

**Genetic Analysis of Salt Tolerance in Asian  
Germplasm of Barley**

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**Genetic Analysis of Salt Tolerance in Asian  
Germplasm of Barley**

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# ABBREVIATIONS

AM: Association mapping  
Chr: Chromosome  
CSSLs: Chromosome segment substitution lines  
DH: Doubled haploid  
LD: Linkage disequilibrium  
LIS: Leaf injury score  
NL: Number of leaves  
NIL: Near-isogenic lines  
MLM: Mixed linear model  
QTL: Quantitative trait locus  
OPA: Illumina oligonucleotide pool assays  
OWB: Oregon Wolfe Barley  
PDW: Whole plant dry weight  
RDW: Root dry weight  
RIL: Recombinant inbred lines  
RL: Root length  
RFW: Root fresh weight  
RDW: Root dry weight  
SFW: Shoot fresh weight  
SDW: Shoot dry weight  
SL: Shoot length  
SNP: Single nucleotide polymorphism  
2R/6R: Two row/ six row type



# CHAPTER 1:

## INTRODUCTION AND THESIS OUTLINE

### 1.1. Barley (*Hordeum vulgare* L.)

#### 1.1.1. Taxonomy and origin

Barley is a monocot cereal crop belonging to the division *Magnoliophyta*, class Liliopsida, order Cyperales, family Poaceae, subfamily of the Pooideae, the tribe Triticeae and genus *Hordeum* (Gallais and Bennerot 1992). *Hordeum* group form a botanical complex that includes 32 species and 45 taxa comprised of diploid, tetraploid and hexaploid. Triploid forms of barley ( $2n = 3x = 21$ ) spontaneously induced, however, are rarely encountered in nature (Singh 2003).

The majorities of these species are perennials and have different systems of reproduction. However, the cultivated barley (*H. vulgare* ssp. *vulgare* L.) and its ancestor (*H. vulgare* ssp. *spontaneum* C. Koch.) are annual and diploid species (Bothmer *et al.* 1995, Nevo and Chen 2010, Pourkheirandish and Komatsuda 2007). Cultivated barley is one of 32 species of the genus *Hordeum*, diploid ( $2n=14$  chromosomes) (Bothmer *et al.* 1995) and largely self-pollinating (Wagner and Allard 1991). The barley genome has haploid genome size of around 5.3 Gbp (Bennett and Leitch 1995).

Barley was first domesticated from its wild progenitor *Hordeum vulgare* ssp. *spontaneum* in the Fertile Crescent region of the Near East about 10,000 years ago. Barley

cultivation reached to Spain ca. 7,000 years BP (Before Present), North-Africa and Ethiopia ca. 8,000 years BP and Northern Europe ca. 6,000 years BP (Nevo 1992). This wide distribution exposed barley to new climatic and edaphic conditions. Due to the initial large genetic variation in barley together with accumulation of new mutations and recombination's, the crop became locally and widely adapted. This was a gradual process over millennia and it is the basis for the creation of a multitude of locally adapted, genetically variable landraces (Bothmer *et al.* 2003).

Barley is an herbaceous plant reaching 60 to 120 cm height and its root system consists of seminal and adventitious roots. The stems are straight having nodes to which one of the leaves attaches (Gomez-Macpherson 2001). In maturity stage, plant comprises a main axis and 2-5 tillers. The leaves are linear, 5 to 15 mm widths and are alternately generated on the sides of tillers. Domesticated barley is classified as either six-rows (6R) or two-rows (2R), depending on the physical arrangement of the spikes on the plant (Goyal and Ahmed 2012).

### **1.1.2. Importance of barley in the world**

Barley is one of the most highly adapted cereals with production climates ranging from sub-arctic to subtropical. Because of its use as feed, food and malt production, barley is grown in many areas of the world for cultural as well as economic reasons (Goyal and Ahmed 2012, Zhang and Li 2009). Europe is the most predominant continent growing barley followed by Asia (Upreti 2005).

Barley is grown on approximately 56 million hectares in the world in average from 2006 to 2008 (FAO 2010). The FAO records the production in 106 countries worldwide, with an average production per year of 143.4 million tons for the same period (Newton *et al.* 2011).

Among cereals, barley ranks fourth in terms of grain production in the world after maize, rice and wheat (Fig.1.1). In 2007, world barley production reached 139 million tons, 3 million tons more than the previous year's results (FAO 2009). Three regions produce more than a half of the world's barley: the European Union (EU) (43%, mainly Spain, Germany and France with about an 8%-share each), the Russian Federation (11%) and Canada (9%) (Fig.1.2). On the other hand, Saudi Arabia is the largest importer of barley in the world with 6 million tons (42% of world importation) in 2007-2008. The importation by Japan, China, Morocco and Tunisia is about 9, 8, 5 and 3%, respectively.

In Asia, barley is mostly cultivated in China, India, Turkey and Middle East. The major states in India cultivating barley are Rajasthan, Haryana and Punjab. 90% of barley grains in India are used as human food, malt (Sun and Gong 2010), beer, whisky industrial alcohol and vinegar (Tiwari 2010).

## **1.2. Salt stress and its mechanisms**

Salt is one of the principal abiotic factors affecting crop yields in arid and semi-arid irrigated areas. Salt impairs crop growth and threatens global food security. It negatively influences the survival and reduces the yield of food crops worldwide up to 70% (Nitin *et al.* 2012).

### **1.2.1. Ionic and osmotic stresses**

Salt inhibits plant growth via two stresses (Fig. 1.4). A rapid, osmotic stress that reduces the plant ability to take up water and inhibit the growth of young leaves (Vysotskaya *et al.* 2010). Shoot and extent root growth is permanently reduced within hours of salt stress and this effect does not appear to depend on sodium concentration but rather is a response to the osmolarity of the external solution (Munns *et al.* 2002). A slower, ionic stress or salt specific stress that may enter the transpiration stream and eventually injury cells in the transpiring leaves (Bohnert and Bressan 2001, Guimaraes 2009, Munns and Tester 2008, Munns 2005). The ionic stress ( $\text{Na}^+$  and/or  $\text{Cl}^-$  specific effects) is superimposed on the osmotic effects and showed greater genetic variation than osmotic effects (Munns *et al.* 2002). Metabolic toxicity of  $\text{Na}^+$  is largely a result of its ability to compete with  $\text{K}^+$  for binding sites essential for cellular function. High  $\text{Na}^+/\text{K}^+$  ratios can disrupt various enzymatic processes in the cytoplasm (Tester and Davenport 2003). Ionic stress is associated with a reduction of chlorophyll content and inhibits photosynthesis, inducing leaf senescence and premature leaf death. Necrosis of older leaves, starting at the tip and margins and working back through the leaf is the results of salt accumulation (Bohnert and Jensen 1996). Growth reductions occur as a result of the shortening of the lifetime of individual leaves (Munns 2002, Tester and Davenport 2003). It starts when salt, especially  $\text{Na}^+$ , accumulates to toxic concentration in the old leaves. The chloride-triggered injury is identifiable by the extensive leaf blade scorching symptoms whereas the accumulation of sodic salts results in leaf mottling and leaf necrosis (Dajic 2006).

In parallel with the osmotic and ionic stress, secondary oxidative stress results following the toxicity induced by the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions at the cell and the formation of reactive oxygen species (ROS) (Chinnusamy and Zhu 2004, Gupta and Huang 2014).

These radicals are produced due to the altered metabolism of the chloroplast and mitochondria during salt stress (Meloni *et al.* 2003). They cause oxidation of various cellular components including membrane lipids, proteins, nucleic acids (Apse and Bumwald 2002); inhibit ion of photosynthesis (Price and Hendry 1991), causing lipid peroxidation, protein denaturation and mutations at the DNA level. In addition, in the presence of salt stress, ROS induce short and long term cell death (Mittler 2002).

### **1.2.2. Mechanisms of salt tolerance at cellular level**

Adaptive strategies of plants exposed to salt are based upon the utilization of one or more of the following major mechanisms (Dajic 2006, Munns 2005):

- 1) Phenological avoidance (related to plants which complete their cycle of growth and development in the most favorable period of the vegetation season);
- 2) Salt avoidance through salt exclusion, which can be achieved by low root permeability for certain ions, especially sodium;
- 3) Salt avoidance through secretion, which is dependent on the presence of special salt glands and bladders;
- 4) Dilution of high salt concentration in plant tissues by succulence and growth, which is among other things, related to the flexibility of cell walls;
- 5) Active accumulation and compartmentation of salts into the vacuoles;
- 6) Biochemical tolerance through adaptations of cell organelles and macromolecular systems to excess of salt, and
- 7) Nutritive tolerance (the capacity for metabolic utilization of potassium and calcium ions in order to mitigate the adverse effects of sodium ions).

To summarize, the mechanisms controlling the osmotic stress are not specific to salt (Munns 2005) because they are caused by factors associated with water stress. The mechanisms controlling Na<sup>+</sup> transport at tissue and cellular levels fall into two categories: 1) Limiting salt

accumulation: exclusion of  $\text{Na}^+$  into tissues and cells and organelles where it can do little damage or by minimizing the amount of  $\text{Na}^+$  entering the plant through its roots and 2) Efficient compartmentalizing of  $\text{Na}^+$  in the vacuoles (Munns 2005, Munns and Tester 2008, Rajendran *et al.* 2009, Tester and Davenport 2003).

The limitation of salt accumulation is done by many mechanisms such as exclusion of  $\text{Na}^+$ ,  $\text{K}^+/\text{Na}^+$  root selectivity, ion retranslocation, allocation, leaching and salt excretion (Dajic 2006).

Salt exclusion is a very efficient but complex way to prevent massive ion uptake in root zone, to enable a lower uptake and to accumulate of salts in the upper parts of the plant, especially in the transpiring organs. Salt sensitive plants, such as beans and maize are the most prominent  $\text{Na}^+$  excluders. In moderately tolerant crops, such as bread wheat, salt tolerance is associated with low rates of sodium transport to the shoots and high  $\text{K}^+/\text{Na}^+$  discrimination (Gorham 1990, Javid *et al.* 2011). For instance, bread wheat excluded more than 98% of the  $\text{Na}^+$  in the soil solution, and consequently  $\text{Na}^+$  concentration build-up in leaves remained less than 50 mM (Javid *et al.* 2011, Munns *et al.* 2006). Barley, on the other hand, excluded less than 98% of the  $\text{Na}^+$  in the soil solution, and the concentrations reached up to 500 mM (Javid *et al.* 2011). Rice may be an exception because of large rates of sodium influx into the roots under salt stress which was ascribed to leakage past the endodermis (Yeo *et al.* 1999). The level of  $\text{Na}^+$  entry into the root through leakage via the apoplast in rice was about ten times greater than this bypass flow in wheat (Garcia *et al.* 1997, Munns *et al.* 2006, Sahi *et al.* 2006).

The strategy of salt exclusion relies on the selective release of  $\text{Na}^+$  into the xylem and its resorption from the xylem stream. Net accumulation of sodium ions in the plant is dependent on the balance between passive influx and active efflux. The salt tolerance in species that exclude salts is achieved by changes between sodium and calcium ions, rather than changes in osmotic potential, since adsorption of calcium ions on membranes of root cells leads to reduced penetration of monovalent cations (Munns *et al.* 1983, Munns 2002).

Export from leaves in the phloem could conceivably help to maintain low salt concentrations (Allu *et al.* 2014). However there appears to be relatively little retranslocation of salt from leaves, in relation to the import in the transpiration stream. This can be seen in the continued presence of salt in leaves long after the salt around the roots is removed (Munns



2005). Estimates of xylem and phloem fluxes indicated that, in barley, phloem export from a leaf was only 10% of the import in the xylem.

Salt excretion is also a very efficient way to prevent excessive concentrations of salts and to build up in photosynthetic tissues (Shu *et al.* 2012). Salt excreters remove salt through glands or bladders (Ben-Hassine *et al.* 2009) or cuticle located on each leaf.

Salt tolerance by compartmentation is very important mechanism that operates in many glycophytes such as *Arabidopsis* (Moller and Tester 2007, Moller *et al.* 2009, Munns and Tester 2008), wheat and barley (Munns 2005, Munns *et al.* 1995).

To avoid deleterious  $\text{Na}^+$  toxicity in the cytoplasm, it must be compartmentalised into cell vacuoles (Munns and Tester 2008). This allows maintenance of optimum cellular levels of  $\text{K}^+$  and  $\text{Ca}^{2+}$  as well as  $\text{Na}^+$  exclusion by the plant. These two activities are known to operate at the plasma membrane and tonoplast levels, as integral components of the ion transport network. This is one of the key physiological criteria of plant salt tolerance, to maintain optimal  $\text{K}^+/\text{Na}^+$  ratio in the cytosol (Tester and Davenport 2003). A higher  $\text{K}^+/\text{Na}^+$  ratio essentially indicates that a plant has not only excluded  $\text{Na}^+$  to some extent but has also maintained a healthy level of  $\text{K}^+$  for normal metabolic activities and injury avoidance under salt (Javid *et al.* 2011).

If  $\text{Na}^+$  and  $\text{Cl}^-$  are sequestered in the vacuole of a cell, organic solutes that are compatible with metabolic activity even at high concentrations (hence 'compatible solutes') must accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole. The compounds that accumulate most commonly are sucrose, proline, and glycine betaine, although other molecules can accumulate to high concentrations in certain species. Accumulation of these compatible solutes, such as proline and mannitol, also occurs under drought stress and sometimes under other stresses. Many studies of genes controlling the synthesis or metabolism of these solutes have indicated their essential role in tolerance to abiotic stresses (Munns and Tester 2008).

Different classes of proteins of uncertain biochemical function (possibly macromolecule protection factors) are synthesized under conditions of salt stress, such as: osmotins, dehydrins, late embryogenesis abundant proteins (LEA) and polyamines, primarily putrescine and spermine (Tester and Davenport 2003). Under salt treatments, a balance between content of the

free and bound polyamines in roots of barley seedlings might be relevant for salt tolerance (Zhao *et al.* 2003).

### 1.2.3. Mechanisms of salt tolerance at molecular level

Salt tolerance is a complex trait and it is most likely controlled by interactions of many of salt responsive genes. Plants recognize a salt stress and condition adaptive response mechanisms (Ashraf *et al.* 2009, Blumwald *et al.* 2000, Hasegawa *et al.* 2000). Table 1.1 shows the reported responses involved in molecular processes such as ion homeostasis (membrane proteins involved in ionic transport), osmotic adjustment and water regime regulation (osmolytes), as well as scavenging of toxic compounds (Blumwald *et al.* 2004).

Fig.1.5 shows the genes and transporters that plants use to maintain a high  $K^+/Na^+$  ratio and have been identified and characterized (Jamil *et al.* 2011, Munns and Tester 2008). These include

- $Na^+/H^+$  antiporters in plasma membranes that remove  $Na^+$  from the cytosol as part of the regulatory SOS pathway (Zhu 2001).
- Vacuolar  $Na^+/H^+$  antiporters (*NHXs*) (Apse *et al.* 1999, Blumwald *et al.* 2000) and energy suppliers of these *NHXs* (like  $H^+$  pumps: HVA/68 and HVP1) (Ligaba and Katsuhara 2010). Compartmentalization is generally carried out by vacuolar membrane  $H^+$  ATPase and  $H^+$  pyrophosphatase proteins (Leidi *et al.* 2010, Rodríguez-Rosales *et al.* 2009, Ye *et al.* 2009).
- High and low affinity  $K^+$  transporters (*HKT*) (Shabala and Cuin 2007, Shabala *et al.* 2010). The *HKT* family consists of two classes which function either as specific  $Na^+$  transporters or  $Na^+$  and  $K^+$  co-transporter (Hauser and Horie 2010, Horie *et al.* 2012). *HKT2:1* was shown to enhance  $Na^+$  uptake and higher  $Na^+$  concentration in xylem sap.

A survey of cell responses to salt stress identified a large number of genes induced by salt. For an example, 218 salt-inducible cDNA clones have been detected in barley roots, of which 133 cDNA clones have homology to known proteins and 24 are identified as genes for signal transduction (Ueda *et al.* 2004).

It has been demonstrated that the proton pump and  $K^+/Na^+$  antiport in vacuolar membranes were important in ion selective absorption and compartmentation of  $Na^+$  in barley seedlings (Garbarino and DuPont 1989).

### 1.3. Morphological responses of plants to salt

Numerous studies show that salt stress affects plant growth. The reduced growth of plants under salt stress depends on the intensity and duration of the stress (Bounaqba 1998). Also, the response of plants to salt varies according to genus, species and variety (Fig. 1.6) (Javid *et al.* 2011), and according to the stage of development. The integrity of cell membranes (Alem *et al.* 2002, Meloni *et al.* 2003), nutritional acquisition (Davenport *et al.* 2003, Zhu 2001), the activities of several enzymes (Zhang and Blumwald 2001), the level of total protein (Chen and Marine 1991), carotenoids (Demiral and Türkan 2005) and the synthesis of cytokinins (Atanassova *et al.* 1997, Dias de Azevedo *et al.* 2004) are some of the processes affected by salt stress leads to a disturbance of general metabolism of plants.

Leaf is more sensitive to salt than root. Effects of short-term exposure (days) are considered differently from long-term exposure (weeks to years). The answer in the short term is probably the water status of the root and we suggest that a signal from the root regulates leaf expansion. The answer to what limits growth in the long term may be the maximum salt concentration tolerated by the fully expanded leaves of the shoot; if the rate of leaf death approaches the rate of new leaf expansion, the photosynthetic area will eventually become too small to support continued growth (Munns and Termaat 1986, Lutts *et al.* 1996).

The higher ratios of toxic salts in leaf apoplasm lead to dehydration and turgor loss, death of leaf cells and tissues (Marschner 1995), changes in plant and decreased efficiency of photosynthesis (Ashraf and Shahbaz 2003, Kao *et al.* 2003, Sayed 2003). Reduced photosynthesis under salt is not only attributed to stomatal closure leading to a reduction of intercellular CO<sub>2</sub> assimilation, but also to non-stomatal factors like reduction in green pigments and leaf area.

The phenotype associated directly with salt tolerance is still unclear and controversial. Although some researchers have noted that leaf chlorosis/necrosis after salt is associated with salt tolerance. The leaf injury imposes a negative influence on cell division and the plant growth. This is an indirect advantage to the plant, as any reduction of leaf expansion reduces the surface area of leaves exposed to transpiration and thereby reduces water loss (Roslyakova *et al.* 2011). Na<sup>+</sup> specific damage results in necrosis of the older leaves, starting at the tips and

margins and working back through the leaf. Growth and yield reductions occur as a result of the shortening of the lifetime of individual leaves (Munns 2002, Tester and Davenport 2003).

#### **1.4. Salt tolerance in barley**

Exclusion of  $\text{Na}^+$  from the shoot has frequently been observed as a central mechanism of salt tolerance in cereal crops such as durum wheat (Munns and James 2003, Sayed 1985, Shahzad *et al.* 2013), bread wheat (Gorham 1990) and barley (Forster 2001, Pritchard *et al.* 2004). Barley cannot exclude 98% of the salt from the transpiration stream. It must also be able to compartmentalize the salt in vacuoles, thereby protecting the cytoplasm from ion toxicity and avoiding buildup in the cell wall which would cause dehydration (Flowers and Yeo 1986). Salt-tolerant wild *Hordeum* species had better  $\text{Na}^+$  excluding ability than cultivated barley (Garthwaite *et al.* 2005). There is a strong correlation between salt exclusion and salt tolerance in cereals such as barley.

Salt tolerance by compartmentation is very important mechanism that operates in barley (Munns *et al.* 1995, Munns 2005, Munns and Tester 2008). Barley sequesters  $\text{Na}^+$  in the vacuole (James *et al.* 2002). This mechanism would avoid toxic effects of salt on photosynthesis and other key cytosolic metabolic processes. It has been demonstrated that the proton pump and  $\text{K}^+/\text{Na}^+$  antiport in vacuolar membranes were important in ion selective absorption and compartmentation of  $\text{Na}^+$  in barley seedlings (Garbarino and DuPont 1989).

In contrast to the  $\text{Na}^+$  excluding ability of roots of rice, barley has the ability to prevent root to shoot  $\text{Na}^+$  translocation at high external NaCl. Phloem export from a leaf was only 10% of the import in the xylem in barley (Munns and Termat 1986).

Leonova *et al.* (2005) found that soil salinization increased the sodium content in barley seedlings as compared to the control plants. In general, salt-susceptible cultivars accumulated more  $\text{Na}^+$  in their shoots than salt-tolerant cultivars; the reciprocal pattern was found in the roots. In contrast to the  $\text{Na}^+$  excluding ability of roots of rice, barley has the ability to prevent root to shoot  $\text{Na}^+$  translocation at high external NaCl.

Roots have a remarkable ability to control their own  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations. Their ion concentrations do not increase with time, and at high salt they can have a much lower  $\text{Na}^+$  and  $\text{Cl}^-$  concentration than the external solution. Barley also has a remarkable low  $\text{Na}^+$

concentration in roots: for example, plants grown at 250 mM NaCl had only 120 mM NaCl in their roots (Munns *et al.* 2006).

## **1.5. Breeding for salt tolerance in barley**

The sequence of breeding for plant tolerance to abiotic stresses consists of several stages (Ahmad and Prasad 2012): (1) conventional breeding and germplasm selection; (2) clarification of the specific molecular mechanism in tolerant and sensitive genotypes; (3) biotechnology-oriented improvement of selection and breeding operations by functional genomics investigations, use of molecular probes and markers for selection among natural and bred populations, and transformation with specific genes; (4) large-scale propagation (seed or vegetative) of the engineered and selected genotypes; and (5) improvement and adaptation of current agricultural practices.

Breeding for salt tolerance is one of the objectives of breeders since the last decades. Considerable improvements in salt tolerance of important crop species have been achieved 2 decades using barley, rice, pearl millet, maize, sorghum, alfalfa, and many grass species. Compared with other cereal crops, barley is highly tolerant to salt. However, barley still suffers from salt toxicity in many areas of the world. On the other hand, dramatic differences can be found among the barley species. The genetic diversification and the adaptability to a broad range of ecological conditions have highly strengthened the salt tolerance in barley. These factors might have raised a rich gene pool with a large variation in adaptation to salt.

### **1.5.1. Barley germplasm selection**

Plant scientists have adopted various strategies to overcome the damage by salt. One of them is to exploit genetic variability of the available germplasm to identify a tolerant genotype that may sustain a reasonable yield on salt-affected soil (Ashraf *et al.* 2006). Conventional breeding techniques such as interspecific hybridization, cross and recurrent selection, rely on existing genetic variability of plants in responses to salt.

Wild barley (Qiu *et al.* 2011, Mano and Takeda 1998, Robinson *et al.* 1999, Suhayda *et al.* 1990, Wu *et al.* 2011), landraces (Al-Dakheel *et al.* 2012, Bockelman *et al.* 2010, Sbei *et al.* 2012, Xue *et al.* 2012) and improved varieties (Bchini *et al.* 2010, Taghipour and Salehi 2000, Wei *et al.* 2003) have been subjected to investigations in search of salt tolerance.

Mano and Takeda (1998) evaluated salt tolerance of 340 accessions of *Hordeum*, consisting of 41 brittle-rachis forms of *Hordeum vulgare* L. subsp. *vulgare* (*H. agriocrithon*) accessions, 154 *H. vulgare* L. subsp. *spontaneum* (*H. spontaneum*) accessions, and 145 accessions of ten other species or subspecies of wild *Hordeum*. They found that the levels of salt tolerance for seed germination in wild *Hordeum* species were generally lower than those in cultivated barley and the NaCl tolerance level of the different species were as follows: *H. agriocrithon* > *H. spontaneum* > other wild *Hordeum* species. In addition, when leaf injury index was used to assess tolerance at the seedling stage, the levels of salt tolerance in wild *Hordeum* species were generally lower than those found in cultivated barley. Most wild *Hordeum* species showed high NaCl tolerance at the seedling stage and were considered to be good sources for salt tolerance breeding.

Since 1997 until today, a large number of biparental populations have been developed at a lot of research centers to identify the genetic basis of barley's salt tolerance (Koval *et al.* 2010, Shavrukov *et al.* 2010). Mini core collections, world wild core collections have been conserved in Gene Banks and international organization to achieve this main goal.

### **1.5.2. Assesment for salt tolerance in barley**

Assesment of a large number of genotypes of a crop is necessary to identify the salt tolerant germplasm for breeding programs to evolve the salt tolerance and high yielding crop varieties (Karan and Subushi 2012). This approach involves understanding the response to salt of plants at different growth stages as reported in different crops involving barley. There are many reports referring to assess for salt tolerance of varieties and lines of crops including barley (Chen *et al.* 2005, Tajbakhsh *et al.* 2006, Wei *et al.* 2003).

Assesment methods have scored a variety of phenotypic parameters including relative water content, germination rate, coleoptile length, stem and radicle length, as well as dry and fresh weight of roots and shoots (Chen *et al.* 2005, Chen *et al.* 2008, Mano and Takeda 1997, Siahisar *et al.* 2009, Tajbakhsh *et al.* 2006, Xue *et al.* 2009). Most indices for salt tolerance are not readily applicable in breeding programs although efforts have recently focused on generating molecular markers associated with these traits.

Assesment under field conditions is limited by the variation due to changeable environmental factors, such as the soil and climate conditions (Tavakkoli *et al.* 2010, Xue *et al.*

2009). Germination and seedling growth in a saline environment are the growth stages that are widely used to assess the salt tolerance of the barley genotypes due to the benefits of their reduced environmental effects (Chen *et al.* 2005, Ellis *et al.* 2002, Mano and Takeda 1997, Shavrukov *et al.* 2010, Taghipour and Salehi 2008). Additionally, a hydroponic system is freed from the difficulties associated with soil-related stress factors and the low narrow sense heritability of salt tolerance.

### 1.5.3. Biparental mapping

Genetic mapping can be done mostly in two ways (1) using the experimental populations also referred to as “biparental” mapping a population that is called QTLs-mapping as well as genetic mapping or gene tagging, and (2) using the diverse lines from the natural populations or germplasm collections that is called LD-mapping or “association mapping.”

Biparental mapping populations that are used for QTLs analysis include F2 and backcrossed populations, recombinant inbred lines (RILs) and doubled haploid lines (DH), although initially F2 population that were actually developed with the aim of constructing linkage maps, was also utilized for QTL mapping for salt tolerance (Graner *et al.* 1991, Li *et al.* 2008). Molecular markers associated with QTLs for salt tolerance in barley were presented in the summarized table (Table 1.2) indicating the cross combinations and the references of published papers.

A number of QTLs affecting salt tolerance were detected on all chromosomes in the cross between *H. spontaneum* and *H. vulgare*, although several QTL clusters were present on chromosomes 1H, 4H, 6H and 7H (Ellis *et al.* 1997). In cultivated barley, QTLs controlling salt tolerance were mapped on chromosomes 1H, 4H, 5H and 6H at germination and on chromosomes 1H, 2H, 5H and 6H at the seedling stage (Mano and Takeda 1997).

In the cross of Derkado x B83-12/21/5, the largest individual effects on salt tolerance was associated with the chromosomal regions around the two dwarfing genes *sdw1* (3H) and *ari-e.GP* (5H). The *sdw1* gene resulted in an overall yield increase, but was only detected as a secondary QTL (Ellis *et al.* 2002). A survey of QTLs for salt tolerance in barley seedlings revealed 12 QTLs for seven traits (Ellis *et al.* 2002). It was reported in several species that QTLs linked to salt tolerance vary with the developmental stage of the plant (Flowers 2004).

Recently, a single locus controlling Na<sup>+</sup> exclusion was on chromosome 7H (Shavrukov *et al.* 2010). QTL analysis using the F2 and F3 populations derived from the cross between CPI-71284-48 (wild barley accession capable of limiting sodium accumulation in the shoots under saline hydroponic growth conditions) and the cultivated barley (*H. vulgare ssp. vulgare*) cultivar Barque (Barque-73, a moderate Na<sup>+</sup> excluder) attributed the control of the Na<sup>+</sup> exclusion trait from CPI-71284-48 to a single locus on the short arm of chromosome 7H, which was named *HvNax3*. The locus reduced shoot Na<sup>+</sup> accumulation by 10-25% in plants grown in 150 mM NaCl. Markers generated using colinearity with rice and *Brachypodium*, together with the analysis of introgression lines and F2 and F3 families, enabled *HvNax3* to be mapped to a 1.3 cM interval. Genes corresponding to rice and *Brachypodium* intervals encode 16 different classes of proteins and include several plausible candidates for *HvNax3*. More recently, fine mapping populations were used to identify a barley locus controlling an environmentally sensitive Na<sup>+</sup> exclusion trait (*HvNax4*) on the long arm of the chromosome 1H.

The *HvNax4* locus lowered shoot Na<sup>+</sup> content by between 12% and 59% (g<sup>-1</sup>DW), depending on the growth conditions in hydroponics and a range of soil types, indicating a strong influence of environment on expression. *HvNax4* was fine-mapped on the long arm of barley chromosome 1H. Corresponding intervals of 200 kb, containing a total of 34 predicted genes, were defined in the sequenced rice and *Brachypodium* genomes. A co-segregating barley gene (*HvCBL4*) with close similarity to Arabidopsis SOS3 was identified. *HvCBL4* was investigated as a candidate for the *HvNax4* gene by mRNA expression profiling and sequencing and, in addition, by constructing the molecular model of *HvCBL4* based on the known crystal structure of SOS3 from *Arabidopsis*.

Many linkage analysis studies have been conducted in barley over the past two decades; only a limited number of detected QTLs for salt tolerance were cloned or tagged at the gene level (Rivandi *et al.* 2010, Shavrukov *et al.* 2010).

#### **1.5.4. Association mapping**

Association mapping (AM), based on linkage disequilibrium (LD), offers an alternative method for QTLs mapping. It utilizes ancestral recombination events to make marker-phenotype associations (Kraakman *et al.* 2006, Thornsberry *et al.* 2001).



The general characteristics of this field of genetics involve the use of unstructured or loosely structured populations that are both phenotypically and genotypically characterized to detect statistical associations between DNA polymorphisms and heritable in traits variation (Nnadozie *et al.* 2007, Wen *et al.* 2009). Association mapping shares much in common with QTL mapping. Both attempt to detect co-segregation of polymorphic genetic markers with genes underpinning trait variation but differ in terms of some key properties (Table 1.3).

Recently, researchers focused on the identification of QTLs for salt tolerance in the barley accessions using worldwide core collections by association analysis due to the limitation in the biparental population created through crossing and limited number of recombination. For example, Eleuch *et al.* (2008) used 22 simple sequence repeat (SSR) markers to investigate the genetic diversity of 48 barley accessions and found some associations with yield and other agronomic traits under salt stress. Wu *et al.* (2011) and Qiu *et al.* (2011) examined tissue dry biomass and the Na<sup>+</sup> and K<sup>+</sup> contents of 188 wild Tibetan barley under 300 mM of NaCl at early stage. Nguyen *et al.* (2013a) evaluated spring barley collection for salt tolerance (200 mM of NaCl) at vegetative stage using a hydroponic system and used to identify quantitative trait loci for salt tolerance by means of an association mapping approach.

## **1.6. Progress and limitations in previous studies**

Salt is one of the principal abiotic factor affecting crop yields in arid and semi-arid irrigated areas (Szabolcs 1989). Almost three quarters of the surface of the earth is covered by salt water and so it is not surprising that salts affect a significant proportion of the world's land surface. Over 800 million hectares of land throughout the world are salt-affected, either by salt (397 million ha) or the associated condition of sodicity (434 million ha) (Babu *et al.* 2007, FAO 2005). This is over 6% of the world's total land area. Most of this salt area is natural (Munns 2005). A significant proportion of recently cultivated agricultural land has become saline due to salt water used for irrigation, inadequate drainage, salt water flooding of coastal land, and salt accumulation in dry areas. Approximately 20% of agricultural land in the world and nearly half of all irrigated land suffer from salt (Flowers and Yeo 1995) and it continues to be a major problem in the arid and semi-arid regions. Salt damage has rapidly extended to broad areas of arable land in the world and is in danger of becoming saline. North Africa, Near East and Asia Pacific are the most severely damaged by salt compared with other areas (Fig. 1.3). The area

affected by salt in South, South East, North, and Central Asia covered 25% of the total salt soil. The major salt problem in Asia is the accumulation of salt in dryland which results from land clearing (Pankova and Konyushkova 2013).

One of the important crops in the worldwide production is barley. It is ranked fourth after rice, wheat and maize. Developing countries accounts for about 18% (26 million tons) of total barley production and 25% (18.5 million hectares) of the total harvested area in the world. Barley grain is mostly used as feed for animals, malt, and food for human consumption. Malt is the second largest use of barley. Farmers also use barley straw as animal feed in West Asia, North Africa, Ethiopia, Eritrea, Yemen and East Asia.

Few studies on salt tolerance have been done in Asian barley germplasm compared with European, Mediterranean basin, American and Australian barley germplasm (Table 1.2). Based on the frequency of markers/genes and DNA polymorphism studies, the Asian barley showed a high level of diversity in landraces, improved varieties and their wild relatives (Cançado 2011, Komatsuda *et al.* 1999, Liu *et al.* 1999, Naeem *et al.* 2011, Russell *et al.* 2014). Asia is known as a secondary center of diversity of barley.

Western type of barley were more evaluated compared to Asian barley. Two sets of barley doubled haploids from Steptoe/Morex (149 DH lines) and Harrington/TR306 (146 DH lines) produced by a modified bulbosum method in the Oregon State University Barley Breeding Program and provided by Hayes (Department of Crop and Soil Science, Oregon State University, Corvallis, OR 973314501, USA (Chen and Hayes 1989), were used to identify QTLs for salt tolerance for physiological and morphological traits at germination and seedling stage (Aminfar *et al.* 2011, Mano and Takeda 1997, Nguyen *et al.* 2013b, Siahisar and Narouei 2010). One hundred and fifty DHs were produced from a cross between Derkado (prostate semi dwarf phenotype *sdw1/Ari.e GP*) and the Scottish Crop Research Institute (SCRI) breeding line B83-12/21/5 (erect semi dwarf phenotype *Sdw1/ari-e GP*) to associate phenotype/genotype for yield and salt tolerance in barley populations segregating for 2 dwarfing genes (Ellis *et al.* 2002). A segregating DH population of 93 lines, developed by anther culture of the F1 hybrid between CM72 (California Mariout 72, six-rowed; salt-tolerant) and Gairdner (An Australian cultivar, two-rowed; salt sensitive were used to identify of QTLs associated with salt tolerance at late growth stage in barley ( Xue *et al.* 2009). Zhou *et al.* (2011) used a total of 172 F1-derived DH lines to identify QTLs for salt tolerance at bottom stage. The materials were

generated from a cross between Yuyaoxiangtian Erleng (YYXT) and Franklin (Shannon 9 Triumph). YYXT is relatively tolerant to salt stress but was originally introduced to Australia because of its superior tolerance to waterlogging. Franklin is Australian malting barley that is generally considered susceptible to salt. Recently, Xue *et al.* (2012) used 188 DH lines from a cross between a Chinese landrace variety, TX9425 (waterlogging and salt tolerant), and a Japanese malting barley, Nasu Nijo (waterlogging and salt sensitive), to identify QTLs associated with the tolerance. The salt tolerance was evaluated with both a hydroponic system and in potting mixture.

Breeding for salt tolerance has been met with limited success due to the complex nature of salt tolerance. Barley shows a complex response to salt tolerance, different levels of sodium tolerance and employs a variety of tolerance mechanisms (ability to minimize Na<sup>+</sup> accumulation in the shoot, sequestration of sodium in vacuoles, discrimination between Na<sup>+</sup> and K<sup>+</sup> accumulation (Moller and Tester 2007, Munns *et al.* 1995, Zhu 2001). As a consequence, many methods and traits were employed to assess for tolerance to salt in barley. No standard trait used for assessment even at seedling stage. Salt tolerance in many crops is known to change with growth stage. Salt tolerance is controlled by several genes with additive effect (Qi *et al.* 2011). New germplasm and molecular tools make it possible to develop better barley varieties faster for salt tolerance, but challenges still remain due to complexity of salt tolerance.

## **1.7. Thesis outline**

In this study we explore the natural variation of barley using a large number of accessions to determine key traits for salt tolerance in barley. Association mapping method is used as a new tool to identify QTLs for salt tolerance in barley.

The overall objectives of the present research are:

1. To understand key traits determining salt tolerance at seedling stage;
2. To provide new tools to better exploit the genetic variation of salt tolerance;
3. To evaluate genetic variation in salt tolerance of Asian barley collection;
4. To identify new QTL associated with salt tolerance.

In the Chapter 1, the literature review devised on 3 parts including the importance of barley in the world, salt stress mechanisms and breeding for salt tolerance in barley. Also, we present the problematic of the research study and the thesis outline to achieve our objectives.

In the Chapter 2, the author evaluated a set of 296 accessions of Asian barley for salt tolerance with 250 mM NaCl in a hydroponic solution. The objectives were to evaluate the genetic variation in salt tolerance of barley and to determine the suitable traits for salt tolerance assessment at seedling stage in barley accessions.

In the Chapter 3, the author analyse the association of salt tolerance with 384 SNP markers to identify QTLs for salt tolerance. The objectives were to determine the population structure of the Asian barley accessions and to identify SNP markers associated with salt tolerance at seedling stage, based on the association analysis.

In the Chapter 4, the author discussed the findings presented in this thesis in relation to the current status and the prospects of breeding for salt tolerance in barley and cereals. The impact of our results on major issues related to trait discovery strategies for salt tolerance. These include phenotyping strategies and association mapping in breeding for salt stress tolerance.

**Table 1.1. Mechanisms of salt tolerance, organized by plant processes and their relevance to the three components of salt tolerance (Munns and Tester 2008).**

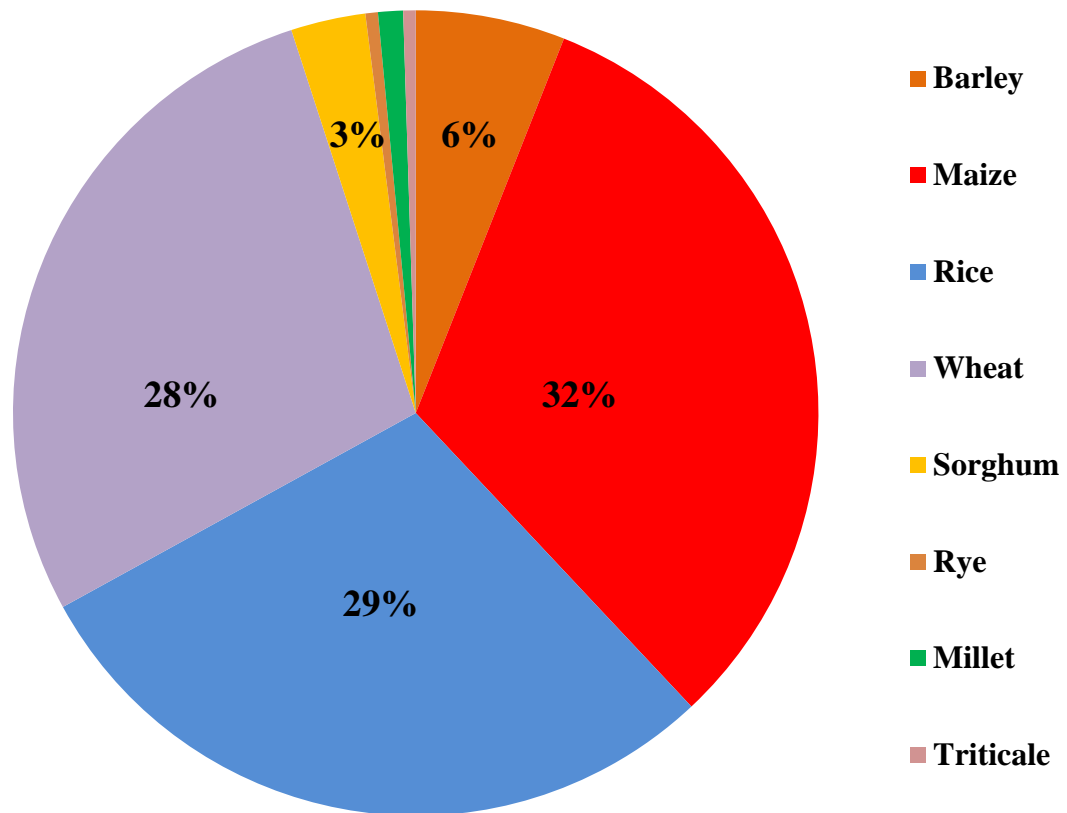
<b>Process involved</b>	<b>Candidates genes</b>	<b>Osmotic stress</b>	<b>Ionic stress</b>	<b>Tissue tolerance</b>
		<b>Osmotic tolerance</b>	<b>Na<sup>+</sup> exclusion</b>	
<b>Sensing and signaling in roots</b>	<i>SOS3, SNRKs</i>	Modification of long-distance signaling	Control of net ion transport to shoot	Control of vacuolar loading
<b>Shoot growth</b>	?	Decreased inhibition of cell expansion and lateral bud development	Not applicable	Delay in premature senescence of old leaves
<b>Photosynthesis</b>	<i>ERAI, PP2C, AAPK, PKS3</i>	Decreased stomatal closure	Avoidance of ion toxicity in chloroplasts	Delay in ion toxicity in chloroplasts
<b>Accumulation of Na<sup>+</sup> in vacuoles</b>	<i>NHX, AVP</i>	Increased osmotic adjustment	Increased sequestration of Na <sup>+</sup> into root vacuoles	Increased sequestration of Na <sup>+</sup> into leaf vacuoles
<b>Accumulation of organic solutes</b>	<i>P5CS, OTS, MTID, M6PR, S6PDH, IMTI</i>	Increased osmotic adjustment	Alteration of transport processes to reduce Na <sup>+</sup> accumulation	Accumulation of high concentration of compatibles solutes in cytoplasm

**Table 1.2. Molecular markers associated with QTLs for salt tolerance in barley.**

<b>Molecular markers</b>	<b>QTLs</b>	<b>Cross</b>	<b>References</b>
<b>Chr. 1H</b>			
GLBI-ABC160	Seedling Salt tolerance	Stephoe*Morex	Mano and Takeda (1997)
ABC160-His4A	Seedling Salt tolerance	Stephoe*Morex	
WG789B-ABR337	Seedling Salt tolerance	Stephoe*Morex	
Drun8-ABC261	Germination salt tolerance	Harrigton*TR306	
HPb-2240-bPb-0631	Spikes per plant	CM72*Gairdner	Xue <i>et al.</i> (2009)
<b>Chr. 2H</b>			
ABG459-Pox	Seedling Salt tolerance	Stephoe*Morex	Mano and Takeda (1997)
ABC152D-Rm5S1	Seedling Salt tolerance	Stephoe*Morex	
His3C-ABC152D	Seedling Salt tolerance	Stephoe*Morex	
P21M12d	Shoot dry weight	Derkado X B83-12/21/5	
bPb-6088-bPb-4377	Dry weight per plant	CM72*Gairdner	Ellis <i>et al.</i> (2002)
Bmag0381-bPb-0827	Grain number per plant	CM72*Gairdner	Xue <i>et al.</i> (2009)
bPb-3536-bPb-1103	Shoot Na <sup>+</sup> conc.	CM72*Gairdner	
<b>Chr. 3H</b>			
bPb-0048-bPb-4564	Plant height	CM72*Gairdner	Xue <i>et al.</i> (2009)
bPb-7989-bPb-4660	Spikes per plant	CM72*Gairdner	Xue <i>et al.</i> (2009)
<b>Chr. 4H</b>			
MWG634-WG622	Germination salt tolerance	Stephoe*Morex	Mano and Takeda (1997)
P17M62f	Shoot dry weight	Derkado X B83-12/21/5	
bPb-1278-bPb-3512	Tiller number	CM72*Gairdner	Ellis <i>et al.</i> (2002)
bPb-1278-bPb-3512	Spikes per line	CM72*Gairdner	Xue <i>et al.</i> (2009)
bPb-0130-bPb-8437	Spike per plant	CM72*Gairdner	
<b>Chr. 5H</b>			
WG889-ABC324	Germination salt tolerance	Stephoe*Morex	Mano and Takeda (1997)
ABC309-MWG632	Germination salt tolerance	Harrigton*TR306	
iEst9-WG908	Seedling Salt tolerance	Stephoe*Morex	
WG364-MWG514B	Seedling Salt tolerance	Stephoe*Morex	
CDO504-ABG712	Seedling Salt tolerance	Harrigton*TR306	
TubA3-MWG740	Seedling Salt tolerance	Harrigton*TR306	
Bmag337	Shoot dry weight	Derkado X B83-12/21/5	Ellis <i>et al.</i> (2002)
<b>Chr. 6H</b>			
ABG387B-ABG458	Germination Salt tolerance	Stephoe*Morex	Mano and Takeda (1997)
BCD340E-KsuD17	Seedling Salt tolerance	Stephoe*Morex	
bPb-6421-bPb-3921	Spikes per line	CM72*Gairdner	
bPb-7323-bPb-2751	Grain Yield	CM72*Gairdner	Xue <i>et al.</i> (2009)
bPb-8889-bPb-7323	Na <sup>+</sup> ;K <sup>+</sup> ratio	CM72*Gairdner	
<b>Chr. 7H</b>			
bPb-1209-bPb-6821	Spikes per line	CM72*Gairdner	Xue <i>et al.</i> (2009)
P40M38b	Tiller number	Derkado X B83-12821/5	Ellis <i>et al.</i> (2002)

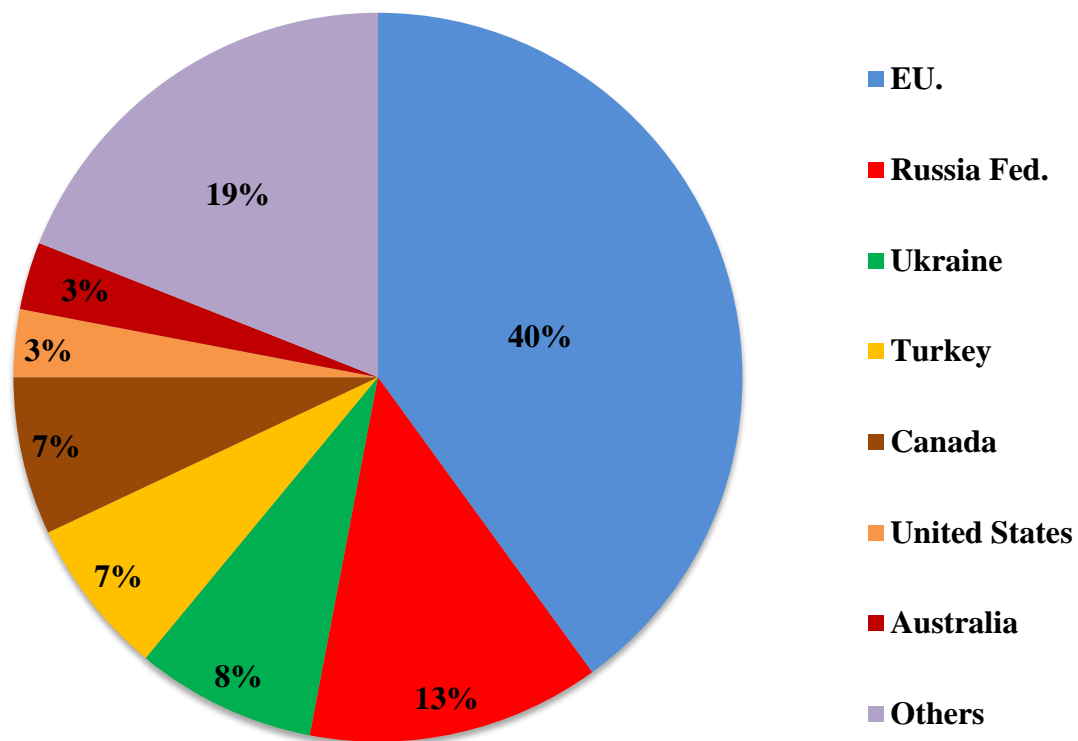
**Table 1.3. Summary of association genetics and conventional QTLs mapping (Nnadozie *et al.* 2007).**

<b>Attribute</b>	<b>QTL mapping</b>	<b>Association genetics</b>
Detection goal	Wide region within specific pedigrees within which a QTL is isolated.	Physically as close as possible to causative sequence
Resolution of causative trait polymorphism	Low- moderate density linkage maps only required	High- disequilibrium within small physical regions requiring many markers
Experimental population for detection	Defined pedigrees, e.g. backcross, F2, RILs, 3 and 2 generations pedigrees/families, half-sub families	Linkge disequilibrium experiments: unrelated individuals (“unstructured population), large numbers of small unrelated families (e.g. transmission disequilibrium tests, TDT)
Marker discovery coasts	Moderate	Moderate for few traits, high for many traits
Extent of inference	Pedigree specific , except where species has high extand LD	Species or subspecies wide
Numbers of markers required for genome coverage	$10^2$ to $10^3$	$10^5$ for small genomes to $10^9$ for large genomes

