

Towards the development of dry direct seeded rice varieties by stacking of multiple QTLs/genes

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ABSTRACT

Green revolution has brought transition in rice cultivation and architecture of plant. Further, the present way of rice cultivation system has been considered to be labor intensive, requires more of fresh water that yields marginal profit. Change in climate and stiff competition for quality water has warrant rice farmers to change their system of cultivation from transplanted to dry direct seeding. The changing climate scenario and reduced water availability has shown marginal increase in area of direct seeded rice. The dry direct seeded rice has changed the way of rice cultivation that requires less water, labor and promotes rice plant to be more efficient for nutrient than the existing one. Therefore, rice plant breeders are involved in developing suitable genotype adaptable for mechanized system of farming from sowing to harvest with increased efficiency towards water and nutrient.

Puddled transplanted rice (TPR) system with stagnant water irrigation is reported to increase the productivity, controls weed growth, and increases nutrient availability (e.g., iron, zinc, and phosphorus). However, the cost of cultivation of transplanted rice is increasing due to more energy for irrigation requirements, higher cost and amount of inputs and makes rice cultivation more expensive and less profitable. Thus, an alternate system of rice production is very much necessary for sustainability and economic security of rice farmers. Additionally, water is becoming precious commodity, groundwater tables are started falling, and wells are going dry. The green revolution in rice came from irrigated land, which further resulted in aquifer gets depleted. Thus, the trend indicates that the India's food production is water based and may burst (Garg and Hassan, 2007) any time. Rice requires ~3,500 L of water to produce 1 kg of rice and most of the rice growing states in India are also concerned about water consumption for urban needs. Thus, water use efficiency (WUE) in rice needs to be enhanced. Dry direct seeded / aerobic rice is one such extensive water-saving technology, which uses less water than conventional flooded rice (Atlin et al., 2006). Additionally, continuous standing water in paddy field

favors nitrogen loss (Zhu and Chen, 2002) and around 80-90% of applied phosphorus is not available to plants (Schindler et al., 2016). Thus, WUE and nutrient use efficiency (NUE) has to be combined for reducing the cost of cultivation and increasing the profit of the farmers.

Dry direct seeded /aerobic rice

Dry direct seeded rice refers to a cultivation of rice in aerated soil, leveled, unpuddled field conditions, where occasional water stagnation may occur under rainfed low land condition. While, aerobic rice refers to cultivation under a well-aerated condition at field capacity. Thus, dry-DSR cultivation practice is becoming popular in irrigated areas, where the problem of water shortage occurs (Kumar and Ladha, 2011). DSR is a water-saving technology which reduces labor requirements, mitigates greenhouse gas emissions, and more importantly the yield of the crop can be compared with that of transplanted rice (Kumar and Ladha, 2011). In China, temperate aerobic rice cultivars under supplementary irrigation showed grain yield of 6 t/ha (Bouman et al., 2005). But, in temperate zone countries such as United States, lowland rice varieties were tested under aerobic condition and 20-30% yield

reduction was observed. The reduced yield under aerobic was due to the reduction in panicles per meter square, spikelets per meter square, poor grain filling, and harvest index (Visperas et al., 2002). Even though, there was reduction in yield, the aerobic system can provide 4-6 t/ha of grain yield even under less rainfall of 400-600mm of water (George et al., 2001). In this review, we have highlighted QTLs and/or genes identified under DSR condition and discussed their utilization in developing new rice varieties suitable for dry DSR for sustainable rice production in climate changing scenarios

