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## Review Article: Plant Breeding for Water Logging Resistance

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### Abstract

Sources of tolerance and reliable trait evaluation methods 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. Plant breeding techniques, conventional or genetic engineering, might be an effective and economic way of developing crops to grow successfully in waterlogged condition. Marker assisted selection (MAS) is a new and more effective approach which can identify genomic regions of crops under stress. The discovery of comprehensive molecular linkage maps enables us to do the pyramiding of desirable traits to improve in waterlogging tolerance through MAS. However, because of genetic and environmental interaction, too many genes encoding a trait, and using undesirable populations, the mapping of QTL was hampered to ensure proper growth and yield under waterlogged conditions. Steady advances in the field of genomics and proteomics over the years will be helpful to increase the breeding programs which will help to accomplish a significant progress in the field crop variety development and also improvement in near future. Waterlogging response of soybean and major cereal crops, such as rice, wheat, barley, and maize and discovery of QTL related with tolerance of waterlogging, development of resistant variety, and in addition, future prospects have also been discussed.

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### Introduction

Water logging occurs over vast regions throughout the world adversely affecting approximately 10% of the global land area (FAO, 2002). It usually occurs when rainfall or irrigation water deposits on the soil surface or sub soil for prolonged period of time. It can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within one or two days (Hossain and Uddin, 2011). In the world of increasing food demand scientists are trying to produce more crop in the barren farmland and in

unfavorable condition. Usually in those areas water, soil condition, and temperature are not favorable for agricultural production. Waterlogging is one of the most hazardous natural occurrences, which can also be called as flood, submergence, soil saturation, anoxia, and hypoxia, which are generally used to describe waterlogging conditions depending upon the moisture or water level on the field. Generally, two types of flooding are present in the field: (1) waterlogging, in which root and some portion of the shoot goes under water, and (2) complete submergence, where the whole plant goes under water. Landraces cultivated by local farmers

include varieties adapted to waterlogging or complete submergence which could be used as a source of genetic material for improving the tolerance of rice to some environmental stresses like waterlogging. Almost a total of over 22 million hectares of lowland rice cultivable area worldwide are vulnerable to flash flooding, representing a total of almost 18% of the global supply (Mohanty and Khush, 1985). To avoid such damage, farmers traditionally cultivate flood-adapted lowlands and landraces which can withstand complete submergence or flooding for 10 days or more and can resume its growth after desubmergence (Catling, 1992). Usually these landraces have total yield not more than 2 t/ha, in contrast to semi dwarf varieties which have yield of 6–8 t/ha. Regrettably, the “mega varieties” which are grown widely in different parts of Asia are prone to flooding and dies within one week. Plant breeders identified this problem in the 1970s, while trying to increase production in the low-lying areas by introducing submergence tolerance traits to high yielding varieties which are popular to local farmer (Mackill *et al.*, 1996).

In case of barley, waterlogging causes chlorophyll, protein, and degradation of RNA, and also reduces the concentration of nutrients like nitrogen, phosphorus, metal ions, and minerals in shoot. After the onset of flood leaf chlorosis starts (Buchanan-Wollaston *et al.*, 2003), root and shoot growth was also affected which results in reduction of accumulation of dry matter and also finally in yield (Malik *et al.*, 2002). Estimated 20–25% yield loss can happen depending upon plant damage; it may be exceed 50% due to waterlogging (Setter *et al.*, 1999).

Maize is also susceptible to waterlogging which causes loss of yield in tropical and subtropical region. Fifteen percent of all maize growing areas of South-East Asia face waterlogging problem, which may lead to yield loss of a range about 25–30% annually (Rathore *et al.*, 2000).

