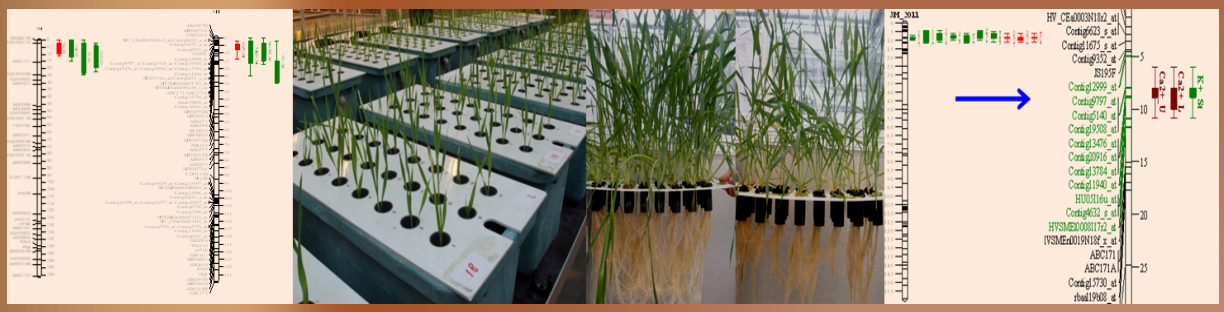
FineMapping and Further Characterisation of a chromosomal Region of 3H with QTLs for Salinity Tolerance in Barley (*Hordeum vulgare* L.)



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Thank you!... Dank u!... Cảm ơn!

Nguyễn Hoàng Hân

Summary

Salinity, a serious environmental problem worldwide, affects over 6% of world's arable land, approximately 800 million hectares (Munns and Tester, 2008). Under salt stress, growth rate and yield of plants will be seriously reduced by osmotic and ionic stress. Thus, research on salt-tolerant plants is of importance for agriculture. Among cereals, barley is considered one of the most salinity tolerant species. The mechanisms underlying salt tolerance and their genetic control are, however, not fully understood. Nevertheless, recent studies have suggested that the regulation of the cellular contents of ions such as Na^+, K^+, Cl^- and Ca^{2+} play a major role (Hasegawa et al., 2000; Tester and Davenport, 2003; and Munns and Tester, 2008). To get a better understanding of the genetic factors influencing salt stress tolerance in barley, the Steptoe x Morex DH population was evaluated for salinity tolerance using a hydroponics system with and without 200 mM NaCl (Nguyen *et al.*, unpublished data). The study found several QTLs for plant growth and ion concentration in both growing conditions, some of which were colocalised in QTL regions on Chromosomes 2H and 3H. It was suggested that QTLs for ion content play important roles in salt tolerance. However, the genetic regions of those QTLs found in Nguyen's study are still rather large, which makes the identification of the genes controlling the QTLs difficult.

This MSc thesis, therefore, aimed at fine mapping and further characterization of a chromosomal region of 3H with QTLs for salinity tolerance. Twenty-two recombinant DH lines and two parents were exposed to control conditions as well as 200 mM NaCl on a hydroponics system. Young barley plants were found to be able to survive 200 mM NaCl conditions after 18 days; however, their growth decreased significantly. Stomatal conductance was very sensitive to salt stress while chlorophyll content was less affected by salinity. Under salinity, Na^+ and Cl^- contents were increased, whereas concentrations of K^+, Ca^{2+} and Mg^{2+} were decreased significantly. Our results show that barley plants were capable of storing Na^+ and Cl^- in older leaves while accumulating more K^+ in young leaves and stem. Correlation analysis results revealed that Na^+ and Cl^- were positively correlated under salt stress. In the same condition, K^+ and Na^+ had a positive correlation in stem and root tissues while a negative correlation between these two ions was found in root tissue in control treatment.

Furthermore, this research confirmed most of the QTLs studied by Nguyen *et al.* (unpublished data). QTLs for Ca^{2+} in young and old leaf tissues and QTL for K^{+} in stem tissue on 3H QTL region in salt stress were finemapped to an interval of 0.891 cM. These QTLs were colocalised in the same region (8.013 - 8.904 cM), but they seemed to be controlled by different genes. Based on our results and literature studies on ion transporter genes, *HKT* and *CAX* family genes were suggested as potential candidates for underlying genes in these QTLs.

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2H	Chromosome 2
3H	Chromosome 3
ANOVA	Analysis of variance
CAX	Ca^{2+} exchanger
CBL10	Calcineurin B-like 10
cds	Coding sequence
DAS	Days after final salt stress reached
DH	Double haploid
EC	Electrical conductivity
Gs	Stomatal conductance
h	heritability
HKT	High-affinity k transporter
L leaf	Older leaves (lower leaves)
M	Morex
NHX	Vacuolar Na^+/H^+ antiporter
NL	Number of leaves
NT	Number of tillers
PH	Plant hight
QTL(s)	Quantitative trait locus (loci)
RDW	Root dry weight
RL	Root length
S	Steptoe
SDW	Shoot dry weight
%SDW	Shoot dry metter
SFW	Shoot fresh weight
Sh/R	Shoot-root ratio
SL	Seedling length
SOS	Salt overly sensitive
TDW	Total dry weight
U Leaf	Young leaves (upper leaves
WUR	Wageningen University and Research Centre

1 Introduction

Salinity affects agriculture worldwide and will become worse in the coming decades (Rajendran *et al.*, 2009). More than 6% of the world's area of arable land, being over 800 million hectares of land is affected by salinity (Munns and Tester, 2008). Salt-affected soils are common in arid and semiarid regions where rainfall is limited and insufficient to filter adverse amounts of salts from the rhizosphere (Rengasamy, 2002; Tester and Davenport, 2003; Rengasamy, 2006). Poor water management in irrigated culture may also cause salinity stress, due to excessive evaporation of water, while leaving salts and other substances behind.

Salinity affects plant growth in two ways: hampering water uptake by the roots, and accumulation of salts in the leaves up to toxic levels (Munns and Tester, 2008). Roots of plants growing on saline soils have a difficulty to take up water because of a high osmotic value of the soil water surrounding the root. For water uptake under these conditions, plants have to accumulate extra soluble salt. This has an immediate adverse effect on cell growth and its metabolism. Na^+, Ca^{2+} and Mg^{2+} cations are the most closely associated with salinity as well as the anions Cl^- and SO_4^{2-} (Tavakkoli *et al.*, 2010). Hasegawa et al. (2000) considered Na^+ and Cl^- to be the most important ions because both are toxic to plants when accumulated at high concentrations (Munns and Tester, 2008).

Although barley is a salinity-tolerant crop, its growth and production are greatly affected by salt stress (Munns and Tester, 2008). Many studies on barley plants under saline conditions have been suggested that the reductions of growth and photosynthesis in barley were mainly linear to Na^+ and Cl^- stress (Tavakkoli *et al.*, 2011). Tavakkoli *et al*. (2011) also concluded that high Na^+ content decreased K^+ and Ca^{2+} uptake and reduced photosynthesis mainly by reducing stomatal conductance. Moreover, a genetic study using offspring of a salt-tolerant (K305) and a salt-sensitive (I743) barley cultivar suggested that the salt-tolerant plants accumulated Na^+ and Cl^- and reduced plant growth less than the salt-sensitive ones whereas K^+ content of tolerant and sensitive plants were not significantly different (Ligaba and Katsuhara, 2010). Therefore, the growth reduction of the salt-sensitive plant is mainly attributed by elevated levels of Na^+ and Cl^- in shoots.

In addtion, it has been suggested that maintenance of ion homeostasis is very important for plant to tolerate salt stress (Niu *et al.*, 1995; Zhu, 2002; Zhu, 2003; Ligaba and Katsuhara, 2010). However, the mechanisms through which barley plants can cope with salinity stress are not fully understood. Ligaba and Katsuhara (2010) conducted that the more expression of K^+-transporters, vacuole H^+-ATPase and organic pyrophosphatase in shoots and Na^+/H^+ antiporters in roots of the tolerant plant could be key factors in salt tolerance. Moreover, a study of Tibetan wild barley suggested that *HvHKT1* and *HvHKT2* mainly control Na^+ and K^+ transport under salt stress, respectively (Qiu *et al.*, 2011). In Qiu *et al.* study, the expression of *HvHKT1* in roots was increased dramatically, while *HvHKT2* was down-regulated significantly.

To get a better understanding of differences in genotypic response to salinity stress in barley Nguyen and his colleagues (unpublished data) studied genetic variation in response to salt stress on hydroponics using a mapping population derived from the cultivars Steptoe x Morex double haploid (DH) population (Kleinhofs *et al.*, 1993). They found highly significant differences among DH lines for various salinity-related traits, in part controlled by QTLs. Interestingly, the QTLs for traits such as plant growth and contents of Na^+, K^+, Na^+/K^+, Ca^{2+}, Mg^{2+} and Cl^- of shoots and roots were mapped on this population (Appendix 1.1). Clustering of QTLs on 2H and 3H was observed, suggesting that these chromosomal regions are very important for understanding the salt-tolerance mechanisms in barley. Thus, the roles of these two QTL regions in relation to salt tolerance need further study on Steptoe and Morex DH population. In addition, the QTL localizations were approximately 13.2 cM and 29.1 cM for 2H and 3H QTL region, respectively. Therefore, they need to be improved.

From that point of view, Vincent Kock did an MSc thesis with focusing on the study on 2H QTL region. He selected a set of recombinant DH lines from the Steptoe x Morex DH population, which showed recombination between haplotypes of Steptoe and Morex in the 2H QTL region. The recombinant lines were tested on a hydroponics system in almost the same way as in the mapping study by Nguyen *et al.* (unpublished). His study confirmed strong QTL for K^+ content in shoot tissue, which detected on 2H by Nquyen's study. He narrowed down the 2H QTL region to 2.2cM; this was six times smaller than the region found in the mapping study with the whole population. In addition, his study also

suggested *KT/HAK/HUP* and *HKT* transporter genes for underlying genes for the QTLs on 2H.

The focus of this study was on the QTL cluster on 3H, using the same experimental conditions as Kock's study. This study aims at confirming the 3H QTL region, including the finemapping for salinity QTLs and identification of ion transporter genes possibly underlying the QTLs on this chromosome.

To achieve these, a new greenhouse experiment was carried out using a small set of 22 recombinant DH lines, Steptoe and Morex. The experiment was carried out on an aerated hydroponics system at two contrasting salt levels (with and without 200 mM NaCl). To study the effects of QTL region on 3H, several measurements of plant traits relevant to salt tolerance were done during the experiment with four replications. Furthermore, concentrations of various ions in plant tissues were determined in two replications.

2 Materials and Methods

2.1 Experimental design

The experiment had a randomized completed block design (RCBD) with 12 main plots of two containers (subplots), on an aerated hydroponics system at Unifarm of Wageningen University and Research Center (WUR Unifarm) used for the two salt treatments (Appendix 2.3). Each container had twenty-four positions to which the twenty-four genotypes were randomly assigned with help of GenStat v.12 (Payne *et al.*, 2009).

Four out of 12 'main plots' were used for measuring various plant traits (2.4). Moreover, in order to get enough material for determining ion composition in various plant tissues, tissue from plants from 6 main plots were pooled for each genotype-treatment combination after drying and weighing the biomass of each plant fraction. As a result, the analysis of ion concentration had two replications.

2.2 Recombinant DH lines

This experiment focused on a detailed study of the QTL region on 3H. The selected DH lines used in this study showed in the QTL region on 3H recombination between the haplotypes from Steptoe and Morex (Appendix 2.2). Therefore, these lines are referred to as recombinant DH lines. These recombinant DH lines were selected out of a set of 139 DHs from the Steptoe x Morex DH mapping population used by Nguyen *et al.* (unpublished). In Nguyen *et al.* study, the Steptoe x Morex DH population was evaluated on a hydroponics system at two salt levels control (0 mM extra NaCl) and salt (200 mM extra NaCl) conditions. They detected QTLs for Ca^{2+} and Mg^{2+} content in shoot tissue in control treatment and QTLs for K^+/Na^+ ratio and Cl^- also in shoot tissue upon salt stress on the short arm of 3H. That was called QTL region on 3H. The QTL region was approximately 29.1 cM (from 0 to 29.1 cM). Appendix 2.1 gave the list of selected recombinant DH lines for this study.

Seeds of the selected recombinant DH lines and their parents were incubated at 37^{o}C for 24 hours before germination. Seeds were germinated on silver sand for seven days, and the seedlings obtained were then transplanted to the aerated hydroponics system at WUR

Unifarm. Plants were allowed to grow for seven days on the system prior to the start of salt stress treatments as described below.

2.3 Stress treatments

The experiment had two salt stress levels: 0 mM extra NaCl (control treatment) and 200 mM additional NaCl (salt treatment). The treatments were assigned to blocks (main plots) as described above. Salt treatment was applied by adding in a period of 4 days in 4 steps of 50mM NaCl to the system up to an end-concentration of 200 mM to avoid salt shock to the plants. Plants were kept growing on these conditions until harvest.

2.4 Plant measurements

A subset of 2 x 4 containers were used to collect data on plant traits as described in detail in the following sections.

2.4.1 Plant height

Plant height (PH) was measured at different time points from the seedling stage to the end of experiment. Plant height at seedling stage (seedling length - SL) was measured at 7 days after germination (just before transplanting onto the hydroponics system). This implied that for each DH per replication data from three plants were collected. The plant height was measured at 0 (7 days after transplanting), at 3 days after final salt stress reached (DAS), at 10 DAS and at 18 DAS from the surface of the container to the tip of longest leaf of the plant, when held upright.

2.4.2 Number of tillers

Tillers of an individual plant were counted at 3, 7 and 18 DAS. Only tillers that were longer than five centimeters were counted.

2.4.3 Number of leaves

At harvest (18 DAS), number of leaves (NL) of the main tiller were counted.

2.4.4 Root length and shoot-root length ratio

Root length (RL) was also measured at harvest time (18 DAS). The longest root of the plant was used to determine the root length.

Shoot-root ratio (Sh/R) was calculated by dividing plant height at 18 DAS by root length of the corresponding plant at harvest.

2.4.5 Chlorophyll content

The hand-held SPAD 502 meter (Minolta, Osaka, Japan) is a device to measure chlorophyll content of leaves in a non-destructive, and an accurate and rapid way (James *et al.*, 2002). Therefore, SPAD meter was applied to measure chlorophyll content of the third as well as the youngest expanding leaves at several times during the experiment. The readings were taken at the bottom, middle, and top of leaves from the main tiller (third or/and the youngest expanding leaf). SPAD values were the average of the three measurements available per leaf. The SPAD readings of the third leaf were collected at 2 and 9 DAS and the ones of the youngest leaf at 9 and 16 DAS. Chlorophyll content was estimated by the formulae $10^{M^{0.256}}$ in which M represented the mean SPAD value (Markwell *et al.*, 1995).

2.4.6 Stomatal conductance

Stomatal conductance was assessed by a leaf porometer (Decagon Devices, Pullman, USA). The measurements were carried out at the middle part of the youngest expanding leaf of the main tillers at 3, 10 and 17 DAS, and they were taken between 10:00 am and 1:00 pm to minimize environmental variation.

The reduction in stomatal conductance to stress was obtained by subtracting the mean of stomatal conductance of individual genotypes in control treatment from the stomatal conductance measured under salt stress.

