Chapter 13
Improvement of Plant Waterlogging Tolerance

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Abstract Sources of tolerance and a reliable trait evaluation method are crucial in breeding for abiotic stress tolerance. Waterlogging is one of the most important abiotic stresses in high rainfall areas. Waterlogging tolerances have been reported in different plant species. However, the complexity of the trait makes it very difficult to evaluate, thus hard to breed for. A reliable screening method can make the breeding programme more successful. This chapter will summarize: genetic resources and genetic behaviour of waterlogging tolerance; different selection criteria; and QTLs controlling the tolerance. The importance of accurate phenotyping in screening for QTLs controlling the tolerance is also discussed.

13.1 Introduction

Waterlogging is one of the most important constraint factors for crop production. Nearly 16% of the total territory of the United States suffers from waterlogging (Boyer 1982). In South-East Asia, 15% of all maize growing areas are affected by waterlogging, causing 25–30% of yield losses every year (Rathore et al. 1998). The yield losses in soybean due to waterlogging can be 17–43% if waterlogged at the vegetative growth stage or 50–56% if waterlogged at reproductive growth stages (Oosterhuis et al. 1990; Scott et al. 1990). In barley, Bandyopadhyay and Sen (1992) reported more than 50% loss in yield after 2 days and 80% loss in yield after 3 days of super-saturation treatment after 6 weeks normal growth in a coastal saline soil. Even for the relatively tolerant wheat (Wang et al. 1996; Ikeda et al. 1955), the average yield losses of 39–44% were also found under waterlogging conditions (Musgrave and Ding 1998; Collaku and Harrison 2002). The inhibition

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of nitrogen uptake and the consequent redistribution of nitrogen within the shoot are important contributory factors in the early senescence of leaves and the retarded growth of shoots in flooded plants (Drew and Sisworo 1977). A decrease in the nitrogen concentration in shoots of seedlings can occur rapidly after the onset of flooding and precede leaf chlorosis (Drew and Sisworo 1977; Wang et al. 1996) and consequently reduces shoot and root growth, dry matter accumulation and final yield (Kozlowski 1984; Drew 1991; Huang et al. 1994a, b; Malik et al. 2001). Roots are also injured by O_2 deficiency and metabolism changes during acclimation to low concentrations of O_2 (Drew 1997).

Waterlogging tolerance is defined in physiological studies as the survival or the maintenance of growth rates under waterlogging at different stages of development relative to nonwaterlogged conditions, whereas the agronomic definition of waterlogging tolerance is the maintenance of relatively high grain yields under waterlogging relative to nonwaterlogged conditions (Setter and Waters 2003). The agronomic definition based on grain yields alludes to the possibility that a waterlogging tolerant variety may possess a mechanism of tolerance associated with escaping from anaerobic conditions through dormancy or slow growth during a stress period, and have a rapid recovery following stress (Setter and Waters 2003). Therefore, evaluation of crop varieties should consider both the physiological performance during waterlogging and their recovery ability after waterlogging. Germplasm evaluation based on grain yield may be confounded because of the possibility that tolerance and recovery mechanisms only partly contributed to the grain yield after the waterlogging stress was terminated. This is especially the case in environments where waterlogging is for a short time, and other environmental factors or stress may also affect the grain yield. Sometimes other stress may even contribute more to the final grain yield than waterlogging stress, unless the waterlogging events are during or close to the grain-filling period.

The most economic way to reduce the damage caused by waterlogging is to introduce waterlogging tolerance into current varieties. To achieve this target, both sources of tolerance and a reliable trait evaluation method are crucial. The understanding of the genetic behaviour of waterlogging tolerance is also needed to make the selection more efficient. This chapter will briefly review genetic resources and genetic studies on waterlogging tolerance, selection criteria for waterlogging tolerance and QTLs controlling waterlogging tolerance related traits.

13.2 Genetic Resources of the Tolerance

Extensive screening of barley germplasm for waterlogging or wet tolerance has occurred in China and Japan. Work by Qiu and Ke (1991) involved screening 4,572 varieties in Shanghai province, China. Waterlogging was imposed at three stages (leaf 3 stage, stem elongation and heading) for 10–15 days each. Calculation of a “damage index” was based on yield of plants in waterlogging treatments expressed as a percentage of yield under nonwaterlogged conditions. Varieties were classified into five groups according to waterlogging induced damage: 0.4% of varieties had 1% damage; 5% had 1–10% damage; 30% had 10–20% damage; 32% had 20–40% damage; the remaining 33% had >40% damage. The majority of the 16 varieties identified with the highest waterlogging tolerance also had very early or medium maturity, indicating that recovery was not the mechanism of tolerance (Qiu and Ke 1991). Recently, a germplasm screening project was conducted in several Chinese universities/institutes to search barley germplasm for waterlogging tolerance (Zhou, Final report on the project of Australia China collaboration on barley germplasm research, unpublished data). According to the loss of yield per plot after waterlogging treatment, the varieties were classified into three groups: tolerant varieties (yield loss less than 25%); medium (yield loss between 25 and 75%) and susceptible varieties (yield loss more than 75%). Landrace barley had a higher percentage of tolerant varieties (64%) than bred varieties (11%). Six-row barley showed a higher percentage (50%) of waterlogging-tolerant varieties than two-row varieties (13%), which was partly because most of the landrace varieties were six-rowed. Naked barleys had a higher proportion of tolerant (42%) than the hulled (18%). Xiao et al. (2007) also found a Chinese landrace variety, Yongjiahong Liuleng, showed the least yield loss after 12 d waterlogging at tillering stage. The tolerance of some varieties was also confirmed in Australia under controlled waterlogging conditions (Fig. 13.1) (Pang et al. 2004; Zhou et al. 2007). Takeda and

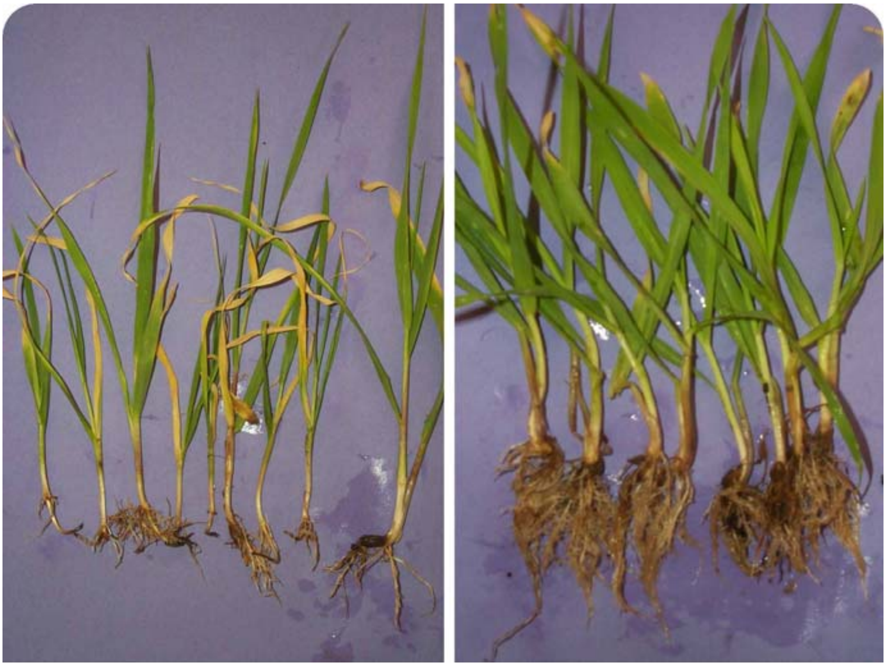

Fig. 13.1 A Chinese variety showed much better waterlogging tolerance than an Australian variety (Zhou et al. 2007)

