

# Quiescence in rice submergence tolerance: an evolutionary hypothesis

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Rice (*Oryza sativa*) varieties differ considerably in their tolerance to submergence, a trait that has been associated with the *SUB1A* gene. Recently, this gene was found in some wild rice species and landraces, which along with *O. sativa*, belong to the AA genome type group. On the basis of geographical and historical data, we hypothesize that *SUB1A-1* from wild species may have been introgressed into domesticated rice. This introgression probably occurred in the Ganges Basin, with the subsequent spread of the *SUB1A-1* to other areas of South Asia due to human migration. The lack of the *SUB1A* gene in diploid CC genome type wild rice showing submergence-tolerant traits suggests the presence of a different survival mechanism in this genetic group.

## Evolution of submergence tolerance in rice

Various hydrological regimes characterize natural and anthropic habitats where flood events vary in extent, depth, frequency, and seasonal incidence. Extreme hydrological regimes include river floodplains, coastal marshes, the Amazon floodplain, as well as the areas where rice is cultivated, which are characterized by submergence of the plants that can reach several meters but can also be absent [1]. Flood-prone environments determine the distribution and abundance of plant species and exert an evolutionary pressure which selects traits that are advantageous under these environmental conditions [1]. The adaptability of a plant to waterlogging or submergence is enhanced by the development of either metabolic or anatomical characteristics [1]. These traits act in synergy to promote plant tolerance to water submergence and represent a source of genetic variations for crop breeding [2].

Rice is a unique crop due to its adaptability to different flooding conditions. The number of varieties, landraces, and wild relatives covering the large area of production is synonymous with an exceptional plasticity to diverse hydrological regimes. One of the many ways that have enabled rice to adapt to flooding has recently been identified as a 'quiescence strategy' [3]. It is characterized by reduced plant elongation during submergence, associated with regrowth when the water recedes. The ethylene responsive factor (ERF) *SUBMERGENCE1A* (*SUB1A*), in its allelic form *SUB1A-1*, is the key determinant of this survival mechanism [4]. *SUB1A-1* is induced by ethylene, a gaseous

plant hormone that becomes entrapped by water submergence [5]. *SUB1A-1* positively regulates the transcription of genes related to the fermentative metabolism, which is required under low oxygen conditions to sustain energy production [6]. *SUB1A-1* also represses genes related to the breakdown of carbohydrates, in order to preserve the sugars needed to fuel regrowth when the water recedes [6]. Remarkably, *SUB1A-1* also restricts shoot elongation, thus preventing the excessive use of carbohydrates to sustain growth, which would in any case be insufficient for the plant to reach the water surface [5]. Limited shoot elongation under submergence is achieved through the positive action of *SUB1A-1* on the gibberellin (GA) signaling repressors SLENDER RICE-1 (SLR1) and SLR1-LIKE (SLRL1) [7]. During the post-hypoxic phase, *SUB1A-1* is involved in acclimation to dehydration, paradoxically occurring as natural progression upon desubmergence, by increasing ABA responsiveness and reactive oxygen species (ROS) detoxification [8]. Some submergence tolerance can also be conferred by the intolerant *SUB1A-2* allele, when highly expressed [9]. This allele differs in terms of the absence of the *SUB1A-1* MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) site [4], which might affect the activation of the protein and the regulation of downstream genes. The introgression of *SUB1A-1* in rice varieties with high yields and grain quality traits, but reduced tolerance to submergence, resulted in the production of new varieties with both a high yield and submergence tolerance [10].

Considerable efforts have been made to characterize the presence/absence of the *SUB1A* gene in *Oryza* spp. [4,9,11–13]. We summarize these findings on the basis of geographical origin and description of the original habitat of the genotypes studied, in order to examine the evolution of *SUB1A-1* and thus the submergence tolerance trait.

## *SUB1A* gene is present in wild and cultivated rice

The genus *Oryza* L. consists of approximately 23 species harboring several distinct genome groups [14]. The *Oryza* spp. that belongs to the AA genome type includes *Oryza sativa* and *Oryza glaberrima* cultivated rice and wild species (i.e., *Oryza rufipogon*, *Oryza nivara*, *Oryza barthii*, *Oryza longistaminata*, *Oryza meridionalis*, and *Oryza glumaepatula*) [15], which can exchange genetic information when sympatric and flowering at the same time [16]. After being originally associated with the *O. sativa* small subgroup of *indica* varieties [4], including the *aus* landrace Flooding Resistant 13A (FR13A), *SUB1A* alleles have also been described in other genotypes including some wild

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species (e.g., *O. nivara* and *O. rufipogon*) [9,12,13] which, together with *O. sativa*, all belong to the AA type genome group. Of the wild species belonging to the AA genomic group, *O. rufipogon* and *O. nivara* are considered to be the most likely ancestors from which *O. sativa* was domesticated [17].