2.4.7 Plant biomass

Plants were harvested at 18 DAS. Shoot fresh weight (SFW) was measured immediately at harvest. The tissues were then dried at 75^{o}C for three days to obtain shoot dry weight (SDW)

and root dry weight (RDW). Total dry weight (TDW) was the sum of SDW and RDW. The shoot dry matter (%SDW) was obtained by dividing SDW by SFW *100%.

2.4.8 Salinity tolerance

Salinity tolerance was calculated as the ratio of biomass under salt stress expressed as percentage of the biomass produced without salt stress as defined by Munns and Tester (2008).

2.5 Analysis of ion concentration in plant tissues

At harvest, plants were separated into young leaves, old leaves, stems and roots tissues. The young leaves were the upper three leaves from the main tiller and two youngest leaves from tillers. The remaining leaves were considered the old leaves. After collecting the dry matter figures, various plant fractions from six plants of each DH were pooled per stress treatment for ion analyses. Tissue samples were grinded to make a finer powder. Approximate 25 (x) milligrams (mg) powder of the young, old leaves and stem dry matter, and about 20 mg of root dry matter were weighed into 10 ml glass tubes. The samples were ashed at 575^{o}C for five hours. The ash samples were then dissolved with 1 ml of 3M formic acid by shaking at 99^{o}C for 30 minutes and after that diluted with 9 ml milliQ water, followed by shaking for 30 minutes at 75^{o}C to help the samples dissolve completely. The products were kept as stock samples. For measurements, 100µl of stock samples were transferred to 10-ml running tubes, which contained 9.9 ml milliQ buffer to get 10-ml running solution. Concentration of ions Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cl^{-}, SO_4^{2-} and PO_4^{3-} were measured by Ion Chromatography (IC) system 850 Professional, Metrohm, Switzerland. The final concentrations in plant tissues were obtained by subtracting the measure of a blank sample from the sample measure from IC, and multiplying by 1000 to account for the dilution factor, and dividing by the amount of x (mg) tissue sample.

2.6 Statistical analysis

Analyses of variance (ANOVAs) of plant traits were carried out for each of the two stress treatments separately. This gave estimates for the least significant differences between DHs (LSD at p=0.05). The statistical analyses were done by GenStat v.12 (Payne *et al.*, 2009).

Heritability (h^2) of traits was calculated by the ratio of variance of genotype and the sum of variance of genotype and environment (1). Environmental variance (s^2_e) was the mean square of residuals (2). The genetic variance (s^2_g) was obtained from mean square (MS) of genotype subtracted to residual MS, then divided to the number of replications (3). Mean square of genotype and MS of residual were obtained from ANOVA results.

$$h^2 = \frac{s^2_g}{s^2_g + s^2_e} \quad (1)$$

$$s^2_e = MS_{residual} \quad (2)$$

$$s^2_g = \frac{MS_{genotype} - MS_{residual}}{n} \quad (3)$$

n: number of replications
n=2 for ion content analysis
n= 4 for greenhouse measurements

Correlations between traits were calculated using the genotypic means for agronomic traits and ion contents in various plant tissues. The correlation matrices were obtained with help of the software package GenStat v.12 (Payne *et al.*, 2009).

2.7 Finemapping analysis

The first step of finemapping was the verification of the QTLs for salinity tolerance on the same chromosomal region of 3H as in the study of Nguyen *et al.* (unpublished). The QTL map was analysed by using the dataset from 22 recombinant DH lines. This was done by MapQTL v.6 (Kyazma). Then, crossing-over point of QTL region on 3H was examined by plot the ion concentration of individual recombinant line against its recombinant site. Finally, finemapping to narrow the QTL region was done by identifying the two nearest recombinant DH lines to the crossing over points for the QTLs.

3 Results

3.1 The variation between recombinant lines and their parents

Genotypic variation between recombinant DH lines and their parents was analysed by ANOVA. The genotypic variation for most of the plant traits and ion contents in the plant tissues under study was significantly different. The following sections describe in more detail the results of genetic variation between the recombinant DH lines, the parental cultivars Steptoe and Morex.

3.1.1 Genotypic variation for agronomic traits

3.1.1.1 Plant height, root length, and shoot-root length ratio

The results of analysis of variation indicated that plant height, root length, and shoot-root length ratio of recombinant DH lines and their parents were considerably different (Table 3.1). The results illustrated that plant height, root length, and shoot-root length ratio were significant differences in both treatments for all measurement time points excluding the plant height that was measured prior to the application of salt stress (PH_0). The differences in plant height between DHs were mainly determined by their genetic constitution. As showed in Table 3.1, heritability (h^2) for seedling length on an individual plant basis was 0.84. The h^2 of plant height measured after the start of salt stress treatment was also high for both treatments, ranging from 0.53 to 0.69. The h^2 estimates for root length and shoot-root length ratio were smaller than the estimates of h^2 for PH, but still equal or greater than 0.3 indicating that the variation is at least in part genetic.

It was also indicated that these traits were significantly affected by salt stress. The effects of salt stress on PH at 3, 10 and 18 DAS were significant at $p<0.01$. Root length and shoot-root length ratio were different between control and salt treatment. However, the results showed that there had no interaction between genotype and salt tress of PH, RL and Sh/R (Table 3.2). This interaction result indicated that plants which had shorter PH or RL in control treatment

were shorter in salt stress. As a result, the interaction of genotype and salt stress was not significant.

Table 3.1 Genotypic variation for plant height, root length and shoot-root length ratio between recombinant DH lines and their parents

Trait	Control (0mM NaCl)						Salt (200mM NaCl)					
(cm)	Mean	Min	Max	LSD (5%)	*p*	h^2	Mean	Min	Max	LSD (5%)	*p*	h^2
SL	10.26	6.15	14.06	1.18	**	0.84	-	-	-	-	-	-
PH_0	18.13	14.75	23.02	4.94	ns	0.24	18.10	15.17	22.80	5.21	ns	0.21
PH_3	37.20	30.73	42.30	4.47	**	0.53	31.49	21.32	36.92	4.59	**	0.63
PH_10	55.80	47.13	64.25	5.23	**	0.61	40.74	31.20	46.62	4.51	**	0.69
PH_18	69.13	57.50	81.50	6.03	**	0.66	45.00	36.25	53.50	5.23	**	0.65
RL_18	60.20	49.00	70.25	11.44	**	0.35	28.41	21.75	35.75	7.41	*	0.30
Sh/R_18	1.18	0.94	1.54	0.28	**	0.41	1.65	1.35	2.41	0.52	*	0.31

SL - seedling length; PH - plant height; RL – root length; Sh/R – shoot-root ratio; 0, 3, 10, and 18 – DAS; Min - minimmun; Max - Maximmun; LSD - least significant differences; and h^2 – heritability

Table 3.2 ANOVA for plant height, root length and shoot-root ratio

Source of variation	d.f.	PH_0	PH_3	PH_10	PH_18	RL_18	Sh/R
Genotype	23	ns	**	**	**	**	**
Salt stress	1	ns	**	**	**	**	**
Genotype*Salt stress	23	ns	ns	ns	ns	ns	ns

d.f.: degree of freedom, **: $p<0.01$, ns: not significant

3.1.1.2 Number of tillers and number of leaves

Genotypic variation in number of tillers and number of leaves is summarized in Table 3.3. As for PH, genotypic variation between the DHs NT and NL is fairly to highly heritable. The h^2 for NL was 0.94 and 0.77 in the control and salt treatment, respectively (Table 3.3).

The effects of salt stress on NT and NL were significant at $p<0.01$ for all time points. The NT that was measured at 3 and 18 DAS had an interaction of genotype and salt stress while the interation of genotype and salt stress for NT at 10 DAS was not significant. The NL also had a significant interaction of genotype and salt stress (Table 3.4).

Table 3.3 Genotypic variation of number of tillers and number of leaves of recombinant lines and their parents

	Control (0mM NaCl)						Salt (200mM NaCl)					
Trait	Mean	Min	Max	LSD (5%)	*p*	h^2	Mean	Min	Max	LSD (5%)	*p*	h^2
NT_3	2.34	1.25	3.00	0.72	**	0.47	1.83	1.25	2.75	0.66	**	0.40
NT_10	3.54	1.50	5.25	0.99	**	0.63	2.13	1.25	3.50	0.84	**	0.58
NT_18	5.26	2.75	8.75	1.94	**	0.60	2.30	1.00	4.50	1.00	**	0.67
NL_18	6.78	6.00	7.00	0.14	**	0.94	6.44	6.00	7.00	0.37	**	0.77

NT - number of tillers; NL - number of leaves; **: $p<0.01$

Table 3.4 ANOVA for number of tillers and number of leaves

Source of variation	d.f.	NT_3	NT_10	NT_18	NL_18
Genotype	23	**	**	**	**
Salt stress	1	**	**	**	**
Genotype*Salt stress	23	**	ns	**	**

d.f.: degree of freedom, **: $p<0.01$, ns: not significant

3.1.1.3 Plant biomass

Table 3.5 summarizes the genotypic variation in plant biomasses. The variation was significant at $p<0.01$ for all plant biomass measures and shoot dry matter content (%SDW). The differences in plant biomass were mostly moderately heritable (h^2 values from 0.37 to 0.53). Shoot dry matter in the control treatment, in addition, was highly heritable ($h^2 = 0.87$).

Plant growth was dramatically affected by salt tress. The influence of salt stress on plant biomass was significant at $p<0.01$ (Table 3.6). Plant biomass reduced significantly under saline conditions (Figure 3.1). An approximately 4-fold reduction was found in SFW. Shoot dry weight observed in the salt treatment was half the level in the control treatment. The reduction of RDW was much smaller, although the influence of salt stress on RDW was significant at $p<0.01$ (Figure 3.1b).

Table 3.6 also showed the interactions of genotype and salt stress of plant biomass and shoot dry matter. The interaction of genotype and treatment of SFW and %SDW was statistically significant at $p<0.01$, whereas RDW had no interaction of genotype and salt tress.

Table 3.5 Genotypic variation for plant biomass of recombinant DH lines and their parents

Trait	Control (0mM NaCl)						Salt (200mM NaCl)					
(gram)	Mean	Min	Max	LSD (5%)	*p*	h^2	Mean	Min	Max	LSD (5%)	*p*	h^2
SFW	18.92	11.81	28.34	6.30	**	0.53	4.72	3.04	6.83	1.58	**	0.50
SDW	1.89	1.27	2.68	0.62	**	0.51	0.82	0.58	1.14	0.28	**	0.41
RDW	0.31	0.16	0.51	0.19	**	0.37	0.23	0.14	0.33	0.09	**	0.44
TDW	2.20	1.47	3.16	0.78	**	0.49	1.06	0.76	1.42	0.35	**	0.41
%SDW	10.04	8.52	12.32	0.63	**	0.87	17.63	15.02	20.97	2.25	**	0.47

**: $p<0.01$; SFW - shoot fresh weight; SDW - shoot dry weight; RDW - root dry weight; TDW - total dry weight; and %SDW - shoot dry matter

Table 3.6 ANOVA for plant biomasses

Source of variation	d.f.	SFW	SDW	RDW	TDW	%DW
Genotype	23	**	**	**	**	**
Salt stress	1	**	**	**	**	**
Genotype*Salt stress	23	**	*	ns	*	**

d.f.: degree of freedom, **: $p<0.01$, *: $p<0.05$, ns: not significant

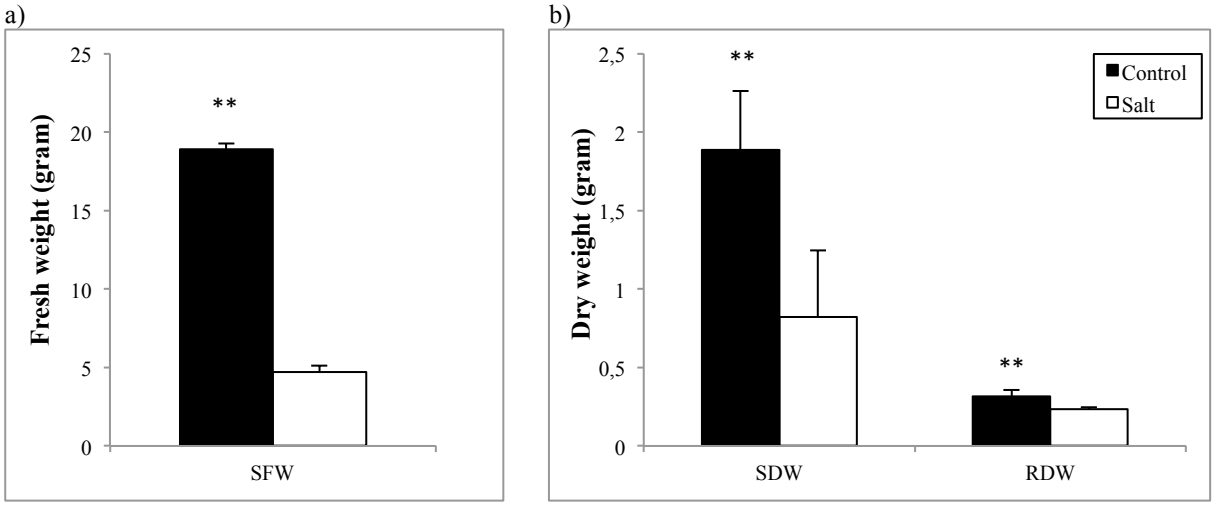


Figure 3.1 Shoot fresh weight (a), shoot dry weight and root dry weight (b) under salt and control conditions (**: $p<0.01$). The error bars indicated standard errors of genotypic means

3.1.1.4 Salt tolerance

Plant salt tolerance was also significantly different among recombinant DH lines and their parents ($p<0.01$, Table 3.7). Even though root length was more sensitive than plant height to salt stress (Figure 4.1), Figure 3.2 showed that the genotypic response to stress with respect to root growth was much stronger than the response for shoot growth.

Table 3.7 Genetic variation of plant biomasses salt tolerance

Salt tolerance (%)	Mean	Min	Max	LSD (5%)	*p*	h^2
SFW_ST	25.37	20.15	40.74	8.61	**	0.40
SDW_ST	44.44	35.40	63.80	15.19	**	0.33
RDW_ST	79.86	45.20	145.90	33.65	**	0.54
TDW_ST	49.14	37.90	71.70	16.45	**	0.37

**: $p<0.01$

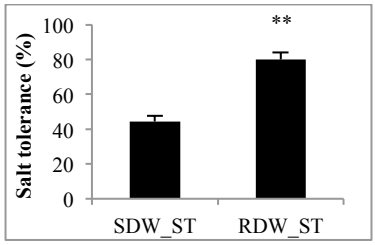


Figure 3.2 Comparison of shoot and root salt tolerance

3.1.2 Genotypic variation for chlorophyll content

Chlorophyll content of the third leaf and the youngest expanding leaf were measured at two times. Table 3.8 showed that the genotypic variation in chlorophyll content of the third and in the youngest expanding leaf was significant. In the control treatment, the genetic variation for this trait in the third and in the youngest leaf at 9 DAS was high (h^2=0.75 and 0.74,

respectively). In the salt treatment, however, the h^2 of chlorophyll content of the youngest leaf at 16 DAS was the greatest (0.78).