Fukuyama (1986) tested 3,457 cultivars (preserved at the Barley Germplasm Center, Okayama University) by submerging 50 sterilized grains of each in deionized water in a test tube for 4 days at 25°C and subsequently determining their germination percentage after 4 days on moistened filter paper at 25°C. The germination percentage ranged from 0 to 100. The collections from China, Japan and Korea contained many tolerant cultivars (average indices 71.6, 66.3, and 60.5, respectively) while those from North Africa, Ethiopia and southwest Asia showed few tolerant cultivars (19.6, 13.8, and 13.2, respectively). The most tolerant cultivars retained complete germinability after 8 days of soaking at 25°C. In a glasshouse experiment, 20 different barley varieties (lines) showed significant differences in waterlogging tolerance based on grain yield and yield components with some varieties including Weisubuzi, Su5078, Tong83-11, Tong88-58 being significantly better than others (Xu et al. 2005). Setter et al. (1999) demonstrated a genetic diversity of waterlogging tolerance in barley exposed to intermittent waterlogging over 4 weeks, and waterlogging tolerance was assessed using leaf chlorosis following waterlogging. According to their results, grain yield of barley was reduced by 51–84% of nonwaterlogged plants, but the order of yield reduction did not coincide with that of leaf chlorosis. Yang et al. (1999) compared the waterlogging tolerance of eight barley dwarf-mutants. The results showed that physiological and biochemical characters such as green leaf number of main stem, fresh weight of plant and activity of superoxide dismutase (SOD) in the flag leaf were greatly changed by waterlogging stress, which also resulted in a decrease in grain yield. The results of that experiment also showed that there is a significant difference of tolerance to waterlogging among the mutants, and 95-39, 95-31 and 95-53 were better than others in waterlogging tolerance.

Genetic differences exist for tolerance to waterlogging in wheat (Davies and Hillman 1988; Thomson et al. 1992; Ding and Musgrave 1995; Huang et al. 1994a, b; McKersie and Hunt 1987; Gardner and Flood 1993). For example, Huang et al. (1994b) showed that there is good genetic diversity for tolerance of wheat to hypoxic solution cultures. In a glasshouse experiment with 14 wheat varieties and several doubled haploid wheat lines, Setter et al. (1999) showed that there was good diversity for waterlogging tolerance based on shoot growth during continuous waterlogging for 4 weeks, and after waterlogging during 3 weeks recovery period following drainage. Davies and Hillman (1988) demonstrated variation in vegetative growth and yield under continuous flooding of 4-week-old plants of various wheat species, with the hexaploid *Triticum macha* and the tetraploid *T. dicoccum* being the most tolerant. Inter-variety differences in wheat seedling survival after 7 days flooding with cold treatments have also been reported by McKersie and Hunt (1987).

Genetic variation was also reported in many other plant species, including oats (Lemons e Silva et al. 2003), cucumber (Yeboah et al. 2008a, b), Soybean (VanToai et al. 1994; VanToai and Nurjani 1996; Sayama et al. 2009; Hou et al. 1995) and maize (Anjos e Silva et al. 2006; Mano et al. 2005c). Mano et al. (2002) screened 46 maize inbred lines collected from Japan and the United States for pregermination flooding tolerance (germination rates of the seeds soaked for 8 days at 25°C) and found wide variations among the lines. The waterlogging tolerance of the other 223 inbred lines also showed wide variation for the tolerance at the seedling stage. Hou et al. (1995) tested 730 soybean varieties from different sources for seed germination after a 4 day soaking at 25°C. A large variation in seed flooding tolerance existed in the soybean germplasm as reflected in the germination rate which ranged from 0 to 100%. Most of the varieties tested were sensitive to seed flooding, and only 4% of the tested varieties had high tolerance (germination rate >90%).

13.3 Selection Criteria

Waterlogging tolerance is likely to be a complex trait affected by several mechanisms and complicated by confounding factors such as temperature, plant development stage, nutrient availability, soil type and sub-soil topography. Direct selection on grain yield has low effectiveness since the heritability of yield after waterlogging has been reported to be very low (Collaku and Harrison 2005). While the ability to produce high seed yield in flooded fields is the ultimate criterion of flooding tolerance, other traits, including leaf color, plant height, root, and shoot biomass, have been used frequently as determinants of flooding tolerance. Burgos et al. (2001) found that the lines derived from a cross between wheat spelt, which survived flooding well, germinated early, emerged fast, preserved their membrane integrity, and that the biggest seed can suffer more from flooding.

In soybean, Sayama et al. (2009) found that pigmented seed coat and small seed weight tended to give a positive effect on seed-flooding tolerance. Githiri et al. (2006) used relative seed weight and 100-grain weight as indications of early vegetative growth stage waterlogging tolerance for soybean in a pot experiment in a vinyl plastic greenhouse. They found that one of the major QTLs for the tolerance was at the similar position to a large QTL for days to the time of flowering, with that the late maturity may have conferred a longer growth period for recovery from flooding stress. A comparison of cross section area of the hilum revealed that the tolerant cultivars tended to have a larger area than the susceptible cultivars, suggesting that an inner space of the hilum can act as a reservoir at the initial stage of inundation, thereby reducing water absorption speed in tolerant cultivars (Muramatsu et al. 2008). Injury ratings (0 being no damage and 9 being 90% or more of the plants dead, Fig. 13.2) after waterlogging were used by Cornelious et al. (2005).

