



Annual Research & Review in Biology

18(1): 1-10, 2017; Article no.ARRB.35616
ISSN: 2347-565X, NLM ID: 101632869

Molecular Breeding of Submergence-tolerant Rice

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Authors' contributions

This work was carried out in collaboration between all authors. Author KVA wrote the first draft of the manuscript. Authors AVU and PIK reviewed the first manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/35616

Editor(s):

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Complete Peer review History: <http://www.sciencedomain.org/review-history/21289>

Mini-review Article

Received 21st July 2017
Accepted 4th October 2017
Published 7th October 2017

ABSTRACT

Rice is a unique crop which is generally grown in submerged soil. However, not all rice varieties according to its genotype can tolerate to complete flooding. The selection using DNA markers is now frequently used in real breeding programs, including for the development of submergence-tolerant rice. This review summarizes the researches devoted the problem of breeding of submergence tolerant rice. Data on mechanisms, adaptation strategies, loci associated with tolerance to this stress are given. We had placed great emphasis on investigations aimed at application of molecular-genetic markers in the breeding of complete flooding tolerant varieties.

Keywords: Submergence tolerance; breeding; DNA-markers; rice.

1. INTRODUCTION

Flooding disturbs the basic physiological processes of plants such as water absorption, respiration, photosynthetic activity. Even a short

period of plant waterlogging significantly reduces crop productivity [1,2,3,4]. Rice (*Oryza sativa* L.) is generally grown in flooded anaerobic soil. However, not all rice varieties according to its genotype can tolerate to complete flooding

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[1,4,5]. In the world, development of the submergence tolerant versions of rice cultivars linked to the fact that large growing areas are prone floods. Flooding is one of the main abiotic stresses in South and South-East Asia. More than 16 million hectares of rice crops in this region are exposed to annual flooding and due to ongoing climate change, this figure will grow [6, 7]. Indeed, in recent times, the areas affected by floods due to sea level rise and extreme weather events have continued to expand. The most vulnerable in this respect are the coastal areas in South and South-East Asia [8]. For example, in Myanmar in 2008, more than 1.7 million hectares of cropland were affected by the devastating cyclone [9]. A year earlier, in Bangladesh, the cyclone destroyed more than 640,000 ha of rice crops. In India more than 5 million hectares of rice field are flooded during of planting season [10]. On the other hand, the major component of social and economic sustainability in these regions is the rice production. So, in Vietnam the coastal areas provide more than 75% of the total rice production in Vietnam, in Myanmar provides about 70%, and in Bangladesh produces about 35% of the national rice production [11]. In total, the annual economic loss due to flooded rice fields is more than US \$ 600 million [9].

Flooding tolerance during seed germination allows the transition from transplanting to direct sowing of seeds [12,13]. Direct-seedling is becoming a popular way, because of its cheapness and convenience. However, not all rice varieties can germinate under submergence conditions, preventing the extensive introduction of direct sowing seeds methods [13]. Russia is the most northerly rice growing area. Here, a complete flooding is also applied for weed control because they cannot overcome deep layer of water [14]. Despite the high toxicity, the use of herbicides is an indispensable part of modern technology for growing the vast majority of crops. However, practice shows that the chemical plant protection in some cases is either inefficient or unprofitable [14]. Proceeding from the aforesaid obtaining rice cultivars which would combine high productivity and tolerance to submergence is an extremely important objective.

2. MECHANISMS OF SUBMERGENCE TOLERANCE IN RICE

Unlike other crops, rice has several mechanisms of submergence tolerance, which were formed in the process of evolution. One of them is the organization of longitudinal interconnected air

cavities or aerenchyma, which allows for transport of oxygen from well-aerated shoots to submerged roots [15,16]. Oxygen diffusing through the aerenchyma to the apical meristem of the root can be used both in the process of respiration and disperse radially into the rhizosphere [17]. To prevent radial diffusion of oxygen into the rhizosphere, under flooding conditions a dense barrier is formed by the suberization and lignification of the peripheral cell layers in root outside to the aerenchyma. It is also believed that this barrier prevents the penetration into the root of toxic substances formed in the soil as a result of anaerobiosis [17,18]. Another mechanism is the formation of a gas films on the surface of submerged leaves [19]. It is shown that the removal of the gas film reduces the partial pressure of oxygen in the roots, and also decreases the efficiency of photosynthesis [19,20]. Despite the above adaptive mechanisms to the low oxygen conditions, many rice varieties are sensitive to complete submergence. Under complete flooding their leaves and stems are moderately elongated, reaching the surface of water, but this elongation depletes the energy reserves, which leads to a decrease in yields and even plant death [21]. Nevertheless, some rice varieties have a high tolerance to this stress factor. As a ruler, such tolerance to complete submergence is realized through two different growth control strategies. One of them is the so-called quiescence strategy, in which the elongation of the roots and shoots of plants is restricted, and thus the necessary energy is preserved to resume growth after lowering the water level [22]. Such rice varieties are resistant to flash-flood for 10 to14 days [23]. In this case, a negative correlation between the elongation of plant vegetative organs and survival under complete flooding conditions can be observed.