Table 3.8 Genetic variation of chlorophyll content of third leaf and the youngest expanding leaf

Chlorophyll content ($\mu M/m^2$)		Control						Salt					
		Mean	Min	Max	LSD (5%)	p	h^2	Mean	Min	Max	LSD (5%)	p	h^2
Third leaf	2 DAS	338	240	417	59	**	0.46	364	294	444	55	**	0.63
	9 DAS	376	301	428	60	**	0.75	399	321	471	53	**	0.61
Youngest leaf	9 DAS	346	284	412	53	**	0.74	360	282	441	53	**	0.56
	16 DAS	400	289	515	70	**	0.46	388	317	467	72	**	0.78

**: $p<0.01$

A study of the effects of salt stress on chlorophyll content showed that chlorophyll content of the third leaf were significantly affected by salt stress ($p<0.01$), whereas that of the youngest expanding leaf at 9 DAS was significant at $p<0.05$ and not significant at 16 DAS (Table 3.9).

On the other hand, the interaction of the chlorophyll content of the third leaf was not significant, whereas it was significant at $p<0.01$ for the chlorophyll content of the youngest expanding leaf (Table 3.9). This result concluded that the youngest expanding leaf chlorophyll content in some extent was lower in control treatment but greater in salt stress or vice versa.

Table 3.9 ANOVA for chlorophyll content measured

Source of variation	d.f.	Third leaf		The youngest expanded leaf	
		2 DAS	9 DAS	9 DAS	16 DAS
Genotype	23	**	**	**	**
Salt stress	1	**	**	*	ns
Genotype*Salt stress	23	ns	ns	**	**

d.f.: degree of freedom, **: P<0.01, *: P<0.05, ns: not significant

3.1.3 Genotypic variation for stomatal conductance

Genotypic variation of stomatal conductance was mainly contributed by environmental conditions even though the stomatal conductance was measued in between 10:00 am and 1:00 pm to minimize the effects of environment. The significant differences of stomatal conductance were analyzed in control and salt treatment, but the h^2 for stomatal conductance was very low (Table 3.10). This resulted in the greater effects of environmental conditions than genotypic variation.

On the other hand, the genotypic variation of reduction of stomatal conductance was significant at $p<0.01$ for all time points. The variation was highly heritable (h^2 from 0.50 to 0.75). This was due to a calculation of the stomatal conductance reduction method as described (see *materials and method*). This calculation method helped to reduce the effects of environmental variance. As a result, although differences of stomatal conductance were by environmental variance, the genotypic variation of stomatal conductance reduction was highly heritable.

In addition, the effects of salt stress on stomatal conductance were significant at $p<0.01$ in all tine points (Table 3.11 and Figure 3.3). However, only stomatal conductance at 3 DAS showed a significant interaction between genotype and salt stress.

Among three time points, the highest stomatal conductance was measured at 3 DAS (21 days after germination) in control treatment. Stomatal conductance slightly reduced in a week later (10 DAS). Then it stayed similar after 10 DAS (Figure 3.3). In contrast, even though stomatal conductance was very sensitive to salt stress, it slightly increased after a rapid reduction observed when the final salt stress was reached (Figure 3.3). The decreases of the stomatal conductance reduction resulted in the decreases of stomatal conductance in control treatment and the increase in salt stress (Figure 3.4). Thus, stomatal conductance under salt stress did recover to some extent with prolonged exposure to stress.

Table 3.10 Genotypic variation for stomatal conductance

	Control						Salt					
Trait (mM/m^2s^2)	Mean	Min	Max	LSD (5%)	*p*	h^2	Mean	Min	Max	LSD (5%)	*p*	h^2
Gs_3	169	123	231	64	*	0.16	60	34	88	27	*	0.18
Gs_10	141	103	198	66	ns	0.00	59	35	79	23	*	0.16
Gs_17	143	76	189	72	*	0.15	71	24	113	42	ns	0.12

Gs: Stomatal conductance; *: $p<0.05$; ns: $p>0.05$

Table 3.11 Analyses of variance for stomatal conductance at three successive timepoints

Source of variation	d.f.	Gs_3	Gs_10	Gs_17
Genotype	23	ns	ns	**
Salt stress	1	**	**	**
Genotype*Salt stress	23	*	ns	ns

d.f.: degree of freedom, **: P<0.01, *: P<0.05, ns: not significant

Table 3.12 Variation of the reduction stomatal conductance

Reduction	Mean	Min	Max	LSD	*p*	h^2
Gs_3	109.33	49.60	177.20	27.45	**	0.75
Gs_10	81.69	46.50	132.70	24.62	**	0.66
Gs_17	71.78	16.90	156.30	41.75	**	0.50

**: $p<0.01$

a)

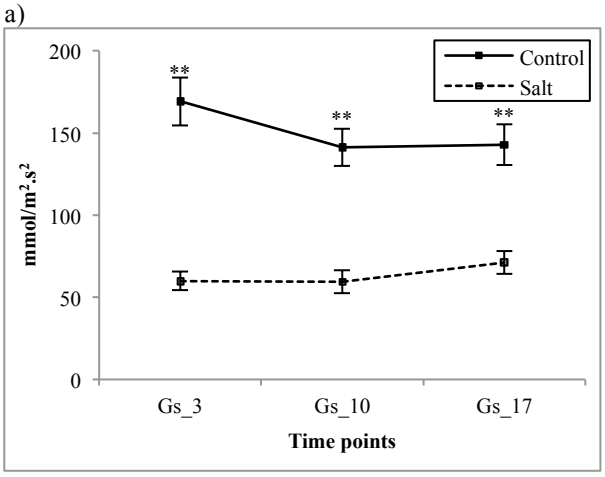


Figure 3.3 Effects of salt stress on stomatal conductance at different time points

b)

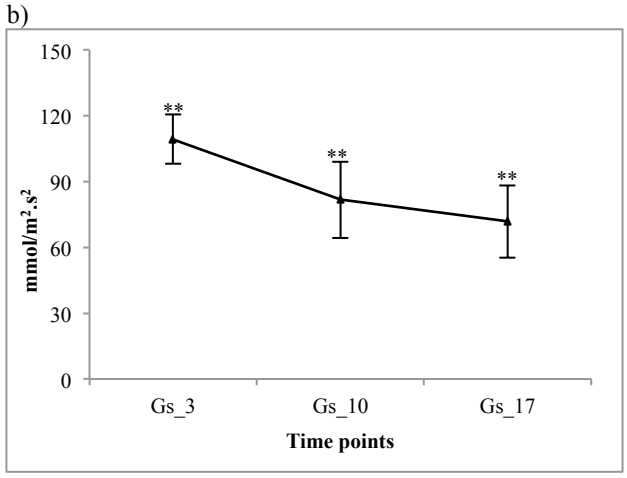


Figure 3.4 Reduction of stomatal conductance atdifferent time points

3.1.4 Genotypic variation in ion concentration in various plant tissues

3.1.4.1 Ion concentration in control treatment

The genotypic variation of ion concentration of plant tissues in control treatment are indicated in Table 3.13. The variation in Cl^- content in the plant parts studied was surprisingly highly heritable. The differences were significant in all plant tissues. However, the genotypic variation in K^+/Na^+ ratio was not significant in all tissues. Ca^{2+} was the second ion showing large genotypic differences in the control treatment. This held true for the leaves and the stem tissues. Concentration of K^+ in the leaves tissues also showed heritable variation (Table 3.13). The concentrations of ions in young leaves tissue tended to show more variation between than the contents in the lower leaves, the stems and the roots. This held for Mg^{2+}, PO_4^{3-} and SO_4^{2-}, in particular.

Table 3.13 Variation in concentration of ions in various fractions of plants from the control treatment

Plant part		Mean	Min	Max	LSD	*p*	h^2
Na^+	U Leaf	5.98	4.04	10.83	4.34	ns	0.06
	L Leaf	7.28	4.48	8.92	4.33	ns	0.21
	Stem	6.99	4.01	10.33	3.17	*	0.39
	Root	11.53	7.73	20.14	7.94	ns	0.15
K^+	U Leaf	58.92	36.70	87.00	18.48	**	0.59
	L Leaf	68.45	40.00	95.30	29.76	*	0.36
	Stem	84.68	53.40	109.00	27.66	ns	0.28
	Root	57.28	41.90	86.90	33.78	ns	0.03
Ca^{2+}	U Leaf	10.83	5.22	18.23	4.31	**	0.81
	L Leaf	22.10	15.18	31.70	7.15	**	0.65
	Stem	6.80	4.18	11.55	4.13	*	0.39
	Root	7.28	4.04	15.37	4.46	ns	0.28
Mg^{2+}	U Leaf	3.25	2.15	6.12	1.86	*	0.39
	L Leaf	5.33	3.35	7.86	2.26	*	0.41
	Stem	3.44	2.25	4.91	1.48	ns	0.13
	Root	3.94	2.78	4.89	2.30	ns	0.11
Cl^-	U Leaf	10.52	7.51	17.82	3.39	**	0.71
	L Leaf	16.71	9.13	32.23	8.62	**	0.56
	Stem	21.65	13.48	30.86	6.67	**	0.53
	Root	4.86	2.40	9.31	2.16	**	0.70
PO_4^{3-}	U Leaf	28.26	20.25	40.76	5.43	**	0.73
	L Leaf	29.47	20.50	42.00	14.85	ns	0.16
	Stem	28.88	21.69	38.15	10.18	ns	0.16
	Root	45.15	34.52	57.62	14.09	ns	0.30
SO_4^{2-}	U Leaf	10.51	8.60	15.55	1.64	**	0.79
	L Leaf	11.12	8.93	14.92	4.19	ns	0.11
	Stem	9.31	6.92	12.30	3.52	ns	0.10
	Root	16.24	12.51	20.72	5.32	ns	0.10
K^+/Na^+	U Leaf	10.67	6.17	16.20	6.55	ns	0.14
	L Leaf	9.83	5.97	17.55	5.79	ns	0.24
	Stem	12.82	10.50	16.63	4.31	ns	0.25
	Root	5.08	2.04	10.02	4.89	ns	0.27

**: P<0.01, *: P<0.05, ns: not significant, U leaf: young leaf, L leaf: old leaf

3.1.4.2 Variation in ion concentration of tissues from plant grown under salt stress

The measurements of ion concentrations observed in different tissues from plants grown under salt stress showed smaller differences in ion concentration than those observed in control treatment. Table 3.14 gives a summary of the genotypic variation in content of ions in tissues of stressed plants. In the control treatment, the differences between DHs in K^+/Na^+ ratio were significant in none of the plant tissues (Table 3.13). The differences in K^+/Na^+ ratios in the young leaves, stems and roots tissues observed in the stressed plants, however, were

significant (at $p<0.01$). Unlike the analyses in non-stressed plants, the genotypic differences in ion contents in the stems from stressed plants tended to be the most significant (Table 3.14).

Table 3.14 Genotypic variation in ion concentration observed in various parts of plants grown under salt stress

		Mean	Min	Max	LSD	*p*	h^2
Na^+	U leaf	51.75	36.60	69.50	16.60	ns	0.30
	L leaf	82.78	64.90	95.10	19.24	ns	0.20
	Stem	53.75	34.10	78.82	12.02	**	0.65
	Root	71.44	54.70	93.20	33.24	ns	0.08
K^+	U leaf	16.68	8.86	25.02	7.11	ns	0.31
	L leaf	13.14	8.65	18.27	6.70	ns	0.06
	Stem	17.13	11.71	21.76	3.51	**	0.65
	Root	20.69	14.21	28.09	10.73	ns	0.05
Ca^{2+}	U leaf	2.23	1.12	3.79	1.40	*	0.41
	L leaf	6.16	3.73	9.08	2.99	ns	0.29
	Stem	2.14	0.92	4.49	2.60	ns	0.04
	Root	5.31	3.42	7.54	3.38	ns	0.06
Mg^{2+}	U leaf	1.45	0.84	2.05	0.64	ns	0.20
	L leaf	2.13	1.45	3.05	1.37	ns	0.03
	Stem	1.50	1.06	2.08	0.43	**	0.61
	Root	2.50	1.22	3.73	1.99	ns	0.08
Cl^-	U leaf	44.22	22.80	69.40	18.95	**	0.45
	L leaf	80.68	58.10	99.80	24.06	ns	0.28
	Stem	58.40	36.70	93.50	22.50	*	0.43
	Root	47.02	35.50	58.10	24.18	ns	0.08
PO_4^{3-}	U leaf	35.60	29.38	47.50	9.64	ns	0.18
	L leaf	40.27	33.46	47.84	13.46	ns	0.15
	Stem	24.27	18.14	27.96	3.48	**	0.55
	Root	47.36	35.40	62.40	21.75	ns	0.07
SO_4^{2-}	U leaf	10.74	6.17	17.48	3.45	**	0.54
	L leaf	10.93	5.62	16.61	3.38	**	0.74
	Stem	6.77	3.99	8.32	2.90	ns	0.05
	Root	16.33	11.89	20.78	6.91	ns	0.00
K^+/Na^+	U leaf	0.32	0.20	0.46	0.08	**	0.72
	L leaf	0.16	0.10	0.24	0.07	ns	0.32
	Stem	0.32	0.23	0.43	0.06	**	0.71
	Root	0.30	0.23	0.37	0.07	**	0.47

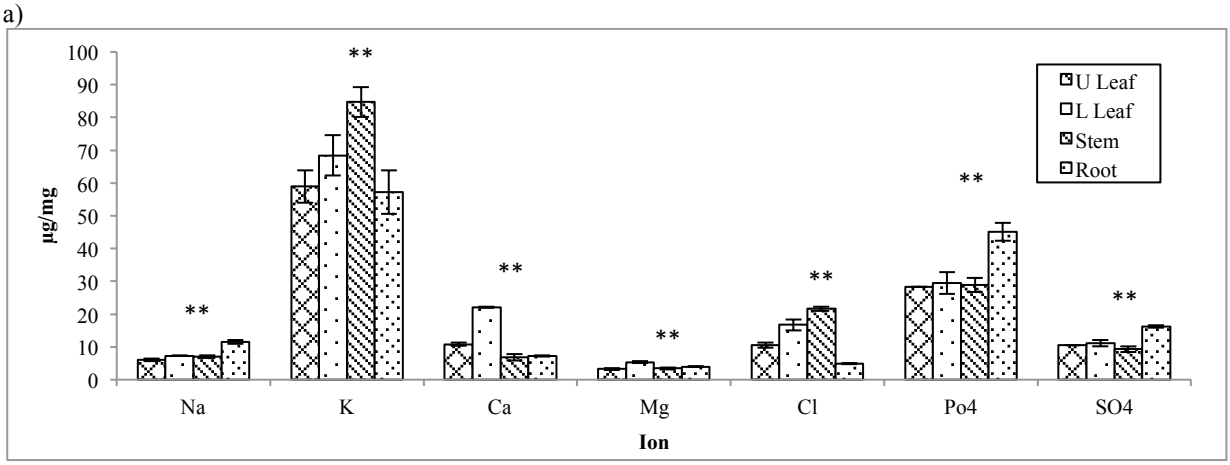
**: P<0.01, *: P<0.05, ns: not significant

3.1.4.3 Variation in distribution in ions over different plant fractions

The distribution of the ions over the different plant parts showed striking differences (Figure 3.5). In the control treatment, the high amounts of Na^+ present in the different plant parts were predominantly found in the roots, whereas in the stressed plants the Na^+ concentrations were

relatively high in the lower leaves. The distribution for Cl^- over the plant tissues from the salt treatment had a similar pattern as the distribution for Na^+ over the plant parts. In the control as well in the stress treatment, however, plants tend to have higher Cl^- concentrations in the shoots than in the roots. K^+ concentration in control and salt treatments showed a different distribution. In control treatment, the stems had significantly higher K^+ content than the other tissues. Under stress, the roots had a higher content than the shoot parts. Ca^{2+} concentration in lower leaves was highest in both treatments although Ca^{2+} contents in the lower leaves and in the roots were not different in salt stress. Moreover, concentration of Mg^{2+} in over plant tissues was also different. In the control treatment, Mg^{2+} was highest in the lower leaves whereas, roots Mg^{2+} was highest in salt stressed plants. PO_4^{3-} and SO_4^{2-} were preferentially allocated to the roots in both treatments.

