the unreliable nature (mention before). The map used was the integrated linkage map of CxE comes from the linkage map of parent C and linkage map of parent E. The population type of CxE is CP, a population resulting from a cross between two heterogeneously heterozygous and homozygous diploid parents.

After the three data files were loaded into a MapQTL project which was the working area for the program, analysis could be start. An interval mapping was done in each trait on all linkage groups. One of the assumptions of interval mapping is that the data residuals behave according to the normal distribution so before the quantitative data were input to the program, normality plots were done for each trait separately, to check for normality and extreme values were excluded. The normality plots were done using summary statistics in Genstat. In interval mapping, a QTL likelihood profile was calculated. The likelihood for the presence of a

segregating QTL was determined for each position on the genome. Also, the genotypic effects of the QTL and the residual variance were calculated. LOD score is a likelihood ratio statistic, which is the 10-base logarithm of the quotient of the likelihood of there is segregating QTL and the likelihood there is no segregating QTL. When the LOD score was higher than the significance threshold somewhere on the linkage group, a segregating QTL was concluded to be there. The significance threshold was considered to be the value of 4 at the beginning according to a mathematical formulae used for this (previous analysis). All quantitative traits that contain linkage groups with LOD score that exceeds the threshold level, which was 4, were selected for further analysis (permutation test). The permutation test was done in order to determine the exact significance threshold in which LOD scores higher than this threshold considered that contain a QTL at that region. By doing the permutation test, the frequency distribution of the LOD was determined based on the actual data of which we were certain that there was no association between the segregating QTL and the marker. The P-value was set at 0.05 and 1000 permutations were done to estimate the significance threshold. The significance threshold was observed at relative cumulative count 0.95 in genome wide. The threshold then was enter in charts output of interval mapping results and areas with LOD score higher than the threshold were assumed to contain a QTL. However, at that areas several QTLs might segregating, some of the residual variance might be determined by other segregating QTLs, so we took into account those QTLs by using the markers that were seem to be associated with a QTL as cofactors. As a result the residual variance was reduced and the power of the test increased. This procedure was done with RMQM Mapping Analysis. The significance threshold used was the same as in interval mapping and the results could be seen in charts.

Results

Tetraploid cultivars in hydroponics (Bintje, Desiree, Russet Burbank, Monalisa, Mondial and Mozart)

Salinity tolerance of a set of six tetraploid cultivars was evaluated in the greenhouse on a hydroponic system. In this thesis, data on ion content were collected in three different tissues (root, stem and leaf). Contents for Na^+, Ca^{2+}, K^+, Mg^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-} ions were measured and significant differences between treatments and replications were done. Additionally, 26 growth data were measured, some of them during the growth in the greenhouse and some other after harvest, to see for significant differences between replications and treatments. Finally, correlations between ions and ions-growth data were done.

Replications differences for ion content

In our statistical analysis, we first tested whether the replications were sufficiently similar. In control leaf, there were significant differences between replications for K^+ (P=0.032) and Na/K ratio (P=0.032) (Table 1). K^+ and PO_4^{3-} ions showed significant differences in control stem with (P=0.015) and (P=0.048) respectively. In control root, replications were not significantly different for all ions.

In salt leaf, Cl^- (P<.001), Na^+ (P<.001) and Na/K (P=0.008) ratio showed significant differences between the two replications, while the ions concentrations were higher for replication 3 for the three ions. In salt stem, significant differences between replications for Cl^- (P=0.02), K^+ (P=0.037) and Na/K (P=0.028) were shown. Additionally, in salt root there were significant differences between replications for Cl^- (P<.001), K^+ (P<.001) and Na^+ (P<.001) with replication 3 had higher ions concentrations for the three ions (table 1). This implies that replication 3 received more NaCl than replication 2 but on the other hand this hypothesis did not confirmed by growth data. Most of the growth data had no significant differences between the two replications.

Table 1. Differences between replications for ion contents (mg/g) using one-way anova in six tetraploid cultivars grown in hydroponics

Control, leaf					Control,stem			
Variate	Repl.2	Repl.3	Range	F value	Repl.2	Repl.3	Range	F value
K	18.11	13.18	10.56-24.13	0.032	45.2	31.7	18.24-54.23	0.015
Na/K	0.817	1.122	0.484-1.307	0.032	0.262	0.394	0.163-0.778	0.177
PO4	22.14	19.38	16.05-26.26	0.162	22	18.61	16.96-26.75	0.048
Salt,leaf					Salt,stem			
Variate	Repl.2	Repl.3	Range	F value	Repl.2	Repl.3	Range	F value
Cl	15.5	34.1	8.285-39.84	<.001	21.2	32.2	12.09-38.93	0.02
K	11.65	9.14	5.451-14.76	0.209	34.4	22	11.88-47.13	0.037
Na	25.9	44.1	16.74-49.39	<.001	23.6	30.1	16.06-37.18	0.066
Na/K	2.32	5.4	1.84-9.06	0.008	0.71	1.61	0.545-3.02	0.028
Salt,root								
Variate	Repl.2	Repl.3	Range	F value				
Cl	17.77	72.66	13.47-76.79	<.001				
K	22.3	51	17.83-68.27	<.001				
Na	23.57	42.95	18.95-46.1	<.001				
Na/K	1.076	0.902	0.58-1.481	0.273				

Treatment effect on ion contents

All ions except Mg^{2+} showed treatment effect in leaves and they had significant differences between treatments. Also, the Ca^{2+}, K^{+}, Mg^{2+} and SO_4^{2-} contents decreased after the salt application. On the other hand, Cl^{-}, Na^{+}, Na/K ratio and PO_4^{3-} contents increased after the salt application.

Ions (Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, Na/K) concentrations were significantly different between control and salt treated plants in stem. All ion contents were higher in salt treated plants than control except K^{+} content. Treatment X genotype interaction was remarkable event shown in SO_4^{2-} ion.

In root, there was a significant treatment effect on Ca^{2+}, Na^{+} content and Na/K ratio with P=0.023, P<0.001, P<0.001 respectively. Na^{+} and Na/K ratio concentrations increased after salt treatment while Ca^{2+} concentration decreased (table 2).

Table 2. Differences between treatments for ion contents (mg/g) using two-way anova and replication as a block factor in six tetraploid cultivars grown in hydroponics

Tissue:leaf

Variate	Control	Salt	F value	Treatment x Cultivar(interaction)
Ca	11.535	8.494	< 0.001	0.05
Cl	0.23	24.803	< 0.001	0.134
K	15.65	10.39	0.003	0.54
Mg	3.974	3.65	0.223	0.538
Na	14.2	35	<.001	0.846
Na/K	0.97	3.86	<.001	0.715
PO4	20.76	23.42	0.034	0.612
SO4	7.49	5.96	<.001	0.867

Tissue:stem

Variate	Control	Salt	F value	Treatment x Cultivar(interaction)
Ca	7.83	8.48	0.439	0.357
Cl	0.75	26.731	< 0.001	0.672
K	38.4	28.2	<.001	0.282
Mg	2.23	4.41	<.001	0.161
Na	11.28	26.85	<.001	0.348
Na/K	0.328	1.16	0.001	0.446
PO4	20.31	22.66	0.072	0.154
SO4	6.12	6.28	0.653	0.038

Tissue: Root

Variate	Control	Salt	F value	Treatment x Cultivar(interaction)
Ca	19.5	12.3	0.023	0.48
Cl	missing values			
K	28.3	36.7	0.142	0.979
Mg	7.69	7.55	0.807	0.911
Na	14	33.3	<.001	0.993
Na/K	0.568	0.989	<.001	0.813
PO4	38	35.1	0.313	0.098
SO4	16.5	16.49	0.985	0.736

Genotypic variation of ion contents

In leaves of control plants significant differences were shown for Ca^{2+} content between cultivars (P=0.024), but no cultivar effect is observed for the other ions. The significant difference for Ca^{2+} content was proven by ANOVA, but significant differences within the cultivars cannot be shown using Bonferroni test because is unbalanced design (for one cultivar there was a missing value in one replication). Bonferroni test was done to check and find the differences between the cultivars (every time that comparisons were done between more than two objects this test was used). In stems of control plants significant differences for K^{+} and SO_4^{2-} contents between the cultivars were seen with P=0.041 and P=0.006 respectively. Moreover, for SO_4^{2-} content in salt treated plants for tissue stem significant differences were shown between the cultivars (P=0.029) while the other ions showed no cultivar effect. The significant differences identified by ANOVA for $SO4^{-2}$ content in salt treated plants for tissue stem and for K^{+} content in control plants and tissue stem, were not in agreement with the Bonferroni test in which no significant differences were found between cultivars for both ions. Additionally, there were significant differences between cultivars for PO_4^{3-} content (P=0.01) in control plants and tissue root (table 3). All the other ions in control plants for tissue root had no cultivar effect and the same can be noticed in salt treated plants and tissue root.

Figures 1-6 (Appendix

) show how the concentrations of ions were set up in different tissues for both control and salt conditions for the six tetraploid cultivars.

Table 3. Differences between cultivars for ion contents (mg/g) using two-way anova and replication as a block factor in six tetraploid cultivars grown in hydroponics. Bonferroni test shows the differences between cultivars. Bonferroni test column show if the bonferroni test was done (yes) and if not (no).

		Leaf	Control					
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	Bon. Test
Ca	11.32	10.86	10.15	10.23	11.87	14.51	0.024	no
		Stem	Control					
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	
K	25.46 a	41.34 a	44.16 a	43.28 a	42.66 a	33.79 a	0.041	yes
SO4	4.918 a	5.922 ab	5.985 ab	7.217 b	6.751 b	5.934 ab	0.006	yes
		Root	Control					
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	
PO4	29.2 ab	40.4 ab	16.2 a	58.4 b	35.8 ab	48.2 ab	0.01	yes
		Stem	Salt					
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	
SO4	4.53 a	5.52 a	4.25 a	10.05 a	6.64 a	6.66 a	0.029	yes

Treatment effect on growth data

There were significant differences in %RDW (percentage of root dry weight), %SDW (percentage of shoot dry weight), CC6 (chlorophyll content at day 6), CC20 (chlorophyll content at day 20), CF6 (chlorophyll fluorescence at day 6), CFu20 (chlorophyll fluorescence of upper leaves at day 20), RDW (root dry weight), RFW (root fresh weight), SDW (shoot dry weight), SFW (shoot fresh weight) and SL (shoot length) between the treatments for tetraploid cultivars grown in hydroponics. All these growth data showed a decrease after the salt application except from CC6 in which there was a slight increase (table 4). R_S_DW (root to shoot dry weight) and R_S_FW (root to shoot fresh weight) showed no significant differences between the treatments. Additionally, significant differences between the replication 2 and 3 under salt conditions for growth data were only shown for CF0, CF6, CF10 and CF13 (table 5).

Differences in replications for growth data

According to table 5 growth data were taken also for replication 4 (2 hydroponics containers left for one more week in standard medium after replication 2 and 3 were harvested), so three replications are existing here. The chlorophyll fluorescence at day 6, 10 and 13 (CF6, CF10, CF13), RDW, SDW, SL growth data in control plants showed significant differences between replications with P=0.002, P<.001, P<.001, P=0.037, P=0.028, P<.001 respectively. However, in salt treated plants the chlorophyll fluorescence at day 0, 6, 10 and 13 (CF0, CF6, CF10, CF13) and SL6 (shoot length at day 6) growth data had significant differences between the replications with P<.001, P=0.002, P<.001, P=0.02, P<.001 respectively.

Cultivar effect on growth data

Lot of agronomic data showed cultivar effect in both control and salt treated plants (table 6). In control plants significant differences were observed between

cultivars for CC0, CC6, CC10, CC13, CC16, CC20, CF13, RFW, RL (root length) growth data with P<.001, P<.001, P<.001, P<.001, P<.001, P=0.008, P<.001, P=0.019, P=0.003 respectively. According to Bonferroni test except from the growth data mention above two more growth data showed significant differences between the cultivars. The two growth data were %SDW and CFu20 but these results were not in agreement with ANOVA results.

According to ANOVA, in salt treated plants, CC0, CC6, CC10, CC13, CC16, CC20, CF0, CF10, CF13, CFu20, R_S_DW (root to shoot dry weight), R_S_FW (root to shoot fresh weight), SDW, SL6 agronomic data had significant differences between cultivars. However, according to bonferroni test significant differences were shown between the cultivars for those and 5 more growth data which were %SDW, RDW, RL, SFW and SL and were not in agreement with ANOVA results.

Figures 7-10 in Appendix show how the amount of Chlorophyll content and Chlorophyll fluorescence is set up in different days for the six tetraploid cultivars.

Table 4. Differences between treatments for growth data in tetraploid cultivars grown in hydroponics using two-way anova (Treatment factor 1: Cultivars, Treatment factor 2: Treatment, Block factor: Replication)

Treatment(means)			
Variate	Control	Salt	F value
%RDW	5.89	4.34	0.015
%SDW	9.57	8.41	0.007
CC6	49.51	50.96	0.007
CC20	45.59	42.92	0.011
CF6	0.81772	0.81403	0.041
CFu20	0.8194	0.8092	0.003
RDW	0.478	0.235	<.001
RFW	9.78	5.99	0.001
SDW	2.85	1.36	<.001
SFW	34.9	19.2	0.003
SL	30.5	26.17	0.022
R_S_DW	0.168	0.1939	0.081
R_S_FW	0.298	0.365	0.182

Table 5. Differences between replications for growth data using one-way anova for control and salt treated tetraploid cultivars grown in hydroponics. Asterisk mean missing values and yes in bonferroni test column mean that this test was done.

Variate	Replication(means) for control					
	2	3	4	Range	F value	Bon. Test
CF0	0.784 a	0.774 a	0.7885 a	0.721-0.803	0.096	yes
CF6	0.8153 a	0.8147 a	0.8232 b	0.8-0.829	0.002	yes
CF10	0.8688 b	0.8065 a	0.8068 a	0.76-0.881	<.001	yes
CF13	0.8175 b	0.8232 b	0.8083 a	0.801-0.836	<.001	yes
RDW	0.571 a	0.385 b	*	0.14-0.936	0.037	yes
SDW	3.26 a	2.43 b	*	0.967-5.194	0.028	yes
SL6	15.83 b	17.75 b	10.71 a	8.0-22	<.001	yes
Variate	Replication(means) for salt					
	2	3	4	Range	F value	Bon. Test
CF0	0.791 b	0.7653 a	0.7868 b	0.718-0.799	<.001	yes
CF6	0.8076 a	0.8124 ab	0.8221 b	0.759-0.827	0.002	yes
CF10	0.8418 b	0.8185 a	0.8153 a	0.797-0.853	<.001	yes
CF13	0.8236 b	0.811 a	0.8186 ab	0.792-0.833	0.02	yes
RDW	0.252 a	0.218 a	*	0.118-0.457	0.347	yes
SDW	1.59 a	1.13 a	*	0.412-2.97	0.071	yes
SL6	12.83 a	11.98 a	18.42 b	9.5-25	<.001	yes

Table 6. Differences between cultivars for growth data (using one-way anova and replication as a block factor) under control and salt conditions. Red colour represent the controversy between outcome of Bonferroni test and one-way anova. In Bonferroni test column yes mean that Bonferroni test was done.

	Cultivars(means) for control							
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	Bon.test
%SDW	11.18 b	8.72 ab	9.85 ab	9.45 ab	10.02 ab	8.19 a	0.225	yes
CC0	50.17 b	45.05 ab	42.62 a	46.12 ab	43.3 a	41.63 a	<.001	yes
CC6	54.73 d	49.88 bc	49.22abc	46.53 ab	50.62 c	46.05 a	<.001	yes
CC10	52.65 c	49.22 bc	48 b	44.27 a	49.97 bc	43.7 a	<.001	yes
CC13	51.63 d	50.02 cd	47.67 bc	44.87 ab	50.32 cd	43.48 a	<.001	yes
CC16	51.93 c	49.8 bc	48.33 b	44.42 a	50.23 bc	44.03 a	<.001	yes
CC20	47.05 bc	48.45 c	43.4 ab	43.95 ab	49 c	41.7 a	0.008	yes
CF0	0.792 a	0.7933 a	0.776 a	0.782 a	0.7793 a	0.7703 a	0.083	yes
CF10	0.8212 a	0.8365 a	0.8345 a	0.8252 a	0.822 a	0.8248 a	0.126	yes
CF13	0.8203 bc	0.8242 c	0.8183 abc	0.8088 a	0.8135 ab	0.813 ab	<.001	yes
CFu20	0.8185 ab	0.827 b	0.8115 ab	0.807 a	0.8285 b	0.824 ab	0.103	yes
RDW	0.442 a	0.588 a	0.578 a	0.409 a	0.448 a	0.404 a	0.707	yes
RFW	7.12 a	12.98 c	10.47 abc	6.86 a	12.58 bc	8.68 ab	0.019	yes
RL	31.75 ab	37 cd	29.5 a	38.5 d	33.75 bc	33.75 bc	0.003	yes
R_S_DW	0.1644 a	0.1962 a	0.1984 a	0.151 a	0.1372 a	0.1605 a	0.628	yes
R_S_FW	0.289 a	0.321 a	0.249 a	0.266 a	0.396 a	0.266 a	0.838	yes
SDW	2.75 a	2.93 a	3.09 a	2.78 a	3.14 a	2.38 a	0.881	yes
SFW	24.6 a	43.2 a	42.5 a	25.5 a	40.1 a	33.5 a	0.588	yes
SL	30 a	29 a	29.75 a	34 a	29.5 a	30.75 a	0.942	yes
SL6	15.83 a	13.42 a	12.92 a	16.5 a	15.08 a	14.83 a	0.144	yes
	Cultivars (means) for salt							
Variate	Bintje	Desiree	Russet.B	Monalisa	Mondial	Mozart	F value	Bon.test
%SDW	8.97 ab	7.87 ab	8.76 ab	7.48 a	8.11 ab	9.26 b	0.161	yes
CC0	48.55 b	45.23 ab	43.85 ab	44.45 ab	45.43 ab	40.33 a	0.009	yes
CC6	54.55 cd	52.92 bcd	49.75 b	50.25 bc	54.85 d	43.47 a	<.001	yes
CC10	51.05 bc	50.43 bc	47.6 b	48.58 bc	53.35 c	42.28 a	<.001	yes
CC13	52.63 de	49.57 cd	44.88 bc	43.07 ab	55.4 e	38.25 a	<.001	yes
CC16	53.08 bc	49.72 bc	44.73 ab	40.2 a	54.3 c	37.5 a	<.001	yes
CC20	49.1 c	47.4 c	37.35 ab	42.4 bc	48.7 c	32.6 a	0.004	yes
CF0	0.7838 ab	0.7905 b	0.7807 ab	0.7822 ab	0.7822 ab	0.7668 a	0.006	yes
CF10	0.8238 ab	0.831 b	0.8233 ab	0.819 a	0.8298 b	0.8242 ab	0.01	yes
CF13	0.8258 b	0.8253 ab	0.8168 ab	0.8127 ab	0.8165 ab	0.8092 a	0.017	yes
CFu20	0.812 bc	0.827 c	0.7975 ab	0.8175 c	0.8105 bc	0.7905 a	0.01	yes
RDW	0.192 ab	0.291 ab	0.243 ab	0.21 ab	0.317 b	0.157 a	0.052	yes
RFW	4.34 a	9.26 a	4.49 a	5.47 a	8.06 a	4.29 a	0.404	yes
RL	31 ab	40 c	27.5 a	37 bc	35 abc	36 abc	0.071	yes
R_S_DW	0.1382 a	0.1715 a	0.1476 a	0.1903 a	0.2057 a	0.3102 b	<.001	yes
R_S_FW	0.241 a	0.291 a	0.177 a	0.418 ab	0.395 ab	0.67 b	0.038	yes
SDW	1.43 b	1.77 b	1.79 b	1.12 ab	1.55 b	0.51 a	0.005	yes
SFW	18.2 ab	30.5 b	25.6 b	14.5 ab	20 ab	6.4 a	0.069	yes
SL	27.75 b	25.75 ab	26.75 ab	27.25 ab	26.5 ab	23 a	0.199	yes
SL6	15.17 ab	13.42 ab	12.08 a	16.3 b	15.17 ab	14.33 ab	0.046	yes

Correlation analysis of ion and growth data

Correlation coefficients (r) among ion contents in different tissues and the growth parameters measured at harvest or measured in different days of growth and also between ion contents in different tissues are shown for control and salt conditions (Appendix 1 and 2 for control and Appendix 3 and 4 for salt treated plants). In some cases positive in other cases negative association are expected between ions and between ions with growth data. Positive and negative values of correlations are exported using two-sided test of correlations different from zero. All values smaller than significant level of 0.05 were chosen for analysis.

