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MOLECULAR BREEDING EFFORTS TO IMPROVE RICE ABIOTIC STRESS TOLERANCE IN BANGLADESH

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SUMMARY

Agriculture is the most important sector of the Bangladesh economy with rice as the staple crop for food security. Rice production in Bangladesh has been threatened by various abiotic stresses: including flooding, drought, and salinity; with flooding as the most severe and frequent problem that affects the country's crop production. Recently, scientists have identified and utilized the *SUB1* gene that confers rice flooding tolerance trait, to many popular varieties in Asia, including Bangladesh. Importantly, scientists in Bangladesh are making efforts to breed submergence-tolerant early maturing rice to cope with flooding, which may provide a means for farmers to improve their rice production. Moreover, drought and salt-tolerant rice lines are also being developed through molecular breeding programs. All of these research advances are providing the means for Bangladesh to improve abiotic stress tolerance to increase rice production, providing more stable food security under stress-prone environments.

Key words: Flood, drought, salinity, *SUB1* gene, early-maturing varieties, marker-assisted backcrossing (MABC)

Key findings: Sub1 lines and other improved varieties tolerant to different abiotic stresses will assist Bangladesh to achieve and sustain food security.

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INTRODUCTION

Bangladesh is a developing country with serious challenges to improve the health and nutrition status of its population. Thirty-seven million people out of the 156.6 million struggle with food security (World Food Programme, 2015). Agriculture remains the most important sector of the economy, accounting for 19.6% to the national gross domestic product (GDP). Bangladesh has rich agricultural lands and natural resources. River deltas bring silt, and mud from the Himalayan Mountains eroded over millions of years nourishing the country into a very fertile agriculture land (Harrison, 2015). Rice, jute, sugarcane, potato, pulses, wheat, tea, and tobacco are the main crops grown in Bangladesh, which account for 72% of the overall agribusiness. Rice is the principal crop with about 28.8 million metric tons produced annually (Headey and Hoddinott, 2016). Traditionally, farmers maintain a sequence in crop rotation by switching out one crop for another after harvesting (Rasul and Thapa, 2004). For instance, they often grow pulses, a grain legume, after rice. Leguminous pulses recover nutrients consumed by rice from the previous season by fixing atmospheric nitrogen into the soil. Thus, the land remains productive without using too much fertilizer. Moreover, they apply cow dung or compost providing additional nutrients in an environmentally-friendly manner. These traditional farming systems also aid in the biological control of pests and diseases (Baten, 2010).

Unfortunately, even with fertile soil and good agricultural practices, Bangladesh still struggles to produce sufficient food for its people. Although, excellent progress has been made in the past few decades, agricultural development is still significantly constrained by various challenges. In particular, crop production is frequently affected by flood, drought, and salinity (Mondal, 2010). Among these abiotic stresses, flooding is the most severe and frequent problem that damages the country's crop production (Tingsanchali and Karim, 2005). Bangladesh is prone to flooding as it is located on the Ganges Delta and many tributaries flowing into the Bay of Bengal. The coastal flooding paired with the bursting of Bangladesh's riverbanks is common and severely affects the landscape and the society. More importantly, about 90% of the country is less than 10 meters (33 feet) above sea level and 80% is in floodplains, rendering Bangladesh at high risk of further widespread damage due to flooding (Center for Excellence in Disaster Management & Humanitarian Assistance, 2015). The flooding, which previously followed the same patterns for decades, has now changed and often comes about 15 days sooner, right at the harvesting season (Islam and Suman, 2008). This subtle change in climate can ruin harvests, threaten the ecosystem, and make the already unstable livelihoods of people more vulnerable. Due to a heavy reliance on farming, many people lose their food supply and income when crops are damaged or destroyed. In this review, we will focus on the utilization of the *SUB1* gene to improve rice flooding tolerance in Bangladesh, and also briefly discuss molecular breeding efforts for other abiotic stresses, such as drought and salinity in Bangladesh agriculture.

Understanding rice flooding tolerance

Because of the well-developed aerenchyma tissues which diffuse oxygen through continuous air spaces throughout the root and the shoot and to avoid anoxia development in roots, rice is the only crop adapted to aquatic environments (Peng and Ismail, 2004).However, complete submergence for long periods due to flooding can dramatically affect rice yield and growth (Septiningsih *et al*., 2013a). Flood water limits gas diffusion and light penetration. Most rice plants will die if it is fully submerged for more than three days. Some local varieties may survive the flood; however, they usually have very poor agronomic performance, low yield, and some other undesirable characteristics. More than 16% of rice-grown areas in the world, including Bangladesh, are affected by flooding (Peng and Ismail, 2004). Among the different types of flooding, flash flooding, in which water completely submerges the crop for up to two weeks, is the most common (Mackill *et al*., 2010).