Different traits have been used as indirect selection indices for waterlogging tolerance in maize. Mano et al. (2005a, b, 2006) used adventitious root formation (Fig. 13.3), leaf injury and dry matter production as indications of waterlogging tolerance of maize. Adventitious root formation was also suggested to provide an alternative for some teosinte to address soil flooding or waterlogging (Bird 2000). Mano et al. (2002) studied pre-germination flooding tolerance and waterlogging tolerance at the seedling stage of maize and found no correlations between them,

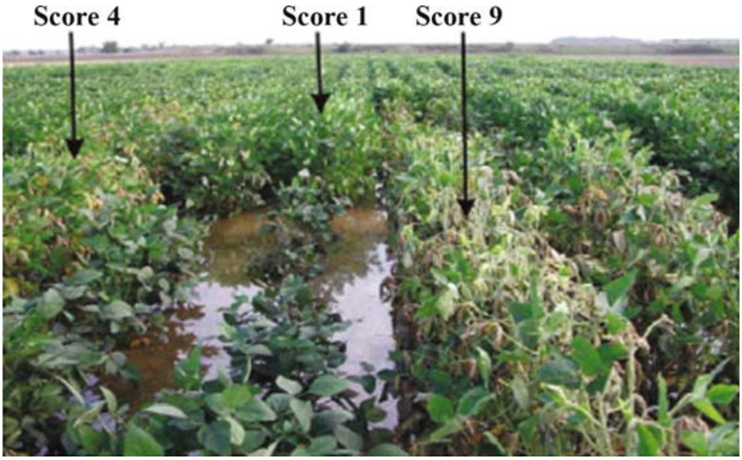

Fig. 13.2 Visual ratings for waterlogging injury of RIL mapping populations of soybean in the field. Injury ratings range from 0 to 9 with 0 being no damage and 9 being 90% or more of the plants dead (Cornelious et al. 2005)

Fig. 13.3 Two-week-old flooded maize inbred lines. *Left*: tolerant with adventitious roots; *right*: susceptible without adventitious roots. Shoots were removed from the plants (Mano et al. 2005b)

i.e. pre-germination flooding tolerance was independent of waterlogging tolerance at the seedling stage.

Among all the different criteria, leaf chlorosis after waterlogging has been one of the major indices used by researchers in different crops such as wheat (Boru et al. 2001; Cai et al. 1996; Cao et al. 1995; Ikeda et al. 1954), soybean (Reyna et al. 2003) and barley (Hamachi et al. 1990). van Ginkel et al. (1992) demonstrated that there is a high negative correlation between leaf chlorosis (or death) and grain yield in wheat. Dead leaf percentage under excess soil moisture was thought to be the best criterion for selection for flooding tolerance in early generations because its heritability values are relatively constant and it is easy to measure (Hamachi et al. 1990) and was correlated with reduction of grain yield/plant and culm length (Hamachi et al. 1989). Wang et al. (2007), using principal components analysis, identified three principal components, two for spike-grain factor and one for the number of green leaves that can be used as selecting indices for waterlogging tolerance. Other indices, such as plant survival and reduction in dry matter accumulation, were also used in barley (Li et al. 2008).

Close relationships have been reported between some physiological traits and waterlogging tolerance. Waterlogging tolerant varieties showed better ability to develop more adventitious roots and larger percentage of aerenchyma (Pang et al. 2004), to uptake K^+ in root mature zone in WL conditions, to maintain larger O_2 uptake in root mature zone in WL conditions (Pang et al. 2006) and to tolerate secondary metabolites associated with WL soil conditions (Pang et al. 2007). These physiological traits cannot be easily used in routine screening program but they are useful criteria for further waterlogging related QTL identification which leads to marker assisted selection.

13.4 Genetic Studies on Waterlogging Tolerance

Waterlogging tolerance is considered to be a quantitative trait, even though some reports have found that the tolerance has been found to be controlled by one dominant gene in common wheat (Cao et al. 1995), Makha wheat (Fang et al. 1997) and maize (Sachs 1993). Most of the early published research in genetic studies on waterlogging tolerance was done in wheat, and almost all of this work measured waterlogging tolerance using leaf chlorosis or leaf/plant death and some other traits (Cao et al. 1992, 1994, 1995; Cai et al. 1996). These researchers indicated that waterlogging tolerance is under genetic control, and is heritable, with a broad sense heritability estimated to be over 70%. They concluded that it is possible to improve waterlogging tolerance in wheat by selecting progeny in early generations based on related traits. Cao et al. (1992, 1995) found that waterlogging tolerance based on leaf chlorosis was controlled by one dominant gene, but tolerance based on traits such as green leaves/main stem, plant height, grains per ear and 1000-grain weight could be controlled by multiple genes in the varieties involved in their study (Cao et al. 1994).

Boru (1996) extended the research of van Ginkel et al. (1992)'s work at CIMMYT by continuing genetic studies involving several of the tolerant wheat varieties. In three waterlogging tolerant wheat genotypes, tolerance was conditioned by four major genes. The three tolerant wheat genotypes used in his study carried different genes, although they all possessed one tolerant gene (*Wt1*) in common. It was proposed that these different genes could control different mechanisms of tolerance to waterlogging, therefore waterlogging tolerance could be substantially improved by combining all tolerance genes into one genotype (Boru 1996). This may not be so where genes are related to the presence of different strategies of growth versus nongrowth during waterlogging. Some of the work in China (Cao et al. 1994) also indicated that additive gene action is the major determinant of the inheritance of waterlogging tolerance. Boru et al. (2001) further studied the inheritance of waterlogging tolerance in wheat by using three tolerant (Prl/Sara, Ducula and Vee/Myna) and two sensitive (Seri-82 and Kite/Glen) spring bread-wheat lines. Leaf chlorosis was used as a measure of waterlogging tolerance. The sensitive by sensitive cross, seri-82 Kite/glen, showed the highest mean values for percentage leaf chlorosis and area under chlorosis progress curve, and the lowest mean values for plant height, biomass, grain yield, and kernel weight. The expression of waterlogging tolerance was not influenced by a maternal effect. The F1 hybrids were intermediate for leaf chlorosis, indicating that tolerance was additive. Quantitative analysis also indicated that additive gene effects mainly controlled waterlogging tolerance in these crosses.

The only early work to evaluate the heritability of waterlogging tolerance based on plant grain yield was conducted by Bao (1997) using 20 wheat varieties. He found that heritability for tolerance to 15 days waterlogging in the field at the tillering stage and the booting stage was 74.7 and 80.2%, respectively. However, Collaku and Harrison (2005) found that grain yield had the lowest heritability ($h^2 = 0.25$) while relatively higher heritabilities were found for kernel weight (0.47), chlorophyll content (0.37) and tiller number (0.31). They suggested that selecting waterlogging tolerance in early generations using relatively highly inheritable traits, such as kernel weight, would be an efficient way as grain yield has a low heritability.