Because of the few cultivars and significant differences between the salt replications without any reliable explanation behind that, correlation analysis results especially under salt conditions should be regarded with care.

Growth parameters were 26 in total and shown in Appendix 9.

Correlation under control conditions

Ions with ions

Correlation analysis between ion contents was shown in appendix 1 under control conditions. Ca^{2+} in leaf showed strong and very strong positive correlations with Mg^{2+} in leaf (r=0.9252) and Na^{+} in stem (r=0.9504) respectively. Ca^{2+} in root showed strong negative correlation with PO_4^{3-} in leaf (r=-0.91) while a very strong positive association with Ca^{2+} in stem (r=0.9853) was observed. Strong negative associations were observed for Ca^{2+} in stem with K^{+} in root (r=-0.9116) and PO_4^{3-} in leaf (r=-0.9061). Correlation analysis exhibited very strong positive association for K^{+} in root with PO_4^{3-} in root (0.9755). Mg^{2+} in stem had very strong negative correlation with PO_4^{3-} in stem (r=0.9634). Additionally, Na^{+} in leaf with SO_4^{2-} in leaf were strong negatively associated (r=-0.9291).

Ions with growth data

Mg^{2+} in stem and PO_4^{3-} in stem exhibited very strong negative associations with CC (chlorophyll content) at most of the days (Appendix 2). Also, Mg^{2+} in stem showed strong negative association with CF13 (r=-0.9244) and strong positive association with R_S_DW (r=0.9066). PO_4^{3-} in stem showed negative correlation with %SDW (r=-0.926). CC20 was very strong negatively correlated with Na^+ in leaf (r=-0.9667) and moderately positive correlated with SO_4^{2-} in leaf (r=0.8944). CF6 showed very strong association with Mg^{2+} in root (r=0.9622). PO_4^{3-} in root was strong negatively correlated with CF13 (r=-0.9147) and very strong positively correlated with RL (r=0.9773). Na^+ in root exhibited very strong association with CFu20 (r=0.9582), while SO_4^{2-} in root showed very strong negative association with CFu20 (r=-0.9507). Strong positive correlations were shown for Ca^{2+} (in root and stem) with SDW and SFW (Appendix 2). K^+ in leaf was very strong positively associated with R_S_FW (r=0.9599) and as a consequence of this Na/K ratio in leaf was strong negatively associated (r=-0.9174) with R_S_FW. K^+ in root was strong positively correlated with RL (r=0.9137) and SL (r=0.9638). Moreover, Na^+ in leaf was very strong positively associated with %RDW (r=0.9862).

Correlations under salt conditions

Ions with ions

Ca^{2+} in root was negatively correlated with PO_4^{3-} in root (r=-0.8922) and Ca^{2+} in stem (r=-0.8186). Ca^{2+} in stem showed strong and moderately positive associations with PO_4^{3-} in root (r=0.9068), Na^+ in stem (r=0.8892) and Mg^{2+} in stem (r=0.8609). Strong positive correlations were shown for Cl^- in stem with PO_4^{3-} in stem (r=0.9386), Mg^{2+} in stem (r=0.9267) and K^+ in stem (r=0.9055). Additionally, K^+ in stem exhibited moderately positive association with Mg^{2+} in stem (r=-0.822) and K^+ in leaf showed moderately negative association with PO_4^{3-} in stem (r=-0.8182). Also, Mg^{2+} in stem was moderately positively correlated with PO_4^{3-} in stem (r=0.8339) and Na^+ in stem (r=0.8826). Moreover, Na^+ in stem showed positive associations with

PO_4^{3-} in stem (r=0.8265) and PO_4^{3-} in root (r=0.8471), while showed moderately negative association with SO_4^{2-} in leaf (r=-0.8575).

Ions with growth data

Moderately negative correlations were observed for Na/K ratio in the leaf with CC6 (r=-0.8881), CF0 (r=-0.8339) and CF13 (r=-0.8396). Na/K ratio in root, Na/K ratio in stem, Na^+ in root and SO_4^{2-} in root showed moderately and strong positive correlations with several days of CC and CF (Appendix 4). The growth trait that exhibited the most associations both negative and positive was CFu20 (Appendix 4). PO_4^{3-} in leaf had moderate negative correlation with CFm20 (r=-0.8238). RDW showed moderately negative correlation with Mg^{2+} in leaf (r=-0.8521). RL was moderately negatively associated with Ca^{2+} in root (r=-0.8673) and was moderately positively associated with Ca^{2+} in stem (r=0.8938) and Na^+ in stem (r=0.871). Also RL was strong positively associated with PO_4^{3-} in root (r=0.9202). R_S_DW had moderately positive association with Cl^- in stem (r=0.8159), strong negative association with K^+ in leaf (r=-0.9165) and strong positive association with PO_4^{3-} in stem (r=0.9255). R_S_FW had moderately negative correlation with K^+ in leaf (r=-0.8619) and moderately positive correlations with Na/K ratio in leaf (r=0.8846) and PO_4^{3-} in stem (r=0.8948). Moreover, SL was strong negatively associated with Na/K ratio in leaf (r=-0.9259) and moderately positively associated with SO_4^{2-} in root (r=0.813). Finally, SL0 was moderately positively correlated with K^+ in stem (r=0.8485) and moderately negatively correlated with Na/K ratio in stem (r=-0.8771).

CxE Population

Salinity tolerance of a set of 96 genotypes of CxE diploid population (two parents and 94 offspring) was evaluated in the greenhouse on a hydroponic system. In this thesis ion content data were collected from three different tissues (root, stem and leaf). We measured contents for Na^+, K^+, Mg^{2+}, Ca^+ Cl^-, SO_4^{2-} and PO_4^{3-} ions and

significant differences between treatments and between tissues for ion contents were found. Additionally, correlation analysis between ion content and ions with growth data was take place in different tissues and between tissues under both control and salt conditions.

Treatment effect on ion contents

In root, there were significant differences between treatments for ion contents for all the ions (P<.001) except SO_4^{2-} ion. In leaf for all the ions significant differences were shown between the treatments for their ion contents (P<.001). In stem control, as mention above (in material and methods), the dissolvability of the samples was poor so the concentrations were not so reliable and this could also proved from Na^+ concentrations which were ranging between 0.0395-0.24, while for the same cation concentrations in root control and leaf control were ranging between 6.174-25.75 and 4.395-14.72 respectively. Previous analysis from 2010 ion data showed that ion concentrations in stem control were normal and chromatographs from current experiment were normal. Therefore, there is no possible explanation justify this. Finally, some ions included in the statistical analysis but it is recommended to be regarded with care. Mg^{2+}, Cl^-, K^+, PO_4^{3-} and SO_4^{2-} contents in stem showed significant differences between the treatments (P<.001). On the other hand, Ca^{2+} in stem had no significant difference between the two treatments for its content (P=0.295).

Mg^{2+}, Ca^{2+}, PO_4^{3-}, concentrations and Na/K ratio in root were significantly decreased after the salt application, while Cl^-, Na^+ and K^+ concentrations in root were significantly increased after the salt application. The same significant increases and decreases were observed in leaf with the only difference that K^+ content reduced in salt treated genotypes. Additionally, the SO_4^{2-} content showed significant decrease in salt treated genotypes in tissue leaf. In tissue stem, Mg^{2+} and Cl^- contents were significantly enhanced by NaCl treatment while K^+, PO_4^{3-} and SO_4^{2-} concentrations were significantly decreased by NaCl treatment (table 7).

Tissue effect for ion contents

Significant differences and concentrations of ions between the different tissues could offer the possibility to make assumptions for possible components of salinity tolerance. All the ions in both control and salt conditions showed significant differences between the tissues for their contents (table 8).

Control conditions

Under control conditions, root Mg^{2+}, Ca^{2+}, and SO_4^{2-} contents were significantly higher than in leaf and stem. Leaf Mg^{2+} and SO_4^{2-} contents were significantly higher than in stem. The Cl^- and PO_4^{3-} concentrations in stem were significantly lower than in root and leaf. Stem K^+ content was significantly higher than in root and leaf. Significantly higher concentration was observed in root Na^+ compared to leaf Na^+.

Salt conditions

Under salt conditions, root Mg^{2+}, Ca^{2+}, Cl^-, Na^+ and SO_4^{2-} concentrations were significantly higher than in leaf and stem. Stem Mg^{2+} and Cl^- had significantly higher content than in leaf. SO_4^{2-} content was significantly higher in leaf than in stem. PO_4^{3-} in stem had significantly lower concentration than in root and leaf. The highest concentration of K^+ was observed in stem followed by root and the lowest concentration of K^+ is shown in tissue leaf (all are significantly different). Finally, stem K/Na ratio was significantly higher than in leaf, while leaf K/Na ratio is significantly higher than in root. Therefore, root Na^+ content was the highest and with stem K^+ content and K/Na ratio being very high, Na^+ may actively excluded from the stem part.

Table 7. Differences between treatments for ion contents (mg/g) in CxE population. Asterisk mean missing values.

Root					
Variate	Control	Range	Salt	Range	F-value
Mg	17.017	8.926-29.19	13.784	5.584-23.58	<.001
Ca	43.747	8.627-94.04	30.826	8.456-84.3	<.001
Cl	0.868	0.592-1.842	66.645	43.4-83.15	<.001
Na	14.554	6.174-25.75	48.093	29.93-70.4	<.001
K	25.53	9.254-43.42	29.86	12.93-50.87	<.001
PO4	22.967	11.05-39.46	18.724	12.32-26.03	<.001
SO4	25.93	14.83-39.53	25.23	15.23-38.69	0.328
K/Na	1.85	0.39-3.422	0.617	0.273-1.029	<.001
Leaf					
Variate	Control	Range	Salt	Range	F-value
Mg	5.572	3.44-8.548	4.362	2.151-8.095	<.001
Ca	15.333	9.357-22.77	11.235	6.378-17.62	<.001
Cl	0.821	0.361-1.706	36.561	7.287-67.31	<.001
Na	9.152	4.395-14.72	20.724	8.065-38.02	<.001
K	27.576	13.02-45.16	24.084	12.94-38.51	<.001
PO4	22.005	17.51-26.03	19.182	14.28-25.86	<.001
SO4	11.97	6.22-17.45	6.29	3.952-9.948	<.001
K/Na	3.243	0.719-6.163	1.258	0.558-2.705	<.001
Stem					
Variate	Control	Range	Salt	Range	F-value
Mg	4.357	2.134-7.481	6.720	2.097-11.91	<.001
Ca	11.418	7.177-16.94	11.774	5-19.33	0.295
Cl	0.709	0.364-1.336	53.523	32.68-82.13	<.001
Na	*	0.0395-0.24	21.71	10.98-39.66	*
K	69.967	36.24-100.5	40.121	11.08-67.06	<.001
PO4	16.967	9.79-26.32	12.283	7.349-19.52	<.001
SO4	6.840	3.796-11.58	4.979	2.158-9.952	<.001
K/Na	*	230.7-2141	1.941	0.431-3.859	*

Table 8. Differences between tissues for ions contents (mg/g) in CxE population

Control							
Variate	Root	Range	Leaf	Range	Stem	Range	F-value
Mg	17.017 c	8.926-29.19	5.572 b	3.44-8.548	4.357 a	2.134-7.481	<.001
Ca	43.75 b	8.627-94.04	15.33 a	9.357-22.77	11.42 a	7.177-16.94	<.001
Cl	0.8678 b	0.592-1.842	0.8207 b	0.361-1.706	0.7093 a	0.364-1.336	0.015
Na	14.553 c	6.174-25.75	9.152 b	4.395-14.72	0.093 a	0.0395-0.24	<.001
K	25.53 a	9.254-43.42	27.58 a	13.02-45.16	69.97 b	36.24-100.5	<.001
PO4	22.97 b	11.05-39.46	22 b	17.51-26.03	16.97 a	9.79-26.32	<.001
SO4	25.93 c	14.83-39.53	11.97 b	6.22-17.45	6.84 a	3.796-11.58	<.001
K/Na	1.8 a	0.39-3.422	3.2 a	0.719-6.163	906.1 b	230.7-2141	<.001

Salt							
Variate	Root	Range	Leaf	Range	Stem	Range	F-value
Mg	13.784 c	5.584-23.58	4.362 a	2.151-8.095	6.72 b	2.097-11.91	<.001
Ca	30.83 b	8.456-84.3	11.23 a	6.378-17.62	11.77 a	5-19.33	<.001
Cl	66.64 c	43.4-83.15	36.56 a	7.287-67.31	53.52 b	32.68-82.13	<.001
Na	48.09 b	29.93-70.4	20.72 a	8.065-38.02	21.71 a	10.98-39.66	<.001
K	29.86 b	12.93-50.87	24.08 a	12.94-38.51	40.12 c	11.08-67.06	<.001
PO4	18.72 b	12.32-26.03	19.18 b	14.28-25.86	12.28 a	7.349-19.52	<.001
SO4	25.23 c	15.23-38.69	6.29 b	3.952-9.948	4.98 a	2.158-9.952	<.001
K/Na	0.617 a	0.273-1.029	1.258 b	0.558-2.705	1.941 c	0.431-3.859	<.001

Correlation analysis of ion and growth data

Correlation coefficients (r) among ion contents in different tissues as well as between three growth parameters and ion contents in different tissues were done under control and salt conditions. Positive and negative values of correlations are exported using two-sided test of correlations different from zero. All values smaller than significant level of 0.05 were chosen for analysis. The samples from tissue stem in control plants were not dissolved well as mention above so correlations between ions in tissue stem under control conditions should be regarded with care. The Na^+ content in stem in control plants was left out from the analysis because it showed completely unreliable values after the ion concentrations were exported from ion chromatography.

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Correlations under control conditions

Ions in leaf and stem tissues

Correlations between ions in leaf and stem tissues are shown in table 9. There was a strong positive correlation between Mg^{2+} in leaf with Ca^{2+} in leaf (r=0.6827) and also Mg^{2+} in leaf had moderately positive correlation with Cl^- in leaf and Cl^- in stem. Additionally, Mg^{2+} in leaf showed weak positive associations with Mg^{2+} in stem, PO_4^{3-} in leaf and SO_4^{2-} in leaf. The correlation analysis for Mg^{2+} in stem exhibited positive correlations with Ca^{2+} in stem, Cl^- in leaf, Mg^{2+} in leaf and SO_4^{2-} in stem while it showed negative association with K^+ in stem. Na^+ in leaf showed negative associations with Ca^{2+} in stem and K^+ in leaf.

Ions in leaf and root tissues

The associations between ions in leaf and root tissues are shown in table 10. Mg^{2+} in leaf is positively correlated with Ca^{2+} in root and both had a negative correlation with K^+ in root. There was a very strong positive association (r=0.8557) between Mg^{2+} in root and Ca^{2+} in root but on the other hand Mg^{2+} in root was negatively associated with PO_4^{3-} and SO_4^{2-} in root. Remarkably, Na^+ in root had weak positive correlations with Cl^- in root and K^+ in leaf. Also, PO_4^{3-} and SO_4^{2-} in root were

positively correlated and both of the ions were positively associated with K^+ in root while showed negative association with Ca^{2+} in root.

Ions in stem and root tissues

Correlations between ion contents in stem and root tissues are shown in table 11. The Ca^{2+} content in root was positively correlated with Cl^- in stem. A negative association was shown between Mg^{2+} in stem with K^+ in root. The SO_4^{2-} in root was negatively correlated with Cl^- in stem.

Ions with growth data

Associations between ions and three growth data are shown in table 12. The chlorophyll content at day 11 (CC11) was negatively correlated with root K/Na ratio (r=-0.4019) and PO_4^{3-} in stem. There was a strong positive association between RDW and SDW (r=0.6243) but SDW showed more positive and negative associations with ion data than RDW. SDW exhibited higher negative association with Cl^- in leaf and Mg^{2+} in stem than RDW with those ions. Also, SDW showed positive correlations with ions K^+, PO_4^{3-} and SO_4^{2-} in root.