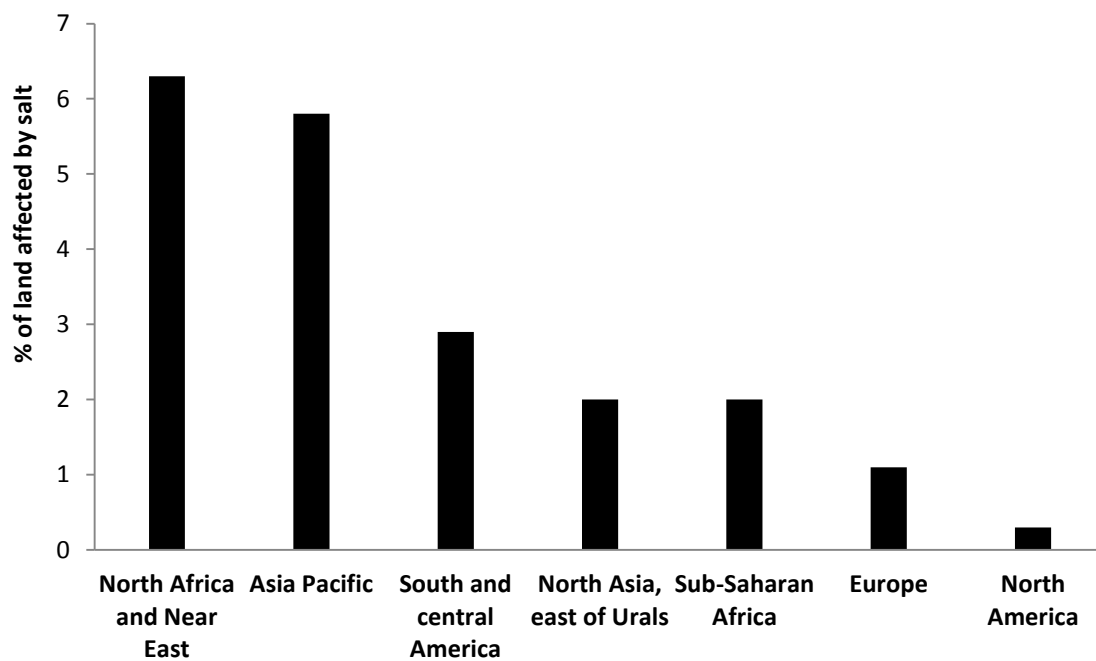


**Fig. 1.1. World barley production compared to other cereals (FAO 2009).**

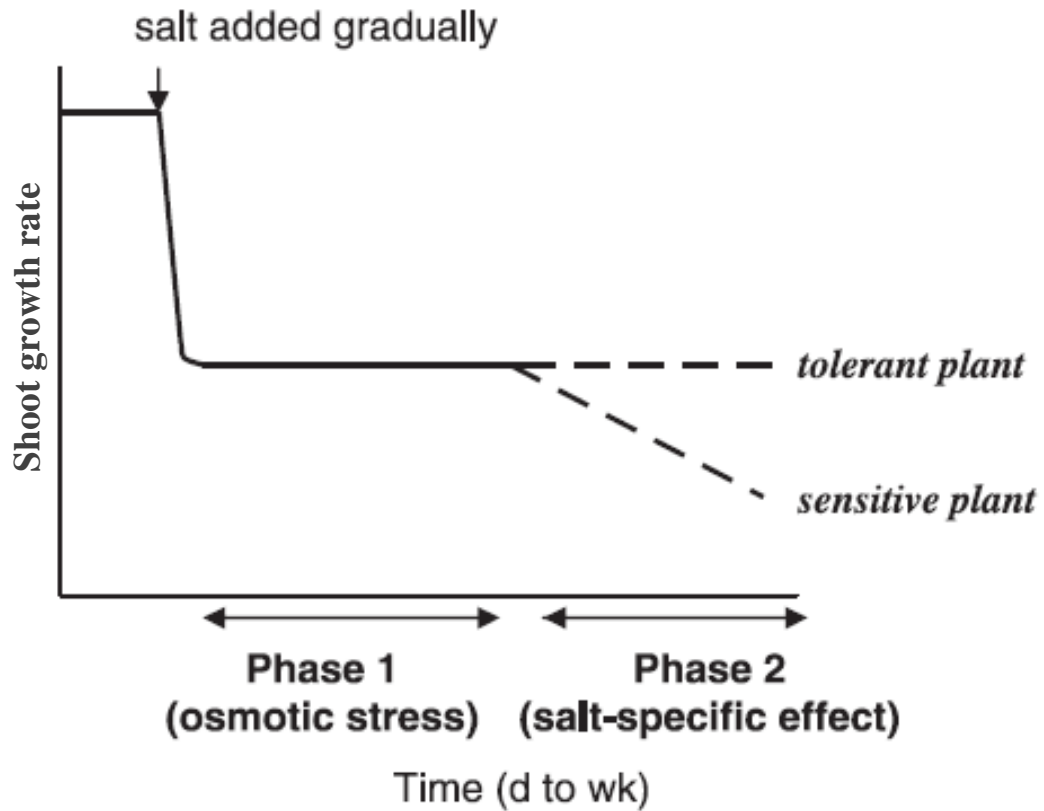




**Fig. 1.2. Global barley production in major countries (FAO 2009).**

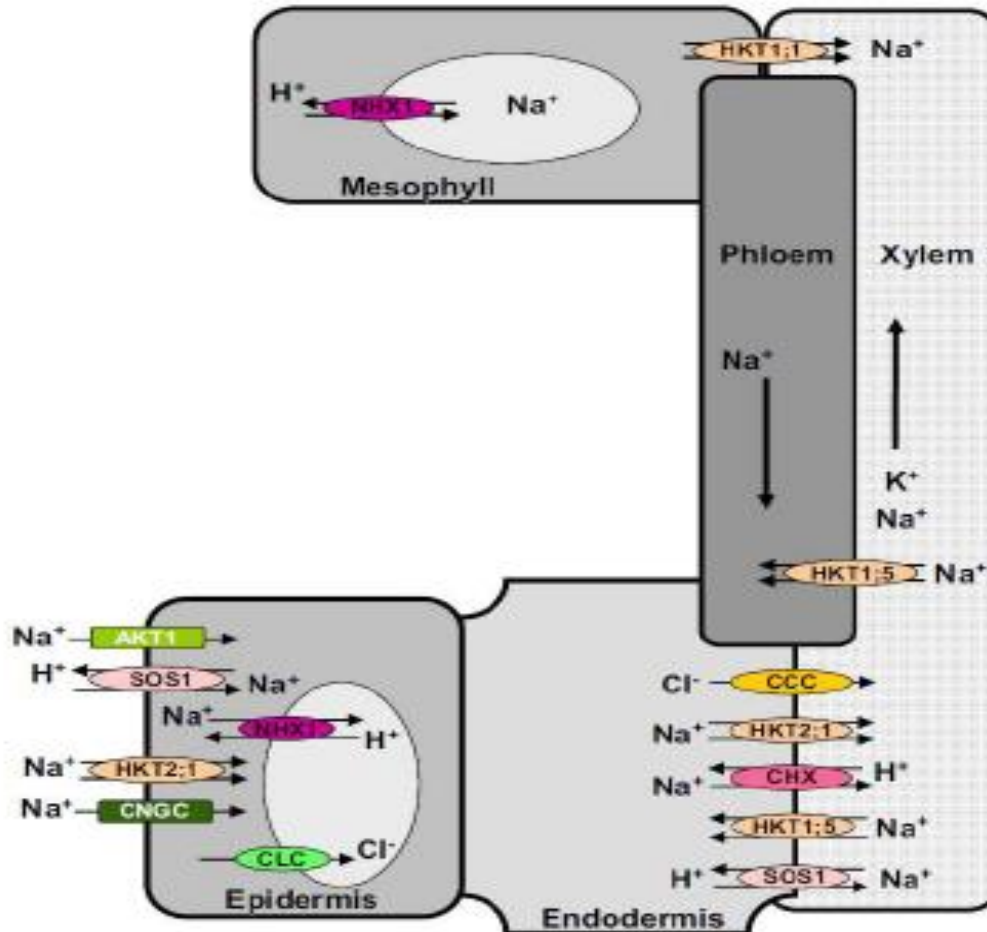


**Fig. 1.3. Distribution of land affected by salt in different areas (FAO 2006).**



**Fig.1.4. Schematic illustration of the two-phase growth response to salt for genotypes that differ in the rate at which salt reaches toxic levels in leaves (Munns 2005).**

With annual species, the timescale is days (d) or weeks (wk). For perianual species, the timescale is weeks or months depending on species and salt level.



**Fig. 1.5. General functions and localization of  $\text{Na}^+$  and  $\text{Cl}^-$  transporters in plant cell (Deinlein *et al.* 2014, Mian *et al.* 2011).**

$\text{Na}^+$  uptake at the soil-root boundary occurs via non-selective cation channels like CNGCs. In halophytes,  $\text{K}^+$  channels such as AKT1 may also be involved in  $\text{Na}^+$  uptake. HKT1:1 helps to control the accumulation of  $\text{Na}^+$  in shoots and retrieval of  $\text{Na}^+$  from xylem. HKT2:1 mediates high affinity uptake of  $\text{Na}^+$  but may also participate in  $\text{Na}^+$  xylem loading. HKT1:5 reduces the xylem  $\text{Na}^+$  concentration and shoot  $\text{Na}^+$  load.  $\text{Na}^+$  efflux into the vacuole and apoplast occurs via antiporter systems: NHX1 at the tonoplast and SOS1 at the plasma membrane. SOS1 may also mediate xylem loading of  $\text{Na}^+$  along with other antiporters such as CHXs. Chloride channels (CLCs) may be involved in compartmentation of  $\text{Cl}^-$  into the vacuole and chloride cation co-transporters (CCCs) may mediate xylem loading of  $\text{Cl}^-$  in the plant. The mechanism and identity of  $\text{Cl}^-$  uptake systems are not known.

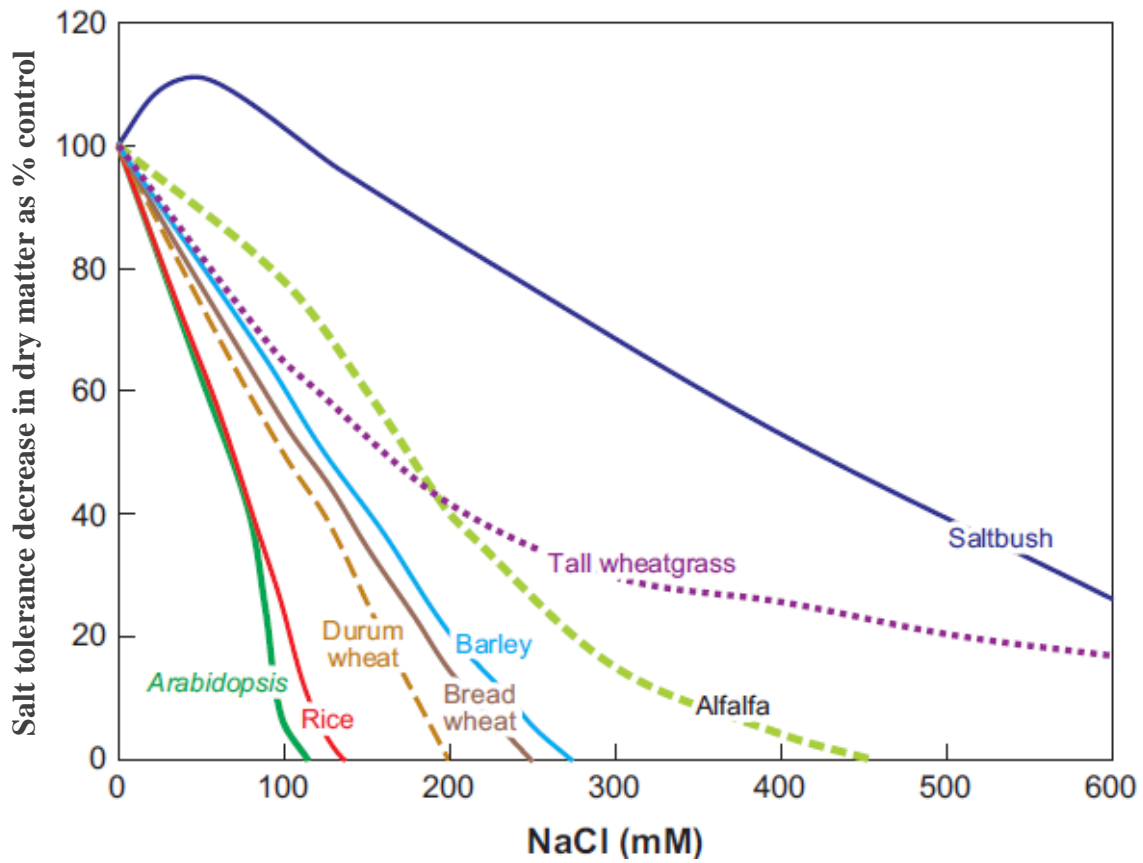


Fig. 1.6. Growth of diverse plant species to a range of salt levels (Javid *et al.* 2011).

# CHAPTER 2:

## ASSESSMENT FOR SALT TOLERANCE IN

### ASIAN BARLEY

#### 2.1. Introduction

Salt stress inhibits plant growth in two ways. First, osmotic stress reduces the plant's ability to take up water and rapidly inhibits the growth of young leaves. Shoot and root growth is permanently reduced within hours of salt stress. This effect does not appear to depend on the sodium concentration but rather is a response to the osmolarity of the external solution. The ionic stress or salt-specific stress may enter the transpiration stream, eventually injuring cells in the transpiring leaves (Munns 2005, Munns and Tester 2008). Necrosis of the older leaves, starting at the tip and margins and working back through the leaf, is the result of salt accumulation. Growth reductions occur as a result of the shortened lifetimes of the individual leaves (Munns 2002, Tester and Davenport 2003). This process begins when salt, especially  $\text{Na}^+$ , accumulates to toxic concentrations in old leaves. The regulatory mechanisms of the osmotic stress are not specific to salt (Munns 2005) because they are caused by factors associated with water stress (Fig. 1.4.). The regulatory mechanisms of  $\text{Na}^+$  transport at both the tissue and cellular levels respond with either  $\text{Na}^+$  exclusion from the tissue and cells or an efficient transportation of  $\text{Na}^+$  into the vacuoles (Munns 2005, Munns and Tester 2008, Tester and Davenport 2003, Rajendran *et al.* 2009).

Among cereals, rice (*Oryza sativa* L.) is the most sensitive and barley (*Hordeum vulgare* L.) is the most tolerant (Munns and Tester 2008) which is considered as an ideal model plant for genetic and physiological studies on salt tolerance due to its short growth period, early

maturing, diploid and self-pollinating characteristics (Bothmer *et al.* 1995). Barley is widely cultivated in saline areas as the most salt-tolerant field crop. The genetic diversification and adaptability to a broad range of ecological conditions contributed to the salt tolerance in barley and to its rich gene pool (Komatsuda *et al.* 1999). These factors might have raised a rich gene pool with a large variation in the plant's adaptations to salt. Many methods have been used to assess the barley germplasm for salt tolerance. Assessment under field conditions is limited by the variation due to changeable environmental factors, such as the soil and climate conditions (Xue *et al.* 2009). Germination and seedling growth in a saline environment are the growth stages that are widely used to access the salt tolerance of the barley genotypes due to the benefits of their reduced environmental effects (Chen *et al.* 2005, Ellis *et al.* 2002, Mano and Takeda 1997, Taghipour and Salehi 2008, Shavrukov *et al.* 2010). Additionally, a hydroponic system is freed from the difficulties associated with soil-related stress factors and the low narrow sense heritability of salt tolerance.

Based on the frequency of markers/genes and DNA polymorphism studies, the Asian barley showed a high level of diversity in landraces, improved varieties and their wild relatives (Cançado 2011, Komatsuda *et al.* 1999, Liu *et al.* 1999, Naeem *et al.* 2011, Russell *et al.* 2014). On the other hand, Western type of barley were more evaluated compared to Asian barley and Asia is known as a secondary center of diversity of barley. Therefore, it was expected to detect novel QTLs for salt tolerance using the Asian germplasm. Also, barley cultivation in Asian areas has been severely damaged and continuously selected for salt tolerance.

Barley accessions belonging to the Asian core collection from Institute of Plant Science and Resources, Okayama University were evaluated at seedling stage for salt tolerance under 250 mM NaCl in an hydroponic solution. The objectives were to evaluate the genetic variation of barley for salt tolerance and to determine the suitable traits for salt tolerance assessment in barley accessions.

## **2.2. Materials and methods**

### **2.2.1. Plant materials**

A total of 296 barley accessions were used in this study, originating from the following distant Asian countries: Bhutan, China, India, Japan, Korea and Nepal (Table 2.1). The set of accessions composed by the improved cultivars and landraces included the six and the two-

rowed types. Spring, winter and facultative growth habits were represented by 139, 98 and 59 accessions, respectively (Table 2.2). The accessions were selected from an Asian core collection preserved at Okayama University. This core collection was initially investigated by Liu *et al.* (1999) to reveal the genetic diversity among barley core collections based on the allelic variations at 6 isozyme loci.

### 2.2.2. Salt tolerance evaluation

The assessment for salt tolerance at seedling stage is previously examined (Ellis *et al.* 2002, Mano and Takeda 1997). To check the method to assess salt tolerance in the Asian barley germplasm at the seedling stage, five accessions (Asan Jungbori, Rogbori, Cheongweon Native, Gho 4, and Lumley 2) were randomly chosen and evaluated for salt tolerance in hydroponic culture at the seedling stage. The accessions numbers are 308, 311, 330, 332 and 367. A completely randomised design with three replications (four plants per accession) for each treatment (0, 150, 200 and 250 mM NaCl) was used. Seeds were sown and germinated for 6 days in growth chamber (Biotron). Seedlings were selected for shoot and root uniformity, transplanted in container that contains the nutrient solution (Table 2.3) and grown for 8 days in growth chamber at 22/20°C (day/night).

The NaCl was added to the nutrient solution at two complete leaf stages (8th day after transplantation). To reduce the effect of osmotic shock, adding salt started by 100 mM and then increased within 3 days to reach the final concentration, 150, 200 and 250 mM of NaCl, respectively. Then the treatment maintained for 16 days. The solution was changed every 7 days and stirred every day. The pH of solution was measured twice a week. The experiment was laid out as a completely randomized design with three replications and four plants per replication.

The leaf injury score (LIS) was recorded. The root length (RL), shoot length (SL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) were recorded and the salt tolerance indexes (STI) were calculated according to the following formula (1).

$$STI = \frac{\text{Value of trait under salt treatment}}{\text{Value of trait under control}} \times 100 \quad \dots\dots\dots (1)$$



The NaCl concentration and the period of treatment were estimated in function of the most reduction of traits under treatments compared to the control.

The assesment of 296 Asian accessions for salt tolerance at the seedling stage was carried out in a growth chamber with a controlled temperature of 24/18°C (day/night) and natural light. The experiment was performed over a split plot design. The experiments repeated 3 times for each treatment (control and 250 mM NaCl). There were 12 plants per accession (four plants per each replication). The containers were painted with a black colour to avoid the light-sensing by the roots.

Seeds were sterilised in 6% of sodium hypochlorite for 10 minutes and germinated in a Petri dish for 5 days in an incubator (22/20°C, day/night). Uniform seedlings were selected and transplanted into a container filled with a hydroponic solution (Otsuka House Nos. 1 and 2, Otsuka Chemical Co., Osaka, Japan) of the following proportions (%): TN (21%), P<sub>2</sub>O<sub>5</sub> (8%), K<sub>2</sub>O (27%), MgO (4.0%), MnO (0.10%), B<sub>2</sub>O<sub>3</sub> (0.10%), CaO (23%), Fe (0.18%), Cu (0.002%), Zn (0.006%) and Mo (0.002%). The salt treatment was started when the second leaves completely developed. To reduce the effect of osmotic stress, the initial salt concentration was set at 150 mM and then increased within 3 days to reach the final concentration of 250 mM NaCl. The treatment was maintained for 17 days. The solution was changed every week. The pH of solution was adjusted to 6.5.

After 26 days from the transplantation the number of leaves (NL), root length (RL) and shoot length (SL) were recorded. Plants were separated into roots and shoots, dried in an oven at 85°C for 72 hours and weighed in order to determine the shoot dry weight (SDW), root dry weight (RDW) and plant dry weight (PDW). The leaf injury score (LIS) was assessed from 1 to 5 with [1: no apparent chlorosis; 2: slight (25% of the leaves showed chlorosis); 3: moderate (50% of the leaves showed chlorosis and some necrosis); 4: severe chlorosis (75% of the leaves showed chlorosis and severe necrosis) and 5: dead (leaves showed severe necrosis and were withered)] as shown in Fig. 2.1 (Lee *et al.* 2008, Valencia *et al.* 2008). No symptoms of necrosis or chlorosis were identified under control in the early vegetative stage.

### **2.2.3. Statistical analysis**

The analysis of variance under control and NaCl treatment conditions for each trait was performed using GLM model analysis in the SAS software version 9.0 (SAS Institute Inc. 2011). Distribution analysis for the LIS and the STI was performed using the JMP V11 software, ver. 9 (SAS Institute Inc. 2011). The Spearman's coefficient correlation ( $r$ ) was determined for all traits with a two-tailed test of significance. Post Hoc Multiple comparisons were used to compare the relationship between salt tolerance and origin of accessions and growth habit type. The equal variance was assumed by Duncan test with a level of significance 0.05 by the software SPSS inc. (2010). Student's t-test with two tailed distribution for two sample equal variances was used to compare categories of accessions, rowed type and seed type.

## **2.3. Results**

### **2.3.1. Verification of experimental conditions for salt treatment**

As shown in the figure (Fig. 2.2), there was a wide difference among accessions in control and salt treatments for all traits RL, SL, RFW, RDW, SFW and SDW. Fresh and dry weights were more affected by salt than other traits specially for the 200 and 250 mM of NaCl. The coefficient of variation within accessions varied from 3.2 to 66% for all trait. the lowest variation within accessions was observed for the SL ( CV  $\approx$  3.2 to 25%) under all traits, while it was higher for the RDW.

Salt tolerance index of the RL, SL, RFW, RDW, SFW and SDW for the 5 accessions (Acc. number: 308, 311, 330, 332 and 367) was presented in the Fig. 2.3. The STI of the traits between control and 150 mM of NaCl was 60- 80% for all traits, except the SDW that was not affected and some accessions showed an amelioration in biomass under 150 mM. A significant reduction of root and shoot biomasses was observed between the 0-200 mM of NaCl and 0-250 mM. The shoot dry weight at 200 and 250 mM of NaCl was between 50-65% and between 58-60% of the control, respectively.

LIS was scored "1" under the control (Fig. 2.4). Under 150 mM of NaCl, the leaves were slightly affected by salt and all accessions showed the score "2". The variation of the LIS among accessions was observed at the treatments 200 and 250 mM of NaCl and necrosis and

chlorosis spots were observed. The coefficient of variation within accessions varied from 16.8 to 24% at the treatment 200 mM while less variation ( from 11 to 18%) under 250 mM of NaCl.

The LIS was recorded after 10 days and 16 days of treatment (Fig. 2.5). A statistically significant variation in injury score were shown between the treatment for 10 days and the treatment for 16 days. There was no significant variation of the responses of accessions under 200 and 250 mM of NaCl for the same period of treatment. According to the preliminary results, we decided to use the treatment with 250 mM of NaCl for 16 days for assesment of salt tolerance at early stage in this study because it allowed the highest inhibition in biomass increase and the lowest coefficient of variation of LIS within accessions.

### **2.3.2. Phenotypic variation among Asian barley accessions**

Phenotypic variation in various growth traits under 250 mM NaCl treatment and control conditions are presented in Table 2.4. The frequency distribution for all traits of barley accessions under control and salt treatment is shown in the Fig. 2.6. The frequency distribution of the LIS is shown in the Fig 2.8. Variations in NL, SL, RL, SDW, RDW and PDW were observed between control and salt stress conditions. Under salt treatment, the NL ranged from 2 to 7, whereas the range under control condition was from 2 to 12. Plant height (SL) ranged from 25 to 50 cm under the control while it ranged from 15 to 38 cm only. The RL varied between control and salt treatment. The SDW ranged from 0.5 to 5 g/plant under control condition, while it decreased to 0-2.3 g/plant. The RDW ranged from 0.3 to 1.5 g/plant under control condition, whereas, it was from 0.2 to 1.2 g/plant. Under both conditions, the values of all of these traits showed a normal distribution, and the large variability among accessions under the control and also under salt treatment (Fig 2.6).

There was a wide difference in the response to salt stress among 296 accessions, indicating a great difference in salt tolerance among Asian landraces and improved cultivars. Significant reductions for NL, SL, RL, SDW, RDW and PDW due to salt stress were observed as 65, 67, 71, 50, 65 and 54%, respectively (Table 2.4). The coefficient of variation among the accessions ranged from 18.3 to 48.7% and from 14.9 to 41% under NaCl treatment and control conditions, showing the highest level of variation. The biomass was affected by NaCl treatment more than the LIS, which showed a 26% variation among accessions. Additionally, the lowest

coefficient of variation within accessions was observed in the LIS, indicating 0-20% in all the accessions.

### **2.3.3. Assessment of salt tolerance**

To assess the environmental interaction effects, ANOVA was performed for both treatments (Table 2.5). Under both conditions, significant variations among accessions, replications and interactions were observed in the traits NL, SL, RL, RDW and PDW, with the exception of SDW and LIS. Under the control, the SDW showed no significant variations among the accessions and interactions, although there was a significant variation of 5% among replications. Under NaCl treatment, SDW showed a non-significant variation among replications. SDW and LIS are only factors without significant variation among accessions, especially under control condition. The SDW ranged from 0.58 to 5.01 g/plant under the control but from 0.28 to 2.80 g/plant under NaCl treatment. A non-significant variation in the LIS was shown among the replications and interactions. The environmental variation, the hydroponic system and even the replications used for this experiment had no influence on the results of the scoring under NaCl treatment.

Salt tolerance was assessed using the leave injury score (LIS) and the STI (SDW) as shown in Fig. 2.7 and Fig. 2.8. The majority of the accessions were categorized as slight to moderately tolerant to NaCl. The average LIS was 2.61 and a standard deviation 0.65. Twenty accessions (Acc. number: 28-104-121-159-188-190-227-228-230-238-253-255-256-259-260-277-325-339-343-344) didn't present necrosis symptoms. A non-significant variation in the LIS was shown among the replications and the interaction. The environmental variation, the hydroponic system and even the replications used for this experiment did not influence the results of the scoring under NaCl treatment. As a result, this trait can be used as a stable trait for assessment for salt tolerance, followed by SDW. LIS is a parameter to assess the salt tolerance for barley germplasm at vegetative stages under 250 mM of NaCl.

A large variation in the response to salt stress was presented by the frequency distribution of the STI (SDW), ranging from 14 to 125% with an average of 54% and a standard deviation of 19 (Fig. 2.7, Table 2.6). The coefficient of variation of the STI (SDW) was 35.8% which was the highest compared to other related traits indicating a great difference in salt tolerance among Asian landraces and improved cultivars.

#### **2.3.4. The correlation between salt tolerance and other traits**

STI (SDW) was found to be strongly associated with biomass production (shoot, root and plant dry weight) under both treatments (Table 2.7). It was also correlated with the RL, SL and NL. STI (SDW) was also found strongly correlated to the STI (SL), STI (RDW), and STI (PDW). LIS was correlated to the NL and the STI (NL) with a correlated coefficient 0.13 and 0.16, respectively. While, LIS is not correlated with the total biomass production under either condition and also not correlated with the salt tolerance indices. No correlation was found between the STI and LIS suggested the genes controlling LIS and STI (SDW) are not linked to one another or one gene do not have pleiotropic effect on other gene. It is most probable due to two different mechanism's controlling the biomass production, necrosis and senescence of leaves under salt treatment.

#### **2.3.5. Classification of accessions by bivariate analysis**

Classification of accessions was obtained based on bivariate analysis obtained by fitting the STI (SDW) to LIS. The accessions were divided into 4 groups consisting of tolerant, slight tolerant, moderate tolerant and susceptible accessions (Fig. 2.9) involving 20, 55, 101 and 120 accessions in each group respectively (Table 2.8). The group one (brown color in Fig. 2.9) presented by 20 tolerant accessions to salt. Among them, 11 accessions (Acc. 188, 190, 227, 228, 230, 238, 253, 255, 256, 259 and 260) were originated from Japan. 101 moderately tolerant accessions are represented by the group 2 (blue color in Fig. 2.9) and are mostly represented by Chinese accessions (42 accessions), Japanese accessions (24 accessions) and Korean accessions (16 accessions). The group 3 represented by 120 susceptible accessions (green color in Fig. 2.9) and is mostly 43 Indian accessions, 40 Chinese accessions and 27 Nepalese accessions. Finally, group 4 represented by 55 slight tolerant accessions (red color in Fig. 2.9). Eleven accessions from Bhutan, 17 accessions from Japan and 16 accessions from Korea are classified as slightly tolerant.

#### **2.3.6. Relationship between salt tolerance and accession characteristics**

The relationship between salt tolerance and some factors such as, origin (Japan, Nepal, China, India, Korea), categories of accessions (landraces/ improved), growth type (spring, winter /facultative), rowed type (two /six rowed type), naked or covered seeds are presented in

the Fig. 2.10. According to the T test, the improved cultivars are more tolerant than landraces (Fig. 2.10A).

The accessions originated from Japan and Bhutan were more tolerant compared to accessions originated from India, China and especially accessions from Nepal showed a significant reduction of biomasses compared to other accessions (Fig. 2.10B). According to the relation between Leaf injury score (LIS) and the origin of accessions (Fig. 2.10F), The accessions originated from Japan showed the lowest LIS, followed by Chinese accessions, indicating that Japanese accessions are more tolerant than accessions originated from others countries.

T tests for the equality of means showed a non-significant variation between winter (Means= 55.2%) and spring (Means= 57.9%) growth habits, with a significant two-tailed value of 0.3. On the other hand, STI (SDW) with a mean value equal to 45.5% for facultative types showed a significant difference at 0.01 levels from both the winter and spring growth habits, respectively.

The two rowed type accessions were tolerant to salt than six rowed type (Fig. 2.10C). The accessions with a facultative growth type were more susceptible than spring and winter type (Fig. 2.10D). There was no significant variation between spring and winter growth type in terms of salt tolerance. Also, there was no relationship between salt tolerance and covered /naked seeds (Fig. 2.10E).

## **2.4. Discussion**

Salt tolerance is a complicated trait. For that reason, different methods (hydroponics, semi-hydroponics, pots, and fields) and many parameters have been previously used to assess barley germplasm for salt tolerance. Assessment under saline field conditions (Xue *et al.* 2009) has various limitations related to the variation induced by such changing environmental factors as soil, heterogeneity and weather conditions. Germination and seedling growth under saline environments are the stages that are widely used to select for salt tolerance of barley genotypes due to their reduced environment effects. Additionally, the hydroponic system is free from the difficulties associated with soil-related stress factors (Chen *et al.* 2005, Ellis *et al.* 2002, Mano and Takeda 1997, Shavrukov *et al.* 2010, Taghipour and Salehi 2008). Consequently, various phenotypic traits have been used for salt tolerance assessments (germination rate, plant height,

root length, biomass production, chlorophyll content, senescence rate, reflectance traits, etc.). The main goal of these studies was to identify determinant traits for the tolerance assessment and least influenced by environmental effects. In this research, the LIS and STI (SDW) were chosen as the determinant traits to assess for salt tolerance at seedling stage. The LIS presented the lowest coefficient of variation within the accessions, and there was no effect of the hydroponic system found, in comparison with the others traits used for assessment. On the whole, stress imposes injuries onto the cellular physiology, resulting in metabolic dysfunction. The leaf injury imposes a negative influence on cell division and the plant growth. Plants prevent the water loss from the cell and protect the cellular proteins by the synthesis of compatibles solutes (Lutts *et al.* 1996). This comprises another specific mechanism to overcome the hyper saline environment, permitting plants to thrive in these conditions by adjusting their internal osmotic status (Roslyakova *et al.* 2011). Decreasing the entry of NaCl into the plants through the  $\text{Na}^+/\text{H}^+$  vacuolar antiporters and sequestering the excess  $\text{Na}^+$  in vacuoles by tonoplast  $\text{Na}^+/\text{H}^+$  antiporters may be the principal mechanisms involved in the protection of plants from salt stress.

By combining the result of the relationship between salt tolerance [STI (SDW) and LIS] and geographical distribution, we concluded that biomass production of the accessions originated from Japan and Bhutan are less affected than others. The Japanese improved varieties are more tolerant than Japanese landraces accessions (Fig. 2.11A). The 2 row types Japanese accessions are improved varieties. The Japanese two-row types showed significantly higher tolerance to NaCl compared with six-row types (Fig. 2.11B). Higher level of tolerance in Japanese two-row type accessions may contribute to higher level of salt tolerance in Japanese accessions compare with other countries. Some allelic regions for salt tolerance may be introduced from 2 row type accessions during breeding for brewing beer program. The 2 row types accessions (Fig. 2.12) especially “New golden”, “Azuma Golden”, “Satsuki Nijo” and “Haruna Nijo” show a high STI (SDW).

Also, the Bhutan accessions which are landraces showed a high level of tolerance to salt, suggesting may a natural selection of some allelic regions of tolerance to salt was conserved from generation to other generation or that tolerance to salt may linked to the origin of accessions and the geographical distribution. Malysheva-Otto *et al.* (2006) and Hadado *et al.* (2009) explained that the high diversity in African and Asian barley accessions was due to

hybridization, natural selection and diversified environments. Malysheva-Otto *et al.* (2006) showed that molecular diversity in barley accessions from various geographic regions worldwide differed with respect to allelic richness, frequency of unique alleles and extent of heterogeneity.

## **2.5 Conclusion**

Two hundred ninety-six Asian barley (*Hordeum vulgare* L.) accessions were assessed for salt tolerance. The experiment was laid out at the seedling stage in a hydroponic solution under control and with a 250 mM NaCl treatment with three replications of four plants each. Salt tolerance was assessed by the salt tolerance indices (STIs) of the number of leaves (NL), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW) and leaf injury score (LIS). This last metric was scored from 1 to 5 according to the severity of necrosis and chlorosis observed on leaves. There was a wide variation in salt tolerance among the Asian barley accessions. The LIS and STI of the SDW were the suitable parameters to assess for salt tolerance at seedling stage because they allow a non-significant variation among accessions under control condition with a non-significant variation among replications under NaCl treatment. Tolerant and susceptible accessions were assessed and used for further study.



**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics.**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
1	TKB64	TKB64	landrace	Okayama U	Bhutan	Drukaldzong	n	6	DL	0	M	*	W
2	TKB69b	TKB69b	landrace	Okayama U	Bhutan	Thinleygang	n	6	DL	0	M	*	S
3	TKB73a	TKB73a	landrace	Okayama U	Bhutan	Khelakha	n	6	DL	0	ME	*	S
5	TKB75a	TKB75a	landrace	Okayama U	Bhutan	Tongsa	n	6	DL	0	M	*	S
6	TKB75c	TKB75c	landrace	Okayama U	Bhutan	Tongsa	n	6	LL	0	M	*	S
7	TKB79a	TKB79a	landrace	Okayama U	Bhutan	Jakar	n	6	DEK	0	MP	*	S
8	TKB80a	TKB80a	landrace	Okayama U	Bhutan	Nangar	n	6	DEK	0	M	*	W
9	TKB80c	TKB80c	landrace	Okayama U	Bhutan	Nangar	n	6	LL	0	M	*	W
11	TKB81c	TKB81c	landrace	Okayama U	Bhutan	Changkha	n	6	DEK	0	M	*	S
12	TKB82b	TKB82b	landrace	Okayama U	Bhutan	Serpuchen	n	6	LL	0	M	*	I
14	TKB82f	TKB82f	landrace	Okayama U	Bhutan	Serpuchen	n	6	LL	0	M	*	S
16	ZDM1187	Dong Ning PI7Hao-1	Improved	ICARDA	China	Heilongjiang	*	6	LL	0	M	S	S
17	ZDM1207	Hu Lan PI 1-2	Improved	ICARDA	China	Heilongjiang	*	6	LL	0	M	S	S
18	ZDM1275	Bai Quan PI 7 Hao	Improved	ICARDA	China	Heilongjiang	*	6	LL	0	MP	S	S
19	ZDM1302	La Lin Luo 1 Hao	Improved	ICARDA	China	Heilongjiang	n	6	DL	0	M	*	S
20	ZDM1386	Ning Cheng Da Mai (1)	Improved	ICARDA	China	Neimeng	n	6	LL	0	M	*	S
21	ZDM1912	Xin Mai 1 Hao	Improved	ICARDA	China	Jiangsu	*	6	LL	0	P	S	S
22	ZDM1980	Jiang Ning 1281	Improved	ICARDA	China	Jiangsu	*	6	LL	0	P	*	I
23	ZDM3727	Gang Tuo Qing Ke 1 hao	Improved	ICARDA	China	Sichuan	n	6	LL	0	M	*	S
24	ZDM380	Tu Da Mai 1	Improved	ICARDA	China	Shandong	*	6	DS	5	MP	*	W
25	ZDM2468	Hu Mai 4 Hao	Improved	ICARDA	China	Shanghai	*	2	DL	0	M	*	S
26	ZDM8252	Jing Pi 198	Improved	ICARDA	China	Beijing	*	6	LL	0	M	*	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
27	ZDM8306	E Dong 85-1	Improved	ICARDA	China	Hubei	*	2	DL	0	M	*	I
28	ZDM8253	Jing Ke 1 Hao	Improved	ICARDA	China	Beijing	n	6	DL	0	ME	*	S
29	ZDM8251	Jing pi C627-6	Improved	Chin Acad As	China	Beijing	*	6	DS	0	M	*	S
30	ZDM8254	Jing luo 2 hao	Improved	Chin Acad As	China	Beijing	n	6	DL	0	M	S	S
31	ZDM8266	Han 85-222	Improved	Chin Acad As	China	Heibe	*	2	DL	0	MP	*	W
32	ZDM8286	Fu 8	Improved	Chin Acad As	China	Nei Mongol	*	2	LL	0	MP	*	S
33	ZDM8271	Hu mai 10 hao	Improved	Chin Acad As	China	Shanghai	*	2	DL	0	ME	*	S
34	ZDM8309	E nong 82-6003	Improved	Chin Acad As	China	Hubei	*	2	DL	0	ME	*	I
35	ZDM8314	E jing 145	Improved	Chin Acad As	China	Hubei	*	2	DL	0	M	*	I
36	OUC001	Vladivostock	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
37	OUC601	Harbin 13-8A	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
38	OUC302	Manchuria Native 1	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
39	OUC003	Fengtien Black	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	*	S
40	OUC603	Pinchiang Chaotung	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	*	S
41	OUC304	Sanchiang Fuchin	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
42	OUC005	Chientao Lungching	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
43	OUC006	Mongolia 6 row	Landrace	Okayama U	China	Mongolia	n	6	LL	0	MP	*	S
44	OUC306	Sanho	Landrace	Okayama U	China	Manchuria	*	6	LL	0	ME	s	S
45	OUC007	Fuchin	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	s	S
46	OUC008	Harbin Native	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	*	S
47	OUC308	Mulan	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	s	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
48	OUC009	Taonan	Landrace	Okayama U	China	Manchuria	*	6	LL	0	MP	s	S
49	OUC609	Tungfeng	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	s	S
50	OUC014	Manchuria 1	Landrace	Okayama U	China	Manchuria	*	6	LL	0	M	*	S
52	OUC015	Hsin Hsien	Landrace	Okayama U	China	Hopei	n	6	DL	0	M	*	S
53	OUC016	Shantung Naked	Landrace	Okayama U	China	Shantung	n	6	DL	0	P	*	W
54	OUC617	Litsun 2	Landrace	Okayama U	China	Tsingtao	*	6	DL	5	P	*	W
55	OUC317	Chiaohsien5	Landrace	Okayama U	China	Tsingtao	*	6	DL	0	P	*	W
56	OUC618	Changtien 1	Landrace	Okayama U	China	Tsingtao	*	6	LL	1	P	*	W
57	OUC318	Pukou 2	Landrace	Okayama U	China	Kiangsu	*	6	LL	0	MP	*	W
58	OUC619	Wuhu	Landrace	Okayama U	China	Anhwei	*	6	LL	5	MP	*	W
59	OUC019	Tatung	Landrace	Okayama U	China	Anhwei	*	6	LL	0	MP	*	W
60	OUC319	Chihchou	Landrace	Okayama U	China	Anhwei	*	6	LL	5	MP	*	W
62	OUC622	Tungliu	Landrace	Okayama U	China	Anhwei	*	6	LL	0	MP	*	W
63	OUC022	Pantse 1	Landrace	Okayama U	China	Kiangsi	n	6	LL	0	M	*	W
64	OUC023	Liussuchiaio 1	Landrace	Okayama U	China	Kiangsi	*	6	LL	5	MP	*	W
65	OUC624	Tawangmiao 1	Landrace	Okayama U	China	Kiangsi	*	6	LL	0	MP	*	W
66	OUC324	Chiuchiang	Landrace	Okayama U	China	Kiangsi	*	6	LL	5	MP	*	W
67	OUC625	Juichang 1	Landrace	Okayama U	China	Kiangsi	*	6	LL	0	MP	*	W
68	OUC627	Mushinchiang 3	Landrace	Okayama U	China	Hupei	*	6	LL	0	MP	*	W
69	OUC628	Yanghsin 3	Landrace	Okayama U	China	Hupei	*	6	LL	0	MP	*	W

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesement and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
70	OUC328	Titienchiao 2	Landrace	Okayama U	China	Hupei	*	6	DL	5	M	*	W
71	OUC329	Paishapu 2	Landrace	Okayama U	China	Hupei	n	6	LL	0	MP	*	W
72	OUC630	Paisha Tayeh 1	Landrace	Okayama U	China	Hupei	*	6	LOB	0	MP	*	W
73	OUC332	Tayeh 4	Landrace	Okayama U	China	Hupei	n	6	LL	0	M	*	W
74	OUC635	Tayeh 11	Landrace	Okayama U	China	Hupei	*	6	LL	5	M	*	W
76	OUC038	Chinniu 1	Landrace	Okayama U	China	Hupei	n	6	LL	0	M	*	W
77	OUC639	Chinniu 3	Landrace	Okayama U	China	Hupei	*	6	LL	5	MP	*	W
78	OUC039	Hsin- antien 1	Landrace	Okayama U	China	Honan	*	6	LL	0	MP	*	W
79	OUC642	Chiaochuang 6	Landrace	Okayama U	China	Honan	*	6	LEK	5	MP	*	W
80	OUC644	Chengchou 5	Landrace	Okayama U	China	Honan	*	6	LEK	5	MP	*	W
81	OUC045	Changchou 1	Landrace	Okayama U	China	Kiangsu	n	6	LL	0	M	*	W
82	OUC646	Suchou 1	Landrace	Okayama U	China	Kiangsu	n	6	LL	0	M	*	W
83	OUC346	Shanghai 1	Landrace	Okayama U	China	Kiangsu	n	6	LL	0	M	*	W
84	OUC047	Shanghai 3	Landrace	Okayama U	China	Kiangsu	n	6	DL	0	M	*	W
85	OUC348	Shanghai 7	Landrace	Okayama U	China	Kiangsu	n	6	DL	0	ME	*	I
86	OUC349	Tohoku Shiro Hadaka	Landrace	Okayama U	China	Manchuria	n	6	DS	0	ME	*	S
87	OUC050	Violaceum Sg Type3	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
90	OUC051	Tibetanum Sh Type 6	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
92	OUC352	Violaceum Sg Type 10	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
94	OUC654	Asiaticum Type 14	Landrace	Okayama U	China	Tibet	n	6	LS	0	M	*	S
95	OUC054	Sikangensa type 15	Landrace	Okayama U	China	Tibet	n	6	LLB	0	M	*	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
98	OUC657	Tibet white 16	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
99	OUC357	Tibet white 25	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
100	OUC059	Tibet violet	Landrace	Okayama U	China	Tibet	n	6	LL	0	M	*	S
101	OUC661	Violaceum 2( china)	Landrace	Okayama U	China	China	*	6	LL	0	MP	*	S
102	OUC662	Itu Native	Landrace	Okayama U	China	Tsingtao	n	6	LL	0	MP	*	S
103	ZDM 100	Guang da mai	Landrace	Chin Acad As	China	Shanxi	n	6	LL	0	M	*	S
104	ZDM 102	Ying chun da mai	Landrace	Chin Acad As	China	Shanxi	n	6	LL	0	M	*	S
105	ZDM 109	Luo ren da mai	Landrace	Chin Acad As	China	Shanxi	*	6	DL	0	M	*	S
106	ZDM 1039	Quan zhi da mai	Landrace	Chin Acad As	China	Shanxi	*	6	DEK	0	P	*	I
107	ZDM 1236	Hai lun pi 4 hao	Landrace	Chin Acad As	China	Heillongjiang	*	6	LL	0	MP	S	S
108	ZDM 1247	Ke shan da mai	Landrace	Chin Acad As	China	Heillongjiang	*	6	LL	0	M	S	S
109	ZDM 1265	Ke shan xi cheng da mai	Landrace	Chin Acad As	China	Heillongjiang	*	6	LL	0	M	S	S
110	ZDM 1456	Ding xi da mai	Landrace	Chin Acad As	China	Gansu	*	6	LL	0	MP	*	S
111	ZDM 2099	Zi gan liu leng	Landrace	Chin Acad As	China	Jiangsu	*	6	DL	0	P	*	I
112	ZDM 2515	Chuan sha wan dai mai	Landrace	Chin Acad As	China	Shanghai	*	6	LL	5	M	*	I
113	ZDM 2523	Zhong da mai	Landrace	Chin Acad As	China	Shanghai	*	6	LL	5	P	*	I
114	ZDM 3447	Liu leng zi da mai	Landrace	Chin Acad As	China	Hubei	*	6	DL	0	MP	*	W
115	ZDM 3515	Lao wu hu xu mai	Landrace	Chin Acad As	China	Hunan	*	2	LL	0	M	*	S
116	ZDM 5202	Wu shen yang cao mai	Landrace	Chin Acad As	China	Nei Mongol	*	2	LL	0	M	S	S
117	ZDM 5204	Wu shen da mai	Landrace	Chin Acad As	China	Nei Mongol	*	6	LL	0	M	*	S
118	ZDM 5205	Dong sheng da mai	Landrace	Chin Acad As	China	Nei Mongol	*	6	LL	0	MP	*	S
120	ZDM 5292	Mii mai	Landrace	Chin Acad As	China	Jiangxi	n	6	LL	3	MP	*	W