Both additive and nonadditive effects were important in the determination of the inheritance of flooding tolerance in maize (Anjos e Silva et al. 2006). A maize F_2 population developed from a waterlogging tolerant variety and a sensitive variety showed transgressive segregation in both directions for most traits under waterlogging conditions, indicating that both parents transmitted favourable alleles for each trait. Broad-sense heritabilities were from 0.28 for root length to 0.82 for total dry weight under waterlogging conditions. Root length was more easily affected by waterlogging stress (Qiu et al. 2007). Hou et al. (1995) found that seed flooding tolerance in soybean was controlled by both additive and dominant genes. A small number of effective factors and high narrow sense heritability in the diallel analysis indicated that selection for tolerance would be effective in early generations. Wang et al. (2008) reported three major genes with the heritability of 0.42 dominating submergence tolerance of soybean. A simple additive model explained the variations of tolerance score, adventitious root formation and waterlogged root dry weight in cucumber. Nonallelic interactions were detected for waterlogged vine length and root length. Complementary epistasis occurred in waterlogged vine length while additive × additive, additive × dominance and dominance × dominance epistastic effects were significant for waterlogged root length. Transgressive segregation was also observed in most of the traits in the F_2

generation. The estimates of narrow-sense heritabilities for tolerance score and adventitious root formation were moderately high ($h_N^2 = 0.54–0.74$) (Yeboah et al. 2008a). In another experiment, they found the broad sense heritability was from 0.43 for adventitious root formation to 0.88 for vine length (Yeboah et al., 2008b).

Hamachi et al. (1989) reported that heterosis for tolerance expressed as reduction in damage was observed in F_1s, and frequency distributions of damage in F_2s showed continuous variation. A 6 × 6 half diallel analysis was conducted in barley from crosses of three waterlogging tolerant Chinese cultivars and three susceptible Australian or Japanese cultivars (Zhou et al. 2007). The waterlogging treatment was imposed starting from the 3-leaf stage. The percentage of yellow leaf was recorded after waterlogging treatment. Three Chinese cultivars showed significantly higher general combining ability for waterlogging tolerance while the variance of specific combining ability was not significant, indicating that the tolerance was mainly controlled by additive effects. High heritability ($h_B^2 = h_N^2 = 0.73$) of waterlogging tolerance indicated that selection in early generations could be very efficient. They concluded that when selections are made in a segregating populatin, the most effective selection strategy is to discard the plants with severe leaf chlorosis (Zhou et al. 2007).

13.5 Marker-Assisted Selection

Even though the heritability was relatively high for leaf chlorosis (Zhou et al. 2007) and early generation selection could be efficient, well-controlled waterlogging conditions are still crucial for the precise evaluation of this trait. In practice, it is very difficult for breeders to control the multiple confounding environmental factors in a field experiment over thousands of barley genotypes. Development of molecular markers associated with barley waterlogging tolerance and marker-assisted selection (MAS) could effectively avoid environmental effects. QTL analysis has proven to be very useful in identifying the genetic components of the variation for important economic traits (Mazur and Tingey 1995). A molecular marker closely linked to the target gene or QTL can act as a “tag” which can be used for indirect selection of the gene(s) in a breeding program (Babu et al. 2004).

13.5.1 QTL Controlling Waterlogging Tolerance

Earlier efforts involved trying to locate the waterlogging tolerance genes onto different chromosomes. Poysa (1984) used substitution lines to study the flooding tolerance of wheat and found that all three substitution lines survived severe flooding stress (7 days) and showed significantly better tolerance than Chinese Spring, but under moderate flooding stress (5 days) only substitution line 5D was better than Chinese Spring. The author suggested that genes controlling resistance to flooding stress are present on all three chromosomes. Taeb et al. (1993) reported that the related species *Thinopyrum elongatum* and *Elytrigia repens* had better waterlogging tolerance than wheat when comparing a number of *Triticeae* species for tiller production, shoot dry matter production and root penetration in waterlogged soil. Tests of a number of wheat-alien amphiploids showed that there was at least partial expression of this exotic genetic variation in a wheat genetic background. The presence of chromosome 2E and 4E of *Th. Elongatum* was associated with a positive effect on root growth in waterlogged conditions. The positive effect of the 4E chromosome addition was mimicked by tetrasomic lines carrying extra doses of wheat homoeologous 4B and 4D, and it was concluded that the beneficial effect contributed by the presence of 4E was probably due to an increased dosage of group 4 chromosomes. However, the positive effect of adding chromosome 2E to wheat could not be reproduced by added doses of chromosomes 2A, 2B, or 2D, suggesting that this alien chromosome carries genes for tolerance not present on its wheat homoeologues. This gene was further located to the long arm of chromosome 2E by testing ditelosomic addition lines (Taeb et al. 1993).

Great effort has been put in identifying quantitative trait loci (QTL) controlling the tolerance. In most instances, waterlogging tolerance related traits were used as indications of the tolerance. In a cross between spelt and wheat (relatively susceptible to waterlogging), five QTLs explaining 41% of the phenotypic variance were found for survival to flooding, which were localized on the chromosomes 2B, 3B, 5A and 7S. Ten QTLs were found for seedling growth index after flooding and they were localized on 2A, 2B, 2D, 3A, 4B, 5A, 5B, 6A and 7S (Burgos et al. 2001).

Six QTLs were found for early vegetative growth stage waterlogging tolerance in soybean (Githiri et al. 2006) in 2 years greenhouse experiments. The major QTL was found in both years and accounted for 30–49% of the variation. Some minor QTLs were also identified but most of them were different from different experiments. Three QTLs on linkage groups A1, D1a and G were reported by Wang et al. (2008), explaining 4.4–7.6% of the total phenotypic variation. Sayama et al. (2009) identified four QTLs for germination rate and normal seedling rate of soybean under waterlogging conditions. Among them, Sft1 on the linkage group H exhibited a large effect on germination rate after a 24-h treatment; Sft2 exhibited the largest effect on seed-flooding tolerance which was involved in seed coat pigmentation. Sft1, Sft3 and Sft4 were independent of seed coat colour and seed weight. From two different populations of soybean, VanToai et al. (2001) identified a single QTL from the tolerant parent which was associated with improved plant growth and grain yield. This QTL was not associated with maturity, normal plant height and grain yield. Lines with the tolerant allele showed 95% higher yielding and 16% taller on average. The QTL was also validated in another population later (Reyna et al. 2003). Further studies were conducted using two populations with 103 and 67 recombinant inbred lines to investigate QTLs controlling waterlogging tolerance in soybean (Cornelious et al. 2005). These two populations contained a common tolerant variety. In each population, one significant QTL was found, explaining

10 and 16% of the phenotypic variation, respectively. The QTL was at different position in two populations but the alleles provide the waterlogging tolerance in both populations were from the tolerant variety.

Adventitious root formation under waterlogging conditions was used to study the waterlogging tolerance of maize and teosinte. QTLs associated with waterlogging tolerance (adventitious root formation) were found on chromosome 4 and 8 from a cross between maize and teosinte with teosinte contributing all the tolerance. In an F_2 population of maize, QTLs controlling adventitious root formation on the soil surface under flooding conditions were identified on chromosomes 3, 7 and 8. The one on chromosome 8 was also identified from a different cross (Mano et al. 2005a). Later, Mano et al. (2006) identified a QTL for flooding tolerance in maize evaluated by either leaf injury or dry weight production after flooding treatment. However, this QTL only explained 10% of phenotypic variation of dry weight production and 14% of leaf injury. A major QTL controlling trait associated with relative shoot dry weight and relative total dry weight of maize was mapped to the same region of chromosome 9 which could be consistently identified in different experiments (Qiu et al. 2007). This QTL was located near a known anaerobic response gene. They also identified many other minor QTLs on chromosomes 1, 2, 3, 4, 6, 7 and 10, explaining 3.9–14.3% of the variation. These minor QTLs were specific to particular traits or environments (Qiu et al. 2007). The major QTL on chromosome 9 for relative shoot dry weight was not found in Anjos e Silva et al. (2005)'s study. Instead, by using a single marker analysis they detected three markers for shoot dry matter under waterlogging conditions. These three markers were located on chromosomes 3, 4 and 5. The markers on chromosomes 3 and 4 were also associated to root dry matter.