Damages of soybean because of waterlogging are chlorosis, necrosis, stunting, defoliation, reduced nitrogen fixation, and plant death which causes yield loss (Reyna *et al.*, 2003) and all of these symptoms occur at various vegetative and reproductive stages of the plant (Linkemer *et al.*, 1998). Only 2 days of flooding can cause 18% of yield loss at late vegetative stage while it may exceed to 26% if flooding occurs at early reproductive stage of soybean (Scott *et al.*, 1989). Flooding regularly affect soybean growth and grain yield around the world including USA (Stanley *et al.*, 1990).

Waterlogging is a major problem for wheat cultivation where around 12% of cultivated soil affected by excess water (Boyer, 1982). About 39–40% yield loss is recorded under water logging condition (Collaku and Harrison 2002). Researchers suggested that it is a combining effect of reduced kernel and tiller numbers which is responsible for reducing yield of wheat in waterlogging (Collaku and Harrison, 2002).

### **Mechanism of Waterlogging Tolerance in Plants**

Lack of oxygen supply for the plant is the main reason of damage in waterlogging condition, which hampers nutrient and water uptake, as a reason the plant shows wilting. In oxygen-deprived condition plants shift its metabolism from aerobic to anaerobic mode. Plants which can withstand waterlogging condition have mechanisms such as increased availability of soluble sugar, aerenchyma formation, greater activity of glycolytic pathway and fermentation enzymes, and involvement of antioxidant defense mechanism to cope with the oxidative stress induced by waterlogging. Ethylene plays an important role in change of mechanisms of plants in deficiency of oxygen. It was also reported that ethylene induces the genes of enzymes associated with aerenchyma formation, glycolysis and fermentation pathway (Hossain and Uddin, 2011).

### **Role of Ethylene in Aerenchyma Formation**

Formation of aerenchyma is initiated by ethylene, which is one of many adaptive features of plants at submergence to avoid anaerobiosis by increasing the availability of oxygen. The soft tissues with large intercellular spaces to render low-resistance internal pathways for the exchange of gases between aerobic shoot to the anaerobic root are called Aerenchyma (Jackson and Armstrong, 1999). This feature has been reported in many species like, *Trifolium subterraneum* (Aschi-Smiti *et al.*, 2003), wheat (Watkin *et al.*, 1998), rice (Justin and Armstrong, 1991), barley (Arikado and Adachi, 1955), maize (Gunawardena *et al.*, 2001), soybean (Bacanamwo and Purcell, 1999), *Vigna luteola*, and *Carex* spp (Sairam *et al.*, 2008). Oxygen leaks out into the roots and surrounding soil through the aerenchyma. As a result, a small oxygenated soil environment formed which might create aerobic environment to microorganisms and prevent the development of potentially toxic soil components such as oxides of Iron, Cu, and Mn (Armstrong and Armstrong, 1988). Synthesis of ethylene was highly increased in

roots under hypoxia condition, simultaneously with the formation of aerenchyma (Visser *et al.*, 1997).

Waterlogging promotes biosynthesis and accumulation of ethylene, followed by induction of RBOH expression. RBOH activity leads to production and accumulation of O<sub>2</sub><sup>-</sup> at the apoplast. The O<sub>2</sub><sup>-</sup> is spontaneously or enzymatically converted to H<sub>2</sub>O<sub>2</sub>, which can easily diffuse into the cytosol through the plasma membrane. Under waterlogged conditions, in the cytosol of stelar cells and cells in the outer cell layers, H<sub>2</sub>O<sub>2</sub> and other ROS are scavenged by constitutively-expressed MT. By contrast, in the cortical cells, the decreased MT expression prevents ROS scavenging, thereby leading to greater ROS accumulation, which activates the subsequent processes of programmed cell death and lysis of the cortical cells (i.e., *Lysigenous aerenchyma* formation). Under aerobic conditions, the RBOH gene is expressed at low level and the MT gene is constitutively expressed in the cortical cells.