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
121		Bilara-2	Improved	Nat Bur Ind	India	India	*	6	LL	0	E	*	S
122		Azad	Improved	Nat Bur Ind	India	India	*	6	LL	0	M	*	S
123		DL 88	Improved	Nat Bur Ind	India	India	*	6	LL	0	M	*	S
125		RD 2052	Improved	Nat Bur Ind	India	India	*	6	LL	0	M	*	S
159	OUI704	Gat	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	I
160	OUI705	Tibba 1	Landrace	Okayama U	India	India	*	6	LL	5	M	*	I
161	OUI706	Sangrambata	Landrace	Okayama U	India	India	*	6	LL	0	M	*	I
162	OUI707	Dul	Landrace	Okayama U	India	India	*	6	LL	0	M	*	I
163	OUI708	Hanswani	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	I
164	OUI409	Kela	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	S
171	OUI417	Sangri	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	I
172	OUI718	Surnji	Landrace	Okayama U	India	India	*	6	LL	0	M	*	S
173	OUI719	Ghaundhar	Landrace	Okayama U	India	India	*	6	LL	0	M	*	S
177	OUI725	Pandukeshwar 2	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	I
179	OUI427	Bijoria	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	S
180	OUI429	Kunjanpur 1	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	S
182	OUI432	Mansinghkanda 4	Landrace	Okayama U	India	India	*	6	DS	0	ME	*	I
183	OUI433	Dhun 1	Landrace	Okayama U	India	India	*	6	LS	0	ME	*	S
184	OUI434	Gauriat 1	Landrace	Okayama U	India	India	*	6	LL	0	ME	*	S
185	OUI436	Timladigi 2	Landrace	Okayama U	India	India	*	6	LL	0	M	*	I
188	OUJ 810	Asahi 19	Improved	Okayama U	Japan	Dainippon Brew	*	2	DL	0	ME	*	S
189	OUJ 516	Satsuki Nijo	Improved	Okayama U	Japan	Sapporo Brew	*	2	LL	0	ME	s	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
190	OIJ 220	Fuji Nijo	Improved	Okayama U	Japan	Kirin Brew	*	2	DL	0	M	*	S
191	OIJ 520	Hinode Hadaka	Improved	Okayama U	Japan	Tottori AES	n	6	LL	0	M	*	W
192	OIJ 820	Kikai Hadaka	Improved	Okayama U	Japan	Tokaikinki AES	n	6	LL	0	M	*	I
193	OIJ 221	Nanpuu Hadaka	Improved	Okayama U	Japan	Shikoku AES	n	6	LL	5	M	*	W
194	OIJ 829	Hoshimasari	Improved	Okayama U	Japan	Kitami AES	*	2	DL	0	M	*	S
195	OIJ 530	Benkeimugi	Improved	Okayama U	Japan	Fukushima AES	*	6	DL	0	ME	*	W
197	OIJ 232	New Golden	Improved	Okayama U	Japan	Tochigo AES	*	2	DL	0	MP	*	S
198	OIJ 832	Azuma Golden	Improved	Okayama U	Japan	Tochigo AES	*	2	DL	0	ME	*	S
199	OIJ 235	Daisen Gold	Improved	Okayama U	Japan	Tottori AES	*	2	DL	0	ME	*	S
200	OIJ 539	Shiratama Hadaka	Improved	Okayama U	Japan	Shikoku AES	n	6	LL	0	MP	*	W
201	OIJ 241	Amagi Nijo 3	Improved	Okayama U	Japan	Kirin Brew	*	2	DL	0	ME	s	S
202	OIJ 246	Senbon Hadaka	Improved	Okayama U	Japan	Shikoku AES	*	6	LL	0	M	*	W
203	OIJ 546	Ishuku Shirazu	Improved	Okayama U	Japan	Kyushu AES	*	2	DL	0	ME	*	S
204	OIJ 846	Kawamizuki	Improved	Okayama U	Japan	Kyushu AES	*	2	DL	0	M	*	S
205	OIJ 247	Haruna Nijo	Improved	Okayama U	Japan	Tochigo AES	*	2	DL	0	ME	*	S
206	OIJ 848	Misato Golden	Improved	Okayama U	Japan	Tochigo AES	*	2	DL	0	ME	*	S
208	OIJ 857	Asamamugi	Improved	Okayama U	Japan	Nagano AES	*	6	DL	5	MP	*	S
210	OIJ 262	Hayate Hadaka	Improved	Okayama U	Japan	Shikoku AES	n	6	LL	0	M	*	W
212	OIJ 606	Mitsukiko 1	Landrace	Okayama U	Japan	Hokkaido	n	6	LL	0	M	*	S
213	OIJ 008	Hosogara 2	Landrace	Okayama U	Japan	Aomari	*	6	LL	0	MP	*	S
216	OIJ 016	Gozen	Landrace	Okayama U	Japan	Akita	*	6	LL	0	M	*	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
217	OIJ 318	Sangatsu	Landrace	Okayama U	Japan	Yamagata	*	6	LL	5	ME	*	W
218	OIJ 324	Zenkoji	Landrace	Okayama U	Japan	Niigata	*	6	DS	0	MP	*	W
219	OIJ 329	Hachikoku	Landrace	Okayama U	Japan	Fukui	*	6	DS	5	M	*	W
220	OIJ 030	Chikurin Ibaraki 3	Landrace	Okayama U	Japan	Ibaraki	*	6	DL	5	M	*	W
221	OIJ 331	Tochigi Bozu 1	Landrace	Okayama U	Japan	Tochigi	*	6	DSB	9	ME	*	W
222	OIJ 632	Bizen Wase 5	Landrace	Okayama U	Japan	Gunma	*	6	DS	5	M	*	W
223	OIJ 334	Honen	Landrace	Okayama U	Japan	Gunma	n	6	DS	0	M	*	W
224	OIJ 636	Hozoroi	Landrace	Okayama U	Japan	Chiba	*	6	DL	5	M	*	S
225	OIJ 641	Dairokkaku	Landrace	Okayama U	Japan	Nagano	*	6	DS	5	M	*	S
227	OIJ 647	Akashinriki	Landrace	Okayama U	Japan	Shizuoka	n	6	LL	0	M	*	S
228	OIJ 657	Kinai Nita Hadaka	Landrace	Okayama U	Japan	Osaka	n	6	LL	5	ME	*	S
229	OIJ 360	Nara Hakumai 1	Landrace	Okayama U	Japan	Nara	n	6	LL	0	M	*	S
230	OIJ 361	Shinrikimugi	Landrace	Okayama U	Japan	Wakayama	n	6	LL	0	M	*	I
231	OIJ 064	Hayakiso 2	Landrace	Okayama U	Japan	Shimane	*	6	DLB	9	ME	*	W
232	OIJ 367	Zairai Tanbo	Landrace	Okayama U	Japan	Okayama	*	6	DL	5	M	*	S
237	OIJ 382	Katano	Landrace	Okayama U	Japan	Saga	*	6	DL	5	M	*	S
238	OIJ 384	Kairyo Ogara	Landrace	Okayama U	Japan	Nagasaki	*	6	DL	5	ME	*	S
239	OIJ 386	Hachikoku	Landrace	Okayama U	Japan	Kumamoto	*	6	DS	5	M	*	W
241	OIJ 092	Kagoshima Kamaore 1	Landrace	Okayama U	Japan	Kagoshima	n	6	LL	0	M	*	I
242	OIJ 693	Nigatsuko	Landrace	Okayama U	Japan	Kagoshima	*	6	LL	5	M	*	S
243	OIJ 715	Tanikaze	Landrace	Okayama U	Japan	Miyazaki	*	6	DL	5	M	*	S
244	OIJ 116	Niho	Landrace	Okayama U	Japan	Kanagawa	*	6	DS	5	M	*	S



**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
245	OIJ717	Yanagiho	Landrace	Okayama U	Japan	Ishikawa	*	6	LSB	5	M	*	S
246	OIJ 719	Shirozasa	Landrace	Okayama U	Japan	Yamanashi	*	6	DL	5	M	*	S
247	OIJ 721	Mitori	Landrace	Okayama U	Japan	Tochigi	*	6	LS	5	M	*	W
248	OIJ 725	Takayama Sangatsu 1	Landrace	Okayama U	Japan	Iwate	*	6	LL	9	MP	*	W
249	OIJ 726	Kesajiro	Landrace	Okayama U	Japan	Niigata	*	6	LL	5	MP	*	W
250	OIJ 730	Kuiamari	Landrace	Okayama U	Japan	Hiroshima	*	6	DSB	5	M	*	W
251	OIJ 740	Murasakimugi	Landrace	Okayama U	Japan	Yamanashi	*	6	DL	5	MP	*	W
252	OIJ 148	Kome Hadaka	Landrace	Okayama U	Japan	Nara	n	6	LL	0	M	*	S
253	OIJ 750	Shiromiyuki	Landrace	Okayama U	Japan	Ehime	n	6	LL	0	M	*	I
254	OIJ 152	Isejiro	Landrace	Okayama U	Japan	Mie	n	6	DS	0	M	*	I
255	OIJ 752	Tanbajiro	Landrace	Okayama U	Japan	Hyogo	n	6	DS	0	M	*	I
256	OIJ 755	Ohichi	Landrace	Okayama U	Japan	Hiroshima	n	6	LL	0	M	*	I
257	OIJ 761	Osome	Landrace	Okayama U	Japan	Hyogo	n	6	DL	0	M	*	W
258	OIJ 771	Saruho	Landrace	Okayama U	Japan	Nagano	n	6	LLB	0	ME	*	I
259	OIJ 776	Takayama	Landrace	Okayama U	Japan	Ehime	n	6	DS	5	M	*	I
260	OIJ 783	Tokushima Mochimugi 1	Landrace	Okayama U	Japan	Tokushima	n	6	LL	0	M	*	W
262		Tapgolbori	Improved	CropES Korea	Korea	WB Res Inst	*	6	DL	1	MP	*	W
263		saeolbori	Improved	CropES Korea	Korea	WB Res Inst	*	6	DL	0	M	*	S
264		Kangbori	Improved	CropES Korea	Korea	Crop Exp Sta	*	6	DL	3	M	*	S
265		Albori	Improved	CropES Korea	Korea	Yeongnam CES	*	6	DL	3	M	*	I
266		Buhobori	Improved	CropES Korea	Korea	Yeongnam CES	*	6	DL	2	M	*	S
267		Namhaebori	Improved	CropES Korea	Korea	Yeongnam CES	*	6	DL	3	M	*	S
268		Alchanbori	Improved	CropES Korea	Korea	Yeongnam CES	*	6	DL	5	MP	*	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
270		Bunong	Improved	CropES Korea	Korea	Crop Exp Sta	*	6	DL	5	M	*	W
271		Hangmi	Improved	CropES Korea	Korea	Crop Exp Sta	*	6	DL	0	M	*	W
272		Suwan # 4	Improved	CropES Korea	Korea	Crop Exp Sta	*	6	DS	5	MP	*	W
273		Samdogjeonbug # 45	Improved	CropES Korea	Korea	Crop Exp Sta	*	6	DS	9	M	*	W
274		Doosan # 8	Improved	CropES Korea	Korea	DoosanFarm Co	*	2	LL	0	M	*	S
275		Hyanmaeg	Improved	CropES Korea	Korea	Crop Exp Sta	*	2	LL	0	ME	S	S
276		Muanbori	Improved	CropES Korea	Korea	WB Res Inst	n	6	DL	0	M	*	I
277		saessalbori	Improved	CropES Korea	Korea	Honan CES	n	6	LS	0	M	*	I
278		Nulssalbori	Improved	CropES Korea	Korea	Honan CES	n	6	LL	0	MP	*	W
279		Naehanssalbori	Improved	CropES Korea	Korea	Honan CES	n	6	DL	0	M	*	W
280		Cheongmaeg	Improved	CropES Korea	Korea	Gyeongnam PRDA	n	6	DL	0	M	*	W
282	OUK 602	Jangheung Naked 2	Landrace	Okayama U	Korea	Jeorandum	n	6	DL	5	MP	*	W
283	OUK 304	Boseong Covered 3	Landrace	Okayama U	Korea	Jeorandum	*	6	DLB	5	MP	*	W
284	OUK 606	Hwasun Covered 2	Landrace	Okayama U	Korea	Jeorandum	*	6	LL	5	MP	*	W
285	OUK 611	Gwangju Covered 5	Landrace	Okayama U	Korea	Jeorandum	*	6	LL	0	MP	*	W
286	OUK 613	Gogdeong Naked 4	Landrace	Okayama U	Korea	Jeorandum	n	6	DL	5	MP	*	W
287	OUK 315	Yeonggwang Naked 1	Landrace	Okayama U	Korea	Jeorandum	n	6	DL	0	MP	*	W
288	OUK 318	Jecheon 5	Landrace	Okayama U	Korea	Gyeongsnagname	*	6	DS	5	M	*	W
289	OUK 320	Jinan Dohadaka	Landrace	Okayama U	Korea	Jeorabug	n	6	DL	0	MP	*	W
290	OUK 024	Buan Waessalbori	Landrace	Okayama U	Korea	Jeorabug	n	6	LL	0	M	*	W
291	OUK 324	Geumseong Native	Landrace	Okayama U	Korea	Jeorabug	n	6	LL	0	MP	*	W
292	OUK 627	Buyong Naked 3	Landrace	Okayama U	Korea	Jeorabug	n	6	LL	5	MP	*	S

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
293	OUK 331	Jeonju Native	Landrace	Okayama U	Korea	South K AES	*	6	DL	5	P	*	W
294	OUK 632	Gyeongyug	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DS	5	M	*	W
295	OUK638	Tongyeong Covered 1	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DL	5	M	*	S
296	OUK 340	Jingyo Naked 1	Landrace	Okayama U	Korea	Gyeongsnagnam	n	6	DL	5	M	*	W
297	OUK 341	Sacheon Naked	Landrace	Okayama U	Korea	Gyeongsnagnam	n	6	DL	0	MP	*	W
298	OUK 043	Masan Naked 1	Landrace	Okayama U	Korea	Gyeongsnagnam	n	6	LL	9	M	*	S
299	OUK 344	Donglae Waedong	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DS	5	M	*	W
300	OUK 647	Namji Milyang Native	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DS	5	MP	*	S
301	OUK 053	Jinju Native	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DL	5	M	*	W
302	OUK 653	Jinju Naked	Landrace	Okayama U	Korea	Gyeongsnagnam	n	6	DL	5	MP	*	W
303	OUK 358	hayang Jecheon 5	Landrace	Okayama U	Korea	Gyeongsnagnam	*	6	DS	5	MP	*	W
304	OUK 661	Pohang Naked 2	Landrace	Okayama U	Korea	Gyeongsnagnam	n	6	DL	0	M	*	W
305	OUK 366	Jeomchon Covered 1	Landrace	Okayama U	Korea	Chungcheongnam	*	6	LL	0	M	*	S
306	OUK 073	Janghang Shirodo	Landrace	Okayama U	Korea	Chungcheongnam	n	6	DL	0	M	*	W
307	OUK 680	Haemi Covered 4	Landrace	Okayama U	Korea	Chungcheongnam	*	6	DL	5	MP	*	W
308	OUK 082	Asan Jungbori	Landrace	Okayama U	Korea	Chungcheongnam	*	6	DL	0	M	*	S
309	OUK 684	Seonghwan Gyeonggi N.3	Landrace	Okayama U	Korea	Chungcheongnam	n	6	DL	0	ME	*	I
310	OUK 385	Neulbori	Landrace	Okayama U	Korea	Chungcheongnam	*	6	DSB	5	M	*	W
311	OUK 386	Rogbori	Landrace	Okayama U	Korea	Chungcheongnam	*	6	DL	0	MP	*	W
312	OUK 389	Shirodo	Landrace	Okayama U	Korea	Chungcheongnam	n	6	DL	5	MP	*	S
313	OUK 690	Ogcheon Seungmaeg	Landrace	Okayama U	Korea	Chungcheongbug	*	6	DS	5	MP	*	W
314	OUK 395	Jugsan Jungbori Native	Landrace	Okayama U	Korea	Gyeonggi	*	6	DS	5	MP	*	W

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
315	OUK 396	Icheon Naked	Landrace	Okayama U	Korea	Gyeonggi	n	6	LL	9	M	*	S
316	OUK 397	Euijeongbu Seungmaeg 1	Landrace	Okayama U	Korea	Gyeonggi	*	6	DL	5	MP	*	W
317	OUK 400	Ongjin Covered 1	Landrace	Okayama U	Korea	Hwanghae	*	6	DL	5	MP	*	W
318	OUK 403	Sariweon Yungmobori 1	Landrace	Okayama U	Korea	Hwanghae	*	6	LL	0	M	*	I
319	OUK 404	Senshutsu 18	Landrace	Okayama U	Korea	West K EAS	*	6	DL	0	P	*	S
321	OUK 711	Hwacheon Native	Landrace	Okayama U	Korea	Gangweon	*	6	LL	0	P	*	S
322	OUK 712	Hong cheon Native	Landrace	Okayama U	Korea	Gangweon	*	6	LS	0	MP	*	W
323	OUK 714	Suncheon Native	Landrace	Okayama U	Korea	Pyonganam	*	6	LL	0	M	s	S
324	OUK 415	Zairai Shiro	Landrace	Okayama U	Korea	Pyonganbug	*	6	LL	0	MP	*	I
325	OUK 417	Pungsan Native	Landrace	Okayama U	Korea	North K EAS	*	6	LL	5	M	*	I
326	OUK 418	Anbyeon Native	Landrace	Okayama U	Korea	Hamgyongnam	*	6	LLB	5	P	*	S
327	OUK 421	Harumaki Domugi	Landrace	Okayama U	Korea	North Korea	*	6	DL	0	ME	*	S
328	OUK 422	Gyeongseong Native	Landrace	Okayama U	Korea	North Korea	*	6	LL	0	M	s	S
329	OUK 436	Gupo Covered 2	Landrace	Okayama U	Korea	Gyeongsangnam	*	6	DS	0	M	*	W
330	OUK 442	Cheongweon Native	Landrace	Okayama U	Korea	Chungcheongbug	*	6	LS	5	M	*	W
331	OUN 308	Gho 1	Landrace	Okayama U	Nepal	Nepal	n	6	DLB	0	M	*	S
332	OUN 309	Gho 4	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
333	OUN 610	Thonje 7	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	ME	*	I
334	OUN 313	Tilman Camp 3	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	M	*	S
335	OUN 615	Annapura B.C.1	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	M	*	S
336	OUN 016	Pisang 1	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	s	S
337	OUN 017	Thangja 1	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	M	*	I

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued).**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
338	OUN 018	Katmandu 1	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
339	OUN 619	Bimtakothi 9	Landrace	Okayama U	Nepal	Nepal	n	6	DL	0	M	*	S
342	OUN 325	Annapurna 1	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	ME	*	S
343	OUN 327	Katmandu 5	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	M	*	I
344	OUN 328	Kakani Bangalow 2	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
345	OUN 630	Macha Khola 1	Landrace	Okayama U	Nepal	Nepal	*	6	LS	0	ME	*	I
346	OUN 031	Birkna Camp 1	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
347	OUN 333	Chame 1	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	ME	*	I
348	OUN 637	Thonje 21	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	ME	*	I
350	OUN 041	Ngyak 6	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
351	OUN 344	Prok 1	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	M	*	I
352	OUN 645	Tsumje 1	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	MP	*	W
354	OUN 647	Dhumpu 1	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
355	OUN 048	Chame 11	Landrace	Okayama U	Nepal	Nepal	n	6	LNK	0	M	*	S
356	OUN 350	Tukucha	Landrace	Okayama U	Nepal	Nepal	n	6	LL	0	ME	*	S
357	OUN 050	Ghara 2	Landrace	Okayama U	Nepal	Nepal	n	6	LLB	0	ME	*	S
360	OUN 353	Sikha1	Landrace	Okayama U	Nepal	Nepal	n	6	DSB	0	M	*	S
361	OUN 356	Ulleri 2	Landrace	Okayama U	Nepal	Nepal	*	6	DS	0	ME	*	S
362	OUN 360	Thomje 4	Landrace	Okayama U	Nepal	Nepal	n	6	DEKB	0	M	*	S
363	OUN 061	Keronja 2	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	M	*	I
364	OUN 664	Sikha 12	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	S
366	OUN 367	Ulleri 10	Landrace	Okayama U	Nepal	Nepal	*	6	LOB	0	ME	*	S
367	OUN 669	Lumley 2	Landrace	Okayama U	Nepal	Nepal	*	6	LL	0	ME	*	I
368	OUN 371	Ulleri 20	Landrace	Okayama U	Nepal	Nepal	*	6	LSB	0	ME	*	S
369	OUN 676	Nepal 5	Landrace	Okayama U	Nepal	Nepal	n	6	LLB	0	ME	*	S
370	OUN 381	Sipche 14	Landrace	Okayama U	Nepal	Nepal	*	6	LS	0	ME	*	I

**Table 2.1. List of barley (*Hordeum vulgare* L.) accessions used for the salt tolerance assesment and their morphological characteristics (Continued)**

BCCEA	ID	Name	Category	Source	Country	Origin	Cov/Na	R.T.	Ear awn	L.S.H.	S.T.	R.H	Spr or Win
371	TKN 14a	TKN 14a	Landrace	Okayama U	Nepal	Gaulen	*	6	DS	0	ME	*	I
372	TKN 14f	TKN 14f	Landrace	Okayama U	Nepal	Gaulen	*	6	DS	0	ME	*	I
373	TKN 21a	TKN 21a	Landrace	Okayama U	Nepal	Hille	n	6	DL	0	M	*	S
374	TKN 22a	TKN 22a	Landrace	Okayama U	Nepal	Hille	*	6	LL	0	ME	*	I
375	TKN 23a	TKN 23a	Landrace	Okayama U	Nepal	Sabat	n	6	LEK	0	ME	*	S
376	TKN 24b	TKN 24b	landrace	Okayama U	Nepal	Sabat	*	6	LL	0	ME	*	I
377	TKN 25b	TKN 25b	landrace	Okayama U	Nepal	Jhilimarang	*	6	DS	0	ME	*	I
378	TKN 29a	TKN 29a	landrace	Okayama U	Nepal	Mohariya	*	6	LSB	0	ME	*	I
379	TKN 33b	TKN 33b	landrace	Okayama U	Nepal	Ghandrung	*	6	LLB	0	ME	*	I
380	TKN 37a	TKN 37a	landrace	Okayama U	Nepal	Ghandrung	*	6	DL	0	ME	*	I

**Legend:**

BCCEA= indicates code number of the Institute of Plant Science and Resources, Okayama University. A total of 296 accessions were chosen from 380 accessions; Cov/Na = Covered (\*) /Naked (n); R.T= 2 Row type (2), 6 row types (6), L= labile or irregular and D= deficiens

L.S.H= Low leaf sheath hair (9=numerous; 5=medium; 1=sparse and 0=absent)

S.T. = Seedling type (E= erect; ME= semi erect; M=Medium; MP=semi prostate; P=prostrate)

Spr/Win/I = spring, winter or facultative type.

	Ear type ( density)	
	Lax	Dense
Long awned	LL	DL
Short awned	LS	DS
(Normal) hooded	LNK	DNK
Elevated hooded	LEK	DEK
Subjected hooded	LSK	DSK
Long awned in the central row, and awletted or awnless in lateral rows	LLB	DLB
Short awned in the central row, and awletted or awnless in lateral rows	LSB	DSB
Awnless or awletted in central and lateral rows	LOB	DOB
Elevated hoods in central row, and awnless in lateral rows	LEKB	DEKB

**Table 2.2. Number of accessions used in this study according to their origin.**

Origin	Categories		Row type		Caryopsis		Growth habit			Total
	Improved	Landrace	Two	Six	Covered	Naked	Spring	Winter	Facultative	
<b>Buthan</b>	0	11	0	11	0	11	7	3	1	<b>11</b>
<b>China</b>	20	75	9	86	65	30	52	34	9	<b>95</b>
<b>India</b>	4	16	0	20	20	0	11	0	9	<b>20</b>
<b>Japan</b>	20	41	12	49	40	21	30	22	9	<b>61</b>
<b>Korea</b>	18	48	2	64	45	21	21	38	7	<b>66</b>
<b>Nepal</b>	0	43	0	43	29	14	18	1	24	<b>43</b>
<b>Total</b>	<b>62</b>	<b>235</b>	<b>23</b>	<b>273</b>	<b>199</b>	<b>97</b>	<b>139</b>	<b>98</b>	<b>59</b>	<b>296</b>

**Table 2.3. Nutrient composition used for the hydroponic culture (%).**

	TN(AN/NN)	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	MgO	MnO	B <sub>2</sub> O <sub>3</sub>	CaO (Ca)	Fe	Cu	Zn	Mo
<b>Otsuka 1</b>	10.0 (1.5/8.2)	8.0	27.0	4.0	0.10	0.10	-	0.18	0.002	0.006	0.002
<b>Otsuka 2</b>	11.0 (NN)	-	-	-	-	-	23.0 (16.4)	-	-	-	-

TN: total nitrogen, AN: ammonium nitrogen, NN: Nitrate nitrogen



**Table 2.4. Variation in various growth traits determined after 17 days of testing with 250 mM of NaCl treatment or the control.**

Trait	Treatment	Min	Max	Means	SD	CV among acc. (%)	CV within acc. (%)
LIS	NaCl	1	5	2.61	0.66	25.63	0-20
SL (cm)	NaCl	11.50	46.40	25.96	4.76	18.33	4-29
SL (cm)	Control	20.60	55	38.25	5.73	14.99	2-21
RL (cm)	NaCl	6.4	43.80	17.06	4.09	23.97	6-44
RL (cm)	Control	8.3	51.60	23.74	6.81	28.69	5-32
NL	NaCl	2	11	4.21	1.36	32.33	0-49
NL	Control	3	15	6.42	1.69	26.39	0-36
SDW (g/plant)	NaCl	0.28	2.80	1.06	0.52	48.68	5-70
SDW (g/plant)	Control	0.58	5.01	2.12	0.87	40.95	6-50
RDW (g/plant)	NaCl	0.12	1.02	0.48	0.21	44.45	9-74
RDW (g/plant)	Control	0.25	1.52	0.73	0.26	36.01	6-64
PDW (g/plant)	NaCl	0.42	3.84	1.54	0.69	45.04	8-74
PDW (g/plant)	Control	0.83	6.33	2.85	1.08	38.01	6-42

LIS: leaf injury score; SL: shoot length; RL: root length; NL: number of leaves; SDW: shoot dry weight; RDW: root dry weight; PDW: plant dry weight; min: minimum value; max: maximum value; Means: average of 12 values; SD: standard deviation; CV: coefficient of variance within and among accessions (%).

**Table 2.5. Analysis of variance summaries (Mean Square) of data on the seedling growth of barley under control and NaCl treatment conditions.**

<b>Treatment</b>	<b>VS</b>	<b>df</b>	<b>NL</b>	<b>SL</b>	<b>RL</b>	<b>SDW</b>	<b>RDW</b>	<b>PDW</b>	<b>LIS</b>
<b>Total</b>	Treatment	1	7777.2 **	381426.4 **	70953.8 **	32.5 **	26.6 **	1074.9 **	-
	Accession	295	28.0 **	254076.3 **	108457.0 **	26.6 **	102.6NS	17.6NS	-
	Replication	2	395.5 **	605.8NS	1674.0 **	3.5 **	10.4 **	222.8 **	-
<b>Control</b>	Accession	295	1436.2 **	274.1**	409.1 **	12.0NS	32.7 **	12.1 **	-
	Replication	2	6114.8 **	7920.7 **	858.1 **	69.3 *	364.7 **	112.8 **	-
	Interaction	590	1022.6 **	49.1**	46.5 **	11.9NS	28.8 **	10.7 **	-
<b>NaCl</b>	Accession	295	1452.7 **	156.4 **	92.9 **	64.9 **	15.4 **	3.7 **	32.4 **
	Replication	2	11559.0 **	206.0 **	1921.0 **	64.2NS	93.7 **	46.0 **	22.7NS
	Interaction	590	1225.0 **	44.2 **	27.6 **	59.4 **	13.9 **	5.2 **	23.8NS
<b>STI</b>	Accession	295	1540.8 **	610.1**	3098.8 **	4381.2 **	4648.6 **	4077.7 **	-
	Replication	2	191539.0 **	19653.6 **	62492.0 **	156745.0 **	164101.0 **	157120.0 **	-

VS: Variance source; df: degree of freedom; NL: Number of leaves; SL: Shoot length; RL: Root length; SDW: Shoot dry weight; RDW: Root dry weight; PDW: Plant dry weight; LIS: Leaf injury score

\*, \*\*, NS: significant at the 0.05, 0.01 levels and non-significant at the 0.05 level.

**Table 2.6. Salt tolerance index (STI) of the related traits.**

<b>Trait</b>	<b>Min</b>	<b>Max</b>	<b>Means</b>	<b>SD</b>	<b>CV among acc. (%)</b>
STI ( SL)	50.36	91.99	68.12	7.61	11.17
STI (RL )	43.27	134.76	74.81	16.42	21.94
STI (NL )	36.84	92.19	66.87	11.13	16.65
STI (SDW)	14.30	125.89	<b>54.57</b>	19.54	<b>35.80</b>
STI ( PDW)	16.46	124.55	58.06	19.35	33.32
STI ( RDW)	22.07	121.75	68.81	20.45	29.72

**Table 2.7. Correlation coefficients (*r*) among the traits, the leaf injury score and salt tolerance index of shoot dry weight under 250 mM of NaCl treatment and the control.**

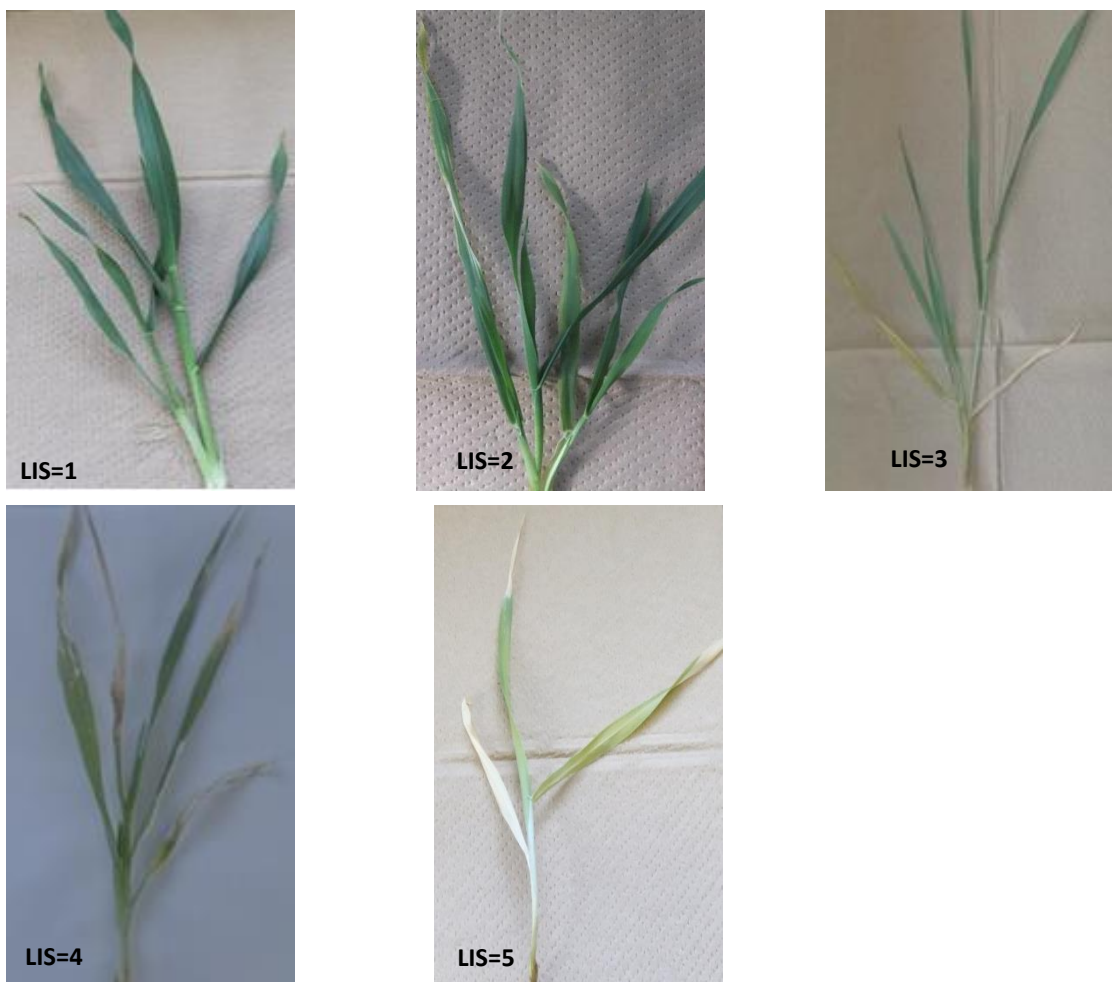
Trait	Treatment	LIS	STI (SDW)	SDW	
				Control	NaCl
LIS	NaCl	1	NS	NS	NS
SL (cm)	Control	NS	-0.33**	0.65**	0.35**
SL (cm)	NaCl	NS	-0.16**	0.42**	0.30**
RL (cm)	Control	NS	-0.16**	0.39**	0.30**
RL (cm)	NaCl	NS	NS	0.26**	0.35**
NL	Control	NS	NS	0.53**	0.42**
NL	NaCl	-0.13*	0.30**	0.24**	0.62**
SDW(g/plant)	Control	NS	-0.62**	1	0.43**
SDW (g/plant)	NaCl	NS	0.40**	0.43**	1
PDW (g/plant)	Control	NS	-0.61**	0.99**	0.43**
PDW (g/plant)	NaCl	NS	0.40**	0.43**	0.99**
RDW (g/plant)	Control	NS	-0.53**	0.88**	0.37**
RDW (g/plant)	NaCl	NS	0.37**	0.37**	0.86**
STI (SL)	-	NS	0.41**	-0.13 *	NS
STI (RL)	-	NS	0.12 *	-0.22**	NS
STI (NL)	-	0.16**	0.22**	-0.31**	0.16**
STI (SDW)	-	NS	1	-0.62**	0.38**
STI (RDW)	-	NS	0.84**	-0.45**	0.43**
STI (PDW)	-	NS	0.99**	-0.59**	0.39**

LIS: Leaf injury score; STI: Salt tolerance index; SDW: Shoot dry weight.

\*, \*\*, NS: significant at the 0.05, 0.01 level and non-significant at the 0.05 level.

**Table 2.8. List of accessions according to the tolerance to salt.**

<b>Salt tolerance</b>	<b>Accessions number</b>
<b>Tolerant:</b> <b>20 accessions</b>	28-104-121-159-188-190-227-228-230-238-253-255-256-259- 260-277-325-339-343-344
<b>Slight tolerant:</b> <b>55 accessions</b>	1-2-3-6-7-8-9-11-14-16-18-19-22-24-25-26-27-29-30-31-32- 197-198-200-204-205-206-212-213-216-217-218-219-220-221- 222-223-225-262-265-266-273-274-275-279-285-286-286-290- 303-307-310-314-319-374
<b>Moderate:</b> <b>101 accessions</b>	5-17-23-35-36-37-38-39-41-42-43-46-49-56-58-64-66-67-68-69- 72-77-82-84-92-98-99-100-101-102-103-106-107-108-109-110- 113-114-116-117-118-120-122-123-125-177-182-184-185-189- 192-194-195-199-201-202-208-224-231-232-237-242-243-244- 247-248-249-250-251-252-254-257-258-264-267-268-278-280- 282-284-288-291-299-323-324-326-327-328-329-330-331-332- 333-334-335-336-351-355-360-364-372-379
<b>Susceptible:</b> <b>120 accessions</b>	12-20-21-33-34-40-44-45-47-48-50-52-53-54-55-57-59-60-62- 63-65-70-71-73-74-76-78-79-80-81-83-84-85-87-90-95-96-105- 112-119-115-160-161-163-164-169-171-172-173-179-180-183- 191-193-229-239-241-245-246-263-270-271-272-276-283-287- 289-292-293-294-295-296-297-298-300-301-302-303-304-305- 306-308-309-311-312-313-315-316-317-318-321-322-337-338- 342-345-346-347-348-350-352-354-356-358-361-362-363-366- 367-368-369-370-371-373-375-376-377-378-380



**Fig. 2.1. Leaf injury scores of barley seedlings under salt stress condition.**

**Legend**

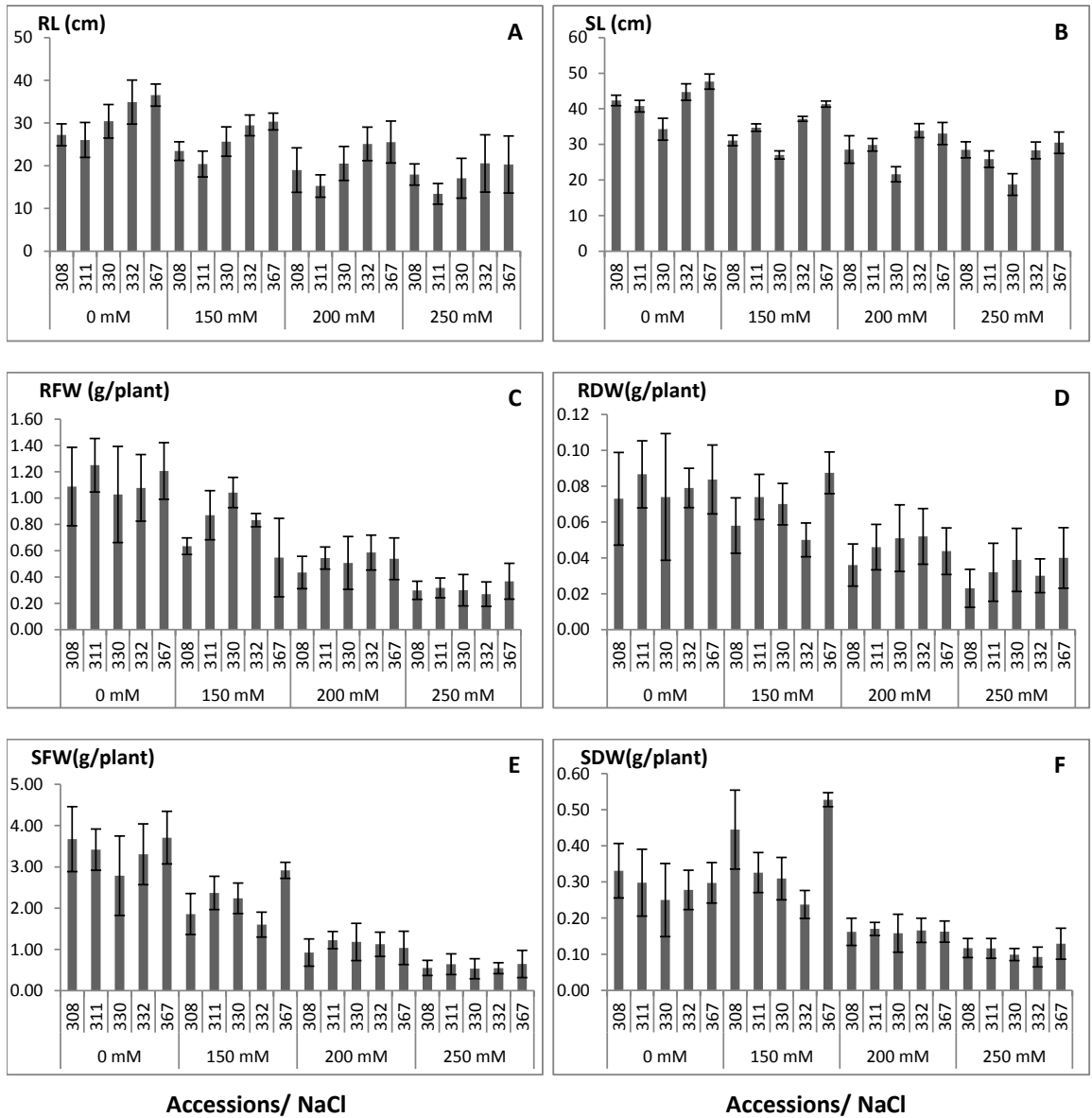
**LIS= 1** (no apparent chlorosis)

**LIS= 2** (slight: 25% of the leaves showed chlorosis);

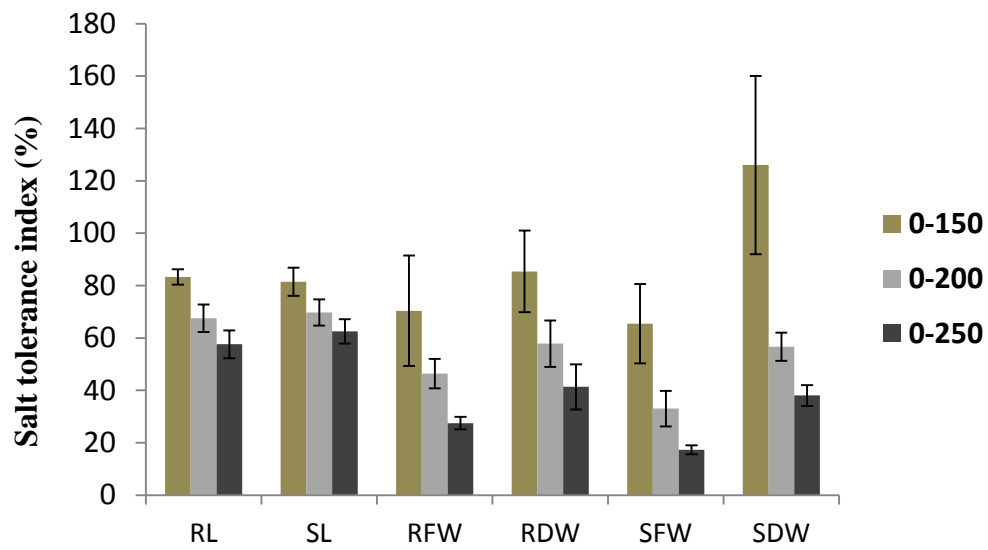
**LIS= 3** (moderate: 50% of the leaves showed chlorosis and some necrosis);

**LIS= 4** (severe chlorosis: 75% of the leaves showed chlorosis and severe necrosis)

**LIS= 5** (dead: leaves showed severe necrosis and were withered)

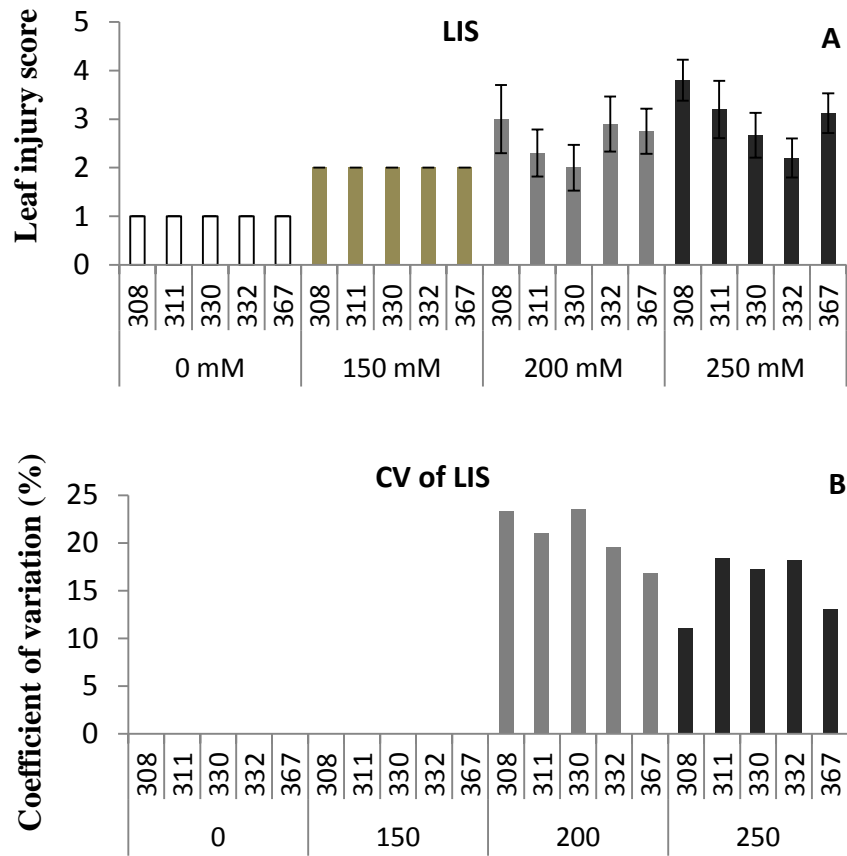


**Fig. 2.2. Root length (A), shoot length (B), root fresh weight (C), root dry weight (D), shoot fresh weight (E) and shoot dry weight (F) of 5 accessions under control, 150, 200 and 250 mM of NaCl.**

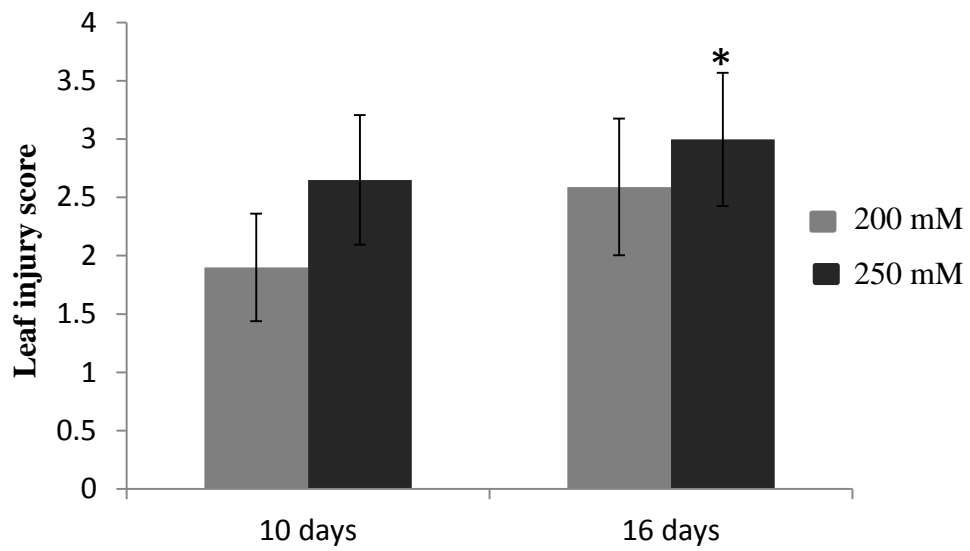


**Fig. 2.3. Salt tolerance index of the traits under treatments 150, 200 and 250 mM of NaCl.**



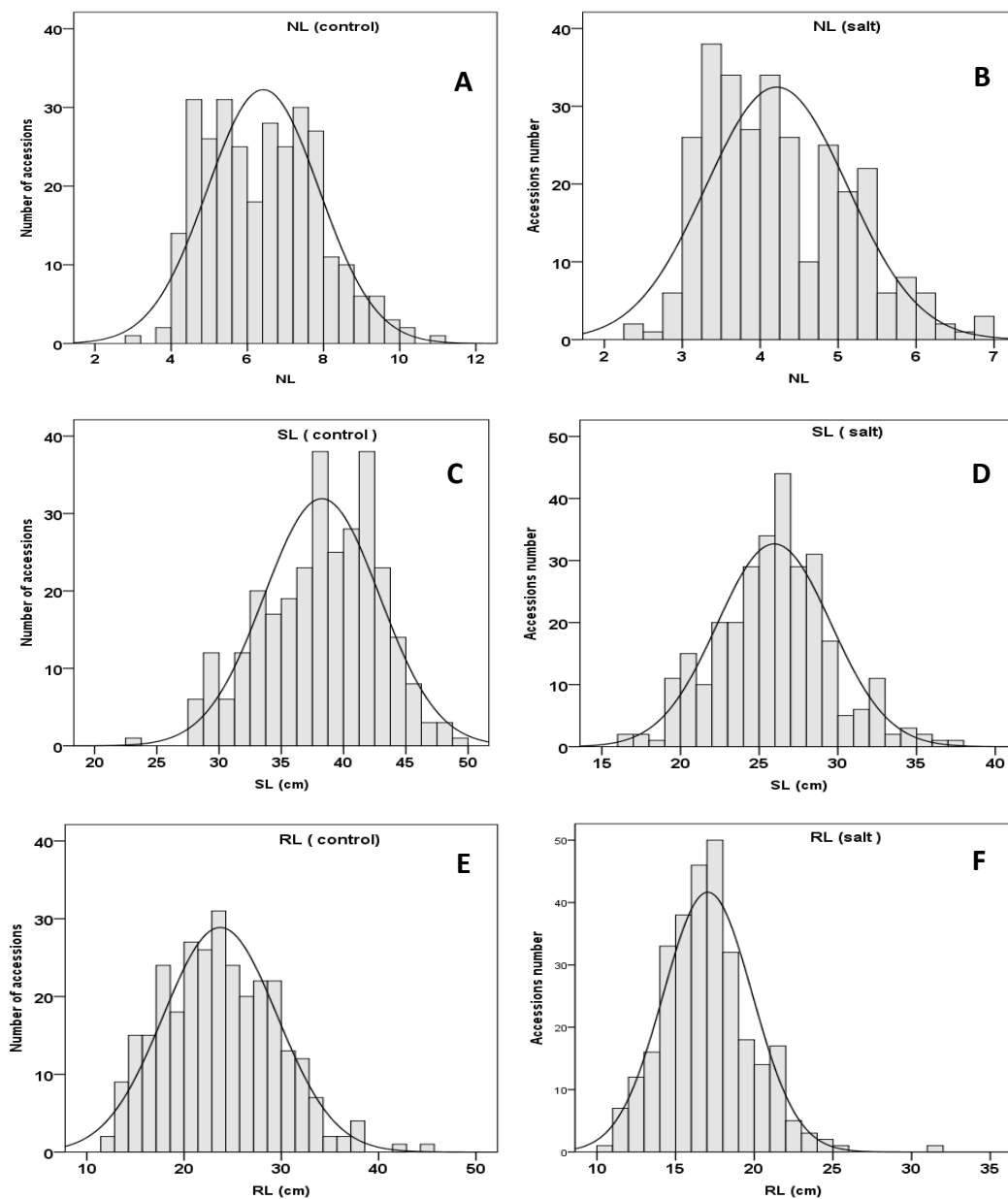


**Fig. 2.4. Leaf injury score (A) and coefficient of variation within accessions (B) under salt treatments.**

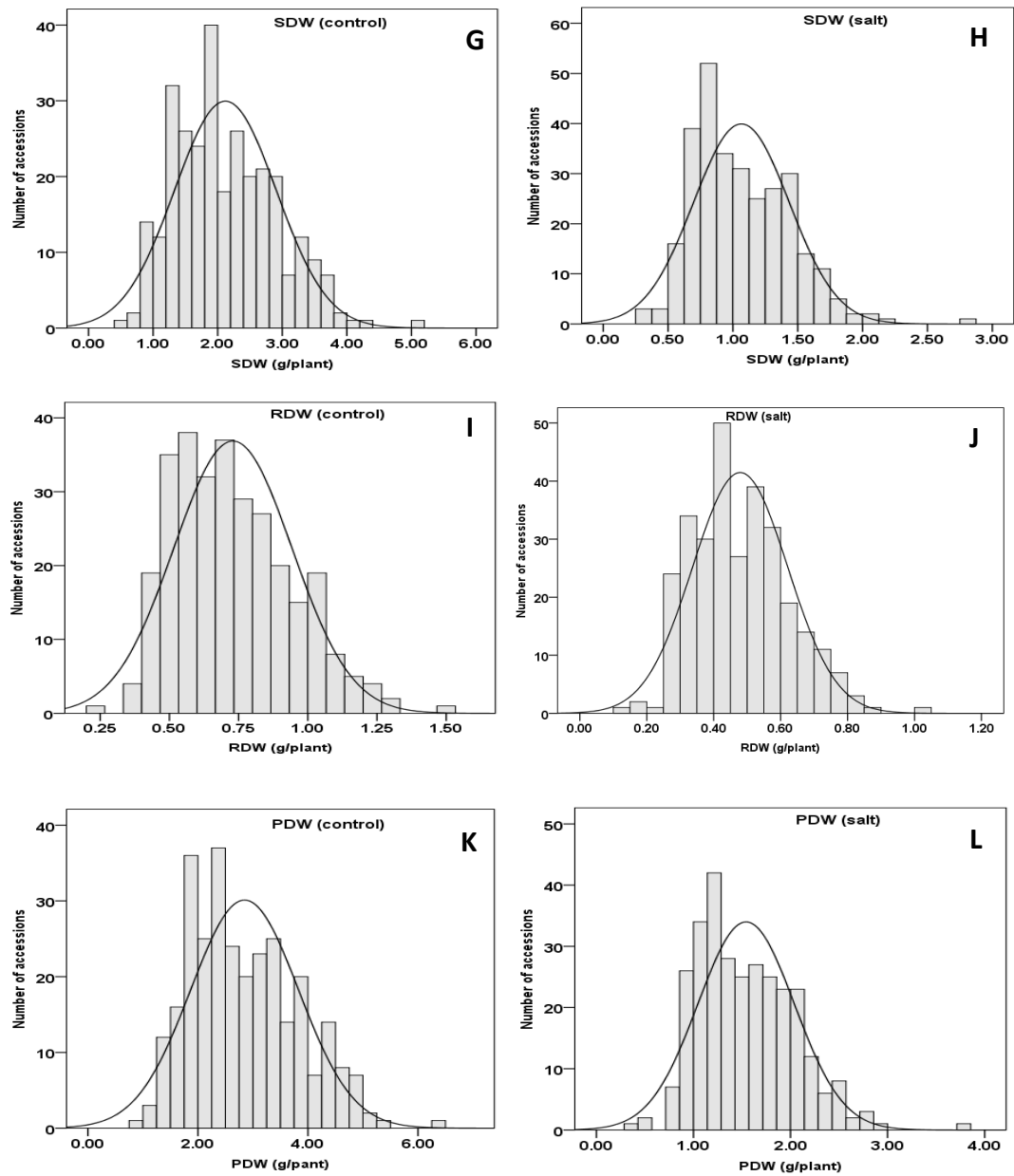


**Fig. 2.5.** Leaf injury score 10 and 16 days after treatment with 200 or 250 mM of NaCl.

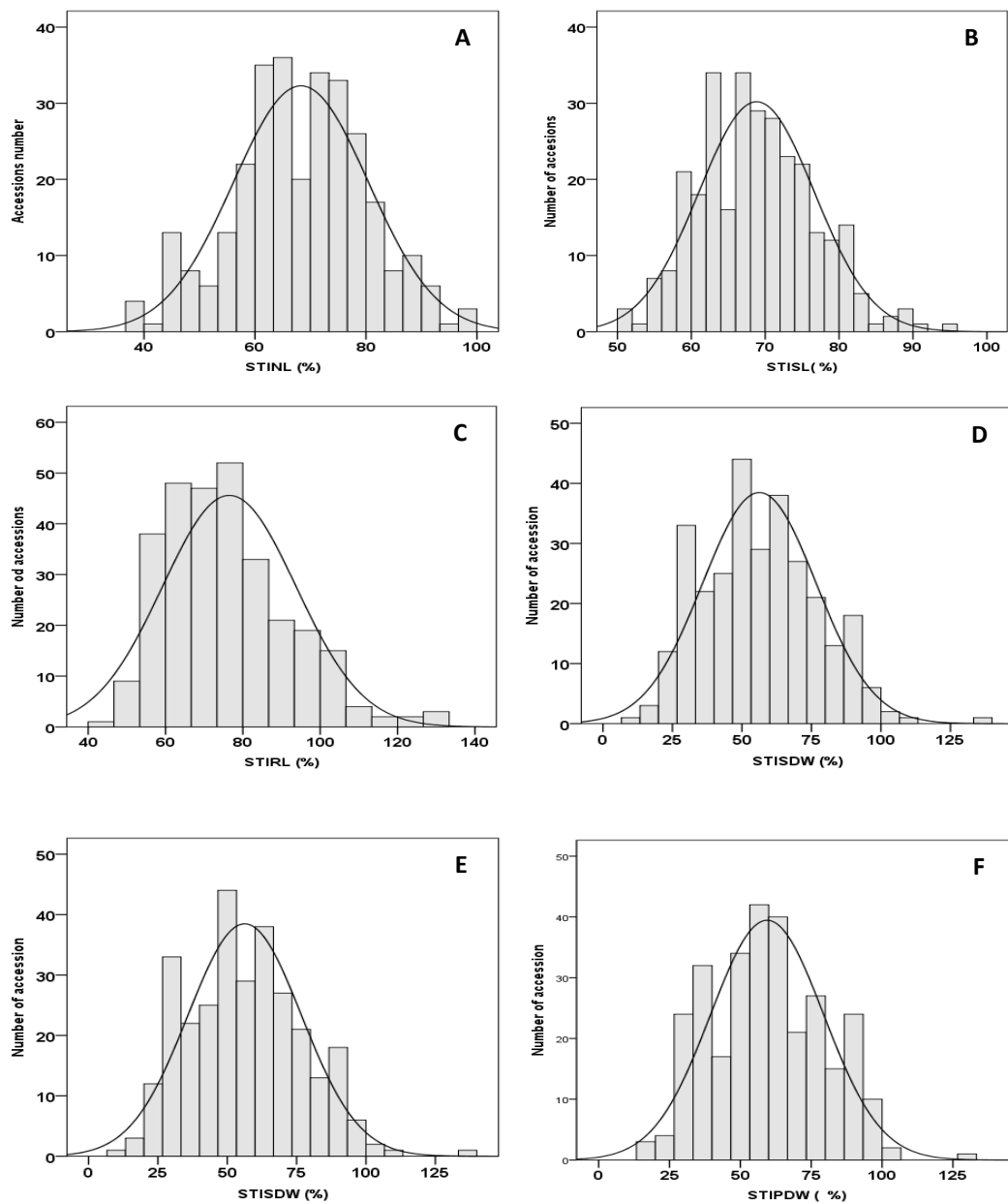
\* Significant at ( $P < 0.05$ ).



