Chapter 4
Molecular Mapping and Breeding for Genes/QTLS Related to Climate Change

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Abstract Through selection by humans, crop plants are adapted to produce optimal yield in the areas where they are cultivated. Climate changes may cause stress to plants, disturb plant growth, and decrease plant yield. Food shortages due to crop failure may cause hunger particularly in poor countries. Therefore, it is important to develop new crop cultivars that can adapt to climate changes. This chapter summarizes quantitative trait loci (QTL) analysis and findings for candidate genes of traits related to tolerance to drought, heat, salinity, macronutrient and micronutrient deficiency, flooding, frost, particularly in major cereal crops. In addition, QTL studies on flowering time of cereal crops are also deliberated. Flowering time is a critical plant phase that determines the transition from the vegetative to the reproductive phase. The optimal time of flowering largely affects the overall yield. Information obtained from QTL analysis has been utilized in the development of stress-tolerant cultivars. Indeed, several stress-tolerant cultivars have been released in stress-prone areas. However, QTLs for most of the traits have not been elucidated. Fortunately, development in sequencing technologies has accelerated elucidation of the genomic regions conferring stress tolerance. This chapter also provides information regarding several recent technologies and approaches in QTL/gene mapping and also in plant breeding.

4.1 Introduction

Through selection by humans, crop plants are adapted to produce optimal yield in the areas where they are cultivated. Climate changes (warmer winters, high temperatures during summer, drought, sea level rise, and increased heavy precipitation), together with its side effects such as high salinity and deficiency of

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macronutrients and micronutrients may cause stress to plants, disturb plant growth, decrease yield, and as a consequence decrease agricultural production (IPCC 2007). Crop failure may cause food shortage and hunger in poor areas. Therefore, it is important to develop new crop cultivars that can adapt to climate changes.

Quantitative trait loci (QTL) analysis has been utilized to determine genomic regions associated with stress-tolerance traits. QTL analysis uses a segregating population derived from two cultivar parents contrasting in stress-tolerance level. Major QTLs for traits such as flowering time, tolerance to high salinity, submergence, or frost/cold were determined quickly, and through fine mapping the candidate genes for QTLs were also identified. However, in more complex traits such as drought and heat stress tolerance, some QTLs identified in one study may not be detected in studies using different mapping populations or stress conditions. To overcome this problem, meta-QTL analysis has been used to identify consensus QTLs across different populations and environments. Along with advances in sequencing technologies and also a decrease in sequencing cost, genomewide association studies (GWAS) became more common in identifying single nucleotide polymorphisms (SNPs) or indels associated with stress tolerance. GWAS enables researchers to identify SNPs and indels without creating mapping populations, but instead utilizing varieties and landraces available in germplasm collections (Mitchell-Olds 2010). This approach shortens the time needed to develop a mapping population. Moreover, GWAS can be applied to minor crop species that have limited availability of genomic information or molecular markers.

The first part of this chapter will describe QTL analysis performed to identify traits involved in tolerance to drought, heat, salinity, submergence, cold and frost, and macronutrient and micronutrient deficiency. For drought, heat, salinity, submergence, and macronutrient and micronutrient deficiency, we will focus mainly on studies conducted on major cereals such as rice, maize, and wheat. For cold and frost, we will focus on studies using temperate grasses such as wheat, barley, perennial ryegrass, and meadow fescue. Flowering QTL studies are also included because flowering is the most critical development stage to determine crop yield. The second part of this chapter will introduce several applications of QTL analysis results through marker-assisted selection (MAS), transgenics, and challenges in developing stress-tolerant cultivars. The third part will deliberate on new technologies that have been adapted to identify QTLs/genes contributing to stress-tolerance traits.

4.2 Molecular Mapping of Genes/QTLs for Tolerance to Stresses

4.2.1 Drought Tolerance

Approximately, more than 40 % of rice growing areas and temperate maize cultivation areas depend on rain as the water source (Campos et al. 2004; Courtois et al. 2009). Crop yield in these areas is vulnerable to drought, and as a consequence, food production may decrease due to prolonged drought. Therefore, the ability to maintain yield level under drought stress is important for cereal crops. A direct approach to identifying drought-tolerant varieties is by evaluating the grain yield under drought stress. QTLs associated with high grain yield under reproductive-stage drought have been identified in rice (Lanceras et al. 2004; Bernier et al. 2007; Kumar et al. 2010; Vikram et al. 2011; Venuprasad et al. 2012), maize (Ribaut et al. 1997; Tuberosa et al. 2002; Swamy et al. 2011), durum wheat (Maccaferri et al. 2008), bread wheat (Li et al. 2007), and many other crops. Another approach is to identify QTLs responsible for traits related to water extraction ability, which generally focuses on root morphology such as deep root length, root thickness, and root dry weight. Some major QTLs for deep root length have been identified in rice (Steele et al. 2006, 2007; Uga et al. 2011) and maize (Trachsel et al. 2009). QTL introgression from the deep-rooting rice varieties "Kinandang Patong" and "Azucena" into the shallow-rooting variety "IR64" has succeeded in improving the root length of rice (Steele et al. 2006, 2007; Uga et al. 2011).

Meta-QTL analysis is utilized to identify consensus QTLs across different mapping populations and different test conditions (Goffinet and Gerber 2000; Veyrieras et al. 2007). This method has been applied in determining consensus QTLs for grain yield (Swamy et al. 2011), maize flowering time (Chardon et al. 2004), soybean nematode resistance (Guo et al. 2006), earliness in bread wheat (Hanocq et al. 2007), and drought tolerance-related QTLs for rice (Courtois et al. 2009; Khowaja et al. 2009) and maize (Hao et al. 2009). Meta-QTL analysis for grain yield under drought stress showed that several QTLs coincide with QTLs for root and leaf morphology, and contain stress-inducible and ABA-response genes, growth and development-related genes, and sugar transport-related genes (Swamy et al. 2011). Several QTLs for grain yield under drought conditions were located near the *Sd1* locus on chromosome 1 of rice (Swamy et al. 2011). *Sd1* is a major locus responsible for semidwarf rice (Monna et al. 2002). It is interesting to note that semidwarf rice has been selected for cultivation because of plant lodging reduction and increase in grain yield (Monna et al. 2002). *Sd1* encodes GA20-oxidase that catalyzes the last step of active GAs biosynthesis (Monna et al. 2002). The relationship between semidwarf characteristics and drought tolerance is also observed in other cereal crops (Swamy et al. 2011). Major QTLs for wheat grain yield under drought were detected near *Rht-b1*, the orthologous locus of *Sd1* on

chromosome 4B (Swamy et al. 2011). Courtois et al. (2009) collected data that consisted of 1,467 QTLs associated with 29 root parameters in rice, including root number, maximum root length, root thickness, and root/shoot ratio. The data was collected from 24 independent papers using more than 10 different mapping populations. The analysis determined 119 consensus QTLs, and found that most QTLs related to root morphology formed a cluster on chromosome 1. Meanwhile, a study on drought tolerance in maize collected QTL data from 22 experiments (Hao et al. 2009). Two hundred and thirty-nine QTLs detected under water stress and 160 QTLs detected under control conditions were compiled. As a result, 39 consensus QTLs under water stress and 36 consensus QTLs under control conditions were identified (Hao et al. 2009). Several genes related to stress response such as genes encoding NCED, a carotenoid cleavage enzyme and CBF1/DREB transcription factors were identified within the meta-QTLs.

Besides water extraction ability, the ability to use water resources is also important. This is called water use efficiency (WUE), which is defined as follows.

From the physiological viewpoint, WUE is defined as moles of carbon gained in photosynthesis in exchange for water used in transpiration. From the farmers' and agronomists' viewpoint, WUE is the yield achieved from the water made available to the crop through precipitation and/or irrigation (Condon et al. 2004). The common method to measure WUE is carbon isotope discrimination (Δ; Farquhar et al. 1989). Δ is the ratio of 12C to 13C (Farquhar et al. 1989). Natural variation in leaf Δ among *Arabidopsis* accessions is related to variation in transpiration efficiency (Masle et al. 2005). QTL analysis of transpiration efficiency in *Arabidopsis* identified the *ERECTA* gene as the transcription efficiency regulator (Masle et al. 2005). The *ERECTA* gene encodes a putative leucine-rich repeat receptor-like kinase (LRR-RLK), which is involved in inflorescence development, stomatal density, epidermal cell expansion, and mesophyll cell proliferation. Lines carrying the *erecta* mutation showed increased stomatal density, and also increased stomatal conductance. High stomatal conductance means higher loss of water to the air. In drought conditions, low stomatal density is important to reduce water loss. Near-isogenic lines (NILs) carrying *ERECTA* from the high transcription efficiency variety showed higher leaf transpiration efficiency and higher plant dry weight/water used ratio (Masle et al. 2005). *ERECTA* is also identified as candidate gene in determining grain yield of rice (Swamy et al. 2011). *ERECTA* is also conserved among angiosperm species; therefore there is a high possibility that it is involved in regulating stomatal density in other species.