Another strategy is the rapid elongation of leaves and internodes under flooding condition [24]. So some varieties can increase the length of the shoot by more than 25 cm per day [25]. This rapid elongation allows the leaf tips to reach the water surface in a short time, which makes it possible for efficient photosynthesis and gas exchange [24]. Both strategies are caused by ethylene response factors [5,26].

Also to mechanisms that impart a high tolerance to flooding, one can include the accumulation of non-structural carbohydrate, a higher level of antioxidant protection, maintaining a high chlorophyll content in the leaves, the ability to

maintain an active fermentative metabolism and etc [27]. It has been shown that tolerant rice plants accumulate 30-40% more non-structural carbohydrates compared to the submergence-susceptible varieties [28]. A greater activity of ADP glucose pyrophosphorylase along with lower activity of fructose 1,6-diphosphatase provides a significant accumulation of non-structural carbohydrates in submergence tolerant rice varieties [29]. These non-structural carbohydrates are utilized during submergence to maintenance the required energy rate [27]. A study of the activity of superoxide dismutase, ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione reductase demonstrates the best antioxidant protection against oxidative damage in submergence tolerant rice plants [30]. In this case, the protection and activity of the photosynthetic system are associated with the high activity of the antioxidant system [31]. For more details on the mechanisms of plant tolerance to flooding are available in the following reviews [21,23,24,32,33,34,35,36].

3. QTLs ASSOCIATED WITH SUBMERGENCE TOLERANCE

As described above, some rice varieties can limit own growth during the complete flooding and resume it after lowering the water level. One of the most famous donors of this submergence tolerance is the traditional Indian rice FR13A. This cultivar was actively used by breeders around the world, since the 70s of last century. However, the genetic basis of FR13A tolerance to water stress has long remained unexplored. In the second half of the 90s, two independent research groups using recombinant inbred lines derived from FR13A described a major QTL (later named *SUBMERGENCE 1* or *SUB1*), and several minor QTLs of submergence tolerance [37]. It was later established that the *SUB1* locus consists of three genes (*Sub1A*, *Sub1B* and *Sub1C*), which encodes ethylene-response factors and are activated under flooding conditions [26]. *Sub1B* and *Sub1C* are present in all cultural forms of *O. sativa*, which have been investigated so far, while *Sub1A* are found only in submergence tolerance rice variety [26,38]. In this case, the accumulation of ethylene in water-covered seedlings of rice is as the initial signal for expression of *Sub1A*, which subsequently leads to a restriction of growth. To conserve energy and carbohydrates, the expression of genes encoding α -amylase and sucrose synthase, involved in the metabolism of starch

and sucrose, are suppressed under flooding in rice plants carrying *Sub1A* [22]. Also, *Sub1A* increases the expression of the *SLR1* and *SLRL1* genes (the key repressors of gibberellin signaling in rice), which negatively regulates the elongation of the shoot during flooding [5]. It was shown by Septiningsih et al. [39], that the level expression of *Sub1A* determines the degree of tolerance to submergence. In practice, this means that to get the maximum tolerance effect in hybrid plants, both parents should carry *Sub1A*. In addition, the study of allelic variants of the *Sub1A* gene revealed the existence of a *Sub1A-1*-tolerant allele and *Sub1A-2*-intolerant allele [38,39]. The *Sub1A* was identified in *O. sativa* subspecies *indica*, as well as in *O. rufipogon* and *O. nivara* [40]. It is noteworthy that *Sub1A* is not represented in *O. sativa* subspecies *japonica*.