### **Role of Ethylene Accumulation in Adventitious Roots Formation**

Portion of the plant stem in flooded condition produces adventitious roots and grows horizontally (diageotropism). This may be the new roots which replace old root systems (Jackson and Drew, 1984). Because of the position of the new roots is close to water surface and they are connected to the stem, close to the formatted aerenchyma, they have more access to oxygen than the old root system. Large air space between these roots enables diffusion of gas between roots and shoots. Adventitious root primordia of deep water rice initiates as a normal plant development but the formation is initiated by the death of nodal epidermal cell covering the tip of primordia which occurred as a result of flood induced ethylene development (Mergemann and Sauter, 2000). This helps adventitious root development and prevents injury to the growing root.

### **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 (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.*, 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 non growth 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 water logging 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 crossseri-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 non additive effects were important in the determination of the inheritance of flooding tolerance in maize (Anjos e Silva *et al.*, 2006). A maize F2 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 favorable 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  $\times$  additive, additive  $\times$  dominance and dominance  $\times$  dominance epistatic effects were significant for waterlogged root length. Transgressive segregation was also observed in most of the traits in the F2 generation. The estimates of narrow-sense heritabilities for tolerance score and adventitious root formation were moderately high ( $hN^2 = 0.54-0.74$ ) (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 F1s, and frequency distributions of damage in F2s showed continuous variation. A 6 $\times$ 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 ( $hB^2 = hN^2 = 0.73$ ) of waterlogging tolerance indicated that selection in early generations could be very efficient. They concluded that when selections are made in a segregating population, the most effective selection strategy is to discard the plants with severe leaf chlorosis (Zhou *et al.*, 2007).

## **Methods of Breeding for Waterlogging Tolerance**

### **Conventional Breeding for Tolerance**

A good number of researches have been done to find out the morphological, physiological, anatomical, molecular, biochemical response of plant to anoxia and hypoxia for at least two decades (Vartapetian and Jackson, 1997). Lack of oxygen has been suggested as main difficulty for plant (Schmitthenner, 1985), during the time and after the flooding plant may be affected by root rot disease which cause reduction of growth and loss of yield (Schmitthenner, 1985), also includes nitrogen deficiency of plant (Fausey *et al.*, 1985), or nutrient imbalance (Barrick and Noble, 1993).

Crops in different low-laying areas were subjected to flooding for various periods. Few landraces and traditional varieties of rice have the mechanism to withstand flooding which was first recognized in 1950s and screened in 1970s. Indian accessions FR13A and FR43B and some Sri-Lankan varieties like, Kurkaruppan, Goda Heenati, and Thavalu have been known for their tolerance against complete submergence while FR13A was found to be 100% tolerant for 7 days of complete submergence of 10 days old seedlings (HilleRisLambers and Vergara, 1982). Because of some poor agronomic characteristics like, photoperiod sensitivity, low yields and poor quality of grain, and tallness, FR13A needs to be crossed to produce submergence tolerant variety as a donor for submergence. Effort of producing such a variety started in 1980s (Mangrauthia *et al.*, 2014) and in mid 1990s a



submergence tolerant IR49830- 7-1-2-2 of FR13A-derived breeding line was produced and the submergence tolerant gene was introduced to high yielding short-to-intermediate statured lines (Mishra *et al.*, 1996). It was found that submergence tolerance was controlled by a single or few major genes with some minor genes with smaller effect (Mohanty and Khush, 1985).

However, because of their lodging-proneness and susceptibility to insect, pests, and disease these traditional submergence tolerant varieties are not suitable for large scale production. For this reason IRRI tried to develop submergence tolerant variety with desirable characteristics. "IR 49830-7-2-2" is such a line which is high yielding and resistant to pest and disease and has been used as donor parent; India developed "Sudhir" which is derived from "FR13A" × "Biraj" crosses (Dana *et al.*, 2013). Despite the above achievements, till date no desirable submergence tolerant variety with high yield has been developed.