Perhaps the stay-green trait is the easiest indicator for evaluation of drought tolerance. The stay-green characteristic has been identified in rice, sorghum, maize, and durum wheat (Xu et al. 2000; Sanchez et al. 2002; Jiang et al. 2004; Zheng et al. 2009; Kumar et al. 2010). Stay-green plants retain their chlorophyll and have delayed leaf senescence under drought conditions, whereas the leaves of normal plants usually senesce. The stay-green characteristic is important particularly during the postflowering stage, because stay-green plants can keep more photosynthetically active leaves to transfer enough nutrition to grains. Fourteen stay-green QTLs were detected in maize (Zheng et al. 2009), and 46 QTLs were detected in a

rice doubled-haploid (DH) line population (Jiang et al. 2004). Three QTLs were identified in a wheat recombinant inbred line (RIL) population derived from a cross between stay-green cultivar "Chirya 3" and nonstay-green cultivar "Sonalika." The QTLs were named *QSg.bhu-1A*, *QSg.bhu-3B*, and *QSg.bhu-7D* (Kumar et al. 2010). However, only *QSg.bhu-1A* was consistently detected over 2 years of experiment. Four stay-green QTLs (*Stg1*, *Stg2*, *Stg3*, and *Stg4*) were identified using three sorghum RIL populations derived from a cross of two inbred lines including "B35," a cultivar resistant to postflowering stress, and "Tx7000," a cultivar sensitive to postflowering stress (Xu et al. 2000). *Stg1* and *Stg2* were located on linkage group A, *Stg3* on linkage group D, and *Stg4* on linkage group J. *Stg1*, *Stg2*, and *Stg3* were consistently identified in different trial locations over 2 years of experiment, whereas *Stg4* was identified in different trial locations over 1 year of experiment (Xu et al. 2000). Further analysis showed that *Stg1*, *Stg2*, and *Stg3* were collocated with three QTLs controlling the chlorophyll content (*Chl1*, *Chl2*, and *Chl3*). Although the candidate genes have not been determined, *Stg1* and *Stg2* contain genes for heat shock proteins, cell membrane ATPase, ABA-responsive genes, and key photosynthetic enzymes (NADP-dependent malate dehydrogenase, chlorophyll a/b binding protein, and Rubisco) (Xu et al. 2000).

4.2.2 Heat Tolerance

By the end of the twenty-first century, global temperature is predicted to increase by about 2–4 °C (IPCC 2007). Higher temperature accelerates plant development, including flowering time. For bioenergy plants, heat stress can reduce biomass production. In cereal crops, heat stress reduces anther dehiscence and pollen fertility rate. It subsequently reduces grain filling, and also the overall yield. Heat stress thresholds differ among plant species and cultivars (Prasad et al. 2006; Wahid et al. 2007). Heat tolerance also varies depending on development stage. Generally, plants are most sensitive during flowering and grain filling rather than in the vegetative stage (Maestri et al. 2002; Prasad et al. 2006; Jagadish et al. 2007).

Several parameters have been used to determine heat tolerance: pollen germination rate, pollen tube length, spikelet sterility, cell membrane thermostability, chlorophyll stability index, canopy temperature depression, grain quality change, and antioxidant level (Matsui et al. 2001; Prasad et al. 2006; Paliwal et al. 2012). Heat tolerance is sometimes related to higher stomatal opening, which is intended to cool plant tissue by releasing more water to air. However, this approach is not beneficial when water is scarce, a condition that usually accompanies heat stress.

Despite the complexity of heat tolerance regulation, several stable QTLs have been identified in wheat (Paliwal et al. 2012). QTLs were identified in a mapping population derived from heat-tolerant hexaploid wheat cultivar "NW1014" and a heat-susceptible "HUW468". QTLs were identified on chromosome 2B, 7B, and

7D for the heat susceptibility index (HSI) for thousand grain weight (HSITGW), HSI for grain fill duration (HSIGFD), and canopy temperature depression (CTD).

The heat shock protein (HSP) has been identified as a candidate gene for heat stress tolerance in rice (Maestri et al. 2002; Ye et al. 2012). Proteomic analysis studying the heat stress effect during anthesis in heat-tolerant rice cultivar "N22" and heat-sensitive rice cultivars "IR64" and "Moroberekan" showed that the heat shock protein content increased in "N22" as a sign of the heat tolerance of "N22" (Jagadish et al. 2010). QTL and molecular analysis also showed HSP as a candidate gene for heat stress tolerance in *Arabidopsis* (Hong and Vierling 2000). HSP101 was mapped to the QTL region for heat stress tolerance in *Arabidopsis* (Hong and Vierling 2000). However, although HSPs are conserved among plant species in terms of high sequence similarity, the specific function in each species may vary (Maestri et al. 2002), therefore further research is needed to elucidate the function of each HSP in heat-stress tolerance.

4.2.3 Salt Tolerance

Approximately 830 million ha worldwide are affected by salinity (Rengasamy 2006), and the extent will increase due to extensive irrigation, drought, and the increasing level of seawater (Pitman and Lauchli 2002). The presence of salt in soil at seed sowing decreases the germination rate (Foolad 1999). A high level of salt in soil during the vegetative stage inhibits plant growth and causes leaf chlorosis, subsequently leading to a decrease in crop yield (Parida and Das 2005; Thomson et al. 2010). High salt inhibits plant growth in two ways (1) concentrations of salt in the soil reduce the ability of plants to absorb water (the osmotic effect of high salinity) (Lauchli and Grattan 2005); (2) when salt exists in plant cells, the cells may be damaged and the metabolic and photosynthetic capacity of the plant declines (Lauchli and Grattan 2005). Studies on *Arabidopsis*, rice, barley, and tomato have used plant condition parameters (root growth, germination rate, or seedling growth) to identify QTLs conferring salt tolerance (Mano and Takeda 1997; Foolad 1997; Foolad and Chen 1999; Foolad et al. 2001; Prasad et al. 2000; Quesada et al. 2002). QTL analysis showed that salt tolerance is controlled by polygenes, and tolerance at the germination stage and the seedling stage is controlled by different mechanisms (Foolad and Chen 1999).

Salt-tolerance during vegetative growth is also associated with the ability of plants to control Na^+ ion concentration in the leaves through exclusion or sequestration of Na^+ (Munns and Tester 2008). Several QTL analyses in rice, wheat, and *Arabidopsis* used the Na^+ concentration or the Na^+:K^+ ratio to identify genes responsible for maintaining low levels of Na^+ in the leaves and enhancing salt tolerance. For example, Gregorio and Senadhira (1993) studied the exclusion of Na^+ ions and increased K^+ in order to maintain a low Na^+:K^+ ratio in the shoots of three salt-tolerant *indica* rice cultivars: "Nona Bokra," "Pokkali," and "SR26."

QTLs for salt tolerance were extensively studied using an RIL population from a cross between the salt-susceptible variety "IR29" and salt-tolerant "Pokkali" (Gregorio 1997). A major QTL called *Salto1* associated with the Na^+:K^+ ratio in shoots and salinity tolerance at the seedling stage was identified in chromosome 1 (Gregorio 1997; Bonnila et al. 2002). QTL analysis using an $F_{2:3}$ population from a cross between the susceptible cultivar "Koshihikari" and salt-tolerant cultivar "Nona Bokra" also identified *Salto1* as a QTL for salt tolerance (Lin et al. 2004). This QTL was designated *Shoot K^+ concentration* (*SKC-1*) because it is associated with shoot K+ ion concentration (Lin et al. 2004). These QTLs are responsible for salt tolerance during plant vegetative growth. Map-based cloning of the *Salto1* region identified the gene *OsHKT1;5* (Ren et al. 2005). *OsHKT1;5* produces a protein that is highly similar to high-affinity K^+ transporters (HKT-type transporters) that play an important role in maintaining Na^+ and K^+ in shoots and leaf blades (Ren et al. 2005). These HKT-type transporters maintain low Na^+ concentration in leaf blades by excluding Na^+ from the xylem sap of leaf sheaths. The proteins also enhance the concentration of K^+ in leaf blades and sheaths.