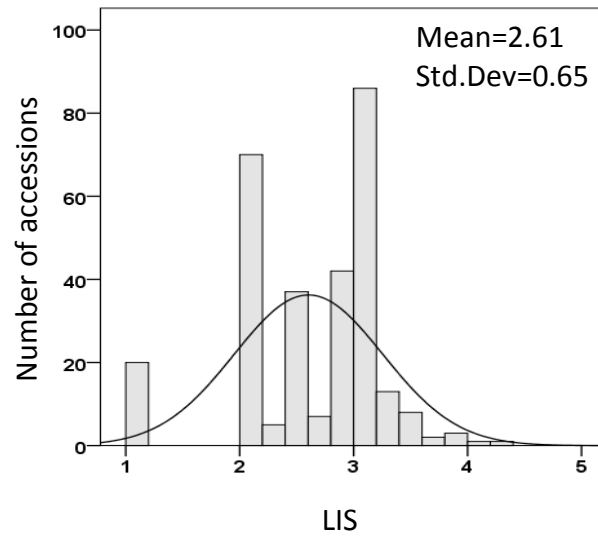
**Fig. 2.6. Frequency distribution of number of leaves (A, B), shoot length (C, D), root length (E, F), shoot dry weight (G, H), root dry weight (I, J) and plant dry weight (K, L) under control ( A, C, E, G, I, K) and salt treatment (B, D, F, H, J, L ) of 296 barley accessions.**



**Fig. 2.6. (Continued) Frequency distribution of number of leaves (A, B), shoot length (C, D), root length (E, F), shoot dry weight (G, H), root dry weight (I, J) and plant dry weight (K, L) under control (A, C, E, G, I, K) and salt treatment (B, D, F, H, J, L) of 296 barley accessions.**



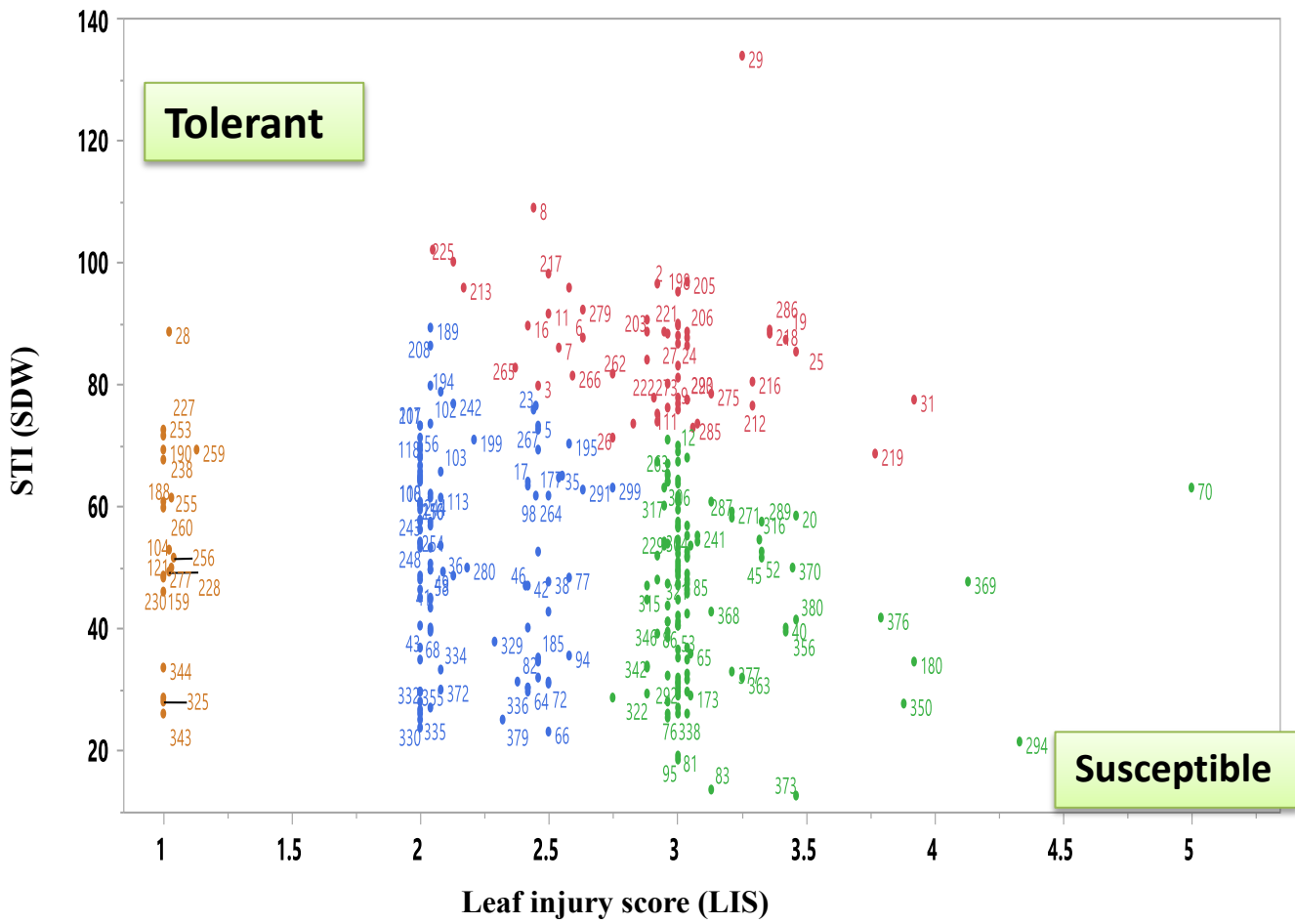
**Fig. 2.7.** The frequency distribution for salt tolerance index (STI) for number of leaves (A), shoot length (B), root length (C), root dry weight (D), shoot dry weight (E) and plant dry weight (F).



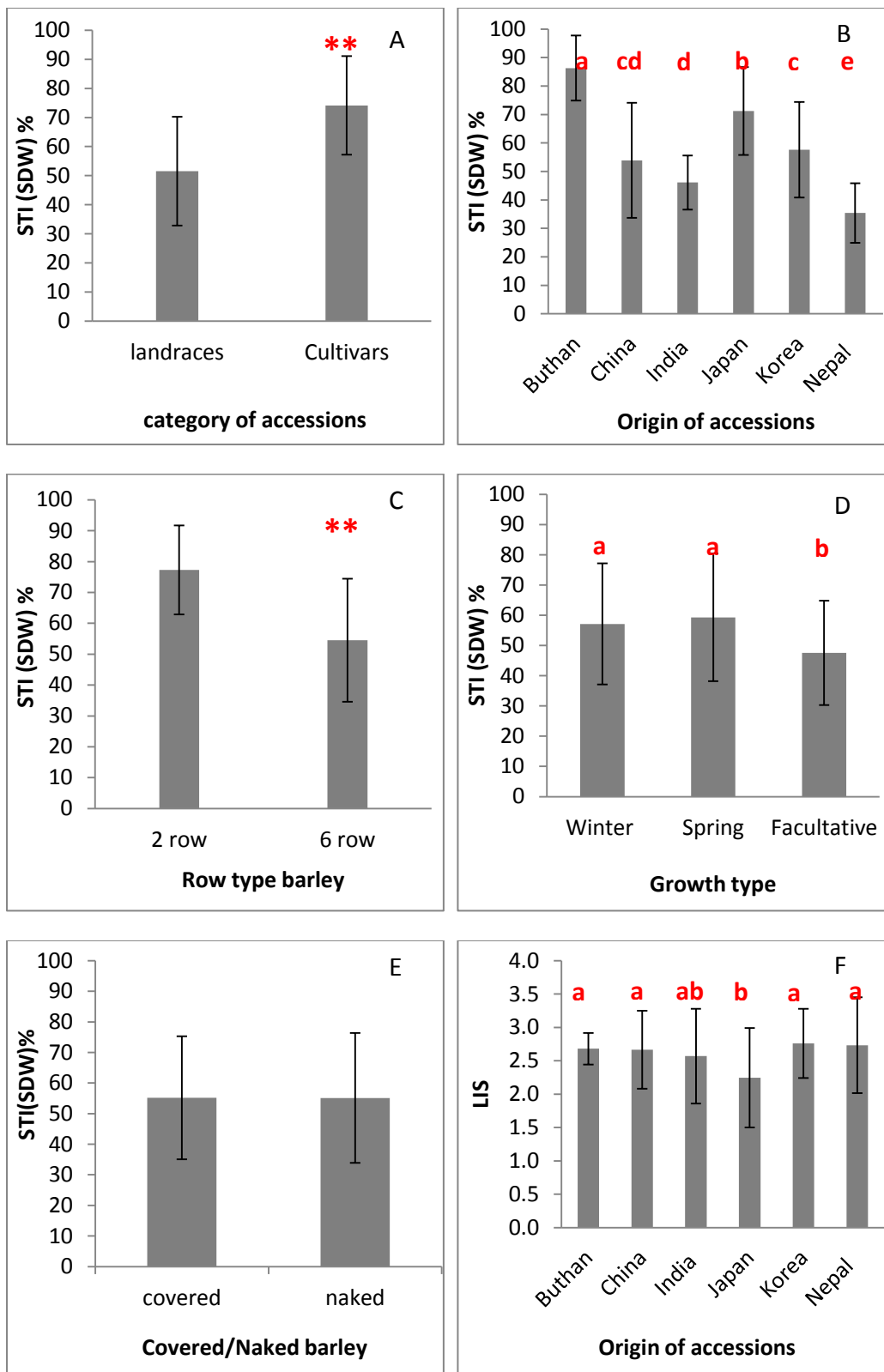
**Fig. 2.8. The frequency distribution for the LIS of 296 Asian barley accessions (average of 12 replicates by accession).**

LIS: leaf injury score

Std.Dev: Standard deviation



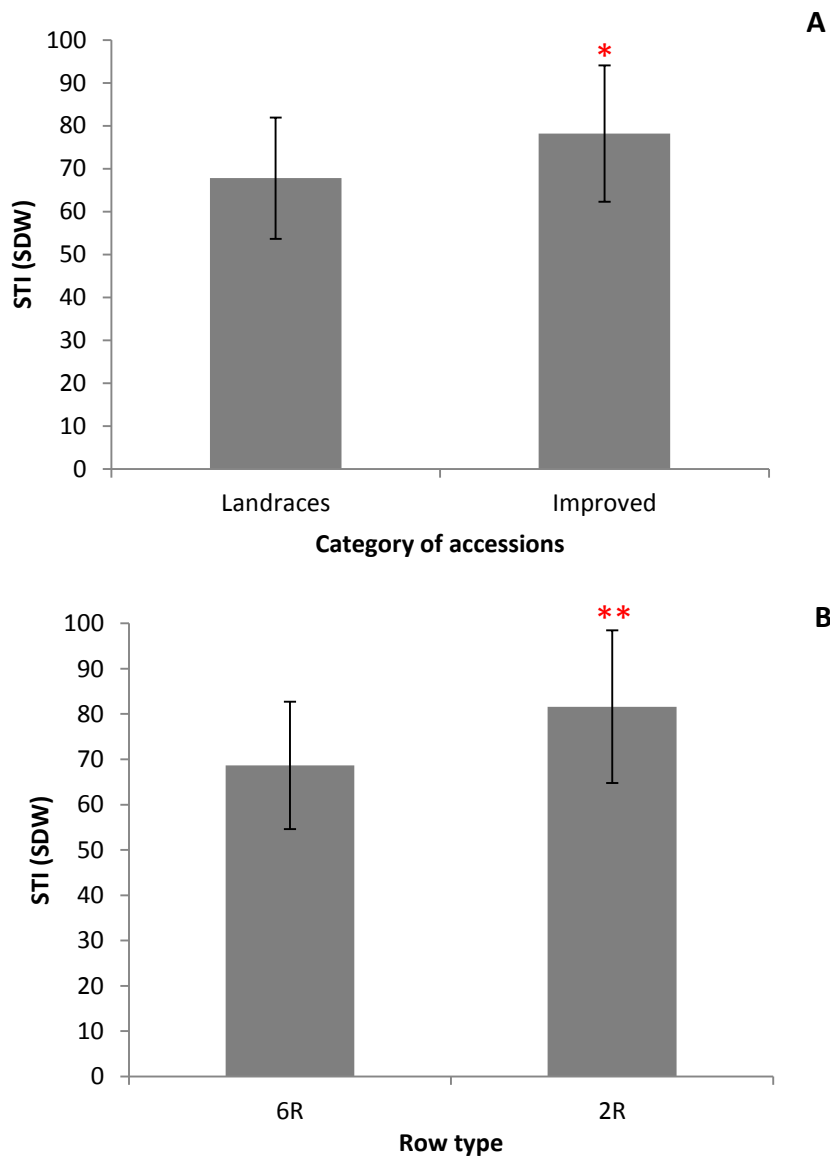
**Fig. 2.9. Bivariate Fit of the STI (SDW) by LIS.**



**Fig. 2.10. Relationship between STI (SDW) and category of accessions (A), origin of accessions (B), row type (C), growth type (D) and grain type (E).**

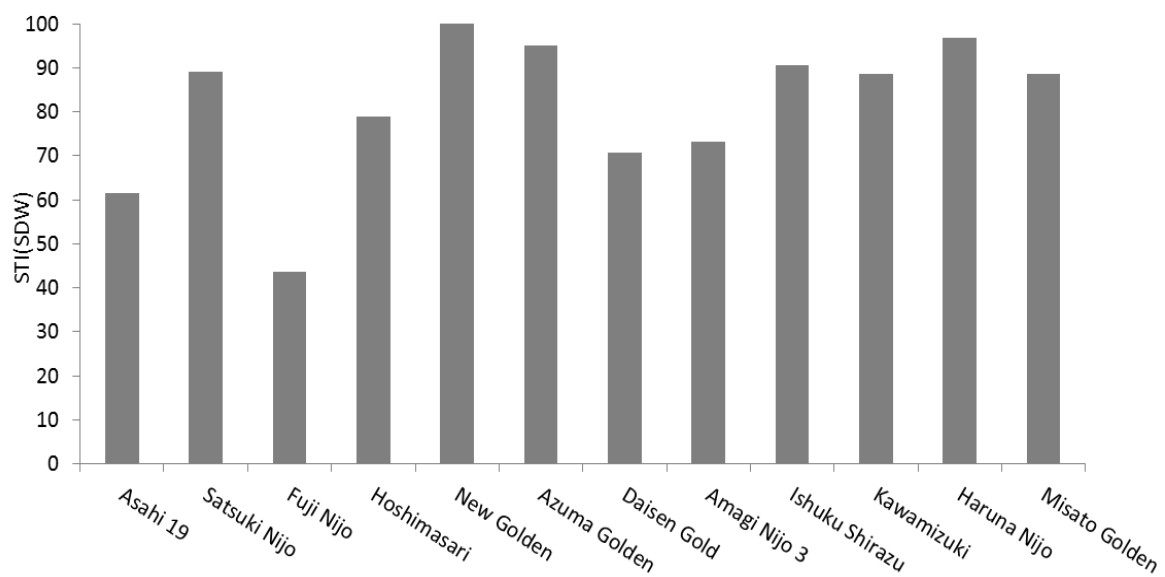
\*\* Significant at ( $P < 0.01$ ). Bars with the same letter are not significantly different ( $P > 0.05$ ).





**Fig. 2.11. Relationship between STI (SDW) and barley traits in Japanese accessions: (A) category of accessions and (B) row type accessions.**

\*, \*\* Significant at ( $P < 0.05$ ) and ( $P < 0.01$ ).



**Fig. 2.12. STI (SDW) of the Japanese two-row type accessions.**

# CHAPTER 3:

## ASSOCIATION MAPPING FOR SALT TOLERANCE IN BARLEY ACCESSIONS

### 3.1. Introduction

In the last decade the generation of molecular markers and their mapping has offered new opportunities for plant breeding. These resources allow the tracking of specific loci and alleles through the identification of markers linked to major genes, analysis of quantitative trait loci (QTLs), positional cloning of genes and characterization of genetic variation in germplasm. In addition, mapped markers can often be used in related species to analyse syntenic relationships.

Identification of QTLs mainly relied on linkage analysis. The linkage analysis requires a mapping population based on the products of one (doubled haploids) or two (F<sub>2</sub>s) cycles of recombinations. The method proved successful detection of QTLs for any traits in a variety of crops such as rice (Harushima *et al.* 1998, Mansuri *et al.* 2012, Subudhi *et al.* 2006, Xu *et al.* 2000), wheat (Boukhatem *et al.* 2002, Randhawa *et al.* 2013, Varshney *et al.* 2006), sorghum (Apotikar *et al.* 2011) and barley (Xue *et al.* 2009, Wu *et al.* 2011).

A large number of barley mapping populations have been developed to map genes and QTLs to control agronomic and quality traits (Forster *et al.* 2004, Prakash and Verma 2006, Witzel *et al.* 2010), including salt tolerance-related traits (Ben-

Hamida *et al.* 2009, Nguyen *et al.* 2013a, Nguyen *et al.* 2013b, Mano and Takeda 1997, Xue *et al.* 2012). Mano and Takeda (1997) identified QTLs controlling salt tolerance at the germination and seedling stages in barley by interval mapping using two doubled haploid (DH) populations derived from the crosses of Steptoe x Morex and Harrington x TR306. They concluded that salt tolerance at germination and at the seedling stage were controlled by different QTLs. Forster (2001) reviewed the positive effects of semi-dwarfing genes on salt tolerance and the wild types and mutants showed significant differences in their responses to salt stress in this study. Advanced mapping populations including near-isogenic lines (NIL) (Marcel *et al.* 2007), chromosome segment substitution lines (CSSLs) and recombinant chromosome substitution lines (RCSLs) (Sato and Takeda 2009) were also developed to facilitate the genetic dissection of salt tolerance.

Bi-parental mapping is the first step for clarifying the genetic basis of quantitative traits in plants. However, its QTL detection is limited only within two haplotypes. In addition, long time is needed for mapping population development (Cerda and Cloutier 2012). Association mapping (AM) does not require mapping populations and it can employ a larger number of haplotypes having natural variation on the target quantitative trait (Kraakman *et al.* 2006, Li *et al.* 2011, Platten *et al.* 2013, Vioni *et al.* 2013). AM is based on genotype-phenotype correlations among individuals in genepool (Cerda and Cloutier 2012, Nnadozie *et al.* 2007). It detects linkage disequilibrium (LD) between markers and traits which have been preserved during the historical recombination accumulated after the domestication of barley (Comadran *et al.* 2009, Li *et al.* 2007). Thus, AM requires high density markers which have enough resolution to detect LD on the genome (Cerda and Cloutier 2012, Forster *et al.* 2000, Pasam *et al.* 2012, Varshney *et al.* 2007, Yu *et al.* 2012). Few studies have been published on the detection of QTLs for salt tolerance in barley based by AM. Recently, Nguyen *et al.* (2013a) detected QTL for salt tolerance in a spring barley collection under 200 mM of NaCl at the vegetative stage by association mapping with SNP markers.

In the present chapter, a collection of Asian barley accessions was used. We analyzed the association of related traits to salt tolerance with SNP markers to detect QTL for salt tolerance. The objectives were to achieve the following: 1) to determine the population structure of the Asian barley collection; 2) to assess the decay of

linkage disequilibrium (LD) between marker loci and to examine how tightly the alleles at two loci are associated in these two distinct regions of the genome and 3) to identify SNP markers associated with salt tolerance at early seedling, based on the association analysis.

## **3.2. Materials and methods**

### **3.2.1 PCR amplification and genotyping**

DNA was extracted from the leaf samples using an automated DNA isolation system (PI 2000, Kurabo Industries Limited, Japan). The DNA concentration for each sample was adjusted to 50 ng/μl.

The reference genetic map was developed by Illumina oligonucleotide pool assays (OPA) using several mapping populations (Close *et al.* 2009). From a total of 1536 SNP detection platform of barley OPA 1 (Close *et al.* 2009), 384 SNPs were selected to avoid redundant or vicinal genetic positions of the reference map (Table 3.1).

PCR, hybridisation and scanning were performed according to the Golden Gate genotyping assay protocol (Illumina Inc.) (Fan *et al.* 2006) with a HiScan microarray scanner (Illumina Inc.) by Sato Kazuhiro group at the Institute of Plant Science and Resources, Okayama University, Japan. SNP basecalling was performed using Genome Studio (Illumina Inc.) with imported cluster positions from previously developed cluster files. The SNPs with rare alleles and poor quality were excluded. The final set of 318 good quality SNPs were used. The distribution of SNPs on each chromosome is presented in Table 3.1.

The largest number of SNPs is on chromosome 2H (67 SNPs), followed by chromosome 5H (62 SNPs), and the lowest number of SNPs was observed on chromosomes 4H (42 SNPs). The average distance between marker loci was 2.81 cM.

### **3.2.2. Population structure**

Population structure was estimated using three different methodologies. First, the Q matrix was calculated using STRUCTURE 2.3.4 software (Pritchard *et al.* 2000). The number of groups/subpopulations (k) was set from 1 to 10 and 10 iterations were performed. An admixture model with a burn-in period of 100,000 and 10,000 Markov Chain Monte Carlo repetitions with correlated allele frequencies

among populations were used. The number of subpopulations ( $k$ ) into accessions was first estimated by calculating the posterior probability  $\ln(P(D))$  and  $L'(k)$  for all possible  $k$  values between 1 and 10. The true ( $k$ ) value was calculated using an ad hoc quantity ( $\Delta k$ ) described by Evanno *et al.* (2005). When  $\Delta k$  had the highest value, the value of  $k$  was the number of clusters. Second, neighbor-joining (NJ) tree of 296 Asian barley accessions was constructed from SNP markers by TASSEL.

Third, the eigenanalysis proposed by Patterson *et al.* (2006) and Nguyen *et al.* (2013a) were also used to investigate the population structure. Eigenanalysis was run with TASSEL using the SNP marker set. A set of significant eigenvectors were obtained and used as covariables to account for population structure.

### **3.2.3. Linkage disequilibrium and association mapping**

Briefly, LD is calculated pairwise between two polymorphic sites; and the most frequently used LD measures are the standardized disequilibrium coefficient ( $D'$ ) and the correlation between alleles at two loci ( $r^2$ ) (Flint-Garcia *et al.* 2003). The  $D'$  is the standardized disequilibrium coefficient which mainly measures recombinational history and is therefore useful to assess the probability of historical recombination for a given population. The  $r^2$  is essentially the correlation between the alleles at two loci; it summarizes both recombinational and mutational history and is useful in the context of association studies. Both parameters vary in the interval from 0 to 1. To test the significance of the LD, we also obtained  $P$ -values that were determined by permutation test to calculate the proportion of permuted gamete distributions that were less probable than the observed gamete distribution under the null hypothesis of independence (Weir 1996).

Linkage was analysed using TASSEL 3.0.163 (Bradbury *et al.* 2007). LD was calculated pairwise between two polymorphic sites and the most frequently used LD parameters were the standardized disequilibrium coefficient ( $D'$ ) and the squared allele frequency correlations ( $r^2$ ) (Flint-Garcia *et al.* 2003). The  $r^2$  between loci were considered to be in significant when  $P < 0.001$ , the rest of  $r^2$  values were not considered as informative.  $P$  values were estimated for all pairs of SNP markers within the same chromosome. The extent and the distribution of LD were visualized by plotting  $r^2$  values against the genetic distance (cM) between markers for full genome and on each chromosome. A critical value of  $r^2$  as an evidence of linkage was

derived from the distribution of the unlinked  $r^2$ . Unlinked  $r^2$  estimates were square root transformed to approximate a normally distributed random variable and then the parametric 95<sup>th</sup> percentile of that distribution was taken as a population-specific critical value of  $r^2$ . The intersection of the LOESS curve fit to syntenic  $r^2$  with this baseline was considered as the estimate of the extent of LD on the chromosome (Brescghello and Sorrells 2006). LD plot showing LD patterns among the SNPs markers genotyped on each chromosome was presented to ensure of markers with the strongest evidence of association.

#### **3.2.4. Phenotypic assessment**

The assessment of 296 accessions of barley for salt tolerance at the seedling stage was presented in Chapter 2. The leaf injury score (LIS) was recorded. The root length (RL), shoot length (SL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) were recorded and the salt tolerance indices were calculated.

#### **3.2.5 Marker/trait association**

A set of 318 from 384 SNP markers, with minor allele frequency (MAF) higher than 0.05, were used to perform association mapping. Marker-trait associations were calculated using the following six models to evaluate the effects of population structure (Q, PC) and kinship (K): (1) naïve without controlling for Q or K, (2) Q model taking into account the population structure Q, (3) PCA model controlling for PC, (4) K model taking into accounts the kinship K, (5) QK model taking into account both Q and K, and (6) PK model taking into account both PC and K (Upadhyaya *et al.* 2013). The naïve, Q, and PCA models were assessed using the generalized linear model (GLM). The K, QK, and PK models were assessed using the mixed linear model (MLM) in TASSEL (Bradbury *et al.* 2007).

The K matrix was generated in TASSEL with all the SNP markers. The  $P$  values obtained from all models were converted into  $-\log_{10}(P)$ .  $P$  value significance thresholds for declaring the presence of positive marker-trait associations were calculated based on a false discovery rate (FDR) (Benjamini and Hochberg 1995). The significance thresholds for FDR at a level of 0.05 as  $-\log_{10}(P)$  were set to 2.44.

### **3.3. Results**

#### **3.3.1. Population structure among Asian barley accessions**

To calculate the Q matrix, the number of subpopulations (J) into accessions was first estimated by calculating the posterior probability  $\ln(P(D))$  and  $L'(K)$  for all possible k values between 1 and 10. The true (k) value was calculated using an ad hoc quantity ( $\Delta k$ ) described by Evanno *et al.* (2005). The  $\Delta k$  (Fig. 3.1) shows a clear peak at the true value  $k=2$ . The number of population was estimated to 2 subpopulations. This was used to generate the Q matrix for both the Q and QK models in this study. The result was consistent with the data obtained from the PCA analysis (Fig. 3.2). PCs 1 and 2 explained most of the variation, suggesting the presence of the major groups in the population. Neighbor-joining method (Fig. 3.3A) also indicated that the accessions were grouped into major two clusters excepting for seven accessions in the third cluster. Therefore, the value  $k=2$  was used.

The two subpopulations (k 1 and k 2) were classified into 210 and 86 accessions, respectively (Table 3.3 B). The used germplasm was grouped according to the need of vernalization. The first subpopulation (J1) involved 36.6% and 63.2% of the spring and combined facultative and winter growth types, respectively. The second subpopulation involved 72.1% of spring growth type and 27.9% of both the facultative and winter growth type. The all accessions with two-rowed type were included in the second subpopulation.

#### **3.3.2. Linkage disequilibrium (LD) decay**

LD analysis was performed using 384 SNPs. Decay of LD over barley genome is presented in Fig. 3.4. The significance threshold is shown with a black line. The significant  $r^2$  value was set at 0.32, 0.22, 0.29, 0.32, 0.28, 0.29, and 0.28 on the chromosome 1H, 2H, 3H, 4H, 5H, 6H and 7H, respectively. The red curve is the LOESS approximation of mean LD for all comparisons. There is no clear decay by showing the decay of LD of all genome and the mean distance between markers was inflated due to a relatively small number of marker pairs that were exceptionally distant. Consistent allele was below 1cM.



In this case, we referred to the LD plot by each chromosome and we check markers linked each other and markers significantly associated with salt tolerance (Fig. 3.7 to Fig. 3.11).

### **3.3.3. Detection of QTLs by GLM model under control and salt stress conditions**

In GLM model, we used three models: the naïve model, the Q model and the PC model. The naïve model has no control on false positives and negatives which can be caused by grouping effect (population structure) or by familial relatedness (relationship of all individuals or kinship). While, the Q model took in account the population structure and control the error due to the population grouping. PCA model control the principal component analysis.

Under control condition, a total of 166, 122 and 117 significant SNP markers were detected by Naïve (Appendix Table 1), Q (Appendix Table 2), and PC (Appendix Table 3) models and associated with 6 different traits. The  $-\log_{10}(P)$  was ranged from 2.5 to 16.28. On the chromosome 1H, the SNP markers number 43, 54 and 56 were highly associated with the RL.

Under salt condition, the naïve model detected 253 significant SNP markers with  $-\log_{10}(P)$  ranging from 2.5 to 17% and associated with 7 different traits (Appendix Table 4). Among them, 83 markers were associated with the SDW. Three SNP markers located on chromosomes 1H, 2 H and 3H were associated with the LIS. In contrast, the Q model detected only 181 significant SNP markers associated with seven traits (Appendix Table 5) with  $-\log_{10}(P)$  ranging from 2.5 to 12.8. A total of 101 SNP markers were associated with the SDW and located on the all barley chromosomes. Only 5 SNP markers were associated with the LIS and located on the chromosomes 1H, 2H and 3H. Only 132 QTLs were detected by the PCA model and most of SNP markers were associated with the SDW and SL (Appendix Table 6). Four markers were associated with the LIS and were the same as detected previously by Q model.

### **3.3.4. Detection of QTLs by MLM models under control and salt stress conditions**

Using MLM models (K, QK and PK), 9 QTLs were detected on the chromosomes 1H (3 QTLs), 2H (2 QTLs), 6 H (1 QTL) and 7H (3 QTLs), respectively and were associated with the 6 traits under control condition (Table 3.3).

The  $-\log_{10}(P)$  was ranged from 2.39 to 4.38. Three QTLs for the RL were detected on the chromosome 1H in the position 62.8, 137.8 and 138.3 cM. Two QTLs for the SDW were located on the chromosomes 2H and 7H. In the same position on the chromosome 7H, 2 QTLs for the RDW and PDW were also detected. Finally, 1 QTL for the NL was also located on the chromosome 7H and fare away with 14.6 cM for the one.

Under salt condition, significant markers-traits association is shown in the Table 3.4. Fifteen SNP markers were associated with morphological traits under salt condition and were distributed on the chromosomes as below; 1H (1 QTL), 2H (4 QTLs), 3 H (3 QTLs) 4H (3 QTLs), 5 H (2 QTLs) and 7H (1 QTL). These SNP markers were associated with the traits LIS (6 QTLs), SL (4 QTLs), NL (3 QTLs) and RL (2 QTLs).

### **3.3.5. Detection of QTLs for salt tolerance index (STI)**

By comparing the QQ plots for all models (Naïve, Q, PCA, K, QK and PK), the expected  $-\log_{10}(P\text{-value})$  vs  $-\log_{10}(P\text{-value})$  showed stable results for the models for K, QK and PK (Fig. 3.5). The minimum  $-\log_{10}(P)$  threshold value for a significant locus was set at 2.44. We detected a large number of significant markers for salt tolerance.

The SNP markers associated with the STI for each trait were mentioned in the Table 3.5. Thirty-four SNP markers were detected by MLM models, associated with the STI and located on the chromosome 1H (9 QTLs), 2H (4 QTLs), 3H ( 8 QTLs), 4H ( 6 QTLs), 5H (3 QTLs), 6H (5 QTLs) and 7 H ( 2 QTLs). The  $-\log_{10}(P)$  was ranged from 2.5 to 4.03. Four QTLs were detected in the nearest position on the chromosome 1H (135.6 to 138.3 cM).

### **3.3.6. Detection of QTLs for salt tolerance related traits**

As mentioned in the Chapter 2, LIS and STI of (SDW) were selected as suitable traits for salt tolerance assessment. Seven QTLs for salt tolerance (Table 3.6, Fig. 3.6) at the seedling stage were detected on chromosomes 1H (2 QTLs), 2H (2 QTLs), 3H (1 QTL), 4H (1 QTL) and 5H (1 QTL). Five QTLs were associated with the LIS and located on different chromosomes and two QTLs were detected and associated with the STI (SDW) on chromosomes 1H and 2H. The  $-\log_{10}(P)$  value ranged from 2.5 to 4.42.

The nucleotide changes caused by salt stress were [A/G] and [A/C] alleles for LIS and STI (SDW), respectively. The nucleotide changes caused by salt stress were [A/G] alleles at markers, 3263-2865, 4434-804, 2236-773, ABC09432-1-1160 and ABC06144-pHv8601 for LIS and [A/C] and [A/G] alleles at markers, 4927-1340 and 1826-229 for STI (SDW), respectively. The allele “G” improved the LIS in positive direction at all QTLs identified on chromosomes 1H, 2H, 4H and 5H. The accessions 227, 230, 238, 253, 255, 260, 277, 325, 339, 343 and 344 improved the allele “G”. The alleles “C” and “G” improved STI (SDW) in positive direction on chromosomes 1H and 2H, respectively. The accessions 8, 11, 14, 29, 30, 197, 198, 203, 205, 203, 217, 221 and 297 improved the allele “C” and the allele “G” for the trait STI (SDW).

Two QTLs were detected at a similar position (137.8 and 140.5 cM) on chromosome 1H. These two markers SNP53 and SNP56 were associated with LIS and SDW. On the other hand, the markers SNP53, SNP54, SNP55 and SNP56 were highly linked as shown in the LD plot on the chromosome 1 H (Fig. 3.7). The marker SNP53 was also associated with the trait STI (PDW) with  $-\log_{10}(P) = 2.48$ . The marker SNP52 and SNP54 were associated with the trait STI (RL) with a  $P$  value 0.0001 and 0.0004 respectively. The marker SNP55 was associated with the LIS with a lowest  $P$  value which was 0.053 and 0.049 according to the QK and PK models. The  $-\log_{10}(P)$  was 1.26-1.30, respectively. On chromosome 2H, the SNP86 was associated with the LIS. The marker SNP86 was not linked to nearest marker SNP85 nor SNP87. The marker SNP90 and SNP94 were associated with the trait STI of SL and RL and were distinct by 6.2 to 17.7 cM.

LIS was associated with SNP markers SNP150 and SNP156 on chromosome 3H. According to the LD plot representing the LD on chromosome 3H (Fig. 3.9), SNP156 was not linked to the markers SNP155 and SNP154. While, it is slightly ( $P < 0.0001$ ) linked to markers SNP151 and SNP153 which was associated also to the LIS but with a lower  $P$  value [ $P = 0.04$  and  $-\log_{10}(P) = 1.3$ ]. SNP156 was highly linked to the SNP146 and to the markers SNP150. To summarize, the markers SNP150, SNP151 and SNP156 which were highly to slightly linked were also associated to the salt tolerance.

The marker SNP203 on chromosome 4H which associated with the LIS was highly linked to the SNP202, SNP204, SNP205, SNP208, SNP209 and SNP210 (Fig. 3.10).

Finally, the marker SNP253 located on the chromosome 5H was associated with the LIS with  $-\log_{10}(P) = 2.54$  but was not linked to markers in nearest position (Fig. 3.11).

The markers associated with salt tolerance on the chromosome 1H, 3H and 4H were also linked to the markers in the same region, indicating that there is a natural selection for populations and landraces may kept those allelic regions underlying an adaptation to salt.

### 3.4. Discussion

A large number of QTLs for salt tolerance have been previously detected in different barley germplasm (Aminfar *et al.* 2011, Eleuch *et al.* 2008, Ellis *et al.* 2002, Mano and Takeda 1997, Nguyen *et al.* 2013a, Nguyen *et al.* 2013b, Rivandi *et al.* 2010, Shavrukov *et al.* 2010, Taghipour and Salehi 2008, Xue *et al.* 2009, Zhou *et al.* 2011). Different mapping populations have been developed to detect QTLs. For example, Mano and Takeda (1997) identified QTLs for salt tolerance at germination and seedling stages using two DH populations derived from the crosses of Steptoe/Morex and Harrington/TR306. Recently, researchers have more focused on identifying QTL for salt tolerance at germination and seedling stage by association analysis using worldwide barley core collections to resolve the limitation of biparental segregating populations. For instance, Qiu *et al.* (2011) and Wu *et al.* (2011) examined tissue dry biomass and the Na<sup>+</sup> and K<sup>+</sup> contents of 188 Tibetan barley accessions under 300 mM of NaCl at early growth stage. Nguyen *et al.* (2013a) evaluated the spring barley collection for salt tolerance (200 mM of NaCl) at the vegetative stage using a hydroponic system and detected QTLs for salt tolerance through an association mapping approach.

In the current study, QTLs for salt tolerance at the seedling stage in Asian barley accessions were detected by the association analysis with 384 SNP marker systems (Table 6). Seven significant QTLs for salt tolerance were mapped on chromosomes 1H (2 QTLs), 2H (2 QTLs), 3H (1 QTL), 4H (1 QTL) and 5H (1 QTL); five and two of these QTLs were associated with LIS and STI (SDW), respectively. Among the seven QTLs, five QTLs had been previously reported (Mano and Takeda 1997, Nguyen *et al.* 2013a, Rivandi *et al.* 2010, Zhou *et al.* 2011). Only two QTLs for LIS were newly detected on chromosomes 3H and 4H through this study.

Two QTLs for LIS and SDW were detected at similar region (137.8 and 140.5 cM) on the long arm of chromosome 1H. These QTLs were previously reported by Mano and Takeda (1997) and closely linked to *HvNax4* (Rivandi *et al.* 2010) which is a gene controlling an environmentally sensitive Na<sup>+</sup> exclusion.

In our study, two QTLs associated with STI (SDW) and LIS were detected at the position 59.9 cM on the short arm of chromosome 2H and 68.2 cM on the long arm of chromosome 2H, respectively. At a similar region (59.2 cM) to these QTLs for STI (SDW) and LIS, QTLs for leaf senescence and shoot length were detected (Nguyen *et al.* 2013a). Zhou *et al.* (2011) also detected QTL for combined injury score and plant survival at the position 48 cM.

On the other hand, other QTL for LIS was mapped at the position 65.1 cM on chromosome 4H and closely linked to the vernalisation gene *VRN-H2* (Zitzewitz *et al.* 2005) at the position 66 cM.

Another QTL for LIS at the position 89.2 cM on chromosome 5H was mapped at an adjacent region including the QTL for salt tolerance reported elsewhere (Mano and Takeda 1997). The *VRN-H1* gene was located at the position 98 cM (Szucs *et al.* 2006) with a distance of 8.8 cM from the QTL for salt tolerance on chromosome 5H.

Newly detected QTLs for LIS on chromosomes 3H and 4H should be confirmed by QTL analysis using segregating mapping population.

### **3.5. Conclusion**

The detection of QTLs for salt tolerance of barley accessions was performed at the seedling stage using the association analysis methods with 384 SNP markers. Seven significant QTL for salt tolerance in terms of STI (SDW) and LIS at the vegetative stage were detected on chromosomes 1H (2 QTLs), 2H (2 QTLs), 3H (1 QTL), 4H (1 QTL) and 5H (1 QTL). Five and two of these QTLs are associated with LIS and with STI of the SDW, respectively. Among them, five QTLs had been corresponded to the previously reported. Two QTLs associated with LIS were newly detected on chromosomes 3H and 4H suggesting that association mapping can identify new QTLs for salt tolerance and explores candidate genes of salt tolerance. The novel QTLs should be confirmed by linkage mapping analysis.