Table 9. Correlations between ion contents in leaf and stem tissues under control conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_leaf	-													
Ca_stem	0.1582	-												
Cl_leaf	0.2455	-0.0638	-											
Cl_stem	0.1619	-0.1144	0.4167	-										
K_Na_leaf	-0.0222	0.2383	0.1647	0.0177	-									
K_leaf	-0.0265	0.1488	0.0912	-0.0683	0.8278	-								
K_stem	0.2347	-0.0433	-0.0689	0.0699	-0.1438	0.0631	-							
Mg_leaf	0.6827	0.0288	0.4932	0.4025	0.0264	-0.1631	-0.023	-						
Mg_stem	0.1405	0.2949	0.459	0.0285	0.2122	0.0167	-0.4203	0.325	-					
Na_leaf	-0.0427	-0.3132	-0.1205	-0.0829	-0.8465	-0.4945	0.1685	-0.1553	-0.2191	-				
PO4_leaf	0.3042	0.2657	0.1368	-0.0364	0.1012	0.1661	-0.012	0.3813	0.2265	0.0396	-			
PO4_stem	0.2048	0.2523	0.0813	-0.0443	-0.0518	-0.1506	0.221	0.1792	0.2299	-0.0017	0.304	-		
SO4_leaf	0.3887	0.0617	0.1978	0.1645	-0.0689	0.0437	0.0823	0.3133	0.0803	0.0473	0.0511	-0.2144	-	
SO4_stem	0.055	0.2665	0.2079	0.1899	0.0521	0.0117	-0.2301	0.1389	0.309	0.002	-0.0014	0.196	0.4787	-
	Ca_leaf	Ca_stem	Cl_leaf	Cl_stem	K_Na_leaf	K_leaf	K_stem	Mg_leaf	Mg_stem	Na_leaf	PO4_leaf	PO4_stem	SO4_leaf	SO4_stem

Table 10. Correlations between ion contents in leaf and root tissues under control conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_leaf	-															
Ca_root	0.2424	-														
Cl_leaf	0.2318	0.1986	-													
Cl_root	0.1747	-0.0589	-0.0228	-												
K_Na_leaf	-0.1758	-0.072	0.122	0.2024	-											
K_Na_root	-0.0699	-0.2468	-0.2444	-0.1073	-0.2045	-										
K_leaf	-0.1368	-0.1456	0.0493	0.1794	0.824	-0.0615	-									
K_root	0.0972	-0.479	-0.3594	0.149	-0.0442	0.744	0.139	-								
Mg_leaf	0.6719	0.3421	0.4847	0.1386	0.0048	-0.2915	-0.1656	-0.3187	-							
Mg_root	0.1575	0.8557	0.1201	-0.166	-0.0365	-0.2272	-0.0136	-0.4001	0.1805	-						
Na_leaf	0.2247	0.0119	-0.0658	-0.1165	-0.8778	0.2317	-0.5517	0.1076	-0.0535	0.0343	-					
Na_root	0.1705	-0.2688	0.0028	0.29	0.3157	-0.5611	0.3251	0.0962	0.0005	-0.1691	-0.2682	-				
PO4_leaf	0.4528	0.1406	0.2893	0.2645	-0.0176	0.0093	-0.0035	0.0316	0.5822	0.0545	0.1132	-0.0283	-			
PO4_root	0.1721	-0.5498	-0.1018	-0.0224	0.0915	0.2877	0.2329	0.5838	-0.0786	-0.45	0.0145	0.2536	0.0199	-		
SO4_leaf	0.3833	0.2333	0.2125	0.186	-0.1302	0.0109	0.0392	0.064	0.3849	0.1756	0.237	0.035	0.2954	-0.0371	-	
SO4_root	0.1365	-0.329	-0.222	0.1569	0.0878	0.4039	0.2356	0.6385	-0.152	-0.2979	-0.0435	0.2361	0.0435	0.5187	0.2051	-
	Ca_leaf	Ca_root	Cl_leaf	Cl_root	K_Na_leaf	K_Na_root	K_leaf	K_root	Mg_leaf	Mg_root	Na_leaf	Na_root	PO4_leaf	PO4_root	SO4_leaf	SO4_root

Table 11. Correlations between ion contents in stem and root tissues under control conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_root	-													
Ca_stem	0.0366	-												
Cl_root	0.0373	0.302	-											
Cl_stem	0.3347	-0.0959	-0.2519	-										
K_Na_root	-0.2234	-0.1274	-0.0998	-0.0113	-									
K_root	-0.4935	0.1074	0.1968	-0.2175	0.7097	-								
K_stem	0.1404	-0.0485	-0.1068	-0.0705	0.2665	0.2732	-							
Mg_root	0.8776	-0.0384	-0.0855	0.23	-0.1788	-0.4136	0.2479	-						
Mg_stem	-0.0855	0.3673	0.1645	-0.0263	-0.4091	-0.3827	-0.4361	-0.1211	-					
Na_root	-0.3415	0.268	0.2665	-0.2223	-0.5261	0.1879	-0.1009	-0.2969	0.1281	-				
PO4_root	-0.6188	0.0323	0.1227	-0.193	0.1905	0.5559	0.2737	-0.5158	-0.0977	0.3859	-			
PO4_stem	0.144	0.2311	-0.1204	-0.0684	-0.115	-0.1738	0.3644	0.1521	0.089	-0.1186	0.1242	-		
SO4_root	-0.4545	0.1115	0.1825	-0.3586	0.3747	0.6921	-0.071	-0.3896	-0.1545	0.3557	0.5695	-0.1603	-	
SO4_stem	0.0818	0.3466	-0.0347	0.1698	-0.0882	-0.1006	-0.2206	0.0296	0.2535	0.0409	-0.0422	0.1283	0.055	-
	Ca_root	Ca_stem	Cl_root	Cl_stem	K_Na_root	K_root	K_stem	Mg_root	Mg_stem	Na_root	PO4_root	PO4_stem	SO4_root	SO4_stem

Table 12. Correlations between ion contents and growth data under control conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

CC11	-		
RDW	-0.015	-	
SDW	-0.1989	0.6243	-
Ca_leaf	0.1189	0.0491	-0.1563
Ca_root	0.0509	0.1509	-0.2824
Ca_stem	-0.0075	0.0776	-0.0908
Cl_leaf	0.0123	-0.4206	-0.5708
Cl_root	0.0062	0.2671	0.0715
Cl_stem	0.1858	-0.1626	-0.2606
K_Na_leaf	-0.0174	-0.076	-0.0435
K_Na_root	-0.4019	0.1808	0.5186
K_leaf	-0.0319	0.2209	0.2186
K_root	-0.2777	0.2713	0.598
K_stem	-0.1796	0.3624	0.3632
Mg_leaf	0.3284	-0.2398	-0.5346
Mg_root	0.0263	0.2653	-0.0732
Mg_stem	0.2523	-0.3439	-0.5253
Na_leaf	-0.0339	0.2153	0.1097
Na_root	0.2237	0.0301	-0.0295
PO4_leaf	0.1529	0.0615	-0.1369
PO4_root	-0.1919	0.0576	0.4294
PO4_stem	-0.391	-0.0693	-0.0586
SO4_leaf	0.1475	0.1466	-0.1134
SO4_root	-0.2404	0.3094	0.5155
SO4_stem	-0.0322	-0.1286	-0.3881
	CC11	RDW	SDW

Correlations under salt conditions

Ions in leaf and stem tissues

Correlations between ion contents in leaf and stem tissues are shown in table 13. The Mg^{2+} in stem showed positive correlations with the most of the ions used in the current experiment. However, it exhibited weak negative correlation (r=-0.2587) with the K/Na ratio in tissue leaf. Similarly, the Na^{+} in leaf was strongly negatively correlated (r=-0.7532) with the K/Na ratio in leaf. Remarkably, for both ions (Mg^{2+} in stem and Na^{+} in leaf) no association was found with K^{+} in leaf. The Cl^{-} in leaf

exhibited weak negative correlation with the K/Na ratio in leaf, however it showed positive association with K^+ in leaf. Additionally, there was a strong positive association between Cl^- and Na^+ content in the leaf and a positive association between Cl^- and Ca^{2+} content in the leaf. In stem, there was a positive association between K^+ and Cl^- contents and between Cl^- and Na^+ while a strong negative correlation (r=-0.7316) was observed between Na^+ and the K/Na ratio but not between Na^+ and K^+ contents. The SO_4^{2-} content in leaf and stem was weakly negatively correlated with the K/Na ratio in leaf and stem respectively. Remarkably, Na^+ in leaf was positively associated with Cl^- in stem.

Ions in leaf and root tissues

Associations between ions in leaf and root tissues are shown in table 14. The Ca^{2+} content in root is positively correlated with Mg^{2+} in root and both of them are negatively correlated with Cl^-, K^+, SO_4^{2-} and PO_4^{3-} contents in root. There was a positive association between Cl^- and Na^+ in root, while there was a negative association between Na^+ and the K/Na ratio in root. In contrast, the Cl^- content was positively correlated with K^+ content in the root. Remarkably, a weak negative correlation was observed between Na^+ in leaf and K^+ in root.

Ions in stem and root tissues

Correlations between ions in stem and root tissues are shown in table 15. There was a weakly negative correlation between Na^+ in stem and the K/Na ratio in root, while a weak positive association was observed between Na^+ in stem and Na^+ in root. Additionally, the Na^+ content in the root was weakly negatively associated with Mg^+ content in stem.

Ions with growth data

Correlations between ions and three growth data are shown in table 16. The CC11 had weak negative associations with Cl^- content in leaf and stem and Na^+ content in leaf while it was positively correlated with Mg^{2+} in root. The RDW and SDW were strongly positively correlated (r=0.6912) but their associations with ion contents were not the same. Most of the associations between RDW and ion

contents for example Ca^{2+} in leaf, Cl^{-} in leaf and root, K^{+} in stem, Mg^{2+} in leaf and PO_4^{3-} in leaf were positive, while most of the associations between SDW and ion contents for example Ca^{2+} in root, K^{+} in leaf, Mg^{2+} in root and stem, Na^{+} in leaf and PO_4^{3-} in stem were negative. All the three growth data examined here were negatively correlated with the SO_4^{2-} content in stem.

Table 13. Correlations between ion contents in leaf and stem tissues under salt conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_leaf	-															
Ca_stem	0.0618	-														
Cl_leaf	0.519	0.2723	-													
Cl_stem	0.3392	0.5508	0.3969	-												
K_Na_leaf	0.1118	-0.1456	-0.2837	-0.2812	-											
K_Na_stem	0.1204	0.0687	-0.1447	0.1496	0.0716	-										
K_leaf	0.285	0.0933	0.5256	0.0124	0.4823	-0.206	-									
K_stem	0.1584	0.3072	-0.0776	0.6001	-0.0336	0.7663	-0.1854	-								
Mg_leaf	0.6242	0.0769	0.4336	0.0745	0.1592	0.1287	0.3201	0.0342	-							
Mg_stem	0.2497	0.294	0.4198	0.306	-0.2587	-0.1325	0.1933	-0.1355	0.1461	-						
Na_leaf	0.1596	0.2246	0.738	0.3847	-0.7532	-0.2405	0.133	-0.073	0.0758	0.4392	-					
Na_stem	0.0045	0.1727	0.1878	0.4023	-0.1648	-0.7316	0.1412	-0.1938	-0.1289	0.0469	0.3446	-				
PO4_leaf	0.3518	0.0682	0.4015	0.1978	-0.1036	0.1007	0.3308	0.085	0.2994	0.4059	0.3207	-0.0817	-			
PO4_stem	-0.0317	0.2676	-0.0098	0.2501	-0.2227	0.135	-0.0865	0.3477	-0.0122	0.1837	0.2517	0.1491	0.2953	-		
SO4_leaf	0.1867	-0.0162	0.2151	-0.0506	-0.2722	-0.1052	0.0365	-0.138	0.0765	0.2686	0.3667	-0.0376	0.334	0.15	-	
SO4_stem	0.061	0.3576	0.2759	0.2965	-0.2189	-0.2835	0.1436	0.0265	-0.0206	0.2547	0.4085	0.448	0.0274	0.4647	0.3181	-
	Ca_leaf	Ca_stem	Cl_leaf	Cl_stem	K_Na_leaf	K_Na_stem	K_leaf	K_stem	Mg_leaf	Mg_stem	Na_leaf	Na_stem	PO4_leaf	PO4_stem	SO4_leaf	SO4_stem

Table 14. Correlations between ion contents in leaf and root tissues under salt conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_leaf	-															
Ca_root	-0.0349	-														
Cl_leaf	0.536	0.1629	-													
Cl_root	0.1176	-0.566	0.0719	-												
K_Na_leaf	0.082	-0.0728	-0.2874	0.125	-											
K_Na_root	0.0915	-0.3844	-0.1315	0.2226	0.193	-										
K_leaf	0.275	0.2643	0.5092	-0.0632	0.4988	-0.0264	-									
K_root	0.0886	-0.5514	-0.1127	0.5531	0.2229	0.8886	-0.0562	-								
Mg_leaf	0.6311	0.1212	0.4512	-0.0307	0.1607	0.0736	0.343	0.0252	-							
Mg_root	-0.0189	0.8981	0.1802	-0.5366	-0.0343	-0.2689	0.3104	-0.4777	0.0961	-						
Na_leaf	0.196	0.237	0.7349	-0.1364	-0.7655	-0.2425	0.0937	-0.2659	0.0986	0.2444	-					
Na_root	-0.006	-0.2069	0.0799	0.5802	-0.0063	-0.4899	-0.0447	-0.0589	-0.13	-0.3154	0.0363	-				
PO4_leaf	0.3622	0.2012	0.4171	-0.1645	-0.1056	-0.0706	0.329	-0.165	0.3221	0.1932	0.3231	-0.1555	-			
PO4_root	0.0488	-0.7698	-0.0307	0.3003	-0.1826	0.2952	-0.3035	0.3915	-0.0033	-0.6938	0.0614	0.1057	-0.089	-		
SO4_leaf	0.2142	-0.052	0.2692	-0.1463	-0.3088	0.1224	0.0337	0.0563	0.0961	0.0281	0.4201	-0.1436	0.3178	0.2324	-	
SO4_root	0.1287	-0.585	-0.0678	0.3532	0.0726	0.121	-0.1303	0.3697	-0.1045	-0.5497	-0.1091	0.4242	-0.1896	0.5089	0.2318	-
	Ca_leaf	Ca_root	Cl_leaf	Cl_root	K_Na_leaf	K_Na_root	K_leaf	K_root	Mg_leaf	Mg_root	Na_leaf	Na_root	PO4_leaf	PO4_root	SO4_leaf	SO4_root

Table 15. Correlations between ion contents in stem and root tissues under salt conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

Ca_root	-															
Ca_stem	0.0467	-														
Cl_root	-0.5984	0.0683	-													
Cl_stem	0.1055	0.5396	0.0166	-												
K_Na_root	-0.432	-0.1929	0.2719	-0.1664	-											
K_Na_stem	-0.1558	0.1277	0.0281	0.2372	0.0987	-										
K_root	-0.5852	-0.1198	0.5779	-0.1313	0.8995	0.0233	-									
K_stem	-0.0043	0.3494	-0.0408	0.6737	-0.0911	0.7611	-0.0905	-								
Mg_root	0.8993	0.012	-0.5461	0.0499	-0.2824	-0.1251	-0.4818	-0.0603	-							
Mg_stem	0.1374	0.2834	-0.1613	0.2897	0.0357	-0.0697	-0.0954	-0.1046	0.2896	-						
Na_root	-0.2056	0.1871	0.5669	0.1231	-0.4573	-0.1728	-0.0439	0.0253	-0.3392	-0.2693	-					
Na_stem	0.2539	0.1347	-0.1069	0.3467	-0.2397	-0.7071	-0.1475	-0.1379	0.1539	-0.0257	0.2443	-				
PO4_root	-0.7611	-0.0918	0.3017	-0.0311	0.3418	0.075	0.419	0.0096	-0.7126	-0.0867	0.0696	-0.1187	-			
PO4_stem	0.4302	0.2646	-0.2592	0.3082	-0.2405	0.1366	-0.2686	0.3703	0.4084	0.1584	0.0102	0.1973	-0.1771	-		
SO4_root	-0.5839	0.1101	0.3831	-0.0029	0.1421	-0.1006	0.3799	0.0003	-0.5723	-0.1413	0.4408	0.1106	0.4894	-0.1207	-	
SO4_stem	0.2705	0.3511	-0.1884	0.2649	-0.2109	-0.2866	-0.167	0.008	0.1915	0.2511	0.134	0.4349	-0.1254	0.4382	0.2138	-
	Ca_root	Ca_stem	Cl_root	Cl_stem	K_Na_root	K_Na_stem	K_root	K_stem	Mg_root	Mg_stem	Na_root	Na_stem	PO4_root	PO4_stem	SO4_root	SO4_stem

Table 16. Correlations between ion contents and growth data under salt conditions. The green highlight represent the positive correlations and the red highlight represent the negative correlations.

CC11	-		
RDW	0.0212	-	
SDW	0.0134	0.6912	-
Ca_leaf	-0.091	0.3372	0.0903
Ca_root	0.1958	-0.1784	-0.5368
Ca_stem	-0.2048	0.1308	-0.0927
Cl_leaf	-0.2808	0.2902	-0.1957
Cl_root	-0.0649	0.3334	0.3919
Cl_stem	-0.3251	0.2096	-0.0546
K_Na_leaf	0.164	-0.011	0.1306
K_Na_root	0.1051	0.1493	0.227
K_Na_stem	0.0402	0.2965	0.2214
K_leaf	-0.0352	0.0276	-0.2582
K_root	0.0073	0.1758	0.3579
K_stem	-0.1921	0.2434	0.1875
Mg_leaf	0.0425	0.2985	-0.0211
Mg_root	0.3245	-0.1336	-0.5205
Mg_stem	-0.0457	-0.0113	-0.3644
Na_leaf	-0.2809	0.0354	-0.3302
Na_root	-0.2052	0.0566	0.186
Na_stem	-0.2122	-0.1899	-0.1315
PO4_leaf	-0.1137	0.2546	-0.1647
PO4_root	-0.1316	0.1262	0.3641
PO4_stem	0.1408	-0.1219	-0.316
SO4_leaf	-0.1041	0.1359	-0.028
SO4_root	-0.2089	-0.0401	0.2438
SO4_stem	-0.2452	-0.2723	-0.4197
	CC11	**RDW**	**SDW**

Tetraploid cultivars and CxE genotypes grown in the field

Salinity tolerance of a set of 25 tetraploid cultivars (5 were from those grown also in hydroponics) and 20 genotypes of CxE population (two parents, the 9 with the best performance and the 9 with the lowest performance in hydroponics) was evaluated in the field. Ion contents (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-}) were measured in two different dates (19-06-10 and 27-07-10) from only leaf tissue. Additionally, yield per plant was measured and significant differences between treatments and performance were found.

Developmental stage effect

Significant differences were made for ion content between the two dates to check if the developmental stage had an effect in ion concentration.

Tetraploid cultivars grown also in hydroponics

In control and salt plants (table 17) significant differences were shown between the two dates. There were no significant differences for Cl^- and Na^+ contents between the two dates in both conditions. The significant differences found for some ions were probably results from different nutritional requirements at different developmental stages of the plant.

CxE genotypes

In control and salt plants (table 17) significant differences were shown between the two dates. There were no significant differences for Cl^- content between the two dates in both conditions. Na^+ content in 27-07-10 was significantly decreased under control conditions, while no significant differences were observed for Na^+ content under salt conditions. The other significant differences could be attributed to different nutritional requirements at different developmental stages of the plant.

Tetraploid cultivars grown only in field

In control and salt plants (table 17) significant differences were shown between the two dates. There were significant differences between the two dates for Cl^- content under salt conditions, while no significant differences were observed for Cl^- content under control conditions. Na^+ content was significantly reduced in 27-07-10 under control conditions, while no significant differences were observed for Na^+ content between the two dates under salt conditions. The other significant differences could be attributed to different nutritional requirements at different developmental stages of the plant.