a)



b)

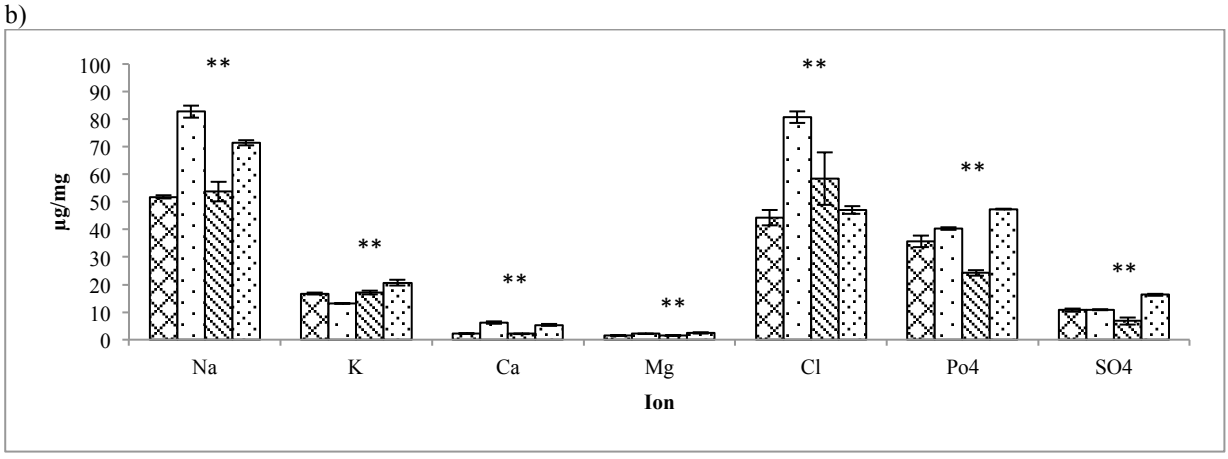


Figure 3.5 Distribution of ion concentration in different plant tissues in control treatment (a) and salt tress (b) (**: P<0.01)

3.1.4.4 Effects of salinity on ion concentration in plant tissues

The effects of salinity on ion concentrations in plant tissues are summarized in Figure 3.6. Concentrations of Na^+ and Cl^- increased significantly due to stress in all plant tissues, whereas the contents of K^+, Ca^{2+} and Mg^{2+} decreased considerably. The increases for Na^+ and the decreases for K^+ upon salt stress led to a significant reduction of K^+/Na^+ ratios in all plant tissues. Moreover, the effects of salt stress on PO_4^{3-} contents of the leaf parts and stems were significant at $P<0.01$, while there has no effects of salt stress on PO_4^{3-} in the roots. However, SO_4^{2-} content in the leaves and the roots was not affected by salt stress.

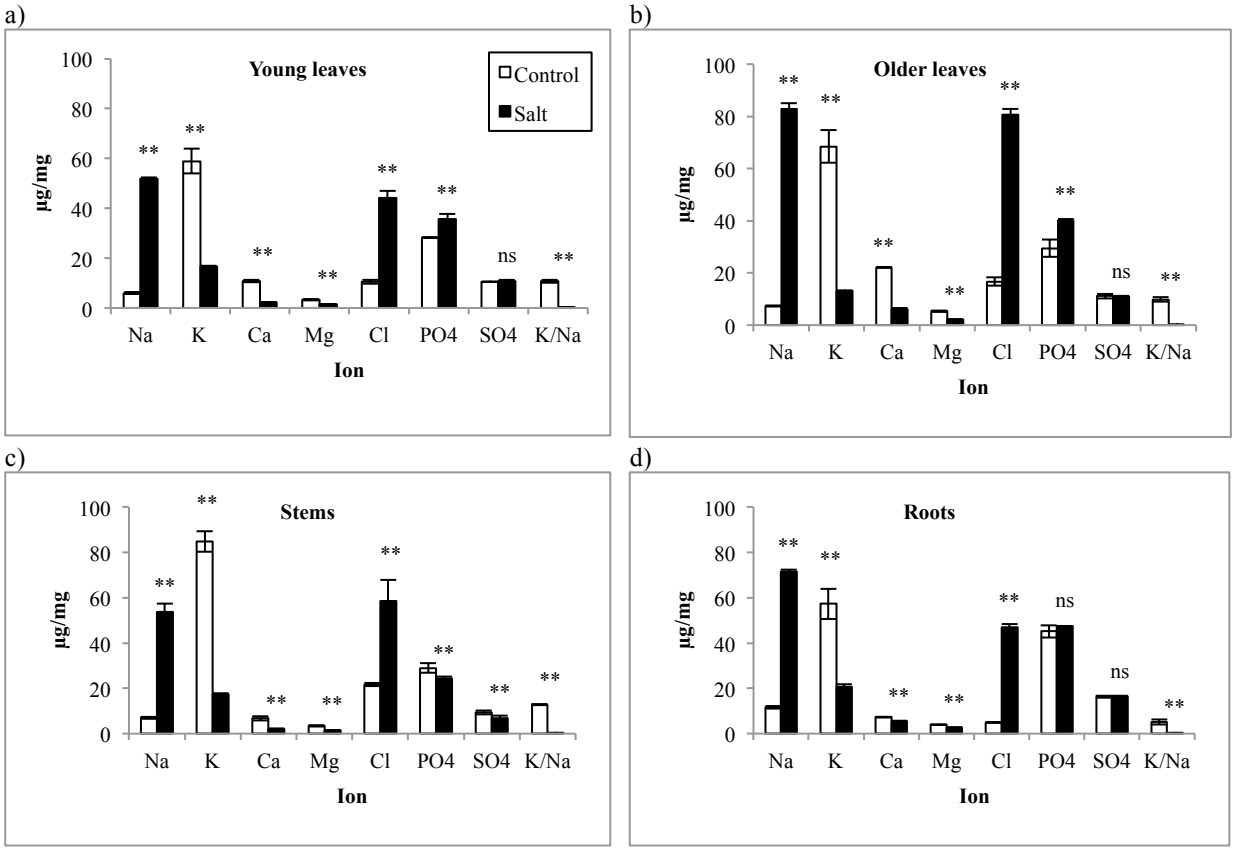


Figure 3.6 Effects of salinity stress on ion cencentration in young leaves (a), older leaves (b), stems (c) and roots (d) (**: P<0.01, ns: not significant)

3.2 Correlation analysis

3.2.1 Relation between chlorophyll content and agronomic traits

The chlorophyll contents of both third and the youngest leaf generally were positively correlated with plant height and the biomass measures although most of the correlation coefficients were not significant (Table 3.15). The correlations of chlorophyll content of the third leaf and agronomic traits revealed that the third leaf at 9 DAS (27 days after germination) was not important for plant growth and development, whereas the third leaf at younger stage (20 days after germination) was more relevant. In addition, the youngest expanding leaf chlorophyll content had significant correlations with plant height and plant biomass, especially the chlorophyll content of the youngest expanded leaf at 16 DAS. In control treatment, however, the correlation of the youngest expanded leaf at 16 DAS was negatively correlated with NT. In conclusion, chlorophyll of the youngest expanded leaf in salt stress was more important for plant growth and development than that of the third leaf.

Table 3.15 Correlation between chlorophyll content and agronomic traits

Traits	Third leaf				The youngest expanding leaf			
	2 DAS		9 DAS		9 DAS		16 DAS	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt
NT_3	+ ns	+ ns	+ ns	+ ns	- ns	0.45*	- ns	+ ns
NT_10	+ ns	+ ns	- ns	+ ns	- ns	+ ns	-0.47*	+ ns
NT_18	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	-0.44*	- ns
NL_18	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns
PH_0	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns
PH_3	0.43*	+ ns	+ ns	+ ns	0.45*	+ ns	0.46*	0.44*
PH_10	0.48*	0.42*	+ ns	+ ns	+ ns	+ ns	0.57**	0.48*
PH_18	0.54**	+ ns	+ ns	+ ns	+ ns	+ ns	0.63**	0.43*
RL_18	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns
Sh_R	+ ns	+ ns	+ ns	- ns	+ ns	- ns	0.57**	+ ns
SFW	+ ns	0.42*	+ ns	+ ns	+ ns	0.55**	- ns	0.43*
SDW	0.52**	0.47*	+ ns	+ ns	+ ns	0.56**	+ ns	0.54**
RDW	0.40*	+ ns	+ ns	+ ns	+ ns	0.46*	+ ns	+ ns
PDB	0.51*	0.46*	+ ns	+ ns	+ ns	0.58**	+ ns	0.50*
%SDW	+ ns	+ ns	+ ns	- ns	+ ns	- ns	0.74**	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

3.2.2 Relation between stomatal conductance and agronomic traits

The correlation of stomatal conductance and plant agronomic traits are summarized in

Table 3.16. Stomatal conductance measured in the control treatment, was not significantly correlated to any of the plant agronomic traits. The stomatal conductance measured at 10 DAS on stressed plants, was negatively correlated with NT. This measure, Gs_10 was positively correlated to PH and %SDW. At 17 DAS, the correlation between stomatal conductance and %SDW was also positive. The correlation between stomatal conductance and RDW at this time point was negative.

Table 3.16 Correlation between stomatal conductance and agronomic traits

Traits	Gs_3		Gs_10		Gs_17	
	Control	Salt	Control	Salt	Control	Salt
NT_3	+ ns	- ns	- ns	- ns	+ ns	- ns
NT_10	+ ns	- ns	- ns	-0.44*	+ ns	- ns
NT_18	+ ns	- ns	- ns	-0.50*	- ns	- ns
NL_18	- ns	+ ns	+ ns	+ ns	+ ns	+ ns
PH_0	+ ns	+ ns	+ ns	+ ns	- ns	+ ns
PH_3	- ns	- ns	- ns	+ ns	- ns	- ns
PH_10	- ns	+ ns	+ ns	0.43*	+ ns	+ ns
PH_18	- ns	+ ns	+ ns	0.54**	+ ns	+ ns
RL_18	- ns	- ns	- ns	+ ns	+ ns	- ns
Sh_R	+ ns	+ ns	+ ns	+ ns	- ns	+ ns
SFW	- ns	- ns	+ ns	+ ns	+ ns	- ns
SDW	- ns	- ns	+ ns	+ ns	+ ns	+ ns
RDW	- ns	- ns	+ ns	- ns	+ ns	-0.45*
TDW	- ns	- ns	+ ns	+ ns	+ ns	- ns
%SDW	+ ns	+ ns	- ns	0.40*	+ ns	0.44*

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

3.2.3 Relations between contents of ions and various plant traits

An analysis of correlation of ion concentration in different plant tissues and greenhouse measurement traits is summarized in Appendix 3. Most of the minerals did not correlate with the plant agronomic traits, except for number of tillers with all ion contents found in the young and old leaves and in the stems in both treatments. Especially, number of tillers in control treatment showed strong negative correlations with Ca^{2+}, Mg^{2+} and PO_4^{2-} in old leaves and with Ca^{2+} in young leaves. In the control treatment, chlorophyll content and Mg^{2+} contents of the plant fractions studied showed all positive correlations except for the fraction with old

leaves. Moreover, the correlations stomatal conductance at 17 DAS had positive correlations with Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-} in control treatment but not correlation in salt stress.

3.2.4 Interrelationships between contents of ions in various plant fractions

3.2.4.1 Contents measured in control treatment

The matrices of coefficients of correlation between ion contents are illustrated in Table 3.17 for different fractions of plant grown under control conditions. The correlations between stem traits were the most striking ones. The significant correlations were always positive, except in some cases for K^+/Na^+.

In young and old leaves, furthermore, the correlations between Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-} contents were all positively significant. In root tissue, however, Ca^{2+} only correlated with SO_4^{2-}; and Mg^{2+} had no correlation with any ion.

3.2.4.2 Contents measured under salt stress

Table 3.18Table 3.18 summarizes the correlations between ion contents in different parts of stressed plant. The correlations between Na^+ or K^+ with the contents of other ions measured in stems and roots were significant. Although the content of Na^+ increased and that of K^+ decreased under salt condition, it was striking that in both plant fraction the correlation was significant. In young and old leaves, the correlations were not significant.

Under salt stress, both Na^+ and Cl^- contents increased dramatically as was shown in Figure 3.6. Their correlations were very high positive in all plant tissues (Table 3.18).

Table 3.17 Matrices of coefficients of correlation between contents of ions of different parts of plant grown in the control treatment

Plant part	Ions	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	PO_4^{2-}	SO_4^{2-}
U leaf	Na^+	-						
	K^+	+ ns	-					
	Ca^{2+}	- ns	+ ns	-				
	Mg^{2+}	+ ns	0.43*	0.58**	-			
	Cl^-	- ns	0.45*	0.69**	0.43*	-		
	PO_4^{3-}	- ns	+ ns	0.83**	0.71**	0.67**	-	
	SO_4^{2-}	- ns	+ ns	0.64**	0.59**	0.7**	0.67**	-
	K^+/Na^+	-0.6**	0.59**	+ ns	+ ns	+ ns	+ ns	+ ns
L leaf	Na^+	-						
	K^+	+ ns	-					
	Ca^{2+}	+ ns	- ns	-				
	Mg^{2+}	+ ns	- ns	0.86**	-			
	Cl^-	+ ns	+ ns	0.74**	0.68**	-		
	PO_4^{3-}	0.46*	+ ns	0.79**	0.7**	0.59**	-	
	SO_4^{2-}	0.53**	+ ns	0.68**	0.71**	0.62**	0.81**	-
	K^+/Na^+	- ns	0.73**	-0.41*	- ns	- ns	- ns	- ns
Stem	Na^+	-						
	K^+	0.81**	-					
	Ca^{2+}	+ ns	+ ns	-				
	Mg^{2+}	0.61**	0.72**	0.72**	-			
	Cl^-	0.65**	0.79**	0.44*	0.72**	-		
	PO_4^{3-}	0.81**	0.89**	0.51*	0.8**	0.69**	-	
	SO_4^{2-}	0.74**	0.81**	0.49*	0.66**	0.71**	0.84**	-
	K^+/Na^+	-0.63**	- ns	- ns	- ns	- ns	- ns	- ns
Root	Na^+	-						
	K^+	-0.65**	-					
	Ca^{2+}	+ ns	+ ns	-				
	Mg^{2+}	- ns	+ ns	+ ns	-			
	Cl^-	- ns	0.56**	+ ns	- ns	-		
	PO_4^{3-}	+ ns	0.53**	+ ns	- ns	0.75**	-	
	SO_4^{2-}	- ns	0.68**	0.41*	+ ns	0.62**	0.76**	-
	K^+/Na^+	-0.86**	0.85**	- ns	+ ns	+ ns	+ ns	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively, -: negatively

Table 3.18 Matrices of coefficients of correlation between contents of ions of different parts of plant grown in the salt treatment