HKT-type transporters are also involved in salt tolerance of wheat and *Arabidopsis* (Huang et al. 2006; James et al. 2006; Rus et al. 2006; Byrt et al. 2007). Two HKT-type transporter genes (*TmHKT7-A2* and *TmHKT1;5-A*) were mapped to *Nax1* and *Nax2,* QTLs for shoot Na^+ exclusion in durum wheat (genome AABB; Huang et al. 2006; James et al. 2006). A major QTL for salt tolerance *Kna1* was also mapped in bread wheat. The genomic region of *Kna1* corresponds to *Nax2* in durum wheat, suggesting that the two regions are orthologs (Byrt et al. 2007). The ortholog of TmHKT7-A2 TmHKT1;5-A, and OsHKT1;5 in *Arabidopsis* is AtHKT1;1 (Rus et al. 2006; Horie et al. 2009). The *athkt1;1* mutant increased Na^+ concentration of the xylem sap and conversely reduced the Na^+ content of the phloem sap (Sunarpi et al. 2005). The mutant also has increased salt sensitivity, while complementation with *AtHKT1;1* restores the salt tolerance of the plants (Sunarpi et al. 2005), indicating that *AtHKT1;1* is involved in salt tolerance in *Arabidopsis*. TmHKT7-A2 TmHKT1;5-A, OsHKT1;5, and AtHKT1;1 are included in class 1 HKT-type transporters (Horie et al. 2009).

Besides *Salto1*, cellular ion homeostasis in rice is also regulated by several other QTLs. Thomson et al. (2010) identified a QTL for shoot Na^+:K^+ ratio in chromosome 9 in "Pokkali"-derived RILs. QTL analysis using an F_2 population derived from a cross between salt-tolerant *japonica* rice mutant, "M-20" and the sensitive original variety "77-170" (Zhang et al. 1999) identified a major gene for salt tolerance on chromosome 12. The gene encodes a plasma membrane H+-ATPase that produces a proton and electrical gradient. The gradient is used as the force for transport and regulation of Na^+ and Cl^- uptake. Koyama et al. (2001) utilized an RIL population derived from a cross of two *indica* cultivars to perform QTL analysis associated with low Na^+ ion uptake and regulation of the Na^+:K^+ ratio. Two QTLs associated with regulation of the Na^+: K^+ ratio were found on chromosome 1 and 4 (Koyama et al. 2001). The QTL on chromosome 1 coincided with the QTL for Na^+ ion uptake. It is unknown whether the QTL is the same as *Salto1* or not, because of the different location due to different markers flanking the QTL

(Koyama et al. 2001). Besides HKT-type transporters, *Nax1*, *Nax2*, and *Kna1* also contain genes encoding intracellular Na^+/H^+ antiporter (NHX) and Salt Overly Sensitive 1 (SOS1). SOS1 is a putative Na^+/H^+ antiporter (Shi et al. 2002), and the overexpression of *SOS1* improved salt tolerance of *Arabidopsis* (Shi et al. 2002).

Recently, a QTL controlling salt tolerance and ABA sensitivity was identified in *Arabidopsis* using an RIL population derived from a cross between "Landsberg *erecta*" (salt and ABA sensitive) and "Shakdara" (salt and ABA resistant) (Ren et al. 2010). The QTL was designated *Response to ABA and Salt 1* (*RAS1*) (Ren et al. 2010). The *RAS1* gene of the sensitive accession "Landsberg *erecta*" encoded a protein with 230 amino acid residues, whereas *RAS1* of the resistant accession "Shakdara" encoded a protein truncated at its C-terminal with only 209 amino acid residues. Knockdown or loss of *RAS1* led to enhanced salt tolerance and ABA insensitivity, whereas introducing *RAS1* to tolerant cultivars led to salt and ABA hypersensitivity (Ren et al. 2010). Therefore, *RAS1* is the negative regulator of salt tolerance.

4.2.4 Flood and Submergence Tolerance

While drought is the major issue in global climate-change-related crop production, flooding becomes a major problem in monsoon areas. Flooding affects rice, maize, wheat, barley, oats, and sorghum (Xu and Mackill 1996; Setter and Waters 2003; Zaidi et al. 2003; Hattori et al. 2009; Promkhambut et al. 2010). Prolonged flooding, particularly flooding that submerges entire plants causes hypoxia in plants, stops photosynthetic activities, and as a result, kills the plants. Two different forms of flood tolerance have been developed by plants. To face deepwater flooding that lasts for several months and involves water from a few to several meters, elongating the internodes to keep the top leaves above the water's surface helps plants to survive. Another type of flood is the flash flood which arrives suddenly but lasts no longer than a few weeks. Seedlings are the most affected by flash floods. Susceptible young seedlings will use all available energy to grow above the water, and as a result, will die after having consumed all energy reserves when the water finally drains. Submergence-tolerant plants will not grow (stunting) to avoid excessive energy consumption. When a flood ends, the tolerant plants will start growing again using their conserved energy.

Research on flood tolerance has been done extensively in rice. Rice is a staple food in the flood-prone areas of South Asia and Southeast Asia. More than 16 million ha of rice lands of the world in lowland and deep-water rice areas are affected by flooding due to complete submergence (http://www.knowledgebank.irri.org). By positional cloning, Hattori et al. (2009) identified two major QTLs *Snorkel1* (*SK1*) and *SK2* for deepwater flood tolerance on chromosome 12. *SK1* and *SK2* encode ethylene-responsive factor type transcription factor (ERF). The ERF domain is also possessed by DREB transcription factors, which are known to

regulate response to drought and high temperature stress. Under low oxygen conditions, ethylene increases. Ethylene then induces the expression of *SK1* and *SK2*. Overexpression of *SK1* and *SK2* in nondeepwater rice resulted in internode elongation, even in nonflood conditions, suggesting that *SK1* and *SK2* regulate the internode elongation. Although the mechanism of how *SK1* and *SK2* promote internode elongation is still unclear, it is suggested that gibberellic acid (GA) may be involved in this method. The reason is under deepwater conditions, uniconazole (a GA biosynthesis inhibitor) inhibits internode elongation in deepwater-tolerant rice (Suge 1987; Hattori et al. 2009). However, no QTLs related to deepwater tolerance are found to contain GA biosynthesis genes. Further research using transgenics is needed to elucidate the relationship of *SK1* and *SK2* with GA.

Using a cross between an *indica* submergence-tolerant rice "IR40931-26" and a sensitive *japonica* rice "PI543851," one major QTL *Submergence1* (*Sub1*) controlling flash-flood tolerance was located near the centromere of chromosome 9 (Xu and Mackill 1996). "IR40931-26" was derived from the strongly submergence-tolerant cultivar "FR13A." *Sub1* contributed to approximately 70 % of the phenotypic variance in submergence tolerance (Xu and Mackill 1996). Using positional cloning, three genes encoding ethylene response factor (AP2/ERF) were located in the *Sub1* region, and were named *Sub1A*, *Sub1B*, and *Sub1C* (Fukao et al. 2006; Xu et al. 2006). *Sub1B* and *Sub1C* are present in all tolerant and susceptible cultivars studied so far, whereas *Sub1A* exists only in the tolerant cultivars (Xu et al. 2006). Two alleles of *Sub1A* exist in the *indica* rice cultivars: *Sub1A-1* in tolerant cultivars and *Sub1A-2* in sensitive cultivars. The contribution of *Sub1A-1* to submergence tolerance was confirmed by expression analysis in the sensitive cultivar "Swarna" (Xu et al. 2006). Recently, new QTLs for submergence tolerance have been found through QTL analysis using mapping populations derived from two moderately tolerant varieties, "IR72" and "Madabaru" (Septiningsih et al. 2012). Several progenies showed higher survival rates than the "FR13A"-derived tolerant variety "IR40931." Four QTLs were identified on chromosome 1, 2, 9, and 12; the QTL on chromosome 9 was *Sub1*. The *Sub1* allele was inherited from "Madabaru," while other QTLs contain tolerant alleles from IR72. These QTLs may be used to enhance submergence tolerance besides *Sub1*.