The transition from seedlings to direct sowing seeds makes it necessary to develop varieties with tolerance of flooding during seed germination. Previously a few QTL of tolerance to anaerobic germination was found on chromosomes 5 and 11 [17]. In another paper, based on a hybrid population obtained by crossing IR 64 and a tolerant donor, Khao all hlan On (Myanmar), it was identified five QTLs on chromosomes 1 (*qAG-1-2*), 3 (*qAG-3-1*), 7 (*qAG-7-2*), and 9 (*qAG-9-1* and *qAG-9-2*) [41, 42]. In this case, as noted by the authors, the QTL located on the long arm of chromosome 9 (*qAG-9-2* or *AG1*) and associated with local modulation of the trehalose-6-phosphate (T6P) level [43] is the most promising for breeding. Another QTL for anaerobic germination tolerance was found on the short arm of chromosome 7 (*qAG7.1* or *AG2*) in a study of the plant population obtained from crossing the sensitive line IR 42 and the tolerant Chinese variety Ma-Zhan Red [44]. Using the 5291 SNP markers on GWAS study, as well as analysis of expression profiles, Zhang et al. [45] revealed the LOC_Os06g03520 gene associated with tolerance of flooding during seed germination. The combination of genome-wide association analysis and biparental QTL mapping approaches allowed researchers to identify a genomic region carrying hexokinase (*HXK6*, LOC_Os01g53930) and associated with coleoptile elongation in submerged rice plants [46]. The same authors conducted RNA-seq analysis of six rice genotypes demonstrating different coleoptile elongation rates under anaerobic conditions [47]. Based on the results of this study, the genes have been identified that

contribute to the anaerobic rice germination, including carbohydrate metabolism, lipid metabolism, ethylene signaling pathways, cell wall growth and elongation.

Identification of genes responsible for internodes elongation under flooding was done in the following studies [48,49,25]. Using the progeny of crossing Taichung65 (T65) with moderate growth and C9285, which is characterized by intensive shoot elongation under flooding, the researchers initially identified 3 major QTLs associated with the trait of deepwater. Among them, the largest effect was noted for the locus located on chromosome 12. Positional cloning and functional analysis using a transgenesis made it possible to identify the genes *SNORKEL1* (*SK1*) and *SNORKEL2* (*SK2*), which regulate the response to deep water flooding. In response to deepwater flooding, the *SK*-genes were expressed at high levels in the leaf blade, leaf sheath and basal part of the stem, including nodes and internodes. The authors also obtained transgenic plants in which the *SK*-genes were driven by the *OsAct1* promoter. The overproduction of *SK1* and *SK2* in such plants resulted to internodes elongation in comparison with the control, even under dry conditions. It has also been shown that *SK*-genes are activated by ethylene and to act as transcription factors. With an *SK*-genes expression, an increase in the content of gibberellic acid and a decrease in abscisic acid are observed. Moreover, during internodes elongation, an increase in the expression level of the expansin involved in the cell wall softening [50,51,52] and a shift in cellulose microfibril orientation are registered [53]. The formation of internodes aerenchyma occurs simultaneously with its elongation and is enhanced by ethylene [54,55]. As in the case of *Sub1A*, *SK*-genes have been identified only in *O. sativa* subspecies *indica*. In addition to the major loci of submergence tolerance (described above), some minor QTLs were also revealed. So in one of the detailed studies [56], using FR13A and Jao Hom Nin as donors, a number of QTLs located on chromosomes 1, 2, 5, 7, 10 and 11 were identified. Major QTL was identified on the long arm of chromosome 1 [42]. In an earlier study, QTLs associated with tolerance were shown on chromosomes 6, 7, 11 and 12 [57]. Additional novel QTLs originating from FR13A on chromosomes 1, 8, and 10 were described in study Gonzaga et al. [58]. It is interesting to note that the presence of these QTLs provides a high survival rate (95 %) of rice under flooding conditions even without *Sub1A*. A summary of

QTLs associated with submergence tolerance is presented in Table 1.