Plant part	Ions	Na	K	Ca	Mg	Cl	PO4	SO4
U leaf	Na^+	-						
	K^+	+ ns	-					
	Ca^{2+}	+ ns	- ns	-				
	Mg^{2+}	+ ns	0.69**	+ ns	-			
	Cl^-	0.90**	+ ns	- ns	+ ns	-		
	PO_4^{3-}	0.46*	0.47*	+ ns	0.57**	+ ns	-	
	SO_4^{2-}	0.54**	+ ns	+ ns	+ ns	+ ns	0.65**	-
	K^+/Na^+	-0.46*	0.75**	- ns	0.49*	- ns	+ ns	- ns
L leaf	Na^+	-						
	K^+	+ ns	-					
	Ca^{2+}	+ ns	- ns	-				
	Mg^{2+}	- ns	- ns	+ ns	-			
	Cl^-	0.91**	- ns	+ ns	- ns	-		
	PO_4^{3-}	+ ns	+ ns	+ ns	0.41*	+ ns	-	
	SO_4^{2-}	0.46*	- ns	+ ns	+ ns	0.42*	+ ns	-
	K^+/Na^+	-0.53**	0.79**	-0.49*	+ ns	-0.56**	- ns	-0.43*
Stem	Na^+	-						
	K^+	0.46*	-					
	Ca^{2+}	+ ns	+ ns	-				
	Mg^{2+}	0.61**	0.67**	+ ns	-			
	Cl^-	0.96**	0.54**	+ ns	0.64**	-		
	PO_4^{3-}	0.49*	0.46*	+ ns	0.49*	0.46*	-	
	SO_4^{2-}	0.82**	0.59**	+ ns	0.73**	0.85**	0.64**	-
	K^+/Na^+	-0.54**	0.47*	+ ns	+ ns	- ns	- ns	- ns
Root	Na^+	-						
	K^+	0.77**	-					
	Ca^{2+}	+ ns	+ ns	-				
	Mg^{2+}	0.72**	0.72**	+ ns	-			
	Cl^-	0.81**	0.51*	0.41*	0.52**	-		
	PO_4^{3-}	0.78**	0.56**	+ ns	+ ns	0.65**	-	
	SO_4^{2-}	0.79**	0.48*	+ ns	0.45*	0.78**	0.74**	-
	K^+/Na^+	- ns	0.54**	- ns	+ ns	- ns	- ns	- ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

3.2.4.3 Interrelationships between ion contents within different fractions of plant

The correlations between ion concentrations in different plant tissues are shown in Table 3.19 to 3.22. The correlations between cations in different plant tissues in control and in salt treatment are given in Table 3.19 and Table 3.20, respectively. Table 3.21 and 3.22 showed the same data for the anions.

Among cations, the correlations of K^+ in different plant tissues were the most considerable. In control treatment, K^+ concentration in the roots had negative correlations with K^+ in the leaves and in the stems. In salt treatment, however, K^+ content in the roots correlated positively with K^+ in the leaves and in the stems. In addition, the correlation of Ca^{2+} in different plant tissues were also important to understand the mechanism how plant transport Ca^{2+} to plant tissues in different conditions. In control treatment, Ca^{2+} in the stems correlated positively with Ca^{2+} in the leaves, whereas Ca^{2+} had the negative correlations with Ca^{2+} in the leaves in salt tress although the correlations were not significant.

Among anions, the correlations of Cl^- in different plant tissues were the most important. Cl^- in the leaves and the stems correlated positively in both treatments. In control treatment, Cl^- in the roots had negative correlations with the leaves and a positive correlation with the stems, whereas their correlations in salt stress were vice versa.

Table 3.19: Correlation of cation in different plant tissues in control treatment

Ions	Na^+			K^+			Ca^{2+}			Mg^{2+}		
Tissues	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem
L_Leaf	- ns			+ ns			0.76**			0.50*		
Stem	0.46*	- ns		0.45*	0.46*		0.53**	0.67**		+ ns	+ ns	
Root	- ns	- ns	- ns	- ns	-0.48*	- ns	- ns	- ns	+ ns	+ ns	- ns	+ ns

Table 3.20: Correlation of cation in different plant tissues in salt treatment

Ions	Na^+			K^+			Ca^{2+}			Mg^{2+}		
Tissues	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem
L_Leaf	+ ns			0.44*			0.64**			+ ns		
Stem	0.55**	+ ns		+ ns	+ ns		- ns	- ns		- ns	- ns	
Root	+ ns	-0.44*	+ ns	+ ns	+ ns	0.48*	+ ns	- ns	+ ns	- ns	- ns	0.52**

Table 3.21 Correlation of anion in different plant tissues in control treatment

Ions	Cl^-			PO_4^{2-}			SO_4^{2-}		
Tissues	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem
L_Leaf	0.62**			+ ns			+ ns		
Stem	0.58**	0.62**		+ ns	- ns		+ ns	+ ns	
Root	+ ns	+ ns	- ns	0.40*	0.41*	- ns	+ ns	- ns	- ns

Table 3.22 Correlation of anion in different plant tissues in salt treatment

Ions	Cl^-			PO_4^{2-}			SO_4^{2-}		
Tissues	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem	U Leaf	L Leaf	Stem
L_Leaf	0.50*			0.56**			0.70**		
Stem	0.74**	+ ns		+ ns	+ ns		+ ns	- ns	
Root	- ns	- ns	+ ns	+ ns	+ ns	0.52**	- ns	- ns	- ns

*: P<.05, ** P<.01, ns: not significant, +: positively and -: negatively

3.3 QTLs for salinity tolerance

QTL mapping was carried out using of genotypic data from this experiment. This resulted in the identification of several QTLs for the different traits (of parts) of plants grown under stress or control conditions on QTL regions 2H and 3H (Figure 3.7). These QTL regions were more or less in the same chromosomal region as the prominent ones identified by Nguyen *et al.* (unpublished).

Nguyen *et al.* study showed QTLs for Ca^{2+} and Mg^{2+} contents in shoot tissues from plants grown in the control treatment on a chromosomal region on 3H. This experiment found QTLs for Ca^{2+} contents in young and old leaves, and in stems and a QTL for Mg^{2+} in old leaves (Figure 3.7a). Therefore, in conclusion, this study confirmed the results of Nguyen and coworkers. Moreover, a QTL for K^+ content in old leaves from plants grown in the control treatment, QTLs for Cl^- and PO_4^{3-} contents in roots of non-stressed plants mapped on a chromosomal region of 3H similar to the one found by Nguyen *et al*. study. However, QTLs for Na^+/K^+ and Cl^- in the shoots from stressed plants were not mapped in this study. In contrast, this study found QTLs for Ca^{2+} content in young and old leaves and a QTL for K^+ content in stems of salt-stress plants (Figure 3.7b). The finemapping of these QTLs for salinity tolerance is described in the next sections.

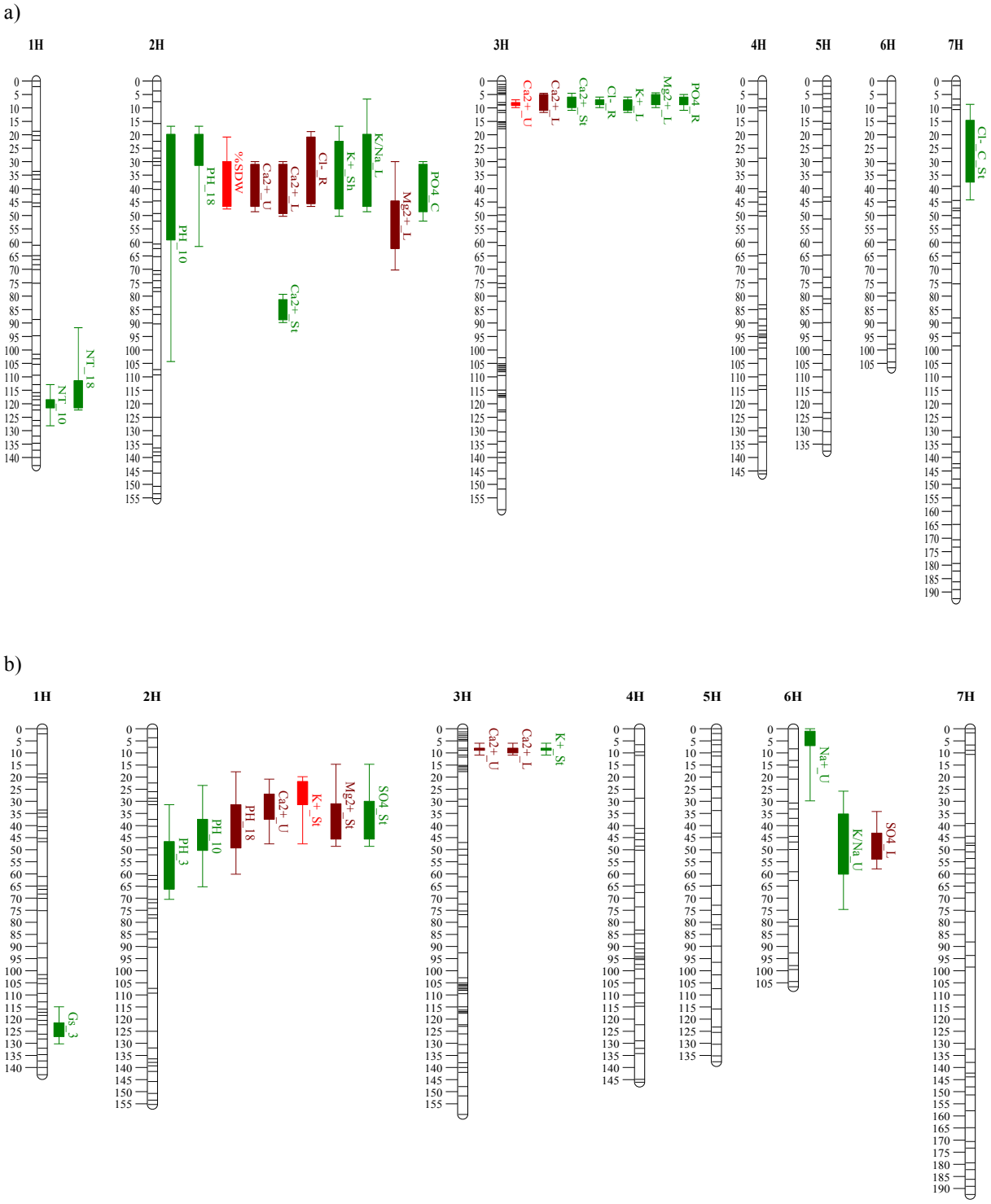


Figure 3.7 QTL mapping of traits of plants grown under control (a) and the stress conditions (b) using data of 22 recombinant DH lines from the SM DH population (green bar: 3.0<LOD <4.5, brown bar: 4.5<LOD <7.0, and red bar: LOD>7.0)

3.4 Finemapping of QTLs for salinity tolerance on 3H

The QTL mapping has shown on 3H a region with strong QTLs for Ca^{2+} content in young and old leaves from stressed plants and one QTL for K^+ in the stems from these plants. These QTLs did co-localize somewhere on the 3H linkage map between 5.0 cM to 11.7 cM (Figure 3.7b). The peak LOD values for these QTLs were at the same map position (data not showed). The following sections show in detail the results on the fine mapping of these QTLs.

3.4.1 QTLs for Ca^{2+} content in young and old leaves

The Ca^{2+} concentration in leaves from SM- and MS- recombinants is plotted against the location of the crossing over point of this QTL region (Figure 3.8). SM recombinants were those genotypes with an S allele (Steptoe allele) on the left side of the QTL region on 3H and with an M allele (Morex alllele) on the right side of the QTL region. MS recombinants were those genotypes that had the opposite S and M alleles in the QTL region. The position of crossing over point was estimated by taking the mean value of all SM or MS recombinants that located on left or right side of the crossing over point (Figure 3.8). Fine mapping was then done by analyzing the allelic differences of the two nearest crossing over point genotypes of the same SM or MS recombinants.

This finemapping approach narrowed the QTL region for Ca^{2+} contents in young and old leaves to an interval of 0.891 cM (Figure 3.9). The results showed that finemap based on SM recombinants were the same for young and old leaves, but different based on MS recombinants. Chromosomal region of leaves Ca^{2+} QTLs was 0.891 cM (interval from 8.013 to 8.904 cM) if the finemap was based on SM recombinants. If the finemap was based on MS recombinants, QTL region of Ca^{2+} in young leaves was finemapped to a region of 0.744 cM (from 8.013 to 8.757 cM) and QTL region of Ca^{2+} in old leaves was narrowed to an interval of 0.144 cM (from 8.757 to 8.904 cM) (Figure 3.9). However, the total of regions of QTLs for Ca^{2+} in young and old leaves that were finemapped by MS recombinants were the same the finemapping regions by SM recombinants. Moreover, concentration of Ca^{2+} in young and old leaves of some MS recombinants may be controlled by other QTL regions such as QTL region on 2H, but the effects of the QTL on Ca^{2+} content in those tissues were clearly observed on

SM recombinants (Figure 3.8). Therefore, the finemap based on the SM recombinants that had longer region was more accurate than based on MS recombinant.

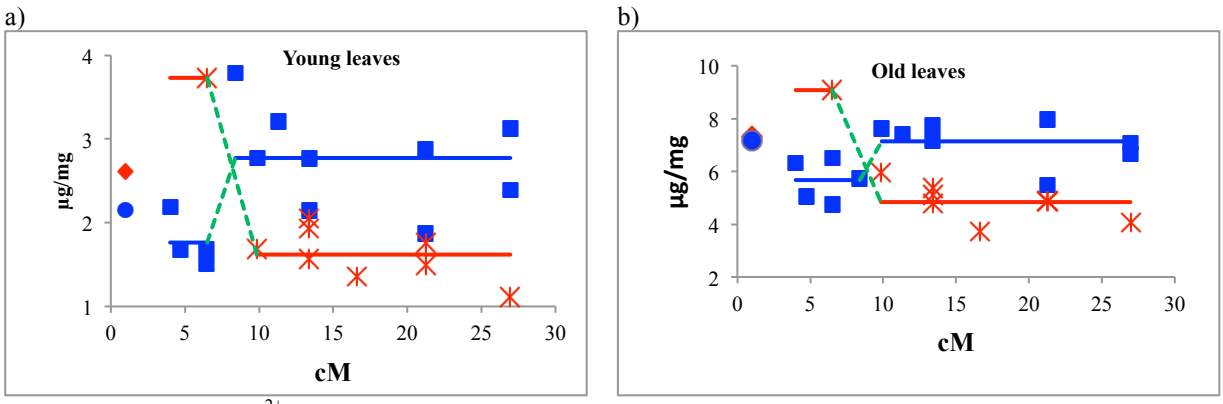


Figure 3.8 Plots of Ca^{2+} contents in young (a) and old (b) leaves against site of recombination on 3H in recombinant DH lines. Red stars: SM recombinants; blue squares: MS recombinants; red lozenge: Steptoe; blue circle: Morex

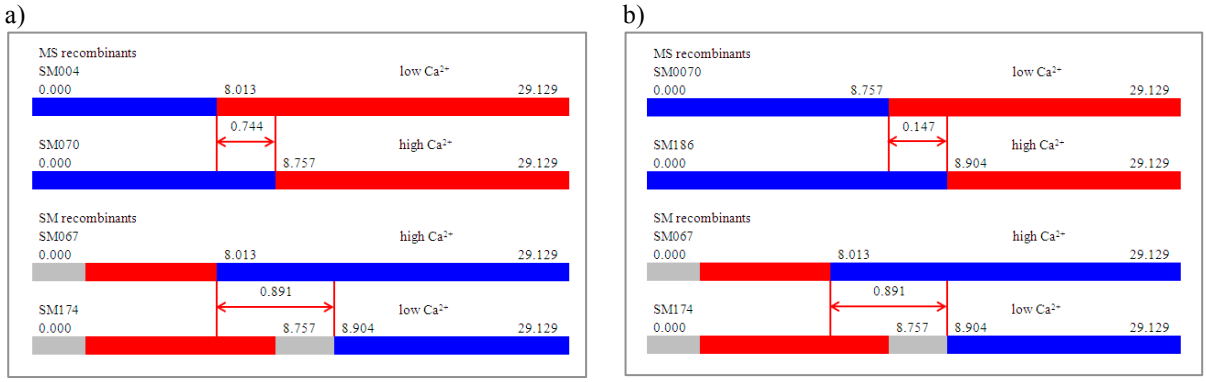


Figure 3.9 Detailed finemapping of QTLs for Ca^{2+} in the young (a) and old (b) leaves. The genetic map was made from short arm of 3H of two nearest SM and MS recombinants crossing over point. The bleu part of the gene map was donated by Morex haplotype, the red part by Steptoe haplotype and the gray by unknown haplotype. Positions between closed red arrows indicated finemap regions in cM

3.4.2 QTL for K+ content in stems

Detailed fine mapping for QTL for K^+ in stems was done as finemap for QTLs for Ca^{2+} in young and old leaves as described above. Figure 3.10 showed the plot of the location of the crossing over point of this QTL region for the content of K^+ in stems from SM- and MS-recombinants. The result indicated that QTL for K^+ in stems was finemapped to a region of

0.891 cM (Figure 3.11), exactly the same region for fine mapping QTLs for Ca^{2+} in the leaves (Figure 3.9). However, concentration of K^+ in the stems of recombinant DH lines were vice versa Ca^{2+} contents in the leaves. If a recombinant had high Ca^{2+} in the leaves, it had low K^+ in the stems and the other way around.