When *Sub1A* is overexpressed, *Alcohol dehydrogenase-1* (*Adh1*) is upregulated (Xu et al. 2006). *Adh1* is enhanced in young root under low oxygen conditions, in response to dehydration, low temperatures and to abscisic acid presence, proving the involvement of *Sub1A* in stress response. Since *Sub1A* is an ERF, the *Sub1A* expression is upregulated in response to increased levels of ethylene under flash-flood conditions. However, *Sub1A* does not promote internode elongation as observed in *SK-1* and *SK-2*, but instead enhances the accumulation of mRNA and protein of two suppressors of GA signaling, *SLENDER RICE1* (*SLR1*) and *SLR1-like 1* (*SLRL1*) (Fukao and Bailey-Serres 2008) that are correlated to inhibition of internode elongation under flood conditions.

Even though *Sub1A* has a different function compared to *SK-1* and *SK-2*, the similarity of their ethylene response domain (ERF domain) is high. *Sub1A* and *SK-1*

shared 63.3 % of similarity in the ERF domain, while *Sub1A* and *SK-2* showed 65 % similarity in the ERF domain. Phylogenetic analysis based on the ERF domain, showed that *SK1*, *SK2*, and *Sub1A* are grouped within the same family group (Hattori et al. 2009). It is interesting to analyze the reasons as to why genes with similar structure have opposing functions, and how the plants evolved genes to adapt to specific environments (e.g., flash floods or deepwater).

4.2.5 *Macronutrient and Micronutrient Deficiency Tolerance*

Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) are major nutrients needed for normal plant growth. In addition, boron (B), copper (Cu), iron (Fe), chlorine (Cl), manganese (Mn), molybdenum (Mo), and zinc (Zn) are also needed in small quantities (micronutrients). Among these major nutrients, many soils lack N, P, and K because plants need them in larger amounts. The next common deficiency, particularly in lowland rice fields, is zinc (Zn) (Neue and Lantin 1994; Quijano-Guerta et al. 2002).

Although NPK deficiency can be alleviated by fertilizer application, soil conditions such as drought, high concentrations of other elements, pH, and soil temperature can decrease the efficacy of fertilizer application. Drought decreases the amount of nutrients diffused in the soil, and as a consequence, lower amounts of nutrients are available for absorption by plant roots (Pinkerton and Simpson 1986; Hu and Schmidhalter 2005). For example, N application in drought-affected areas did not increase yield as much as application in well-irrigated areas (Smika et al. 1965; reviewed in Hu and Schmidhalter 2005). High concentrations of salt (Na^+) in the soil inhibit the absorption rate of K, because K^+ absorption competes with Na^+. The presence of aluminum (Al) and Fe in the soil decreases the amount of P that can be utilized by plants, because P forms a complex with Al and Fe that cannot be absorbed by plants. This phenomenon is stronger in acid soil with pH less than 6. Moreover, P deficiency often occurs in areas that are highly eroded, or contain high calcium carbonate ($CaCO_3$) because P and Ca form an insoluble complex. Low temperature during the early growing season also inhibits P availability. Since nutrient deficiency often occurs in soil that is affected by drought, salinity, and acidity, development of crop cultivars with high mineral use efficiency will save fertilizer costs and benefit the farmers.

QTL analysis for N use efficiency (NUE) has been performed in the model plant *Arabidopsis* (Loudet et al. 2003) and major crops such as rice, wheat, and maize (Hirel et al. 2001; Obara et al. 2004; Le Gouis et al. 2008). NUE can be measured as shoot dry matter, total N, nitrate, or free-amino acid contents (Hirel et al. 2001; Loudet et al. 2003; Mickelson et al. 2003; Obara et al. 2004; Le Gouis et al. 2008). As in water use efficiency, mineral use efficiency is also regulated by a large number of genes. Some genes show effects only under certain development stages,

or under mineral deficiency. Therefore, QTL analysis under deficiency conditions and QTL analysis under normal conditions may give different results. For example, an NUE study in *Arabidopsis* showed that eight QTLs related to shoot dry matter were detected in nonlimiting N conditions (N+), whereas under N-deficient conditions (N−) only four QTLs were detected (Loudet et al. 2003). Similarly, nine QTLs were related to free amino acid content in N+ conditions but only five QTLs controlled the free amino acid content in N− conditions (Loudet et al. 2003). In temperate maize, the number of QTLs related to grain yield, N content, and N use efficiency were also lower when tested in N− conditions compared to N+ conditions (Hirel et al. 2001, 2007; Gallais and Hirel 2004; Coque and Gallais 2006). Among the QTLs, a QTL for grain yield on chromosome 5 colocalized with a gene encoding cytosolic glutamine synthetase (GS; *gln4* locus) (Hirel et al. 2001). Other grain yield QTLs collocated with GS genes; for example *gln1* and *gln2* on chromosome 1, and *gln3* on chromosome 4 (Gallais and Hirel 2004). The coincidence of NUE QTLs with GS genes was also observed in rice (Obara et al. 2001, 2004; Yamaya et al. 2002) and wheat (Coque et al. 2008). One cytosolic GS gene (*GS1*) was located in a QTL region on the long arm of chromosome 2, which contributed to one-spikelet weight of rice (Obara et al. 2001). Unlike rice and maize, wheat cytosolic *GS* was associated with N content increase in the grain but did not correlate to grain yield components (Habash et al. 2007). There is a high possibility that *GS* is the candidate gene for NUE QTLs in rice, maize, and wheat, because of its central role in plant N metabolism. GS catalyzes the condensation of glutamate and ammonia to form glutamine. Glutamate and glutamine are the N donors in biosynthesis of amino acids, nucleic acids, chlorophyll, plant hormones, and secondary metabolism products. In addition, in higher plants, N is mainly transported as glutamine and asparagine. In addition to the *GS* gene, QTL mapping for grain yield and grain protein yield on a doubled-haploid population from a cross between N stress-tolerant wheat and N stress-sensitive wheat identified 233 QTLs clustered into 82 genome regions (Laperche et al. 2007). Meta-QTL analysis and factorial regression on the result showed that three QTLs were collocated with dwarfing gene (*Rft-B1*), photoperiod sensitivity gene (*Ppd-D1*), and the awns inhibitor gene (*B1*) (Laperche et al. 2007).

Long root hairs, greater root surface area, and deep roots are associated with higher phosphorus uptake efficiency (PUE) under low P conditions. Root morphology is used as a parameter to identify high PUE because P is not mobile in the soils. QTLs for P deficiency-induced root elongation have been identified in *Arabidopsis* (Reymond et al. 2006), wheat (Su et al. 2006), and rice (Shimizu et al. 2004, 2008). Shimizu et al. (2008) found that a QTL for root elongation under phosphorus deficiency (REP) detected on the long arm of chromosome 6 of rice (*qREP-6*) is associated with an increase of tiller number and shoot P content. In contrast, a major QTL responsible for P content in rice tiller was not associated with seedling root growth (Wissuwa et al. 2005). This QTL was mapped to chromosome 12 of rice, and designated *Phosphorus uptake 1* (*Pup1*; Wissuwa et al. 2002). NILs carrying the *Pup1* segment from high PUE parent Kasalath showed higher P uptake

(threefold) compared to Nipponbare (Wissuwa and Ae 2001). The finding suggests that factors other than root morphology are also involved in PUE.

One example of QTL analysis for micronutrient deficiency tolerance is discussed here: tolerance to Zn deficiency. Zn deficiency has been associated with a wide range of soil conditions: high pH soils, peatland, saline soils, and soils with high magnesium and calcium content (Neue and Lantin 1994). Zn deficiency causes leaf bronzing, and plant mortality when severe (Wissuwa et al. 2006), and retarded pollen development in maize (Sharma et al. 1987). Wissuwa et al. (2006) identified two major QTLs for tolerance to Zn deficiency on chromosome 2 and 12 of rice. The QTLs were designated *Zinc-deficiency-induced mortality*-2 (*Zmt-2*) and *Zmt-12*. However, these QTLs did not relate to leaf bronzing. Only one minor QTL was associated with both plant mortality and leaf bronzing (Wissuwa et al. 2006).

4.2.6 Cold and Frost Stress Tolerance

Temperate plants recognize low but nonfreezing temperatures in autumn and winter, develop cold acclimation during winter, and begin growth when temperature rises again as a sign of spring. The length and low temperature level needed as a signal for cold acclimation are variable among and within plant species. If winter temperatures are warmer than usual, plants may not adequately acclimate and may be damaged by frost in spring. A warmer spring may also cause earlier and sparse flowering in wheat and barley, reducing crop yield.