Treatment effect for ion contents

In date 19-06-10, significant differences are shown in table 18. Na^+ content showed significant decrease in salt treated plants in all types of plant material. Cl^- content had no significant differences between treatments in all types of plant material. Salt treated plants might not perceive any salt stress was the most possible explanation. Significant differences for other ions could be attributed to different nutritional requirements at different developmental stages of the plant.

In date 27-07-10, significant differences are shown in table 18. Both Na^+ and Cl^- contents had no significant differences between treatments. Significant differences for other ions could be attributed to different nutritional requirements at different developmental stages of the plant.

Table 17. Differences between dates for ion contents (mg/g) in tetraploid cultivars and CxE population grown in the field.

TETRAPLOID CULTIVARS GROWN ALSO IN HYDROPONICS						
	Control			Salt		
Variate	19/6/2010	27/7/2010	F value	19/6/2010	27/7/2010	F value
Ca	31.2	34.9	0.395	28.7	32.3	0.261
Cl	2.64	1.97	0.295	2.59	1.86	0.232
K	30.1	20.4	0.069	33.3	17.1	0.004
Mg	5.69	7.12	0.21	4.56	4.67	0.873
Na	7.11	5.3	0.091	4.9	4.82	0.864
Na/K	0.24	0.288	0.42	0.153	0.309	0.012
PO4	5.98	3.56	<.001	5.49	3.5	<.001
SO4	5.19	4.63	0.22	4.51	4.08	0.202
CxE GENOTYPES						
	Control			Salt		
Variate	19/6/2010	27/7/2010	F value	19/6/2010	27/7/2010	F value
Ca	35.3	29.4	0.041	29.694	30.452	0.79
Cl	2.001	2.047	0.927	1.855	3.029	0.182
K	16.41	9.48	<.001	16.071	13.386	0.111
Mg	7.92	5.38	<.001	3.801	3.199	0.208
Na	6.57	4.76	<.001	4.432	4.536	0.765
Na/K	0.455	0.561	0.086	0.29	0.379	0.017
PO4	6.63	5.29	0.03	5.496	4.309	0.027
SO4	5.72	6.05	0.414	4.595	4.555	0.905
TETRAPLOID CULTIVARS GROWN ONLY IN FIELD						
	Control			Salt		
Variate	19/6/2010	27/7/2010	F value	19/6/2010	27/7/2010	F value
Ca	35.5	39.9	0.153	34.4	34.5	0.971
Cl	2.4	2.05	0.285	2.62	1.91	0.024
K	32.3	20.3	<.001	30.97	15.7	<.001
Mg	8.47	8.79	0.753	4.47	4.43	0.962
Na	8.27	6.42	0.014	6.08	5.69	0.505
Na/K	0.27	0.392	0.028	0.207	0.434	<.001
PO4	6.54	4.35	<.001	6.59	3.54	<.001
SO4	5.484	4.915	0.006	5.06	4.24	<.001

Table 18. Differences between treatments for ion contents (mg/g) in tetraploid cultivars and CxE population grown in the field.

TETRAPLOID CULTIVARS GROWN ALSO IN HYDROPONICS						
	19/6/2010			27/7/2010		
Variate	Control	Salt	F value	Control	Salt	F value
Ca	31.2	28.7	0.462	34.9	32.3	0.535
Cl	2.64	2.59	0.933	1.97	1.86	0.861
K	30.1	33.3	0.51	20.4	17.1	0.435
Mg	5.69	4.56	0.146	7.12	4.67	0.042
Na	7.11	4.9	0.032	5.3	4.82	0.451
Na/K	0.24	0.153	0.014	0.288	0.309	0.764
PO4	5.98	5.49	0.255	3.56	3.5	0.871
SO4	5.19	4.51	0.072	4.63	4.08	0.206

CxE GENOTYPES						
	19/6/2010			27/7/2010		
Variate	Control	Salt	F value	Control	Salt	F value
Ca	35.3	29.7	0.02	29.372	30.452	0.749
Cl	2.001	1.855	0.746	2.047	3.029	0.268
K	16.41	16.07	0.837	9.48	13.386	0.021
Mg	7.92	3.8	<.001	5.377	3.199	0.002
Na	6.57	4.43	<.001	4.763	4.536	0.585
Na/K	0.455	0.29	<.001	0.561	0.379	0.005
PO4	6.63	5.5	0.013	5.290	4.309	0.16
SO4	5.72	4.59	<.001	6.052	4.555	0.003

TETRAPLOID CULTIVARS GROWN ONLY IN FIELD						
	19/6/2010			27/7/2010		
Variate	Control	Salt	F value	Control	Salt	F value
Ca	35.5	34.4	0.638	39.9	34.5	0.204
Cl	2.4	2.62	0.492	2.05	1.908	0.642
K	32.3	31	0.56	20.3	15.7	0.055
Mg	8.47	4.47	<.001	8.79	4.43	<.001
Na	8.27	6.08	0.006	6.42	5.69	0.169
Na/K	0.27	0.207	0.061	0.392	0.434	0.577
PO4	6.54	6.59	0.863	4.35	3.54	0.003
SO4	5.48	5.06	0.05	4.915	4.241	0.001

Differences for yield per plant

CxE genotypes

There were no significant differences between treatments for yield per plant in:

1. 20 CxE genotypes (all together in comparison)
2. High performing genotypes
3. Low performing genotypes

Also, no significant differences were observed between high and low performing genotypes for yield per plant under both (control and salt) conditions (table 19).

Tetraploid cultivars grown also in hydroponics

There were no significant differences between treatments for yield per plant but a reduction for yield per plant was observed in salt treated plants (table 19).

Tetraploid cultivars grown only in field

The yield per plant (table 19) was significantly reduced in salt treated plants (P=0.002).

Table 19. Differences between treatment and performance for yield per plant in tetraploid cultivars and CxE genotypes grown in field

Differences between treatments for yield per plant for all CxE genotypes grown in field		
Control	Salt	F value
0.868	1.026	0.417
Differences between high and low performing CxE genotypes for yield per plant under control conditions		
High	Low	F value
0.75	0.94	0.393
Differences between high and low performing CxE genotypes for yield per plant under salt conditions		
High	Low	F value
0.82	1.00	0.582
Differences between treatments for yield per plant in high performing CxE genotypes		
Control	Salt	F value
0.75	0.82	0.765
Differences between treatments for yield per plant in low performing CxE genotypes		
Control	Salt	F value
0.94	1.00	0.861
Differences between treatments for yield per plant in 5 tetraploid cult. (also grown in hydr.)		
Control	Salt	F value
1.616	1.403	0.088
Differences between treatments for yield per plant in 20 tetraploid cult. (only in field)		
Control	Salt	F value
1.508	1.269	0.002

QTL mapping

The current study shows the QTL analysis of ion data in 2008 for CxE diploid potato population. Additionally, comparisons are made with the QTL analysis of ion data in 2009 that was done in similar experiment with the same genotypes used from CxE diploid potato population. In Appendix 5 and 6 the QTLs are placed on the chromosomes using the program MapChart.

QTL analysis of ion data

The steps that were used for QTL analysis were:

1. Load the three files in to the program (genotype file, map file and quantitative data file).
2. Find a significance threshold to use for the LOD score by mathematical method (this is value 4 and was determined for previous analysis).
3. Interval mapping. Select the quantitative data that contain regions in chromosomes with LOD score more than 4.
4. Permutation test to find the significance threshold to be used for the current experiment.
5. Interval mapping.
6. RMQM mapping by using the markers in region exceed the significance threshold as cofactors and take over the role of nearby QTLs.

QTL analysis of ion data under salt conditions

Na-leaf

The permutation test in Na-leaf shows that LOD scores more than the significance threshold of 4,5 somewhere in a linkage group lead to conclusion that there was a segregating QTL. A QTL was found in position 37,434 for Na-leaf (table 20) with LOD value of 4,55 in chromosome 7 (hereafter, CE7). The %explained which is the variance explained by the QTL effect is 20,6. Moreover, the mean genotypic information coefficient (hereafter, GIC_m) was 0,97. The GIC_m shows the power of interval mapping and is the marker information content. It can take values from 0-1 (the highest the more marker information the more power of interval mapping). After the use of the linked marker at that position as a cofactor by rMQM mapping the QTL effect was still there and no new QTLs revealed.

A segregating QTL was also detected for Na-leaf in 2009 in CE7 (table 21) between positions 29,006-53,203. The estimated map position of the QTL, which is the position with the largest LOD on the linkage group, was at 38,968. After the use of two-LOD support interval, a 95% confidence interval was constructed for the area

contains the QTL and this area was between 36,706-46,052. The two-LOD support interval can be estimated by taking the two positions left and right of the point estimate, that have a LOD value of two less than the maximum and a roughly 95% confidence interval around this point estimate is obtained. The two-LOD support interval is useful for very large areas showing a segregating QTL in a linkage group.

SO_4-leaf

In CE2 (chromosome 2) a QTL was found which had strong effect in position 102,904 for SO4-leaf (table 20). The significance threshold after the permutation test (hereafter, PT) was 4,5 and the LOD score for this QTL was 5,56. Also, the GIC_m is 0,966 which is high and the % of the variance explained by the QTL effect is 24,1 which is also high. In rMQM mapping the linked marker at that position was used as cofactor but nothing new came out and the QTL was still there. No QTL for SO4-leaf was detected after QTL analysis of ion data in 2009.

Mg-stem

For Mg-stem the significance threshold after PT was determined at 4,4. The interval mapping showed 4 QTLs. Two different areas in chromosome 3 and two different areas in chromosome 4 exceeded the significant threshold. The positions in CE3 were 46,098-78,344 and 86,396-108,129, while the positions in CE4 are 0-3,306 and 5,968. Combinations of flanking markers used as cofactors in rMQM mapping for all the areas in the two chromosomes were done. The combinations were used because program couldn't use all the markers as cofactors at once. The number of combinations of different markers was 11. The five out of eleven combinations show that no QTLs were existing in CE4, and both QTLs in CE3 were there. Three combinations showed 4 QTLs one in each predefined area but the second area in CE4 has the QTL in position 17,577 instead of position 5,968. One combination showed only one QTL in CE4 in position 17,577 and both QTLs in CE3 and finally only one combination of markers used as cofactors confirmed the same results as interval mapping. All these lead to the conclusion that QTLs in CE4 might be artificial, while there were very strong QTLs in CE3. Next step was to load the parental maps (C and E) as map files instead of integrated CxE map to check the real order of the markers.

Finally, the order of markers proved to be different in the parental maps compare with integrated map, so a marker or markers were placed in wrong linkage groups, and this lead to the conclusion that there was only one segregating QTL with very strong effect in CE3 between positions 46,098-108,129 (table 20). The estimated position of the QTL on the map is at position 68,19 with LOD score of 8,77. Using the two-LOD support interval the QTL has 95% chances to be between positions 62,036-74,887.

The same QTL was confirmed from ion data QTL analysis in 2009. In the same chromosome between 45,098-110,001 was the area found to contain the segregating QTL (table 21). After the use of two-LOD support interval the QTL had 95% confidence interval to be between positions 66,708-68,685. The estimated position on the map was at the same position (68,19), the same as in current study.

K/Na-leaf, Cl-leaf, Ca-stem and K/Na-root

In CE3 for K/Na leaf the LOD score exceeded the predefined significance threshold (4,7) in position 87,396-91,396 but despite that the % of variance that explained by the QTL effect was high (22,2-22,4) the GIC_m were too low (0,641-0,71) and there were no marker scores at that area so the QTL found at that area considered as an artificial QTL (table 20). However, in QTL analysis in 2009 for K/Na leaf a QTL was detected in CE7 instead of CE3 between positions 28,006-45,435 (table 21) with GIC_m and % of variance explained by the QTL effect being high. The estimated position of the QTL on the map was at position 41,802.

Furthermore, QTL analysis in 2009 was showed a segregating QTL for Cl-leaf in CE3 with very high GIC_m and high % of variance explained by the QTL effect (table 21). The estimated position on map was at position 18,383. In the current study, LOD values were observed in CE7 for Cl-leaf that was just under the significance threshold (table 20).

Additionally, in 2009 a segregating QTL was detected for Ca-stem in CE11 (table 21) at estimated map position 8,86. The area contain the LOD values that exceeded significance threshold were between positions 8-10,86. The current study showed LOD values just under significance threshold for Ca-stem in CE11 (table 20).

Moreover, a segregating QTL was detected at 88,206-89,444 area for K/Na root in CE8 in 2009 (table 21). The estimated map position was at 89,444. In the current study LOD scores just under significance threshold were observed for K/Na root in CE1 (table 20).

Table 20. Identification of QTLs under salt conditions in 2008.

Year	Trait	LOD PT	Group	Position	Locus	LOD	% Expl.	GIC_m
2008	Na-leaf	4,5	CE7	37,434	PotSNP730	4,55	20,6	0,97
2008	SO4-leaf	4,5	CE2	102,904	PotSNP128	5,56	24,1	0,966
2008	Mg-stem	4,4	CE3	62,036		7,1	29,4	0,975
2008	Mg-stem	4,4	CE3	62,175	PotSNP131	7,27	30	0,98
2008	Mg-stem	4,4	CE3	62,908	PotSNP423	7,69	31,4	0,986
2008	Mg-stem	4,4	CE3	63,064	PotSNP1074	7,8	31,8	0,988
2008	Mg-stem	4,4	CE3	63,698	PotSNP502	8,14	32,9	1
2008	Mg-stem	4,4	CE3	64,698		8,29	33,4	0,984
2008	Mg-stem	4,4	CE3	65,698		7,64	31,2	1
2008	Mg-stem	4,4	CE3	65,708	PotSNP1001	7,63	31,2	1
2008	Mg-stem	4,4	CE3	66,708		7,85	31,9	0,963
2008	Mg-stem	4,4	CE3	67,708		7,89	32,1	0,971
2008	Mg-stem	4,4	CE3	67,753	PotSNP75	7,89	32,1	0,972
2008	Mg-stem	4,4	CE3	68,118	PotSNP87	7,84	31,9	0,986
2008	Mg-stem	4,4	CE3	68,19	bch_E	8,77	34,9	0,978
2008	Mg-stem	4,4	CE3	68,685	Sti013-B	8,54	34,2	0,969
2008	Mg-stem	4,4	CE3	68,991	StCHY2	7,62	31,2	0,983
2008	Mg-stem	4,4	CE3	69,991		7,91	32,1	0,902
2008	Mg-stem	4,4	CE3	70,991		6,94	28,8	0,885
2008	Mg-stem	4,4	CE3	72,56		7,78	31,7	0,86
2008	Mg-stem	4,4	CE3	72,887	StPho1a-MspI540	7,97	32,3	0,885
2008	Mg-stem	4,4	CE3	73,887		7,78	31,7	0,88
2008	Mg-stem	4,4	CE3	74,887		6,98	29	0,91
2008	K/Na-leaf	4,7	CE3	89,396		4,8	22,4	0,665
2008	K/Na-leaf	4,7	CE3	90,396		4,79	22,4	0,651
2008	K/Na-leaf	4,7	CE3	88,396		4,78	22,4	0,685
2008	K/Na-leaf	4,7	CE3	91,396		4,75	22,2	0,641
2008	K/Na-leaf	4,7	CE3	87,396		4,74	22,2	0,71
2008	Cl-leaf	4,5	CE7	35,107	PotSNP447	4,36	19,6	0,969
2008	Cl-leaf	4,5	CE7	37,434	PotSNP730	4,34	19,5	0,97
2008	Ca-stem	4,5	CE11	34,246		4,01	18	0,942
2008	Ca-stem	4,5	CE11	33,246		3,71	16,8	0,885
2008	K/Na-root	4,5	CE1	68,29	E32M61-12h1	4,29	19,7	0,864
2008	K/Na-root	4,5	CE1	65,873	PotSNP464	4,27	19,6	0,842

Table 21. Identification of QTLs under salt conditions in 2009.

Year	Trait	LOD PT	Group	Position	Locus	LOD	% Expl.	GIC_m
2009	Na-leaf	4,5	CE7	36,706	PotSNP138	5,76	25,3	0,938
2009	Na-leaf	4,5	CE7	37,434	PotSNP730	6,03	26,3	0,97
2009	Na-leaf	4,5	CE7	38,434		8,53	35,1	0,886
2009	Na-leaf	4,5	CE7	38,968	E39/M60-3c7	7,86	32,8	0,971
2009	Na-leaf	4,5	CE7	39,968		8,14	33,8	0,937
2009	Na-leaf	4,5	CE7	40,968		7,75	32,5	0,939
2009	Na-leaf	4,5	CE7	41,802	E39/M60-14c7	7,11	30,2	0,957
2009	Na-leaf	4,5	CE7	42,802		7,93	33,1	0,921
2009	Na-leaf	4,5	CE7	43,802		8,34	34,4	0,921
2009	Na-leaf	4,5	CE7	44,802		8,14	33,8	0,957
2009	Na-leaf	4,5	CE7	45,435	PotSNP712	7,76	32,5	0,999
2009	Na-leaf	4,5	CE7	46,052	E32M61-25c7	6,27	27,2	0,986
2009	Mg-stem	4,5	CE3	66,708		12,66	46,6	0,963
2009	Mg-stem	4,5	CE3	67,708		13,48	48,7	0,971
2009	Mg-stem	4,5	CE3	67,753	PotSNP75	13,5	48,8	0,972
2009	Mg-stem	4,5	CE3	68,118	PotSNP87	13,66	49,2	0,986
2009	Mg-stem	4,5	CE3	68,19	bch_E	14,55	51,3	0,978
2009	Mg-stem	4,5	CE3	68,685	Sti013-B	14,42	51	0,969
2009	K/Na leaf	4,6	CE7	28,006		4,8	22,4	0,899
2009	K/Na leaf	4,6	CE7	29,006		5	23,2	0,883
2009	K/Na leaf	4,6	CE7	30,006		5,15	23,9	0,875
2009	K/Na leaf	4,6	CE7	31,006		5,25	24,3	0,876
2009	K/Na leaf	4,6	CE7	32,006		5,28	24,4	0,885
2009	K/Na leaf	4,6	CE7	33,006		5,23	24,2	0,903
2009	K/Na leaf	4,6	CE7	34,006		5,12	23,7	0,929
2009	K/Na leaf	4,6	CE7	35,006		4,94	23	0,963
2009	K/Na leaf	4,6	CE7	35,107	PotSNP447	4,92	22,9	0,969
2009	K/Na leaf	4,6	CE7	36,107		5	23,3	0,937
2009	K/Na leaf	4,6	CE7	36,706	PotSNP138	5,04	23,4	0,938
2009	K/Na leaf	4,6	CE7	37,434	PotSNP730	5,14	23,8	0,97
2009	K/Na leaf	4,6	CE7	38,434		6,21	28	0,886
2009	K/Na leaf	4,6	CE7	38,968	E39/M60-3c7	5,59	25,6	0,971
2009	K/Na leaf	4,6	CE7	39,968		6,3	28,4	0,937
2009	K/Na leaf	4,6	CE7	40,968		6,49	29,1	0,939
2009	K/Na leaf	4,6	CE7	41,802	E39/M60-14c7	6,15	27,8	0,957
2009	K/Na leaf	4,6	CE7	42,802		6,33	28,5	0,921
2009	K/Na leaf	4,6	CE7	43,802		6,11	27,6	0,921
2009	K/Na leaf	4,6	CE7	44,802		5,53	25,4	0,957
2009	K/Na leaf	4,6	CE7	45,435	PotSNP712	5,08	23,6	0,999
2009	Cl leaf	4,4	CE3	18,383	PotSNP1141	5,32	22,9	0,99
2009	Ca-stem	4,5	CE11	8		4,79	21,1	0,83
2009	Ca-stem	4,5	CE11	8,86	PotSNP114	4,78	21,1	0,897
2009	Ca-stem	4,5	CE11	9,86		4,69	20,7	0,867
2009	Ca-stem	4,5	CE11	10,86		4,54	20,1	0,85
2009	K/Na root	4,5	CE8	88,206		4,6	20,6	0,942
2009	K/Na root	4,5	CE8	88,551	E39/M60-46e8	4,71	21	0,954
2009	K/Na root	4,5	CE8	89,444	PDVsg_R1	4,92	21,8	0,965

QTL analysis of ion data under control conditions

K-leaf

In K-leaf as a quantitative trait for QTL analysis no QTL was detected after interval mapping and PT value of 4,5. However, after the use of combinations of different flanking markers as cofactors by rMQM mapping , QTLs were observed in CE5 and CE8. In CE8 a QTL was found (table 22) in position 70,844 and 17,2% of the variance is explained by the QTL effect. In CE5 two area exceeded the significant threshold, so parental maps were used again instead of integrated CxE map, as map files and the order of the markers was different between parental maps compare to integrated map, so a marker or markers were placed in wrong linkage groups, that means markers were placed in different linkage groups in CxE integrated map compared to single parent maps, and this cause the appearance of two areas contain a QTL. Therefore, one QTL was observed in CE5 in position 46,335-53,929 and the estimated position of the QTL on the map was at position 53,929 and 19,5% of the variance is explained by the QTL effect (table 22). The LOD score was 5,33 after rMQM mapping. In QTL analysis in 2009, no QTLs were detected for K-leaf.