4. MARKER-ASSISTED SELECTION FOR RICE SUBMERGENCE TOLERANCE

Due to the rapid development of molecular biology, there is a real opportunity to analyze the inheritance of traits in their relations with specific DNA sequences. The term marker-assisted selection (MAS) was proposed for this new approach [59]. Currently, one of the most widely used MAS directions is marker-assisted backcrossing (MABC). The aim of MABC is the introgression of the target QTL from agronomic non-standard donors into elite varieties. MABC significantly exceeds to conventional backcrossing in accuracy and efficiency [60]. An important step for breeding of submergence tolerant rice was the identification of the locus *Submergence 1A* or *Sub1A* [26]. DNA sequence polymorphism both directly and around of the *Sub1A* QTL allowed to develop and implement in the breeding programs effective molecular markers for controlling the transfer of the *Sub1A* region [26,38,39,61]. Using the developed markers for *Sub1A* introgression into mega varieties, the breeders in the shortest time obtained submerging tolerance rice without loss of productivity and nutritional qualities [39,62]. So under the control of the SSR marker RM23805, the *Sub1A* was introduced in the susceptible variety OM1490. A distinctive feature of the allele of RM23805 locus inherited from the donor of tolerance (IR64-Sub1A) was the presence on the electropherogram a 230 bp amplification product, while the allele obtained from the susceptible OM1490 is 240 bp. All lines with introgressed *Sub1A* revealed a higher survival under flooding compared to the recurrent parent [63]. In another study, the *Sub1A* gene was introgressed into the high-yielding Indian variety, Swarna [61]. The crossing scheme was as follows. Initially, the high-yield variety Swarna was crossed with the *Sub1A* donor, IR49830-7. Next, F₁s were backcrossed with Swarna. From BC₁F₁, individual plants were isolated which were analyzed for the presence of the donor allele using the RM219 marker distally flanking the *Sub1* locus.

In the second backcross generation used the same selection strategy for individual plants with the desired combination of alleles at the target loci, including the selection of recombinants between *Sub1* and the nearest proximal marker RM316. The results of this study demonstrated

that a sensitive mega variety can be efficiently transformed to a submergence tolerant variety in three backcross generations without loss of yield potential [61]. The successful introgression *Sub1A* locus, derived from the IR64, was made into the popular Vietnamese rice variety - AS996. Herewith *Sub1A* inheritance was confirmed by using tightly linked markers ART5 and SC3 [64]. The IR64-Sub1 was also chosen for the introgression of the submerging tolerance QTL into another elite Vietnamese rice variety, Bactrim 7. However, in this case, the markers ART5 and RM23877 showed a higher efficiency in controlling the transfer of the sought locus [65]. To date, the *Sub1A* gene has been introgressed into mega varieties such as Samba Mahsuri and

CR1009 (India), IR64 (Philippines, IRR), Thudakkam 1 or TDK1 (Laos), BR11 (Bangladesh). According to the results of field trials, these improved mega varieties showed higher survival rates in flood conditions than the recurrent parent. Herewith, under normal conditions, *Sub1A* did not affect on the other important agronomic traits [6,62,66]. The development of highly productive varieties with an introgressed *Sub1A* locus promotes the further incorporation of *Sub1A* into new elite varieties. This is due to the fact that donor parents are already well adapted to the region of cultivation and possess many impotent agronomic traits [67].

Table 1. A summary of QTL for submergence tolerance traits in rice

Loci ^A	Marker interval	Ch	Trait	Phenotypic variance explained (%)	LOD	Reference
<i>QTL_{ch9}</i> , <i>qSUB9.1</i> (<i>SUB1</i>)	RM337- RM464; id9001352-SC3	9	High survival, lowest elongation	69.00	36.9	[26,37,56]
<i>qSUB1.1</i>	id1000556-id1003559	1	High survival	20.20	5.0	[58]
<i>qSUB4.1</i>	id4010621-id4012434	4	High survival	12.40	3.0	[58]
<i>qSUB8.1</i>	id08005815-id8007472	8	High survival	13.00	3.1	[58]
<i>qSUB10.1</i>	id10005538-RM25835	10	High survival	15.80	3.9	[58]
<i>qAG-5</i>	RM405-RM249	5	Anaerobic germination	15.51	3.8	[41]
<i>qAG-7-2</i>	RM21868-RM172, seq- rs3583	7	Anaerobic germination	19.43	9.7	[42,45]
<i>qAG-7-1, AG2</i>	RM3583-RM21427	7	Anaerobic germination	30.30	14.5	[44]
<i>qAG-9-2, AG1</i>	RM3769-RM105, seq- rs4216	9	Anaerobic germination	33.49	20.3	[42,43,45]
<i>qAG-11</i>	RM21-RM22, seq-rs5125	11	Anaerobic germination	10.99	3.0	[41,42,45]
<i>qAG-1-2</i>	RM11125-RM104; id29187939id32847451	1	Anaerobic germination	17.89	9.8	[42,46]
<i>qAG-3-1</i>	RM7097-RM520	3	Anaerobic germination	18.19	5.7	[42]
<i>qAG-9-1</i>	RM8303-RM5526	9	Anaerobic germination	23.58	16.4	[42]
<i>qTIL12 (SK1 and SK2)</i>	RM6386- RM235	12	Internode elongation	27.00	6.2	[25,48,49]
<i>qTIL1</i>	RM6840- RM259	1	Internode elongation	22.00	4.4	[48,49]
<i>QTL_{ch1}</i>	A330402-RM104	1	High survival	15.80	5.4	[56]
<i>QTL_{ch2}</i>	G45-RM250	2	Total shoot elongation	24.00	7.3	[56]
<i>QTL_{ch5}</i>	R2289-C1018	5	High tolerance	28.30	12.3	[56]
<i>QTL_{ch7}</i>	RM214-RM234	7	High tolerance	11.70	4.4	[56]
<i>QTL_{ch10-1}</i>	RM222-RM216	10	High tolerance	18.00	9.5	[56]