Genomic regions responsible for frost tolerance in wheat were identified using chromosome substitution lines of frost-sensitive "Chinese Spring" and frost-tolerant "Cheyenne" substitution lines (Sutka 1981; Galiba and Sutka 1988). The most effective region in chromosome 5A was designated *FROST RESISTANCE-A1* (*Fr-A1*) (Sutka and Snape 1989; Galiba et al. 1995). Another major QTL, *Fr-A2*, was mapped to chromosome 5, approximately 30 cM apart from *Fr-A1* (Vagujfalvi et al. 2003; Baga et al. 2007). Major frost-tolerance QTLs in barley, *FR-H1* and *FR-H2*, are orthologous to *Fr-A1* and *Fr-A2* of wheat (Francia et al. 2004, 2007). *Fr-A1* cosegregated with vernalization QTL *VRN-1* in most genetic studies (Roberts 1986; Hayes et al. 1993; Sutka and Snape 1989; Limin and Fowler 2002; 2006). Although Galiba et al. (1995) suggested that *Fr-A1* is a gene closely linked to *VRN-1*, Dhillon et al. (2010) suggested that *Fr-A1* is a pleiotropic effect of *VRN-1* because wheat with mutation in *VRN-1* showed higher frost tolerance than wheat with active *VRN-1*. Wheat NILs carrying a winter wheat type of *VRN-1* allele showed higher cold tolerance than lines carrying the *VRN-1* allele for spring growth habit (Limin and Fowler 2006).

The *Fr-A2* locus contains a cluster of *C-repeat binding factor* (*CBF*) genes (Vagujfalvi et al. 2003; Baga et al. 2007). *CBF* genes belong to the *DREB* group, because the genes encode transcription factors that bind to the conserved core sequence CCGAC (c-repeat) in the promoters of genes involved in dehydration and cold response. Eleven *CBF* genes form a cluster within the *Fr-A2* locus, and the

order of orthologs in barley is conserved (Skinner et al. 2005; Miller et al. 2006). Higher *CBF* expression was observed in lines carrying the frost-tolerant *FR-2* allele compared to those carrying the frost-sensitive allele (Xue 2003; Skinner et al. 2005; Vagujfalvi et al. 2005). Among *CBF* genes, *TaCBF14*, *TaCBF15*, and *TaCBF16* transcript levels were more than fourfold higher in the frost-tolerant lines (Vagujfalvi et al. 2005). *CBFs* regulated two cold-regulated genes: *COLD RESPONSIVE PROTEIN 14b* (*COR14b*) and *WHEAT COLD SHOCK 120* (*WCS120*) (Vagujfalvi et al. 2003; Francia et al. 2004; Knox et al. 2008; Galiba et al. 2009). These genes were mapped to the *Fr-A2* locus through expression-QTL analysis. Meanwhile, *HvCBF2* and *HvCBF4* showed the highest expression in frost-tolerant barley, but the genes were not orthologous to *TaCBF14*, *TaCBF15*, and *TaCBF16* (Stockinger et al. 2007; Galiba et al. 2009). The difference in expression level of *CBF* genes may be related to variation in frost tolerance between species (Galiba et al. 2009).

CBF genes also play a role in cold acclimation of perennial ryegrass (*Lolium perenne*) and meadow fescue (*Festuca pratensis*). Four *LpCBF* genes of perennial ryegrass were mapped to linkage group 5 in a position orthologous to the *CBF* clusters in wheat and barley (Tamura and Yamada 2007). One *CBF* gene (*FpCBF6*) was identified in meadow fescue (Alm et al. 2011). This gene was mapped to chromosome 5F and was found to be an ortholog of *Fr-H2* (Alm et al. 2011). Interestingly, although meadow fescue had two QTLs on chromosome 5F, which corresponded to *FR-A1* and *FR-A2*, its vernalization gene *FpVRN1* is located on chromosome 4F, instead of chromosome 5F as in wheat and barley (Alm et al. 2011). This fact supports the hypothesis that different genes on chromosome 5F, not *VRN1*, are involved in cold acclimation.

Cold tolerance is also related to tolerance to drought stress and low ion leakage. Dehydrin is a protein that is involved in cold and drought tolerance by protecting the cell membrane from damage. Dehydrin (*Dhn*) genes are collocated with a cold-tolerance QTL in meadow fescue (Alm et al. 2011). A QTL for electrical conductivity was detected on linkage group 4 in perennial ryegrass (Yamada et al. 2004).

4.2.7 Flowering Time Regulation

Flowering is an important transition step from the vegetative phase to the reproduction phase. Plants use photoperiod and temperature to determine flowering time. Understanding how photoperiod and temperature interact to determine flowering time is important to ensure optimal plant yield. In high latitude areas with four seasons, flowering too late in winter may kill flowers, or not give enough time for grain filling. In drought-prone areas, early flowering rice cultivars are preferred by farmers to escape drought that may occur during the grain-filling period (reviewed in Kamoshita et al. 2008). For biomass crops, late flowering or nonflowering types are chosen to reduce nutrient transfer from stems and leaves to flowers. The next

section summarizes flowering regulation in major crops, including annual crops such as rice and maize and overwintering crops such as winter wheat and barley.

4.2.7.1 Flowering Regulation in Annual Crops

QTL studies on flowering time in rice have been performed mainly using the segregating population derived from a cross between the *japonica* cultivar "Nipponbare" and the *indica* cultivar "Kasalath." "Nipponbare" is sensitive to photoperiod, whereas "Kasalath" is not (Yano et al. 2001). Fourteen QTLs related to flowering time were identified, and six of them have already been fine-mapped. These QTLs were designated *Heading date*1 (*Hd1*)—*Hd14*; based on a definition of flowering in rice because flowering means the start of heading in rice (Yano et al. 1997, 2001; Lin et al. 1998; Yamamoto et al. 1998). Among the 14 QTLs, *Hd1*, *Hd3a*, *Hd3b*, and *Hd6* were related to photoperiod sensitivity (Yano et al. 2001). *Hd1* codes a GATA1-type protein, which is an ortholog of *CONSTANS* (*CO*) in *Arabidopsis* (Putterill et al. 1995). Epistatic interaction was observed between *Hd1* and *Hd3a* (Lin et al. 2000). *Hd3a* showed a high similarity with the *Arabidopsis Flowering time T* (*FT*) gene (Kojima et al. 2002). Under short-day conditions, *Hd1* promotes flowering by upregulating the expression of *Hd3a*. This is in contrast to the *CO-FT* relationship in *Arabidopsis*, because *CO* upregulates *FT* under long-day conditions (Kobayashi et al. 1999; Onouchi et al. 2000; Samach et al. 2000). Meanwhile, the candidate gene for *Hd3b* has not yet been determined, but it is associated with late heading under long-day conditions (Monna et al. 2002). *Hd6* delays flowering under long-day periods and encodes the α-subunit of protein kinase CK2 (CK2α; Takahashi et al. 2001). *Arabidopsis* CK2 interacts with and phosphorylates circadian clock-associated 1 protein (CCA1) (Sugano et al. 1998). Among the *Hd* genes, phenotypic variation in the flowering time of rice was explained mainly by allelic variation of *Hd1* (Takahashi et al. 2009). In addition, *Hd1* and *Hd2* may be involved in flowering response to temperature (Nakagawa et al. 2005).

Besides the *Hd1* pathway, rice flowering time is also controlled by *Early heading date1* (*Ehd1*) (Doi et al. 2004). Under long-day conditions, *Ehd1* promotes flowering (Doi et al. 2004). *Ehd1* is preferentially expressed under short-day conditions even under the absence of *Hd1* (Doi et al. 2004). *Ehd1* encodes β-type response regulator and regulates the expression of *FT*-like genes and several MADS-box genes (*OsMADS14*, *OsMADS15*, and *OsMADS1*) (Doi et al. 2004). *Ehd1* encodes *OsMADS14* and *OsMADS15*, which are orthologs of *Arabidopsis AP1*, which acts downstream of *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) and *LEAFY* (*LFY*). Redundancy and antagonistic function of *Hd1* and *Ehd1* may relate to the ability of rice to adapt to a broad range of environments with different temperature and photoperiod requirements for development (Izawa 2007; Takahashi et al. 2009; Ebana et al. 2011).

Another photoperiod pathway gene in rice, *Ghd7,* colocalizes with a QTL for major effects underlying traits related to plant height, heading date, and number of

grains per panicle (Xue et al. 2008). *Ghd7* encodes a CCT-domain protein whose enhanced expression under long-day conditions suppresses *Ehd1* and *Hd3a*, delays heading date and as a consequence increases plant height and panicle size. Five allelic variations of *Ghd7* exist in rice and wild rice (*Ghd7-1*, *Ghd7-2, Ghd7-3*, *Ghd7-0*, and *Ghd7-0a*). Among them, *Ghd7-0* and *Ghd7-0a* were nonfunctional and were found in rice varieties grown in cool areas and with short growing periods (Xue et al. 2008).