Other major attempts include development of two population of DHL by IRRI using double haploid lines (DHL) by using cross between submergence tolerant and intolerant cultivars (Sarkarung *et al.*, 1995). Intermediate height high-yielding rice varieties like "Jagannath" (OUAT) and "Pankaj" (IRRI) in India in 1969 and "Mahsuri" in Malaysia in 1971 was a landmark in lowland rice breeding. In early 80's two varieties "Savitri" and IR42 developed by Central Rice Research Institute (CRRI) and IRRI, respectively, proposed as high yielding and resistant to insect, pests, and diseases but not as tolerant to submergence as FR13A. Nowadays, scientists are using these varieties as a base material for the development of submergence tolerant variety.

Nowadays accumulated CO<sub>2</sub> in the root zone of soybean plant has been considered as the main reason of injuries of plant. Breeding for flooding tolerant soybean cultivar aims at developing a variety which can give maximum grain yield from a flooded field; where as other attributes such as plant height, root and shoot biomass, leaf color, and so forth are also considered as major determinants (Daugherty and Musgrave, 1994). Application of that knowledge to produce flooding tolerant soybean variety is not yet successful which may be very useful for growers. Genetic viability for soybean flood tolerance has been discovered (VanToai *et al.*, 1994), marker assisted selection (MAS) has been successfully used to breed crops with genetically improved QTLs (Stuber, 1994).

Various species of wheat were studied under continuous flooding conditions to determine difference of growth and yield. Waterlogging tolerant cultivars of wheat were identified (Collaku and Harrison, 2002) which unlocks the potential of waterlogging tolerant variety research of wheat. Presence of *ADH* gene in wheat which is also found in barley and rice are associated with waterlogging tolerance to ensure the presence of tolerance mechanism in wheat. All possible crosses between waterlogging tolerant cultivars of spring wheat were made and suggested that waterlogging is controlled by a few number of genes (Boru *et al.*, 2001). Another experiment was conducted to find out the narrow-sense of heritability for grain yield and yield components of 80 families of soft red winter wheat population of F<sub>2</sub> in waterlogging condition to provide selection criteria for further breeding. The plant was kept on five week of waterlogging and REML (Restricted Maximum Likelihood) was used to find out the genetic variance components. Kernel weight was found to be the highest heritability (0.47) along with chlorophyll content (0.37), and tiller number (0.31) and the lowest heritability were grain yield ( $h^2 = 0.25$ ) attributes. Kernel weight and grain yield was found to be highly correlated. Selection for a relatively highly heritable trait, as kernel weight, would be an effective way to improve waterlogging tolerance in early generations, as grain. Total yield increase of 17% could be done by selecting on the basis of the index: grain yield-kernel weight-tiller number (Collaku and Harrison, 2005).

### **Marker Assisted Breeding for Submergence Tolerance**

Since in mid 1990s major genes responsible for waterlogging tolerance have been identified (Table 1) and it become easy for the researcher to concentrate on modification or use of those genes to develop new waterlogging tolerant crop varieties like rice, wheat, maize, soybean, barley, and so forth. In the case of rice introgression of *Sub1* gene to specific varieties by marker assisted backcrossing (MAB) for various land types and choice of the farmer and addition of new varieties through genetic engineering became possible.

### **Identification of QTL Associated With Waterlogging Tolerance**

Genetic control of submergence tolerance was unknown until mid-1990s. It was suggested as a quantitative trait (Mohanty *et al.*, 1982). *Sub1* the major QTL responsible for submergence tolerance was found out by molecular

mapping on chromosome 9, which contributes almost 70% of phenotypic variation in tolerance (Xu and Mackill, 1996). Furthermore, it was also suggested that the major chromosome 9 along with other minor QTL is not responsible for more than 30% of the submergence tolerance of rice (Toojinda *et al.*, 2003). *Sub1* was mapped to a 0.16-cM region with circa 3,000 F2 progeny on 9th Chromosome (Xu *et al.*, 2000) and using 4,022 F2 individuals a fine-scale physical mapping of *Sub1* also done, which further specified the position of locus 0.075 cM (Xu *et al.*, 2006) using two different types of varieties, the resistant indica Teqing and tolerant FR13A derivative IR40931-26.