Like Ca^{2+} content in the leaves, concentration of some MS recombinants may controlled by other QTL regions, whereas the effect of this QTL on stems K^+ content were strongly detected. The finemapping based on the SM recombinants also showed a larger region than the used of MS recombinants (Figure 3.11). As a result, the chromosomal region of 0.891 cM was finemapped based on SM recombinants.

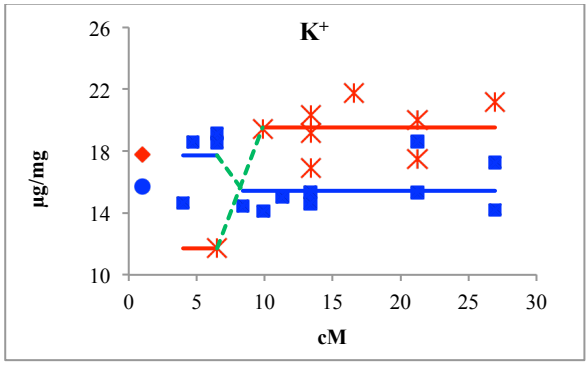


Figure 3.10 Plots of Ca^{2+} contents in young (a) and old (b) leaves against site of recombination on 3H in recombinant DH lines. Red stars: SM recombinants; blue squares: MS recombinants; red lozenge: Steptoe; blue circle: Morex

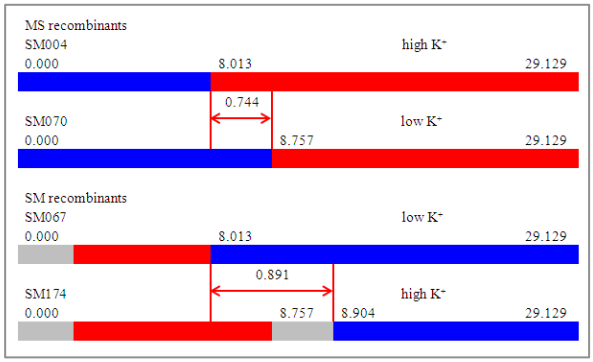


Figure 3.11Detailed finemapping of QTL for K^+ in stems. The genetic map was made from short arm of 3H of two nearest SM and MS recombinants crossing over point. The bleu part of the gene map was donated by Morex haplotype, the red part by Steptoe haplotype and the gray by unknown haplotype. Positions between closed red arrows indicated finemap regions in cM

4 Discussion

4.1 Growth and development of plant under salinity conditions

Plant growth and development are considerably reduced under salt stress. Our results indicate that the longer plants grow in salt tress the greater of salinity on plant height and number of tillers are (Figure 4.1). The reductions of plant height and number of tillers lead to the reduction of shoot dry weight. Figure 4.3 shows that the reduction of NT is greater than the reduction of PH, whereas dry weight of a tiller of salt stressed plant is greater than dry weight of a non-stressed plant (Figure 4.2). Therefore, the reduction of SDW mainly results in the reduction of NT.

Root dry weight is also reduced greatly although the reduction of RDW is less than the reduction of SDW. The reduction of RDW may be caused by the reduction of root length. Figure 4.3 shows that RL is decreased over 50% while the reduction of RDW is around 20%. This indicates that roots of plants in salt stress are more tolerant than other tissues. For example, the ratio of shoot and root length in salt stress is greater than in control treatment, but the ratio of shoot dry weight and root dry weight is vice versa (Figure 4.2). It means that root length is reduced more than plant height, but root dry weight is decreased less than shoot dry weight under salt stress.

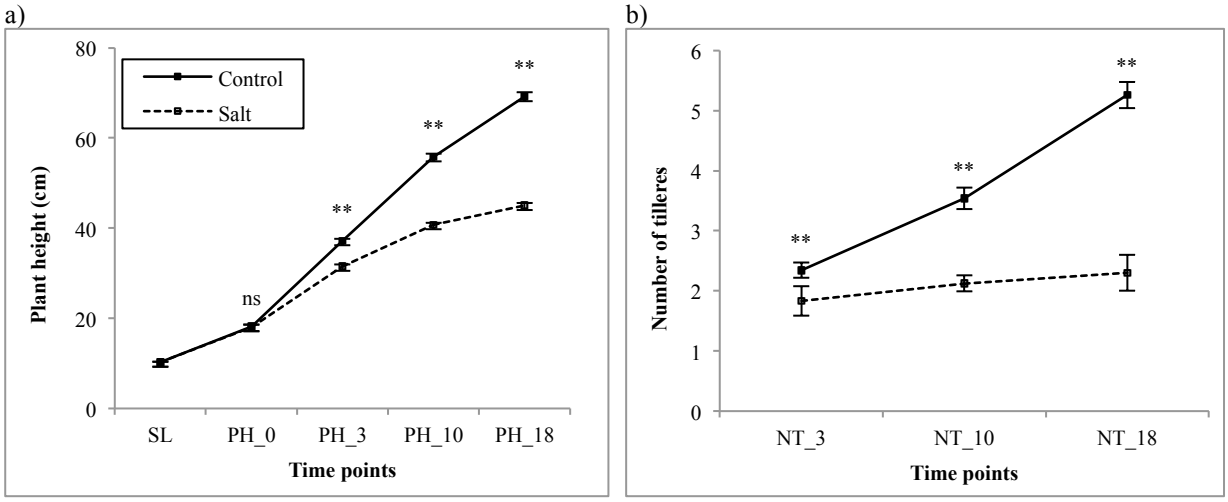


Figure 4.1 Effects of salinity on plant height (a) and number of tillers (b)

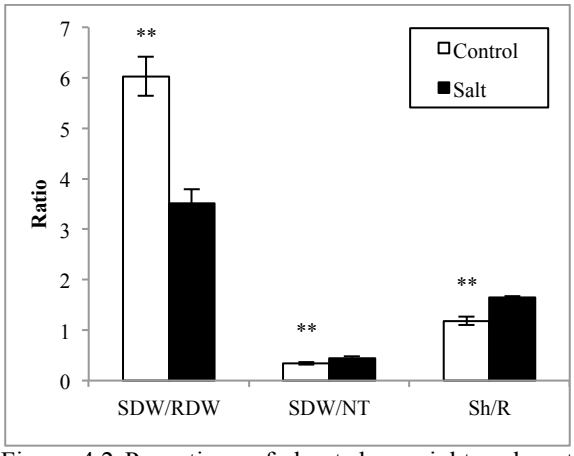


Figure 4.2 Propotions of shoot dry weight and root dry weight (SDW/RDW), shoot dry weight and number of tillers (SDW/NT), and plant height and root length (Sh/R)

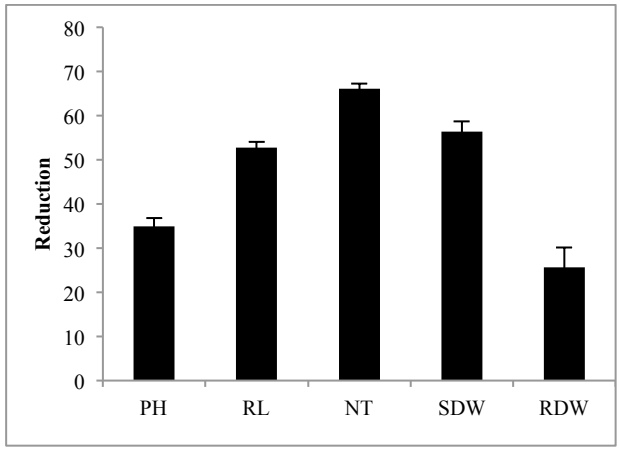


Figure 4.3 Reduction of plant height (PH), root length (RL), number of tillers (NT), shoot dry weight (SDW) and root dry weight (RDW)

4.2 Salt stresses and components of salinity tolerance

The two important stresses of salt stress on plant are (i) osmotic induced rapid stress and (ii) slow ionic stress. Osmotic stress starts immediately after the salt concentration around the root zone increased to the threshold level, the level of salt stress that plants can grow well at below that level and adverse affect at above that level. The threshold level differs from species. In barley, the threshold of salt stress is approximate 100 mM (Rao *et al.*, 2006; Munns and Tester, 2008). The effects of osmotic stress can be easily seen by the reduction of stomatal conductance (Figure 3.3 and Figure 3.4). The later effects of osmotic stress are the reduction of plant growth such as shorter PH and RL, and fewer NT that lead to the reduction of plant biomasses (Figure 4.3). Osmotic stress may affect on total leaf area (Munns and Tester, 2008). In fact, leaves in salt tress are smaller and shorter, especially the flag leaf (data not shown). Ionic stress, however, comes later when salt accumulated to toxic concentrations in the leaves.

Plants have three distinct types of salt tolerance: (i) tolerance to osmotic stress, (ii) Na^+ exclusion from leaf blades, and (iii) tissue tolerance (Munns and Tester, 2008; Rajendran *et al.*, 2009). Osmotic tolerance would result in greater leaf growth and stomatal conductance; the second component of salt tolerance is the ability of Na^+ exclusion by roots insures that Na^+ does not accumulate to toxic concentrations in the leaves; and finally, tissue tolerance is the capability of accumulating Na^+ and Cl^- in the older leaves (Munns and Tester, 2008).

Our results indicate that the osmotic tolerance and the tissue tolerance play important roles for salinity tolerance in barley. New leaves are continuously growing even though the effects of salt tress on NL are significant. The minimum and the maximum of number of leaves of both stressed and non-stressed plants are the same (Table 3.3). Moreover, an increase of stomatal conductance can be seen 10 days after applying 200 mM NaCl (Figure 4.3). It suggests that osmotic tolerance may cause these phenomena in the ways that plants may involve in synthesising compatible solutes such as proline, trehalose, mannitol, glycine betaine and osmoprotectant compounds like dehydrins.

Furthermore, this study shows that tissue tolerance may be important key factors in salinity tolerance. The accumulations of Na^+ in the young, old leaves, stems and roots are 7, 10, 6 and 5 times greater in the salt stress than in control treatment, respectively (Figure 4.4). Cl^- accumulation is also induced in salt stress, especially the accumulation of Cl^- in the roots. In addition, barley plants accumulate significantly more Na^+ and Cl^- in the old leaves than in the young leaves, the stems, and the roots (Figure 3.5b). The ability of storing toxic ions (Na^+ and Cl^-) in old leaves helps minimize the effects of salt stress on other important tissues such as the photosynthesizing younger leaves. The high toxic ions in old leaves cause high rate of leaf senescence of salt stressed plant. However, in some recombinant DH lines, the old leaves are green until harvest (18 DAS) (data not shown). These results confirm the importance of tissue tolerance in plant salt tolerance.

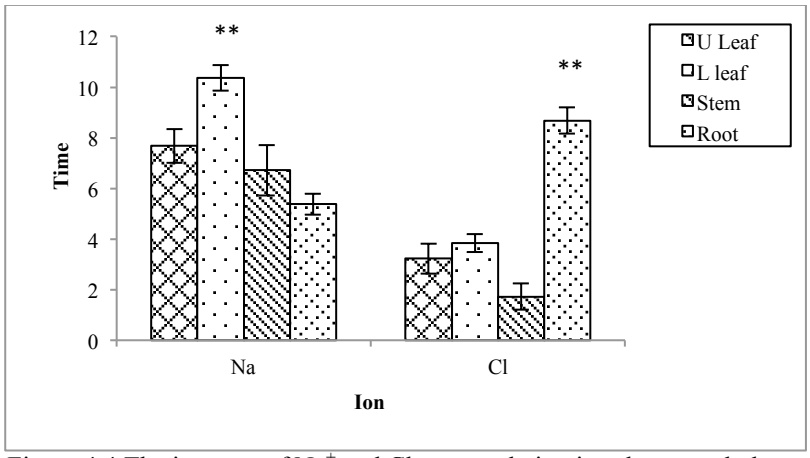


Figure 4.4 The increase of Na^+ and Cl^- accumulation in salt stressed plant

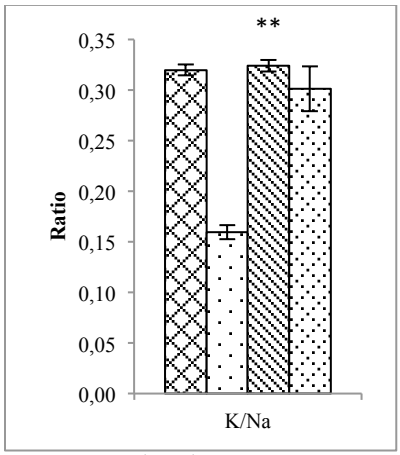


Figure 4.5 K^+/Na^+ ratio of salt stress plant

4.3 QTLs for salinity tolerance on chromosome 3H

4.3.1 Finemapping

The confirmation of QTL region on 3H indicates that this QTL region plays essential roles in ions up taking and distribution in plant tissues. In this experiment, the QTL region is smaller and all QTLs in control and salt treatment colocalize in the same region (Figure 3.7). Salinity QTLs for Ca^{2+} in young and old leaves and a QTL for K^+ in stem are finemapped to an interval of 0.891 cM (Figure 3.9 and Figure 3.11). This result of finemapping is based on the two SM recombinants that located nearest crossing over point although finemapping based on MS recombinants results smaller interval (Figure 3.8 – 3.11). That is due to the concentrations of Ca^{2+} in young, old leaves and K^+ stems of some MS recombinants might be controlled by other QTL regions such as QTL region on 2H. Moreover, some of twenty-two 3H recombinants that selected based on the QTL region of Nguyen *et al.* study are not the recombinants when they are considered in the new QTL region of this study. This result leads to the concentration of ion of some MS recombinant lines are not affected by 3H QTL region. In contract to MS recombinants, the QTLs on Ca^{2+} concentration in the leaves and K^+ in the stems, finemapping based on SM recombinants are more accurate than using MS recombinants although the 3H QTL region has strong effects on SM recombinants. SM recombinants are significantly separated into two groups even though the group on the left of crossing over point has only a recombinant (Figure 3.8 and 3.10). It is supposed that unique recombinant line might be an outline. However, ion concentrations were analyzed with 2 replications with 6 biological replications for one analyse. Therefore, I conclude that this is not an outline. Therefore, finemapping that based on SM recombinants might give an accurate result than that based on MS recombinants.