Numerous flowering genes in maize have been identified. One of the major genes is *Vegetative to Generative Transition1* (*Vgt1*) (Salvi et al. 2007). *Vgt1* contains a *cis*-regulatory element of the floral repressor gene *ZmRap*2.7, which is located ~70 kb downstream of *Vgt1*. Unlike *Hd1* or *Ehd1*, *Vgt1* does not correlate with photoperiod (Chardon et al. 2004). Nevertheless, allele variation of *Vgt1* is highly correlated with geographical origin (Ducrocq et al. 2008).

4.2.7.2 Flowering Regulation in Plants Requiring Vernalization

Winter perennial plants require some period of low temperature during winter to initiate flowering. The process is called vernalization. Temperature and the length of cold exposure required for vernalization vary among different species and also among varieties within species (Kim et al. 2009). Changes in winter and spring temperature affect the flowering time of plants. For example, high temperature in spring accelerates the heading time of winter wheat (Hu et al. 2005). In addition, if winter temperature is high, winter wheat varieties that require long vernalization will not receive sufficient cold temperatures. As a result, the amount of flower/heading will reduce. The vernalization process has been extensively studied in *Arabidopsis*, winter wheat, and barley (Von Zitzewitz et al. 2005; Distelfeld et al. 2009; Kim et al. 2009). Besides vernalization, flowering of wheat and barley is also regulated by photoperiod (Turner et al. 2005; Hemming et al. 2008; Li and Dubcovsky 2008). Some of the genes involved in the vernalization pathway are also involved in the photoperiod response pathway (for a review see Kim et al. 2009).

The vernalization process in wheat and barley involves the three genes *VRN1*, *VRN2*, and *VRN3*. *VRN1*, *VRN2*, and *VRN3* of wheat were mapped using a segregating population derived from a cross between winter wheat that requires vernalization and spring wheat that does not require vernalization (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003, 2004b, 2006). *VRN1* was isolated by map-based cloning of the diploid wheat *Triticum monococcum* and mapped to the long arm of chromosome 5A (Yan et al. 2003). On the basis of gene expression pattern analysis, *VRN1* was also mapped to chromosome 5B and 5D of the hexaploid wheat *T. aestivum* (Danyluk et al. 2003; Trevaskis et al. 2003). The locations of *VRN1* on chromosome 5B and 5D correspond to the location on chromosome 5A (Danyluk et al. 2003; Trevaskis et al. 2003). *VRN2* was also mapped using the diploid wheat *T. monococcum*; it is mapped on the long arm of chromosome 5A

(Yan et al. 2004b). Meanwhile, using the hexaploid wheat *T. aestivum*, *VRN3* was mapped to the short arm of chromosome 7B (Yan et al. 2006).

VRN1 encodes a MADS-box transcription factor that resembles floral meristem identity genes *AP1* and *FRUITFULL* (*FUL*) in *Arabidopsis* (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003). *VRN2* encodes a CCT-domain protein of which the homolog in *Arabidopsis* is still unknown (Yan et al. 2004b). *VRN3* is the homolog of the *Arabidopsis FT* gene, which promotes flowering (Yan et al. 2006). In vernalization-requiring wheat cultivars, *VRN2* suppresses *VRN3* expression during summer and autumn. During winter, low temperature induces the expression of *VRN1*. Accumulated *VRN1* promotes inflorescence initiation, and also suppresses *VRN2*. As a result, *VRN3* repression is removed and flowering is induced (Trevaskis et al. 2007). The photoperiod pathway of flowering induction in wheat and barley also involves *VRN3* and *VRN1*. Under long-day conditions, a photoperiod gene *Photoperiod-1* (*Ppd1*) activates *VRN3* (Turner et al. 2005). *VRN3* interacts with bZIP transcription factor FD, and in turn, activates *VRN1* (Li and Dubcovsky 2008). However, as described above, in winter wheat, *VRN2* prevents *Ppd1* activity by repressing *VRN3*.

Mutation in *VRN3* is related to the vernalization requirement of wheat cultivars. Wheat cultivars carrying dominant *VRN3* alleles showed early flowering, and increased *FT* gene expression due to the insertion of a retroelement in the promoter region (Yan et al. 2003). Meanwhile, a dominant *VRN2* allele is responsible for winter growth habit, whereas a dominant *VRN1* allele is responsible for spring growth habit. In the diploid wheat *T. monococcum*, the *VRN2* locus contains two tandemly located zinc finger-CCT-domain genes (*ZCCT1* and *ZCCT2*) (Yan et al. 2004a, b). Both genes are able to confer the vernalization requirement. Recessive cultivars with mutations encompassing all ZCCT genes or in the conserved amino acids of the CCT domains showed spring growth habit (Distelfeld et al. 2009). Allelic variations at the *VRN2* locus of the B genome are associated with variation in heading time of tetraploid wheat, although the effect was not seen after the vernalization process (Distelfeld et al. 2009).

VRN1 genes exist on each genome of hexaploid wheat: *VRN-A1*, *VRN-B1*, and *VRN-D1*. A single dominant *VRN* allele is sufficient to confer spring growth habit, which means the cultivar does not need vernalization to induce flowering (Stelmakh 1987; Allard et al. 2012). Allelic variations were found in *VRN-A1*, *VRN-B1*, and *VRN-D1* (Yan et al. 2004a; Fu et al. 2005). Whereas the allelic variation of *VRN-B1* and *VRN-D1* is determined only by deletion within the first intron, the allelic variations of *VRN-A1* showed an indel within the promoter sequence and also deletion within the first intron of the sequence (Yan et al. 2004a; Fu et al. 2005). Yan et al. (2004a) found at least five allelic variations of *VRN-A1*, with two major types *Vrn-A1a* and *Vrn-A1b*. The *Vrn-A1a* type has duplication in the promoter region, flanked by host direct duplication (HDD). The *Vrn-A1b* type has a 20-bp deletion in the 5′-UTR region and two nucleotide substitutions in the HDD region (Yan et al. 2004a). Interestingly, the *Vrn-A1a* type was dominant in hexaploid spring wheat cultivars released in the United States and Argentina between 1970 and 2004, and also dominant in the spring wheat cultivar from CIMMYT

(Yan et al. 2004a). The results suggest that *Vrn-A1a*-type cultivars spread to Argentina and the United States might be related to the introduction of the semidwarf germplasm from CIMMYT in the 1970s. *Vrn-A1a* is also dominant in the Canadian spring wheat cultivars that show a short growing period (Iqbal et al. 2007). Although the dominant allele of *VRN1* may not be involved in the vernalization process, a different combination of dominant *VRN-A1*, *VRN-B1*, and *VRN-D1* loci affected the precocity (early maturing) of spring wheat cultivars (Stelmakh 1992, 1998). It is also shown that plants with three dominant *Vrn1* alleles need less time to anthesis compared to plants with only one dominant *Vrn1* allele (Allard et al. 2012). Moreover, the time to anthesis is shortened in plants carrying *Vrn-A1* compared to *VRN-B1* or *VRN-D1* (Stelmakh 1992, 1998; Allard et al. 2012). Perhaps allelic variation of *Vrn-A1* is also related to the level of plant precocity.

Vernalization in barley is regulated by *VRN-H1*, *VRN-H2*, and *VRN-H3* (Karsai et al. 2005; Yan et al. 2006; Cockram et al. 2007; Szücs et al. 2007; Wang et al. 2010). The barley *VRN-H1* gene is mapped on the long arm of chromosome 5H, which corresponds to *VRN-A1*, *VRN-B1*, and *VRN-D1* in hexaploid wheat (Galiba et al. 1995; Laurie et al. 1995; Dubcovsky et al. 1998; Barrett et al. 2002; Iwaki et al. 2002; Yan et al. 2003). The *VRN-H2* gene has been mapped in the distal part of chromosome arm 4HL in barley, and corresponds to *VRN2* in wheat (Laurie et al. 1995). *VRN-H3* is mapped to chromosome 7H and an ortholog of *Arabidopsis FT* (Yan et al. 2006). The alleles for spring growth habit (*Vrn-H1*, *vrn-H2*, and *Vrn-H3*) are epistatic to the alleles for winter growth habit (Takahashi and Yasuda 1971). Therefore, only plants possessing a combination of *vrn-H1*, *Vrn-H2*, and *vrn-H3* showed a winter growth habit (Takahashi and Yasuda 1971; Saisho et al. 2011). Facultative spring barley genotypes, which are cold tolerant but unresponsive to vernalization have a dominant *Vrn-H1* allele and recessive *vrn-H2* (Von Zitzewitz et al. 2005). As in wheat, mutation in the first intron of *VRN-H3* caused increased expression and response to vernalization (Yan et al. 2006).