Scientists are making efforts to understand how plants cope with flooding and breed submergence-tolerant rice with the pioneering work done at the International Rice Research Institute (IRRI) in the Philippines. Previously, several submergence-tolerant rice varieties had been conventionally bred and released to the market. However, due to the lack of some important agricultural traits, these submergence-tolerant rice varieties were not widely adopted by the farmers. For example, the variety "Popoul", derived from a highly flood tolerant line IR49830-7-1-2-1, was released in Cambodia in 1999. But this variety did not have the grain quality that local people preferred (reviewed in Septiningsih *et al*., 2013a).

Groundbreaking work was initiated in the mid-1990s with the identification of a major quantitative trait locus (QTL) named *Submergence 1* (*SUB1)* that controls rice submergence tolerance during vegetative stage (Xu and Mackill, 1996).The genes underlying the *SUB1* locus were isolated as three ethylene response factors (ERFs) through an international collaboration of the research groups at IRRI, the University of California at Davis, and the University of California at Riverside (Xu *et al*., 2006). Further it was confirmed that *SUB1A* is the primary contributor to submergence tolerance (Xu *et al*., 2006; Septiningsih *et al*., 2009). Importantly, expression of *SUB1A*, which is up-regulated rapidly in response to submergence, conferred robust tolerance to flooding during the vegetative stage in rice (Xu *et al*., 2006). This work not only revealed an important mechanism for how plants control tolerance to environmental stress, but also sets the stage for generating flood-tolerant rice varieties.

SUB1A confers rice tolerance to flash flooding during vegetative stage. Different sets of genes control flooding tolerance during seed germination (termed as anaerobic germination or AG), an important trait for direct-seeded rice (Angaji *et al*., 2010; Septiningsih *et al*., 2013b). A gene called *OsTPP7*, encoding a trehalose-6-phosphatephosphatase (TPP), which confers AG tolerance in a tolerant rice donor from Myanmar, Khao Hlan On, was isolated and functionally characterized (Kretzschmar *et al*., 2015). The *OsTPP7* gene could potentially enhance the rate of starch mobilization and growth in anaerobic germination tolerant plants. Understanding the mechanism of anaerobic germination tolerance and developing AG-tolerant varieties are important tasks, since farmers are increasingly shifting from transplanted to direct-seeded rice. The *OsTPP7* gene could be transferred to high-yielding varieties to enhance the practice of direct seeding for a more sustainable rice industry (Kretzschmar *et al.*, 2015; Toledo *et al*., 2015). However, unlike the case of the large-effect *SUB1* gene, the *OsTPP7* gene has only a medium effect for AG tolerance. Therefore, it needs to be pyramided with other QTLs or genes to achieve a higher level of tolerance for flood-prone areas.

Improving rice flooding tolerance by introducing *SUB1* genes

Scientists at IRRI have introduced the *SUB1A* gene into eight popular rice varieties including: Swarna-Sub1, S. Mahsuri-Sub1, IR64-Sub1, TDK1-Sub1, CR1009-Sub1, BR11 Sub1, Ciherang-Sub1, and PSB Rc18-Sub1 in Asia through marker-assisted backcrossing (MABC; Neeraja *et al*., 2007; Septiningsih *et al*., 2009; 2015; Iftekharuddaula *et al*., 2011), including a widely used variety from Bangladesh, BR11. Scientists in Bangladesh are further developing more tolerant varieties by introducing this gene into their local rice varieties with high yield, good quality, and adoption to local climate (Iftekharuddaula *et al*., 2016a). Molecular markers are linked to traits and can be used to track beneficial alleles from one generation to another. The first generation of Sub1 varieties were developed within two to three generations of backcrossing after crossing the two parents followed by one generation of selfing (Septiningsih *et al*., 2009). However, the more recent Sub1-varieties have been developed using a faster approach, with one generation of backcrossing and one generation of selfing (Septiningsih *et al*., 2015). MABC is widely practiced by breeders and accepted by the public for improving various traits of different crops by introgressing one or a few major genes in an elite variety and is much faster than conventional breeding, which can take up to eight generations.

On the other hand, genetic engineering also provides an alternative way to introduce a gene into a crop variety, resulting in a genetically modified organism (GMO). Genetic engineering is similar to molecular breeding in that they both change the genetic material of an organism. However, they differ in that genetic engineering is done through direct introduction of a foreign gene into the host genome through transformation techniques without making crosses. In some cases, genetic engineering can provide a faster and more powerful alternative to conventional breeding for specific traits (Wang *et al*., 2003). Notably, the genetically engineered plants have been introduced to Bangladesh and the country is giving farmers more opportunity to grow GMO plants, including rice (https://interactive.aljazeera.com/aje/2016/gmo-eggplants-aubergines-bangladesh/index.html#139). However, in the case of submergence tolerant rice, molecular breeding is currently the best option, since the variety can be developed and released quickly using marker-assisted backcrossing (Septiningsih *et al*., 2013a).