4.3.2 QTL for K^+ in stem

K^+, an important mineral for plant growth and development, is the important osmoticum in plants, provides a beneficial physicochemical environment for proteins and acts as a co-factor for a number of enzymes (Leigh and Wyn Jones, 1984). Recent studies have reported that maintenance of a high cytosolic K^+/Na^+ ratio appears to be critical to plant salt tolerance

(Shabala, 2000; Tester and Davenport, 2003). Wei et al. (2003) concluded that the maintenance of high K^+ to Na^+ ratio, especially in young growing and recently expanded tissues appeared to be an important characteristic of salt tolerance in barley cultivar. Our results indicate that barley accumulates Na^+ more in the old leaves than in the young leaves and the stems, whereas the accumulation of K^+ is greater in the young leaves and the stems than in the old leaves (Figure 3.5b) although the K^+ content of salt stressed plant is reduced significantly (Figure 3.6). As a result, the ratios of K^+/Na^+ of the young leaves and the stems are significant higher than that of the old leaves (Figure 4.5). The maintenance of high K^+/Na^+ in the young leaves and the stems may be the key to salt-tolerant plants. It may help the plants minimize the effects of toxic ions on photosynthesis in young leaves.

K^+ transport across plant membranes is mediated by at least seven families of cation transporters (Shabala and Cuin, 2008). In general, two major groups are distinguished: (i) K^+ permeable channels and (ii) K^+ transporters. Under saline conditions, the expression of K^+ transporters may play important roles in transporting K^+ into the plants. Two families of K^+ transporters have been identified: (a) KUP/HAK transporters and (b) HKT transporters

KUP/HAK transporters

KUP/HAK transporters are involved in both high and low affinity of K^+ accumulation in plants (Santa-Maria *et al.*, 1997). KUP/HAK transporters are a large family with 13 members in Arabidopsis (Maser *et al.*, 2001) and 17 members in rice (Banuelos *et al.*, 2002). In barley, five members of HAK are identified (Santa-Maria *et al.*, 1997). KUP/HAK transporters might mediate low-affinity Na^+ influx at high external Na^+ concentration (Shabala and Cuin, 2008) while mediating high-affinity K^+ uptake (Santa-Maria *et al.*, 1997; Vallejo *et al.*, 2005). However, KUP/HAK transporters would be blocked by Na^+ when Na^+ is present at high concentration (Santa-Maria *et al.*, 1997; Kim *et al.*, 1998).

Studying the expression of KUP/HAK in barley is important to understand the roles of this gene family in K^+ transport in salinity conditions. Under salt stress, K^+ and Na^+ concentrations in the roots and the stems were significantly correlated (Table 3.18). K^+ and Na^+ in the leaves were also correlated positively although the correlations were not significant. Therefore, the

study genes expression of other family genes which have been more important for transporting both K^+ and Na^+ in salt stress such as HKT family.

HKT transporters

The main function of *HKT* is K^+-, Na^+- symport under high-affinity conditions (Schachtman and Schroeder, 1994; Rubio *et al.*, 1995). Several studies have suggested that expressing *HKT1* transporter may also mediate low-affinity Na^+ transport into root tissue (Laurie *et al.*, 2002), especially under high Na^+ concentration condition (Kader *et al.*, 2006; (Rubio *et al.*, 1995). In barley, *HvHKT1* and *HvHKT2* control Na^+ and K^+ transport in root tissues, respectively (Qiu *et al.*, 2011). The study on Tibetan wild barley species indicated that root *HvHKT1* is up-regulated while the root *HvHKT2* is down-regulated under 300 mM NaCl (Qiu *et al.*, 2011). Qiu *et* al. (2011) also concluded that Na^+ concentration of stressed plants increases considerably while the content of K^+ decreases considerably. Our study revealed that the increase of Na^+ concentration and the decrease of K^+ of salt stressed plants were found for all plant tissues (Figure 3.6). In addition, concentrations of K^+ and Na^+ of salt stressed plants were positively correlated (Table 3.18).

Under saline conditions, barley plants are able to accumulate higher Na^+ in the old leaves than in the young leaves and the stems while K^+ concentrations in the young leaves and the stems are higher than in the old leaf (Figure 3.5b). These differences might result from the variation of expression of *HvHKT* family genes in different plant tissues. For instance, the expression of *HvHKT1* may be more in old leaves than in the young leaves and the stems, whereas the expression of *HvHKT2* could be more in the young leaves and the stems than in the old leaves. Therefore, study gene expression on *HvHKT* family genes is essential for understanding K^+/Na^+ homeostasis in barley under salt stress.

Three *HvHKT* genes have been identified: *HvHKT1/HvHKT2;1*, *HvHKT2/HvHKT1;1* and *HvHKT1;5* (Haro *et al.*, 2005; Sato *et al.*, 2009; Platten et al., 2007 unpublished). The functions of *HvHKT1;1* and *HvHKT2;1* are known to control root K^+ and Na^+ transport in Tibetan wild barley under salinity stress, respectively (Qiu *et al.*, 2011). Under 300mM NaCl root *HvHKT2;1* is up-regulated while root *HvHKT1;1* is down-regulated (Qiu *et al.*, 2011).

Their regulations in cultivated varieties and in different plant tissues such as old leaves, young leaves and stems, however, are unknown. Therefore, the study of gene expression of these three *HvHKT* genes is important for further identification gene(s) for salinity tolerance in barley.

Because of the function of *HKT* genes, they might be the good potential candidate genes for underlying genes for K^+ QTL. K^+, Na^+ symporter (*HKT*) is accidentally correspond to our study that K^+ and Na^+ were correlated significantly in stem and in the root tissues under salt stress (Table 3.22). Therefore, the identification *HKT* as a candidate for K^+ QTL is more reliable than other transporter genes.

4.3.3 QTLs for calcium in young leaf and older leaf tissues

Ca^{2+}, an essential second messenger in plant signaling networks, plays structural roles in the cell wall and membrane and regulates plant growth and development (Hepler, 2005; White, 2000). Plants growing with an adequate Ca^{2+} in natural habitats have shoot Ca^{2+} concentration between 0.1 and 5% of shoot dry weight (Marscher, 1995). These values depend on the availability of Ca^{2+} in the environment and the Ca^{2+} requirements of different plant species. Our results reveal that although the uptake of Ca^{2+} under salt stress is reduced significantly (Figure 3.6), the concentration of Ca^{2+} is higher than 0.2% of dry weight in the young leaves and the stems, 0.5% in the older leaves and the roots (Figure 3.5b). These amounts of Ca^{2+} in barley plants may be adequate for plant growth under salt stress due to no symptom of Ca^{2+} deficiency is observed in the young leaves of stressed plant such as “tipburn” of the young leaves.

Ca^{2+} can enter the cell via a number of Ca^{2+}-permeable channels and efflux from cytosol is achieved through Ca^{2+}-ATPases and H^+/Ca^{2+} exchangers (*CAXs*) (White and Broadley, 2003). The Ca^{2+}-permeable channels that are found in many plant membranes can be activated by hyper-polarisation, depolarisation or ligand binding (White, 2000; White and Broadley, 2003). Ca^{2+}-ATPases with high affinity but low capacity for Ca^{2+} transport (Evans and Williams, 1998), are responsible for maintaining cytosolic Ca^{2+} homeostasis in the resting cell, whereas

CAXs, lower affinity but higher capacities for Ca^{2+} transport, are likely to remove Ca^{2+} from cytosol during Ca^{2+} signalling (Hirschi, 2001).

A number of studies suggested that *CAXs* contribute to cytosolic Ca^{2+} homeostasis (Allen *et al.*, 2000). Eleven genes encoding putative *CAXs* have been identified in the Arabidopsis genome (Hirschi, 2001). Over-expression of *AtCAX1* showed higher content of Ca^{2+} in shoot than control plants in several species such as tobacco, carrots, potatoes and tomatoes (Hirschi, 1999; Park *et al.*, 2005a; Park *et al.*, 2005b; Park *et al.*, 2004). In barley, two *HvCAX* genes have been identified based on sequences of EF446604.1 and AB218888.1 (*Hordeum vulgare*, complete cds) (Katsuhara and Yoshihisa, 2005; Zhang and Kleinhofs, 2007 unpublished), encoding proteins of 411 and 462 amino acids. Both *HvCAX* genes are predicted as transmembrane proteins that have 10 to 12 transmembrane domains. However, their function in salinity tolerance remains to be established.

Under salinity conditions, increasing cytosolic Ca^{2+} concentration has at least two effects in salt tolerance, (i) a decrease of the inhibitory effects on growth and (ii) a direct inhibitory effect on Na^+ entry into plant cells (Bressan *et al.*, 1998). It is suggested that the inhibition of Na^+ entry into a plant cell might play an important role in plant salt tolerance. The increase of cytosolic Ca^{2+} may contribute to stimulation of the SOS (salt overly sensitive) pathway. In this pathway, a calcium sensor, *SOS3*, senses cytosolic calcium changes elicited by salt stress. *SOS3* activates and interacts with a protein kinase, *SOS2*. This SOS3/SOS2 complex activates the Na^+/H^+ antiporter encoded by SOS1 gene (Mahajan *et al.*, 2008). As a result, the activated *SOS1* prevents the entry of Na^+ in salt tress such as de-activating *HKT* genes. Moreover, *SOS2* may interact with CBL10 (calcineurin B-like 10), which has been shown to have similar functions as SOS3 (Guo *et al.*, 2007). The complex of CBL10/SOS2 regulates both extrusion of Na^+ ions from the cytosol, and sequestration of Na^+ into the vacuole by activating *NHX* transporters, which pump Na^+ into the vacuole (Mahajan *et al.*, 2008). Salt tolerance may result in the storage of high Na^+ concentrations into the vacuole while the plants continuously take up Na^+.

Our results might indicate that the roles of Ca^{2+} are more important for the expression of *NHX* transporters than the SOS pathway. It is known that plants induce the *SOS* pathway to efflux

Na^+ out cytosal and prevent Na^+ influx into the cells while expression of *NHX* genes allowing high Na^+ content in the cells by storing it in the vacuole as well as the less affected tissues such as the old leaves. As shown in Figure 3.6, the concentration of Na^+ increases significantly in all plant tissues. Moreover, content of Na^+ in the older leaves is dramatically higher than in the young leaves and the stems (Figure 3.5b). The different expression level of *NHX* may result in these differences.

Furthermore, Ca^{2+} content differs significantly between plant tissues (Figure 3.5). Ca^{2+} concentration in the old leaves is significant larger than Ca^{2+} in the young leaves and the stems. This higher Ca^{2+} content in the old leaves may cause activation of the vacuolar Na^+ transporters (*NHX* transporters), facilitating storage of Na^+ in the old leaves. Genes involving in Ca^{2+} transport into the plant tissues may be expressed in different levels for different plant tissues. I have mentioned several Ca^{2+} transporter genes. Among them, two *HvCAX* genes may be responsible for the differences of Ca^{2+} transport in plants under saline condition. The high concentration of Ca^{2+} in old leaves could be related to the high expression of *HvCAX(s)* in this tissue. Gene expression of *HvCAXs* in different plant tissues may answer this question.

5 Conclusions

5.1 Genetic variation analysis

Plant agronomic traits were highly significant between recombinant DH lines and their parents. The differences were mostly caused of genotypic variation (highly heritable). A similar result was found for chlorophyll content in the third leaf and the youngest expanded leaf. For stomatal conductance, the differences appeared to be controlled by environmental conditions rather than genotypic variation, but the differences of reduction of stomatal conductance were highly heritable.

Dry weight of shoot tissue was more affected by salt stress than dry weight of root tissue, whereas salt stress affected root length more than plant height. In addition, the reduction of number of tillers may be considerable. Therefore, the reduction of number of tillers may be mostly responsible for the reduction of SDW.

Ion concentration between plant tissues differed considerably. Under salt stress Na^+ and Cl^- concentrations in plants were increased dramatically, whereas content of K^+, Ca^{2+} and Mg^{2+} was reduced significantly. Moreover, Na^+ and Cl^- concentrations in low leaves were significantly higher than in the young leaves and the stems while K^+ in the young leaves and the stems was higher than in the older leaves.

5.2 Correlation analysis

Chlorophyll content had correlations with plant height and plant biomass, but it was not correlated with number of tillers.

The study of correlations of ion distribution in plant tissues helps understand the roles of the ions in salinity tolerance. In salt treatment, Na^+ and Cl^- had positive correlations for all plant tissues. Although in salt tress content of Na^+ in plant tissues was increased, whereas K^+ was decreased, the positive correlations of K^+ and Na^+ were examined for all plant tissues.

5.3 Mapping recombinant DH lines

This study confirmed most QTLs in control and salt treatments from Nguyen *et al.* study. The QTL region on 3H was smaller and all QTLs colocalized in the same region of an interval of 5.049 to 11.652 cM.

5.4 Finemapping QTLs for salinity

Three QTLs for salinity were finemapped to an interval of 0.891 cM. They were colocalised in the same region, but these QTLs may be controlled by different genes.

From literatures and our results, *HKT* family gene may play important roles in K^+ QTL and *CAX* family genes may be potential candidate genes for Ca^{2+} QTL under salt stress. Therefore, these two family genes were identified as potential candidate genes for ion content of salt QTLs on 3H QTL region.

Furthermore, *NHX* genes may play important roles for storing high Na^+ concentration in the plants by transport Na^+ into the vacuole that helps the plants tolerate to salt with high levels. It would be a good candidate for salinity tolerance in barley. Therefore, study gene expression of this family gene should give a better understand of the mechanisms that plants deal with salt stress.

6 Future Work

Due to the results and conclusions of this study, we suggest continuing study on Steptoe x Morex DH population as following.