**Table 3.1. SNP markers used for genotyping 296 barley accessions.**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP1	1H	8670-388	0.8	84	G	C	[G/C]
SNP2	1H	337-641	1.0	348	A	G	[A/G]
SNP3	1H	6195-2137	1.5	164	A	G	[A/G]
SNP4	1H	3101-111	3.8	344	T	C	[T/C]
SNP5	1H	7372-1253	8.8	383	A	G	[A/G]
SNP6	1H	2609-350	15.4	286	T	C	[T/C]
SNP7	1H	2496-1916	18.1	324	A	G	[A/G]
SNP8	1H	6792-1945	20.9	174	T	C	[T/C]
SNP9	1H	1906-429	23.9	213	T	C	[T/C]
SNP10	1H	6081-850	26.6	261	A	C	[A/C]
SNP11	1H	3751-1136	33.6	183	G	C	[G/C]
SNP12	1H	2151-1310	37.0	304	A	G	[A/G]
SNP13	1H	5381-1950	41.0	292	T	C	[T/C]
SNP14	1H	6720-641	43.3	316	T	C	[T/C]
SNP15	1H	2407-1771	47.5	264	A	G	[A/G]
SNP16	1H	3444-1044	49.7	274	T	C	[T/C]
SNP17	1H	4511-1878	50.6	192	T	A	[T/A]
SNP18	1H	8224-561	52.5	101	T	C	[T/C]
SNP19	1H	7284-710	54.7	176	T	C	[T/C]
SNP20	1H	2577-1122	57.0	166	A	G	[A/G]
SNP21	1H	8486-1964	59.7	314	C	G	[C/G]
SNP22	1H	2459-237	62.8	86	A	G	[A/G]
SNP23	1H	9638-619	64.9	52	A	T	[A/T]
SNP24	1H	3675-2615	65.5	152	T	C	[T/C]
SNP25	1H	5547-294	66.7	315	C	G	[C/G]
SNP26	1H	1386-2088	69.5	290	T	G	[T/G]
SNP27	1H	1016-376	73.9	169	A	G	[A/G]
SNP28	1H	5772-1176	75.5	139	A	G	[A/G]
SNP29	1H	4005-530	77.3	320	A	G	[A/G]
SNP30	1H	3406-221	80.3	161	A	G	[A/G]
SNP31	1H	ABC00697-pHv3016	84.7	11	A	G	[A/G]
SNP32	1H	3204-811	86.2	254	A	G	[A/G]
SNP33	1H	3404-2470	88.2	61	T	C	[T/C]
SNP34	1H	ABC08077-pHv131-02	91.0	25	G	C	[G/C]
SNP35	1H	3087-1763	92.8	146	A	G	[A/G]
SNP36	1H	8867-459	95.4	62	T	C	[T/C]
SNP37	1H	2935-1634	96.9	119	G	C	[G/C]
SNP38	1H	3702-982	99.2	224	T	C	[T/C]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP39	1H	1497-628	101.5	79	A	G	[A/G]
SNP40	1H	4625-1413	105.1	259	T	G	[T/G]
SNP41	1H	3786-2204	106.6	160	T	C	[T/C]
SNP42	1H	4962-1295	108.3	105	T	A	[T/A]
SNP43	1H	5690-1045	112.5	131	T	C	[T/C]
SNP44	1H	9105-497	114.8	74	T	C	[T/C]
SNP45	1H	5048-1685	116.3	265	T	C	[T/C]
SNP46	1H	2633-498	117.8	351	A	C	[A/C]
SNP47	1H	6026-1949	121.1	154	G	C	[G/C]
SNP48	1H	4978-1030	125.3	98	T	C	[T/C]
SNP49	1H	4393-1078	127.1	379	A	G	[A/G]
SNP50	1H	7381-1292	129.6	9	T	C	[T/C]
SNP51	1H	5555-438	131.9	116	A	G	[A/G]
SNP52	1H	ABC05061-1-1-159	135.6	88	T	G	[T/G]
SNP53	1H	4927-1340	137.8	322	A	C	[A/C]
SNP54	1H	4057-2114	138.3	21	T	C	[T/C]
SNP55	1H	4592-118	139.8	306	A	G	[A/G]
SNP56	1H	3263-2865	140.5	71	A	G	[A/G]
SNP57	2H	2582-767	6.5	226	A	G	[A/G]
SNP58	2H	ABC02329-1-20-250	7.1	26	T	C	[T/C]
SNP59	2H	3453-1974	9.3	2	T	C	[T/C]
SNP60	2H	3452-1355	10.9	202	T	C	[T/C]
SNP61	2H	12224-363	15.2	7	A	C	[A/C]
SNP62	2H	4277-1901	18.2	201	T	C	[T/C]
SNP63	2H	ABC01004-sfp18-05	19.3	51	T	C	[T/C]
SNP64	2H	1865-396	21.6	110	G	C	[G/C]
SNP65	2H	2029-1143	26.5	382	A	C	[A/C]
SNP66	2H	7747-1056	28.4	296	T	C	[T/C]
SNP67	2H	7032-201	29.2	335	A	G	[A/G]
SNP68	2H	5652-419	31.0	208	T	C	[T/C]
SNP69	2H	ABC05236-1-10-217	32.2	89	T	G	[T/G]
SNP70	2H	1447-464	37.3	112	T	C	[T/C]
SNP71	2H	2964-382	39.1	358	A	G	[A/G]
SNP72	2H	4410-284	41.7	248	T	G	[T/G]
SNP73	2H	2651-1774	44.1	102	A	G	[A/G]
SNP74	2H	1341-841	45.6	145	A	C	[A/C]
SNP75	2H	ABC02350-pHv759-04	49.0	111	T	C	[T/C]
SNP76	2H	ABC01899-1-1-301	50.6	35	A	G	[A/G]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP77	2H	ABC20402-1-3-298	53.5	196	A	G	[A/G]
SNP78	2H	2580-1456	55.0	108	A	G	[A/G]
SNP79	2H	946-2500	55.7	66	A	G	[A/G]
SNP80	2H	8889-842	57.5	199	T	C	[T/C]
SNP81	2H	4630-1036	58.9	147	T	C	[T/C]
SNP82	2H	1826-229	59.9	34	A	G	[A/G]
SNP83	2H	ABC08774-1-1-752	62.8	68	T	G	[T/G]
SNP84	2H	2634-2228	63.5	60	A	C	[A/C]
SNP85	2H	ABC03181-1-1-164	66.1	64	A	G	[A/G]
SNP86	2H	4434-804	68.2	194	A	G	[A/G]
SNP87	2H	2284-1738	70.5	82	A	G	[A/G]
SNP88	2H	7187-382	71.6	3	T	C	[T/C]
SNP89	2H	334-1164	73.0	121	A	C	[A/C]
SNP90	2H	5573-1170	74.4	32	T	C	[T/C]
SNP91	2H	4377-571	75.2	109	T	C	[T/C]
SNP92	2H	1946-698	78.0	307	T	G	[T/G]
SNP93	2H	6117-1507	82.8	132	A	G	[A/G]
SNP94	2H	2371-950	85.9	301	T	C	[T/C]
SNP95	2H	3469-1152	88.7	354	A	G	[A/G]
SNP96	2H	8632-1809	90.1	273	G	C	[G/C]
SNP97	2H	2020-539	93.5	195	A	G	[A/G]
SNP98	2H	11591-265	95.6	220	T	C	[T/C]
SNP99	2H	11660-365	98.6	376	T	G	[T/G]
SNP100	2H	7236-1384	101.8	244	A	G	[A/G]
SNP101	2H	8523-316	103.7	310	T	C	[T/C]
SNP102	2H	4266-387	105.8	27	T	G	[T/G]
SNP103	2H	ABC04580-1-4-420	108.6	217	A	G	[A/G]
SNP104	2H	111-499	112.9	129	T	C	[T/C]
SNP105	2H	3180-1771	115.1	368	T	C	[T/C]
SNP106	2H	7576-818	117.9	134	T	G	[T/G]
SNP107	2H	3256-1196	120.8	339	T	C	[T/C]
SNP108	2H	ABC16258-1-1-77	121.5	231	A	T	[A/T]
SNP109	2H	3271-1422	125.5	23	T	C	[T/C]
SNP110	2H	9291-1322	126.4	126	A	G	[A/G]
SNP111	2H	ABC01791-1-1-110	127.1	24	A	G	[A/G]
SNP112	2H	6652-209	129.3	234	C	G	[C/G]
SNP113	2H	285-2932	130.0	384	A	G	[A/G]
SNP114	2H	4241-445	133.9	364	T	C	[T/C]



**Table 3.1 SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP115	2H	3608-2133	137.5	127	C	G	[C/G]
SNP116	2H	ABC10785-1-1-82	139.7	283	T	C	[T/C]
SNP117	2H	4879-1560	143.6	38	T	C	[T/C]
SNP118	2H	ABC17314-1-1-226	145.0	99	T	C	[T/C]
SNP119	2H	2052-792	147.9	336	T	C	[T/C]
SNP120	2H	1283-332	151.4	163	A	C	[A/C]
SNP121	2H	6419-1680	155.3	33	T	C	[T/C]
SNP122	2H	3450-692	156.7	73	A	G	[A/G]
SNP123	2H	9426-490	158.2	334	T	C	[T/C]
SNP124	3H	5029-1423	0.0	247	T	C	[T/C]
SNP125	3H	4715-810	2.3	72	C	G	[C/G]
SNP126	3H	ConsensusGBS0194-1	6.0	137	A	G	[A/G]
SNP127	3H	5945-748	8.9	94	T	C	[T/C]
SNP128	3H	1499-290	10.8	69	C	G	[C/G]
SNP129	3H	3646-1984	12.5	369	A	C	[A/C]
SNP130	3H	1440-1148	16.3	279	A	C	[A/C]
SNP131	3H	4443-1835	19.2	270	A	G	[A/G]
SNP132	3H	573-552	22.7	128	T	G	[T/G]
SNP133	3H	3413-1488	24.2	143	A	G	[A/G]
SNP134	3H	4701-2395	26.9	238	T	C	[T/C]
SNP135	3H	5646-568	28.4	188	T	C	[T/C]
SNP136	3H	3688-1291	32.8	193	A	G	[A/G]
SNP137	3H	4593-2007	37.2	50	A	G	[A/G]
SNP138	3H	4844-1737	39.5	227	T	C	[T/C]
SNP139	3H	15141-257	42.1	237	A	G	[A/G]
SNP140	3H	4105-1417	46.3	260	A	C	[A/C]
SNP141	3H	2391-566	47.1	269	T	C	[T/C]
SNP142	3H	ABC13089-1-2-478	51.7	366	C	G	[C/G]
SNP143	3H	1630-1150	54.4	207	T	C	[T/C]
SNP144	3H	2861-1941	56.4	251	T	C	[T/C]
SNP145	3H	1746-1527	59.9	173	A	C	[A/C]
SNP146	3H	5038-1035	63.0	10	T	C	[T/C]
SNP147	3H	4184-393	65.5	67	A	G	[A/G]
SNP148	3H	ABC19175-1-2-375	69.6	313	A	G	[A/G]
SNP149	3H	4149-219	72.3	87	T	C	[T/C]
SNP150	3H	2315-702	74.8	357	T	C	[T/C]
SNP151	3H	1176-1547	78.5	302	A	C	[A/C]
SNP152	3H	12280-797	80.9	361	T	C	[T/C]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP153	3H	8722-512	81.7	365	A	G	[A/G]
SNP154	3H	11116-257	86.0	266	T	C	[T/C]
SNP155	3H	8020-87	88.8	36	T	C	[T/C]
SNP156	3H	2236-773	91.9	267	A	G	[A/G]
SNP157	3H	5224-1560	93.4	12	T	C	[T/C]
SNP158	3H	3791-1525	98.5	326	T	A	[T/A]
SNP159	3H	6302-250	101.4	42	T	C	[T/C]
SNP160	3H	ABC11028-1-1-64	104.5	303	T	A	[T/A]
SNP161	3H	10114-1946	107.6	287	C	G	[C/G]
SNP162	3H	7241-553	111.4	103	A	G	[A/G]
SNP163	3H	2500-1514	114.0	219	A	G	[A/G]
SNP164	3H	4025-300	117.1	100	T	A	[T/A]
SNP165	3H	ABC13753-1-2-167	120.6	300	T	G	[T/G]
SNP166	3H	76-1059	123.7	243	C	G	[C/G]
SNP167	3H	5260-462	126.3	29	T	C	[T/C]
SNP168	3H	2335-1614	130.2	294	A	G	[A/G]
SNP169	3H	3718-1026	131.6	123	A	C	[A/C]
SNP170	3H	3340-1042	134.3	371	T	G	[T/G]
SNP171	3H	11657-398	137.3	252	A	G	[A/G]
SNP172	3H	5008-2402	141.5	8	A	C	[A/C]
SNP173	3H	7772-223	148.9	28	A	G	[A/G]
SNP174	3H	7818-967	150.4	289	G	C	[G/C]
SNP175	3H	13750-348	155.1	93	A	G	[A/G]
SNP176	3H	7803-483	160.1	221	A	C	[A/C]
SNP177	3H	4403-885	162.2	150	A	G	[A/G]
SNP178	3H	ConsensusGBS0271-2	164.3	363	T	C	[T/C]
SNP179	3H	4643-867	167.8	359	T	C	[T/C]
SNP180	3H	ConsensusGBS0632-3	169.3	241	T	G	[T/G]
SNP181	3H	5399-139	172.4	191	T	C	[T/C]
SNP182	3H	8752-523	173.2	329	A	C	[A/C]
SNP183	4H	1996-652	3.7	144	T	A	[T/A]
SNP184	4H	ABC14522-1-8-350	5.6	49	A	G	[A/G]
SNP185	4H	2533-773	8.3	353	A	G	[A/G]
SNP186	4H	3551-2537	12.0	85	A	G	[A/G]
SNP187	4H	5149-1645	19.5	204	T	C	[T/C]
SNP188	4H	2055-947	21.6	170	G	C	[G/C]
SNP189	4H	1595-1107	24.6	236	T	C	[T/C]
SNP190	4H	1001-1187	28.2	350	A	G	[A/G]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP191	4H	6589-1211	33.4	218	A	G	[A/G]
SNP192	4H	2769-1245	36.4	122	T	C	[T/C]
SNP193	4H	1227-1323	40.4	245	A	T	[A/T]
SNP194	4H	1180-70	42.5	135	A	G	[A/G]
SNP195	4H	5726-414	44.9	133	G	C	[G/C]
SNP196	4H	5475-1355	46.4	356	T	C	[T/C]
SNP197	4H	5013-1834	48.5	277	A	C	[A/C]
SNP198	4H	2028-1571	50.4	158	A	G	[A/G]
SNP199	4H	7942-948	52.8	136	T	G	[T/G]
SNP200	4H	587-1438	55.6	106	A	G	[A/G]
SNP201	4H	5848-1413	59.4	75	T	C	[T/C]
SNP202	4H	6326-1032	62.1	268	A	G	[A/G]
SNP203	4H	ABC09432-1-1-160	65.1	325	A	G	[A/G]
SNP204	4H	3549-743	68.2	17	T	C	[T/C]
SNP205	4H	3416-692	72.1	291	T	C	[T/C]
SNP206	4H	1375-2534	76.0	180	G	C	[G/C]
SNP207	4H	9149-1316	77.3	233	T	C	[T/C]
SNP208	4H	1523-1136	81.7	216	A	T	[A/T]
SNP209	4H	4986-1214	84.3	77	A	G	[A/G]
SNP210	4H	4564-604	87.5	175	A	C	[A/C]
SNP211	4H	4039-1686	89.4	39	A	G	[A/G]
SNP212	4H	4361-1867	92.4	240	A	G	[A/G]
SNP213	4H	4919-1051	96.6	115	T	C	[T/C]
SNP214	4H	4535-1366	98.6	319	G	C	[G/C]
SNP215	4H	41-695	100.7	43	A	G	[A/G]
SNP216	4H	2614-1522	103.1	177	T	C	[T/C]
SNP217	4H	5692-310	106.0	209	A	C	[A/C]
SNP218	4H	1561-1053	108.7	16	T	C	[T/C]
SNP219	4H	ABC12417-1-1-46	111.7	258	G	C	[G/C]
SNP220	4H	ABC02813-1-4-326	113.9	347	G	C	[G/C]
SNP221	4H	6689-135	116.9	95	A	C	[A/C]
SNP222	4H	2878-574	119.8	285	T	G	[T/G]
SNP223	4H	ABC08009-1-2-304	121.8	130	T	G	[T/G]
SNP224	4H	954-1377	123.3	378	T	C	[T/C]
SNP225	5H	5206-787	2.1	239	G	C	[G/C]
SNP226	5H	3417-1451	2.8	370	T	C	[T/C]
SNP227	5H	1582-63	6.4	178	T	A	[T/A]
SNP228	5H	7310-996	9.3	373	T	C	[T/C]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP229	5H	3356-243	17.4	299	T	C	[T/C]
SNP230	5H	8785-443	21.3	113	A	G	[A/G]
SNP231	5H	4753-1091	25.2	186	C	G	[C/G]
SNP232	5H	4532-675	27.7	48	A	G	[A/G]
SNP233	5H	8377-1022	31.0	20	T	C	[T/C]
SNP234	5H	4684-775	34.3	214	A	G	[A/G]
SNP235	5H	421-528	37.1	308	T	C	[T/C]
SNP236	5H	5732-1104	40.0	198	G	C	[G/C]
SNP237	5H	7681-229	43.1	70	C	G	[C/G]
SNP238	5H	5250-214	46.2	253	A	G	[A/G]
SNP239	5H	9209-298	48.8	341	T	G	[T/G]
SNP240	5H	5850-2347	51.0	78	T	C	[T/C]
SNP241	5H	8320-955	53.2	151	T	C	[T/C]
SNP242	5H	ConsensusGBS0654-4	56.8	197	T	C	[T/C]
SNP243	5H	1732-491	57.4	155	A	C	[A/C]
SNP244	5H	ABC09365-1-3-378	60.7	235	A	C	[A/C]
SNP245	5H	8561-968	63.3	149	T	C	[T/C]
SNP246	5H	4234-1944	65.5	92	T	G	[T/G]
SNP247	5H	65-778	68.4	153	A	G	[A/G]
SNP248	5H	264-571	70.5	76	T	C	[T/C]
SNP249	5H	5799-578	75.4	19	A	G	[A/G]
SNP250	5H	1896-1435	80.0	271	T	C	[T/C]
SNP251	5H	11931-389	84.5	337	A	C	[A/C]
SNP252	5H	3928-513	87.4	380	T	C	[T/C]
SNP253	5H	ABC06144-pHv86-01	89.4	211	A	G	[A/G]
SNP254	5H	ABC11984-1-2-158	94.4	171	A	C	[A/C]
SNP255	5H	3200-242	95.8	184	A	G	[A/G]
SNP256	5H	3333-1209	99.6	342	A	G	[A/G]
SNP257	5H	5004-375	102.1	205	T	C	[T/C]
SNP258	5H	ABC14689-1-9-399	104.5	141	A	G	[A/G]
SNP259	5H	3398-163	108.0	275	T	G	[T/G]
SNP260	5H	ABC11221-1-3-410	111.7	298	A	G	[A/G]
SNP261	5H	3478-1024	113.1	6	T	C	[T/C]
SNP262	5H	ABC08402-1-2-97	117.5	107	T	C	[T/C]
SNP263	5H	139-1263	122.4	190	A	G	[A/G]
SNP264	5H	ConsensusGBS0531-1	125.8	22	T	C	[T/C]
SNP265	5H	ConsensusGBS0234-1	128.0	375	T	C	[T/C]
SNP266	5H	211-259	130.1	167	A	G	[A/G]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP267	5H	5571-640	135.7	263	T	C	[T/C]
SNP268	5H	1394-1222	137.2	222	C	G	[C/G]
SNP269	5H	ConsensusGBS0704-2	142.2	14	A	G	[A/G]
SNP270	5H	ABC04352-pHv108-01	145.4	59	A	G	[A/G]
SNP271	5H	3883-616	147.4	54	T	C	[T/C]
SNP272	5H	2611-1846	150.3	179	T	C	[T/C]
SNP273	5H	4050-2006	153.5	225	T	C	[T/C]
SNP274	5H	ConsensusGBS0451-1	155.1	309	T	G	[T/G]
SNP275	5H	603-72	159.1	210	T	C	[T/C]
SNP276	5H	5757-248	161.6	4	A	T	[A/T]
SNP277	5H	2290-796	166.6	30	C	G	[C/G]
SNP278	5H	4845-123	169.5	53	A	C	[A/C]
SNP279	5H	ABC09278-1-4-69	171.7	278	T	C	[T/C]
SNP280	5H	552-188	175.9	156	T	C	[T/C]
SNP281	5H	3362-644	177.7	157	C	G	[C/G]
SNP282	5H	2144-852	181.4	293	T	C	[T/C]
SNP283	5H	6851-867	187.4	44	A	G	[A/G]
SNP284	5H	4658-1237	189.6	96	A	G	[A/G]
SNP285	5H	2978-938	192.0	223	T	A	[T/A]
SNP286	5H	2726-852	195.4	57	T	C	[T/C]
SNP287	6H	1692-742	0.0	200	A	G	[A/G]
SNP288	6H	5159-579	1.3	46	A	G	[A/G]
SNP289	6H	ConsensusGBS0136-7	3.1	212	G	C	[G/C]
SNP290	6H	7185-370	6.1	340	A	G	[A/G]
SNP291	6H	5993-2383	9.1	374	T	A	[T/A]
SNP292	6H	ConsensusGBS0346-1	12.5	189	A	C	[A/C]
SNP293	6H	1769-545	17.0	187	T	A	[T/A]
SNP294	6H	1240-844	21.7	168	T	C	[T/C]
SNP295	6H	6719-1166	24.4	47	A	G	[A/G]
SNP296	6H	4611-178	28.4	120	C	G	[C/G]
SNP297	6H	5771-91	31.7	37	A	G	[A/G]
SNP298	6H	3164-1386	34.4	83	T	C	[T/C]
SNP299	6H	3580-331	42.4	232	A	C	[A/C]
SNP300	6H	4445-1911	44.8	65	T	C	[T/C]
SNP301	6H	3378-619	48.7	118	T	C	[T/C]
SNP302	6H	1009-1089	52.8	117	T	C	[T/C]
SNP303	6H	5232-2041	55.7	249	T	C	[T/C]
SNP304	6H	ABC02895-1-4-231	58.0	138	T	C	[T/C]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP305	6H	2298-1526	60.2	317	A	G	[A/G]
SNP306	6H	ConsensusGBS0369-1	63.3	305	C	G	[C/G]
SNP307	6H	3348-395	64.4	333	A	G	[A/G]
SNP308	6H	4235-1617	67.0	352	T	C	[T/C]
SNP309	6H	4064-1724	70.0	215	A	T	[A/T]
SNP310	6H	1096-1482	72.5	330	T	G	[T/G]
SNP311	6H	7361-937	77.9	282	T	C	[T/C]
SNP312	6H	ConsensusGBS0239-5	81.2	295	T	C	[T/C]
SNP313	6H	ABC06682-1-1-311	83.9	345	T	A	[T/A]
SNP314	6H	5965-1387	85.2	284	A	G	[A/G]
SNP315	6H	4641-266	88.9	63	T	C	[T/C]
SNP316	6H	1969-1322	90.2	355	A	G	[A/G]
SNP317	6H	578-587	93.1	165	A	C	[A/C]
SNP318	6H	5124-1707	96.7	311	C	G	[C/G]
SNP319	6H	10425-725	97.4	262	G	C	[G/C]
SNP320	6H	2562-1191	101.4	331	C	G	[C/G]
SNP321	6H	428-1519	105.6	124	T	C	[T/C]
SNP322	6H	2389-526	110.3	181	T	C	[T/C]
SNP323	6H	2152-1547	112.3	250	T	C	[T/C]
SNP324	6H	4396-567	118.4	91	T	G	[T/G]
SNP325	6H	1852-509	119.0	372	A	C	[A/C]
SNP326	6H	ABC08038-1-3-160	121.2	288	A	G	[A/G]
SNP327	6H	617-167	124.9	185	T	C	[T/C]
SNP328	6H	6523-1691	126.9	90	A	G	[A/G]
SNP329	6H	3363-1795	129.4	18	A	C	[A/C]
SNP330	7H	2132-1261	0.0	45	A	T	[A/T]
SNP331	7H	7172-1536	1.9	242	G	C	[G/C]
SNP332	7H	ABC07611-1-5-315	4.1	80	T	C	[T/C]
SNP333	7H	3359-1118	6.8	206	T	C	[T/C]
SNP334	7H	2148-498	9.8	172	A	G	[A/G]
SNP335	7H	1773-358	12.4	228	G	C	[G/C]
SNP336	7H	6394-944	15.0	257	A	G	[A/G]
SNP337	7H	9672-758	17.2	140	T	C	[T/C]
SNP338	7H	4275-1288	19.3	230	C	G	[C/G]
SNP339	7H	1073-916	21.1	367	T	C	[T/C]
SNP340	7H	3187-1073	25.7	360	C	G	[C/G]
SNP341	7H	2916-576	27.5	246	T	C	[T/C]
SNP342	7H	8365-454	29.8	55	A	G	[A/G]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

Number	Chr	Marker name	MapInfo	Index	AA	BB	SNP
SNP343	7H	1404-64	31.8	381	A	G	[A/G]
SNP344	7H	5777-354	34.8	15	A	G	[A/G]
SNP345	7H	ABC03024-1-3-368	37.6	346	T	A	[T/A]
SNP346	7H	398-1244	41.9	323	A	G	[A/G]
SNP347	7H	2585-2901	42.6	97	T	C	[T/C]
SNP348	7H	ConsensusGBS0356-1	46.2	321	T	A	[T/A]
SNP349	7H	239-776	49.7	159	A	G	[A/G]
SNP350	7H	1792-372	52.8	1	A	G	[A/G]
SNP351	7H	4679-1594	55.6	40	A	C	[A/C]
SNP352	7H	12239-662	56.8	114	T	G	[T/G]
SNP353	7H	943-3107	60.7	81	A	G	[A/G]
SNP354	7H	11912-654	63.7	56	A	G	[A/G]
SNP355	7H	4475-478	68.5	276	A	G	[A/G]
SNP356	7H	ABC14535-1-1-75	70.4	343	A	G	[A/G]
SNP357	7H	1735-1424	73.8	349	A	G	[A/G]
SNP358	7H	2924-1189	77.9	125	C	G	[C/G]
SNP359	7H	ABC09320-1-1-112	79.6	148	C	G	[C/G]
SNP360	7H	ConsensusGBS0250-2	82.3	272	A	G	[A/G]
SNP361	7H	3731-103	84.9	142	A	G	[A/G]
SNP362	7H	2462-971	88.0	58	A	G	[A/G]
SNP363	7H	977-1377	98.5	182	T	C	[T/C]
SNP364	7H	4791-1541	101.3	327	C	G	[C/G]
SNP365	7H	1800-1101	104.8	104	T	A	[T/A]
SNP366	7H	4838-494	107.9	203	A	G	[A/G]
SNP367	7H	6541-1329	111.0	256	A	C	[A/C]
SNP368	7H	3900-611	112.5	338	A	G	[A/G]
SNP369	7H	1789-782	116.3	318	C	G	[C/G]
SNP370	7H	ABC10197-1-1-101	122.1	31	A	G	[A/G]
SNP371	7H	2378-498	125.2	297	T	C	[T/C]
SNP372	7H	7216-297	129.9	280	T	C	[T/C]
SNP373	7H	6628-1302	133.8	162	T	G	[T/G]
SNP374	7H	6868-595	136.6	229	T	C	[T/C]
SNP375	7H	7023-448	139.7	5	A	T	[A/T]
SNP376	7H	13108-412	141.8	362	C	G	[C/G]
SNP377	7H	382-2624	143.7	13	T	C	[T/C]
SNP378	7H	93-413	147.5	312	T	C	[T/C]
SNP379	7H	5595-297	149.8	255	A	T	[A/T]
SNP380	7H	12368-207	157.0	328	G	C	[G/C]

**Table 3.1. SNP markers used for genotyping 296 barley accessions (Continued).**

<b>Number</b>	<b>Chr</b>	<b>Marker name</b>	<b>MapInfo</b>	<b>Index</b>	<b>AA</b>	<b>BB</b>	<b>SNP</b>
SNP381	7H	6316-858	159.3	281	T	C	[T/C]
SNP382	7H	8923-707	161.4	377	T	C	[T/C]
SNP383	7H	1437-687	161.5	41	A	G	[A/G]
SNP384	7H	2457-2346	166.6	332	G	C	[G/C]

**Legend**

Number = SNP marker numbers used for genotyping analysis.

Chr = chromosome 1H, 2H, 3H, 4H, 5H, 6H and 7H.

Marker name = names according to the HarvEST#32 assembly.

SNP = Single nucleotide polymorphism.



**Table 3.2. Q matrix and subpopulations (J) for 296 barley accessions.**

<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>	<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>
86	0.999	0.001	1	255	0.999	0.001	1
85	0.999	0.001	1	254	0.999	0.001	1
84	0.999	0.001	1	253	0.999	0.001	1
83	0.999	0.001	1	252	0.999	0.001	1
82	0.999	0.001	1	251	0.999	0.001	1
81	0.999	0.001	1	245	0.999	0.001	1
80	0.999	0.001	1	244	0.999	0.001	1
79	0.999	0.001	1	241	0.999	0.001	1
78	0.999	0.001	1	239	0.999	0.001	1
77	0.999	0.001	1	238	0.999	0.001	1
76	0.999	0.001	1	237	0.999	0.001	1
74	0.999	0.001	1	232	0.999	0.001	1
73	0.999	0.001	1	231	0.999	0.001	1
72	0.999	0.001	1	230	0.999	0.001	1
71	0.999	0.001	1	229	0.999	0.001	1
70	0.999	0.001	1	228	0.999	0.001	1
69	0.999	0.001	1	227	0.999	0.001	1
68	0.999	0.001	1	225	0.999	0.001	1
67	0.999	0.001	1	224	0.999	0.001	1
66	0.999	0.001	1	223	0.999	0.001	1
65	0.999	0.001	1	222	0.999	0.001	1
64	0.999	0.001	1	221	0.999	0.001	1
63	0.999	0.001	1	220	0.999	0.001	1
62	0.999	0.001	1	22	0.999	0.001	1
60	0.999	0.001	1	219	0.999	0.001	1
330	0.999	0.001	1	218	0.999	0.001	1
329	0.999	0.001	1	217	0.999	0.001	1
306	0.999	0.001	1	192	0.999	0.001	1
292	0.999	0.001	1	191	0.999	0.001	1
291	0.999	0.001	1	120	0.999	0.001	1
290	0.999	0.001	1	114	0.999	0.001	1
289	0.999	0.001	1	113	0.999	0.001	1
288	0.999	0.001	1	112	0.999	0.001	1
286	0.999	0.001	1	111	0.999	0.001	1
277	0.999	0.001	1	110	0.999	0.001	1
276	0.999	0.001	1	59	0.998	0.002	1
273	0.999	0.001	1	58	0.998	0.002	1
260	0.999	0.001	1	56	0.998	0.002	1
259	0.999	0.001	1	53	0.998	0.002	1
258	0.999	0.001	1	326	0.998	0.002	1
257	0.999	0.001	1	319	0.998	0.002	1
256	0.999	0.001	1	309	0.998	0.002	1

**Table 3.2. Q matrix and subpopulations (J) for 296 barley accessions (Continued).**

<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>	<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>
304	0.998	0.002	1	39	0.923	0.077	1
302	0.998	0.002	1	295	0.914	0.086	1
299	0.998	0.002	1	325	0.893	0.107	1
298	0.998	0.002	1	50	0.891	0.109	1
287	0.998	0.002	1	311	0.877	0.123	1
285	0.998	0.002	1	279	0.874	0.126	1
250	0.998	0.002	1	200	0.866	0.134	1
210	0.998	0.002	1	103	0.857	0.143	1
57	0.997	0.003	1	104	0.848	0.152	1
321	0.997	0.003	1	322	0.821	0.179	1
312	0.997	0.003	1	21	0.813	0.187	1
212	0.997	0.003	1	193	0.811	0.189	1
242	0.996	0.004	1	20	0.807	0.193	1
297	0.993	0.007	1	90	0.803	0.197	1
54	0.99	0.01	1	92	0.799	0.201	1
278	0.989	0.011	1	270	0.798	0.202	1
282	0.987	0.013	1	105	0.783	0.217	1
118	0.987	0.013	1	9	0.78	0.22	1
296	0.984	0.016	1	52	0.778	0.222	1
271	0.983	0.017	1	268	0.773	0.227	1
102	0.971	0.029	1	264	0.77	0.23	1
307	0.97	0.03	1	23	0.764	0.236	1
202	0.97	0.03	1	19	0.763	0.237	1
106	0.968	0.032	1	87	0.757	0.243	1
343	0.967	0.033	1	99	0.746	0.254	1
294	0.965	0.035	1	94	0.741	0.259	1
55	0.964	0.036	1	339	0.74	0.26	1
301	0.963	0.037	1	337	0.735	0.265	1
316	0.957	0.043	1	315	0.73	0.27	1
300	0.954	0.046	1	100	0.727	0.273	1
283	0.953	0.047	1	1	0.722	0.278	1
317	0.951	0.049	1	216	0.716	0.284	1
272	0.946	0.054	1	43	0.715	0.285	1
24	0.941	0.059	1	8	0.713	0.287	1
117	0.941	0.059	1	6	0.713	0.287	1
310	0.934	0.066	1	352	0.709	0.291	1
293	0.934	0.066	1	265	0.708	0.292	1
284	0.932	0.068	1	7	0.704	0.296	1
313	0.931	0.069	1	2	0.702	0.298	1
280	0.931	0.069	1	3	0.7	0.3	1
303	0.929	0.071	1	362	0.695	0.305	1
324	0.926	0.074	1	98	0.686	0.314	1

**Table 3.2. Q matrix and subpopulations (J) for 296 barley accessions (Continued).**

<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>	<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>
355	0.686	0.314	1	263	0.498	0.502	2
348	0.681	0.319	1	160	0.498	0.502	2
335	0.671	0.329	1	377	0.483	0.517	2
12	0.67	0.33	1	372	0.483	0.517	2
331	0.669	0.331	1	368	0.481	0.519	2
14	0.669	0.331	1	171	0.481	0.519	2
266	0.665	0.335	1	379	0.48	0.52	2
247	0.664	0.336	1	101	0.479	0.521	2
351	0.663	0.337	1	342	0.474	0.526	2
208	0.658	0.342	1	332	0.463	0.537	2
5	0.655	0.345	1	246	0.461	0.539	2
356	0.652	0.348	1	122	0.458	0.542	2
38	0.649	0.351	1	361	0.457	0.543	2
243	0.644	0.356	1	378	0.456	0.544	2
357	0.638	0.362	1	376	0.456	0.544	2
373	0.635	0.365	1	370	0.453	0.547	2
375	0.63	0.37	1	347	0.453	0.547	2
11	0.614	0.386	1	336	0.453	0.547	2
371	0.61	0.39	1	184	0.453	0.547	2
369	0.61	0.39	1	262	0.452	0.548	2
195	0.609	0.391	1	95	0.451	0.549	2
360	0.608	0.392	1	185	0.448	0.552	2
364	0.606	0.394	1	172	0.448	0.552	2
338	0.6	0.4	1	380	0.446	0.554	2
267	0.6	0.4	1	121	0.441	0.559	2
159	0.597	0.403	1	346	0.436	0.564	2
161	0.586	0.414	1	183	0.436	0.564	2
26	0.571	0.429	1	327	0.435	0.565	2
28	0.561	0.439	1	248	0.434	0.566	2
374	0.553	0.447	1	180	0.427	0.573	2
162	0.553	0.447	1	308	0.421	0.579	2
350	0.543	0.457	1	344	0.417	0.583	2
333	0.542	0.458	1	31	0.411	0.589	2
164	0.538	0.462	1	173	0.4	0.6	2
354	0.536	0.464	1	367	0.395	0.605	2
177	0.536	0.464	1	123	0.351	0.649	2
363	0.535	0.465	1	115	0.319	0.681	2
182	0.518	0.482	1	305	0.307	0.693	2
163	0.51	0.49	1	125	0.298	0.702	2
366	0.507	0.493	1	249	0.288	0.712	2
334	0.504	0.496	1	29	0.272	0.728	2
345	0.5	0.5	2	18	0.213	0.787	2

**Table 3.2. Q matrix and subpopulations (J) for 296 barley accessions (Continued).**

<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>	<b>Accessions</b>	<b>Q1</b>	<b>Q2</b>	<b>J</b>
30	0.211	0.789	2	197	0.002	0.998	2
323	0.181	0.819	2				
213	0.177	0.823	2				
318	0.14	0.86	2				
34	0.133	0.867	2				
35	0.115	0.885	2				
116	0.095	0.905	2				
206	0.085	0.915	2				
194	0.046	0.954	2				
44	0.044	0.956	2				
274	0.031	0.969	2				
179	0.028	0.972	2				
33	0.025	0.975	2				
45	0.019	0.981	2				
32	0.012	0.988	2				
40	0.009	0.991	2				
188	0.008	0.992	2				
190	0.007	0.993	2				
17	0.007	0.993	2				
49	0.005	0.995	2				
46	0.005	0.995	2				
275	0.004	0.996	2				
205	0.004	0.996	2				
204	0.004	0.996	2				
203	0.004	0.996	2				
189	0.004	0.996	2				
109	0.004	0.996	2				
107	0.004	0.996	2				
48	0.003	0.997	2				
47	0.003	0.997	2				
42	0.003	0.997	2				
41	0.003	0.997	2				
37	0.003	0.997	2				
36	0.003	0.997	2				
328	0.003	0.997	2				
25	0.003	0.997	2				
199	0.003	0.997	2				
16	0.003	0.997	2				
108	0.003	0.997	2				
27	0.002	0.998	2				
201	0.002	0.998	2				
198	0.002	0.998	2				

**Table 3.3. Significant SNP markers associated with morphological traits under control condition detected by MLM models.**

Model	Trait	Marker	Chr	Distance (cM)	<i>P</i>	$-\log_{10}(P)$	$R^2$ (%)	Genetic Var	Residual Var	$-2\text{LnLikelihood}$
K	SL	SNP105	2H	115.1	0.0011	2.94	4.92	37.19	9.37	1653.27
QK	SL	SNP105	2H	115.1	0.0013	2.90	4.84	37.18	9.36	1642.68
PK	SL	SNP105	2H	115.1	0.0011	2.97	5.01	38.47	9.33	1636.63
K	SDW	SNP123	2H	158.2	0.0039	2.41	10.56	0.01	0.00	-713.88
K	RL	SNP22	1H	62.8	0.0015	2.83	3.88	35.90	12.31	1721.20
QK	RL	SNP22	1H	62.8	0.0014	2.87	3.95	35.99	12.32	1711.13
PK	RL	SNP22	1H	62.8	0.0014	2.87	3.97	36.84	12.29	1704.14
K	SL	SNP314	6H	85.2	0.0014	2.86	3.56	37.19	9.37	1653.27
QK	SL	SNP314	6H	85.2	0.0010	3.02	3.80	37.18	9.36	1642.68
PK	SL	SNP314	6H	85.2	0.0015	2.82	3.54	38.47	9.33	1636.63
K	SL	SNP344	7H	34.8	0.0008	3.08	3.93	37.19	9.37	1653.27
QK	SL	SNP344	7H	34.8	0.0003	3.50	4.58	37.18	9.36	1642.68
PK	SL	SNP344	7H	34.8	0.0006	3.23	4.18	38.47	9.33	1636.63
K	SDW	SNP348	7H	46.2	0.0040	2.39	2.90	0.01	0.00	-713.88
K	PDW	SNP348	7H	46.2	0.0025	2.60	3.22	0.01	0.01	-581.89
K	RDW	SNP348	7H	46.2	0.0006	3.23	4.19	0.00	0.00	-1465.62
QK	PDW	SNP348	7H	46.2	0.0007	3.18	4.09	0.01	0.01	-584.59
QK	RDW	SNP348	7H	46.2	0.0007	3.14	4.05	0.00	0.00	-1464.92
PK	RDW	SNP348	7H	46.2	0.0003	3.55	4.67	0.00	0.00	-1461.69
PK	PDW	SNP348	7H	46.2	0.0016	2.80	3.51	0.01	0.01	-585.34
PK	SDW	SNP348	7H	46.2	0.0028	2.56	3.13	0.01	0.00	-716.48
K	NL	SNP353	7H	60.7	0.0011	2.95	8.47	2.59	1.26	1004.90
QK	NL	SNP353	7H	60.7	0.0010	2.99	8.64	2.64	1.25	997.29
PK	NL	SNP353	7H	60.7	0.0014	2.86	8.27	2.83	1.21	993.34
K	RL	SNP53	1H	137.8	0.0025	2.60	3.19	35.90	12.31	1721.20
QK	RL	SNP53	1H	137.8	0.0027	2.57	3.16	35.99	12.32	1711.13
PK	RL	SNP53	1H	137.8	0.0027	2.57	3.17	36.84	12.29	1704.14
K	RL	SNP54	1H	138.30	0.0000	4.43	6.03	35.90	12.31	1721.20
QK	RL	SNP54	1H	138.30	0.0000	4.37	5.94	35.99	12.32	1711.13
PK	RL	SNP54	1H	138.30	0.0000	4.38	5.98	36.84	12.29	1704.14

$-\log_{10}$  of *P*-values determined for K, PK and QK models with 2.5 as threshold value for strong association on each chromosome (chr) with the proportion of the phenotypic variation [ $R^2$  (%)], genetic variance (Genetic Var), residual variance (Residual Var) and log neperien likelihood ( $-2\text{LnLikelihood}$ ).

**Table 3.4. Significant SNP markers associated with traits detected under salt condition detected by MLM models.**

Model	Trait	Marker	Chr	Distance (cM)	<i>P</i>	$-\log_{10}(P)$	$R^2$ (%)	GeneticVar	ResidualVar	$-2\text{LnLikelihood}$
K	NL	SNP84	2H	63.5	0.001	2.88	3.61	0.86	0.41	677.61
PK	NL	SNP84	2H	63.5	0.001	3.14	4.01	0.86	0.41	666.86
QK	NL	SNP84	2H	63.5	0.002	2.82	3.51	0.88	0.41	670.77
K	SL	SNP57	2H	6.5	0.002	2.69	3.31	9.19	6.60	1491.42
PK	SL	SNP57	2H	6.5	0.002	2.68	3.30	9.24	6.84	1475.41
QK	SL	SNP57	2H	6.5	0.002	2.81	3.47	9.06	6.80	1479.97
K	SL	SNP205	4H	72.1	0.002	2.62	4.30	9.19	6.60	1491.42
K	NL	SNP196	4H	46.4	0.001	3.16	3.99	0.86	0.41	677.61
PK	NL	SNP196	4H	46.4	0.001	3.06	3.85	0.86	0.41	666.86
QK	NL	SNP196	4H	46.4	0.001	3.09	3.89	0.88	0.41	670.77
PK	SL	SNP184	4H	5.6	0.001	2.93	3.71	9.24	6.84	1475.41
QK	SL	SNP184	4H	5.6	0.002	2.76	3.43	9.06	6.80	1479.97
K	RL	SNP180	3H	169.3	0.001	3.29	4.28	5.11	4.77	1374.20
PK	RL	SNP180	3H	169.3	0.001	3.02	3.84	4.71	4.82	1357.50
QK	RL	SNP180	3H	169.3	0.001	3.12	3.99	4.94	4.77	1363.72
K	NL	SNP166	3H	123.7	0.000	3.76	4.92	0.86	0.41	677.61
PK	NL	SNP166	3H	123.7	0.000	3.91	5.16	0.86	0.41	666.86
QK	NL	SNP166	3H	123.7	0.000	3.66	4.77	0.88	0.41	670.77
K	RL	SNP128	3H	10.8	0.001	2.89	4.78	5.11	4.77	1374.20
PK	RL	SNP128	3H	10.8	0.002	2.80	4.59	4.71	4.82	1357.50
QK	RL	SNP128	3H	10.8	0.002	2.77	4.53	4.94	4.77	1363.72
K	SL	SNP105	2H	115.1	0.001	2.89	4.59	9.19	6.60	1491.42
PK	SL	SNP105	2H	115.1	0.000	3.33	5.47	9.24	6.84	1475.41
QK	SL	SNP105	2H	115.1	0.001	3.11	5.00	9.06	6.80	1479.97

**Table 3.4. Significant SNP markers associated with traits under salt condition detected by MLM model (Continued).**

Model	Trait	Marker	Chr	Distance (cM)	<i>P</i>	$-\log_{10}(P)$	$R^2$ (%)	Genetic Var	Residual Var	$-2\text{LnLikelihood}$
QK	LIS	SNP56	1H	140.5	0.0030	2.52	4	0.09	0.38	573.19
PK	LIS	SNP56	1H	140.5	0.0007	3.13	5	0.14	0.38	547.75
K	LIS	SNP56	1H	140.5	0.0018	2.74	4	0.06	0.39	577.45
QK	LIS	SNP86	2H	68.2	0.0010	2.99	5	0.09	0.38	573.19
PK	LIS	SNP86	2H	68.2	0.0013	2.90	5	0.14	0.38	547.75
K	LIS	SNP86	2H	68.2	0.0011	2.95	5	0.06	0.39	577.45
QK	LIS	SNP156	3H	91.9	0.0013	2.89	5	0.09	0.38	573.19
PK	LIS	SNP156	3H	91.9	0.0000	4.42	8	0.14	0.38	547.75
K	LIS	SNP156	3H	91.9	0.0024	2.61	4	0.06	0.39	577.45
PK	LIS	SNP150	3H	74.8	0.0032	2.49	4	0.14	0.38	547.75
PK	LIS	SNP203	4H	65.1	0.0027	2.57	4	0.14	0.38	547.75
PK	LIS	SNP253	5H	89.4	0.0032	2.50	4	0.14	0.38	547.75

$-\log_{10}$  of *P*-values determined for K, PK and QK models with 2.5 as threshold value for strong association on each chromosome (chr) with the proportion of the phenotypic variation [ $R^2$  (%)], genetic variance (Genetic Var), residual variance (Residual Var) and log neperien likelihood ( $-2\text{LnLikelihood}$ ).