K/Na-leaf

Additionally, a QTL was found in CE5 for K/Na leaf in position 53,929 with LOD score 4,54. The significance threshold after PT is 4,4. GIC_m was 0,989 with 20,1% of variance explained by the QTL effect (table 22). No QTLs were detected for K/Na leaf in QTL analysis of 2009.

PO_4-root

A segregating QTL for PO4-root was revealed in QTL analysis of 2009, at position 51,198 in CE2 (table 23), while the QTL analysis in the current study showed LOD scores just under significance threshold for PO4-root in CE4 (table 22).

Table 22. Identification of QTLs under control conditions in 2008.

Year	Trait	LOD PT	Group	Position	Locus	LOD	% Expl.	GIC_m
2008	K-leaf	4,5	CE8	70,844	PotSNP104	5	17,2	
2008	K-leaf	4,5	CE5	46,335		4,6	17,1	
2008	K-leaf	4,5	CE5	47,335		4,72	17,5	
2008	K-leaf	4,5	CE5	47,649	E32M61-32c5	4,73	17,5	
2008	K-leaf	4,5	CE5	48,649		4,92	18,2	
2008	K-leaf	4,5	CE5	49,296	PotSNP1144	4,94	18,2	
2008	K-leaf	4,5	CE5	50,221	PotSNP1152	4,52	16,9	
2008	K-leaf	4,5	CE5	50,221	PotSNP1146	4,51	16,8	
2008	K-leaf	4,5	CE5	52,012		4,9	18,1	
2008	K-leaf	4,5	CE5	52,579	R1	4,87	18	
2008	K-leaf	4,5	CE5	53,423	PotSNP1145	5,31	19,4	
2008	K-leaf	4,5	CE5	53,929	PotSNP1143	5,33	19,5	
2008	K/Na-leaf	4,4	CE5	53,929	PotSNP1143	4,54	20,1	0,989
2008	K/Na-leaf	4,4	CE5	53,423	PotSNP1145	4,49	19,9	0,985
2008	PO4-root	4,6	CE4	77,049	PotSNP59	4,11	18,2	0,931
2008	PO4-root	4,6	CE4	76,863		4,06	18	0,932
2008	PO4-root	4,6	CE4	81,592		4,05	18	0,901
2008	PO4-root	4,6	CE4	77,049	PotSNP1007	4,04	18	0,936
2008	PO4-root	4,6	CE4	77,049	PotSNP1006	4,04	18	0,935

Table 23. Identification of QTLs under control conditions in 2009.

Year	Trait	LOD PT	Group	Position	Locus	LOD	% Expl.	GIC_m
2009	PO4 root	4,6	CE2	51,198	E32M61-6e2	4,92	22	0,939

Discussion

Tetraploid cultivars and CxE diploid potato population grown in hydroponics

The results in the current experiment exhibited significant treatment and organ effect on most of the ions. Genotypes of CxE population grown in hydroponics showed variation in different tissues for their ion contents. This is consistent with (Jefferies, 1996) who proposed genetic variation in potato cultivars for salt tolerance. The same conclusion cannot be drawn for the six tetraploid cultivars grown in hydroponics for their ion contents because replications were a problem (probably one replication receive more NaCl than the other), so we didn't have the statistical power to find differences if they exist. Variation is shown for cultivars grown in hydroponics only for growth data (figure 7-10) in appendix.

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; Tarakcioglu and Inal, 2002; Karimi et al., 2005). Plant growth reduction is seen after salt application (table 4) and this confirm the existence of salt stress.

CxE population

Differences in ion contents

In CxE population the addition of NaCl significantly increases the Na^+ and Cl^- contents in all tissues. Experimental evidence also indicated the enhancement of these two ions after the application of NaCl (Gadallah, 1999; Ferreira et al., 2001; Khan et al., 2001; Karimi et al., 2005; Parida and Das, 2005; Roychoudhury et al., 2008). This increase can be attributed to large amount of Na^+ and Cl^- on the growth medium that create large electrochemical gradient for passive entry of these ions into the plants (Sun et al., 2009). In salt treated plants, Cl^- and Na^+ contents in root are significantly higher than stem and leaf. Cl^- content of stem is significantly higher than in leaf under salt conditions. The concentration of Na^+ in stem is not

significantly different from that of leaf in salt treated plants, while root Na^+ content is significantly higher than leaf and stem Na^+ content. K^+ content in stem is almost twice higher than Na^+ content in stem and with the K/Na ratio being very high (1.941) it's indicated that may be a mechanism function for salinity tolerance like exclusion of Na^+ from the stem part and flux of excessive Na^+ from stem to root (table 8). This is in agreement with (Juan et al., 2005) that report that a strong evidence for enhance salt tolerance is control of Na^+ accumulation and high shoot K/Na ratio, in experiment done for 10 commercial cultivars of tomato subjected to salinity stress (100 mM NaCl). Additionally, Na^+ content in leaf did not accumulated, therefore it may excluded from leaf part and flux to root part because of the high content of root in Na^+ ions. (Shaterian et al., 2005) reported that Na^+ in leaves did not accumulated and generally was excluded from leaf tissues in leaves of late maturing diploid potato clones. Additionally, (Aghaei et al., 2009) report that NaCl induced a more rapid reduction of K^+ in root than in shoot, suggesting that K^+ might be replaced by Na^+ This is not in line with the current experiment that shows a significant increase in root K^+ content after salt application (table 7). Moreover, (Behdani et al., 2008)) conduct a study with two legumes showed K^+ content increase after increasing salt levels in leaf and stem. This result is not in line with results in the current experiment (show that K^+ content is significantly decrease in leaf and stem after salt application) but this increase may be due to the fact that experiment with legumes was done in low levels of salinity in the glasshouse (80 mM NaCl) and of course is a completely different crop. Mg^{2+} shows a significant increase in stem after salt application while it is significantly reduced in leaf and root. The increase of Mg^{2+} content in stem with the combination of QTL analysis that a QTL is found for Mg-stem under salt conditions show a strong evidence that there is a genetic factor influencing Mg^{2+} content in stem under salt stress. Ca^{2+} shows no significant differences in stem after addition of NaCl. This is in agreement with (Ehsanzadeh et al., 2009) that observed that Ca^{2+} in stem and leaf was not affected in 8 genotypes of tetraploid wheat grown in hydroponics. Apparently, a high content of Na^+ cation did not alter the uptake and accumulation of Ca^{2+} cation.

Correlation analysis under control conditions

Most of the associations were weak so the interpretation of the findings should be regarded with care. The correlation analysis between ion contents under control conditions revealed lot of associations between ions. The Na^+ in root was weakly positively associated with Cl^- in root but also with K^+ in leaf. There was a positive correlation between Mg^{2+} in leaf and Ca^{2+} in root and both were negatively associated with K^+ in root. Similar results were observed between these ions in a segregating population in *Arabidopsis thaliana* (Buescher et al., 2010). A strong positive association as expected was shown between RDW and SDW.

Comment [GL2]: Repeat of results

Correlation analysis under salt conditions

Comment [GL3]: You are maki

Comment [GL4]: Ng an abservation, but you do not discuss the observation. You draw conclusions directly, without discussing how likely it is, and whether there is more evidence for this....

Under salt conditions there was not an association between Na^+ content and K^+ content in all tissues. The SO_4^{2-} content in leaf and stem were positively correlated with Na^+ in leaf and stem respectively and also were negatively correlated with the K/Na ratio in leaf and stem showing that SO_4^{2-} is possibly involved in sodium homeostasis under salt stress and has an antagonistic relationship with the K/Na ratio. Additionally, the SO_4^{2-} content in stem was negatively correlated with all three growth data used for correlations and thus it might be a good indicator of traits associated with salt stress. The positive correlation between Na^+ and Cl^- ions in all tissues indicate possibly mechanisms with similar exclusion capacity for the two ions. The Mg^{2+} content in stem exhibited weak negative association with the K/Na ratio in leaf showing that also Mg^{2+} might play a role under salinity conditions. Negative associations between chlorophyll content at day 11 (CC11) and ions (Cl^-, Na^+) shows that accumulation of these ions may affect Chlorophyll ~~affects its~~ content (Stepien and Johnson, 2009; Tavakkoli et al., 2011). SDW showed negative correlations with the two ions (Na^+, Cl^-) while RDW did not. Thus, despite that RDW and SDW were strongly positively correlated SDW seems to be negatively affected much more than RDW (Bernstein and Kafkafi, 2002).

Comment [GL5]: CC11 is not growth data....
Are correlations strong? Why would this be? Any indications form literature on role of SO4?

Comment [GL6]: Does it?

Comment [GL7]: What is consequence, what is cause?

Comment [GL8]: So what did the people in this reference do? Same thing as we? You need to do more than this.

Tetraploid cultivars grown in hydroponics

Differences between replications

Differences between replications for Na^+ and Cl^- ion contents were observed in the different organs of the cultivars. Possibly, those differences are due to concentration of salt applied in the two replications. The most possible explanation is that replication 3 received higher concentration of NaCl than replication 2 (table 1).

Differences between treatments for growth data

SDW (Shoot Dry Weight), SFW (Shoot Fresh Weight), RDW (Root Dry Weight) and RFW (Root Fresh Weight) were significantly reduced after salt application and this is in line with (Evers et al., 1998) who reported that root and shoot growth decreased with increasing salt concentration in cultivar Bintje grown in vitro. R_S_DW (Root to Shoot Dry Weight) and R_S_FW (Root to Shoot Fresh Weight) were increased after salt application. This is in agreement with (Frary et al., 2010) that demonstrated the ability of a wild tomato cultivar to maintain root growth during salinity by increasing root to shoot ratio and finally come to conclusion that roots were shown to be an important factor in tolerance.

RL (Root Length) was not significantly different between the treatments (table 4) while SL (Shoot Length) was significantly reduced after salt application. This is in line with (Aghaei et al., 2008) who reported that the lengths of roots were less severely affected than those of the shoots by increasing salt treatment in two potato cultivars grown in MS media with different concentrations of NaCl.

Correlation analysis under salt conditions

Correlation analysis in the current thesis was based on only six cultivars with bad replications, so correlations may be coincidence and additionally should be regarded with care. However, some correlations are discussed here.

Na^+ and Cl^- ions were significantly increased after salt treatment. Positive correlations of Na^+ in stem with Ca^{2+} and Mg^{2+} and PO_4^{3-} in stem are observed. Additionally, positive correlations of Cl^- in stem with Mg^{2+} and PO_4^{3-} in stem are

observed. Therefore, it is indicated that Ca^{2+} and Mg^{2+} and PO_4^{3-} ions may have an association with salt stress (Appendix 3).

Phenotypic characteristics and evaluation of phenotypic and physiological responses are indicators of salt tolerance in diploid potato clones (Shaterian et al., 2007). Ca^{2+} and Na^{+} in stem (Appendix 4) are positively associated with RL (Root Length). Cl^{-} and PO_4^{3-} in stem are positively correlated with R_S_DW (Root to Shoot Dry Weight). Moreover, PO_4^{3-} in stem is positively correlated with R_S_FW (Root to Shoot Fresh Weight). Therefore, RL, R_S_DW and R_S_FW are indicators of traits associated with salt tolerance.

Tetraploid cultivars and CxE genotypes grown in a test field

CxE genotypes

The analysis of ion contents in CxE genotypes grown in field reveals significant differences between the treatments. In 19/06/10 Na^{+} content significantly decrease in salt treated plants and Cl^{-} content shows no significant differences between treatments. In 27/07/10 no significant differences are observed between treatments for both Na^{+} and Cl^{-} contents. Therefore, plants did not perceive any salt stress and significant differences of the other ions may be due to nutritional requirements at different developmental stages of the plant. Additionally, yield per plant show no significant differences between treatments, so this is one more reason to support the previous hypothesis that plants did not perceive any salt stress.

Tetraploid cultivars grown also in hydroponics

Tetraploid cultivars grown also in hydroponics reveal significant differences in the analysis of ion contents when these cultivars grown in field. In 19/06/10 Na^{+} content significantly decrease in salt treated plants and Cl^{-} content shows no significant differences between treatments. In 27/07/10 no significant differences are observed between treatments for both Na^{+} and Cl^{-} contents. Therefore, plants did not perceive any salt stress and significant decreases of the other ions may be

due to nutritional requirements at different developmental stages of the plant. A decrease is observed for yield per plant between treatments in salt treated plants, but yield per plant in control and salt treated plants is statistically the same, so the previous hypothesis is supported that did not perceive any salt stress.

Tetraploid cultivars grown only in field

The 20 commercial tetraploid cultivars grown only in field showed significant differences in the analysis of ion contents between treatments. In 19/06/10 Na^+ content significantly decrease in salt treated plants and Cl^- content shows no significant differences between treatments. In 27/07/10 no significant differences are observed between treatments for both Na^+ and Cl^- contents. Therefore, plants seem that did not perceive any salt stress and significant decreases of the other ions may be due to nutritional requirements at different developmental stages of the plant. However, there is significant decrease for yield per plant in salt treated plants and this is an indication of the existence of salt stress. The fact that Na^+ and Cl^- contents did not increased in salt treated plants might be due to perceive of only osmotic stress by the plants. It also might be due to the soil conditions. Soil type might affect the ion uptake in salt treated plants and not in control plants because we had two different places (two different blocks) for plant growth, one for control plants and one for salt treated plants.

QTLs detected for ion data

The regions within genomes that contain genes associated with quantitative traits are known as quantitative trait loci (Collard et al., 2005).

QTLs under salt conditions

For ion data of 2008 (current experiment) a QTL is identified for Na^+ in leaf in chromosome 7. This QTL was confirmed by the QTL analysis for ion data of 2009. EST that contains sequence data from a number of organisms were used for correlated the markers used in the linkage map for find candidate genes. PotSNP730 is the only common marker placed in regions contains the QTLs for Na^+ in leaf in both years.

There is no information from this marker for a responsible gene. The identified QTL in 2009 did not relate with any biomass or physiological data. For Na^+ in tissue leaf at vegetative stage three QTLs were identified in CSR27/M148 F_2 population in rice consist of 200 genotypes and also seven QTLs were identified for Na^+ in leaf in reproductive stage (Ammar et al., 2009). Those results reveal that some QTLs were stage/tissue specific (they also analysed Na^+ concentration in stem) and some of them were located on the same marker interval. So, some of those QTLs were common or very tightly linked work at different tissue/growth stages and these QTLs are important in breeding for salt tolerance. Also, in tomato in C population for Na^+ in leaf QTLs were detected in chromosomes 1, 7 and 8 (Villalta et al., 2008). For the same study, QTLs for fruit yield are collocated in chromosomes 1 and 8. As far as we are interested for breeding for salt tolerance, Na^+ in leaf after its relation to fruit yield showed linkage with salinity tolerance. One of these QTLs might involve with LeNHX3 because they were very close. In Arabidopsis salt tolerance conferred by NHX encoding genes is related to Na^+ inclusion. Moreover, in a commercial tomato hybrid variety grafted on P population of RILs, QTLs were identified for Na^+ in leaf in chromosomes 1 and 5 (Asins et al., 2010) and these QTLs were collocated with QTLs for water content providing the association of these traits under salinity.

In both years, a QTL for Mg^{2+} in stem is identified in chromosome 3. In 2009 no related traits identified for Mg^{2+} in stem. However, one gene that may be responsible and corresponds to Mg^{2+} in stem coming from a common marker (PotSNP87) in both years is a homologue to UPGAST1_LYCES (P27057) GAST1 protein precursor.

A QTL is detected for SO_4^{2-} in leaf but only in analysis of 2008 ion data and this absence of detection in 2009 ion data may be attributed to different external environmental conditions between the two years.

QTLs under control conditions

For ion data of 2008 QTLs were identified for K^+ in leaf (chromosome 5 and 8) and for K/Na ratio in leaf in chromosome 5. In CE5, QTL for K^+ in leaf is collocated

with QTL for K/Na ratio in leaf. (Villalta et al., 2008)) found a QTL in P population in tomato for K^+ in leaf in chromosome 1 and also a QTL was found in C population in chromosome 5. Also in P population QTLs for Na^+ in leaf, Na^+ in stem and K^+ in leaf were detected in the same region in chromosome 7, showing the relation of those traits and that the genes at these QTLs seems to control the sodium concentration of the aerial part of the plant in absence of salinity.

Conclusion

- There is treatment dependent variation between the six tetraploid Cultivars grown in hydroponics for their growth data but not for their ion contents. The different tissues have different ion contents under specific growing condition. The application of NaCl in to the growth medium significantly increased Na^+ and Cl^- contents, and Na/K ratio in the tissues of cultivars. The salinity stress significantly increases Mg^{2+} in stem, while decrease significantly K^+ in leaf and stem. Salt stress significantly decreases Ca^{2+} in leaf and root. A PO_4^{3-} and SO_4^{2-} content significantly decreases in leaf after salt application. Shoot and root growth were significantly decreased in salt treated plants, however root to shoot ratio was not affected.

