

Genetic Resources of Wild Rice (*Oryza rufipogon*) for Biotic and Abiotic Stress Tolerance Traits

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Abstract

Rice (*Oryza sativa* L.) is the most important staple food crop of the world; nearly half of the global population depend on it for majority of their dietary intake. Many stresses (biotic and abiotic) have critically affected rice production throughout the world due to global warming, changing climatic conditions and in addition non-durability of biotic resistance gene (s) incorporated into cultivars. Yield increase is not as per the required rate and becomes yield rate is in stagnation. Primary reason of yield stagnation is due to the narrow genetic base in the released varieties. Minimum number of parental lines were utilised to develop new crop varieties which ultimately leads to narrow genetic base. The narrow genetic base in the improved varieties is going to be a main bottleneck for crop improvement program which shield the yield increase. Genetic bottleneck during domestication causes erosion of the genetic diversity in the well adapted cultivars which leads to yield stagnation. Yield plateaus can be surmount through genetic gain by combining the yield related genes/QTLs from different genetic resources of rice germplasm both from local landraces (CLR) and crop wild relatives (CWR). Wild species are the reservoir of genetic diversity with wide adaptability and tolerance to many biotic and abiotic stresses. It is utmost necessary to characterize and conserve rice germplasm for evaluation and effective use of the genetic diversity prevailed in the rice gene pool. Genetic variability in respect to biotic/abiotic resistance is inadequate in the genetic resources of cultivated rice; however, these traits specific genes are available in the unexplored wild species of *Oryza* which are considered as rich source of agronomically important traits including biotic/abiotic traits. Therefore, breeders are trying to identify and transfer of these valuable genes from wild *Oryza* species to improve varieties through pre-breeding method and with the assistance of molecular breeding technology.

Keywords: Rice landraces, Wild rice species, Basmati rice, Biotic and abiotic stress tolerance traits, MAS

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Introduction

Rice (*Oryza sativa* L.) is most important and staple food crop because more than half of the world's population (>3.5 billion) depends on it for their livelihood (Yang and Zhang 2010; Hu et al. 2014; Qian et al. 2016). It is needed to produce double amount of rice by 2050 to feed the more than 9 billion people in this world (Ray et al. 2013; Arbelaez et al. 2015). Total world production was 748 Mt from 163.1 million hectares with productivity of 4.6 tons/hectare (t/ha) in 2016 of which 676.5 million tons was produced by Asian countries. India needs to produce 150 Mt rice by

2030 to feed the increased population with yield increase rate 4.2 t/h (from present rate 3.2 t/h). Production rate in the released varieties has come to the plateaus due to narrow genetic base in the parental lines used in breeding programs (Khush 1997; 2005; Khush et al. 1990; Tanksley and McCouch 1997; Tian et al. 2006). Genetic bottleneck during domestication also causes erosion of the genetic diversity in the well adapted cultivars which leads to yield stagnation (Tanksley and McCouch 1997). Yield plateaus can be surmount through genetic gain by combining the yield related genes/QTLs from different genetic resources of rice germplasm both from local landraces (CLR) and