In addition to *VRN* genes, *Ppd* genes are also involved in regulation of winter wheat and barley flowering time. In hexaploid wheat, *Ppd-1* genes exist on chromosome 2A, 2B, and 2D (*Ppd-A1, Ppd-B1*, and *Ppd-D1*, respectively). The major source of photoperiod insensitivity in wheat is the semidominant *Photoperiod-D1a* (*Ppd-D1a*) allele (Worland 1996; Beales et al. 2007; Yang et al. 2009). Mutation in *Ppd-D1a* is likely to be caused by a 2-kbp deletion of a regulatory region that led to misexpression of the pseudo-response regulator (PRR) gene family (Beales et al. 2007). Varieties carrying the *Ppd-D1a* allele show early flowering regardless of photoperiod length (Worland 1996). The allele was probably introduced from the Japanese variety "Akakomugi," which was used to breed semidwarf varieties (Guedira et al. 2010). Mutation in the regulatory region of the *PRR* gene or a closely linked gene of the *Ppd-B1* locus is also thought to confer photoperiod insensitivity (Beales et al. 2007). No mutation in *Ppd-A1* has been reported in hexaploid wheat, but mutation in *Ppd-A1* and alteration in photoperiod sensitivity is reported in tetraploid wheat (Wilhelm et al. 2009).

The ortholog of *Ppd-1* in barley is *Ppd-H1*, which is a major determinant of long-day response (Laurie et al. 1995). Missense recessive mutations in the CCT

domain of barley *Ppd-H1* cause late flowering under long-day photoperiod conditions (Turner et al. 2005). Another photoperiod QTL, *Ppd-H2* contains the barley homolog of *FT* (Faure et al. 2007; Kikuchi et al. 2009).

4.3 Application of QTL Analysis Knowledge in Breeding Stress-Tolerant Crop Cultivars

Despite the complexity of regulation of stress-tolerance traits, several new cultivars have been developed for tolerance to high salinity, submergence, and drought stress (Table 4.1). Rice cultivars with submergence tolerance have been applied in the field. Using marker-assisted backcrossing (MABC), which needs less time than conventional MAS, a tolerant allele of *Sub1A* has been introgressed to rice megavarieties, such as "Swarna," "Samba Mahsuri," "IR64," "Thadokkam 1," "CR1009," and "BR11" (Septiningsih et al. 2009). Among those varieties, "Swarna-Sub1" was released in India, Indonesia, and Bangladesh; "BR11-Sub1" was released in Bangladesh; and "IR64-Sub1" was released in the Philippines and Indonesia during 2009–2010 (Bailey-Serres et al. 2010). A field test in India demonstrated that "Swarna-Sub1" showed a higher yield than "Swarna" under submergence stress (Sarkar et al. 2009).

However, stress-tolerant cultivars still face challenges. In most cases, stress occurs in combination, i.e., drought occurs with heat stress, high salinity with nutrient deficiency, etc. Or, during a season change, drought may occur after flood. Therefore, cultivars developed for certain stress types must be tested against other stresses that may occur in the field. Fukao et al. (2011) tested near isogenic lines with a tolerant allele of *Sub1A* under drought stress which occurs frequently after a flood. They found that *Sub1A,* the QTL for submergence tolerance, also contributes to enhanced drought tolerance in rice. *Sub1A* keeps plants dormant and conserves energy during the submergence period, and promotes tillering after water has receded. In drought conditions, *Sub1A* enhanced recovery from drought at the vegetative stage through reduction of leaf water loss and lipid peroxidation. Through microarray data analysis, *Sub1A* enhanced the expression of genes related to ABA responsiveness, genes associated with acclimation to dehydration, and those that suppress the accumulation of reactive oxygen species (ROS) by increasing ROS-scavenging enzymes (Fukao et al. 2011).

Another approach is to overexpress *DREB1* genes to enhance plant tolerance. *DREB1* is known as a universal stress defense mechanism that can be found across species. It confers tolerance to drought, high salinity, and low temperature (Kasuga et al. 1999; Shinozaki and Yamaguchi-Shinozaki 2000; Ito et al. 2006; Lata and Prasad 2011). Overexpression of the *DREB1A* gene from *Arabidopsis* enhanced drought tolerance in wheat (Pellegrineschi et al. 2004). In another study, overexpression of *DREB1*/*CBF*-type transcription factors enhanced tolerance to drought, high salinity, and low temperature in rice (Ito et al. 2006). However, both

Table 4.1 Examples of breeding crops with increased stress tolerance through MAS breeding

Crops	Tolerance to	Locus/markers/ genes/	Reported in	Note
Durum wheat	High salinity	*Nax*2 (contain *TmHKT1;5-A)*	Munns et al. (2012)	25 % higher yield and reduction in leaf Na^+ in NILs with *TmHKT1;5-A* in saline soils trial
Rice	Submergence	*Sub1A-1*	Siangliw et al. (2003), Neeraja et al. (2007), Septiningsih et al. (2009), Iftekharuddaula et al. (2010)	Introgressed to megarice varieties Swarna, BR11, IR64; Thai jasmine rice KDML105
Rice	High salinity	*Salto1*	Thomson et al. (2010)	High salt-tolerant RIL (FL478) as breeding donor
Wheat	Drought	–	Condon et al. (2004)	Two varieties for high water use efficiency: Drysdale and Rees
Rice	Drought	Chromosome 9 (RM242-RM201)	Steele et al. (2006)	Increase root length of upland variety, Kalinga III

studies showed that while *DREB1*-overexpressing plants grew better than wild type under stressed conditions, they performed worse under control conditions. Constitutively expressed *DREB1* genes keep plants under a "stressed" condition, and uses energy for stress tolerance rather than relocating nutrients to grains. Pellegrineschi et al. (2004) used an rd29A promoter that works specifically under stress conditions to effectively enhance *DREB1* expression under drought, and suppress its expression under normal conditions.

Choosing the right parental pair is important in MAS breeding. Sometimes stress tolerance results from epistatic interaction between two parents; particularly in drought and heat tolerance that are regulated by complex interactions of genes. Although considerable work has been performed to elucidate the genetic basis of both traits, it is still difficult to identify a specific genomic region that is responsible for conferring drought or heat tolerance. Interaction between genes involved in stress tolerance must also be considered while combining several genes (gene pyramiding) to enhance the stress tolerance of a plant.

4.4 Future Approaches to Mapping QTLs/Genes Associated with Stress Tolerance Traits

We can see from the description above that despite enormous effort to identify QTLs related to stress-tolerance traits, candidate genes have been determined only for a few (salt tolerance, submergence tolerance, flowering time, cold and frost tolerance). To accelerate the identification of stress tolerance-related genes, researchers have developed several technologies and also strengthened international collaborations.

4.4.1 Genomewide Association Studies and Genomic Selection

As an alternative to traditional QTL mapping, GWAS has been more commonly used in mapping QTLs/genes related to stress-tolerance traits. The first advantage of GWAS over traditional QTL mapping is that it does not need an a priori hypothesis about candidate gene location. GWAS utilizes SNPs that exist in the genome. By comparing the percentage of certain nucleotide types between stress-resistant and stress-sensitive populations, the relation between a SNP and a phenotype can be determined. This method represents a shortcut compared to the usual method of QTL mapping, which sometimes stumbles on the lack of molecular markers: create a linkage map, QTL mapping, fine-mapping of the QTL region, identification of the candidate gene and lastly, determination of the nucleotide polymorphisms responsible for stress tolerance. The second advantage of GWAS is that it analyzes varieties and without creating mapping population as in QTL mapping, which may take time especially if we want to analyze perennial plants. The third advantage of GWAS is that it can detect evolutionary history or geographical accumulation of natural variation.

Currently, there are two types of GWAS depending on the population type used in the analysis—a population-based approach and a family-based approach (Mitchell-Olds 2010). Population-based GWAS uses populations of unrelated individuals to examine associations between SNPs and phenotypes. Conversely, family-based GWAS uses pedigrees derived from crosses among different founding genotypes. Population-based GWAS uses unrelated individuals, therefore it takes advantage of historical recombination events that have accumulated over thousands of generations. However, a recombinant type may accumulate in a certain population through natural or artificial selection, and create false-positives in the analysis results. Family-based GWAS has a complementary advantage/disadvantage compared to population-based GWAS. It eliminates the false-positive that occurs because of population structure, but recognizes fewer recombination events.