Comment [GL9]: No conclusion, but repeat from results

- There is treatment dependent variation between the genotypes of CxE diploid population for their ion contents. The different tissues have different ion contents under specific growing condition. The addition of NaCl in the growth medium significantly increased Na^+ and Cl^- contents in the tissues of genotypes. The salinity stress significantly decreases K/Na ratio and PO_4^{3-} content in all organs, it also significantly decreases K^+ content in stem and leaf while significantly increases K^+ content in root, and significantly increases Mg^{2+} content in stem while significantly decreases Mg^{2+} content in leaf and root. Additionally, salt stress significantly decreases SO_4^{2-} content in stem and leaf. The correlation analysis showed that the SO_4^{2-} content was associated with Na^+ content and the K/Na ratio under salt conditions. QTL analysis reveal three QTLs under salt conditions for ion contents (Na-leaf, Mg-stem, SO_4-leaf), while three QTLs are detected for ion contents under control conditions (K-leaf, K/Na-leaf).
- The treatment effect in cultivars and CxE genotypes grown in field is not clear. Therefore, no specific conclusion can be drawn. The most likely explanation is that plants did not perceive salt stress.

Recommendations

Plants grown in 120mM NaCl in hydroponics might have been under severe stress. Because plant responses to salt stress have been shown to be affected by the intensity of the stress, the experiment in hydroponics should be more informative by screening for different salinity level. An intermediate salinity level can be 80mM NaCl.

Comment [GL10]: Why do you think so?

Comment [GL11]: We have done this.

Twenty four tetraploid cultivars can be used for screening for salinity tolerance and of course the 96 genotypes of CxE diploid population used in the current thesis. The 24 tetraploid cultivars should be selected based on their tolerance to salinity (8 sensitive, 8 with moderate tolerance and 8 tolerant). Three replications can be used for the cultivars and two for the CxE segregating population for both conditions (control, salt). One hydroponic container contains 12 plants therefore, 44 hydroponics containers in total will be needed for the experiment. In more details, 12 hydroponic containers for the tetraploid cultivars and 32 for the CxE population. The experimental design for this experiment will be the split- plot design, because it is more suitable to separate the treatments (control, salt) in each replication. The environmental conditions (light intensity, day length, temperature, humidity) will be recorded.

Comment [GL12]: Why two?

The same tetraploid cultivars grown in hydroponics should be grown in the field including 20 CxE genotypes. The CxE genotypes must be selected according to their performance in terms of growth data under salt stress, so the 10 best performing and the 10 worst performing can be selected (similar to a bulk segregant analysis scheme). The field tests can be executed by a potato breeding company. The collaboration between university and the company will benefit both sides. Lot of new potato varieties are introduced to the market every year by each potato breeding company, so the company will add some varieties to be screened for salinity tolerance and at the end the analyses will be done by the university. Three replications of each genotype can be used to control better the environmental variation in the field. The location of the field test contain the salt-treated plants should be as close to the field test with control plants as possible, in order to share

Comment [GL13]: This is what we did.

the same weather conditions. The same environmental conditions (light intensity, day length, temperature, humidity) as done in hydroponics will be recorded.

In the field test containing the salt treated plants the electrochemical gradient of the contaminated water should be estimated. Salinity tolerance of the investigated genotypes can be screened at two developmental stages (vegetative, reproductive) of the plants, because plant responses to salt stress have been shown to be affected by the age/developmental stage of the plants. Replication and blocking can be used to overcome the confounding effects of field variability. The soil heterogeneity that is possible to exist within blocks of the genotypes can be estimated by the use of a check cultivar in different positions in the field. Leaves can be collected at both developmental stages for ion analysis. Chlorophyll content can be measured at both developmental stages of the plants for correlations with ion contents and finally yield per plant will be measured in order to see for correlation with the physiological data.

Comment [GL14]: How?

Comment [GL15]: Did we not do that?

Comment [GL16]: Done

Correlation analysis and significant differences for ion contents and growth data can be done between the 24 tetraploid cultivars and the CxE genotypes grown in field with the same genotypes from hydroponics under both control and salt conditions. Moreover the developmental stage effect will be determined. Subsequently, QTL mapping can take place to find associations between phenotypes and genotypes. Prior the QTL analysis, a reexamination of marker positions in the integrated linkage map used in the current thesis is necessary because some markers were found to be placed on wrong linkage groups. The correct order of the markers will allow more precise localization of the QTLs.

Comment [GL17]: Have you done this?

Comment [GL18]: vague

Because salinity tolerance is known to interact with other environmental conditions, it's crucial to grow CxE and tetraploid cultivars under salinity conditions for a second year because environmental conditions (light intensity, humidity and temperature) might be different.

Comment [GL19]: done

The same number of replications and plant material (24 tetraploid cultivars, CxE population) as grown in hydroponics in the first year will be used with the same salinity level (80mM). The field tests should be repeated as was done in the first year in order to check for environmental variation between the two years. Significant

differences and correlation analysis can be done for ion contents and growth data. Subsequently, QTL mapping can take place to find associations between phenotypes and genotypes.

A combined data analysis from the two previous years will be done. Because the environmental conditions data were recorded, at this point you can conclude how the environmental conditions affect the genotype performance under salinity. Thus, the GxE interaction will be determined, the QTLs found will be compared and the stability of each genotype will be inferred. Moreover the developmental stage effect will be determined.

In addition, the results from the salinity level in the current thesis can be compared with that of 80mM NaCl salinity level.

A different segregating population can be screened for salinity tolerance and the design will be the same as CxE population in hydroponics. QTL mapping should be done for ion contents and growth data for comparison with the QTLs found in CxE population in the last years in order to find differences or similarities of genetic architecture of salt stress tolerance.

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Appendices

Appendix 1. Correlation analysis between ion data under control conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

	Ca_leaf	Ca_root	Ca_stem	K_leaf	K_root	K_stem	Mg_leaf	Mg_root	Mg_stem	Na_K_leaf	Na_K_root	Na_K_stem	Na_leaf	Na_root	Na_stem	PO4_leaf	PO4_root	PO4_stem	SO4_leaf	SO4_root	SO4_stem
Ca_leaf	-																				
Ca_root	-0,2146	-																			
Ca_stem	-0,2083	0,9853	-																		
K_leaf	0,1689	0,0146	0,1826	-																	
K_root	0,0053	-0,8957	-0,9116	-0,1876	-																
K_stem	-0,5378	0,0427	-0,0048	-0,3037	0,4005	-															
Mg_leaf	0,9252	-0,351	-0,3831	-0,0987	0,0944	-0,6261	-														
Mg_root	-0,8107	0,5609	0,5034	-0,3652	-0,1999	0,7439	-0,8316	-													
Mg_stem	0,3388	-0,6171	-0,6581	-0,22	0,8165	0,4937	0,2935	-0,182	-												
Na_K_leaf	-0,2239	0,1058	-0,0616	-0,979	0,1425	0,4508	-0,0142	0,5038	0,2484	-											
Na_K_root	-0,1499	0,94	0,9807	0,3293	-0,9453	-0,1602	-0,3308	0,3657	-0,7401	-0,2309	-										
Na_K_stem	0,944	-0,1907	-0,1434	0,3846	-0,1281	-0,7504	0,871	-0,9013	0,0853	-0,4678	-0,0283	-									
Na_leaf	0,142	-0,1178	-0,2551	-0,7485	0,4203	0,5856	0,1975	0,2572	0,7255	0,8085	-0,4254	-0,1884	-								
Na_root	0,4203	0,4704	0,5838	0,7895	-0,5988	-0,3635	0,108	-0,2671	-0,3066	-0,7207	0,6796	0,5475	-0,4975	-							
Na_stem	0,9504	-0,1545	-0,1111	0,3952	-0,0014	-0,4051	0,7655	-0,7413	0,3748	-0,4043	-0,0476	0,9047	0,0651	0,6064	-						
PO4_leaf	0,5689	-0,91	-0,9061	0,0019	0,8061	-0,1118	0,6295	-0,7145	0,7637	-0,0998	-0,8712	0,4791	0,2854	-0,2511	0,517	-					
PO4_root	0,0312	-0,8625	-0,8476	0,0059	0,9755	0,4274	0,0399	-0,2171	0,8292	-0,0266	-0,8666	-0,0844	0,3346	-0,4253	0,0905	0,7944	-				
PO4_stem	0,4924	-0,4396	-0,4793	-0,1613	0,6362	0,4324	0,3842	-0,2087	0,9634	0,2141	-0,5627	0,2257	0,7422	-0,0996	0,5471	0,6839	0,671	-			
SO4_leaf	0,0148	-0,2424	-0,0954	0,7855	-0,0919	-0,596	-0,0235	-0,5003	-0,4447	-0,8797	0,0911	0,3227	-0,9291	0,3958	0,0871	0,0823	-0,003	-0,5052	-		
SO4_root	-0,7458	-0,2359	-0,3206	-0,6278	0,5131	0,6991	-0,5524	0,638	0,2409	0,6442	-0,4433	-0,8685	0,4331	-0,8745	-0,8048	-0,0407	0,4057	0,0387	-0,4147	-	
SO4_stem	-0,3334	-0,3651	-0,3249	0,1794	0,6654	0,7964	-0,4921	0,3016	0,6327	-0,0772	-0,3859	-0,4476	0,2619	-0,1605	-0,1226	0,276	0,7705	0,5316	-0,1038	0,4604	-
	Ca_leaf	Ca_root	Ca_stem	K_leaf	K_root	K_stem	Mg_leaf	Mg_root	Mg_stem	Na_K_leaf	Na_K_root	Na_K_stem	Na_leaf	Na_root	Na_stem	PO4_leaf	PO4_root	PO4_stem	SO4_leaf	SO4_root	SO4_stem

Appendix 2. Correlation analysis of ion data with growth data under control conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

Comment [GL20]: of cultivars?

	CC0	CC6	CC10	CC13	CC16	CC20	CF0	CF6	CF10	CF13	CF16	CFm20	CFu20
Ca_leaf	-0,2493	-0,3042	-0,3813	-0,4352	-0,201	-0,3486	0,269	-0,6974	-0,6897	-0,3825	-0,391	-0,5601	0,5731
Ca_root	-0,4621	0,3708	0,4746	0,4447	0,5205	0,1016	-0,6242	0,7016	0,3383	0,765	0,3343	0,3599	0,2489
Ca_stem	-0,4219	0,441	0,5207	0,5056	0,6003	0,2426	-0,4864	0,6698	0,2151	0,7446	0,4731	0,488	0,371
K_leaf	0,0802	0,3553	0,219	0,2941	0,454	0,7429	0,7427	-0,1745	-0,7844	-0,1146	0,796	0,6939	0,8261
K_root	0,1613	-0,6478	-0,7042	-0,662	-0,7812	-0,3178	0,3473	-0,3466	-0,1242	-0,854	-0,2873	-0,3669	-0,4801
K_stem	-0,5069	-0,6002	-0,5223	-0,4737	-0,6025	-0,3652	-0,4308	0,7026	0,4144	-0,3134	0,1662	0,0584	-0,5573
Mg_leaf	0,0034	-0,2374	-0,2947	-0,3702	-0,2118	-0,4201	0,2045	-0,8144	-0,4699	-0,2987	-0,6644	-0,7374	0,3099
Mg_root	-0,324	-0,0119	0,1256	0,1453	-0,0092	-0,0746	-0,7088	0,9622	0,7726	0,4005	0,2684	0,3274	-0,5251
Mg_stem	-0,3987	-0,9548	-0,9842	-0,9701	-0,9644	-0,671	0,0763	-0,2202	-0,2461	-0,9244	-0,3537	-0,5755	-0,2594
Na_K_leaf	-0,2501	-0,4242	-0,2739	-0,3416	-0,4909	-0,7755	-0,8378	0,3432	0,8093	0,1147	-0,6932	-0,6275	-0,8108
Na_K_root	-0,3024	0,5646	0,6163	0,6066	0,7224	0,3912	-0,3219	0,5464	0,0842	0,7575	0,5399	0,5664	0,5031
Na_K_stem	-0,0172	-0,0006	-0,1065	-0,1481	0,0917	-0,0326	0,4659	-0,7743	-0,78	-0,2303	-0,2157	-0,3293	0,7299
Na_leaf	-0,5876	-0,8674	-0,7734	-0,8197	-0,865	-0,9667	-0,6144	0,1791	0,3566	-0,4086	-0,6339	-0,7587	-0,5651
Na_root	-0,3541	0,2758	0,2049	0,2222	0,478	0,4074	0,2831	-0,0014	-0,6672	0,1396	0,599	0,4522	0,9582
Na_stem	-0,3846	-0,3385	-0,4342	-0,4584	-0,1945	-0,2297	0,3588	-0,5698	-0,8293	-0,4663	-0,117	-0,3479	0,7175
PO4_leaf	0,1555	-0,5685	-0,6746	-0,6692	-0,6404	-0,3339	0,5496	-0,7826	-0,5293	-0,8673	-0,4456	-0,5691	-0,032
PO4_root	0,0803	-0,6515	-0,7291	-0,6689	-0,7509	-0,2194	0,4497	-0,3142	-0,2765	-0,9147	-0,1032	-0,2292	-0,3218
PO4_stem	-0,5886	-0,9672	-0,9911	-0,9903	-0,9163	-0,7249	-0,0021	-0,1852	-0,3308	-0,8653	-0,3296	-0,5951	-0,0778
SO4_leaf	0,6753	0,6716	0,5333	0,5907	0,6354	0,8944	0,8571	-0,4506	-0,5569	0,0719	0,5245	0,6064	0,5425
SO4_root	0,1646	-0,2569	-0,1682	-0,145	-0,4206	-0,2423	-0,3693	0,421	0,7499	-0,0761	-0,1942	-0,0809	-0,9507
SO4_stem	-0,2686	-0,5699	-0,6033	-0,5093	-0,5845	-0,0483	0,197	0,297	-0,1239	-0,6786	0,3958	0,229	-0,2372

Appendix 2 (continue). Correlation analysis of ion data with growth data under control conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

Comment [GL21]: Cultivars?

	%RDW	%SDW	RDW	RFW	RL	R_S_DW	R_S_FW	SDW	SFW	SL	SL0	SL6
Ca_leaf	0,2256	-0,5021	0,0926	-0,1615	-0,0534	0,5145	-0,0911	-0,3965	-0,1503	-0,1985	-0,1907	0,8234
Ca_root	-0,264	0,0736	0,6267	0,8064	-0,791	-0,3114	0,176	0,9752	0,9299	-0,7615	0,8601	-0,255
Ca_stem	-0,3893	0,115	0,572	0,8837	-0,7465	-0,3867	0,3409	0,9788	0,9342	-0,8017	0,7858	-0,2849
K_leaf	-0,6847	0,1155	-0,1729	0,5437	0,1752	-0,349	0,9599	0,0888	0,147	-0,337	-0,3661	-0,1064
K_root	0,5374	-0,3148	-0,3078	-0,7099	0,9137	0,5199	-0,2988	-0,8473	-0,7325	0,9638	-0,8404	-0,0768
K_stem	0,5482	-0,4529	0,5245	0,1123	0,4623	0,4084	-0,1955	0,1332	0,2631	0,599	-0,1518	-0,7954
Mg_leaf	0,278	-0,3108	-0,1294	-0,4659	-0,1025	0,4472	-0,3486	-0,54	-0,3956	-0,0986	-0,1311	0,9562
Mg_root	0,1272	0,0481	0,4918	0,3931	-0,1594	-0,1409	-0,1214	0,6594	0,5732	0,0628	0,4663	-0,8138
Mg_stem	0,8212	-0,8024	0,2259	-0,4561	0,7524	0,9066	-0,3889	-0,6601	-0,3508	0,7881	-0,6758	0,0241
Na_K_leaf	0,7289	-0,222	0,35	-0,3941	-0,1742	0,4019	-0,9174	0,0392	0,0217	0,3242	0,406	-0,0354
Na_K_root	-0,5437	0,2156	0,439	0,8944	-0,7512	-0,4952	0,472	0,9389	0,8717	-0,8777	0,7324	-0,2086
Na_K_stem	-0,1009	-0,2278	-0,1247	-0,0782	-0,1313	0,2153	0,147	-0,3414	-0,1832	-0,3676	-0,1772	0,8407
Na_leaf	0,9862	-0,7343	0,5576	-0,3662	0,1985	0,8574	-0,8114	-0,2154	0,0037	0,53	-0,0056	0,0108
Na_root	-0,4968	-0,143	0,3074	0,7785	-0,2983	-0,157	0,739	0,4344	0,5756	-0,7107	0,1107	0,095
Na_stem	0,1538	-0,5956	0,2075	0,0616	0,064	0,5166	0,1474	-0,302	0,0007	-0,2021	-0,2953	0,6213
PO4_leaf	0,4377	-0,3951	-0,3623	-0,7209	0,6985	0,5892	-0,2376	-0,9645	-0,782	0,635	-0,8155	0,4697
PO4_root	0,4608	-0,3771	-0,2488	-0,5551	0,9773	0,5166	-0,1112	-0,7991	-0,6405	0,9235	-0,9198	-0,1575
PO4_stem	0,8243	-0,926	0,4145	-0,2862	0,599	0,9686	-0,3484	-0,5216	-0,1554	0,5982	-0,5501	0,1019
SO4_leaf	-0,8558	0,6161	-0,7229	0,1085	0,1095	-0,6719	0,7695	-0,1474	-0,3078	-0,2621	-0,3384	0,1039
SO4_root	0,3984	0,1188	-0,0838	-0,4135	0,365	0,0544	-0,4828	-0,1248	-0,2534	0,7043	-0,0784	-0,5639
SO4_stem	0,3174	-0,4237	0,1711	0,0346	0,8541	0,3669	0,194	-0,2354	-0,0585	0,7276	-0,6771	-0,7011

Appendix 3. Correlation analysis between ion data under salt conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