**Table 3.5. Significant SNP markers associated with salt tolerance related traits (STI).**

Model	Trait	Marker	Chr	Distance (cM)	<i>P</i>	$-\log_{10}(P)$	$R^2$ (%)	Genetic Var	Residual Var	$-2\text{LnLikelihood}$
QK	STIPDW	SNP53	1H	137.8	0.0006	3.22	5	121.98	228.95	2462.11
K	STIPDW	SNP53	1H	137.8	0.0007	3.17	5	121.32	228.85	2474.25
QK	STIPDW	SNP133	3H	24.2	0.0028	2.55	4	121.98	228.95	2462.11
K	STIPDW	SNP133	3H	24.2	0.0022	2.67	4	121.32	228.85	2474.25
QK	STIRDW	SNP210	4H	87.5	0.0029	2.54	4	154.75	276.80	2515.38
QK	STIRL	SNP54	1H	138.3	0.0001	4.00	6	165.92	129.22	2314.99
PK	STIRL	SNP54	1H	138.3	0.0033	2.48	3	220.17	96.44	2180.93
K	STIRL	SNP54	1H	138.3	0.0001	3.91	6	163.61	129.29	2326.98
QK	STIRL	SNP53	1H	137.8	0.0015	2.84	5	165.92	129.22	2314.99
K	STIRL	SNP53	1H	137.8	0.0015	2.82	5	163.61	129.29	2326.98
QK	STIRL	SNP52	1H	135.6	0.0004	3.41	5	165.92	129.22	2314.99
K	STIRL	SNP52	1H	135.6	0.0006	3.26	5	163.61	129.29	2326.98
QK	STIRL	SNP49	1H	127.1	0.0020	2.70	4	165.92	129.22	2314.99
K	STIRL	SNP49	1H	127.1	0.0023	2.63	4	163.61	129.29	2326.98
QK	STIRL	SNP48	1H	125.3	0.0002	3.63	6	165.92	129.22	2314.99
K	STIRL	SNP48	1H	125.3	0.0004	3.42	6	163.61	129.29	2326.98
QK	STIRL	SNP43	1H	112.5	0.0025	2.61	4	165.92	129.22	2314.99
K	STIRL	SNP43	1H	112.5	0.0020	2.70	4	163.61	129.29	2326.98
QK	STIRL	SNP4	1H	3.8	0.0011	2.95	5	165.92	129.22	2314.99
K	STIRL	SNP4	1H	3.8	0.0012	2.91	5	163.61	129.29	2326.98
QK	STIRL	SNP36	1H	95.4	0.0001	3.88	6	165.92	129.22	2314.99
K	STIRL	SNP36	1H	95.4	0.0003	3.58	6	163.61	129.29	2326.98
QK	STIRL	SNP22	1H	62.8	0.0001	4.01	7	165.92	129.22	2314.99
K	STIRL	SNP22	1H	62.8	0.0001	4.09	7	163.61	129.29	2326.98



**Table 3.5. Significant SNP markers associated with salt tolerance related traits (STI) (Continued).**

<b>Model</b>	<b>Trait</b>	<b>Marker</b>	<b>Chr</b>	<b>Distance</b>	<b><i>P</i></b>	<b><math>-\log_{10}(P)</math></b>	<b><math>R^2</math> (%)</b>	<b>Genetic Var</b>	<b>Residual Var</b>	<b>-2LnLikelihood</b>
QK	STIRL	SNP94	2H	85.9	0.0024	2.63	4	165.92	129.22	2314.99
QK	STIRL	SNP102	2H	105.8	0.0030	2.53	4	165.92	129.22	2314.99
QK	STIRL	SNP156	3H	91.9	0.0015	2.83	5	165.92	129.22	2314.99
K	STIRL	SNP156	3H	91.9	0.0023	2.64	4	163.61	129.29	2326.98
QK	STIRL	SNP146	3H	63.0	0.0013	2.88	5	165.92	129.22	2314.99
K	STIRL	SNP146	3H	63.0	0.0015	2.83	5	163.61	129.29	2326.98
QK	STIRL	SNP143	3H	54.4	0.0009	3.06	5	165.92	129.22	2314.99
K	STIRL	SNP143	3H	54.4	0.0010	3.01	5	163.61	129.29	2326.98
QK	STIRL	SNP134	3H	26.9	0.0021	2.69	4	165.92	129.22	2314.99
K	STIRL	SNP134	3H	26.9	0.0022	2.66	4	163.61	129.29	2326.98
PK	STIRL	SNP174	3H	150.4	0.0003	3.46	6	220.17	96.44	2180.93
QK	STIRL	SNP163	3H	114.0	0.0027	2.58	4	165.92	129.22	2314.99
QK	STIRL	SNP161	3H	107.6	0.0001	3.84	6	165.92	129.22	2314.99
K	STIRL	SNP161	3H	107.6	0.0003	3.58	6	163.61	129.29	2326.98
QK	STIRL	SNP211	4H	89.4	0.0028	2.55	4	165.92	129.22	2314.99
QK	STIRL	SNP204	4H	68.2	0.0003	3.56	6	165.92	129.22	2314.99
K	STIRL	SNP204	4H	68.2	0.0003	3.50	6	163.61	129.29	2326.98
QK	STIRL	SNP194	4H	42.5	0.0001	4.03	7	165.92	129.22	2314.99
K	STIRL	SNP194	4H	42.5	0.0002	3.75	6	163.61	129.29	2326.98
QK	STIRL	SNP184	4H	5.6	0.0009	3.06	5	165.92	129.22	2314.99
K	STIRL	SNP184	4H	5.6	0.0008	3.08	5	163.61	129.29	2326.98
QK	STIRL	SNP274	5H	155.1	0.0025	2.60	4	165.92	129.22	2314.99
PK	STIRL	SNP247	5H	68.4	0.0014	2.86	4	220.17	96.44	2180.93
QK	STIRL	SNP244	5H	60.7	0.0005	3.31	5	165.92	129.22	2314.99
K	STIRL	SNP244	5H	60.7	0.0007	3.19	5	163.61	129.29	2326.98

**Table 3.5. Significant SNP markers associated with salt tolerance related traits (STI) (Continued).**

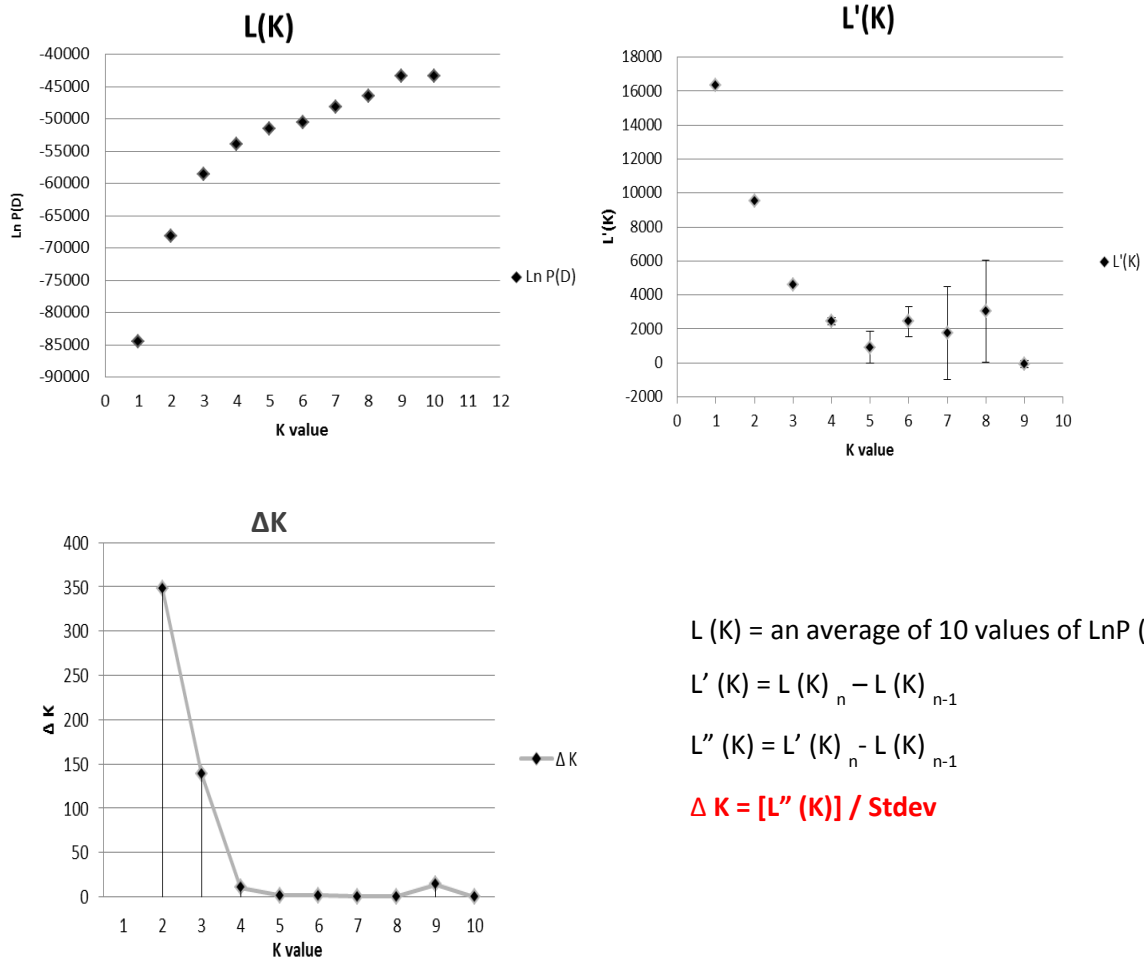
Model	Trait	Marker	Chr	Distance (cM)	$P$	$-\log_{10}(P)$	$R^2$ (%)	Genetic Var	Residual Var	$-2\text{LnLikelihood}$
QK	STIRL	SNP322	6H	110.3	0.0005	3.32	5	165.92	129.22	2314.99
K	STIRL	SNP322	6H	110.3	0.0007	3.16	5	163.61	129.29	2326.98
QK	STIRL	SNP316	6H	90.2	0.0006	3.25	5	165.92	129.22	2314.99
K	STIRL	SNP316	6H	90.2	0.0008	3.10	5	163.61	129.29	2326.98
QK	STIRL	SNP302	6H	52.8	0.0011	2.94	5	165.92	129.22	2314.99
K	STIRL	SNP302	6H	52.8	0.0013	2.89	5	163.61	129.29	2326.98
QK	STIRL	SNP296	6H	28.4	0.0022	2.67	4	165.92	129.22	2314.99
K	STIRL	SNP296	6H	28.4	0.0021	2.69	4	163.61	129.29	2326.98
QK	STIRL	SNP383	7H	161.5	0.0006	3.24	5	165.92	129.22	2314.99
K	STIRL	SNP383	7H	161.5	0.0008	3.10	5	163.61	129.29	2326.98
QK	STIRL	SNP366	7H	107.9	0.0004	3.45	6	165.92	129.22	2314.99
K	STIRL	SNP366	7H	107.9	0.0006	3.26	5	163.61	129.29	2326.98
QK	STISDW	SNP53	1H	137.8	0.0002	3.82	6	125.25	231.52	2465.82
PK	STISDW	SNP53	1H	137.8	0.0029	2.54	3	311.55	185.52	2345.10
K	STISDW	SNP53	1H	137.8	0.0002	3.77	6	124.61	231.41	2477.99
PK	STISDW	SNP82	2H	59.9	0.0038	2.54	3	311.55	185.52	2345.10
QK	STISL	SNP90	2H	74.4	0.0020	2.70	4	45.44	34.85	1933.23
K	STISL	SNP90	2H	74.4	0.0020	2.71	4	44.58	34.89	1943.90
QK	STISL	SNP208	4H	81.7	0.0008	3.12	5	45.44	34.85	1933.23
K	STISL	SNP208	4H	81.7	0.0008	3.11	5	44.58	34.89	1943.90
PK	STISL	SNP184	4H	5.6	0.0010	3.01	4	47.00	25.47	1818.98
K	STISL	SNP184	4H	5.6	0.0031	2.51	4	44.58	34.89	1943.90
QK	STISL	SNP310	6H	72.5	0.0016	2.79	4	45.44	34.85	1933.23
PK	STISL	SNP310	6H	72.5	0.0035	2.46	3	47.00	25.47	1818.98
K	STISL	SNP310	6H	72.5	0.0012	2.92	5	44.58	34.89	1943.90
K	STISL	SNP336	7H	15.0	0.0028	2.55	4	44.58	34.89	1943.90

$-\log_{10}$  of  $P$ -values determined for K, PK and QK models with 2.5 as threshold value for strong association on each chromosome (chr) with the proportion of the phenotypic variation [ $R^2$  (%)], genetic variance (Genetic Var), residual variance (Residual Var) and log neperien likelihood ( $-2\text{LnLikelihood}$ ).

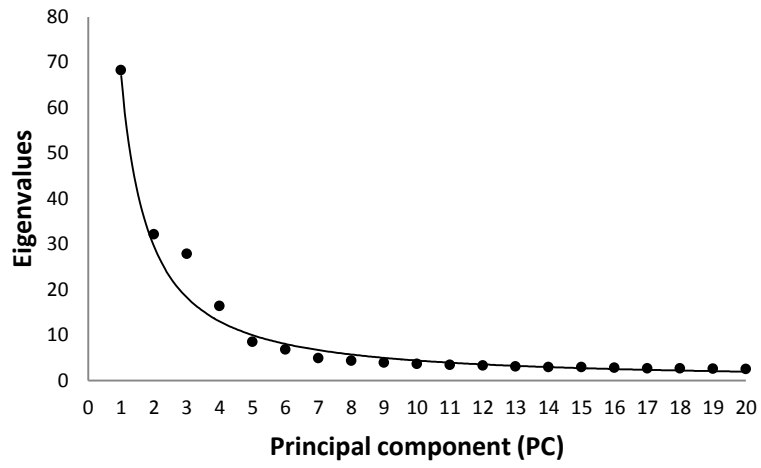
**Table 3.6. QTLs associated with salt tolerance [ $-\log_{10}(P) > 2.44$ ] and their characteristics on barley chromosomes.**

Traits	Chr	Marker	Position (cM)	alleles	(+)	$-\log_{10}(P)$	$R^2$ (%)	Previous reports
<b>STI (SDW)</b>	1H	4927-1340	137.8	[A/C]	C	2.54	6	Mano and Takeda (1997), Rivandi et al. (2010)
<b>STI (SDW)</b>	2H	1826-229	59.9	[A/G]	G	2.54	3	Nguyen et al. (2013a), Zhou et al. (2011)
<b>LIS</b>	1H	3263-2865	140.5	[A/G]	G	3.12	5	Mano and Takeda (1997), Rivandi et al. (2010)
<b>LIS</b>	2H	4434-804	68.2	[A/G]	G	2.9	5	Nguyen et al. (2013a)
<b>LIS</b>	3H	2236-773	91.9	[A/G]	G	4.42	8	
<b>LIS</b>	4H	ABC09432-1-1160	65.1	[A/G]	G	2.57	4	
<b>LIS</b>	5H	ABC06144-pHv8601	89.4	[A/G]	G	2.5	4	Mano and Takeda (1997)

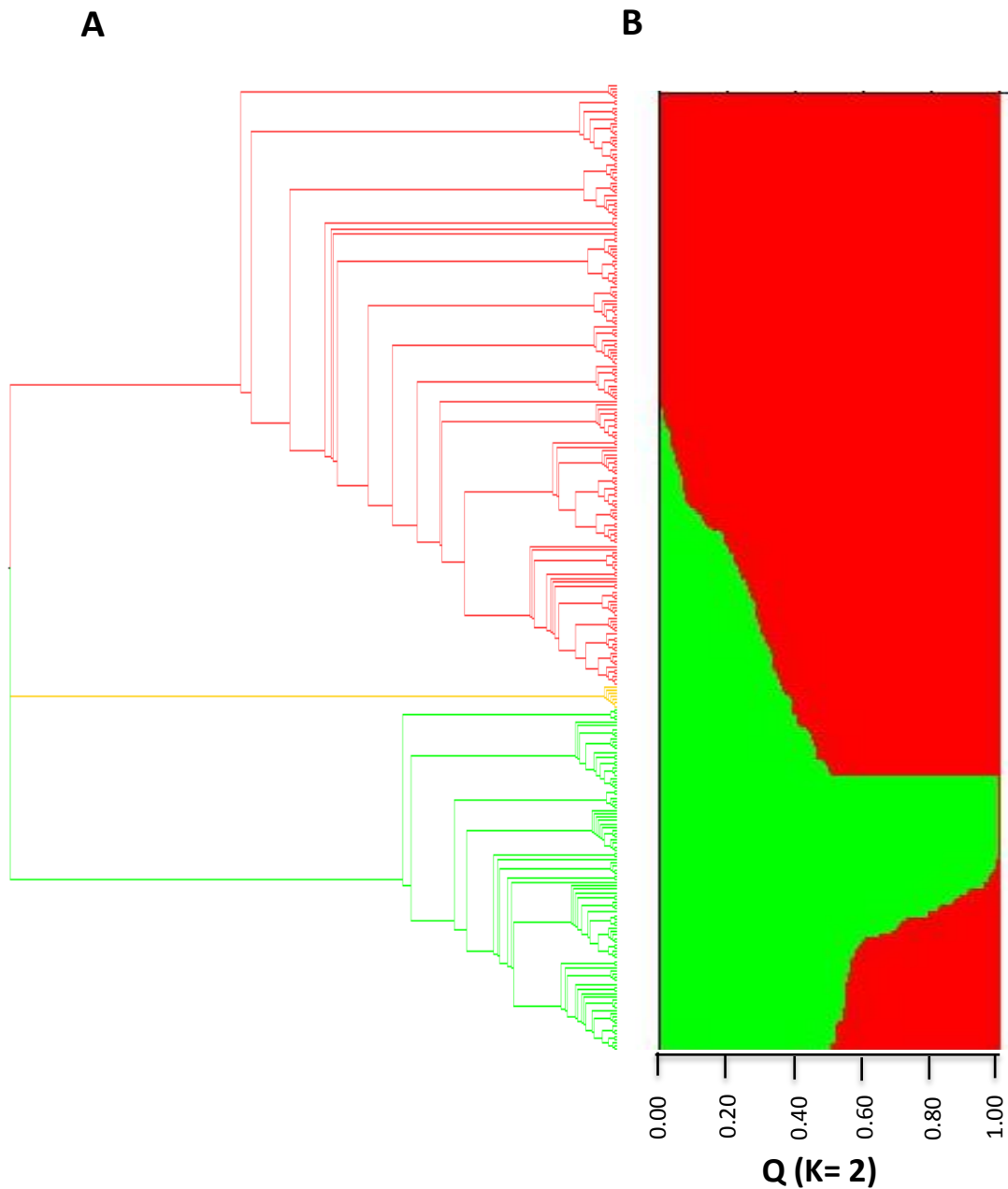
$-\log_{10}$  of  $P$ -values determined for K, PK and QK models with 2.44 as threshold value for strong association on each chromosome (chr).



**Fig.3.1. L (K), L' (K) and ΔK methods for population structure of Asian barley based on the genetic diversity detected by 384 SNP markers.**



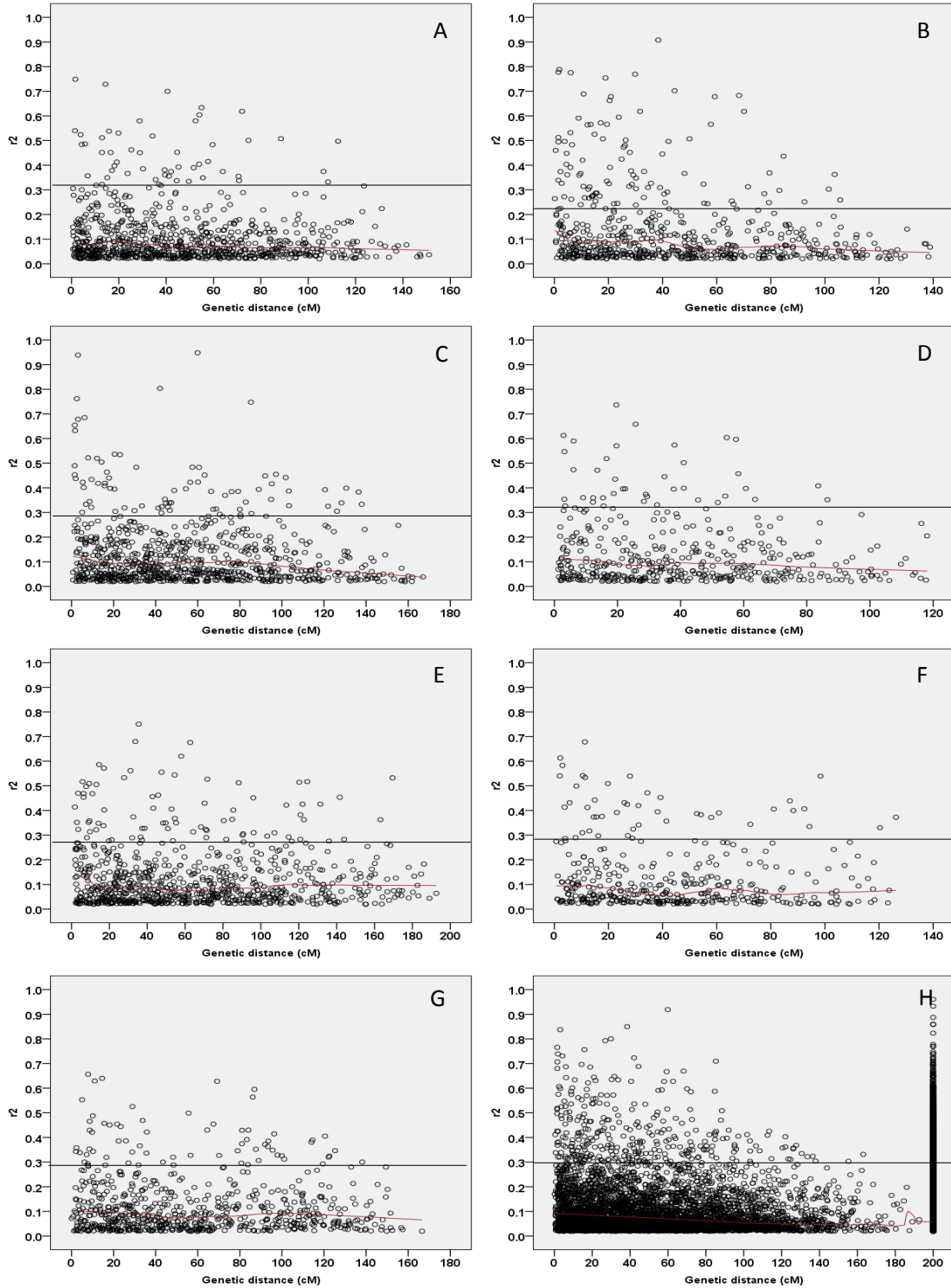
**Fig. 3.2. Eigenanalysis based on the 384 SNP markers of 296 barley accessions. Plot shows 20 significant axes (PCs) with Eigenvalue expressed by PCs.**



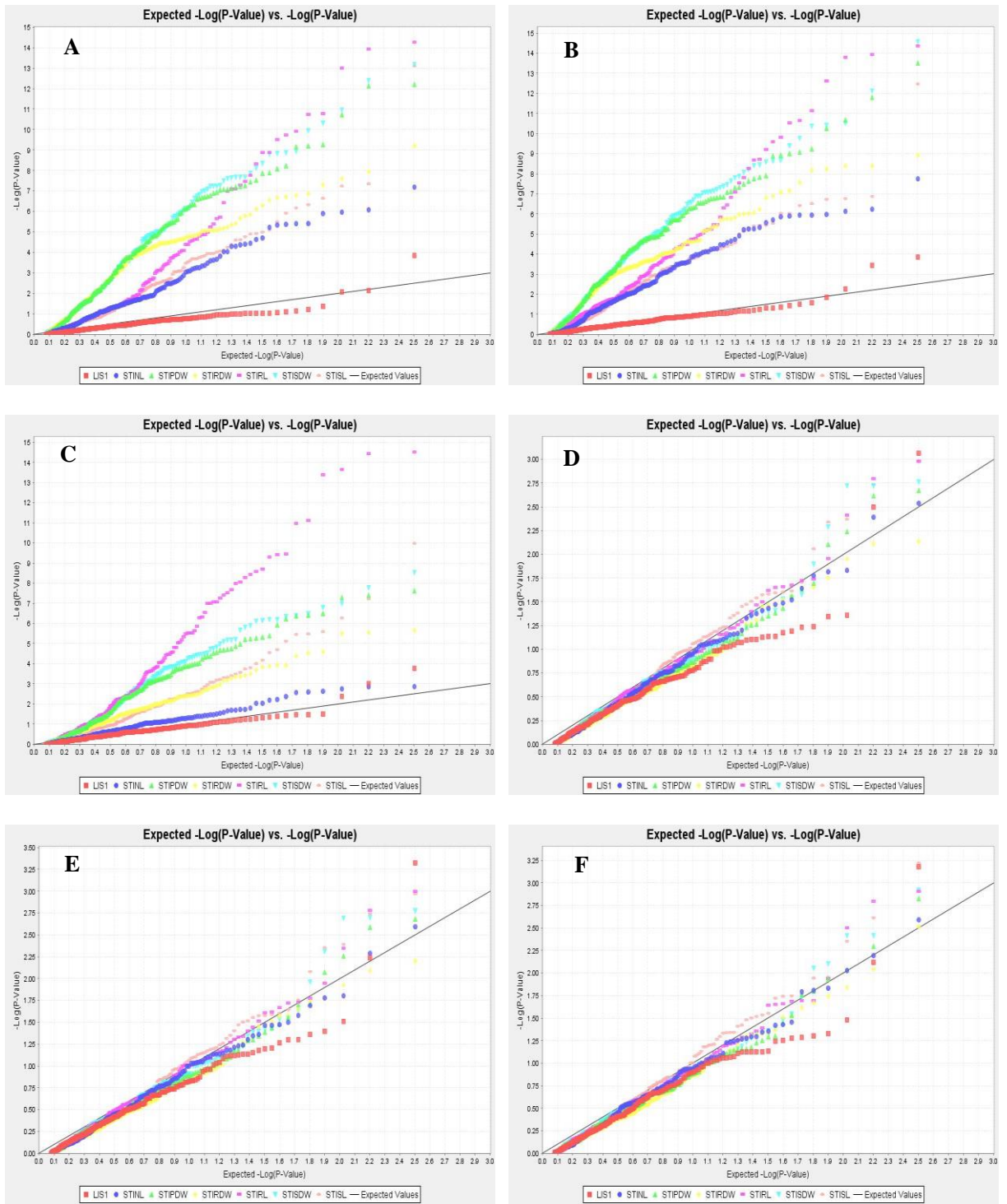
**Fig.3.3. Population structure of Asian barley accessions constructed with the (A): neighbor joining method (NJ) and (B) Q method.**

(A) Neighbor-joining (NJ) tree of 296 Asian barley accessions were constructed from 384 SNP markers and developed with TASSEL.

(B) Summary of plots of estimate of Q for K=2 in Asian barley accessions using STRUCTURE software. Each individual is represented by a single vertical divided in to K colored segments, where K is the number of cluster assumed with the length proportional to each of the K inferred cluster.

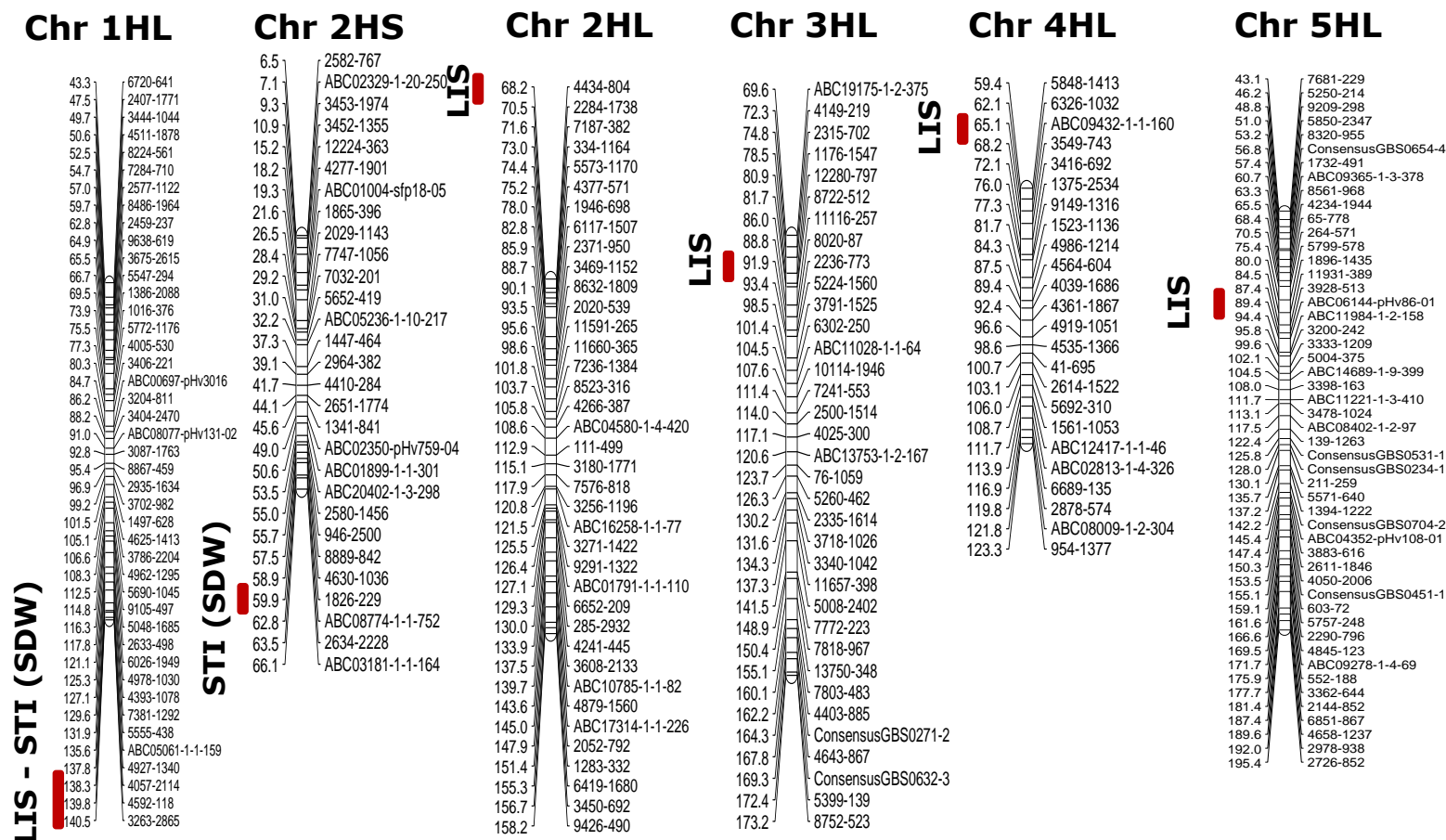


**Fig. 3.4.** Linkage disequilibrium decay on the chromosomes 1H (A), 2H (B), 3H (C), 4H (D), 5H (E), 6H (F), 7H (G) and in the full length genome (H). The horizontal line indicates the 95<sup>th</sup> percentile distribution of unlinked  $r^2$ . The LOESS fitting curve (red line) illustrates the LD decay.



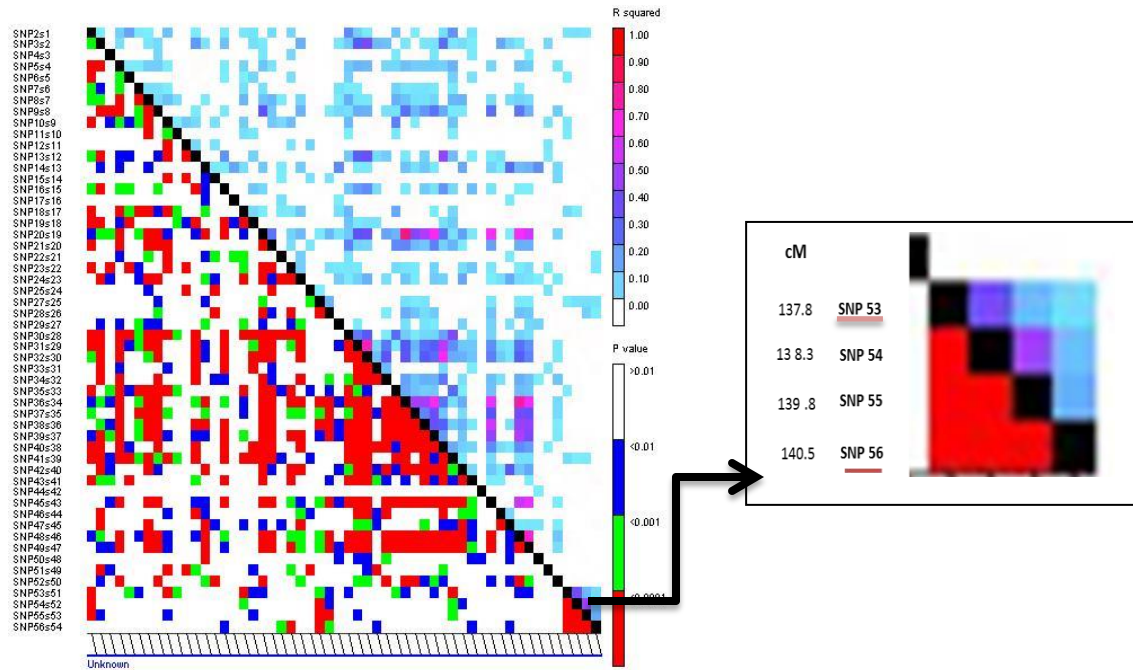
**Fig. 3.5. QQ plot for the traits LIS and STI of each trait in the naïve (A), Q (B), PC (C), K (D), QK (E) and PK (F) models.**



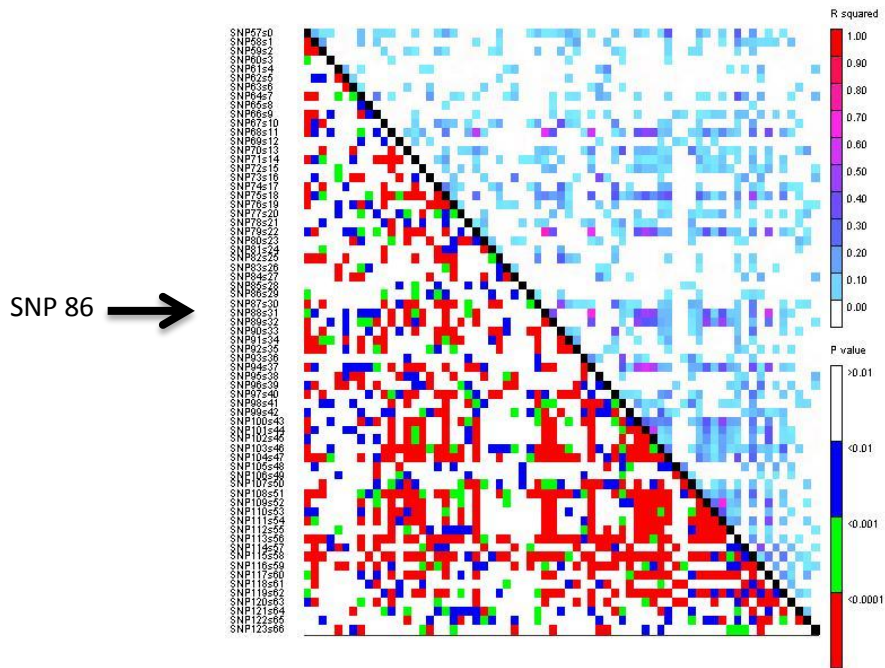


**Fig. 3.6. Chromosome locations of QTL for salt tolerance associated with different traits in the Asian barley accessions (in the right of the chromosome: distance in (cM); in the left: SNP marker names).**

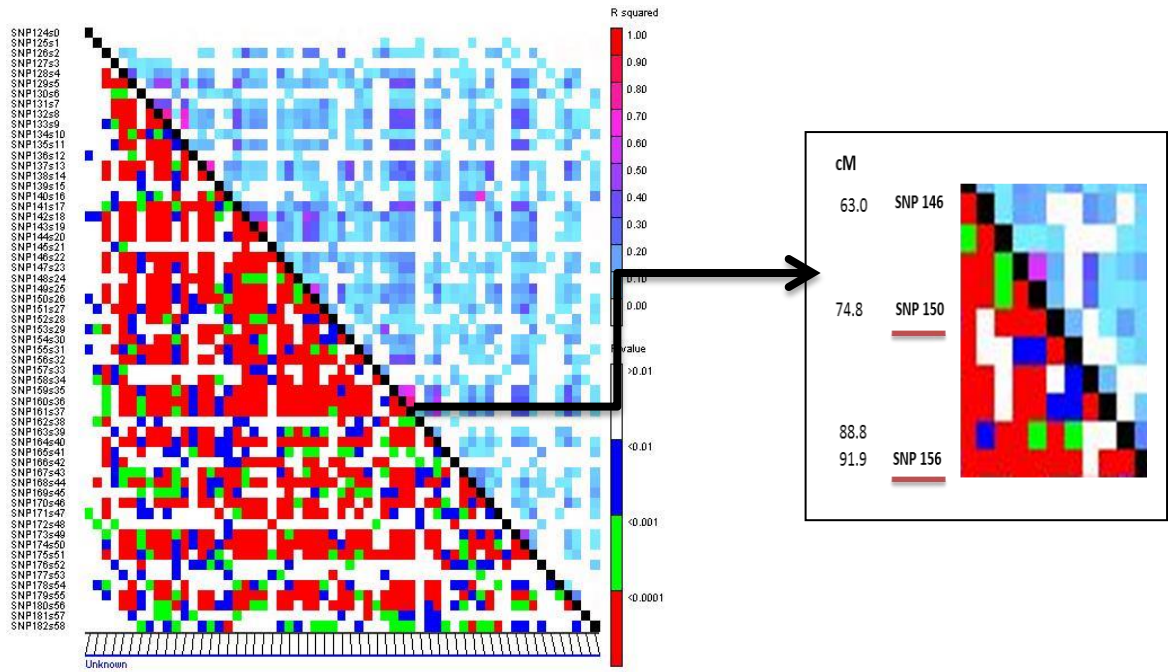
 The SNP markers associated with salt tolerance



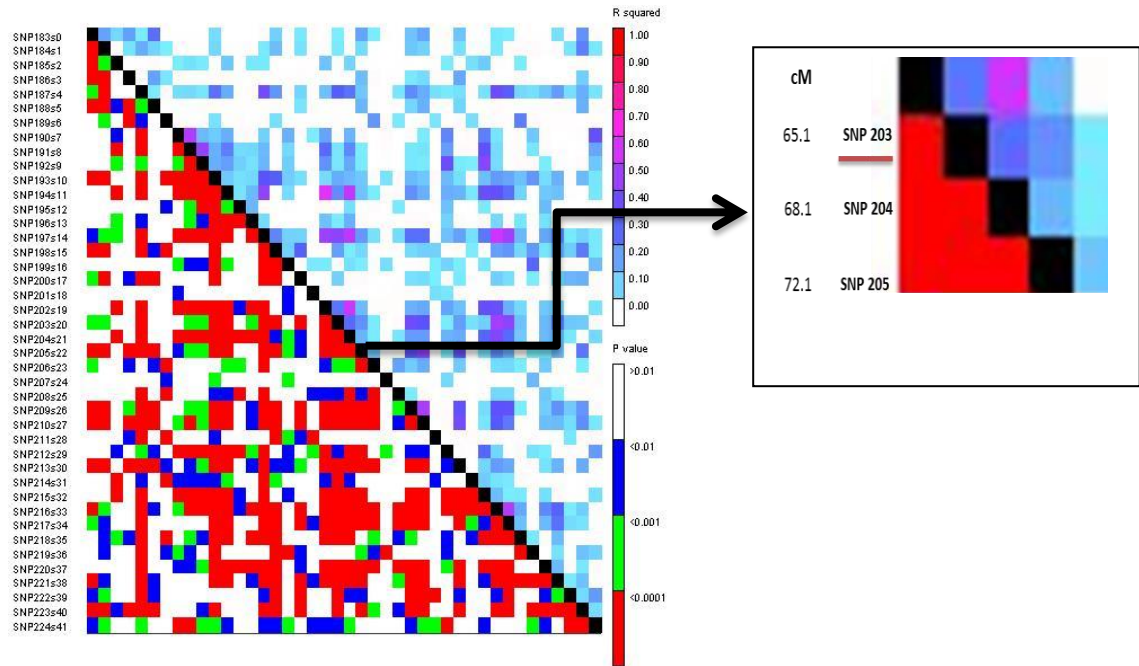
**Fig. 3.7.** LD plot on the chromosome 1H generated by 56 SNP markers and markers significantly associated with salt tolerance trait. The most significantly associated SNP markers (SNP 53 and SNP 56) are underlined.



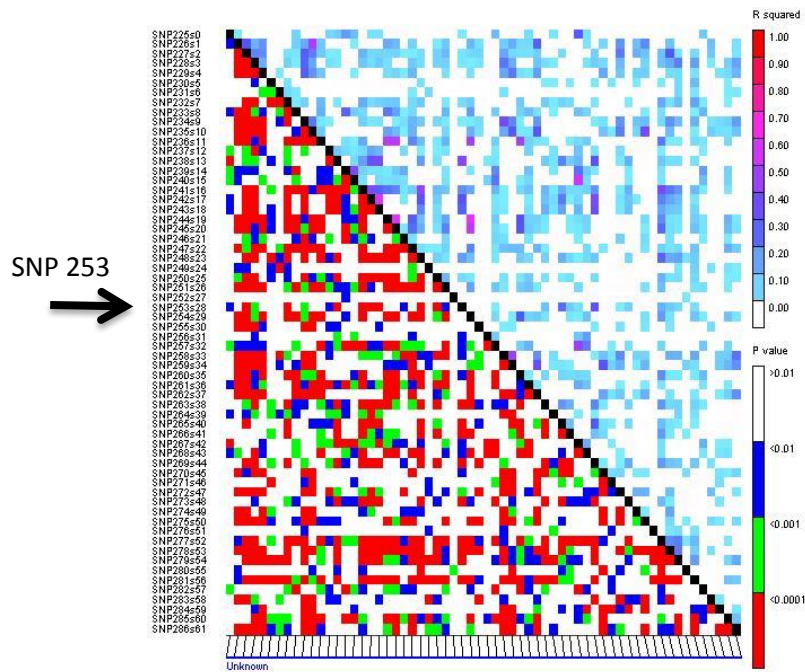
**Fig. 3.8. LD plot on the chromosome 2H generated by 66 SNP markers.**



**Fig. 3.9.** LD plot on the chromosome 3H generated by 59 SNP markers.



**Fig. 3.10.** LD plot on the chromosome 4H generated by 43 SNP.



**Fig. 3.11.** LD plot on the chromosome 5H generated by 62 SNP markers.

## **CHAPTER 4:**

### **DISCUSSION AND PERSPECTIVE**

Barley is one of the oldest domesticated grain crops .It has been cultivated for over 8000 years. Barley is ranked fourth in the worldwide grain production after rice, wheat and maize, and ranked second in the industrial use of grain and is projected to total 27.4 million tonnes in 2008. A significantly high value use of barley is for malting to produce malt as a raw material for the production of beer (Goyal and Ahmed 2012, Zhang and Li 2009). Barley is also one of the most important forage cereal crops.

During the last 2 decades, the exploitation of the diversity of germplasm collected and conserved in the gene banks is an issue to improve barley tolerance for abiotic stress. Improving barley tolerance is a necessity due to weather change, infernal increase in the world population that was estimated to be 9 billion in 2050, and to maintain a balance between world consumption and world production. Farmers are starting facing to various problems in order to find suitable cultivars which can grow under the harsh conditions with capacity of high yield and good resistance to abiotic stresses. Development of crop plants tolerant to salt stress is very important to meet the growing food demand. It has been suggested to exploit naturally occurring inter and intra-specific genetic variability by hybridization of selected salt tolerant genotypes with high yielding genotypes adapted with target environment (Munns *et al.* 2006). Although considerable progress has been made in achieving this goal through conventional breeding, this progress is not satisfactory in view of current demand to increase crop productivity in saline environment (Flowers 2004).

The existence of a large amount of genetic diversity in cultivars, landraces and wild barley are under exploitation by many researchers (Ellis *et al.* 2002, Mano *et al.* 1996, Mano and Takeda 1996).

#### **4.1. Analysis of the variation in salt tolerance of barley**

To examine the variation in salt tolerance, 296 Asian barley accessions involved in a core collection were provided by Okayama University. The seedlings were grown for 17 days under control or 250 mM of NaCl conditions. The traits to assess for salt tolerance showed a high variation in response to salt stress. The salt tolerance index of the shoot dry weight [STI (SDW)] and the leaf injury score (LIS) were chosen as suitable traits to assess for salt tolerance at seedling stage in Asian germplasm because non significance of variation among replication under salt and control treatment for this traits and the highest variation among accessions for this two traits.

LIS can be used as trait to assess salt tolerance in barley. The LIS was previously used as trait for salt assesment by Mano and Takeda (1997) and Zhou *et al.* (2011) to analyze the necrosis in the affected leaf by salt. In this study, the leaf injury score (LIS) is considered as a criterion to assess salt tolerance in barley genotypes at seedling stage. However, LIS is complex physiological trait related to ion concentration or quantity and to osmosis. Salt affects almost all processes of the plant, because of the osmotic effects by high ionic concentrations, competitive interference with nutrient uptake and toxic effects on the plant tissue (Flowers and Yeo 1986). In many reports (Gregorio *et al.* 1997, Kanawapee *et al.* 2012, Platten *et al.* 2013, Suriya-arunroj *et al.* 2005, Wahid 2004), LIS was positively correlated with sodium contents in shoot but not with sodium contents in root and  $\text{Na}^+/\text{K}^+$  ratio under salt stress, but negatively correlated with survival percentage and chlorophyll contents. For example, the visual injury score was highly correlated with leaf  $\text{Na}^+$  concentration ( $r=0.66$ ) across all cultivar groups of *O. sativa*, and *O. glaberrima* (Platten *et al.* 2013). The variation among accessions on the LIS is due to the difference of sodium/chloride concentration in plant cells.

Numerous physiological studies on the mechanisms of tolerance during the vegetative stage have been published (Platten *et al.* 2013), most of which showed an inverse relation between shoot  $\text{Na}^+$  content and/or  $\text{Na}^+/\text{K}^+$  ratio and plant survival and grain yield.



Sequestering the excess  $\text{Na}^+$  in vacuoles by tonoplast  $\text{Na}^+/\text{H}^+$  antiporters may be the principal mechanisms involved in the protection of plants. On the whole, stress imposes injuries onto the cellular physiology, resulting in metabolic dysfunction. The leaf injury imposes a negative influence on cell division and the plant growth. This is an indirect advantage to the plant, as any reduction of leaf expansion reduces the surface area of leaves exposed to transpiration and thereby reduces water loss.

LIS can be used as trait of assessment and also in genetic studies. But we don't know which mechanism is behind LIS: exclusion of  $\text{Na}^+$  or localization of  $\text{Na}^+$  in vacuoles. Physiological and biochemical approaches are needed to better understand regulatory mechanisms on salt tolerance in barley.

In this study, a wide range of variation was observed among accessions in term of STI (SDW), STI (RDW), and STI (PDW) and also in term of STI (RL). The biomass production of barley accessions was in significant correlation with the most of traits. In fact, the variation in plant biomass responses to salt was considered to provide the best means of initial selection for salt tolerant genotypes by previous reports (Maggio *et al.* 2007, Krishnamurthy *et al.* 2007, Veatch *et al.* 2008), prior to the evaluation on the basis of specific traits. Krishnamurthy *et al.* (2007) concluded that substantial variation in early vegetative stage for salt tolerance among sorghum genotypes and several relatively salt tolerant and sensitive sorghum genotypes for biomass production were identified. Albacete *et al.* (2008) proved that salt reduced shoot biomass in tomato by 50–60% and photosynthetic area by 20–25% due to both decreasing leaf expansion and delaying leaf appearance, while root growth was less affected, and the root/shoot ratio increased. Shoot growth in salinized tomato plant is controlled by hormonal changes. Abscisic acid (ABA) and amino carboxylic acid (ACC) concentrations strongly increased in roots, xylem sap, and leaves after 1 and 15 days of NaCl treatment (Cramer and Quarrie 2002).

By combining the result of the relationship between salt tolerance [STI (SDW) and LIS] and geographical distribution, we concluded that biomass production of the accessions originated from Japan and Bhutan did not highly affected compared to others. The Japanese accessions include the 2 row type which was significantly tolerant to salt compared to the 6 row type. We suggested that the level of tolerance into 2 rows participates in the tolerance to

salt in Japanese accessions. The 2 row type Japanese accessions may be produced as elite varieties by the Beer Brewing Company or Sapporo Japan, third largest company to develop new barley varieties to improve the quality of beer. The Buthan accessions are in total landraces. We concluded that the tolerance to salt is highly linked to the breeding program and the geographical distribution. Malysheva-Otto *et al.* (2006) and Hadado *et al.* (2009) explained that the high diversity in African and Asian barley accessions is due to hybridization and natural selection and diversified environments. Malysheva-Otto *et al.* (2006) showed that molecular diversity in barley accessions from various geographic regions worldwide differed with respect to allelic richness, frequency of unique alleles and extent of heterogeneity.

#### **4.2. Detection of QTLs for salt tolerance by association mapping**

Biparental QTL mapping for salt tolerance has resulted in the detection of several genomic regions with candidate genes controlling salt tolerance-related traits (Nguyen *et al.* 2013a, Mano and Takeda 1997, Xue *et al.* 2009). However, the QTLs found with biparental mapping strategies often have not lead to the identification of candidate genes for crop improvement, mainly because of the low resolution of QTL mapping due to genetic linkage blocks as a consequence of the small number of recombination events between the two parental genomes.

In this study, association mapping was performed to identify QTLs controlling salt tolerance at seedling stage using 296 Asian accessions, originated from 6 distinct countries, grown under salt stress with 384 SNP markers. Seven significant QTLs for salt tolerance at the vegetative stage were located on chromosomes 1H (2 QTLs), 2H (2 QTLs), 3H (1 QTL), 4H (1 QTL) and 5H (1 QTL). Five and two of these QTLs are associated with LIS and with STI of the SDW, respectively. Among the QTLs, five QTLs had been previously reported elsewhere (Mano and Takeda 1997, Nguyen *et al.* 2013a, Rivandi *et al.* 2010, Zhou *et al.* 2011) on the chromosomes 1H, 2H and 5H. The QTLs for LIS and SDW detected at similar positions (137.8 and 140.5 cM) on the long arm of the chromosome 1H, were previously reported by Mano and Takeda (1997) using the (ABC261) RFLP marker at germination stage derived from the cross of Harrington/TR 306 and were found to closely linked the gene called *HvNax4* (Rivandi *et al.* 2010), which is a gene controlling an environmentally sensitive Na<sup>+</sup>

exclusion, suggesting a highly correlation between sodium exclusion and necrosis and /or chlorosis in leaves caused by salt.

Two QTLs associated with the biomass production (SDW) at LIS were detected on the chromosome 2H at the position 59.9 and 68.2 cM, respectively. A QTL associated with the senescence rating and SL was detected at the position 59.2 cM by Nguyen *et al.* (2013a). Additionally, Zhou *et al.* (2011) detected a QTL for a combined injury score and plant survival traits at the position 48 cM using Dart markers and DH population. The *vrs1* gene was at position 82.4 cM on chromosome 2H with an interval of 14 cM from the QTL associated with the LIS (68.2 cM). This interval indicated clearly no relation between *vrs1* and salt tolerance.

One QTL associated with the LIS was detected on chromosome 4H at the position 65.1 cM. This QTL found to closely link the vernalisation loci *VRN-H2* (Zitzewitz *et al.* 2005) which located at the position 66 cM suggesting that salt tolerance appear to be in interacting with vernalisation requirement.

The QTL for LIS on chromosome 5H at position 89.2 cM was mapped at a similar position to a QTL for same trait by Mano and Takeda (1997). The *VRN-H1* gene for hibernation was located at the position 98 cM with an interval 95-101 cM from the marker HvPhyC and HvBM5A, respectively (Szucs *et al.* 2006), and with an interval of 8.8 cM of the QTL detected for salt tolerance.

Overall, two QTLs associated with LIS were newly detected on chromosomes 3H and 4H suggesting that association mapping can identify new QTLs and explores candidate genes of salt tolerance.

### **4.3. Final goal and perspective**

There are many reports on research for the salt tolerance at early stage in barley but very little attention has been given to the reproductive stage salt tolerance. The conventional methods of plant selection for salt tolerance are not easy because of large environment effects. However, salt assessment under controlled condition has the benefit of reduced environmental effects. However, yield is the complex end product of many factors which jointly or singly influence the seed yield. Estimates of grain yield bring another complexity to the salt response, not just because the crops must be grown in uncontrolled environments for

long periods of time, but because the conversion of shoot biomass to grain biomass is complex.

The question posed is that the germplasm assessed for salt tolerance at seedling stage is applied to improve the salt tolerance at late stage? There are two opinions regarding the application of assessment at seedling stage to improve the grain production. There is no common understanding in this issue. Some researcher's achievement is not to contribute the susceptible genotypes detected at seedling stage to improve grain production. These materials will die at seedling stage if used by farmers. If so, farmers have to sow a large amount of seeds. Researchers indicate that some tolerant genotypes selected at seedling stage cannot continue growing at late stages and so, no seed production.

Munns and James (2003) explained that the assessment based on leaf injury is particularly prone to interference by other factors or by the high pH typical of many salt soils. Another major factor influencing leaf senescence is nitrogen availability. In the field, a salt soil will almost certainly at some stage be a drying soil, so as nitrogen becomes less available, remobilization from old leaves will induce premature senescence, something that does not occur in hydroponics. In the field, additional traits become important, such as those conferring water use efficiency. As a further program in salt tolerance in Asian barley, we have the plan to plant the same material used in this experiment and evaluate it for salt tolerance in grain production. One of the focuses in future study is on validation of QTLs for salt tolerance at maturity stage in association with barley production.