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crop wild relatives (CWR). So, it is utmost necessary to characterize and conserve rice germplasm for evaluation and effective use of the genetic diversity prevailed in the rice gene pool. Also needed to reprogram future breeding program to improve and sustain genetic diversity, broadening the genetic base for improvement of agronomically important traits with the help of marker assisted selection (MAS). Breeder could introgress these agronomically important genes/QTLs using knowledge of molecular breeding techniques such as MAS to widen the genetic base for the improvement of yield potentiality as well as quality (Likian and Graner 2012; McCouch et al. 2012; Li and Zhang 2013; Ahmad et al. 2015; Agarwal et al. 2016; Babu et al. 2017; Singh et al. 2018). Genetic variability, heritability and genetic advance and QTLs mapping in rice has been studied in details to analyse the yield and yield components for crop improvement (Kalyan et al. 2017; Tefera et al. 2017; Sandeep et al. 2018; Laxmi and Chaudhari 2019; Roy and Shil 2020). Out of 950 million hectare arable land, 250 million hectare irrigated land is affected by salinity (Shahbaz and Ashraf 2013). Rice is the most sensitive cereal crops to salinity which is highly affected by saline soil conditions and considered as one of the major abiotic stresses (Eynard et al. 2015). More than 163 million hectare lands are used for rice cultivation over 100 countries mainly in South Asia and South-East Asia, because the region is climatically suitable for rice cultivation. Rice is the most important food crop, more than half ($\frac{1}{2}$) of the world's population depends on it for their sustainable livelihood. Population growth is increasing day by day and it will reach more than > 9 billion by 2050, and to feed the overpopulation we need to produce nearly double amount of food grains to fulfil the demand (Mammadov et al. 2018). Yield increase is not as per the required rate and becomes yield rate is in stagnation. Primary reason of yield stagnation is due to the narrow genetic base in the released varieties. Minimum number of parental lines were utilised to develop new crop varieties which ultimately leads to narrow genetic base. The narrow genetic base in the improved varieties is going to be a main bottleneck for crop improvement program which shield the yield increase.

Wild Rice as a Reservoir of Agronomically Important Traits

Many stresses (biotic and abiotic) have critically affected rice production throughout the world due to global warming, changing climatic conditions and in

addition non-durability of biotic resistance gene (s) incorporated into cultivars (Normile 2008). Previously it was considered that tolerance traits are negatively correlated with yield trait (Strauss et al. 2002; Wise 2007). Although reality is that transfer of diverse resistance traits into cultivars is not always easy task. Sexually compatible donor wild rice species can be used in conventional breeding process otherwise transgenic technique is to be employed to introgress the desired gene (s) from CWR to cultivars. Some poor qualities unwanted traits can be introgressed from the CWR during conventional breeding process due to linkage drag as a result complication arises in the varietal development. There is a hope to use modern biotechnological approaches such as DNA based molecular markers to eliminate the linkage drag. Thus, marker-assisted backcross breeding (MABB) has been employed as competent technique to quickly eliminate the linkage drag with a minimum number of generations (Peng et al. 2014; Vishwakarma et al. 2014). Different kinds of tools and techniques are being used to characterize and dissect the genetic traits prevailed in the wild rice species for introgression into the cultivars such as chromosomal assignment, monosomic alien addition lines, disomic introgression lines, chromosome segment substitution lines (CSSL), and backcross inbred lines (BIL) (Ali et al. 2010; Jena 2010). Wild species of *Oryza* are the genetic resources of many important traits (Yang et al. 2012; Sanchez et al. 2013) such as resistance to biotic and abiotic stresses (salinity, submergence, aluminium toxicity and drought) (Figure 1).

Wide Hybridization to Introgress Wild Gene into Cultivars

Development of CSSL and NIL lines through Pre-Breeding

Huge number of cultivated rice germplasm exist in the world but harbour a limited genetic diversity due to common parents and origin within single species *Oryza sativa* (Zheng et al. 2017). Due to narrow genetic base in these cultivated varieties, they are prone to attack of diseases and pest and less tolerance to abiotic stresses in this climate change scenario which leads to yield loss as a whole. It is needed to broaden the genetic base of the cultivated germplasm to make them sustainable and more efficient in yield potentiality (Zheng et al. 2017). Pre-breeding is one of the prime important approaches to utilize the wild germplasm of rice for

introgression of novel genes / QTLs / chromosomal segment with important traits to widen the genetic base of the cultivar as well as gaining popularity in rice improvement program. Whole genome sequencing (WGS) research has recognized that a large number of genetic loci have been selected and improved during hybridization and breeding (Huang et al. 2012; Xie et al. 2015; Zheng et al. 2017).