Figure 1 shows a map of wild and landrace rice accessions belonging to the AA genome type investigated to date for the presence/absence of *SUB1A* and with the International Rice Germplasm Collection (IRGC) number of the International Rice Research Institute (IRRI). The map shows the presence of various wild rice species harboring the *SUB1A-1* allele around the basins of the Ganges and Brahmaputra rivers, which include areas of North India, Nepal, and Bangladesh. One was originally reported to be a hybrid swarm, thus probably a natural population of wild and cultivated rice interspecific hybrids. Most archeological evidence indicates the Yangtze Valley in China as the original area of rice cultivation by humans [18–21]. In addition, genetic evidence suggests that the Ganges Valley in India was a second site of potential rice domestication [22]. A phylogeographic study also showed that the southern Himalayas was the domestication region of the *indica* group from the *O. rufipogon* progenitor [23]. Migrants could have disseminated the seeds of submergence-tolerant plants from this area to other parts of Asia, as shown by the presence of landraces with the *SUB1A-1* gene in Sri Lanka, Vietnam, and Thailand (Figure 1).

Interestingly, the presence of the intolerant allele *SUB1A-2* has also been identified in *O. sativa* genotypes from China, Cuba, Nigeria, and Guinea-Bissau. The only accession of the African cultivated rice *O. glaberrima* investigated shows the absence of *SUB1A* and was collected in Senegal [13]. To date, only a few AA rice accessions from Africa have been analyzed, making it difficult to formulate a hypothesis as to how *SUB1A* introgressed in this area.

Previous surveys of AA rice genotypes revealed that the *SUB1A* gene was sometimes, but not always, present in *O. nivara*, *O. rufipogon*, and *O. sativa* [9,11–13]. This supports the theory that submergence tolerance was introgressed from divergent populations of wild relatives into cultivated *O. sativa* [13]. Wild rice species belonging to the AA genome group evolved in areas characterized by different water regimes, with *O. nivara* occurring in seasonally dry habitats, and *O. rufipogon* in permanently wet ones [14]. However, in some places *O. nivara* and *O. rufipogon*, which are cross-compatible, are sympatric in areas of rice cultivation. Despite their ecological distinction, *O. nivara* and *O. rufipogon* have similar morphological characteristics and a relatively low genetic differentiation, thus *O. nivara* is also considered as a subspecies or an ecotype of *O. rufipogon* [14,24]. One hypothesis is that *O. nivara* evolved from an *O. rufipogon* ancestor after a habitat shift [17]. The transfer of the *SUB1A* gene may thus have been successful in environments where flash flooding occurs at the rice seedling stage [25].

Gene flows from AA genome type wild rice species toward nearby *O. sativa* could have occurred with the introgression of key domestication alleles [26]. However, gene flows from cultivated rice could also have contributed

to the genetic diversity of wild relatives in sympatric regions [21]. Studies on the genetic variation of *O. rufipogon* have shown a higher genetic diversity of the wild rice populations growing adjacent to cultivated rice fields than those found some distance away, thus indicating that gene flows occur from cultivated to wild rice [27,28]. The absence of wild rice harboring *SUB1A* in Sri Lanka, where *SUB1A*-containing *O. sativa* landraces are present, suggests that either they have only been recently introduced in Sri Lanka or that *SUB1A* was introgressed from wild rice species to *O. sativa* during domestication in the Indian Basin, and then transferred to Sri Lanka.