Furthermore, a group of scientists from the Bangladesh Rice Research Institute (BRRI) have investigated allelic diversity in the *SUB1* region across 300 local rice accessions, mostly from the *indica* and *aus* sub groups collected from submergence-prone zones in Bangladesh (Iftekharuddaula *et al*., 2016b). They have identified eight accessions that have high level of submergence tolerance. Importantly, novel submergence tolerant genes may control the tolerance from several of these varieties (Iftekharuddaula *et al*., 2016b), and which could potentially provide novel genetic resources to breed rice with higher tolerance to submergence during vegetative stage.

Avoiding flooding by breeding early maturing rice

As previously mentioned, recent changes in climate patterns can result in flooding during harvesting in the dry-season (*boro*). The challenge is that the early monsoon rains may occur before the rice spikelets are fully mature and ready for harvest. However, the planting season cannot be moved earlier because the weather would be too cold for rice to grow then. If the farmers harvest rice plants 10 to 15 days before the start of rainy season, this will avoid the flooding damage. Scientists from PhilRice have developed seven varieties of early maturing rice, one of which is the Sahod Ulan 2. In the rain-fed lowland and drought-prone areas, Sahod Ulan 2 yields about 30% more than regular rice (http://www.philrice.gov.ph/philrice-releases-early-maturing-varieties/). Sahod Ulan 2 matures in 110 days and is also resistant to blast, bacterial leaf and sheath blights, white and yellow stem borers, and green leafhopper (PhilRice, 2011). It will be necessary for Bangladesh to breed high-yielding, early maturing rice varieties adapted to stress-prone environments. Importantly, Bangladeshi scientists have initiated the development of early maturing submergence-tolerant rice lines by introgressing *SUB1* gene into an early maturing rice variety, BRRI dhan33, for the wet season (*T. aman*) to avoid cold spell and diseases later in the season (Iftekharuddaula *et al*., 2016a). Yield tests under both flooded and non-flooded (normal) conditions showed that the converted lines significantly had higher yield compared to the original variety.

Improving rice drought tolerance

Surprisingly, in a flooded country like Bangladesh, drought is another problem in the northwestern region (Shahid and Behrawan, 2008). Flood and drought often come hand in hand, though at different times during the same season. The previously mentioned *SUB1A* gene that makes rice flood-tolerant also makes it more tolerant to drought stress in greenhouse experiments (Fukao *et al*., 2011). It seems paradoxical, but scientists revealed that plants sometime use the similar mechanisms to deal with different abiotic stresses (Zhu, 2016). Flood and drought stress induce a large set of similar genes, including *SUB1A*. The *SUB1A* gene enhances drought tolerance through reduction of leaf water loss (Fukao *et al*., 2011). Therefore, by using *SUB1A*, scientists can hit two birds with one stone but these needs to be tested further in the field. Meanwhile, scientists from IRRI have developed high-yielding varieties that are tolerant to both submergence and drought through molecular breeding by combining *SUB1* with major QTLs for drought tolerance in the same genetic backgrounds (Kumar *et al*., 2014). These varieties have great potential in rainfed areas where flood comes early in the season and drought comes later during reproductive stage, which are common problems, in Bangladesh.

Improving rice salinity tolerance

In addition to flooding and drought, salinity is another threat for Bangladesh agricultural production (Rabbani *et al*., 2013). Nestled at a point where tidal waves from the Indian Ocean flow into the Bay of Bengal, 21% of land in Bangladesh has been intoxicated with excessive salt, especially during the dry season when fresh water level in the rivers becomes low. Winds and currents cause saline water to mix with upstream rivers, and flooding and increased water levels further deteriorate the situation (Devnath, 2014). The excessive salt causes browning of leaves and stunted growth, which severely reduces the yield. A major QTL named *SalTol* has been introgressed into popular varieties for several Asian countries, including Bangladesh (Gregorio *et al*., 2013; Dr. Abdelbagi Ismail, IRRI, personal communication). The introgressed-lines performed better than the original lines under mild stress conditions. In addition, through collaboration of IRRI and Bangladeshi scientists, several high-yielding rice with tolerance to salinity has been developed (Gregorio *et al*., 2013).

CONCLUSION

The rice crop in Bangladesh is frequently destroyed by devastating floods, which causes more than half of the farmers to lose their source of income. This dire situation has been further worsened by other abiotic stress problems, such as drought and salinity. In order to solve these challenging issues, the country has partnered with various research institutions and non-profit organizations to improve rice performance against abiotic stresses. The *SUB1A* gene has provided a promising avenue to develop rice varieties tolerant to water inundation; while major QTLs for drought and salinity have also been proven to be effective under stress conditions. An integrative approach of combining tolerance traits to different abiotic stresses into the same genetic backgrounds through molecular breeding, and shortening the breeding cycle if necessary, will prevent a great deal of yield losses. With such improved varieties, it is hoped that Bangladesh can further improve the lives of its people into the future.

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