Detail analysis of ERFs related to *Sub1* locus indicates the duplication of *Sub1B* leads to the development of *Sub1A* which may happen after the *indica* rice domestication (Fukao *et al.*, 2009). Based on variations of nucleotide in the protein coding region the *Sub1A* is divided in two allelic forms, for example, *Sub1A-1* and *Sub1A-2* in submergence tolerant and intolerant accessions of *indica* and *aus* lines (Xu *et al.*, 2006). FR13A consists of *Sub1A-1* which is only found in tolerant varieties, on the other hand, *Sub1A-2* allele could be found in nontolerant *indica* accessions. Both of the alleles encode identical proteins. The difference is, intolerant allele contains Pro186 and, in the tolerant allele it is Ser186. In case of expression, *Sub1A-1* promotes rapid, prolonged, and pronounced transcript accumulation in leaves of 2- to 4-weeks-old plants in case of submerged condition; on the other hand *Sub1A-2* promotes a lower level of transcript induction (Xu *et al.*, 2006). Detailed analysis of different rice accessions with different *Sub1* haplotype showed that submergence tolerance is associated with variable levels of *Sub1A* transcript in internodes and nodes at the heading stage of the plant (Singh *et al.*, 2009).

For the identification of QTL associated with waterlogging tolerance of barley different parameters was set like leaf chlorosis, plant survival rate, and biomass reduction. Then compare the QTLs identified in two seasons in two different populations using a composite map prepared by the analysis of different molecular markers like SSRs, RFLP, and DArT (Diversity Array Technology). In two barley double haploids (DH) population twenty QTLs associated with waterlogging tolerance was identified and they were validated by different replication of experiments or by different location trials. It was suggested that most of the QTLs were associated with leaf chlorosis and plant survival while some of them

affected multiple waterlogging related traits. Such as QTL, *Qwt4-1* contributed to overcome different waterlogging related stress like leaf chlorosis and reduction of plant biomass (Li *et al.*, 2008).

In order to identify QTL associated with waterlogging tolerance in maize, two maize inbred lines were crossed. That is, “HZ32” (highly waterlogging-tolerant) and “K12” (highly waterlogging sensitive) (Tang *et al.*, 2005) were crossed for developing F2 mapping population. In the maize growing season of years 2004 and 2005, two experiments were conducted, namely, EXP.1 and EXP.2, respectively, with the same F2:3 families along with parents and F1 hybrid with a treatments of controlled (without flooding) and waterlogging. One hundred seventeen SSR markers with average space of 11.5 cM were used for the preparation of F2 population genotyping which was a base-map of 1710.5 cM length. By the use of composite interval mapping (CIM), QTL associated with different plant characters like, root dry weight, root length, plant dry weight, plant height, shoot dry weight, and waterlogging tolerance coefficient were identified in both the experiments, EXP.1(2004) and EXP.2 (2005) in control and waterlogging condition, respectively. In EXP.1, 25 and in EXP.2, 34 QTL were identified where the effects of discovered QTLs were moderate with a range of 3.9–37.3%. Some major QTLs were identified in two chromosomes 4 and 9 and in both experiments associated with root dry weight, shoot dry weight, total dry weight, plant height, and their waterlogging tolerance coefficient. Chromosome 1, 2, 3, 6, 7, and 10 was also identified (Qiu, 2007) as secondary QTLs associated with tolerance.