Chromosome segment substitution lines (CSSLs) are the genetic construct of wide hybridization in pre-breeding method and can be used as novel genetic stocks to be exploited in breeding program and genomic analysis services to identify and detect the characteristic features of agronomically important traits for crop improvement program (Balakrishnan et al. 2018).



Figure 1. Wild rice (*Oryza rufipogon* Griff.) is considered as immediate progenitor ancestor of cultivated rice (*O. sativa* L.). Wild rice is growing in natural habitat condition at marshy ditches of Raiganj, Uttar Dinajpur, West Bengal and reservoir of many agronomically important genes/QTLs.

Linkage Mapping and QTL Analysis

Linkage map will be constructed based on genotypic data of 150 plants of BC2F1 lines using 200 polymorphic SSR markers on all 12 chromosomes. The genetic linkage maps are to be prepared using the Software MapDisto v.1.7.5 (Lorieux 2012; <http://mapdisto.free.fr/MapDisto/>) and/or IciMapping software v4.1 (www.isbreeding.net). Recombination fraction is transferred to estimate map distance using the Kosambi mapping function (Kosambi 1943; Lorieux 2012). Genotyping by sequencing (GBS, Elshire et al. 2011) is to be conducted in the final generation of CSSL population BC4F2 for fine mapping of the quantitative traits (QTLs).

Improvement of Basmati Rice Varieties Using Marker Assisted Selection

Marker assisted backcross breeding (MABC) has been utilized for the introgression of disease resistance genes such as Xa13 and Xa21 (bacterial blight), genes for Blast, BPH (brown plant hopper) resistance genes and several abiotic stress tolerance components have been introgressed into a number of Basmati rice varieties. Marker assisted backcross breeding (MABC) has been utilized for the introgression of these genes into various rice cultivars Pusa Basmati 1, Pusa Basmati 1121 and Pusa Basmati 6 (Singh et al. 2012; Singh et al. 2013; Singh and Gopalakrishnan 2016). Wild rice *Oryza rufipogon* and *O. nivara* were used for yield enhancement in elite cultivars through introgression line development and QTL mapping (Sudhakar et al., 2012; Swamy et al., 2012). Yield improvement has been achieved through wide crossing by using wild rice *Oryza rufipogon* as a donor parent in elite cultivars (Thalapathi et al., 2012; Thalapathi et al., 2015). Utilization of “hidden genes” from wild species has emerged as a novel option for enrichment of genetic diversity for productivity traits. Alien gene has been introgressed into popular rice variety Pusa44 for yield enhancement (Gaikwad et al., 2014) from *O. rufipogon*.

Fungal Blast Disease Resistance Rice Varieties

Biotic stress such as blast disease is continued to be the constraint in rice production and becoming a severe problem worldwide in this global warming and climate change scenario (Umakanth et al.

2017). Blast is measured as the most severe and economically crucial disease caused by a fungal pathogen *Magnaporthe oryzae* (*M. oryzae*) (Wang et al. 2014). One of the most identifiable major biotic stresses is the blast disease caused by *Magnaporthe oryzae* (Umakanth et al. 2017). Although 100 major blast resistance genes (R-genes) have been identified, mapped and their tightly linked DNA markers are available (Miah et al. 2013), only one major gene (Pb1) has been reported for neck blast (Hayashi et al. 2010). In case of blast control, identification of resistance gene (termed as R-gene) is in prime importance, thus many R-genes (more than 100) and about 350 QTLs have been investigated from wild species of rice (Wang et al. 2014; Ashkani et al. 2015; Vasudevan et al. 2015). Some major R gene clusters viz. Piz, Pik, and Pita, were identified and mapped to chromosomes 6, 11, and 12, respectively and some of these are cloned (Wang et al. 2014). Cloned R-genes have been extensively studied at molecular level and have been found to encode nucleotide binding site-leucine-rich repeat (NBS-LRR) proteins (Wang et al. 2014). These types of R genes for blast tolerance have been characterized from wild rice species *O. minuta*, *O. australiensis*, *O. rufipogon*, and *O. rhizomatis*, and considered as precious germplasm to harbor blast resistance R-genes (Wang et al. 2014). These R genes have been transferred into susceptible varieties and confirmed the effectiveness against rice blast severe attack (Sharma et al. 2012). Durability is not so long if single R-gene has been introgressed, it will break by the various pathotypes within a minimum time span. Therefore, it is suggested to make stacking of broad spectrum R-genes to develop more durable resistance varieties with overlapping resistance spectra (Sharma et al. 2014). Therefore, it is necessary to explore new gene pool for R-genes from wild species to get ready before transfer to cultivars. Crop landraces are genetically more dynamic and adaptive equilibrium with both the environment and pathogens (Harlan 1975). Many potential landraces of rice are being replaced by high yielding varieties to meet the food requirements (Umakanth et al. 2017). Despite being less productive they are known to have a high genetic variability for several biotic stresses (Hanamaratti et al. 2008), so they can be explored for rice improvement. Genetic diversity among the rice populations has been precisely assessed by using advance marker technology along with morphological traits (Kumbhar et al. 2015). Molecular markers systems have been successfully exploited by others in rice germplasm