In cucumber, Yeboah et al. (2008b) used a set of 112 $F_{2:3}$ lines derived from the cross between two inbred lines PW0832 (tolerant) to PW0801 (susceptible) to evaluate waterlogging tolerance traits: tolerance score, adventitious root formation (Fig. 13.4), waterlogged shoot dry weight and waterlogged vine length. A total of 14 QTLs were detected for the different waterlogging traits. The QTL for the waterlogged traits accounted for 7.9–33.2% of the phenotypic variations.

There are few reports on waterlogging tolerance related QTL research in barley. Li et al. (2008) selected two double haploid populations (crosses between tolerant and susceptible varieties) to investigate the QTLs for waterlogging tolerance. Leaf chlorosis was chosen as the main indicator for waterlogging tolerance, and

Fig. 13.4 Variations among flooded F_2 plants of cucumber in adventitious root formation (ARF). Numbers 0, 1, 2 and 3 are the score ratings (Yeboah et al. 2008a, b)

plant biomass reduction and plant survival were also recorded. Twenty QTLs for waterlogging tolerance related traits were found in the two barley double haploid (DH) populations. Several of these QTLs were validated through replication of experiments across seasons or by co-location across populations. Some of these QTLs affected multiple waterlogging tolerance related traits. A consensus map (Wenzl et al. 2006) was used to compare QTLs from two different populations and summarized seven QTLs for waterlogging tolerance. These seven QTLs were located on all the different chromosomes except 6H. Among them, the QTL on 4H (Q_{wt}4-1) contributed not only to reducing barley leaf chlorosis, but also increasing plant biomass under waterlogging stress, whereas other QTLs controlled both leaf chlorosis and plant survival.

13.5.2 Accurate Phenotyping is Crucial in Identifying QTLs for Waterlogging Tolerance

Breeding for stress tolerance such as waterlogging tolerance controlled by multiple genes is difficult because of low heritability, variability among stress treatments, and the difficulty of screening a large number of lines in the field or under controlled conditions. These factors make it difficult for breeders to manipulate quantitative traits. Marker assisted selection could be very effective. Molecular markers give unambiguous, single sit genetic differences that can easily be scored and mapped in most segregating populations (Kearsey 1998). However, QTL analysis depends on the fact that the linkage between markers and QTL is such that the marker locus and the QTL will not segregate independently, and differences in the marker genotypes will be associated with different trait phenotypes (Kearsey 1998). The success of MAS depends on the development of reliable markers (accurate QTL location). Accurate phenotyping is imperative to the success of the QTL “genetic dissection” approach.

Genotyping and phenotyping errors are the two major reasons that reduce the accuracy of QTL results. As the development of new techniques, for example DArT technology (Wenzl et al. 2004), and the construction of consensus maps (Varshney et al. 2007; Wenzl et al. 2006), genotyping in barley have become more and more accurate. This leaves accurate phenotyping the major barrier to accurately locate QTLs controlling quantitative traits which are easily affected by environment. To increase phenotyping accuracy, we need to use highly reliable screening systems which are known to differentiate resistant from susceptible lines; to conduct analysis on the means of repeated screens rather than single trials and to ensure that repeatability of the screen is as high as possible. This section will use barley as an example to discuss the importance of accurate phenotyping in QTL analysis.

A same DH population from the cross between Yerong (waterlogging tolerant) and Franklin (waterlogging sensitive) was screened for waterlogging tolerance in glasshouse pot experiments (Li et al. 2008) and big tank experiments (Fig. 13.5) outside, during the normal barley growing season. In pot experiments, leaf

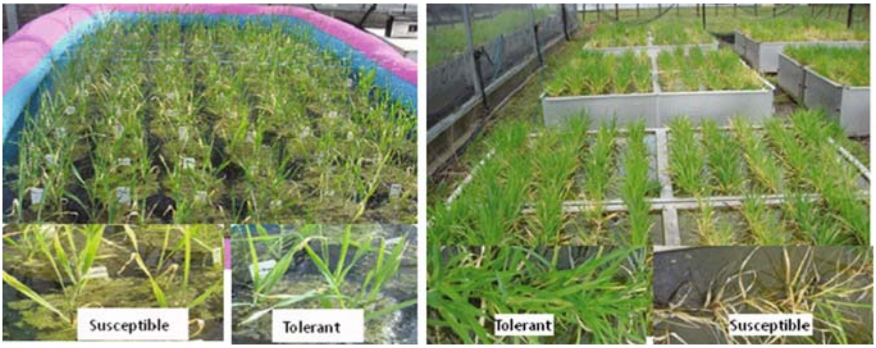

Fig. 13.5 Pot experiments (*left*) and tank experiments used to screen barley DH population for waterlogging tolerance. Much greater differences between tolerant varieties and susceptible varieties were shown in tank experiments

chlorosis, biomass reduction and plant survival were scored after waterlogging treatments. In tank experiments, one combined score system (plant healthiness which is a combined score of leaf chlorosis, plant survival after waterlogging, $0 = $ no affected and $10 = $ all died, Fig. 13.5) was used.

Pot experiments revealed six QTLs controlling waterlogging tolerance related traits. These QTLs explained 5–22% of the phenotypic variation with the biggest contribution from the QTL on 4H (Li et al. 2008). In contrast, only four significant QTLs were identified in the tank experiments. These three QTLs explained a total of more than 45% of the phenotypic variation (Fig. 13.6). In both pot and tank experiments, the QTL on 4H explained the greatest phenotypic variation. Figure 13.7 shows the effectiveness of using closely linked markers to select for waterlogging tolerance. As can be seen from the Figure, when selecting only one single major marker on 4H, the average score of the lines with this marker was 3.5 (more tolerant) which was significantly lower than the average score of 5.4 (more susceptible) from the lines without this marker. When all three markers can be selected, the score of all the lines showed tolerance or medium tolerance to waterlogging with the average score of 2.9. In contrast, when none of the three markers was selected, most of the lines were very susceptible with the average score of 7.4.