	Ca_leaf	Ca_root	Ca_stem	Cl_leaf	Cl_root	Cl_stem	K_leaf	K_root	K_stem	Mg_leaf	Mg_root	Mg_stem	Na_K_leaf	Na_K_root	Na_K_stem	Na_leaf	Na_root	Na_stem	PO4_leaf	PO4_root	PO4_stem	SO4_leaf	SO4_root	SO4_stem
Ca_leaf	-																							
Ca_root	-0,2966	-																						
Ca_stem	-0,0465	-0,8186	-																					
Cl_leaf	0,732	-0,6069	0,2552	-																				
Cl_root	-0,3968	-0,4065	0,6491	-0,3668	-																			
Cl_stem	-0,3852	-0,2406	0,6823	-0,1103	0,2761	-																		
K_leaf	0,577	-0,1369	-0,1162	0,2592	0,2041	-0,7019	-																	
K_root	0,5417	-0,6087	0,7172	0,438	0,2679	0,4567	0,1815	-																
K_stem	-0,6351	-0,0115	0,5634	-0,4017	0,4686	0,9055	-0,5911	0,2411	-															
Mg_leaf	0,8131	0,1797	-0,2475	0,4043	-0,5375	-0,1659	0,3388	0,4799	-0,3284	-														
Mg_root	-0,123	0,5398	-0,7287	-0,5303	-0,0236	-0,7723	0,3799	-0,6598	-0,5493	-0,1373	-													
Mg_stem	-0,2109	-0,4668	0,8609	-0,0446	0,5084	0,9267	-0,4325	0,6889	0,822	-0,1175	-0,7468	-												
Na_K_leaf	0,0212	-0,1334	0,1909	0,5125	-0,5115	0,5241	-0,6067	0,1242	0,3028	0,1241	-0,7705	0,2852	-											
Na_K_root	-0,3526	0,0351	-0,3326	-0,2647	-0,0668	-0,3743	-0,2182	-0,6792	-0,3791	-0,6116	0,5772	-0,4388	-0,2637	-										
Na_K_stem	0,5519	-0,6736	0,1864	0,5166	-0,0032	-0,376	0,3366	0,246	-0,6405	0,023	0,0906	-0,1548	-0,2161	0,4255	-									
Na_leaf	0,7653	-0,412	0,1339	0,8817	-0,2366	-0,2942	0,5943	0,4206	-0,4259	0,5258	-0,3575	-0,1794	0,2696	-0,4993	0,3018	-								
Na_root	0,2432	-0,6505	0,2756	0,2639	0,1219	-0,1473	0,0308	0,1588	-0,4015	-0,2352	0,0817	0,0296	-0,2076	0,6025	0,9234	-0,0499	-							
Na_stem	-0,4075	-0,6279	0,8892	-0,0809	0,6439	0,8014	-0,4667	0,4415	0,7143	-0,5193	-0,5875	0,8826	0,1803	-0,027	0,0702	-0,3059	0,3233	-						
PO4_leaf	-0,0464	0,5461	-0,0939	-0,4296	0,1032	0,2623	0,0758	0,2541	0,4495	0,4644	-0,0385	0,252	-0,1323	-0,7191	-0,7334	-0,1159	-0,7672	-0,1499	-					
PO4_root	0,0292	-0,8922	0,9068	0,2124	0,6622	0,4551	-0,0222	0,6335	0,2938	-0,32	-0,4626	0,6997	-0,0636	-0,006	0,519	0,0236	0,6319	0,8471	-0,3362	-				
PO4_stem	-0,3772	-0,3574	0,6534	0,0124	0,1455	0,9386	-0,8182	0,3405	0,7581	-0,2861	-0,7603	0,8339	0,625	-0,1072	-0,1554	-0,31	0,1029	0,8265	-0,0625	0,5095	-			
SO4_leaf	0,7583	0,3698	-0,6564	0,2694	-0,6284	-0,6717	0,5353	0,0168	-0,746	0,8228	0,3915	-0,633	-0,1948	-0,1571	0,1973	0,4267	-0,0824	-0,8575	0,2014	-0,5606	-0,7082	-		
SO4_root	0,5354	0,0704	-0,1408	-0,1355	0,0022	-0,2483	0,3917	0,4071	-0,3405	0,5755	0,3418	-0,0288	-0,5793	-0,097	0,3556	-0,0645	0,2928	-0,2124	0,3107	0,081	-0,3359	0,5956	-	
SO4_stem	-0,2731	-0,2854	0,6657	-0,356	0,5408	0,7869	-0,4265	0,5607	0,7113	-0,1428	-0,3986	0,8903	-0,0454	-0,1929	-0,0718	-0,4976	0,1842	0,8073	0,2878	0,6514	0,7013	-0,5219	0,2887	-
	Ca_leaf	Ca_root	Ca_stem	Cl_leaf	Cl_root	Cl_stem	K_leaf	K_root	K_stem	Mg_leaf	Mg_root	Mg_stem	Na_K_leaf	Na_K_root	Na_K_stem	Na_leaf	Na_root	Na_stem	PO4_leaf	PO4_root	PO4_stem	SO4_leaf	SO4_root	SO4_stem

Appendix 4. Correlation analysis of ion data with growth data under salt conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

Comment [GL22]: Cultivars?

	CC0	CC6	CC10	CC13	CC16	CC20	CF0	CF6	CF10	CF13	CF16	CFd20	CFm20	CFu20
Ca_leaf	0,0548	0,2207	0,0637	0,0629	0,1705	0,2377	0,0269	0,0539	0,0913	0,4544	0,6239	-0,3366	0,5421	0,3099
Ca_root	-0,3142	-0,0987	-0,3537	-0,1555	-0,2289	-0,2833	-0,3604	-0,1351	-0,5014	-0,1191	-0,5052	-0,5862	-0,7643	-0,6578
Ca_stem	0,2411	-0,0859	0,081	-0,2113	-0,0727	-0,0406	0,3489	-0,3168	0,2429	-0,136	0,088	0,924	0,2555	0,5053
Cl_leaf	-0,3244	-0,295	-0,1766	-0,1491	-0,182	-0,0967	-0,3087	0,4316	0,6021	-0,0965	0,376	0,0551	0,7359	0,0751
Cl_root	0,5792	0,3597	0,3778	0,0344	0,2737	0,1879	0,7606	-0,326	-0,3974	0,2322	-0,0829	0,5846	-0,0535	0,574
Cl_stem	-0,1385	-0,4614	-0,3472	-0,472	-0,4793	-0,4205	-0,1343	-0,5837	0,2384	-0,5757	-0,3327	0,8763	-0,3509	-0,0629
K_leaf	0,2991	0,5023	0,2595	0,1089	0,3641	0,2878	0,4624	0,1681	-0,5054	0,6673	0,2956	-0,4112	0,3404	0,3996
K_root	0,2412	0,0828	-0,0156	-0,2697	-0,0421	0,0238	0,301	-0,5703	0,0155	0,1757	0,3344	0,5406	0,2122	0,529
K_stem	-0,1436	-0,4342	-0,3996	-0,5636	-0,5193	-0,5272	-0,0555	-0,5981	-0,0861	-0,5809	-0,6178	0,8153	-0,6072	-0,1767
Mg_leaf	-0,1778	0,0107	-0,3125	-0,2724	-0,1639	-0,1048	-0,2114	-0,3155	-0,1811	0,2253	0,2322	-0,3615	-0,0329	-0,0616
Mg_root	0,3944	0,6368	0,5027	0,6067	0,6109	0,5215	0,3425	0,232	-0,5035	0,5947	0,1523	-0,7812	-0,0981	0,0745
Mg_stem	0,1511	-0,1873	-0,1136	-0,3531	-0,2446	-0,1964	0,1947	-0,6709	0,1138	-0,2813	-0,1082	0,9277	-0,1771	0,2831
Na_K_leaf	-0,7835	-0,8881	-0,6929	-0,5412	-0,7591	-0,6567	-0,8339	0,1551	0,7191	-0,8396	-0,2944	0,3643	0,0261	-0,5726
Na_K_root	0,3324	0,3602	0,6458	0,8298	0,6073	0,6039	0,155	0,5394	0,3263	0,216	0,3448	-0,3391	0,324	0,1377
Na_K_stem	0,5478	0,5564	0,7451	0,7328	0,7285	0,7899	0,4351	0,3661	0,4332	0,607	0,9364	-0,1409	0,9231	0,7293
Na_leaf	-0,3451	-0,2297	-0,2955	-0,3404	-0,2478	-0,2244	-0,2141	0,3511	0,1801	0,0149	0,1722	-0,0807	0,5349	0,0165
Na_root	0,6522	0,5642	0,8394	0,8291	0,7744	0,8411	0,4912	0,2475	0,4941	0,5135	0,8786	0,0324	0,7894	0,7471
Na_stem	0,2984	-0,0739	0,1766	-0,0416	-0,0051	0,0285	0,322	-0,354	0,2978	-0,2341	0,0047	0,9301	0,0657	0,4081
PO4_leaf	-0,1226	-0,0829	-0,4888	-0,6082	-0,4017	-0,4492	-0,0117	-0,7678	-0,7526	-0,0516	-0,511	0,0402	-0,8238	-0,2532
PO4_root	0,5647	0,2697	0,484	0,2166	0,34	0,3776	0,5961	-0,213	0,2801	0,1952	0,4385	0,7355	0,4836	0,7798
PO4_stem	-0,1346	-0,4644	-0,2113	-0,2575	-0,3668	-0,2792	-0,2023	-0,3479	0,5323	-0,5945	-0,1595	0,8285	-0,1166	-0,0371
SO4_leaf	-0,0489	0,2787	-0,0185	0,1251	0,1711	0,1896	-0,1204	0,0674	-0,2514	0,4791	0,3617	-0,8153	0,1048	-0,0595
SO4_root	0,6719	0,7777	0,5006	0,4232	0,6148	0,6367	0,5698	-0,5324	-0,4417	0,8301	0,6346	-0,3501	0,0278	0,5899
SO4_stem	0,4807	0,1818	0,2039	-0,0136	0,1048	0,143	0,4445	-0,8063	-0,0734	0,0325	0,0785	0,7147	-0,2646	0,4557

Appendix 4 (continue). Correlation analysis of ion data with growth data under salt conditions. Green highlight represent positive correlations. Red highlight represent negative correlations

Comment [GL23]: Cultivars?

	%RDW	%SDW	RDW	RFW	RL	R_S_DW	R_S_FW	SDW	SFW	SL	SL0	SL6
Ca_leaf	-0,5771	0,2439	-0,4154	-0,1808	0,0426	-0,4881	-0,2508	0,1283	0,1029	0,148	-0,809	-0,5386
Ca_root	0,2246	0,4532	-0,5527	-0,7532	-0,8673	-0,1592	-0,1355	-0,2401	-0,3507	0,2807	0,4922	0,3898
Ca_stem	-0,2496	-0,6629	0,4126	0,5742	0,8938	0,4037	0,3604	-0,0608	0,0863	-0,3248	0,0533	0,057
Cl_leaf	-0,2848	0,3792	-0,1131	0,0702	0,357	0,0203	0,1813	0,0343	0,0261	-0,505	-0,7028	-0,6039
Cl_root	0,2544	-0,8839	0,6293	0,6229	0,3847	-0,0859	-0,2682	0,441	0,5582	0,2669	0,2365	-0,01
Cl_stem	-0,3558	-0,3639	-0,0287	0,0419	0,6376	0,8159	0,8091	-0,7106	-0,6009	-0,4891	0,6257	0,7133
K_leaf	0,1456	-0,0637	0	0,0534	-0,3111	-0,9165	-0,8619	0,7957	0,7642	0,5211	-0,615	-0,8058
K_root	-0,7346	-0,4385	-0,1873	0,0941	0,6399	0,0257	0,1801	-0,1881	-0,082	-0,0277	-0,2242	-0,0008
K_stem	-0,0293	-0,4142	0,0202	-0,0171	0,3728	0,6727	0,5995	-0,5333	-0,4399	-0,349	0,8485	0,7103
Mg_leaf	-0,6682	0,3724	-0,8521	-0,675	-0,235	-0,3601	-0,0718	-0,2727	-0,3211	0,1696	-0,3438	-0,0916
Mg_root	0,4194	0,0357	0,1489	-0,009	-0,6969	-0,673	-0,7742	0,5628	0,4817	0,7513	-0,1363	-0,1972
Mg_stem	-0,4462	-0,6243	0,0986	0,2392	0,7717	0,5901	0,5841	-0,4748	-0,3328	-0,2803	0,4231	0,5336
Na_K_leaf	-0,2087	0,5295	-0,3198	-0,2813	0,3073	0,7772	0,8846	-0,7155	-0,7246	-0,9259	0,1696	0,234
Na_K_root	0,3664	-0,0176	0,6302	0,5114	-0,0444	0,0242	-0,1652	0,3011	0,2766	0,1727	-0,1742	-0,0512
Na_K_stem	-0,2783	-0,2045	0,4609	0,6446	0,4474	-0,2835	-0,278	0,4463	0,4811	0,1956	-0,8771	-0,5466
Na_leaf	-0,1074	0,3839	-0,2576	-0,1216	0,0473	-0,3089	-0,1353	0,2516	0,2194	-0,2971	-0,6445	-0,7535
Na_root	-0,2663	-0,3873	0,6265	0,7785	0,583	-0,0365	-0,1035	0,2989	0,3637	0,1777	-0,6297	-0,247
Na_stem	-0,1668	-0,7193	0,5385	0,6249	0,871	0,6269	0,5035	-0,2202	-0,0707	-0,3024	0,3235	0,3876
PO4_leaf	-0,2119	-0,0771	-0,6774	-0,6926	-0,3819	-0,1559	-0,0455	-0,3298	-0,3343	0,2837	0,5581	0,4433
PO4_root	-0,285	-0,7836	0,6226	0,8066	0,9202	0,2261	0,147	0,1448	0,2943	-0,065	-0,1924	-0,0474
PO4_stem	-0,3424	-0,2801	0,1324	0,2078	0,7458	0,9255	0,8948	-0,703	-0,5984	-0,6012	0,4715	0,6514
SO4_leaf	-0,3251	0,5102	-0,6464	-0,5693	-0,5743	-0,6748	-0,4614	0,1093	0,0052	0,429	-0,5452	-0,3507
SO4_root	-0,6467	-0,3391	-0,2774	-0,1005	-0,0652	-0,5862	-0,464	0,093	0,1124	0,813	-0,3539	0,0472
SO4_stem	-0,513	-0,786	0,1499	0,2785	0,6483	0,4151	0,3766	-0,4076	-0,2672	0,1105	0,4285	0,676

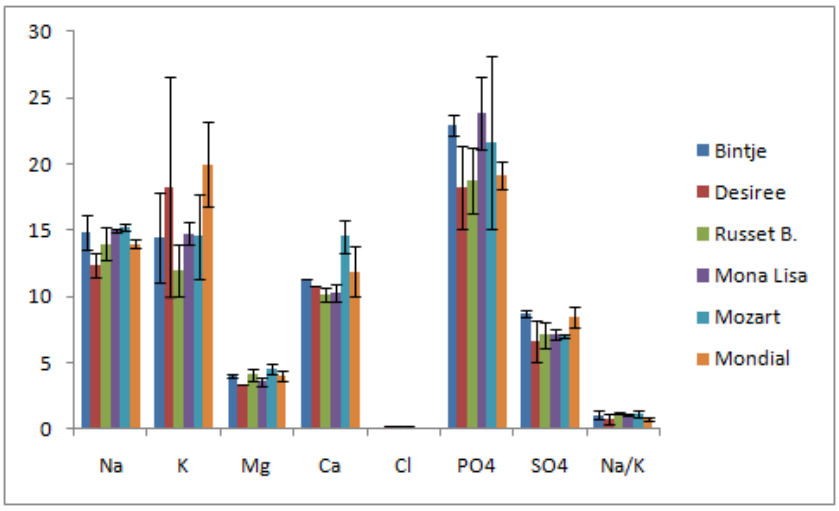


Figure 1. Concentrations of ions in leaves under control conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

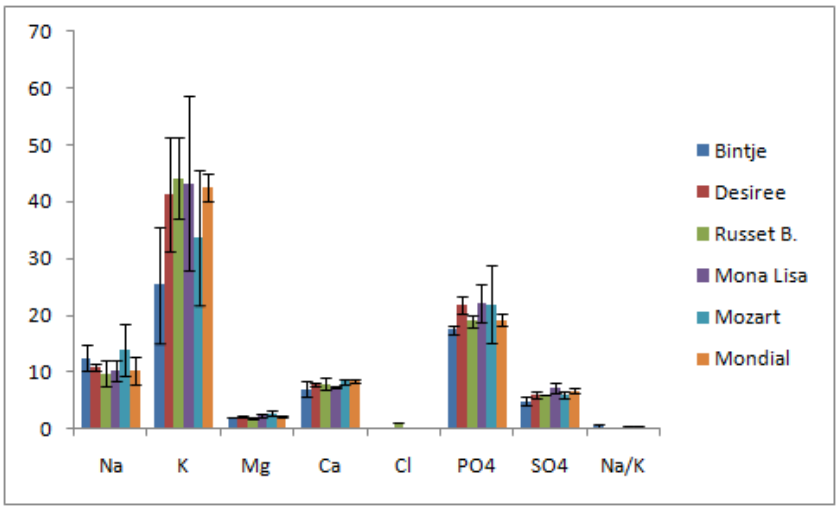


Figure 2. Concentrations of ions in stems under control conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

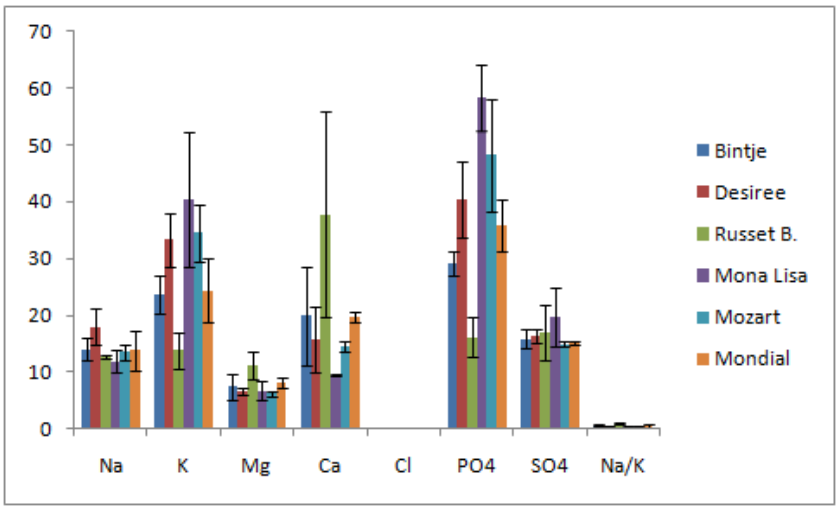


Figure 3. Concentrations of ions in roots under control conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

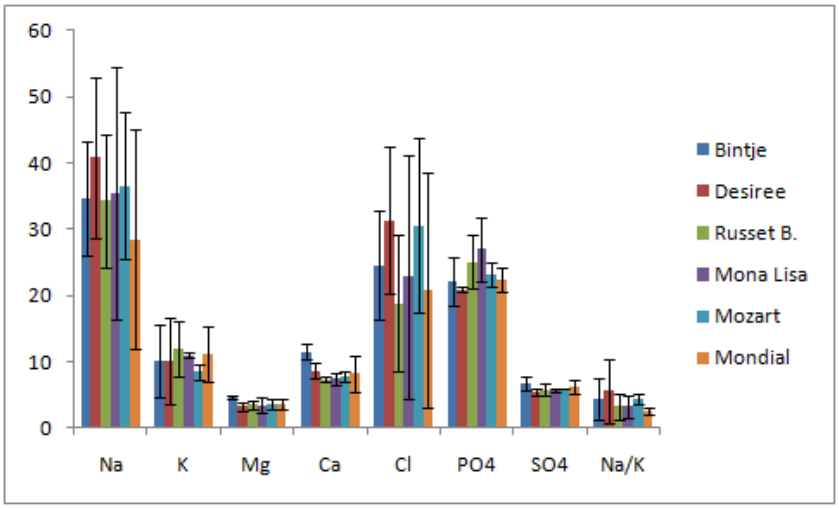


Figure 4. Concentrations of ions in leaves under salt conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