To determine the QTL associated with waterlogging tolerance of soybean, two hundred eight lines of two recombinant inbred (RI) populations, “Archer” × “Minsoy” and “Archer” × “Noir I”, were put into two different experimental setup, that is, one with control (without waterlogging) and other with waterlogged condition, and the plants of waterlogged setup were put into two weeks of flooding at early flowering stage. A single QTL was identified which was responsible for better plant growth and grain yields, 11–18% and 47–180%, respectively, in waterlogging environment linked to marker sat\_064 from the parent Archer (VanToai *et al.*, 2001). In another experiment two populations, that is, A5403 × Archer (Population 1) and P9641 × Archer (Population 2) were used to identify QTLs associated in waterlogging tolerance where 103 and 67 F6:11 RI lines, respectively, were used for mapping population. The

performance of both the populations was tested on the flooded field setup and significant variations were observed, however, no transgressive tolerant segregants were found in both populations. Whole sample was divided into sensitive and tolerant bulk for the purpose of further molecular study.

In molecular genetics studies, two methods, named, SMA (single marker analysis) and CIM (composite interval mapping), were used for the identification of the QTL associated with waterlogging tolerance. Out of 912 SSR markers, 17 markers (population 1) and 15 markers (in population 2) were found associated with waterlogging tolerance in SMA. Most of the markers were closely related with *Rps* gene or QTL responsible for the resistance to *Phytophthora* (*Phytophthora sojae* Kaufmann and Gerdemann). In CIM five markers, that is, Satt59, Satt160, Satt269, Satt252, and Satt485 were found associated with waterlogging tolerance (Cornelius *et al.*, 2005) in both populations. This analysis also suggested that most of the genes come from the parent Archer.

### Manipulation of QTL for Developing Waterlogging Tolerant Variety

Identification of the SUB1 QTL enables the scientist to introgress this gene by MAB into the popular high-yielding varieties of rice (MacKill, 2006). Mapping of the Sub1 allele reveals SNP (single-nucleotide polymorphisms) within *Sub1A* and *Sub1C*, which is useful for the breeders for molecular markers and in precision breeding (Septiningsih *et al.*, 2009). By the use of MAB, *Sub1A* has been already inserted to modern varieties of different countries; BR11, Swarna, Samba Mahsuri IR64, CR1009 and Thadokkam 1 (TDK1) are some of the examples of those varieties (Septiningsih *et al.*, 2009). SSR markers, which were polymorphic between two parents, were generally used for the background conformation or recurrent parent genome conformation also well combined with the Sub1 region originated from FR13A on chromosome 9. Normally newly developed Sub1 lines shows more significant waterlogging tolerance compared to parents (Septiningsih *et al.*, 2009) which is found out by evaluation. These studies show the opportunities of the insertion of the Sub1 region from developed tolerant

variety through MAB to produce tolerant varieties with a diverse genetic background. Furthermore, the effect of *Sub1* on other agronomical characteristics of plant such as, grain quality, growth, maturation, and grain production was determined in IR64 Sub1, Swarna Sub1, and Samba Mahsuri Sub1. Further research of the abovementioned submergence tolerant varieties and their original parents revealed that insertion of *Sub1* gene does not alter field performances including quality and yield of grain under normal growth condition (Singh *et al.*, 2009).

Complete waterlogging of susceptible varieties at the different growing stage considerably reduces the yield attributes like, number of panicles, total number of grains per panicle, and grain filling percentage of the plant and also flowering and maturity may be delayed, resulting in a remarkable decline in yield. On the other hand, a Sub1 rice variety minimizes the total yield reduction by flood and produces more yield than the intolerant varieties at submerged condition (Singh *et al.*, 2009). One of the main advantages of using this approach is that the newly developed Sub1 varieties retain almost all agronomical characteristics of the recurrent parent, especially in terms of yield and quality. Sub1 varieties produced from FR13A-derived varieties have almost the same yield, agronomical, and grain quality characteristics as recurrent parent varieties when grown under regular condition, but, when subjected to flooding for 1 to 2 weeks, Sub1 varieties showed a remarkable advantages in terms of yield than the susceptible ones (Singh *et al.*, 2009). Mega varieties which has *Sub1* gene, can be adopted by the farmer easily, in addition, these new varieties can replace the traditional landraces with low yielding, currently which has been used by the farmers in flood prone areas. Some tolerant lines were evaluated and adopted in low lying areas in more than ten countries in South and Southeast Asia, in field trials as the preferences of the farmers (Manzanilla *et al.*, 2011).