Grain yield and Spikelet fertility

The rice crop displays extensive genetic variation for aerobic adaptation. There are number of quantitative trait loci (QTL) reported for grain yield under both favorable irrigated and unfavorable upland ecology (Venuprasad et al., 2009). In aerobic condition, a consistent and strong QTL *qDTY6.1* was mapped to a 2.2 cM region between RM19367 and RM3805 for grain yield (Venuprasad et al., 2012). The *qDTY6.1* had a large effect on grain yield under favorable aerobic ($R^2 \leq 66\%$) and irrigated lowland ($R^2 \leq 39\%$) conditions, but not under drought stress. Therefore, *qDTY6.1* can be effectively used for rice grain yield under aerobic environment. Further, several QTLs were identified for grain yield such as *qGY1.1*, *qGY6.1*, *qGY8.1*, *qGY9.1*, and *qGY10.1*, found to be consistent across different mapping populations and also under different drought severities (Sandhu et al., 2015). On the other hand, QTLs *qGY8.1* (R^2 value of 34.0%), *qGY2.1* (R^2 value of 22.8%) and *qGY2.2* (R^2 value of 43.2%) were identified for grain yield under aerobic condition in multiple mapping populations. Ye et al. (2012) have identified two major QTLs *qHTSF1.1* ($R^2 = 12.6\%$) and *qHTSF4.1* ($R^2 = 17.6\%$), in BC1F1 and F2 progeny generated from the cross IR64 x N22. Later, they claimed that *qHTSF4.1* consistently increased spikelet fertility in different rice backgrounds. Therefore, it could be an important source for enhancing spikelet fertility in rice at the flowering stage.

Nitrogen and phosphorus use efficiency

Nitrogen is a major nutrient required for improving grain yield, root development, regulation of flowering time, and also grain quality. Several QTLs were identified

and reported for N use efficiency in rice. The genes involved in nitrogen metabolism such as glutamine synthase and NADH glutamate synthetase were reported to increase the nitrogen uptake in rice (Obara et al., 2001). The QTL Dense and Erect Panicle 1 (DEP1) coding for heteromeric G protein was reported to alter the panicle architecture and regulate N use efficiency in rice (Huang et al., 2009). Thus, marker assisted introgression of DEP1 could effectively used for increasing the yield and NUE of rice. Phosphorus is considered as one of the major nutrient essential for proper root development of rice crop (Bovill et al., 2013). The major QTL for low phosphorus tolerance phosphorus uptake 1 (PUP1) was characterized to be a protein kinase gene (Gamuyao et al., 2012). The PSTOL1 gene increases the root biomass of rice crop under low P condition. The expression of several phosphorus transporter genes has also been reported to play a major role in P uptake and redistribution in rice. PT2 and PT6 gene are high affinity phosphorus transporters found to be upregulated under low P condition. Till date, around 133 P associated QTLs have been reported for phosphorus use efficiency (PUE) (Mahender et al., 2017). Recently purple acid phosphatase (PAP21b) gene from Dular genotype was characterized to provide low phosphorus tolerance through enhancing the availability of P present in organic sources (Mehra et al., 2017).

Anaerobic germination, root hair density and lodging resistance

Anaerobic germination is considered as one of the important traits for direct seeded rice, where heavy rainfall occurs after sowing of seeds. Two major QTLs were identified for anaerobic germination in 9th chromosome of rice (*qAG9.1*, *qAG9.2*) (Angaji et al., 2010; Kretzschmar et al., 2015). These QTLs were reported to provide 33% phenotypic variance and can be effectively used in breeding program for uniform germination under water logged condition. Recently, two lodging resistance QTLs such as *qLDG3.1* and *qLDG4.1* were reported by Dixit et al. (2015) can be utilized in breeding program, as occasional flooding in lowland could damage the crop stand in DSR condition. Higher root hair density facilitates greater uptake of nutrients from soil under dry direct seeded condition. *QRHD1.1* and *RHD5.1* have been identified for greater root hair density in rice (Sandhu et al., 2015). Thus,

incorporation of these additional traits would assist in increasing the grain yield/ productivity of dry direct seeded rice.