Other advantages and disadvantages for each approach have been reviewed in Mitchell-Olds (2010).

Recently, Atwell et al. (2010) reported GWAS of 200 *Arabidopsis* inbred lines for more than 200,000 SNPs. Genotype data of 200 *Arabidopsis* inbred lines have been stored (http://www.1001genomes.org) as part of a project to discover the whole-genome sequence variation of 1,001 strains (accessions) of *Arabidopsis*. In 2010, whole-genome sequences of 80 accessions were completed (Cao et al. 2011; Schneeberger et al. 2011). Once the genotyping data for these accessions is completed, researchers can use the data to map the trait of interest, therefore reducing the time and effort involved in genotyping. Atwell et al. (2010) have already mapped QTLs related to 107 phenotypes, including flowering, cold tolerance, seed dormancy, salt tolerance, and pathogen resistance traits.

The family-based GWAS approach has been used in maize, which has a high level of outcrossings and large effective population size. Maize geneticists collaborated to develop a maize nested association mapping (NAM) population that consists of 25 groups of inbred lines derived from 25 parents crossed to a fully sequenced B73 genotype (Buckler et al. 2009; McMullen et al. 2009). Each group consists of 200 inbred lines. The NAM population has been used to characterize flowering regulation in maize (Buckler et al. 2009).

Markers identified through GWAS can be used in genomic selection. Genomic selection uses all markers across an entire genome on a population to explain total genetic variance rather than the few markers used in MAS (Meuwissen et al. 2001). The Genomic selection method is centered on a "training population." In the training population, marker genotypic and phenotypic data of each individual are determined. Then, the effect of each marker to phenotype is calculated (= marker effect). On the basis of the marker effect, we can predict the breeding value of an individual by testing its marker genotypes (see for review, Heffner et al. 2009). Genomic selection is useful particularly in breeding programs that want to utilize small-effect QTLs or gene pyramiding.

4.4.2 Marker Transferability Between Species for QTL Mapping in Minor Crops

How about QTL mapping in minor crop species? For minor crops, often called orphan crops, (Armstead et al. 2009), the limited availability of molecular markers and known genome sequence can hinder construction of a linkage map that covers the entire genome, and as a consequence, some QTLs may not be detected. Besides constructing a genome library enriched with SSR sequences, molecular markers from species in the same family can be used in linkage map construction for a minor crop of interest (Wang et al. 2010). A set of 210 SSR markers developed from

wheat, rice, sorghum, and maize were evaluated for their transferability to minor grass species (*Eleusine coracana*, *Paspalum vaginatum*, *Cynodon dactylon*, and *Festuca arundinacea*) (Wang et al. 2005; Saha et al. 2006; Wang et al. 2010). The transfer rate of SSR markers was correlated with the phylogenetic relationship between species. In other study, SSR markers from the temperate grass model plant *Brachypodium distachyon* were tested for transferability to *Miscanthus sinensis*, a potential bioenergy crop (Zhao et al. 2011). Fifty-seven SSR markers chosen to evenly represent location across the *B. distachyon* genome were tested. Out of these *B. distachyon* SSR markers, 86.0 % are transferable to *M. sinensis* (Zhao et al. 2011).

4.4.3 Comparative Genome Analysis

Comparative analysis of linkage maps between different plant species shows that they maintain the same physical localization of genetic loci on the same chromosomal region (synteny) even though they are divergent species. QTL and fine mapping analyses showed that gene sets associated with traits are conserved between species. For example, genes involved in cold acclimation and flowering time are conserved between wheat, barley, and to some extent with perennial ryegrass and meadow fescue. Comparative genome analysis accelerates the discovery of key genes involved in stress tolerance. It is possible to use the sequence information of a gene that has been identified in one species to isolate the same gene in another species, especially in those that do not have sufficient genome information.

4.4.4 International Collaboration for Genetic Information

Currently, information about QTL analysis and mapping populations is stored in public Web sites, therefore researchers can utilize the information for their breeding programs. Availability of molecular marker sequences, linkage maps, and whole-genome sequence data accelerates identification of candidate genes. In 2008, the US Department of Energy Joint Genome Institute released whole-genome sequence information for several major crops and model plants, and has improved the data since then. Currently, whole-genome sequence information is available for 34 species, including *Arabidopsis*, rice, soybean, cotton, maize, sorghum, grape, rapeseed, apple, and poplar (http://www.phytozome.org). The Web site provides not only whole-genome sequence information but also the physical map of genes or putative genes. This information enables researchers to BLAST markers sequence flanking their QTL regions, extract gene information in genomic regions between the flanking markers, and predict the candidate gene (Ducrocq et al. 2009; Jordan

Table 4.2 Databases providing information on genetic and germplasm stock in *Arabidopsis*, and crops belong to the grass family

Database	Species	URL
GrainGenes	Triticeae/*Avena*	http://wheat.px.usda.gov
Gramene	Gramineae	http://www.gramene.org
MaizeGDB	Maize	http://www.maizegdb.org
NBRP	Plant species	http://www.nbrp.jp
Oryzabase	Rice	http://www.nig.ac.jp/labs/PlantGen/english/oryzabase-e/index.html
Panzea	Maize and teosinte	http://www.panzea.org
PlantGDB	Plant species	http://www.plantgdb.org
Phytozome	Plant species	http://www.phytozome.org
Rice Genome Annotation Project	Rice	http://rice.plantbiology.msu.edu/
TAIR	*Arabidopsis*	http://www.arabidopsis.org

et al. 2010). Other web sites also provide genomic information for general plant species or specific to plant species/family (Table 4.2).

Compilation of QTL data in one Web site helps breeders to select molecular markers suitable for their breeding program. Information for the maize NAM population and agronomical traits' QTLs can be obtained from http://www.panzea.org; while for wheat, barley, rice, wild rice, rye, oat, pearl millet, and several other grass species information can be obtained from http://www.gramene.org. The database not only provides information about traits and the related molecular markers, but also the neighboring markers, which can be used in MABC, and segregating populations used for QTL mapping, which is helpful in providing information about parental species.

Germplasms are also an important factor in the development of stress-tolerant cultivars. For example, *Triticum dicoccoides* and *Hordeum spontaneum*, the wild relatives of wheat and barley showed potential as donors for drought and salt tolerance (Nevo and Chen 2010). Wild rice, *Oryza rufipogon* can also serve as a drought-tolerance donor to cultivated rice (Zhang et al. 2006). Meanwhile, genetic variations in flowering time loci *Ppd-H1* and *Ppd-H2* that only exist in wild barley, *H. spontaneum*, can be used as a source for novel allelic variation for photoperiod response in barley (Cockram et al. 2011), which can adapt to warmer climate in high latitudes. Landraces are also potential sources to improve stress tolerance, for example Indian landraces "Pokkali" and "Nona Bokra" that showed salt tolerance. Several programs have been conducted to collect germplasms and characterize the agronomical traits of the stocks, such as NBRP (http://www.nbrp.jp), Oryzabase (a site for rice germplasm that is sponsored by NBRP), Graingenes (list of wheat, barley, and oat genetic stocks, collection owned by several institutions).

Fast progress in bioinformatics technologies and the development of next-generation sequence is accelerating genome sequencing of plant species. Besides genomic analysis, many tools have been developed to identify genes through transcriptome, proteome, and metabolome profilings (see for review, Mochida

and Shinozaki 2010). Knowledge about genetics, transcriptional regulation, metabolic pathways, and also informatics are needed to analyze and interpret the vast amounts of data generated by these analyses. The iPlant Collaborative (http://www.iplantcollaborative.org) provides a network for researchers to learn about various tools for data storage, management and analysis; and sharing knowledge in plant science research analyses.

4.5 Conclusion

QTL analysis has been useful in the identification of genes responsible for traits related to stress tolerance. In several cases, such as high salinity and submergence tolerance, candidate genes for major QTLs have been characterized and introgressed to widely cultivated varieties. With the advance in genome sequencing technologies using next-generation sequencers, QTL/gene mapping and identification are being accelerated and it is becoming possible to perform QTL analysis on minor crops, which often have limited molecular markers. In addition, breeding techniques are also improved, such as MABC and genomic selection. This knowledge will improve our approaches to the development of superior crop cultivars that are able to adapt to present and future climate changes.

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