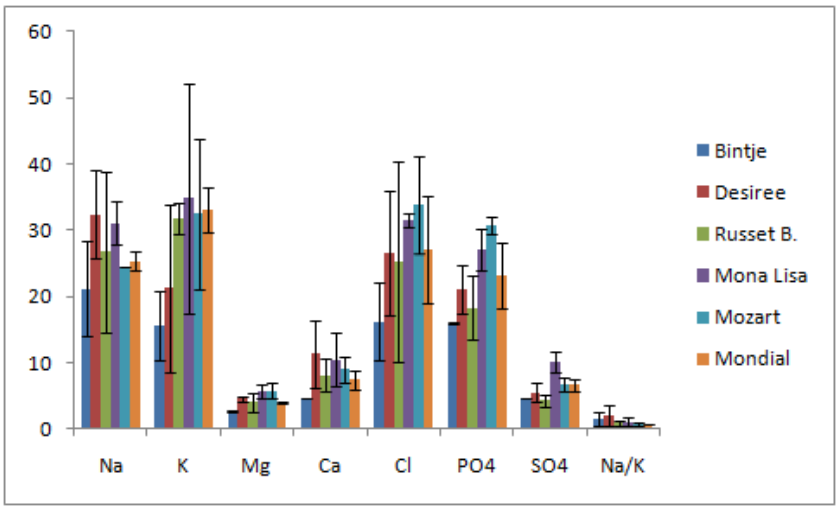


Figure 5. Concentrations of ions in stems under salt conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

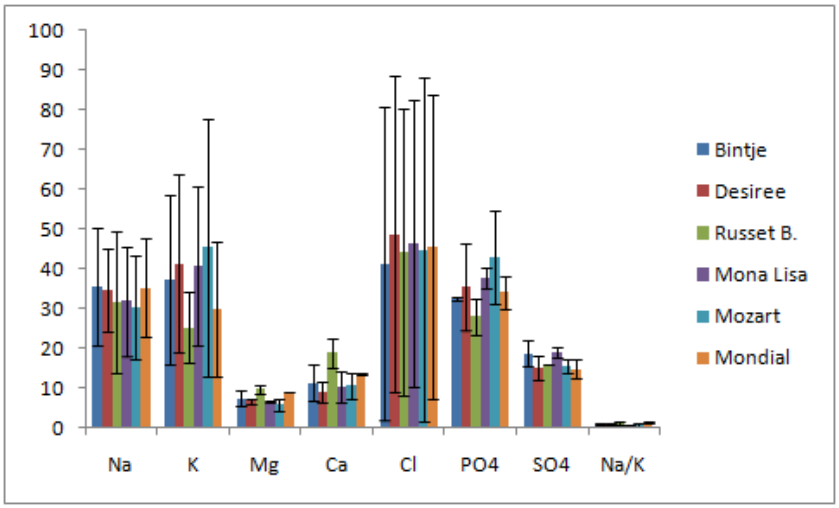


Figure 6. Concentrations of ions in roots under salt conditions for the six tetraploid cultivars grown in hydroponics. SE (standard error) was high as indicated by the high variation in cultivars for ions.

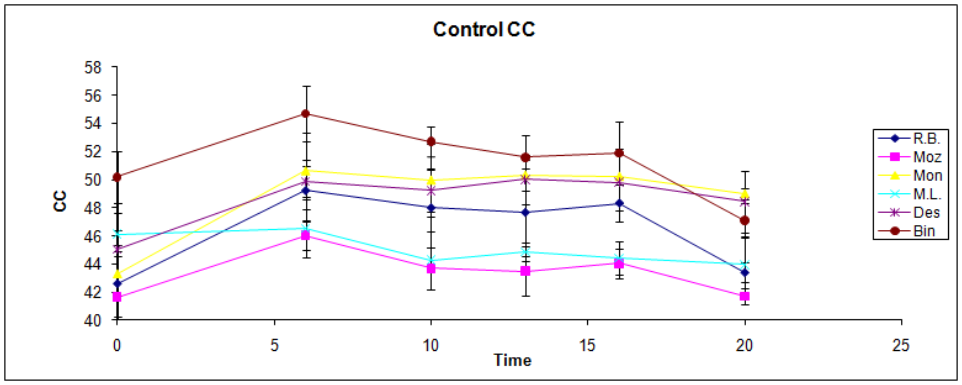


Figure 7. Chlorophyll content at different days under control conditions for the six tetraploid cultivars grown in hydroponics (R.B. stands for Russet Burbank, Moz stands for Mozart, Mon stands for Mondial, M.L. stands for Mona Lisa, Des stands for Desiree and Bin stands for Bintje)

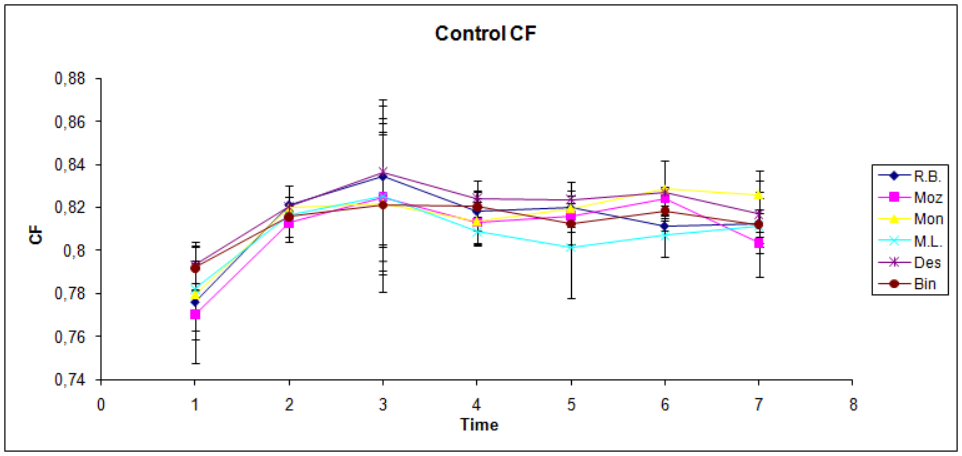


Figure 8. Chlorophyll fluorescence at different days under control conditions for the six tetraploid cultivars grown in hydroponics (R.B. stands for Russet Burbank, Moz stands for Mozart, Mon stands for Mondial, M.L. stands for Mona Lisa, Des stands for Desiree and Bin stands for Bintje)

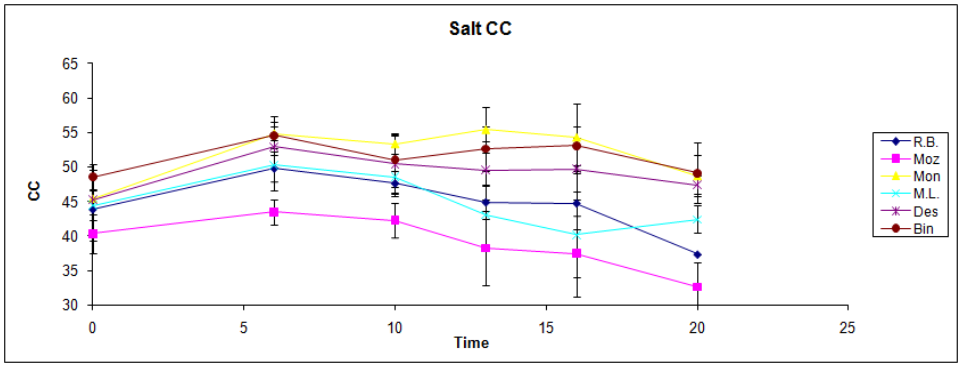


Figure 9. Chlorophyll content at different days under salt conditions for the six tetraploid cultivars grown in hydroponics (R.B. stands for Russet Burbank, Moz stands for Mozart, Mon stands for Mondial, M.L. stands for Mona Lisa, Des stands for Desiree and Bin stands for Bintje)

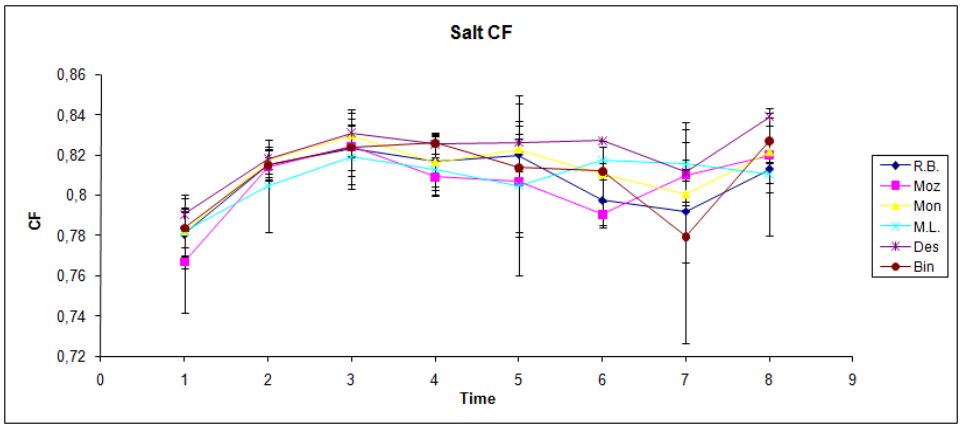
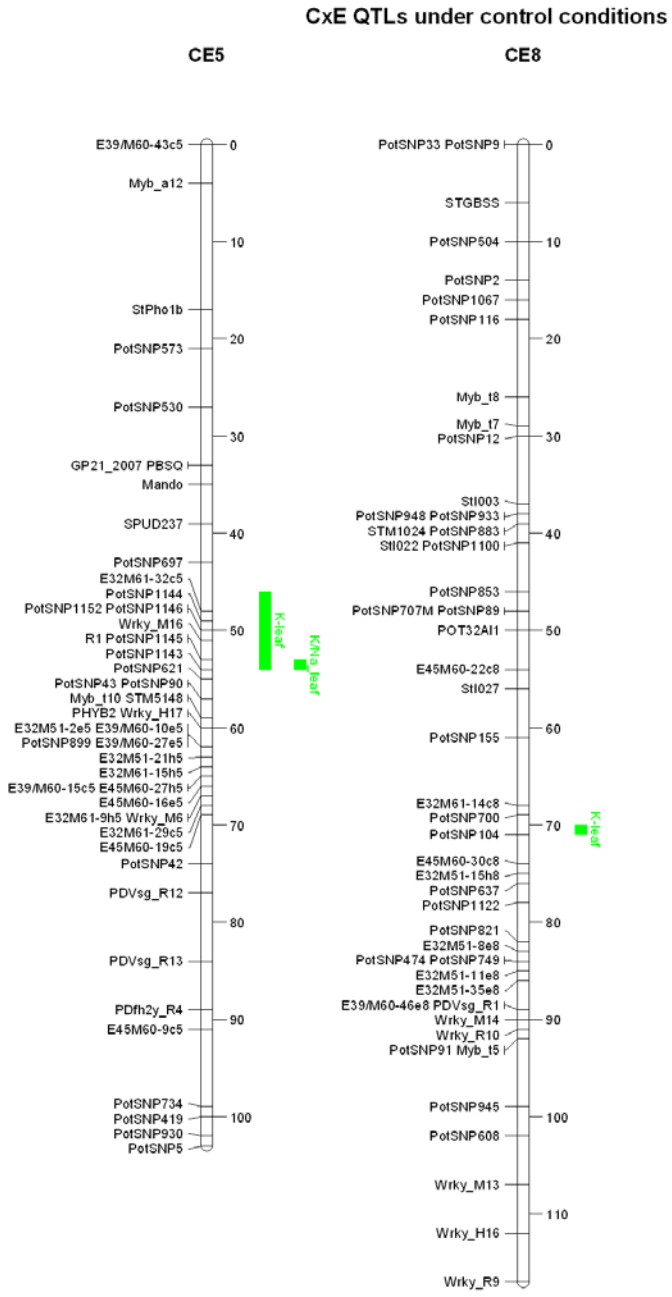
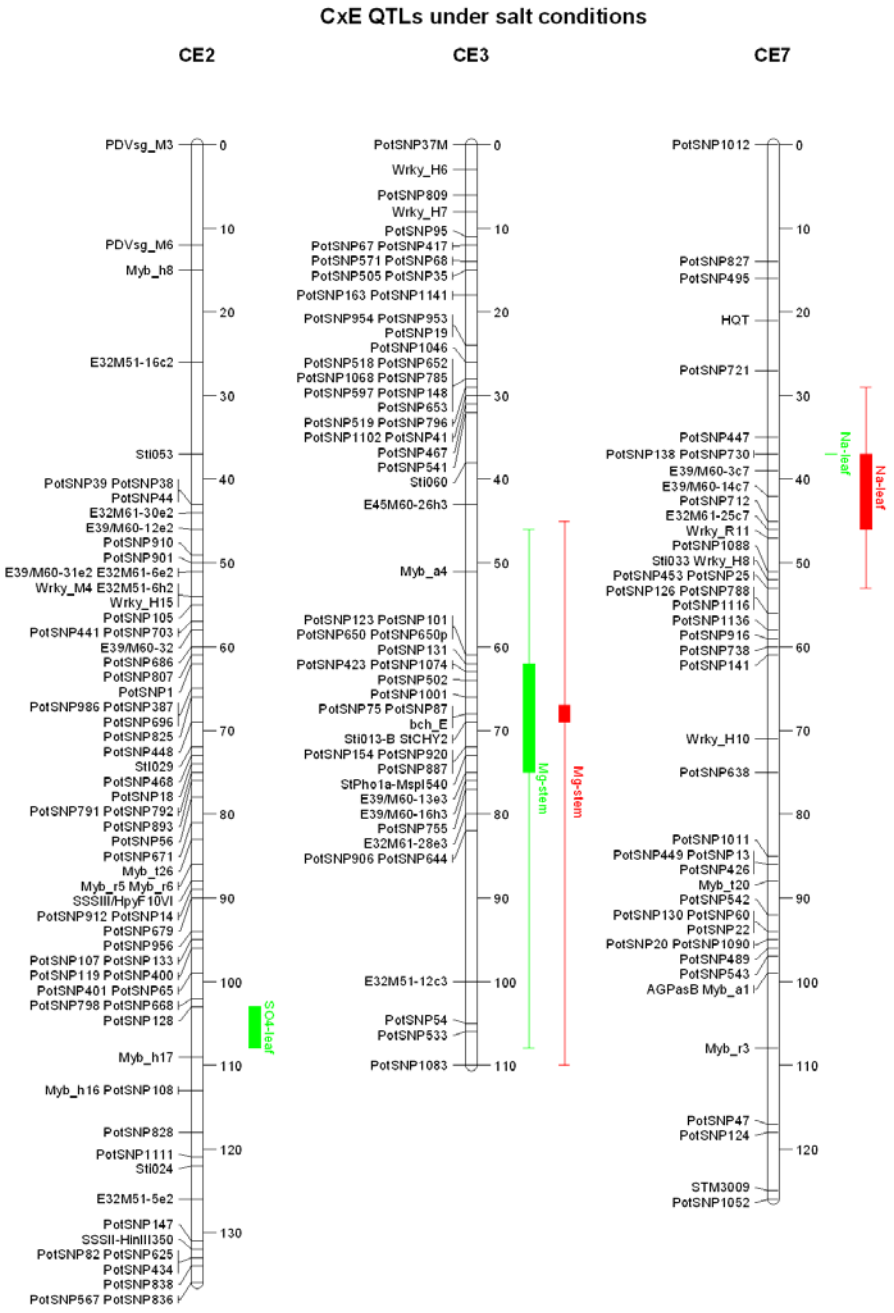


Figure 10. Chlorophyll fluorescence at different days under salt conditions for the six tetraploid cultivars grown in hydroponics (R.B. stands for Russet Burbank, Moz stands for Mozart, Mon stands for Mondial, M.L. stands for Mona Lisa, Des stands for Desiree and Bin stands for Bintje)

Appendix 5. QTLs found under control conditions for ion data of 2008 placed in the chromosomes using MapChart



Appendix 6. QTLs found under salt conditions placed in the chromosomes using MapChart. Red colour represent QTLs found for ion data of 2009 and green colour represent QTLs found for ion data of 2008



Appendix 7. List of CxE population

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

Appendix 8. List of CxE genotypes and tetraploid cultivars grown in field

Tetraploid cultivars only in field	CxE genotypes	Tetraploid cultivars also in hydroponics
Annabelle	CE 027	Bintje
Charlotte	CE 072	Desirre
Diamant	CE 082	Monalisa
Marabel	CE 159	Mondial
Marfona	CE 218	Russetburbank
Melody	CE 634	
Miranda	CE 640	
Nadina	CE 656	
Nicola	CE 659	
Spunta	CE 681	
CMK2002-204-008	CE 689	
CMK2004-006-019	CE 702	
CMK2004-075-048	CE 709	
CMK2004-614-002	CE 719	
Fontane	CE 728	
Ladybritta	CE 732	
Ladyrosetta	CE 777	
Musica	CE 786	
Ramos	P-C	
Soprano	P-E	

Appendix 9. List of abbreviations for growth data used in correlation analysis

CC0	Chlorophyll Content at day 0
CC6	Chlorophyll Content at day 6
CC10	Chlorophyll Content at day 10
CC11	Chlorophyll Content at day 11
CC13	Chlorophyll Content at day 13
CC16	Chlorophyll Content at day 16
CC20	Chlorophyll Content at day 20
CF0	Chlorophyll Fluorescence at day 0
CF6	Chlorophyll Fluorescence at day 6
CF10	Chlorophyll Fluorescence at day 10
CF13	Chlorophyll Fluorescence at day 13
CF16	Chlorophyll Fluorescence at day 16
CFd20	Chlorophyll Fluorescence of down leaves at day 20
CFm20	Chlorophyll Fluorescence of middle leaves at day 20
CFu20	Chlorophyll Fluorescence of upper leaves at day 20
%RDW	Percentage of Rood Dry Weight
%SDW	Percentage of Shoot Dry Weight
RDW	Rood Dry Weight
RFW	Rood Fresh Weight
RL	Root Length
R_S_DW	Root to Shoot Dry Weight
R_S_FW	Root to Shoot Fresh Weight
SDW	Shoot Dry Weight
SFW	Shoot Fresh Weight
SL	Shoot Length
SL0	Shoot Length at day 0
SL6	Shoot Length at day 6