Pest and disease prevalence under DSR system

The pest profile and the extent of damage caused by them vary significantly in DSR compare to the TPR system. The major biotic stresses under DSR system are rice blast, rice root-knot nematode, brown leaf spot and termites. Rice blast, the major disease in both the systems, is more severe in the water limiting conditions of DSR (Farooq et al., 2011). Imparting blast resistance has been one of the major breeding objectives in aerobic rice worldwide especially in countries like Brazil, where a significant proportion of rice area belongs to the DSR system (Bresseghele et al., 2006). Still, 100% yield loss due to blast has been reported in the Brazilian upland rice variety BRS colosso (Prabhu et al., 2009). In India, there are several reports of marker-assisted introgression of blast resistance genes into the background of popular rice varieties. Singh et al. (2011) developed improved Pusa 6A, Pusa 6B, and PRR78, the parental genotypes of rice hybrid Pusa RH 10 by transferring *Pi54* genes for blast resistance. Introgression of blast resistance genes *Pi1*, *Pi2* and *Pi33* into rice variety ADT43 was carried out at Tamil Nadu Agricultural University, Coimbatore. Scientists from Indian Agricultural Research Institute, New Delhi developed and released a blast-resistant variety Pusa Basmati 1637 through the transfer of *Pi9* gene through MAS. Bacterial blight (BB), though not severe as in the TPR system, is also capable of reducing grain yield under the DSR system. The variety, Improved Samba Mahsuri was developed by Indian Institute of Rice Research, Hyderabad, by introgressing BB resistance genes in the background of the popular variety Samba Mahsuri (BPT 5204). Three varieties have been released from NRRI, Cuttack with improved BB

resistance by marker-assisted pyramiding of BB resistance genes. They are Improved Lalat, Improved Tapaswini and CR Dhan 800 in the genetic background of popular rice varieties Lalat, Tapaswini and Swarna, respectively. Improved Lalat and Improved Tapaswini were developed through marker-assisted selection by pyramiding of four BB resistance genes (*Xa4*, *xa5*, *xa13* and *Xa21*) and were released and notified for Odisha in 2012. Subsequently, CR Dhan 800 with three bacterial blight resistant genes *Xa21*, *xa13* and *xa5* in the background of Swarna was released in 2016.

The yield decline observed under consecutive cultivation of aerobic rice in the same crop fields (Peng et al. 2006) is attributed partly to the buildup of rice root-knot nematodes (*Meloidogyne graminicola*) and soil borne fungi, mainly the oomycetes (Verbeek et al., 2016). Based on the experiments conducted using the upland cultivar Apo, Kreye et al. (2009), reported that the rice root-knot nematodes being the major factor leading to yield decline in the DSR system in the Philippines. Three different species of the oomycete fungi, *Pythium*, has been associated with yield reduction in aerobic rice in the Philippines (Van Buyten et al. 2013). Brown spot of rice caused by the fungus *Helminthosporium oryzae* is more severe in upland rice compared to the TPR system (Savary et al., 2005). In addition to these factors, weed infestation especially at the seedling stage and the resultant yield loss is found to be more severe in the DSR systems (Singh et al., 2006). Yield loss in the range of 20-80% has been reported under DSR system (Rao et al., 2007). Development of herbicide-resistant weeds, due to the increased use of herbicides, and emergence of weedy rice are two main constraints observed in areas where DSR replaced the TPR system (Kaur and Singh, 2017). Therefore, breeding rice varieties for direct seeded system combining high yield, early vigour and strong weed competitiveness is an alternative to avoid

Table 1. Rice varieties released from ICAR-NRRI for dry direct/aerobic condition.

S.No.	Variety	Parentage	Duration (days)	Grain yield (t/ha)
1	CR Dhan 200 (Pyari)	CR 2624-IR 55423-01	115-120	4.0
2	CR Dhan 201	IRRI 76569-259-1-2-1/CT 6510-24-1-2	110-115	3.8
3	CR Dhan 202	IRRI 148/IR 78877-208-B-1-1	110	3.7
4	CR Dhan 203 (Sachala)	IR78877-208-B-1-1/IRRI 132	110-115	4.0
5	CR Dhan 205	N22/Swarna	105-110	4.2
6	CR Dhan 206	Brahmannaki/NDR 9930077	105-110	4.2
7	CR Dhan 207 (SRIMATI)	IR71700-247-1-1-2/IR57514-PMI 5-B-1-2	110 -115	4.5
8	CR Dhan 209 (Priya)	IR72022-46-2-3-3-2/IRRI 105	110 -115	4.5

Table 2. List of donors and QTLs/genes of different traits suitable for dry direct seeded rice