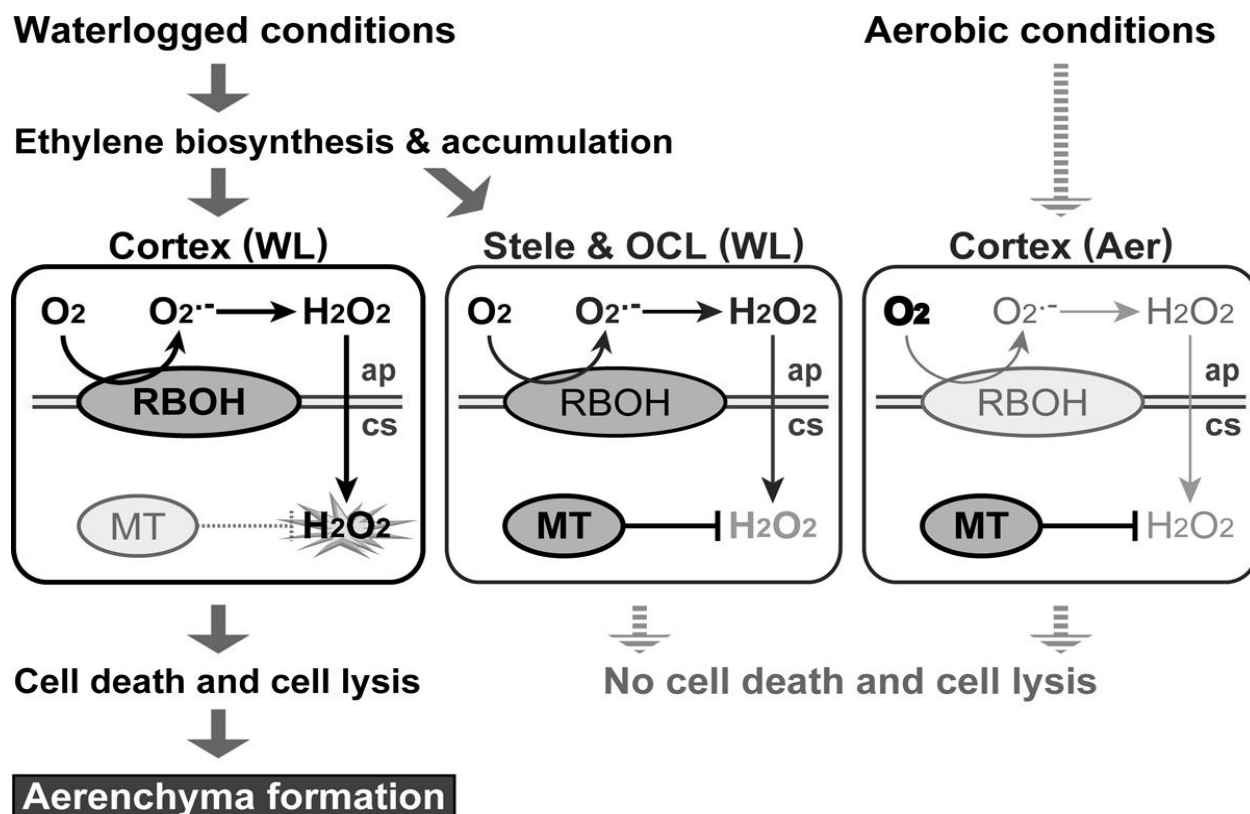
The good yield performance of newly developed varieties, showing the better performance of *Sub1* against flooding or submergence of rice. Those performances of the newly developed varieties have influenced a lot of rice improvement programs in Asia and Southeast Asia to perform rapid seed multiplication and dissemination schemes.