Globally, two main approaches are being used to improve salt tolerance in barley: (i) the exploitation of natural genetic variations, either through direct selection in stressful environments or through mapping quantitative trait loci and subsequent marker-assisted selection; and (ii) the generation of transgenic plants to introduce novel genes and this need gene cloning before.

# Abstract

Salt is one of abiotic stress factors affecting crop production and productivity in arid and semi-arid areas. Almost three quarters of the surface of the earth are covered by salt water and Africa, Near East and Asia are the most severely damaged areas by salt. Salt affected-soil in Asia covered around 25% of the total salt soil.

Among cereals, barley (*Hordeum vulgare* L.) is the most tolerant crop which is considered as an ideal model plant for genetic and physiological studies on salt tolerance due to its short growth period, early maturing, diploid and self-pollination characteristics. Barley is widely cultivated in saline areas as one of the most salt-tolerant field crops. However, barley still suffers from salt toxicity in many areas of the world. On the other hand, dramatic differences can be found among the barley species. The genetic diversification and the adaptability to a broad range of ecological conditions have highly strengthened the salt tolerance in barley. These factors might have raised a rich gene pool in the level of diversity in response to salt

Based on the frequency of markers/genes and DNA polymorphism studies, the Asian barley showed a high level of diversity in landraces, improved varieties and their wild relatives. Asia is known as a secondary center of diversity of barley. Therefore, it was expected to detect novel QTL for salt tolerance using Asian germplasm. Also, barley cultivation in Asia has been severely affected by salt and continuous selection for salt tolerance has taken place in Asia.

The objectives in this study were 1) to evaluate the variation in salt tolerance of Asian accessions of barley, 2) to determine the suitable traits to evaluate salt tolerance and 3) to detect SNP markers associated with salt tolerance at seedling stage by association mapping.

Two hundred ninety-six Asian barley (*Hordeum vulgare* L.) accessions were assessed to detect QTLs underlying salt tolerance by association analysis using a 384 single nucleotide polymorphism (SNP) marker system. The experiment was laid out at the seedling stage in a hydroponic solution under 250 mM NaCl treatment and the control with three replications of four plants each. Salt tolerance was assessed by the leaf injury score (LIS) and salt tolerance indices (STIs) of the number of leaves (NL), shoot length (SL), root length (RL), shoot dry

weight (SDW) and root dry weight (RDW). LIS was scored from 1 to 5 according to the severity of necrosis and chlorosis observed on leaves. There was a wide variation in salt tolerance among the Asian barley accessions. The LIS and STI (SDW) were the most suitable traits for evaluating salt tolerance. The accessions originated from Japan and Bhutan were more tolerant than accessions from other countries. The Japanese two-row type showed significantly higher tolerance to NaCl compared with six-row type. Higher level of tolerance in Japanese two-row type may contribute to higher level of salt tolerance in Japanese accessions compared with other countries. Tolerant and susceptible accessions were classified by a bivariate fit of the STI (SDW) by LIS.

Association was estimated between markers and traits to detect QTLs for LIS and STI (SDW). Seven significant QTLs were detected on chromosomes 1H (2 QTLs), 2H (2 QTLs), 3H (1 QTL), 4H (1 QTL) and 5H (1 QTL). Five QTLs were associated with LIS and 2 QTLs with STI (SDW). Five QTLs were previously reported. Two QTLs associated with LIS were newly detected on chromosomes 3H and 4H. The QTLs detected on chromosomes 4H and 5H were mapped closely to the vernalisation loci *VRN-H2* and *VRN-H1*, respectively. The allele “G” improved the LIS in positive direction at 5 QTLs detected on chromosome 1H, 2H, 3H, 4H and 5H. The allele “C” and “G” improved the STI (SDW) in positive direction on chromosomes 1H and 2H, respectively.

There are many reports on the salt tolerance at early stage in barley but very little attention has been given to the reproductive stage. The lack of economically viable methods of assessment salt tolerance in the field remains an obstacle to breeders. However, yield is the complex end product of many factors which jointly or singly influence the grain yield. A study on salt tolerance in barley germplasm can be undertaken in further study. One of the focuses in future study is on validation of QTLs for salt tolerance at maturity stage in association with yield in barley.

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**Appendix Table 1: QTLs obtained by Naïve model under control condition.**

Trait	Marker	$-\log_{10}(P)$	$R^2$	Trait	Marker	$-\log_{10}(P)$	$R^2$
RL	SNP54	15.10	20.33	RL	SNP378	5.97	7.97
RL	SNP235	11.38	15.34	SDW	SNP304	5.91	7.92
RL	SNP304	11.37	15.47	RDW	SNP226	5.89	7.91
RL	SNP153	11.20	15.40	SDW	SNP53	5.76	7.64
RL	SNP21	10.52	14.15	RL	SNP313	5.70	7.64
RL	SNP53	10.45	14.15	SL	SNP150	5.69	7.98
RL	SNP283	9.91	13.51	RDW	SNP150	5.64	7.90
RL	SNP56	9.70	13.52	NL	SNP244	5.63	7.41
RL	SNP43	9.58	14.17	SL	SNP311	5.62	7.41
RL	SNP303	8.80	12.42	NL	SNP39	5.58	7.39
RL	SNP226	8.58	11.71	NL	SNP245	5.56	7.33
RL	SNP275	8.54	11.50	RL	SNP55	5.55	7.37
NL	SNP34	8.34	11.38	RL	SNP344	5.53	7.47
SL	SNP166	8.18	10.97	NL	SNP48	5.52	7.26
RL	SNP95	8.11	10.98	NL	SNP52	5.50	7.22
RL	SNP177	7.91	10.63	RL	SNP210	5.49	7.28
RL	SNP126	7.76	10.50	NL	SNP217	5.42	7.07
RL	SNP368	7.69	10.40	SL	SNP149	5.42	7.18
RL	SNP91	7.69	10.44	PDW	SNP150	5.41	7.55
NL	SNP37	7.56	10.15	NL	SNP312	5.40	7.08
RL	SNP150	7.16	10.16	RL	SNP310	5.38	7.15
RL	SNP249	7.15	9.71	NL	SNP102	5.37	7.06
NL	SNP194	6.92	9.35	RL	SNP381	5.31	6.91
NL	SNP36	6.85	9.13	PDW	SNP53	5.30	6.99
NL	SNP94	6.83	9.13	PDW	SNP30	5.27	8.78
RDW	SNP304	6.83	9.22	NL	SNP28	5.26	6.94
RL	SNP149	6.80	9.14	NL	SNP337	5.25	6.92
NL	SNP218	6.77	9.22	SDW	SNP186	5.07	6.71
SL	SNP178	6.46	8.70	RL	SNP231	5.06	7.18
PDW	SNP304	6.37	8.57	PDW	SNP178	5.05	6.68
RL	SNP296	6.37	8.45	SDW	SNP150	5.04	7.00
RL	SNP272	6.33	9.03	NL	SNP160	5.03	6.61
SL	SNP210	6.24	8.35	SDW	SNP178	5.00	6.60
RL	SNP205	6.19	12.08	NL	SNP138	4.99	6.50
SL	SNP53	6.12	8.15	NL	SNP196	4.91	6.39
NL	SNP20	6.08	8.09	NL	SNP49	4.91	6.43
RL	SNP186	6.05	8.11	SDW	SNP311	4.89	6.38
RDW	SNP30	6.02	10.11	RL	SNP278	4.83	6.27
RL	SNP361	5.98	8.04	NL	SNP384	4.83	6.29

**Appendix Table 1: QTLs detected by Naïve model under control (continued).**

Trait	Marker	$-\log_{10}(P)$	$R^2$	Trait	Marker	$-\log_{10}(P)$	$R^2$
SDW	SNP30	4.79	7.91	SDW	SNP227	4.08	5.30
RL	SNP188	4.78	6.27	SDW	SNP123	4.08	16.39
PDW	SNP186	4.78	6.29	RL	SNP307	4.07	5.16
NL	SNP161	4.74	6.15	SDW	SNP272	4.06	5.58
PDW	SNP311	4.74	6.16	SDW	SNP166	4.05	5.16
RL	SNP118	4.71	6.19	SDW	SNP41	4.04	5.21
SL	SNP272	4.71	6.57	RDW	SNP235	4.03	5.15
NL	SNP216	4.65	6.06	PDW	SNP272	4.02	5.52
RL	SNP171	4.64	6.11	SL	SNP30	4.01	6.51
RL	SNP311	4.59	5.96	NL	SNP241	4.00	5.17
PDW	SNP149	4.58	5.98	PDW	SNP41	3.99	5.14
SDW	SNP142	4.57	5.97	NL	SNP366	3.98	5.06
NL	SNP88	4.55	5.85	PDW	SNP235	3.95	5.05
NL	SNP178	4.53	5.92	RL	SNP339	3.95	5.06
RDW	SNP149	4.52	5.89	PDW	SNP166	3.93	4.99
NL	SNP319	4.51	5.80	SL	SNP209	3.88	4.93
NL	SNP45	4.49	5.79	SL	SNP227	3.87	5.00
RDW	SNP210	4.48	5.84	NL	SNP202	3.85	4.90
NL	SNP197	4.44	5.79	SL	SNP76	3.83	4.86
PDW	SNP142	4.43	5.77	RDW	SNP206	3.78	4.81
RL	SNP142	4.39	5.70	SDW	SNP210	3.77	4.82
NL	SNP163	4.36	5.66	SDW	SNP183	3.75	4.83
SDW	SNP149	4.35	5.65	RDW	SNP303	3.74	4.98
SL	SNP105	4.31	6.83	NL	SNP166	3.73	4.71
RL	SNP33	4.28	5.52	RL	SNP48	3.73	4.71
RL	SNP369	4.26	5.63	SDW	SNP235	3.73	4.72
RDW	SNP178	4.22	5.48	SL	SNP188	3.70	4.72
RL	SNP219	4.21	5.43	RL	SNP80	3.70	4.69
NL	SNP204	4.18	5.38	RDW	SNP310	3.69	4.72
RL	SNP152	4.18	5.44	NL	SNP339	3.68	4.67
NL	SNP9	4.18	5.35	RL	SNP227	3.68	4.71
NL	SNP115	4.17	5.41	PDW	SNP275	3.65	4.62
NL	SNP40	4.15	5.52	SL	SNP366	3.64	4.58
PDW	SNP227	4.13	5.38	SL	SNP304	3.61	4.60
SL	SNP54	4.13	5.36	RL	SNP352	3.60	4.53
SL	SNP218	4.13	5.41	SDW	SNP275	3.59	4.52
NL	SNP117	4.12	5.25	NL	SNP116	3.57	4.54
NL	SNP137	4.11	5.31	RDW	SNP35	3.57	4.56

**Appendix Table 1: QTLs detected by Naïve model under control (continued).**

Trait	Marker	$-\log_{10}(P)$	$R^2$	Trait	Marker	$-\log_{10}(P)$	$R^2$
NL	SNP148	4.11	5.32	SL	SNP142	3.26	4.08
PDW	SNP210	4.09	5.28	PDW	SNP351	3.25	4.08
NL	SNP179	3.53	4.45	SDW	SNP345	3.24	4.07
PDW	SNP183	3.53	4.52	SL	SNP186	3.24	4.07
SL	SNP232	3.53	6.76	PDW	SNP188	3.23	4.04
SL	SNP171	3.53	4.50	RDW	SNP21	3.18	3.93
SL	SNP143	3.53	4.42	PDW	SNP345	3.18	3.98
SL	SNP348	3.52	4.49	RL	SNP237	3.17	3.93
NL	SNP291	3.51	4.41	SL	SNP350	3.16	3.95
RDW	SNP227	3.51	4.47	RDW	SNP272	3.15	4.18
RL	SNP209	3.50	4.38	PDW	SNP95	3.15	3.92
NL	SNP254	3.49	4.40	SDW	SNP95	3.14	3.91
NL	SNP75	3.48	4.38	RDW	SNP142	3.13	3.90
PDW	SNP226	3.48	4.42	NL	SNP230	3.13	6.80
SL	SNP41	3.45	4.35	RL	SNP60	3.13	4.03
NL	SNP358	3.45	4.55	NL	SNP154	3.12	3.87
SL	SNP90	3.43	4.26	RDW	SNP53	3.12	3.87
NL	SNP351	3.43	4.33	SDW	SNP76	3.12	3.84
SDW	SNP188	3.42	4.32	SDW	SNP206	3.12	3.86
SL	SNP303	3.41	4.48	RL	SNP194	3.11	3.89
NL	SNP5	3.40	4.34	PDW	SNP18	3.11	3.88
NL	SNP220	3.40	4.30	RL	SNP22	3.10	4.08
SL	SNP351	3.40	4.30	SDW	SNP54	3.10	3.87
PDW	SNP206	3.39	4.26	SL	SNP60	3.10	3.99
RDW	SNP311	3.38	4.22	PDW	SNP65	3.10	3.86
PDW	SNP123	3.36	13.34	RDW	SNP249	3.09	3.86
NL	SNP277	3.36	4.17	NL	SNP320	3.09	3.78
PDW	SNP249	3.34	4.23	RDW	SNP41	3.08	3.82
PDW	SNP126	3.34	4.20	SL	SNP253	3.07	3.83
SDW	SNP65	3.34	4.21	RL	SNP124	3.07	3.83
SDW	SNP18	3.33	4.19	RL	SNP274	3.06	3.78
SL	SNP144	3.31	4.15	NL	SNP30	3.04	4.77
SDW	SNP110	3.29	4.17	SL	SNP18	3.04	3.78
SDW	SNP351	3.28	4.13	NL	SNP68	3.04	3.75
RL	SNP5	3.28	4.17	RL	SNP244	3.03	3.71
RL	SNP213	3.28	4.65	NL	SNP159	3.02	3.71
NL	SNP174	3.27	4.15	RDW	SNP186	3.01	3.74
RDW	SNP126	3.27	4.09	SL	SNP369	3.01	3.80
SDW	SNP249	3.26	4.12	RL	SNP170	3.01	3.71

**Appendix Table 1: QTLs detected by Naïve model under control (continued).**

Trait	Marker	$-\log_{10}(P)$	$R^2$	Trait	Marker	$-\log_{10}(P)$	$R^2$
RL	SNP41	3.00	3.71	SL	SNP287	2.77	3.37
SL	SNP34	3.00	3.73	SDW	SNP348	2.76	3.39
RL	SNP36	2.99	3.65	PDW	SNP21	2.76	3.33
PDW	SNP76	2.96	3.62	NL	SNP349	2.76	3.68
RL	SNP94	2.96	3.62	RDW	SNP153	2.76	3.41
SL	SNP231	2.96	3.93	SL	SNP203	2.76	3.35
RL	SNP147	2.95	3.62	PDW	SNP54	2.76	3.37
RL	SNP35	2.95	3.66	NL	SNP182	2.76	3.34
RL	SNP293	2.94	3.65	RDW	SNP166	2.75	3.32
SL	SNP217	2.94	3.56	SL	SNP226	2.75	3.37
NL	SNP289	2.93	3.86	PDW	SNP366	2.75	3.31
RL	SNP175	2.92	3.58	RDW	SNP366	2.74	3.31
NL	SNP32	2.91	3.57	PDW	SNP106	2.74	3.33
PDW	SNP35	2.91	3.60	SL	SNP175	2.73	3.32
RL	SNP27	2.90	3.57	SDW	SNP8	2.73	3.35
PDW	SNP303	2.90	3.72	NL	SNP100	2.71	3.32
SDW	SNP378	2.90	3.56	SL	SNP283	2.71	3.30
SL	SNP6	2.88	3.58	NL	SNP368	2.70	3.28
RL	SNP202	2.88	3.52	SL	SNP207	2.69	3.33
PDW	SNP310	2.86	3.52	NL	SNP129	2.68	3.24
PDW	SNP8	2.85	3.52	RL	SNP92	2.68	3.25
RDW	SNP157	2.85	3.54	SDW	SNP153	2.67	3.28
NL	SNP248	2.84	3.47	SL	SNP118	2.67	3.24
NL	SNP343	2.83	8.32	NL	SNP185	2.66	3.23
SDW	SNP171	2.83	3.49	NL	SNP348	2.65	3.23
RL	SNP158	2.82	3.56	SDW	SNP106	2.65	3.20
SL	SNP345	2.82	3.46	RL	SNP37	2.65	3.18
NL	SNP156	2.81	3.43	NL	SNP38	2.64	3.19
PDW	SNP378	2.81	3.43	PDW	SNP171	2.64	3.21
RDW	SNP278	2.81	3.39	RL	SNP382	2.63	3.14
PDW	SNP153	2.80	3.47	RDW	SNP106	2.62	3.16
RL	SNP84	2.80	3.45	SL	SNP278	2.61	3.12
RL	SNP292	2.80	3.44	RL	SNP77	2.61	3.12
RL	SNP343	2.79	8.18	RDW	SNP95	2.60	3.14
PDW	SNP348	2.78	3.42	SDW	SNP366	2.60	3.11
SDW	SNP226	2.78	3.41	RL	SNP178	2.60	3.14
SL	SNP206	2.77	3.37	SDW	SNP35	2.60	3.15
NL	SNP180	2.77	3.38	SDW	SNP303	2.59	3.26
RDW	SNP8	2.77	3.40	PDW	SNP283	2.59	3.13
RDW	SNP351	2.58	3.12	SDW	SNP237	2.58	3.08



**Appendix Table 2: QTLs detected by Q model under control.**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP43	15.74	22.46	SDW	SNP53	5.13	6.60
RL	SNP54	13.49	17.74	RL	SNP186	5.11	6.60
RL	SNP91	10.84	14.41	RL	SNP378	5.00	6.43
RL	SNP56	9.97	13.62	NL	SNP28	4.99	6.35
RL	SNP303	9.82	13.46	NL	SNP358	4.95	6.65
RL	SNP53	9.64	12.73	RL	SNP272	4.94	6.78
RL	SNP153	9.59	12.90	SL	SNP178	4.92	6.21
RL	SNP304	9.58	12.73	RL	SNP219	4.86	6.18
RL	SNP235	9.37	12.27	RL	SNP293	4.84	6.23
RL	SNP113	9.17	12.22	RDW	SNP150	4.82	6.52
RL	SNP177	8.93	11.71	NL	SNP194	4.79	6.12
RL	SNP21	8.19	10.63	RL	SNP198	4.78	6.06
RL	SNP283	8.18	10.81	PDW	SNP53	4.69	5.98
RL	SNP152	7.69	10.17	PDW	SNP304	4.66	5.97
RL	SNP296	7.66	10.00	NL	SNP36	4.64	5.83
RL	SNP361	7.29	9.63	NL	SNP94	4.62	5.82
RL	SNP205	7.01	13.73	SL	SNP232	4.42	8.52
RL	SNP275	6.80	8.82	RL	SNP55	4.41	5.57
RL	SNP104	6.73	8.90	RDW	SNP226	4.32	5.53
RL	SNP16	6.70	8.70	SDW	SNP304	4.26	5.41
RL	SNP249	6.69	8.78	RL	SNP352	4.25	5.31
RL	SNP226	6.63	8.72	SDW	SNP186	4.21	5.34
NL	SNP34	6.59	8.65	SDW	SNP372	4.21	5.34
RL	SNP95	6.46	8.45	SDW	SNP113	4.19	5.32
RL	SNP372	5.98	7.81	RDW	SNP303	4.14	5.49
RL	SNP126	5.84	7.57	RL	SNP3	4.13	5.24
NL	SNP218	5.80	7.61	SL	SNP60	4.12	5.19
RL	SNP368	5.76	7.47	PDW	SNP372	4.10	5.19
NL	SNP37	5.74	7.36	SL	SNP84	4.09	5.09
RL	SNP33	5.72	7.35	RL	SNP208	4.08	5.11
RL	SNP231	5.62	7.76	SDW	SNP110	4.06	5.14
SL	SNP166	5.55	6.97	SL	SNP303	4.04	5.19
RL	SNP252	5.43	11.29	PDW	SNP178	4.03	5.09
SL	SNP314	5.43	6.90	RL	SNP92	4.00	5.01
RL	SNP149	5.37	6.90	SDW	SNP178	3.99	5.04
RL	SNP150	5.32	7.17	PDW	SNP110	3.98	5.04
RDW	SNP304	5.30	6.89	RL	SNP307	3.98	4.88
SL	SNP53	5.30	6.68	RDW	SNP30	3.97	6.15
NL	SNP52	5.20	6.59	SDW	SNP123	3.97	15.12

**Appendix Table 2: QTLs detected by Q model under control (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
NL	SNP20	3.93	4.88	PDW	SNP123	3.25	12.19
PDW	SNP186	3.92	4.93	NL	SNP138	3.24	3.89
PDW	SNP113	3.90	4.91	SL	SNP218	3.22	3.91
NL	SNP336	3.90	4.90	NL	SNP163	3.22	3.90
NL	SNP196	3.89	4.78	SDW	SNP311	3.19	3.86
RL	SNP60	3.87	4.96	RDW	SNP149	3.18	3.88
RL	SNP310	3.85	4.82	PDW	SNP280	3.17	8.64
PDW	SNP150	3.84	5.04	SL	SNP31	3.17	3.81
RL	SNP313	3.80	4.76	SL	SNP113	3.17	3.80
NL	SNP337	3.73	4.62	NL	SNP142	3.16	3.82
RL	SNP381	3.69	4.48	SDW	SNP272	3.16	4.15
RL	SNP344	3.66	4.59	SDW	SNP280	3.15	8.61
NL	SNP312	3.65	4.46	SDW	SNP30	3.15	4.78
NL	SNP102	3.65	4.46	NL	SNP49	3.15	3.79
NL	SNP39	3.62	4.45	SDW	SNP314	3.10	3.78
RL	SNP302	3.62	4.47	NL	SNP315	3.09	3.72
RL	SNP118	3.62	4.47	PDW	SNP272	3.09	4.04
RL	SNP66	3.60	4.43	SL	SNP177	3.08	3.63
SL	SNP105	3.57	5.14	PDW	SNP285	3.06	3.71
NL	SNP244	3.56	4.33	RDW	SNP372	3.05	3.73
RL	SNP265	3.55	4.37	RDW	SNP110	3.04	3.72
NL	SNP116	3.54	4.37	RL	SNP80	3.03	3.63
NL	SNP48	3.47	4.21	RL	SNP110	3.03	3.68
RL	SNP22	3.46	4.53	PDW	SNP249	3.01	3.66
RL	SNP210	3.46	4.23	RDW	SNP210	3.00	3.63
PDW	SNP30	3.46	5.29	PDW	SNP311	2.98	3.57
NL	SNP245	3.45	4.19	SDW	SNP303	2.97	3.74
NL	SNP178	3.43	4.22	SL	SNP43	2.97	3.85
RL	SNP188	3.43	4.21	NL	SNP185	2.97	3.55
RL	SNP57	3.42	4.19	SL	SNP86	2.95	9.11
NL	SNP217	3.42	4.13	SL	SNP54	2.95	3.48
SDW	SNP150	3.40	4.40	SL	SNP210	2.95	3.46
RL	SNP373	3.39	8.75	SDW	SNP249	2.94	3.56
RDW	SNP178	3.36	4.17	RL	SNP27	2.90	3.48
SL	SNP231	3.34	4.36	SL	SNP57	2.89	3.39
SDW	SNP285	3.34	4.10	PDW	SNP149	2.87	3.43
SL	SNP92	3.33	3.99	RL	SNP103	2.84	3.74
RDW	SNP91	3.32	4.11	PDW	SNP187	2.84	3.37
PDW	SNP303	3.31	4.23	SL	SNP272	2.83	3.55
RL	SNP77	3.28	3.96	SL	SNP42	2.82	3.28
NL	SNP384	3.27	3.95	PDW	SNP314	2.82	3.37

**Appendix Table 2: QTLs detected by Q model under control (continued).**

<b>Trait</b>	<b>Markers</b>	<b>-log<sub>10</sub>(P)</b>	<b>R<sup>2</sup></b>
NL	SNP148	2.80	3.33
SL	SNP110	2.79	3.30
RDW	SNP249	2.78	3.35
RL	SNP171	2.78	3.32
NL	SNP216	2.76	3.27
RL	SNP278	2.74	3.21
RDW	SNP187	2.73	3.24
SDW	SNP187	2.73	3.22
NL	SNP160	2.73	3.20
RL	SNP193	2.72	3.26
SL	SNP187	2.71	3.13
SDW	SNP142	2.71	3.21
RL	SNP213	2.69	3.67
SL	SNP311	2.67	3.08
RDW	SNP53	2.67	3.17
NL	SNP45	2.67	3.11
RL	SNP292	2.67	3.16
RL	SNP124	2.65	3.16
SDW	SNP149	2.65	3.13
SDW	SNP108	2.65	3.15
RL	SNP146	2.65	3.11
SL	SNP207	2.64	3.12
SL	SNP56	2.64	3.14
SDW	SNP43	2.63	3.45
NL	SNP88	2.58	2.98
SDW	SNP166	2.58	3.01
NL	SNP219	2.58	3.01
RDW	SNP280	2.58	6.86
RL	SNP221	2.57	8.77
PDW	SNP108	2.56	3.02
NL	SNP9	2.53	2.92
RL	SNP108	2.53	2.97
NL	SNP319	2.52	2.89
RDW	SNP31	2.52	2.98
PDW	SNP43	2.52	3.27
NL	SNP361	2.52	2.95
RL	SNP58	2.52	2.97
RDW	SNP235	2.51	2.93
SL	SNP195	2.51	2.86

**Appendix Table 3: QTLs detected by PCA model under control.**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP43	16.28	23.37	NL	SNP34	5.05	6.42
RL	SNP54	12.87	17.02	RL	SNP60	4.94	6.60
RL	SNP361	11.33	14.99	RL	SNP149	4.90	6.28
RL	SNP56	10.69	14.51	SDW	SNP113	4.88	6.30
RL	SNP113	10.36	13.80	RL	SNP258	4.81	6.22
RL	SNP177	10.33	13.72	RL	SNP150	4.73	6.26
RL	SNP91	10.26	13.69	NL	SNP218	4.72	5.96
RL	SNP153	10.01	13.45	RL	SNP64	4.66	5.97
RL	SNP235	9.60	12.75	SL	SNP232	4.64	9.18
RL	SNP303	9.33	12.89	PDW	SNP113	4.63	5.93
RL	SNP304	9.30	12.35	RL	SNP381	4.63	5.90
RL	SNP152	8.68	11.55	NL	SNP28	4.58	5.74
RL	SNP53	8.60	11.41	SL	SNP60	4.56	5.99
RL	SNP21	8.29	10.99	PDW	SNP178	4.56	5.81
RL	SNP296	7.68	10.13	SDW	SNP178	4.50	5.74
RL	SNP283	7.59	10.08	RL	SNP22	4.48	6.10
RL	SNP249	6.95	9.21	RL	SNP33	4.46	5.66
RL	SNP205	6.78	13.49	RL	SNP186	4.40	5.63
RL	SNP231	6.69	9.57	RL	SNP286	4.30	5.46
SL	SNP218	6.49	8.51	NL	SNP52	4.27	5.31
RL	SNP16	6.01	7.83	RL	SNP272	4.22	5.75
RL	SNP307	5.99	7.80	RL	SNP378	4.21	5.33
RL	SNP352	5.87	7.63	RL	SNP219	4.20	5.33
RL	SNP275	5.80	7.54	RL	SNP208	4.17	5.30
RL	SNP226	5.75	7.52	RDW	SNP150	4.14	5.47
SL	SNP84	5.75	7.51	SDW	SNP285	4.13	5.24
RL	SNP95	5.74	7.49	SL	SNP303	4.13	5.44
RL	SNP104	5.72	7.52	RL	SNP55	4.12	5.20
SL	SNP53	5.71	7.42	RDW	SNP304	4.11	5.19
RL	SNP372	5.57	7.22	SDW	SNP218	4.08	5.18
RL	SNP368	5.50	7.12	RL	SNP293	4.08	5.17
SL	SNP314	5.46	7.08	PDW	SNP218	4.07	5.14
RL	SNP66	5.40	7.04	SDW	SNP53	4.06	5.13
RL	SNP252	5.24	10.83	SDW	SNP123	3.98	15.82
RL	SNP92	5.23	6.77	SL	SNP105	3.96	6.03
SL	SNP166	5.19	6.69	RL	SNP298	3.96	10.86
RL	SNP118	5.17	6.70	PDW	SNP285	3.86	4.84
RL	SNP126	5.13	6.60	SL	SNP231	3.86	5.28
NL	SNP37	5.07	6.42	RDW	SNP178	3.85	4.81
SL	SNP178	5.07	6.51	SL	SNP92	3.84	4.81

**Appendix Table 3: QTLs detected by PCA model under control (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
PDW	SNP304	3.75	4.68	RL	SNP48	3.09	3.74
NL	SNP194	3.61	4.47	RL	SNP344	3.08	3.79
SL	SNP272	3.57	4.74	NL	SNP3	3.07	3.67
SDW	SNP186	3.55	4.45	SDW	SNP249	3.04	3.72
SDW	SNP372	3.55	4.41	SL	SNP54	3.04	3.68
RDW	SNP303	3.55	4.59	RDW	SNP226	3.04	3.69
NL	SNP366	3.55	4.32	SL	SNP210	3.03	3.66
PDW	SNP361	3.52	4.35	SL	SNP57	3.03	3.65
PDW	SNP53	3.52	4.36	SDW	SNP311	3.02	3.68
RL	SNP58	3.51	4.36	RDW	SNP30	3.02	4.52
PDW	SNP150	3.49	4.51	NL	SNP178	3.02	3.59
RL	SNP198	3.47	4.28	RDW	SNP91	3.02	3.67
SDW	SNP304	3.47	4.30	NL	SNP219	3.01	3.58
PDW	SNP372	3.47	4.28	RL	SNP313	2.99	3.62
NL	SNP94	3.45	4.19	RDW	SNP113	2.98	3.61
NL	SNP36	3.45	4.19	PDW	SNP348	2.97	3.57
NL	SNP187	3.44	4.17	PDW	SNP352	2.96	3.57
RL	SNP57	3.42	4.21	RL	SNP116	2.95	3.56
SDW	SNP361	3.41	4.21	SDW	SNP348	2.93	3.53
NL	SNP265	3.37	4.09	SL	SNP43	2.92	3.90
RL	SNP364	3.34	4.26	NL	SNP337	2.91	3.48
SDW	SNP110	3.31	4.10	SDW	SNP314	2.91	3.52
RL	SNP188	3.31	4.05	RDW	SNP249	2.91	3.51
NL	SNP358	3.30	4.14	RL	SNP339	2.90	3.50
NL	SNP166	3.28	3.97	PDW	SNP303	2.90	3.65
RDW	SNP361	3.28	4.01	RL	SNP194	2.89	3.53
NL	SNP336	3.26	3.99	SL	SNP186	2.89	3.49
PDW	SNP123	3.25	12.73	RL	SNP3	2.89	3.48
SL	SNP177	3.23	3.95	SL	SNP96	2.87	3.43
SL	SNP31	3.23	3.95	SL	SNP195	2.85	3.41
SL	SNP113	3.23	3.96	SL	SNP116	2.83	3.40
NL	SNP196	3.22	3.86	RL	SNP77	2.83	3.39
PDW	SNP110	3.21	3.95	NL	SNP20	2.82	3.31
RL	SNP210	3.19	3.88	NL	SNP102	2.82	3.32
RL	SNP185	3.17	3.89	PDW	SNP280	2.78	7.54
NL	SNP184	3.17	3.80	PDW	SNP106	2.77	3.30
RDW	SNP218	3.17	3.87	RL	SNP114	2.76	3.35
SDW	SNP150	3.15	4.02	PDW	SNP311	2.75	3.28
SDW	SNP352	3.15	3.84	RDW	SNP210	2.74	3.26
PDW	SNP249	3.13	3.83	SL	SNP311	2.74	3.26
PDW	SNP186	3.10	3.80	SDW	SNP280	2.74	7.44

**Appendix Table 3: QTLs detected by PCA model under control (continued).**

<b>Trait</b>	<b>Markers</b>	<b><math>-\log_{10}(P)</math></b>	<b><math>R^2</math></b>
RDW	SNP149	2.72	3.23
NL	SNP312	2.71	3.17
SL	SNP187	2.71	3.20
RDW	SNP106	2.70	3.20
SL	SNP56	2.68	3.24
RL	SNP27	2.67	3.22
SDW	SNP106	2.66	3.15
RL	SNP302	2.65	3.14
PDW	SNP314	2.65	3.14
RL	SNP380	2.63	3.11
NL	SNP39	2.63	3.05
SDW	SNP303	2.63	3.25
RL	SNP80	2.62	3.10
RL	SNP310	2.62	3.11
RL	SNP213	2.61	3.60
NL	SNP384	2.60	3.04
RL	SNP290	2.59	3.05
RDW	SNP372	2.59	3.05
NL	SNP244	2.58	2.99
SDW	SNP272	2.58	3.32
RDW	SNP348	2.56	3.01
PDW	SNP30	2.55	3.73
SL	SNP34	2.55	3.00
SL	SNP42	2.54	3.00
SDW	SNP43	2.53	3.34
SL	SNP110	2.53	2.99
NL	SNP245	2.52	2.91
NL	SNP91	2.52	2.94
RL	SNP124	2.51	3.03
PDW	SNP149	2.51	2.93
RL	SNP264	2.51	2.96
NL	SNP308	2.50	2.88

**Appendix Table 4: QTLs detected by Naïve model under salt treatment.**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SL	SNP310	16.97	22.66	RL	SNP378	11.50	15.59
RL	SNP235	16.32	21.66	PDW	SNP20	11.49	15.54
SL	SNP272	15.31	21.75	SDW	SNP88	11.46	15.35
SL	SNP304	15.01	20.21	PDW	SNP111	11.36	15.41
PDW	SNP89	13.98	18.95	PDW	SNP245	11.35	15.30
RL	SNP354	13.76	18.68	SDW	SNP100	11.35	15.49
SL	SNP210	13.66	18.43	RDW	SNP236	11.33	15.28
SDW	SNP89	13.58	18.44	RDW	SNP111	11.29	15.32
RL	SNP153	13.49	18.44	RDW	SNP197	11.29	15.31
PDW	SNP36	13.33	17.84	SDW	SNP20	11.25	15.22
PDW	SNP94	13.27	17.81	RDW	SNP245	11.23	15.15
PDW	SNP194	13.27	17.97	PDW	SNP45	11.22	15.08
PDW	SNP236	13.17	17.68	PDW	SNP202	11.09	14.96
NL	SNP218	13.15	17.99	SDW	SNP204	11.00	14.84
SDW	SNP36	12.90	17.28	RDW	SNP204	11.00	14.83
PDW	SNP48	12.87	17.24	RDW	SNP109	10.98	14.76
SDW	SNP194	12.85	17.44	SL	SNP226	10.98	15.00
SDW	SNP94	12.85	17.26	PDW	SNP260	10.93	14.94
RL	SNP304	12.83	17.41	SDW	SNP339	10.85	14.69
SDW	SNP236	12.82	17.23	RL	SNP21	10.82	14.55
SDW	SNP48	12.41	16.65	PDW	SNP339	10.70	14.48
PDW	SNP109	12.34	16.55	SDW	SNP202	10.63	14.34
PDW	SNP88	12.33	16.48	SDW	SNP245	10.57	14.27
RDW	SNP88	12.30	16.45	SDW	SNP161	10.53	14.17
RL	SNP158	12.29	17.08	SDW	SNP111	10.52	14.28
PDW	SNP197	12.23	16.56	RDW	SNP45	10.50	14.12
SL	SNP227	12.16	16.58	SDW	SNP115	10.49	14.30
RDW	SNP89	12.09	16.49	SDW	SNP160	10.49	14.20
RL	SNP95	11.91	16.14	PDW	SNP115	10.48	14.28
SDW	SNP197	11.85	16.07	PDW	SNP160	10.48	14.19
RL	SNP234	11.84	16.05	PDW	SNP319	10.48	14.05
SL	SNP235	11.72	15.79	PDW	SNP216	10.39	14.07
RDW	SNP36	11.70	15.71	SDW	SNP45	10.39	13.97
RDW	SNP194	11.69	15.90	PDW	SNP39	10.38	14.05
RDW	SNP94	11.68	15.73	SDW	SNP102	10.37	13.99
SDW	SNP109	11.67	15.67	PDW	SNP161	10.37	13.94
PDW	SNP100	11.61	15.85	PDW	SNP138	10.32	13.89
RDW	SNP48	11.57	15.54	PDW	SNP102	10.30	13.90
PDW	SNP204	11.57	15.59	RDW	SNP100	10.27	14.03
SDW	SNP260	11.51	15.71	PDW	SNP32	10.22	13.84

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RDW	SNP319	10.19	13.67	SDW	SNP173	9.26	12.53
RDW	SNP202	10.14	13.69	RDW	SNP217	9.22	12.31
SL	SNP126	10.12	13.75	PDW	SNP137	9.21	12.50
SDW	SNP216	10.10	13.67	RDW	SNP244	9.21	12.38
PDW	SNP49	9.99	13.53	PDW	SNP75	9.18	12.42
SDW	SNP32	9.98	13.50	RDW	SNP137	9.17	12.44
SL	SNP95	9.95	13.52	SL	SNP344	9.09	12.54
PDW	SNP40	9.95	13.88	NL	SNP197	9.06	12.29
RDW	SNP216	9.94	13.46	SDW	SNP217	9.02	12.04
SDW	SNP39	9.94	13.45	RL	SNP275	9.01	12.15
SL	SNP313	9.92	13.57	NL	SNP34	8.95	12.22
RDW	SNP32	9.89	13.39	SDW	SNP75	8.93	12.08
PDW	SNP244	9.87	13.28	PDW	SNP141	8.90	12.00
SL	SNP150	9.85	14.06	RL	SNP180	8.88	12.05
SDW	SNP49	9.79	13.25	RL	SNP1	8.81	11.92
SL	SNP142	9.78	13.29	PDW	SNP337	8.80	11.90
RL	SNP142	9.78	13.28	PDW	SNP316	8.80	11.82
SDW	SNP319	9.76	13.09	RDW	SNP160	8.64	11.67
RL	SNP368	9.73	13.22	SDW	SNP316	8.61	11.56
SDW	SNP218	9.69	13.34	SDW	SNP141	8.57	11.55
SL	SNP153	9.67	13.31	RDW	SNP102	8.55	11.52
SDW	SNP138	9.67	13.00	PDW	SNP291	8.54	11.50
SDW	SNP40	9.66	13.47	RDW	SNP49	8.49	11.48
RDW	SNP39	9.64	13.05	RDW	SNP312	8.49	11.39
SL	SNP378	9.63	13.08	NL	SNP217	8.48	11.31
RDW	SNP20	9.63	13.03	NL	SNP277	8.43	11.28
SL	SNP149	9.58	13.01	RDW	SNP103	8.41	12.56
RDW	SNP40	9.55	13.32	RL	SNP149	8.39	11.38
RDW	SNP138	9.49	12.76	SDW	SNP337	8.38	11.32
PDW	SNP218	9.47	13.03	SDW	SNP137	8.35	11.32
RDW	SNP115	9.42	12.83	NL	SNP160	8.35	11.27
PDW	SNP103	9.39	14.03	PDW	SNP277	8.34	11.15
SL	SNP311	9.39	12.67	PDW	SNP320	8.33	11.14
SDW	SNP103	9.38	14.01	NL	SNP116	8.32	11.31
PDW	SNP254	9.37	12.69	SL	SNP186	8.26	11.23
SDW	SNP244	9.36	12.59	RDW	SNP141	8.25	11.11
PDW	SNP217	9.35	12.50	SDW	SNP213	8.24	12.57
SDW	SNP254	9.33	12.63	SDW	SNP320	8.23	11.01
RL	SNP345	9.31	12.68	RL	SNP205	8.21	16.09
RL	SNP379	9.30	12.63	RL	SNP84	8.21	11.19
PDW	SNP173	9.27	12.54	RL	SNP313	8.20	11.18



**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RDW	SNP117	8.20	10.96	PDW	SNP312	7.44	9.95
SDW	SNP291	8.17	10.99	SDW	SNP129	7.43	10.00
RDW	SNP291	8.15	10.97	RDW	SNP260	7.42	10.08
SDW	SNP277	8.15	10.89	SL	SNP380	7.40	9.96
RL	SNP259	8.14	11.10	SL	SNP25	7.38	10.06
RDW	SNP161	8.14	10.91	SDW	SNP159	7.37	9.89
RDW	SNP320	8.09	10.82	PDW	SNP31	7.36	10.01
NL	SNP216	8.09	10.91	SDW	SNP196	7.36	9.84
RDW	SNP316	8.07	10.81	RL	SNP35	7.36	10.00
NL	SNP52	8.06	10.81	RDW	SNP179	7.35	9.86
PDW	SNP117	7.97	10.65	RL	SNP272	7.33	10.53
RDW	SNP337	7.95	10.73	PDW	SNP135	7.33	9.83
RDW	SNP339	7.95	10.73	SDW	SNP117	7.31	9.74
RDW	SNP156	7.93	10.70	RDW	SNP254	7.31	9.83
RDW	SNP277	7.93	10.59	SL	SNP73	7.29	9.84
RL	SNP41	7.91	10.71	SDW	SNP31	7.29	9.90
SL	SNP41	7.86	10.64	SL	SNP171	7.27	9.88
SDW	SNP381	7.83	10.42	RDW	SNP135	7.27	9.74
PDW	SNP68	7.82	10.55	SDW	SNP68	7.26	9.76
RDW	SNP75	7.80	10.51	RL	SNP227	7.25	9.85
PDW	SNP213	7.80	11.88	PDW	SNP196	7.23	9.66
RDW	SNP173	7.75	10.45	PDW	SNP38	7.20	9.68
PDW	SNP179	7.70	10.35	SDW	SNP377	7.17	10.04
RL	SNP380	7.67	10.34	RL	SNP333	7.16	9.65
RDW	SNP68	7.67	10.34	SDW	SNP179	7.14	9.57
RL	SNP73	7.63	10.31	SL	SNP345	7.10	9.61
SDW	SNP203	7.63	10.28	SDW	SNP135	7.10	9.51
NL	SNP161	7.62	10.20	SDW	SNP361	7.08	9.60
PDW	SNP159	7.58	10.18	NL	SNP115	7.07	9.56
SL	SNP275	7.57	10.17	NL	SNP40	7.06	9.77
RL	SNP64	7.56	10.25	RDW	SNP31	7.04	9.54
RL	SNP150	7.56	10.74	NL	SNP37	7.01	9.39
RDW	SNP384	7.55	10.14	PDW	SNP381	6.98	9.25
RDW	SNP218	7.52	10.29	RL	SNP278	6.94	9.25
RDW	SNP9	7.51	10.05	RL	SNP126	6.91	9.30
PDW	SNP129	7.49	10.09	SL	SNP369	6.89	9.47
NL	SNP196	7.46	9.98	NL	SNP241	6.88	9.29
NL	SNP358	7.46	10.52	RL	SNP360	6.88	9.48
RL	SNP166	7.46	9.97	RL	SNP344	6.86	9.39
SL	SNP278	7.45	9.97	RL	SNP226	6.86	9.29
PDW	SNP203	7.45	10.03	SL	SNP84	6.84	9.27

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP175	6.82	9.15	NL	SNP156	5.97	7.94
SL	SNP21	6.79	9.05	SL	SNP354	5.97	8.02
SDW	SNP38	6.78	9.09	PDW	SNP329	5.91	7.83
SL	SNP30	6.72	11.33	SL	SNP158	5.89	8.08
NL	SNP32	6.65	8.91	NL	SNP270	5.88	7.84
PDW	SNP289	6.63	9.51	RL	SNP369	5.87	7.99
RL	SNP189	6.62	8.92	PDW	SNP384	5.86	7.75
RDW	SNP38	6.58	8.80	NL	SNP376	5.85	7.75
SL	SNP143	6.54	8.69	PDW	SNP248	5.84	7.76
SL	SNP183	6.52	8.83	RL	SNP258	5.83	7.80
RDW	SNP159	6.50	8.67	NL	SNP173	5.81	7.71
SDW	SNP312	6.48	8.61	SDW	SNP21	5.79	7.64
PDW	SNP156	6.48	8.66	NL	SNP320	5.79	7.61
RDW	SNP129	6.47	8.65	PDW	SNP323	5.77	7.89
RDW	SNP248	6.47	8.65	SL	SNP8	5.76	7.72
RL	SNP8	6.46	8.73	RL	SNP206	5.75	7.62
RL	SNP186	6.45	8.68	RDW	SNP213	5.73	8.60
SL	SNP118	6.44	8.66	PDW	SNP112	5.72	7.64
SL	SNP166	6.38	8.47	RDW	SNP241	5.72	7.64
RL	SNP310	6.38	8.57	NL	SNP220	5.72	7.63
RDW	SNP289	6.37	9.11	SDW	SNP156	5.71	7.57
SL	SNP144	6.36	8.52	RL	SNP10	5.69	7.57
PDW	SNP377	6.35	8.83	SDW	SNP376	5.66	7.48
NL	SNP194	6.32	8.49	SL	SNP368	5.66	7.53
SDW	SNP289	6.28	8.97	RL	SNP210	5.66	7.53
SDW	SNP193	6.27	8.48	SDW	SNP205	5.66	10.99
NL	SNP75	6.26	8.36	NL	SNP20	5.65	7.49
RDW	SNP203	6.26	8.35	SDW	SNP209	5.62	7.40
NL	SNP102	6.25	8.32	NL	SNP186	5.57	7.43
RDW	SNP108	6.25	8.39	NL	SNP248	5.56	7.36
NL	SNP245	6.15	8.17	PDW	SNP21	5.55	7.29
RL	SNP311	6.15	8.17	SDW	SNP323	5.54	7.56
PDW	SNP9	6.14	8.13	NL	SNP129	5.54	7.33
NL	SNP159	6.14	8.15	SDW	SNP215	5.53	7.27
NL	SNP36	6.13	8.12	RL	SNP229	5.50	7.25
PDW	SNP193	6.11	8.25	PDW	SNP322	5.48	7.20
NL	SNP94	6.10	8.10	SDW	SNP248	5.46	7.21
PDW	SNP361	6.04	8.12	SL	SNP209	5.45	7.16
SDW	SNP329	6.02	7.99	SDW	SNP233	5.44	7.17
NL	SNP326	5.98	7.98	NL	SNP163	5.44	7.21
NL	SNP204	5.97	7.92	SDW	SNP112	5.42	7.20

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP53	5.40	7.14	PDW	SNP34	4.87	6.44
SL	SNP105	5.39	8.69	RDW	SNP314	4.87	6.42
NL	SNP141	5.39	7.09	SL	SNP360	4.87	6.55
NL	SNP103	5.37	7.86	SL	SNP234	4.86	6.39
RL	SNP2	5.36	10.19	SDW	SNP149	4.84	6.36
RDW	SNP196	5.34	7.00	SDW	SNP326	4.84	6.36
SL	SNP184	5.34	7.05	SDW	SNP147	4.83	6.32
PDW	SNP209	5.34	7.00	SDW	SNP384	4.82	6.28
SDW	SNP322	5.29	6.93	PDW	SNP3	4.81	6.35
RDW	SNP112	5.27	7.00	RL	SNP247	4.80	6.28
NL	SNP174	5.27	7.06	SDW	SNP3	4.79	6.33
SL	SNP205	5.26	10.18	SL	SNP303	4.78	6.53
NL	SNP329	5.26	6.91	NL	SNP316	4.76	6.18
SDW	SNP9	5.24	6.86	SDW	SNP37	4.75	6.18
RDW	SNP323	5.21	7.08	PDW	SNP349	4.74	6.78
SL	SNP76	5.19	6.78	PDW	SNP326	4.74	6.21
NL	SNP203	5.16	6.79	SL	SNP274	4.73	6.18
PDW	SNP215	5.15	6.73	SL	SNP258	4.73	6.21
SL	SNP178	5.15	6.82	SL	SNP336	4.72	6.27
RDW	SNP329	5.13	6.72	SDW	SNP187	4.70	6.11
PDW	SNP233	5.09	6.67	SDW	SNP360	4.70	6.30
PDW	SNP37	5.07	6.64	SDW	SNP241	4.69	6.15
PDW	SNP376	5.05	6.62	RL	SNP270	4.68	6.12
RDW	SNP34	5.03	6.67	RDW	SNP193	4.68	6.18
NL	SNP360	5.00	6.75	NL	SNP319	4.67	6.03
PDW	SNP241	5.00	6.60	SL	SNP370	4.66	6.10
NL	SNP244	5.00	6.51	RL	SNP171	4.66	6.14
SDW	SNP118	4.98	6.58	PDW	SNP108	4.66	6.11
SDW	SNP358	4.98	6.86	SL	SNP213	4.65	6.88
SL	SNP188	4.98	6.55	RDW	SNP147	4.65	6.06
PDW	SNP147	4.97	6.52	NL	SNP108	4.64	6.09
RL	SNP157	4.97	6.63	SL	SNP162	4.62	6.12
RDW	SNP37	4.97	6.50	RDW	SNP5	4.62	6.12
RDW	SNP322	4.97	6.47	PDW	SNP149	4.61	6.03
NL	SNP135	4.95	6.47	NL	SNP31	4.61	6.06
RDW	SNP154	4.95	6.49	PDW	SNP187	4.60	5.97
NL	SNP39	4.92	6.45	PDW	SNP72	4.60	5.94
RL	SNP274	4.91	6.43	RL	SNP370	4.59	5.99
SL	SNP53	4.89	6.41	SL	SNP379	4.58	5.99
SL	SNP175	4.89	6.41	RDW	SNP3	4.58	6.02
PDW	SNP205	4.89	9.41	NL	SNP364	4.57	6.19