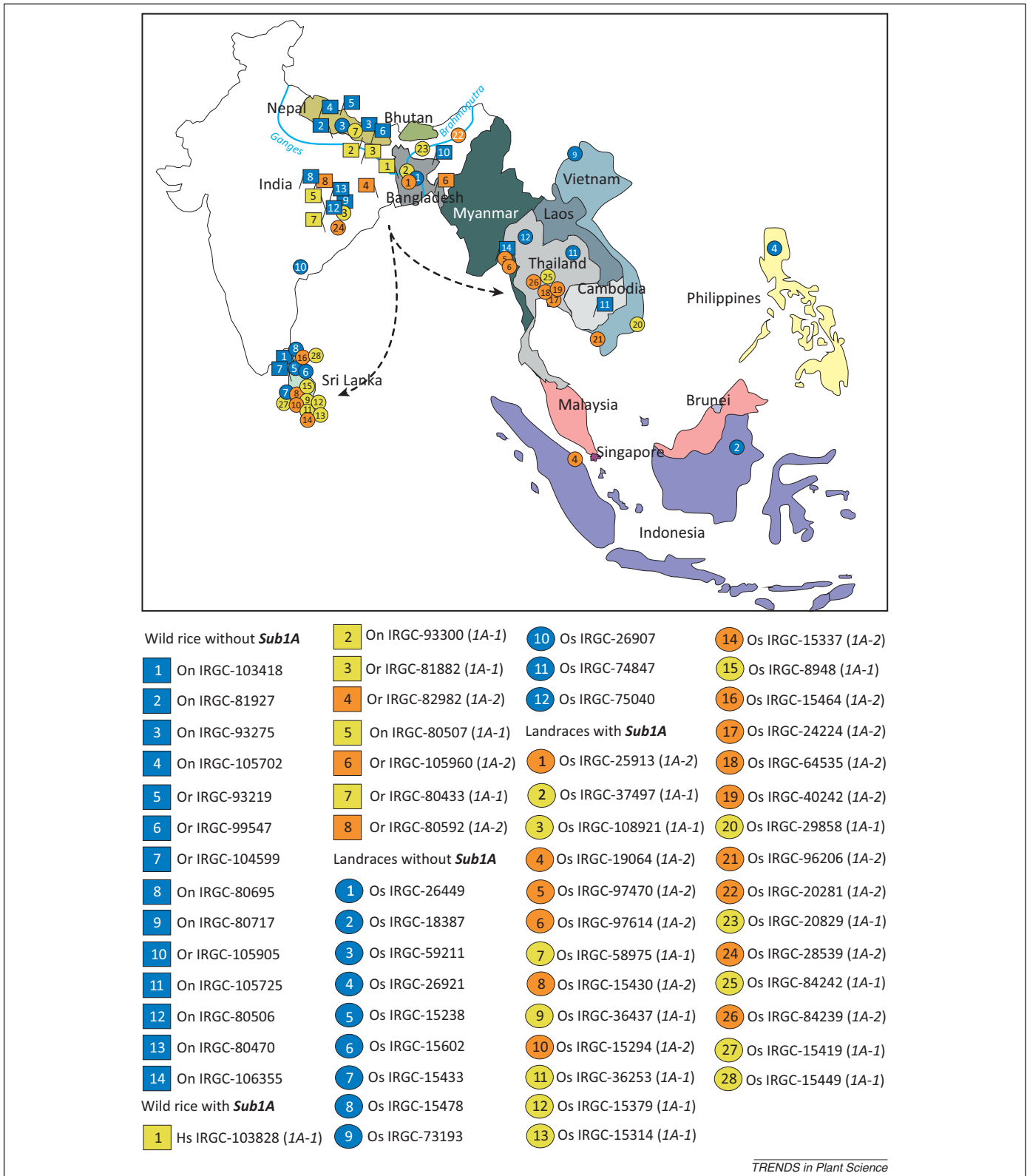
The introgression of *SUB1A* in cultivated rice could have been a relatively recent event. Together with *SUB1A* (whose presence in rice cultivars is variable), other genes encoding ERFs are invariably present in the *SUB1* region, namely *SUB1B* and *SUB1C* [6]. Phylogenetic analysis has provided evidence that *SUB1A* and *SUB1B* diverged from *SUB1C* during the differentiation between the AA and CC genome complexes [13], and that *SUB1A* resulted from the duplication of *SUB1B* [11]. The recent evolution of *SUB1A* is also suggested by the occurrence of small allelic variations in this gene (*SUB1A-1* and *SUB1A-2* only) compared with the highly polymorphic *SUB1B* and *SUB1C* (nine and seven alleles, respectively) [4].

#### ***SUB1A* is absent in submergence-tolerant wild rice belonging to the CC genome group**

To date, the *SUB1A* gene has not been found in diploid wild relatives belonging to the CC type genome group *Oryza officinalis*, *Oryza rhizomatis*, and *Oryza eichingeri* which, however, have been shown to survive flooding by reducing growth under submergence [13]. Only *SUB1C*-like genes have been found in these genotypes, suggesting that this ERF may be more primitive than other *SUB1* genes [13].