As mentioned above, the evaluation of waterlogging tolerance can be affected by many environmental factors, which include soil properties, temperature, water level, time of waterlogging treatment and barley development stage when waterlogging treatment starts. The difference between a pot experiment and a tank experiment is that the tank environment is closer to actual field conditions and the environment can be better controlled while variation among pots was unavoidable. Relatively longer times of waterlogging treatment resulted in greater differences between tolerant (quite healthy), medium tolerant (survived but not as healthy as the tolerant ones) and susceptible (dead) ones (Fig. 13.5). It is not surprising that QTLs identified from the tank experiment would be more reliable than those from pot experiment.

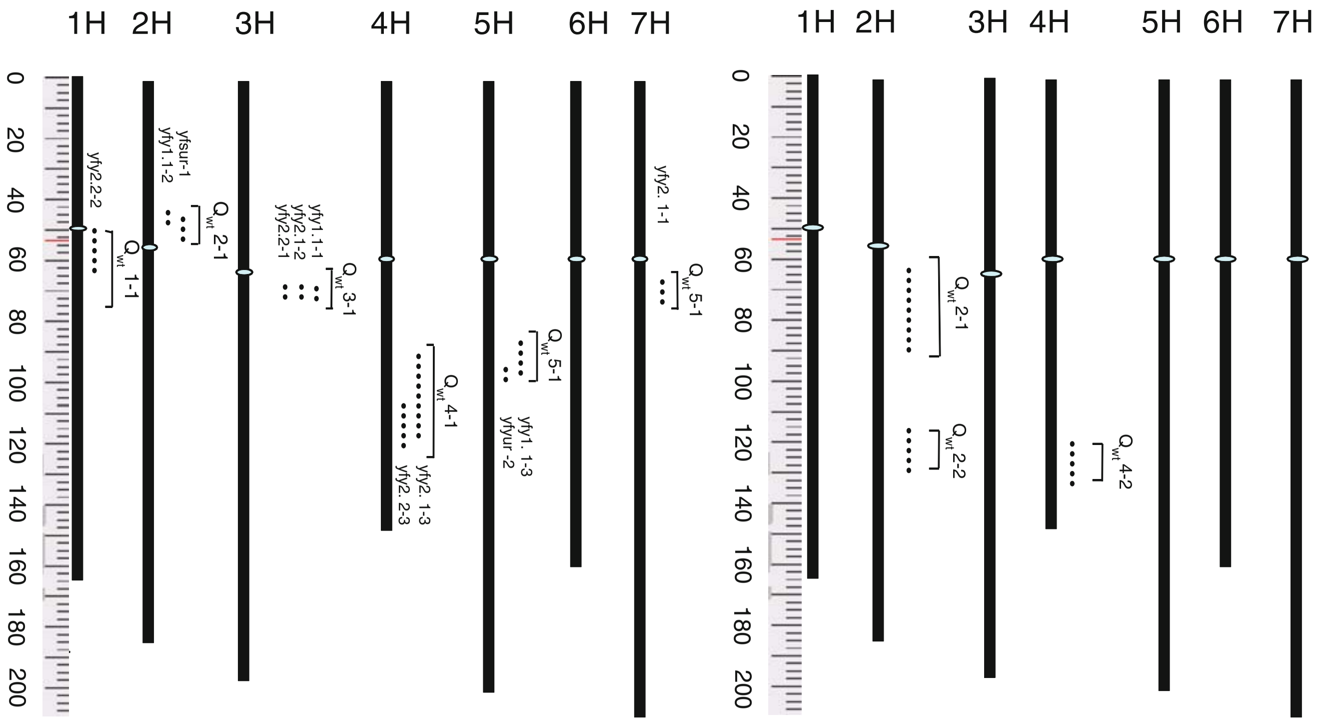

Fig. 13.6 The Franklin/Yerong chromosomes showing the locations of QTLs for waterlogging related trait(s) analyzed in pot experiments (Li et al. 2008) (*left*) and big tank experiments (*right*). A general name (such as Q_{wt}1-1) was given to each chromosome region associated with waterlogging tolerance, the first number was the chromosome number and the second number was the serial number of regions identified on that chromosome. yfy = leaf chlorosis; yfsur = survival rate

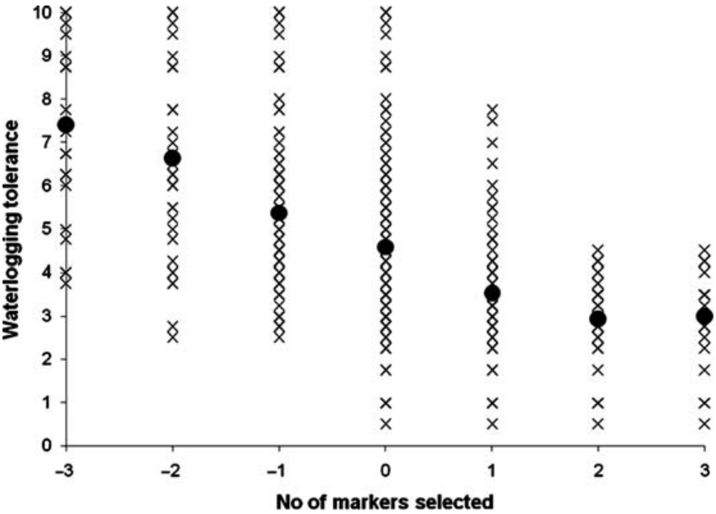

Fig. 13.7 The effectiveness of selecting molecular marker(s) to improve waterlogging tolerance as evaluated in the tank experiments. Waterlogging damage: 0 = very tolerant; 10 = very susceptible. No of markers selected: 3 and −3: three most important markers were selected and unselected, respectively; 2 and −2: three most important markers were selected and unselected, respectively; 1 and −1: only one most important marker was selected and unselected, respectively; 0: no marker was selected. *times*: original data; *filled circle*: average value

In conclusion, waterlogging tolerance exists in different plant species and is genetically inherited which is most likely controlled by several QTLs. Since the heritability of waterlogging tolerance was low and the evaluation of the tolerance can be very hard and easily affected by environmental conditions, the use of MAS could be very effective in selecting tolerance related traits. However, the effectiveness of MAS relies on the accurate location of the QTLs and closely linked markers. Among all the factors that affect the accuracy of QTL locations, phenotyping is the most important one, which needs very reliable screening facilities and selection indices.