**Table.1** QTL identification of water logging tolerance in different crops

Species	QTL/chromosome No./genes	Marker used	Method	References
Rice ( <i>Oryza sativa</i> L.)	Sub1 (Ch. 9)	SSR	MAS	(Xu <i>et al.</i> , 2006)
Barley ( <i>Hordeum vulgare</i> L.)	Qwt4-1	SSR	MAS	(Li <i>et al.</i> , 2008)
Barley ( <i>Hordeum vulgare</i> L.)	<i>tfy2.1-1</i> , <i>tfy1.1-2</i> , <i>tfy1.2-1</i>	RFLP	MAS	(Li <i>et al.</i> , 2008)
Barley ( <i>Hordeum vulgare</i> L.)	<i>tfy1.1-3</i> , <i>tfsur-2</i> <i>tfsur-1</i> , <i>tfy1.1-1</i> , <i>tfmas</i> , <i>tfy2.1-2</i>	DArT	MAS	(Li <i>et al.</i> , 2008)
Maize ( <i>Zea mays</i> L.)	Ch. 4, 9	SSR	CIM	(Qiu <i>et al.</i> , 2007)
Maize ( <i>Zea mays</i> L.)	Ch. 1–3, 6, 7, and Ch. 10	SSR	CIM	(Qiu <i>et al.</i> , 2007)
Soybean [ <i>Glycine max</i> (L.) Merr.]	<i>Rps</i>	SSR	SMA	( VanToai <i>et al.</i> , 2001)
Soybean [ <i>Glycine max</i> (L.) Merr.]	<i>Rps</i>	SSR	CIM	(Cornelius <i>et al.</i> , 2005)

SMA: Single marker analysis, \*CIM: Composite interval mapping, \*MAS: Marker assisted selection.

**Fig.1** Model of *Lysigenous aerenchyma* formation.





## Plant Genetic Engineering Techniques for Water Logging Tolerance in Rice

Transformation of gene of rice was firstly done by protoplast based method through using micro projectile gun, and with the advance of technology with time Agro bacterium mediated gene transfer of rice is also available. The regulatory sequences of controlling expression of transgenes in rice are available now, so as a result, the transgene can be either normally expressed or expressed in response to a specific stimulus or artificially (including induced anaerobic stress, through the use of promoters from genes which strongly respond to waterlogging). Transgene rice with specific attributes like, tolerance to virus, insects. Pests, salt, low temperature, and flood can be produced (Mukhopadhyay *et al.*, 1997). Identification/isolation/cloning of associated genes with waterlogging stress considered as limiting factor (Mukhopadhyay *et al.*, 1997), as a result scientists focused on carbohydrate metabolism because reduced O<sub>2</sub> supply hampers normal respiration resulting in decrease in ATP synthesis. It has been reported that, respiration pathway switch to fermentation pathway from oxidation during oxygen-deprived (anaerobiosis) condition. The inclusion of ethanolic fermentation pathway is considered to be an important component of responses which are elicited in rice and other plants against flooding stress (Minhas, and Grover, 1999). Ethanolic fermentation, that is, pyruvate to ethanol is a relatively simpler process involving two enzymes, that is, PDC or pyruvate decarboxylase and ADH or alcohol dehydrogenase. In the study, tillers of transformed plants show higher PDC activities and ethanol production than untransformed plants and consequently ethanol production of tillers of transgenic plants was positively correlated with survival after submergence. Scientist of CISRO also made considering progress to change in the ADH level in rice (Dennis *et al.*, 2000). However, no attempts directed at making transgenes by using those have been taken.

### Future Prospects

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. Steady advances in the field of genomics and proteomics over the years will be helpful to increase the breeding programs which will help to accomplish a significant progress in the field crop variety development and also improvement in near future.

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