A=QTL names were taken from original sources and in some cases are designed with the abbreviation of the trait name and chromosome number, SUB=Submergence, AG=Anaerobic germination, SK=Snorkels, TIL=Total internode elongation length, Ch=Chromosome, LOD=Logarithm of odds score

Marker-assisted selection for anaerobic germination tolerant rice was begun with detection of the AG1 locus in the variety Khao Hlan On [43,68]. The introgression QTL AG1 was successfully introduced into such Asian varieties as IR64, IR64-Sub, PSB Rc18-Sub1 and PSB Rc82. There are also studies on the introgression of the AG2 locus. In this case, Chinese traditional variety Ma-Zhan Red is used as a donor [48]. The Toledo et al. [69] research focused on the transfer AG1 locus into the Ciherang variety carrying the *Sub1A* gene. Introgression of QTL AG1 and restoration of the Ciherang genome were carried out in two backcross generation followed by self-pollination. To control the inheritance of *Sub1A*, SSR markers RM8300 and ART5 were used. The AG1 transfer, depending on the population to which the donor belonged, was monitored using two to three markers. So for the IR64-AG1c: IR93312-30-101-20-3-66-6-14 population, the TPP_GE5 and HPP400_410_3 markers were used, whereas for IR64-AG1: IR93312-30-101-20-13-64-13, markers used were TPP_GE5, Drebdups6bp and Drebdws4bp. The selection of genetic similarity to Ciherang from the first and second backcross generations was performed using 26 SSR markers uniformly located in polymorphic regions between IR64 and Ciherang. It was shown that most of the major QTLs of anaerobic germination are obtained from different tolerant donors, located in different regions of the rice genome, and their contributions are additive. In this connection, it is suggested that, unlike the QTL *Sub1A*, the introduction of only one QTL into an elite rice variety will not give the desired level of tolerance to flooding at the germination stage [69,70]. In this case, the best strategy for increasing stress tolerance is QTL pyramiding.

The cooperation of the Laboratory of Molecular Genetics of the Southern Federal University and the Laboratory of Selection, Seed and Rice Cultivation of the Agricultural Research Center [71,72,73] made it possible to validate of SSR markers associated with submergence tolerance QTLs *Sub1A* in rice with the aim of introgression of this QTL into the elite rice variety of Russia [14]. It was shown that only RM7481 of 7 studied microsatellite markers (RM219, RM316, RM444, RM464, RM7481, RM8303, RM23877) is effective to control the transfer of the *Sub1A* region into the Russian rice variety. For validation of marker RM7481 the hybrids by crossing Russian variety Novator with *Sub1A* gene donors (BR-11, Inpara-3, CR-1009, TDK-1) were obtained. According to the results of

molecular genetic screening with RM7481, the F₂ plants carrying different allelic variants *Sub1A* locus were identified. Investigation of the second generation hybrids tolerance to deepwater stress showed that the most viable samples under submergence were *Sub1A* locus donor lines and hybrid plants (F₂) carrying the *Sub1A* locus in a homozygous state.

5. CONCLUSION

The consequence of the negative impact on agricultural crops is a decrease in their yield. The introduction of tolerance loci (QTLs) into highly productive variety adapted to certain agroclimatic conditions, as well as the pyramiding of several QTLs in one genotype, are considered being the most promising directions to abiotic and biotic stress tolerance breeding. The use of specific DNA markers associated with such tolerance provides a clear control the inheritance of the target locus, which ultimately reduces the time and cost of breeding. As a result, marker assisted selection is now increasingly used as a high-tech tool in real breeding programs. This review had placed great emphasis on researches aimed at application of molecular-genetic markers in the breeding of submergence tolerant rice varieties. The summarized information will facilitate further investigations of physiological and biochemical mechanisms of submergence tolerance and developing multiple-stress-tolerant rice varieties.

ACKNOWLEDGEMENTS

This work was supported by the Grant of President of Russian Federation no. MK-6123.2016.11. Analytical works were carried out on the equipment of the Centers for collective use of Southern Federal University "High Technology".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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