References

Anjos e Silva S D dos, Sereno MJC de M, Lemons e Silva CF, Oliveira AC de, Barbosa Neto JF (2005) Genetic parameters and QTL for tolerance to flooded soils in maize. Crop Breed Appl Biotechnol 5:287–293

Anjos e Silva SD dos, Sereno MJC de M, Lemons e Silva CF, Barbosa Neto JF (2006) Combining ability of maize genotypes for flooding tolerance. Ciencia Rural 36:391–396

Babu R, Nair SK, Prasanna BM, Gupta HS (2004) Integrating marker-assisted selection in crop breeding – prospects and challenges. Curr Sci 87:607–619

Bandyopadhyay BK, Sen HS (1992) Effect of excess soil water conditions for a short period on growth and nutrition of crops on coastal saline soil. J Indian Soc Soil Sci 40:823–827

Bao XM (1997) Study on identification stage and index of waterlogging tolerance in various wheat genotypes (*Triticum aestivum* L.). Acta Agric Shanghai 13:32–38

Bird RMcK (2000) A remarkable new teosinte from Nicaragua: growth and treatment of progeny. Maize Gen Coop Newsl 74:58–59

Boru G (1996) Expression and inheritance of tolerance to waterlogging stresses in wheat (*Triticum aestivum* L.). PhD thesis, Oregon State University

Boru G, van Ginkel M, Kronstad WE, Boersma L (2001) Expression and inheritance of tolerance to waterlogging stress in wheat. Euphytica 117:91–98

Boyer JS (1982) Plant productivity and environment. Science 218:443–448

Burgos MS, Messmer MM, Stamp P, Schmid JE (2001) Flooding tolerance of spelt (*Triticum spelta* L.) compared to wheat (*Triticum aestivum* L.) – a physiological and genetic approach. Euphytica 122:287–295

Cai SB, Cao Y, Fang XW (1996) Studies on the variability and combining ability of waterlogging tolerance in common wheat. Jiangsu J Agric Sci 12:1–5

Cao Y, Cai SB, Wu ZS, Zhu W, Fang XW, Xiong EH (1995) Studies on genetic features of waterlogging tolerance in wheat. Jiangsu J Agric Sci 11:11–15

Cao Y, Cai SB, Zhu W, Fang XW (1992) Genetic evaluation of waterlogging resistance in the wheat variety Nonglin 46. Crop Genet Res 4:31–32

Cao Y, Cai SB, Zhu W, Xiong EH, Fang XW (1994) Combining ability analysis of waterlogging tolerance and main agronomic traits in common wheat. Scientia Agric Sinica 27:50–55

Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. Crop Sci 42:444–450

Collaku A, Harrison SA (2005) Heritability of waterlogging tolerance in wheat. Crop Sci 45:722–727

Cornelious B, Chen P, Chen Y, de Leon N, Shannon JG, Wang D (2005) Identification of QTLs underlying water-logging tolerance in soybean. Mol Breed 16:103–112

Davies MS, Hillman GC (1988) Effects of soil flooding on growth and grain yield of populations of tetraploid and hexaploid species of wheat. Ann Bot 62:597–604

Ding N, Musgrave ME (1995) Relationship between mineral coating on roots and yield performance of wheat under waterlogging stress. J Exp Bot 46:939–945

Drew MC (1991) Oxygen deficiency in the root environment and plant mineral nutrition. In: Jackson MB et al (eds) Plant life under oxygen deprivation. Academic, The Hague, pp 301–316

Drew MC (1997) Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annu Rev Plant Physiol Plant Mol Biol 48:223–250

Drew MC, Sisworo EJ (1977) Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. New Phytol 79:567–571

Fang XW, Cao Y, Cai SB, Xiong EH, Zhu W (1997) Genetic evaluation of waterlogging tolerance in *Triticum macha*. Jiangsu J Agric Sci 13:73–75

Gardner WK, Flood RG (1993) Less waterlogging damage with long season wheats. Cereal Res Comm 21:337–343

Githiri SM, Watanabe S, Harada K, Takahashi R (2006) QTL analysis of flooding tolerance in soybean at an early vegetative growth stage. Plant Breed 125:613–618

Hamachi Y, Furusho M, Yoshida T (1989) Heritability of wet endurance in malting barley. Jpn J Breed 39:195–202

Hamachi Y, Yoshino M, Furusho M, Yoshida T (1990) Index of screening for wet endurance in malting barley. Jpn J Breed 40:361–366

Hou FF, Thseng FS, Wu ST, Takeda K (1995) Varietal differences and diallel analysis of pregermination flooding tolerance in soybean seed. Bull Res Inst Bioresour (Okayama University) 3:35–41

Huang BR, Johnson JW, Nesmith S, Bridges DC (1994a) Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply. J Exp Bot 45:193–202

Huang BR, Johnson JW, Nesmith S, Bridges DC (1994b) Root and shoot growth of wheat genotypes in response to hypoxia and subsequent resumption of aeration. Crop Sci 34:1538–1544

Ikeda T, Higashi S, Kawaide T (1955) Studies on the wet-injury resistance of wheat and barley varieties. (II) Varietal difference of wet-injury resistance of wheat and barley. Bull Division Plant Breed Cultivation, Tokai-Kinki, National Agricultural Experiment Station 2:11–16

Ikeda T, Higashi S, Kawaide T, Saigo S (1954) Studies on the wet-injury resistance of wheat and barley varieties. (I) Studies on the method of testing wet-injury resistance of wheat and barley varieties. Bull Division Plant Breed Cultivation, Tokai-Kinki, National Agricultural Experiment Station 1:21–26

Kearsey MJ (1998) The principles of QTL analysis (a minimal mathematics approach). J Exp Bot 49:1619–1623

Kozlowski TT (1984) Extent, causes, and impact of flooding. In: Kozlowski TT (ed) Flooding and plant growth. Academic, London, pp 9–45

Lemons e Silva CF, Mattos LAT de, Oliveira AC de, Carvalho FIF de, Freitas FA de, Anjos e Silva SD dos (2003) Flooding tolerance in oats. Crop Breed Appl Biotechnol 5:29–42

Li HB, Vaillancourt R, Mendham NJ, Zhou MX (2008) Comparative mapping of quantitative trait loci associated with waterlogging tolerance in barley (*Hordeum vulgare* L.). BMC Genomics 9:401

Malik AI, Colmer TD, Lambers H, Schortemeyer M (2001) Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. Aust J Plant Physiol 28:1121–1131

Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005a) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays ssp. huehuetenangensis*) seedlings. Euphytica 142:33–42

Mano Y, Muraki M, Fujimori M, Takamizo T (2005b) Varietal difference and genetic analysis of adventitious root formation at the soil surface during flooding in maize and teosinte seedlings. Jpn J Crop Sci 74:41–46

Mano Y, Muraki M, Komatsu T, Fujimori M, Akiyama F, Takamizo T (2002) Varietal difference in pre-germination flooding tolerance and waterlogging tolerance at the seedling stage in maize inbred lines. Jpn J Crop Sci 71:361–367

Mano Y, Muraki M, Takamizo T (2006) Identification of QTL controlling flooding tolerance in reducing soil conditions in maize (*Zea mays* L.) seedlings. Plant Prod Sci 9:176–181

Mano Y, Omori F, Muraki M, Takamizo T (2005c) QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. Breed Sci 55:343–347

Mazur BJ, Tingey SV (1995) Genetic mapping and introgression of genes of agronomic importance. Curr Opin Biotechnol 6:175–182

McKersie BD, Hunt LA (1987) Genotypic differences in tolerance of ice encasement, low temperature flooding, and freezing in winter wheat. Crop Sci 27:860–863