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SDW	SNP72	4.56	5.89	NL	SNP138	4.20	5.38
RL	SNP11	4.56	5.91	PDW	SNP360	4.20	5.56
SDW	SNP34	4.55	5.98	PDW	SNP5	4.19	5.50
SDW	SNP174	4.55	6.02	PDW	SNP118	4.19	5.44
NL	SNP48	4.54	5.87	PDW	SNP358	4.19	5.67
RL	SNP6	4.53	5.98	SL	SNP287	4.19	5.42
NL	SNP88	4.53	5.84	RL	SNP339	4.18	5.39
RDW	SNP349	4.53	6.46	PDW	SNP30	4.18	6.81
NL	SNP148	4.51	5.91	PDW	SNP144	4.15	5.36
SL	SNP229	4.49	5.82	RL	SNP80	4.11	5.29
PDW	SNP174	4.47	5.91	SDW	SNP116	4.11	5.32
RDW	SNP318	4.46	5.77	NL	SNP293	4.11	5.34
SDW	SNP325	4.45	6.44	RDW	SNP381	4.11	5.21
NL	SNP384	4.44	5.75	NL	SNP202	4.10	5.26
RL	SNP96	4.44	5.76	RDW	SNP72	4.09	5.22
SDW	SNP349	4.43	6.29	PDW	SNP143	4.07	5.19
NL	SNP90	4.43	5.68	RL	SNP367	4.06	5.23
RL	SNP30	4.42	7.25	NL	SNP185	4.04	5.21
NL	SNP254	4.40	5.70	RDW	SNP104	4.03	5.25
NL	SNP297	4.38	5.69	RDW	SNP209	4.03	5.14
RL	SNP54	4.38	5.71	RDW	SNP187	4.02	5.13
NL	SNP373	4.34	13.51	NL	SNP71	4.00	5.17
RL	SNP5	4.33	5.70	SDW	SNP108	3.98	5.14
RL	SNP144	4.33	5.62	RDW	SNP253	3.98	5.13
SDW	SNP143	4.32	5.56	NL	SNP260	3.95	5.11
SDW	SNP8	4.32	5.65	SDW	SNP307	3.95	4.99
SL	SNP295	4.31	5.90	NL	SNP114	3.94	5.15
SDW	SNP144	4.31	5.59	SDW	SNP5	3.94	5.13
RL	SNP76	4.30	5.53	SL	SNP35	3.94	5.10
SDW	SNP227	4.30	5.62	RDW	SNP21	3.94	5.01
PDW	SNP325	4.27	6.16	SL	SNP1	3.94	5.04
SDW	SNP163	4.27	5.53	SL	SNP125	3.94	5.21
NL	SNP179	4.26	5.48	PDW	SNP8	3.93	5.09
NL	SNP306	4.25	5.46	NL	SNP117	3.92	4.96
NL	SNP281	4.24	8.28	NL	SNP236	3.92	4.99
NL	SNP291	4.24	5.46	SL	SNP19	3.89	4.98
NL	SNP137	4.24	5.49	NL	SNP68	3.88	4.95
SL	SNP333	4.23	5.48	SL	SNP11	3.87	4.93
RDW	SNP306	4.23	5.44	PDW	SNP318	3.87	4.93
RDW	SNP326	4.22	5.47	PDW	SNP163	3.87	4.96
SDW	SNP30	4.21	6.87	NL	SNP95	3.87	4.95

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SDW	SNP1	3.86	4.93	PDW	SNP212	3.54	4.47
RL	SNP287	3.86	4.95	SL	SNP364	3.54	4.66
SL	SNP64	3.84	4.94	NL	SNP62	3.53	4.52
SDW	SNP268	3.84	4.99	NL	SNP339	3.53	4.46
PDW	SNP170	3.82	4.87	LIS1	SNP56	3.53	4.60
SL	SNP351	3.81	4.88	RDW	SNP149	3.50	4.43
SDW	SNP70	3.80	4.86	RL	SNP209	3.50	4.37
RL	SNP143	3.80	4.80	RL	SNP125	3.49	4.55
SL	SNP18	3.79	4.87	RDW	SNP220	3.48	4.42
RDW	SNP174	3.79	4.91	SDW	SNP290	3.48	4.40
NL	SNP253	3.79	4.86	NL	SNP100	3.48	4.43
NL	SNP45	3.78	4.78	RDW	SNP212	3.47	4.37
RDW	SNP215	3.77	4.77	PDL	SNP86	3.47	11.56
PDW	SNP180	3.77	4.81	PDW	SNP306	3.47	4.35
NL	SNP312	3.77	4.76	PDW	SNP314	3.45	4.37
SL	SNP259	3.76	4.84	RL	SNP262	3.44	4.34
RL	SNP283	3.76	4.81	PDW	SNP65	3.44	4.36
RL	SNP18	3.74	4.78	SDW	SNP51	3.44	4.33
NL	SNP9	3.74	4.72	SDW	SNP273	3.42	4.32
SDW	SNP148	3.72	4.76	NL	SNP310	3.40	4.30
PDW	SNP227	3.72	4.77	SL	SNP223	3.40	4.30
SL	SNP283	3.71	4.74	NL	SNP337	3.40	4.26
SDW	SNP180	3.70	4.71	RDW	SNP377	3.40	4.44
PDW	SNP116	3.67	4.69	SL	SNP147	3.38	4.25
SDW	SNP65	3.67	4.69	RDW	SNP325	3.38	4.74
SDW	SNP170	3.67	4.65	NL	SNP214	3.38	4.22
RL	SNP37	3.66	4.63	NL	SNP187	3.38	4.22
PDW	SNP1	3.66	4.65	RDW	SNP133	3.37	5.00
RDW	SNP30	3.65	5.86	PDW	SNP70	3.37	4.24
RDW	SNP28	3.64	4.61	PDW	SNP150	3.37	4.47
SL	SNP121	3.62	4.71	RDW	SNP119	3.35	4.30
SDW	SNP114	3.62	4.67	NL	SNP131	3.33	4.16
NL	SNP346	3.62	4.59	SDW	SNP150	3.33	4.41
NL	SNP96	3.59	4.54	RDW	SNP170	3.32	4.16
RDW	SNP233	3.59	4.53	SL	SNP2	3.32	6.01
NL	SNP73	3.58	4.54	SDW	SNP212	3.31	4.15
PDW	SNP268	3.56	4.58	RDW	SNP144	3.31	4.15
NL	SNP272	3.56	4.81	SDW	SNP318	3.30	4.11
PDW	SNP119	3.56	4.60	SDW	SNP270	3.28	4.11
SDW	SNP119	3.55	4.60	PDW	SNP290	3.27	4.10
SL	SNP201	3.55	4.50	SL	SNP10	3.27	4.10

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SL	SNP208	3.27	4.08	NL	SNP28	3.03	3.74
RDW	SNP376	3.27	4.06	PDW	SNP154	3.03	3.74
NL	SNP16	3.27	4.08	RDW	SNP143	3.03	3.71
RDW	SNP198	3.26	4.05	NL	SNP97	3.03	3.75
RL	SNP128	3.26	5.62	LIS1	SNP268	3.02	3.79
PDW	SNP273	3.26	4.09	RL	SNP48	3.02	3.70
PDW	SNP104	3.25	4.11	PDW	SNP253	3.02	3.75
PDW	SNP148	3.25	4.08	RDW	SNP167	3.01	3.72
RDW	SNP280	3.25	9.25	PDW	SNP28	3.01	3.70
NL	SNP198	3.24	4.03	NL	SNP301	3.01	3.73
RDW	SNP180	3.21	4.02	SL	SNP211	2.99	3.70
NL	SNP259	3.20	4.03	NL	SNP230	2.99	6.45
PDW	SNP307	3.19	3.92	NL	SNP365	2.98	3.70
RDW	SNP150	3.19	4.19	RL	SNP381	2.98	3.62
SDW	SNP353	3.18	9.55	LIS1	SNP86	2.98	9.76
PDW	SNP280	3.17	9.01	SDW	SNP346	2.97	3.67
RL	SNP211	3.17	3.96	RDW	SNP69	2.97	6.12
NL	SNP378	3.17	3.95	NL	SNP3	2.97	3.69
SDW	SNP280	3.16	8.97	NL	SNP215	2.97	3.63
RDW	SNP71	3.15	3.96	NL	SNP168	2.97	3.64
SL	SNP80	3.15	3.92	SL	SNP262	2.95	3.64
NL	SNP221	3.15	11.42	SDW	SNP347	2.95	6.48
PDW	SNP114	3.15	3.99	NL	SNP112	2.94	3.63
SDW	SNP306	3.13	3.87	PDL	SNP56	2.94	3.72
RL	SNP351	3.13	3.90	RL	SNP286	2.93	3.60
RL	SNP188	3.12	3.89	NL	SNP166	2.92	3.56
PDW	SNP133	3.12	4.56	PDW	SNP334	2.92	3.69
NL	SNP49	3.12	3.86	SDW	SNP92	2.92	3.59
SDW	SNP133	3.12	4.56	NL	SNP300	2.91	3.56
RL	SNP237	3.11	3.84	PDW	SNP210	2.91	3.58
RL	SNP213	3.11	4.37	SL	SNP110	2.91	3.61
NL	SNP69	3.11	6.44	PDW	SNP51	2.90	3.56
SDW	SNP371	3.09	3.83	SL	SNP165	2.89	3.61
SL	SNP139	3.07	3.77	NL	SNP350	2.89	3.56
NL	SNP38	3.07	3.79	RDW	SNP334	2.87	3.61
SDW	SNP210	3.06	3.79	NL	SNP379	2.85	3.50
NL	SNP193	3.06	3.83	RDW	SNP353	2.85	8.45
PDW	SNP353	3.05	9.10	NL	SNP289	2.85	3.73
PDW	SNP347	3.04	6.71	SDW	SNP334	2.85	3.57
PDW	SNP371	3.04	3.75	RDW	SNP361	2.84	3.51
RDW	SNP110	3.03	3.79	PDW	SNP270	2.83	3.47

**Appendix Table 4: QTLs detected by Naïve model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP303	2.83	3.62	SL	SNP250	2.56	15.72
RDW	SNP308	2.83	3.42	RDW	SNP273	2.56	3.09
SDW	SNP104	2.82	3.49	NL	SNP371	2.56	3.07
RL	SNP25	2.82	3.49	RL	SNP147	2.55	3.05
RDW	SNP371	2.82	3.44	PDW	SNP192	2.55	3.03
SDW	SNP300	2.82	3.42	SDW	SNP192	2.54	3.02
PDW	SNP198	2.80	3.39	NL	SNP318	2.54	3.03
RL	SNP228	2.77	3.39	NL	SNP238	2.53	3.09
RL	SNP349	2.77	3.70	NL	SNP87	2.52	3.03
SDW	SNP253	2.77	3.39	RDW	SNP52	2.52	2.99
NL	SNP292	2.76	3.39	RL	SNP315	2.50	3.00
SL	SNP136	2.76	3.43	RDW	SNP360	2.50	3.07
SDW	SNP314	2.75	3.37	RL	SNP285	2.50	3.00
RL	SNP244	2.75	3.32	NL	SNP233	2.50	2.98
NL	SNP323	2.75	3.44				
RL	SNP83	2.74	3.35				
SL	SNP328	2.73	3.28				
PDW	SNP346	2.72	3.31				
NL	SNP242	2.72	3.29				
PDW	SNP73	2.70	3.29				
RDW	SNP347	2.70	5.86				
NL	SNP189	2.70	3.29				
RDW	SNP1	2.70	3.26				
NL	SNP290	2.69	3.27				
SDW	SNP42	2.68	3.24				
NL	SNP351	2.68	3.26				
RL	SNP49	2.67	3.22				
PDW	SNP300	2.65	3.19				
PDW	SNP42	2.65	3.19				
SDW	SNP73	2.64	3.19				
SDW	SNP368	2.62	3.17				
RL	SNP220	2.61	3.16				
SDW	SNP303	2.61	3.28				
NL	SNP104	2.60	3.18				
NL	SNP151	2.60	3.16				
SDW	SNP198	2.59	3.11				
RL	SNP366	2.59	3.09				
RL	SNP7	2.58	3.10				
SDW	SNP235	2.58	3.08				
NL	SNP232	2.57	4.70				
RDW	SNP363	2.57	3.07				

**Appendix Table 5: QTLs detected by Q model under salt treatment.**

Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>	Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>
SL	SNP310	12.81	16.09	SDW	SNP36	7.54	9.23
NL	SNP218	12.06	16.19	NL	SNP34	7.46	9.92
SL	SNP272	10.86	14.38	NL	SNP160	7.43	9.77
RL	SNP32	10.60	13.00	SDW	SNP94	7.42	9.10
NL	SNP142	10.46	13.90	SDW	SNP109	7.41	9.09
SL	SNP304	10.41	13.12	PDW	SNP358	7.40	9.63
RL	SNP235	10.10	12.33	SDW	SNP48	7.35	9.00
SL	SNP208	9.90	12.44	NL	SNP277	7.33	9.56
SDW	SNP361	9.90	12.44	SDW	SNP360	7.32	9.26
NL	SNP358	9.47	13.24	RL	SNP158	7.31	9.13
NL	SNP197	9.46	12.60	NL	SNP186	7.30	9.67
RL	SNP378	9.21	11.35	RDW	SNP88	7.28	8.90
PDW	SNP89	9.15	11.41	RL	SNP84	7.12	8.76
SDW	SNP89	8.85	11.04	PDW	SNP88	7.09	8.61
SL	SNP210	8.68	10.83	NL	SNP40	7.01	9.51
RL	SNP354	8.58	10.55	RDW	SNP111	6.93	8.57
PDW	SNP361	8.58	10.76	SDW	SNP260	6.89	8.55
SDW	SNP358	8.50	11.14	RDW	SNP109	6.87	8.41
RL	SNP153	8.41	10.51	SL	SNP235	6.86	8.48
NL	SNP216	8.40	11.14	PDW	SNP111	6.84	8.41
NL	SNP116	8.32	11.07	PDW	SNP100	6.80	8.44
SL	SNP32	8.16	10.22	NL	SNP272	6.77	9.52
PDW	SNP236	8.06	9.89	SL	SNP226	6.77	8.47
SDW	SNP218	8.05	10.17	RL	SNP379	6.76	8.20
RL	SNP95	7.94	9.75	RDW	SNP48	6.70	8.17
PDW	SNP109	7.94	9.74	SL	SNP227	6.69	8.34
PDW	SNP194	7.84	9.74	NL	SNP95	6.67	8.78
PDW	SNP36	7.84	9.59	SL	SNP95	6.66	8.29
PDW	SNP218	7.83	9.86	RDW	SNP194	6.66	8.27
SL	SNP57	7.83	9.79	PDW	SNP360	6.66	8.38
RL	SNP304	7.83	9.64	RDW	SNP236	6.64	8.13
NL	SNP217	7.83	10.20	PDW	SNP258	6.63	8.17
SDW	SNP236	7.80	9.57	SDW	SNP100	6.62	8.21
NL	SNP52	7.75	10.15	RDW	SNP36	6.57	8.01
PDW	SNP94	7.74	9.49	RDW	SNP94	6.56	8.03
SL	SNP378	7.68	9.63	PDW	SNP45	6.53	7.93
PDW	SNP48	7.68	9.39	NL	SNP270	6.52	8.57
RDW	SNP89	7.67	9.58	SL	SNP186	6.51	8.13
SDW	SNP258	7.62	9.46	SL	SNP40	6.48	8.27
SDW	SNP194	7.57	9.42	SDW	SNP102	6.46	7.86



**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
NL	SNP161	6.45	8.38	PDW	SNP49	5.62	6.81
PDW	SNP197	6.44	7.91	SDW	SNP202	5.61	6.77
NL	SNP196	6.41	8.31	RDW	SNP138	5.61	6.77
SDW	SNP88	6.39	7.73	NL	SNP37	5.58	7.21
PDW	SNP102	6.36	7.72	SDW	SNP204	5.56	6.71
PDW	SNP260	6.35	7.84	SL	SNP115	5.55	6.85
RDW	SNP245	6.35	7.76	SL	SNP281	5.55	9.85
NL	SNP96	6.27	8.19	SDW	SNP92	5.54	6.75
NL	SNP360	6.27	8.41	RL	SNP21	5.53	6.54
PDW	SNP245	6.27	7.62	SL	SNP372	5.51	6.80
NL	SNP115	6.25	8.20	RL	SNP40	5.49	6.79
PDW	SNP20	6.24	7.60	RL	SNP189	5.49	6.59
SL	SNP43	6.24	8.44	SDW	SNP49	5.49	6.64
PDW	SNP138	6.22	7.52	SDW	SNP196	5.48	6.60
SDW	SNP111	6.18	7.56	PDW	SNP319	5.48	6.56
SDW	SNP197	6.12	7.50	SL	SNP216	5.47	6.70
SDW	SNP20	6.09	7.41	RL	SNP3	5.44	6.58
RDW	SNP45	6.08	7.39	RL	SNP360	5.44	6.63
SDW	SNP339	6.04	7.35	SDW	SNP39	5.43	6.58
RDW	SNP218	6.03	7.54	PDW	SNP73	5.42	6.61
NL	SNP310	6.03	7.95	NL	SNP259	5.41	7.06
PDW	SNP204	5.96	7.23	PDW	SNP337	5.40	6.52
PDW	SNP202	5.95	7.20	RDW	SNP319	5.39	6.47
SL	SNP303	5.95	7.66	SDW	SNP290	5.38	6.54
RL	SNP31	5.95	7.22	SL	SNP313	5.37	6.63
NL	SNP32	5.93	7.71	RL	SNP73	5.36	6.42
RDW	SNP197	5.89	7.25	SL	SNP71	5.36	6.62
PDW	SNP339	5.88	7.14	PDW	SNP196	5.35	6.43
SDW	SNP45	5.87	7.09	RDW	SNP202	5.34	6.44
SL	SNP84	5.86	7.27	NL	SNP241	5.33	6.92
SL	SNP31	5.84	7.26	PDW	SNP112	5.29	6.42
RDW	SNP100	5.82	7.19	SL	SNP73	5.27	6.44
SL	SNP25	5.81	7.23	SDW	SNP73	5.27	6.43
SL	SNP126	5.81	7.13	RDW	SNP39	5.24	6.35
SL	SNP153	5.78	7.26	NL	SNP158	5.24	6.95
RL	SNP103	5.77	7.64	NL	SNP304	5.24	6.80
PDW	SNP39	5.75	6.98	SDW	SNP113	5.24	6.43
SDW	SNP138	5.71	6.89	NL	SNP73	5.14	6.64
SDW	SNP245	5.66	6.84	PDW	SNP290	5.11	6.18
RL	SNP275	5.65	6.71	PDW	SNP244	5.10	6.09
RDW	SNP204	5.64	6.84	RDW	SNP137	5.10	6.18

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
NL	SNP309	5.09	10.01	PDW	SNP216	4.69	5.63
SDW	SNP337	5.07	6.10	SL	SNP187	4.69	5.65
RL	SNP234	5.06	5.99	RDW	SNP244	4.69	5.58
RDW	SNP142	5.03	6.08	PDW	SNP380	4.66	5.56
SL	SNP16	5.03	6.09	RDW	SNP216	4.65	5.60
SL	SNP159	5.02	6.07	PDW	SNP160	4.62	5.51
PDW	SNP137	5.01	6.03	NL	SNP354	4.61	5.94
RL	SNP259	4.99	5.97	SDW	SNP381	4.59	5.40
RDW	SNP102	4.99	5.97	RDW	SNP40	4.58	5.69
SDW	SNP112	4.98	6.03	NL	SNP326	4.58	5.86
RL	SNP316	4.98	5.84	SL	SNP105	4.57	6.73
RL	SNP368	4.96	5.88	RL	SNP253	4.57	5.41
SDW	SNP319	4.94	5.88	PDW	SNP40	4.55	5.62
NL	SNP64	4.93	6.37	NL	SNP345	4.54	5.83
NL	SNP364	4.92	6.63	PDW	SNP32	4.54	5.42
SL	SNP320	4.91	5.91	RDW	SNP32	4.54	5.45
SDW	SNP228	4.90	5.92	RL	SNP281	4.53	8.15
RDW	SNP312	4.88	5.82	RDW	SNP49	4.53	5.40
NL	SNP194	4.87	6.30	PDW	SNP161	4.50	5.31
RDW	SNP20	4.87	5.85	SL	SNP118	4.50	5.43
NL	SNP281	4.87	9.57	RDW	SNP171	4.49	5.42
NL	SNP75	4.86	6.21	SL	SNP108	4.48	5.43
RDW	SNP112	4.84	5.85	NL	SNP159	4.48	5.66
PDW	SNP254	4.83	5.78	RDW	SNP336	4.47	5.39
PDW	SNP92	4.83	5.80	PDW	SNP379	4.46	5.34
SDW	SNP115	4.82	5.83	RDW	SNP380	4.45	5.32
SDW	SNP254	4.82	5.77	SL	SNP104	4.45	5.41
RDW	SNP73	4.82	5.84	SDW	SNP216	4.45	5.30
SL	SNP275	4.80	5.77	NL	SNP297	4.44	5.66
RL	SNP180	4.80	5.65	NL	SNP320	4.44	5.57
NL	SNP315	4.79	6.14	NL	SNP36	4.43	5.58
RDW	SNP337	4.78	5.74	RDW	SNP384	4.42	5.23
SDW	SNP161	4.75	5.64	RL	SNP306	4.42	5.18
SDW	SNP244	4.74	5.64	RL	SNP269	4.42	5.16
NL	SNP102	4.74	6.04	SL	SNP150	4.42	5.56
PDW	SNP115	4.73	5.70	NL	SNP245	4.41	5.57
RL	SNP186	4.73	5.65	NL	SNP378	4.41	5.61
SDW	SNP379	4.71	5.68	NL	SNP103	4.40	6.22
PDW	SNP228	4.70	5.65	RL	SNP187	4.39	5.13
SDW	SNP160	4.70	5.61	RL	SNP53	4.39	5.16
RL	SNP173	4.70	5.52	SDW	SNP96	4.39	5.20

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>	Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>
SDW	SNP32	4.37	5.21	NL	SNP234	4.00	5.04
RL	SNP71	4.37	5.15	RDW	SNP115	4.00	4.76
NL	SNP94	4.37	5.51	RL	SNP367	3.99	4.60
NL	SNP185	4.36	5.55	SL	SNP149	3.99	4.72
NL	SNP163	4.36	5.54	PDW	SNP117	3.99	4.62
SL	SNP311	4.36	5.17	NL	SNP229	3.98	4.97
SDW	SNP137	4.36	5.18	SL	SNP53	3.98	4.73
SDW	SNP380	4.35	5.15	SL	SNP162	3.97	4.75
RL	SNP320	4.32	5.04	SL	SNP156	3.96	4.71
RDW	SNP361	4.31	5.20	RL	SNP265	3.96	4.58
RDW	SNP333	4.30	5.13	RDW	SNP217	3.95	4.61
SDW	SNP40	4.29	5.27	PDW	SNP312	3.95	4.59
RL	SNP270	4.29	5.02	NL	SNP20	3.95	4.94
NL	SNP204	4.28	5.39	RL	SNP11	3.94	4.55
NL	SNP379	4.27	5.41	NL	SNP262	3.94	4.95
NL	SNP156	4.27	5.39	NL	SNP114	3.93	5.05
RDW	SNP117	4.26	5.00	PDW	SNP217	3.92	4.55
SDW	SNP270	4.25	5.07	SDW	SNP307	3.92	4.54
SL	SNP110	4.25	5.13	RDW	SNP272	3.91	4.99
SL	SNP142	4.25	5.07	NL	SNP141	3.91	4.87
NL	SNP174	4.24	5.50	PDW	SNP103	3.90	5.04
NL	SNP126	4.24	5.38	PDW	SNP333	3.90	4.58
RDW	SNP360	4.24	5.16	SDW	SNP377	3.89	4.70
NL	SNP376	4.24	5.33	NL	SNP248	3.88	4.85
NL	SNP153	4.23	5.45	NL	SNP313	3.87	4.89
SL	SNP277	4.22	4.97	PDW	SNP381	3.87	4.46
PDW	SNP113	4.20	5.04	SL	SNP220	3.86	4.59
SDW	SNP116	4.20	5.02	PDW	SNP142	3.86	4.52
RL	SNP345	4.20	4.89	RL	SNP205	3.85	6.35
PDW	SNP96	4.16	4.90	RL	SNP303	3.83	4.60
RL	SNP156	4.15	4.86	RDW	SNP339	3.82	4.48
NL	SNP336	4.15	5.32	SDW	SNP173	3.82	4.45
NL	SNP173	4.14	5.22	SL	SNP316	3.81	4.45
NL	SNP235	4.12	5.19	RL	SNP43	3.81	4.90
SL	SNP314	4.09	4.89	NL	SNP129	3.80	4.74
SDW	SNP345	4.07	4.82	SL	SNP197	3.80	4.49
NL	SNP171	4.07	5.19	RDW	SNP258	3.78	4.46
NL	SNP220	4.07	5.15	PDW	SNP345	3.77	4.42
SDW	SNP103	4.04	5.26	RDW	SNP358	3.76	4.65
NL	SNP258	4.03	5.10	SDW	SNP145	3.75	5.95
RDW	SNP9	4.03	4.72	PDW	SNP116	3.74	4.40

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>	Trait	Markers	-Log <sub>10</sub> (P)	R <sup>2</sup>
PDW	SNP75	3.74	4.38	PDW	SNP53	3.45	3.99
SDW	SNP259	3.74	4.42	SL	SNP329	3.45	4.01
PDW	SNP173	3.73	4.33	SDW	SNP333	3.45	4.00
SDW	SNP217	3.73	4.31	NL	SNP290	3.45	4.26
PDW	SNP270	3.72	4.36	SL	SNP125	3.45	4.13
SL	SNP360	3.72	4.47	RL	SNP10	3.44	3.93
PDW	SNP145	3.72	5.88	SL	SNP160	3.42	3.97
RDW	SNP196	3.71	4.31	NL	SNP228	3.42	4.23
SDW	SNP114	3.70	4.49	RDW	SNP179	3.41	3.93
RL	SNP308	3.69	4.21	NL	SNP189	3.41	4.22
SDW	SNP55	3.69	4.31	SDW	SNP303	3.41	4.12
SL	SNP205	3.68	6.68	PDW	SNP291	3.40	3.89
RL	SNP338	3.68	4.23	PDW	SNP259	3.39	3.95
SDW	SNP233	3.68	4.25	RL	SNP302	3.38	3.83
NL	SNP203	3.68	4.57	PDW	SNP233	3.37	3.84
PDW	SNP336	3.67	4.31	NL	SNP244	3.37	4.10
SL	SNP344	3.67	4.38	SL	SNP178	3.36	3.93
RL	SNP166	3.65	4.15	NL	SNP62	3.36	4.19
RL	SNP272	3.65	4.46	RL	SNP90	3.36	3.79
RL	SNP326	3.64	4.17	RDW	SNP254	3.36	3.87
SDW	SNP75	3.63	4.23	SL	SNP183	3.36	3.95
PDW	SNP55	3.61	4.21	RL	SNP174	3.36	3.86
RDW	SNP260	3.60	4.23	SL	SNP60	3.35	4.00
RL	SNP277	3.60	4.06	RDW	SNP290	3.35	3.88
LIS1	SNP156	3.60	4.57	NL	SNP31	3.35	4.16
NL	SNP275	3.60	4.44	RDW	SNP28	3.35	3.86
SL	SNP171	3.59	4.23	SDW	SNP2	3.34	5.26
PDW	SNP179	3.59	4.14	NL	SNP39	3.34	4.09
RDW	SNP68	3.58	4.13	RL	SNP198	3.33	3.77
PDW	SNP68	3.58	4.11	NL	SNP148	3.32	4.09
NL	SNP329	3.57	4.40	SL	SNP11	3.31	3.80
RDW	SNP369	3.52	4.21	RDW	SNP186	3.31	3.85
RDW	SNP156	3.52	4.10	PDW	SNP289	3.30	4.19
SDW	SNP117	3.52	4.02	RL	SNP35	3.30	3.76
LIS1	SNP56	3.52	4.59	SDW	SNP53	3.29	3.79
RDW	SNP295	3.51	4.31	RDW	SNP19	3.29	3.79
SDW	SNP152	3.49	4.08	RL	SNP313	3.29	3.75
RL	SNP373	3.48	10.22	SL	SNP30	3.28	4.86
RDW	SNP95	3.47	4.05	RL	SNP362	3.28	3.66
RDW	SNP228	3.47	4.03	SL	SNP364	3.27	3.95
SDW	SNP205	3.46	6.13	SDW	SNP142	3.26	3.75

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SDW	SNP91	3.26	3.79	NL	SNP316	3.07	3.69
RDW	SNP160	3.25	3.73	RL	SNP159	3.07	3.40
RDW	SNP291	3.24	3.70	SDW	SNP323	3.06	3.71
SDW	SNP312	3.24	3.68	RL	SNP371	3.06	3.41
PDW	SNP377	3.24	3.81	SL	SNP217	3.06	3.45
RDW	SNP289	3.22	4.10	SDW	SNP364	3.05	3.68
NL	SNP135	3.22	3.91	PDW	SNP91	3.05	3.50
RL	SNP310	3.22	3.66	SDW	SNP289	3.04	3.83
SL	SNP166	3.22	3.67	SDW	SNP316	3.04	3.41
PDW	SNP323	3.22	3.92	NL	SNP319	3.03	3.62
PDW	SNP303	3.21	3.85	SDW	SNP206	3.02	3.43
PDW	SNP114	3.21	3.81	RL	SNP142	3.02	3.36
PDW	SNP141	3.21	3.65	NL	SNP292	3.02	3.67
SDW	SNP179	3.21	3.65	SDW	SNP141	3.01	3.39
SDW	SNP68	3.19	3.61	RL	SNP329	3.01	3.34
RDW	SNP53	3.19	3.65	PDW	SNP72	3.01	3.37
NL	SNP343	3.18	7.99	PDW	SNP384	3.00	3.36
SDW	SNP291	3.18	3.61	NL	SNP108	3.00	3.64
SDW	SNP118	3.17	3.63	SDW	SNP72	2.99	3.34
NL	SNP157	3.16	3.93	SDW	SNP277	2.99	3.33
SL	SNP287	3.16	3.65	RL	SNP125	2.99	3.42
LIS1	SNP268	3.16	4.00	SL	SNP21	2.99	3.37
SDW	SNP10	3.14	3.61	SL	SNP274	2.98	3.41
NL	SNP384	3.14	3.79	RL	SNP80	2.98	3.29
SL	SNP375	3.14	36.77	NL	SNP18	2.97	3.60
PDW	SNP2	3.14	4.87	SDW	SNP213	2.97	3.74
PDW	SNP307	3.14	3.51	NL	SNP11	2.97	3.56
RL	SNP263	3.14	3.51	LIS1	SNP86	2.96	9.80
RL	SNP64	3.13	3.54	NL	SNP254	2.96	3.57
PDW	SNP369	3.13	3.68	PDW	SNP272	2.96	3.62
RDW	SNP103	3.12	3.91	RDW	SNP328	2.96	3.32
RDW	SNP379	3.12	3.59	RDW	SNP277	2.95	3.30
NL	SNP214	3.12	3.77	PDW	SNP38	2.95	3.30
RL	SNP1	3.12	3.47	NL	SNP48	2.95	3.52
PDW	SNP316	3.11	3.50	NL	SNP206	2.94	3.54
NL	SNP293	3.10	3.79	SDW	SNP354	2.94	3.35
SL	SNP345	3.09	3.54	RL	SNP8	2.94	3.28
PDW	SNP277	3.09	3.46	RL	SNP301	2.94	3.28
SDW	SNP336	3.08	3.53	RL	SNP350	2.93	3.27
RL	SNP168	3.08	3.41	PDW	SNP364	2.93	3.52
NL	SNP344	3.08	3.83	RL	SNP226	2.93	3.29

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RL	SNP274	2.93	3.26	SDW	SNP64	2.73	3.07
NL	SNP69	2.92	5.99	RDW	SNP96	2.73	3.05
RL	SNP97	2.92	3.26	SDW	SNP234	2.73	3.04
RL	SNP33	2.92	3.22	NL	SNP133	2.72	3.90
SL	SNP379	2.91	3.32	PDW	SNP28	2.72	3.01
RL	SNP216	2.91	3.24	SL	SNP188	2.72	3.06
NL	SNP71	2.91	3.52	NL	SNP179	2.71	3.20
NL	SNP88	2.90	3.44	RL	SNP333	2.71	2.96
SDW	SNP268	2.88	3.28	SL	SNP8	2.70	3.04
PDW	SNP9	2.87	3.19	NL	SNP295	2.70	3.42
NL	SNP183	2.87	3.48	RDW	SNP316	2.69	2.97
SL	SNP285	2.87	3.25	RDW	SNP162	2.69	3.04
SDW	SNP320	2.86	3.20	RDW	SNP34	2.68	3.03
PDW	SNP320	2.86	3.18	RDW	SNP18	2.68	3.01
RDW	SNP141	2.84	3.18	NL	SNP112	2.68	3.19
RL	SNP238	2.84	3.23	SDW	SNP369	2.68	3.08
NL	SNP90	2.84	3.36	RL	SNP75	2.68	2.94
RDW	SNP320	2.83	3.16	SL	SNP326	2.68	2.99
NL	SNP28	2.82	3.37	RDW	SNP185	2.67	2.98
SDW	SNP309	2.82	4.96	SDW	SNP38	2.67	2.94
NL	SNP138	2.82	3.34	SL	SNP371	2.67	2.99
NL	SNP15	2.82	3.36	PDW	SNP19	2.67	2.96
RDW	SNP161	2.81	3.13	RDW	SNP173	2.67	2.95
SDW	SNP315	2.81	3.14	PDW	SNP206	2.66	2.95
SL	SNP133	2.81	3.63	PDW	SNP185	2.65	2.94
SL	SNP354	2.80	3.19	RDW	SNP345	2.65	2.96
SL	SNP184	2.80	3.14	NL	SNP367	2.65	3.13
RL	SNP126	2.80	3.09	NL	SNP370	2.64	3.13
RL	SNP211	2.79	3.09	SDW	SNP193	2.64	2.97
RDW	SNP323	2.79	3.33	LIS1	SNP230	2.64	5.61
RDW	SNP378	2.78	3.15	RL	SNP376	2.64	2.88
PDW	SNP234	2.78	3.11	SL	SNP211	2.64	2.96
RL	SNP119	2.77	3.10	RL	SNP54	2.64	2.87
RL	SNP287	2.76	3.05	PDW	SNP129	2.64	2.91
SL	SNP278	2.76	3.08	SL	SNP158	2.63	3.01
PDW	SNP205	2.76	4.72	SL	SNP165	2.63	2.99
SL	SNP250	2.75	17.21	SDW	SNP129	2.63	2.90
PDW	SNP354	2.75	3.10	PDW	SNP268	2.63	2.95
SDW	SNP215	2.75	3.03	RDW	SNP38	2.63	2.90
NL	SNP137	2.74	3.26	SL	SNP41	2.62	2.92
RDW	SNP75	2.73	3.06	NL	SNP306	2.62	3.08

**Appendix Table 5: QTLs detected by Q model under salt treatment (continued).**

<b>Trait</b>	<b>Markers</b>	<b>-log<sub>10</sub>(P)</b>	<b>R<sup>2</sup></b>
PDW	SNP295	2.62	3.04
RDW	SNP72	2.62	2.87
SDW	SNP146	2.61	2.89
PDW	SNP10	2.61	2.90
SL	SNP295	2.61	3.05
RDW	SNP135	2.61	2.88
NL	SNP260	2.60	3.10
RDW	SNP92	2.59	2.87
SL	SNP369	2.58	2.89
RL	SNP104	2.57	2.83
PDW	SNP378	2.57	2.86
PDW	SNP159	2.57	2.80
NL	SNP35	2.56	3.04
RDW	SNP55	2.56	2.83
RDW	SNP145	2.55	3.88
PDW	SNP146	2.54	2.80
PDW	SNP152	2.54	2.82
PDW	SNP315	2.53	2.78
SL	SNP87	2.53	2.81
SL	SNP75	2.53	2.81
NL	SNP291	2.53	2.95
NL	SNP361	2.53	2.99
SL	SNP146	2.52	2.79
SL	SNP263	2.52	2.79
SL	SNP151	2.52	2.80
PDW	SNP213	2.51	3.06
NL	SNP274	2.51	2.93
PDW	SNP118	2.51	2.75
RDW	SNP175	2.51	2.76
PDW	SNP193	2.51	2.79
RDW	SNP248	2.51	2.76

**Appendix Table 6: QTLs detected by PCA model under salt treatment.**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
SL	SNP208	11.18	13.63	SL	SNP307	4.92	5.77
SL	SNP310	9.65	11.77	PDW	SNP88	4.89	5.42
SL	SNP210	8.94	10.81	PDW	SNP21	4.86	5.39
SL	SNP272	8.18	10.63	SL	SNP358	4.86	5.94
SL	SNP118	8.15	9.92	PDW	SNP102	4.85	5.38
SL	SNP57	7.72	9.30	SL	SNP303	4.82	5.89
SL	SNP105	7.14	10.12	SDW	SNP304	4.82	5.36
SL	SNP304	7.00	8.45	PDW	SNP269	4.82	5.33
NL	SNP166	6.83	7.66	NL	SNP196	4.80	5.26
SL	SNP96	6.73	8.07	RDW	SNP48	4.74	5.36
SL	SNP43	6.72	8.85	SDW	SNP339	4.69	5.18
PDW	SNP89	6.26	7.08	RDW	SNP111	4.68	5.29
SL	SNP25	6.10	7.29	SDW	SNP109	4.65	5.13
SDW	SNP361	6.04	6.81	PDW	SNP235	4.64	5.13
SDW	SNP89	5.99	6.74	SDW	SNP8	4.64	5.15
SDW	SNP303	5.86	6.85	SDW	SNP269	4.63	5.10
SL	SNP114	5.77	7.02	RW	SNP74	4.60	6.86
PDW	SNP303	5.60	6.53	PDW	SNP45	4.58	5.05
SL	SNP227	5.56	6.62	PDW	SNP350	4.58	5.06
PDW	SNP236	5.50	6.16	SDW	SNP301	4.57	5.04
PDW	SNP48	5.49	6.14	PDW	SNP339	4.51	4.97
SL	SNP60	5.45	6.63	SL	SNP178	4.49	5.22
PDW	SNP36	5.43	6.07	RDW	SNP245	4.49	5.05
PDW	SNP94	5.43	6.07	PDW	SNP301	4.47	4.91
SDW	SNP236	5.31	5.93	RDW	SNP109	4.47	5.03
SDW	SNP21	5.30	5.93	RDW	SNP194	4.46	5.11
PDW	SNP194	5.30	6.01	NL	SNP37	4.45	4.84
SL	SNP315	5.26	6.20	RDW	SNP36	4.45	5.01
SDW	SNP235	5.24	5.86	RDW	SNP94	4.45	5.01
RDW	SNP89	5.23	6.04	PDW	SNP138	4.45	4.89
SDW	SNP48	5.22	5.82	NL	SNP142	4.45	4.85
SDW	SNP36	5.19	5.78	SL	SNP66	4.44	5.21
SDW	SNP94	5.19	5.78	RDW	SNP236	4.40	4.94
RDW	SNP88	5.14	5.87	PDW	SNP111	4.39	4.81
NL	SNP52	5.13	5.65	SL	SNP184	4.34	5.03
SL	SNP218	5.11	6.05	SDW	SNP88	4.31	4.72
PDW	SNP109	5.10	5.68	PDW	SNP245	4.30	4.71
SDW	SNP194	5.09	5.76	SL	SNP285	4.30	5.00
PDW	SNP361	5.01	5.56	PDW	SNP100	4.27	4.69
SDW	SNP102	4.98	5.54	NL	SNP220	4.26	4.63



**Appendix Table 6: QTLs detected by PCA model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
RDW	SNP45	4.25	4.75	RDW	SNP319	3.69	4.06
SL	SNP372	4.22	4.87	PDW	SNP304	3.66	3.95
SDW	SNP260	4.19	4.63	RDW	SNP102	3.65	4.02
RL	SNP43	4.19	4.93	RW	SNP230	3.64	7.40
SL	SNP352	4.18	4.83	SL	SNP231	3.63	4.40
SDW	SNP205	4.18	7.35	SDW	SNP337	3.63	3.91
RDW	SNP350	4.16	4.67	RDW	SNP328	3.62	3.98
SDW	SNP100	4.13	4.53	SL	SNP311	3.62	4.13
SDW	SNP350	4.13	4.52	RDW	SNP202	3.61	3.97
PDW	SNP196	4.12	4.49	RL	SNP177	3.61	3.70
SL	SNP226	4.12	4.79	PDW	SNP112	3.60	3.88
PDW	SNP202	4.10	4.46	NL	SNP218	3.59	3.82
PDW	SNP319	4.08	4.44	SL	SNP51	3.59	4.09
PDW	SNP8	4.05	4.43	RDW	SNP100	3.59	3.96
SDW	SNP45	4.03	4.37	NL	SNP15	3.58	3.79
SDW	SNP138	4.02	4.37	RDW	SNP19	3.56	3.91
RDW	SNP138	4.01	4.46	SL	SNP150	3.56	4.15
PDW	SNP20	4.00	4.34	RDW	SNP336	3.56	3.98
SL	SNP220	3.99	4.61	SL	SNP126	3.54	4.00
SL	SNP92	3.99	4.61	RDW	SNP137	3.54	3.88
RDW	SNP303	3.97	4.62	NYL	SNP230	3.53	7.75
SL	SNP314	3.95	4.56	SDW	SNP91	3.51	3.81
RDW	SNP269	3.94	4.37	SL	SNP116	3.51	3.98
PDW	SNP337	3.90	4.25	RDW	SNP97	3.49	3.83
SDW	SNP20	3.90	4.22	RDW	SNP39	3.49	3.82
SDW	SNP92	3.88	4.22	SDW	SNP39	3.49	3.72
SDW	SNP372	3.88	4.19	DWYL	SNP55	3.49	4.17
PDW	SNP49	3.86	4.18	SL	SNP235	3.45	3.90
SL	SNP205	3.85	7.21	RDW	SNP337	3.44	3.78
SDW	SNP202	3.84	4.14	RDW	SNP384	3.42	3.77
SL	SNP378	3.83	4.40	NL	SNP366	3.39	3.56
SDW	SNP111	3.82	4.13	SDW	SNP112	3.39	3.62
SL	SNP149	3.81	4.34	PDW	SNP244	3.38	3.59
LIS1	SNP156	3.80	4.94	NL	SNP84	3.38	3.59
SDW	SNP245	3.79	4.09	SDW	SNP381	3.37	3.58
PDW	SNP39	3.76	4.05	PDW	SNP137	3.37	3.58
SDW	SNP49	3.76	4.06	SL	SNP186	3.36	3.82
SDW	SNP319	3.76	4.05	PDW	SNP91	3.33	3.59
PDW	SNP260	3.73	4.07	RL	SNP180	3.31	3.34
SDW	SNP113	3.72	4.03	SL	SNP16	3.30	3.70
SL	SNP271	3.69	4.24	PDW	SNP205	3.30	5.70

**Appendix Table 6: QTLs detected by PCA model under salt treatment (continued).**

Trait	Markers	$-\log_{10}(P)$	$R^2$	Trait	Markers	$-\log_{10}(P)$	$R^2$
PDW	SNP97	3.29	3.49	PDW	SNP233	2.94	3.05
PDW	SNP92	3.29	3.50	SDW	SNP97	2.94	3.06
RDW	SNP301	3.29	3.56	RDW	SNP20	2.92	3.11
SDW	SNP151	3.28	3.50	SL	SNP183	2.92	3.23
SL	SNP95	3.27	3.68	NL	SNP102	2.90	2.99
RL	SNP235	3.27	3.31	PDL	SNP86	2.90	9.53
RL	SNP21	3.27	3.31	PDW	SNP113	2.87	3.00
SDW	SNP233	3.26	3.45	PDW	SNP336	2.86	3.03
RDW	SNP312	3.26	3.52	RDW	SNP117	2.86	3.03
RDW	SNP112	3.23	3.51	RW	SNP307	2.86	3.51
LIS1	SNP56	3.23	4.18	PDW	SNP19	2.86	2.95
NL	SNP276	3.21	4.25	SDW	SNP238	2.85	3.08
RL	SNP128	3.19	4.23	SL	SNP275	2.84	3.11
SL	SNP152	3.19	3.57	SDW	SNP204	2.83	2.93
RL	SNP208	3.19	3.23	PDW	SNP340	2.82	2.93
PDW	SNP204	3.16	3.32	PDW	SNP338	2.82	2.91
SDW	SNP153	3.15	3.37	SL	SNP286	2.82	3.09
RDW	SNP204	3.15	3.39	SDW	SNP137	2.81	2.91
PDW	SNP372	3.15	3.30	RL	SNP146	2.79	2.75
SL	SNP313	3.14	3.50	RL	SNP378	2.78	2.74
RDW	SNP244	3.13	3.36	SL	SNP153	2.77	3.08
PDL	SNP156	3.12	3.95	RL	SNP361	2.76	2.71
RL	SNP303	3.12	3.25	SDW	SNP338	2.75	2.83
SL	SNP367	3.11	3.45	SDW	SNP218	2.73	2.81
SDW	SNP254	3.10	3.26	RDW	SNP196	2.72	2.86
PDW	SNP254	3.09	3.25	RDW	SNP21	2.71	2.86
SDW	SNP368	3.09	3.24	NL	SNP10	2.71	2.75
SDW	SNP244	3.09	3.23	RDW	SNP9	2.70	2.84
LIS1	SNP268	3.08	3.96	SL	SNP146	2.70	2.93
SDW	SNP152	3.08	3.24	SL	SNP84	2.70	2.95
RDW	SNP28	3.07	3.29	PDW	SNP381	2.70	2.77
SL	SNP58	3.07	3.41	SL	SNP278	2.70	2.92
SL	SNP110	3.07	3.44	PDL	SNP56	2.68	3.37
SL	SNP71	3.07	3.39	NL	SNP358	2.68	2.90
NL	SNP336	3.03	3.18	RL	SNP153	2.68	2.67
SL	SNP361	3.03	3.34	SL	SNP171	2.68	2.94
NYL	SNP166	3.01	3.69	SL	SNP142	2.68	2.91
RDW	SNP49	3.01	3.23	SDW	SNP377	2.67	2.86
SL	SNP344	2.99	3.35	RDW	SNP339	2.67	2.81
PDW	SNP151	2.98	3.13	PDW	SNP368	2.66	2.71
RL	SNP205	2.95	4.76	SL	SNP276	2.65	3.65

**Appendix Table 6: QTLs detected by PCA model under salt treatment (continued).**

<b>Trait</b>	<b>Markers</b>	<b>-log<sub>10</sub>(P)</b>	<b>R<sup>2</sup></b>
SL	SNP295	2.65	3.00
PDW	SNP238	2.64	2.81
PDW	SNP218	2.63	2.69
SL	SNP33	2.63	2.83
PDW	SNP349	2.62	2.93
DWYL	SNP358	2.62	3.09
SDW	SNP35	2.60	2.66
SL	SNP165	2.60	2.87
SL	SNP195	2.59	2.79
SL	SNP121	2.58	2.80
SDW	SNP82	2.58	2.63
SDW	SNP340	2.58	2.63
SL	SNP125	2.57	2.84
NYL	SNP268	2.56	3.12
SDW	SNP268	2.56	2.62
RW	SNP372	2.55	3.06
PDW	SNP117	2.53	2.56
SL	SNP30	2.53	3.56
RDW	SNP295	2.53	2.73
NL	SNP136	2.52	2.57
RDW	SNP333	2.52	2.61
PDW	SNP180	2.52	2.55
LIS1	SNP86	2.52	8.16
RDW	SNP263	2.51	2.61
SDW	SNP180	2.51	2.54
SL	SNP159	2.51	2.69