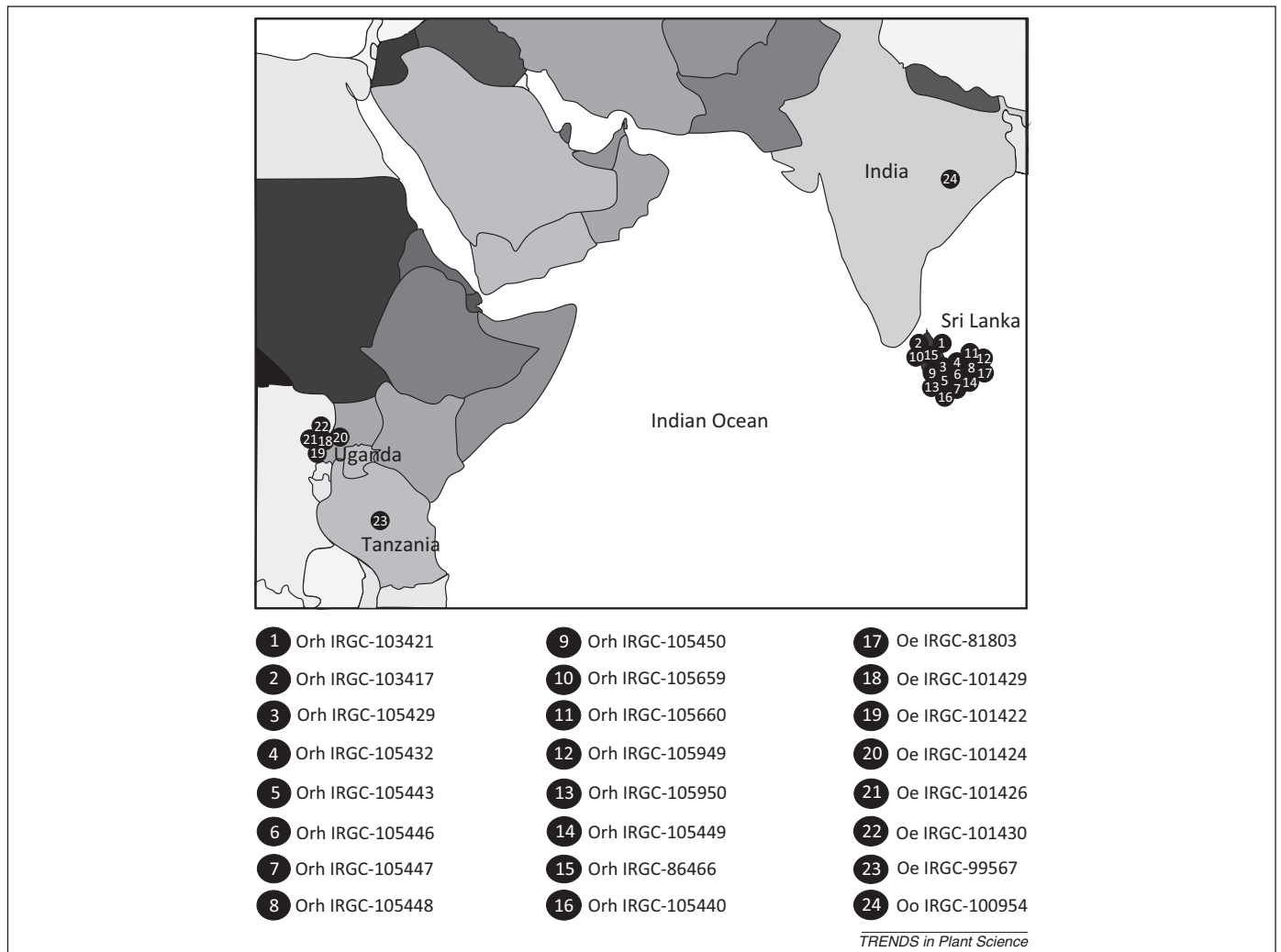
Wild rice belonging to the diploid CC genome type and not harboring the *SUB1A* gene have shown a disjointed distribution in Africa and Asia (Figure 2). A recent hypothesis suggested that *O. eichingeri* from Africa may have been introduced to Sri Lanka a very long time ago, because the accessions from the two locations are highly genetically divergent [24]. This species shows morphological and genetic variations in relation to geographical derivation and habitat, thus exhibiting a high degree of intraspecific variation [14]. In South Asia, wild CC and AA genotypes have been found in the same geographical region [24]. Many of the CC genotypes investigated to date have been collected in Sri Lanka where *O. sativa* landraces harboring *SUB1A* are also present (Figures 1 and 2). However, trials of breeding programs with *O. eichingeri* have reported a very low level of success in crosses with *O. sativa* [15]. Moreover, in Sri Lanka the habitat of *O. rhizomatis* is confined to seasonally dry zones [24], making the genetic exchange between AA and CC rice relatively difficult.