characterization (Zhang et al. 2011; Liu et al. 2015; Nachimuthu et al. 2015; Anandan et al. 2016). Among the molecular markers SSRs aid in accurately estimating the genetic diversity among rice germplasm and are accounted to be more efficient than single-nucleotide polymorphic markers (SNPs) (Singh et al. 2013; Nachimuthu et al. 2015).

Bacterial blight (BB) resistance rice varieties

Leaf blight is a devastating rice disease caused by bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo), yield loss goes as high as 75% in India, Indonesia, and the Philippines (Shakiba and Eizenga 2014). To date near about 41 resistance genes have been identified and out of which eight genes are very extensively characterized for use in breeding program (Ellur et al. 2016). Wild species are the main sources of resistance genes to be used in breeding program. One important bacterial blight resistance gene Xa21, was isolated through positional cloning approach from wild rice species, and found to encode a kinase-like receptor protein (Song et al. 1995). It has been reported that Xa21 is the first gene tagged with DNA markers and used extensively to develop resistant varieties through MAS. More durable resistance gene Xa23, a single dominant gene has been identified from wild rice *O. rufipogon*, with broad spectrum resistance efficacy and considered most promising gene highly resistant to majority of the Xoo strains (Zhang et al. 2001; Zhang and Xie 2014).

A new resistance gene, Xa38, has been recognized from *O. nivara* and its exploitation in breeding strategies is expected in near future (Ellur et al. 2016).

Conventional Breeding for the Development of Virus resistance rice varieties

It was reported that approximately 20 different types of viruses can infect rice and a majority of them use insects as vectors for their transmission. Among these two are more important because they cause significant damage to rice production such as rice grassy stunt disease; caused by rice grassy stunt virus (RGSV), and rice tungro disease; caused by two different variant- rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Insect brown planthopper (BPh) is the vector for transmission of RGSV. Infected plant shows stunted growth with a few panicles with deformed grains

sometimes no panicle. After screening of rice germplasm both cultivated and wild species, only wild rice *O. nivara* (AA genome, $2n=24$) was identified as a source of virus resistant trait of single dominant gene, Gs. It was the first successful report of transfer of agronomically useful gene (Gs) resistance to RGSV disease from wild rice species to cultivar (Khush et al. 1977). Other wild rice species *O. longistaminata* and *O. rufipogon* reported to be found as a source of resistance gene against tungro-virus diseases and has been used as donor to develop resistant varieties (Khush et al. 2004).

Conclusion

Wild rice germplasm is the good source of agronomically important traits and can be utilized all of these untapped hidden genes associated with many biotic and abiotic stress tolerance traits. Characterization and conservation of these wild *Oryza* species is utmost important for future food security purpose. Climate resilient and disease resistant improved rice varieties may be developed utilizing wild rice species in conventional breeding program.

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