Muramatsu N, Kokubun M, Horigane A (2008) Relation of seed structures to soybean cultivar difference in pre-germination flooding tolerance. Plant Prod Sci 11:434–439

Musgrave ME, Ding N (1998) Evaluating wheat cultivars for waterlogging tolerance. Crop Sci 38:90–97

Oosterhuis DM, Scott HD, Hampton RE, Wullschleger SD (1990) Physiological responses of two soybean [*Glycine max* (L.) *Merr*] cultivars to short-term flooding. Environ Exp Bot 30:85–92

Pang JY, Cuin T, Shabala L, Zhou MX, Mendham NJ, Shabala S (2007) Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology in barley roots. Plant Physiol 145:266–276

Pang JY, Mendham N, Zhou MX, Newman I, Shabala S (2006) Microelectrode ion and O_2 flux measurements reveal differential sensitivity of barley root tissues to hypoxia. Plant Cell Environ 29:1107–1121

Pang JY, Zhou MX, Mendham NJ, Shabala S (2004) Growth and physiological responses of six barley genotypes to waterlogging and subsequent recovery. Aust J Agric Res 55:895–906

Poysa VW (1984) The genetic control of low temperature, ice-encasement, and flooding tolerances by chromosomes 5A, 5B, and 5D in wheat. Cereal Res Comm 12:135–141

Qiu FZ, Zheng YL, Zhang ZL, Xu SZ (2007) Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize. Ann Bot 99:1067–1081

Qiu JD, Ke YA (1991) Study of determination of wet tolerance of 4572 barley germplasm resources. Acta Agric Shanghai 7:27–32

Rathore TR, Warsi MZK, Singh NN, Vasal SK (1998) Production of Maize under excess soil moisture (Waterlogging) conditions. 2nd Asian regional maize workshop PACARD, Laos Banos, Phillipines, 23–27 February 1998, p 23

Reyna N, Cornelious B, Shannon JG, Sneller CH (2003) Evaluation of a QTL for waterlogging tolerance in Southern soybean germplasm. Crop Sci 43:2077–2082

Sachs MM (1993) Molecular genetic basis of metabolic adaptation to anoxia in maize and its possible utility for improving tolerance of crops to soil waterlogging. In: Jackson MB, Black CR (eds) Interacting stresses on plants in a changing climate. Springer-Verlag GmbH, Berlin, pp 375–393

Sayama T, Nakazaki T, Ishikawa G, Yagasaki K, Yamada N, Hirota N, Hirata K, Yoshikawa T, Saito H, Teraishi M, Okumoto Y, Tsukiyama T, Tanisaka T (2009) QTL analysis of seed-flooding tolerance in soybean (*Glycine max* [L.] *Merr*.). Plant Sci 176:514–521

Scott HD, DeAngulo J, Wood LS, Pitts DJ (1990) Influence of temporary flooding at three growth stages on soybeans grown on a clayey soil. J Plant Nutr 13:1045–1071

Setter TL, Burgess P, Waters I, Kuo J (1999) Genetic Diversity of barley and wheat for waterlogging tolerance in Western Australia. In 9th Australian Barley technical symposium, Melbourne, Australia. pp 2.17.1–2.17.7

Setter TL, Waters I (2003) Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. Plant soil 253:1–34

Taeb M, Koebner RMD, Forster BP (1993) Genetic variation for waterlogging tolerance in the *Triticeae* and the chromosomal location of genes conferring waterlogging tolerance in *Thinopyrum elongatum*. Genome 36:825–830

Takeda K, Fukuyama T (1986) Variation and geographical distribution of varieties for flooding tolerance in barley seeds. Barley Genet Newsl 16:28–29

Thomson CJ, Colmer TD, Watkin ELJ, Greenway H (1992) Tolerance of wheat (*Triticum aestivum* cvs. Gamenya and Kite) and triticale (*Triticosecale* cv. Muir) to waterlogging. New Phytol 120:335–344

van Ginkel M, Rajaram S, Thijssen M (1992) Waterlogging in wheat: germplasm evaluation and methodology development. Seventh regional wheat workshop for eastern, central and southern Africa, Nakuru, Kenya, 16–19 September 1991

VanToai TT, Beuerlein JE, Schmitthenner AF, Martin SK St (1994) Genetic variability for flooding tolerance in soybeans. Crop Sci 34:1112–1115

VanToai TT, Martin SK St, Chase K, Boru G, Schnipke V, Schmitthenner AF, Lark KG (2001) Identification of a QTL associated with tolerance of soybean to soil waterlogging. Crop Sci 41:1247–1252

VanToai TT, Nurjani N (1996) Screening for flooding tolerance of soybean. Soyb Genet Newsl 23:210–213

Varshney RK, Marcel TC, Ramsay L, Russell J, Röder MS, Stein N, Waugh R, Langridge P, Nike RE, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. Theor Appl Genet 114:1091–103

Wang F, Zhao TJ, Yu DY, Chen SY, Gai JY (2008) Inheritance and QTL analysis of submergence tolerance at seedling stage in soybean [*Glycine max* (L.) *Merr*.]. Acta Agron Sinica 34: 748–753

Wang SG, He LR, Li ZW, Zeng JG, Chai YR, Hou L (1996) A comparative study on the resistance of barley and wheat to waterlogging. Acta Agron Sinica 22:228–232

Wang J, Zhou MX, Xu RG, Lu C, Huang ZL (2007) Studies on selecting indices and evaluation methods for waterlogging tolerance in barley (*Hordeum vulgare* L.). Scientia Agric Sinica 40:2145–2152

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc Nat Acad Sci 101:9915–9920

Wenzl P, Li HB, Carling J, Zhou MX, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang JP, Raman R, Smith PK, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206–227

Xiao YP, Wei K, Chen JX, Zhou MX, Zhang GP (2007) Genotypic difference in growth inhibition and yield loss in barley under waterlogging stress. J Zhejiang University (Agriculture and Life Science). 33:525–532

Xu RG, Lu C, Huang ZL, Huang ZR, Xu JH, Gong ZS (2005) Identification on Waterlogging Tolerance of Barley. Barley Sci 2:11–15

Yang J, Shen Q, Wang N, Li X, Yang W (1999) Wet endurance of Barley dwarf mutants. Acta Agriculturae Nucleatae Sinica 13:147–151

Yeboah MA, Chen XH, Liang GH, Gu MH, Xu CW (2008a) Inheritance of waterlogging tolerance in cucumber (*Cucumis sativus* L.). Euphytica 1620:145–154

Yeboah MT, Chen XH, Chen RF, Alfandi M, Liang GH, Gu MH (2008b) Mapping Quantitative Trait Loci for Waterlogging Tolerance in Cucumber Using SRAP and ISSR Markers. Biotechnology 7:157–167

Zhou MX, Li HB, Mendham NJ (2007) Combining ability of waterlogging tolerance in barley (*Hordeum vulgare* L.). Crop Sci 47:278–284