One genetic analysis determined that the *SUB1C* allele, which is present in *O. officinalis*, *O. rhizomatis*, and *O. eichingeri*, does not contribute to submergence tolerance in rice [29]. Moreover, the heterologous ectopic expression of rice *SUB1C* in *Arabidopsis thaliana* showed a limited contribution of this gene to the submergence stress response [30]. Overall, this supports the idea of a molecular



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**Figure 1.** Location of the original collection sites of AA genome type wild genotypes (square symbols) and landraces (circle symbols), with or without *SUB1A* genes [9,11–13]. Wild genotypes and landraces without either *SUB1A-1* or *SUB1A-2* are represented by blue symbols. Genotypes with the tolerant allele *SUB1A-1* are indicated as '1A-1' in the key and yellow squares or circle symbols in the map, while those harboring the intolerant allele *SUB1A-2* are indicated with '1A-2' in the key and orange squares or circles in the map. The accessions shown represent those investigated to date for the presence/absence of *SUB1A* and with the International Rice Germplasm Collection (IRGC) number of the International Rice Research Institute (IRRI). The source country and collection site of the AA genome type wild rice accessions were studied using the original collection forms of the International Rice Information System (IRIS) from the IRRI (<http://www.irri.org>), whereas landraces were investigated with information obtained by the System-wide Information Network for Genetic Resources (SINGER), now part of the Plant Genetic Resource Gateway (GENESYS, <http://www.genesys-pgr.org>). The passport data of each accession was checked to locate the site where the sample was originally collected before being sent to the seed bank. The collection site included a wild zone or a field border for the wild species and the germplasm location before being sent to the gene bank for landraces. Rice species with unspecified or other statuses (e.g., improved cultivar and crosses) were not included in the analysis, and neither were the wild accessions lacking the original IRRI collection form. The dotted arrows represent the directions in which domesticated rice may have spread.



**Figure 2.** Location of the original collection sites of diploid CC genome type wild genotypes not harboring the *SUB1A* gene [13]. The source country and collection site of the CC genome type wild rice accessions were studied using the original collection forms of the International Rice Information System (IRIS) from the International Rice Research Institute (IRRI) (<http://www.irri.org>). For some of these accessions, even if the country source was indicated, the typology of the original collection site was unknown.

mechanism of submergence tolerance which may be unique to the CC type genome group and which still needs to be found. The rice varieties Madabaru and IR72 have recently been shown to harbor novel submergence tolerance quantitative trait loci (QTLs), which are independent of the ethylene-mediated *SUB1A* pathway [31].

### Concluding remarks

Various studies have highlighted that the presence of *SUB1A* alleles is highly variable in several wild and landrace accessions of rice belonging to the AA genome group. Analysis of the geographical distribution of wild rice and landrace data, together with the molecular analysis for the presence of *SUB1* genes, suggests that the introgression of *SUB1A-1* from wild into cultivated rice originated around the Ganges Basin. The presence of *SUB1A-1* in *O. sativa* landraces present in other Asian regions suggests that early settlers may have brought with them the seeds of submergence-tolerant plants.

Submergence-tolerant traits have also been shown to be present in some diploid CC type wild rice accessions which, however, lack *SUB1A*. Only *SUB1C*-like genes have been found in these genotypes; therefore, it is possible that they

harbor a distinctive molecular mechanism of submergence tolerance, which is still unknown. In South Asia, these genotypes are found in the same geographic region where AA rice species grow; however, success in intergenomic crosses is very low.

Novel QTLs associated with submergence-tolerant traits might be useful in enhancing the level of survival. Indeed, the hybridization of wild and cultivated rice with the introgression of alien genes can give rise to new interesting variations, due to the high level of genetic diversity. Rice breeding programs that could overcome the problem of cross-compatibility barriers would be a breakthrough in rice enhancement.

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