- Gene expression analysis for each of the candidate genes using plant tissues from the current experiment to show association between 'QTL alleles' and gene expression
- Map candidate genes for salinity tolerance
- Over-express or knock-out the candidate genes for salinity tolerance traits

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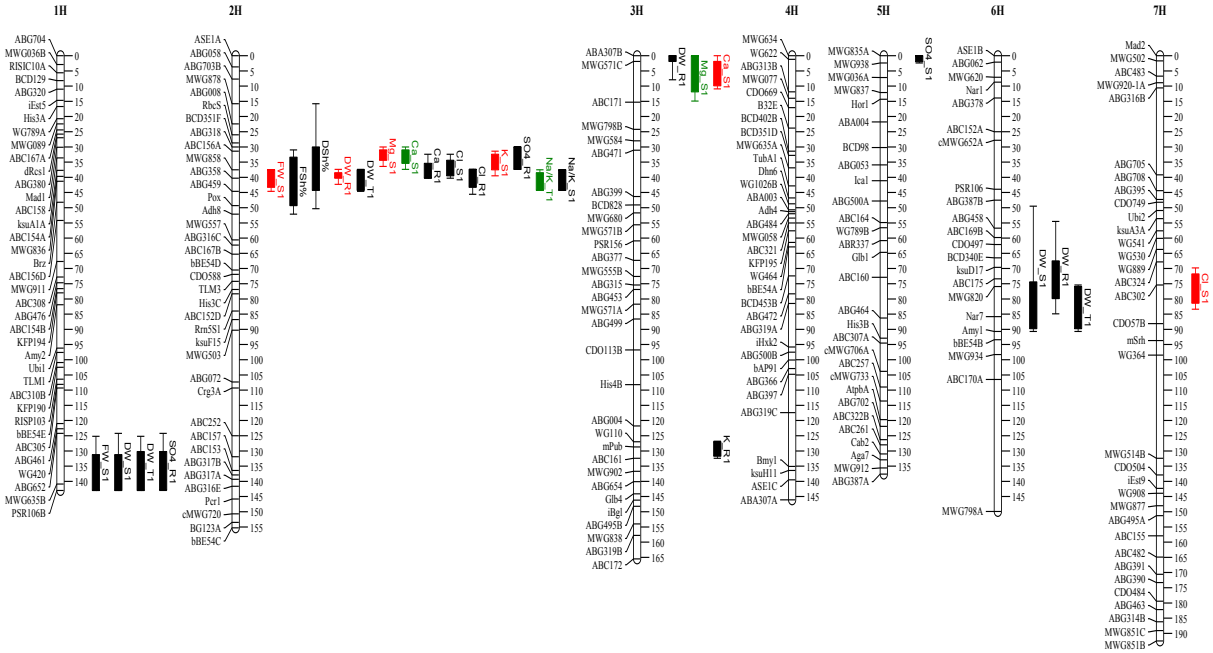
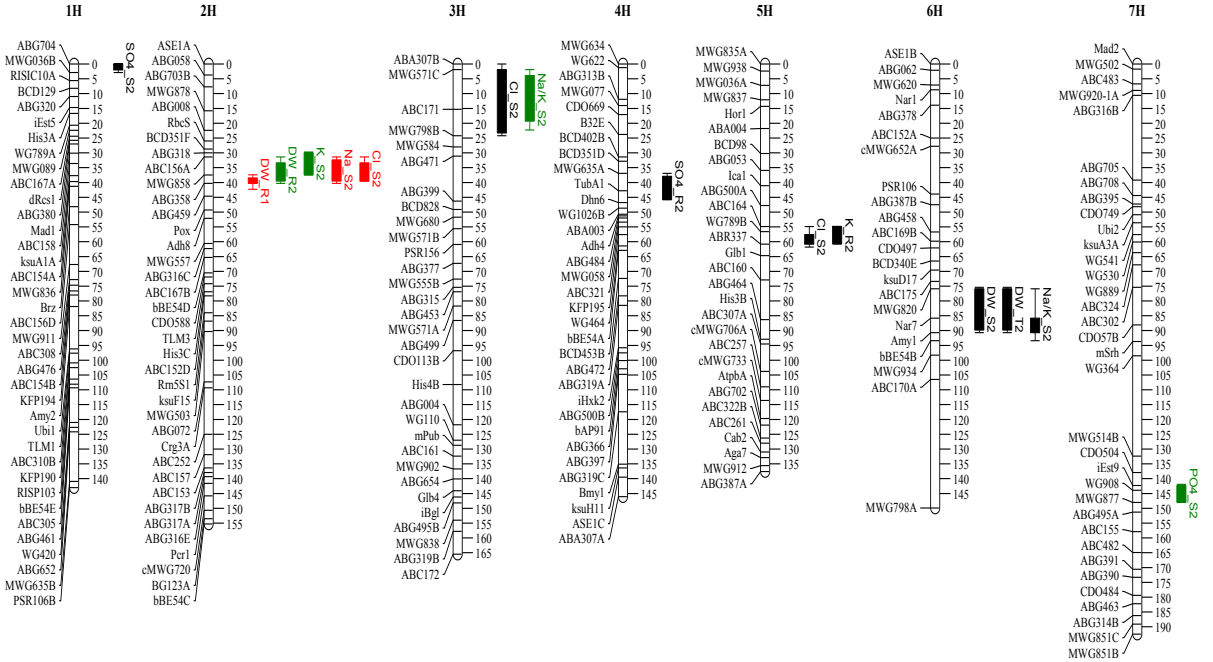
Appendix 1

a



b



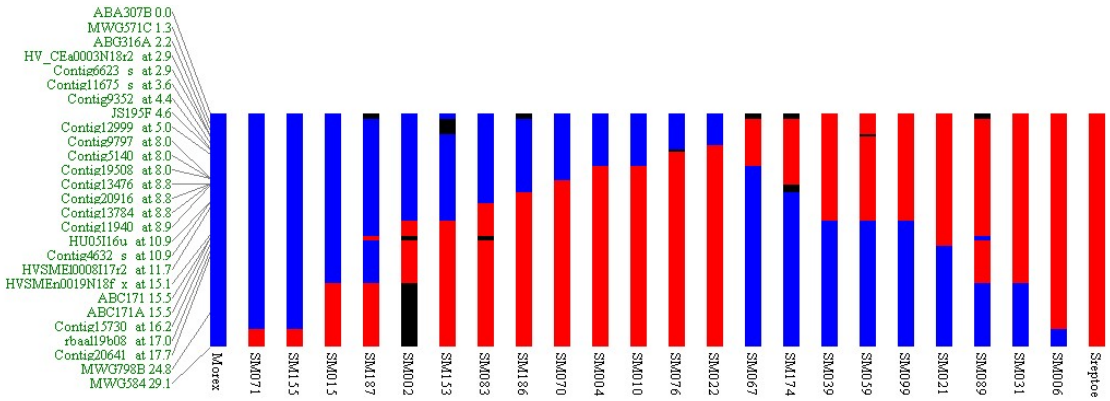
Appendix 1.1. Putative QTLs of Steptoe/Morex DH population growing on control treatment (a) and in salt stress (b)

Appendix 2

Appendix 2.1 Plant materials of the experiments and their recombinant types in 3H QTL region

Type of recomninant	No.	Genotype	Haplotype in left border	Haplotype in right border	Recombinant position
MS	1	SM002	M	S	13.40
	2	SM004	M	S	6.50
	3	SM010	M	S	6.50
	4	SM015	M	S	21.25
	5	SM022	M	S	4.00
	6	SM070	M	S	8.40
	7	SM071	M	S	26.95
	8	SM076	M	S	4.70
	9	SM083	M	S	11.30
	10	SM153	M	S	13.40
	11	SM155	M	S	26.95
	12	SM186	M	S	9.90
	13	SM187	M	S	21.25
SM	14	SM006	S	M	26.95
	15	SM021	S	M	16.60
	16	SM031	S	M	21.25
	17	SM039	S	M	13.40
	18	SM059	S	M	13.40
	19	SM067	S	M	6.50
	20	SM089	S	M	21.25
	21	SM099	S	M	13.40
	22	SM174	S	M	9.85
Parents	23	Steptoe	S	S	-
	24	Morex	M	M	-

M: Morex haplotype, S: Steptoe haplotype



Appendix 2.2 Genetic map the QTL region on 3H of recombinant DH lines, Steptoe and Morex (blue: M haplotype, red: S haplotype, black: unknown haplotype)

Container No.	Control								Salt							
1	24	2	1	8	20	14	5	7	23	21	12	13	9	3	6	4
	9	19	18	12	23	17	6	21	5	15	19	7	16	14	22	1
	4	22	10	16	11	3	15	13	10	20	11	17	2	18	24	8
2	10	22	1	15	18	2	3	23	5	21	15	11	13	2	14	9
	21	11	20	13	7	24	14	17	10	1	20	4	7	19	8	17
	5	19	16	4	8	12	6	9	18	16	6	24	23	22	3	12
3	12	16	2	24	8	21	23	4	8	17	7	12	5	9	21	16
	1	22	6	13	18	19	7	15	2	22	4	23	13	10	6	11
	10	3	11	9	5	17	14	20	20	14	3	1	24	15	18	19
4	8	20	17	24	6	14	10	22	9	10	20	5	8	11	19	15
	15	16	5	21	4	9	2	19	22	14	12	2	23	16	17	24
	13	1	23	12	11	7	3	18	21	18	13	6	7	4	1	3
5	4	3	7	10	23	8	12	6	20	6	9	1	14	2	8	19
	5	11	14	9	17	19	13	20	24	13	5	17	11	16	18	12
	24	2	16	1	18	15	22	21	7	21	3	4	10	15	23	22
6	13	21	2	10	19	8	6	24	4	23	3	13	18	8	11	9
	1	3	12	7	14	9	18	16	5	1	24	22	17	20	6	2
	11	22	20	15	4	17	23	5	12	10	16	19	15	14	7	21
7	8	12	19	14	13	10	5	20	6	18	13	9	1	8	4	16
	9	17	21	4	22	3	6	24	5	3	22	20	21	23	12	24
	18	2	7	23	15	11	16	1	19	7	17	2	15	14	10	11
8	11	5	19	12	23	9	4	2	12	14	13	1	2	23	22	8
	15	17	10	14	1	21	7	18	4	11	15	7	20	19	10	6
	13	22	6	3	8	24	20	16	3	17	18	16	5	21	9	24
9	3	13	10	18	20	6	21	14	13	11	23	17	8	6	21	15
	23	12	7	1	11	9	8	19	1	10	16	9	12	18	14	24
	2	17	4	24	5	15	16	22	7	2	4	3	5	22	19	20
10	12	5	1	22	4	15	11	14	9	19	4	11	10	20	12	7
	23	2	3	21	18	9	7	17	13	21	6	3	15	18	24	16
	16	19	13	6	8	24	10	20	2	8	23	17	22	5	1	14
11	23	19	16	11	4	1	3	24	19	8	3	6	23	12	15	7
	20	7	17	15	6	5	12	14	16	1	4	18	20	21	14	9
	8	22	10	9	18	13	2	21	13	11	22	5	17	24	2	10
12	2	14	11	22	4	16	6	8	8	9	14	5	23	2	4	11
	23	3	10	21	17	15	18	19	7	19	24	6	15	18	3	1
	1	24	13	9	7	5	20	12	20	21	13	12	22	16	10	17

Appendix 2.3 Randomized completed block design (RCBD) with twelve main plots of two sub-plots. Twenty-four positions in a sub-plot were randomly assigned to 24 corresponded genotypes in Appendix 2.1 with help of GenStat v.12

Appendix 3.

Appendix 3.1 Correlation of ion content in young leaf tissue and other traits

	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-		PO_4^{2-}		SO_4^{2-}		K^+/Na^+	
Trait	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
NT_18	+ ns	- ns	- ns	- ns	-0.41*	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns
PH_18	+ ns	0.4*	+ ns	+ ns	+ ns	- ns	0.4*	+ ns	+ ns	+ ns	+ ns	0.42*	+ ns	+ ns	+ ns	- ns
RL_18	- ns	+ ns	- ns	+ ns	+ ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	+ ns
SFW	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns
SDW	+ ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns
%SDW	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	0.44*	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns
RDW	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns
TDW	+ ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns
SPAD_2	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns
SPAD_9_1	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	- ns	- ns	- ns	- ns	+ ns	- ns	+ ns	+ ns
SPAD_9_2	+ ns	- ns	+ ns	- ns	+ ns	- ns	0.53**	+ ns	+ ns	- ns	+ ns	- ns	0.53**	- ns	+ ns	+ ns
SPAD_16	+ ns	- ns	0.41*	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns
Gs_3	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	- ns
Gs_10	+ ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	- ns
Gs_17	- ns	+ ns	+ ns	+ ns	0.55**	- ns	+ ns	+ ns	0.43*	+ ns	0.52**	+ ns	0.44*	+ ns	+ ns	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

Appendix 3.2 Correlation of ion content in older leaf tissue and other traits

	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-		PO_4^{2-}		SO_4^{2-}		K^+/Na^+	
Trait	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
NT_18	- ns	- ns	+ ns	- ns	-0.41*	- ns	-0.52*	+ ns	- ns	- ns	- ns	- ns	-0.45*	- ns	+ ns	+ ns
PH_18	+ ns	+ ns	- ns	+ ns	- ns	- ns	+ ns	- ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns
RL_18	- ns	- ns	- ns	0.41*	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns	+ ns	- ns	- ns	+ ns	+ ns
SFW	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns
SDW	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns
%SDW	+ ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	- ns	+ ns	- ns	- ns	+ ns	+ ns	- ns	- ns
RDW	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	+ ns	+ ns
TDW	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns
SPAD_2	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	+ ns
SPAD_9_1	+ ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns
SPAD_9_2	+ ns	- ns	- ns	+ ns	- ns	+ ns	+ ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns
SPAD_16	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns
Gs_3	- ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	- ns	+ ns
Gs_10	+ ns	+ ns	0.42*	+ ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	0.46*	+ ns	+ ns	+ ns	+ ns	- ns
Gs_17	- ns	- ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	- ns	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

Appendix 3.3 Correlation of minerals content in stem tissue and other traits

	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-		PO_4^{2-}		SO_4^{2-}		K^+/Na^+	
Trait	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
NT_18	+ ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	- ns	+ ns
PH_18	- ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	- ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns
RL_18	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	0.43*	+ ns	- ns	- ns
SFW	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	+ ns
SDW	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	+ ns
%SDW	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns
RDW	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	- ns
TDW	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	+ ns
SPAD_2	- ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	- ns	- ns	+ ns	- ns
SPAD_9_1	+ ns	- ns	- ns	- ns	- ns	+ ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	- ns	- ns	+ ns	+ ns
SPAD_9_2	- ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns
SPAD_16	+ ns	- ns	+ ns	- ns	+ ns	+ ns	0.44*	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns
Gs_3	+ ns	0.41*	- ns	+ ns	- ns	- ns	+ ns	+ ns	+ ns	+ ns	- ns	0.53**	- ns	+ ns	- ns	- ns
Gs_10	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns
Gs_17	- ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	0.47*	+ ns	+ ns	- ns	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively

Appendix 3.4 Correlation of minerals content in root tissue and other traits

	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-		PO_4^{2-}		SO_4^{2-}		K^+/Na^+	
Trait	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
NT_18	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns	+ ns	- ns	+ ns
PH_18	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns
RL_18	0.43*	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	+ ns
SFW	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns
SDW	+ ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	- ns
%SDW	- ns	- ns	+ ns	- ns	- ns	- ns	+ ns	- ns	+ ns	-0.51*	+ ns	- ns	- ns	- ns	+ ns	+ ns
RDW	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns	- ns	+ ns
TDW	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns	- ns
SPAD_2	+ ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	- ns	- ns	+ ns	+ ns	+ ns	- ns	- ns	+ ns
SPAD_9_1	+ ns	- ns	- ns	+ ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	+ ns
SPAD_9_2	- ns	- ns	+ ns	- ns	- ns	- ns	0.49*	- ns	+ ns	- ns	+ ns	+ ns	+ ns	+ ns	+ ns	- ns
SPAD_16	- ns	- ns	- ns	- ns	+ ns	- ns	0.45*	- ns	- ns	- ns	- ns	- ns	- ns	- ns	+ ns	- ns
Gs_3	- ns	+ ns	+ ns	+ ns	- ns	- ns	- ns	- ns	+ ns	- ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns
Gs_10	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	- ns	- ns	- ns	+ ns	+ ns	- ns	+ ns	+ ns	+ ns
Gs_17	- ns	- ns	+ ns	+ ns	- ns	- ns	- ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	- ns	+ ns	+ ns

*: $p<.05$, ** $p<.01$, ns: not significant, +: positively and -: negatively