S.No	Donors	Trait	QTLs/genes	References
1	IR93312-30-101-20-13-66-6	Anaerobic germination	qAG9.1, qAG9.2,	Angaji et al.(2010), Kretzschmar et al. (2015)
2	IR94226-B-177-B	Nodal root Early vigour	qNR5.1 qEUV9.1 qRHD1.1	Sandhu et al. (2015) Sandhu et al. (2015) Sandhu et al. (2015)
3	IR 94226-B-177-B, WAB 880-1-27-9-2	High nutrient uptake (N,P, Fe, Zn)	qNR5.1	Sandhu et al. (2015)
4	IR94225-B-82-B	Grain yield DS	qGYDS1.1, qGYDS6.1, qGYDS9.1, qGYDS10.1	Sandhu et al. (2015)
5	IR91648-B-289-B	Lodging resistance	qLDG3.1, qLDG4.1	Dixit et al. (2015)
8	IR 91648-B-32-B	Early uniform emergence	qEUE11.1	Dixit et al. (2015)
9	Abhaya	Gall Midge	Gm4	Shrivastava et al. (1993)
10	Rathu Hennati/IR64	BPH	BPH3, BPH17	Jairin et al. (2007), Sun et al. (2005)
11	IRBL9	Blast	Pi9	Qu et al. (2006)
12	Aganni	Gall midge	Gm8	Kumar et al. (2000)

herbicide tolerance in weeds and environment pollution. Studies on QTL for seedling vigour and related traits have been reported in rice and well reviewed by Mahender et al. (2015). Recently, Anandan et al. (2016) has reported pleiotropic effect of marker RM341 on chromosome 2 for shoot dry weight on 28 DAS, vigour index on 14 and 28 DAS.

Research on DSR at ICAR-National Rice Research Institute

Aerobic rice breeding was started in the year 2007 at NRRI, Cuttack through hybridizing high yielding irrigated rice varieties with drought tolerant lines, aerobic rice germplasm and other exotic donors with the support of the Asian Development Bank (ADB). The work has resulted in development of promising genotypes and several segregating populations. Also, the fixed lines from IRRI, Philippines were evaluated and a promising variety Apo was identified from AICRIP Varietal Improvement Programme during 2012-13 and released as CR Dhan 200 (CR 2624-IR55423-01; IET 21214) by the Odisha State Sub-Committee on Crop Standards, Notification, and Release of Varieties. Subsequently, seven aerobic varieties were released through CVRC and SVRC from the work of NRRI, Cuttack (Table 1).

Upland rice is known for significant P uptake ability under deficient condition. Therefore, a large scale phenotyping trial under P deficient soil (6 ppm/kg) was conducted at NRRI, Cuttack using 70 upland rice genotypes and wild species (*O. nivara* and *O. rufipogon*) along with two tolerant checks (Kasalath and Dular) and one susceptible check (IR 36). It resulted

in identification of eight upland genotypes and four wild species exhibited superiority over positive checks. The presence of *PUP1* locus was genotyped and the genotypes AC100062, AC100117, Dular, Sekri, Brown gora and AC 100117 had similar allelic pattern with Kasalath (NRRI annual report 2014-15). Similarly, Pandit et al. (2016) genotyped 96 upland cultivars and landraces for better P-uptake and found 46 genotypes possessed the *Pup1* locus. The genotypes N22, Dinoroda, Bowde, Bamawpyan, Tepiboro, Karni, Lalsankari, Surjamukhi, Hazaridhan, and Kalinga III were positive for two closest flanking and two gene specific *Pup1* markers.

To develop high yielding variety over the existing varieties (Table 1) suitable for dry-DSR, a comprehensive breeding program was initiated at NRRI, Cuttack in collaboration with IRRI, for introgression of 19 reported QTLs of ten different traits (Table 2). The QTLs taken for the crossing program includes, QTLs for anaerobic germination (*qAG_{9.1}*, *qAG9.2*), early uniform emergence (*qEUE_{11.1}*), early vigour (*qEUV_{9.1}*), higher nutrient uptake through higher root length density (*qNR_{5.1}*, *qRHD_{1.1}*), nodal root (*qNR5.1*), lodging resistance (*qLDG_{3.1}*, *qLDG_{4.1}*), grain yield (*qGYD_{1.1}*, *qGYD_{6.1}*, *qGYD_{9.1}*, *qGYD_{10.1}*), gall midge (*Gm4*, *Gm8*), brown plant hopper (*BPH3*, *BPH17*) and blast (*Pi9*). The 19 QTLs were spread across 12 advanced breeding lines, used as donors for the study. Pair wise-crossing was attempted and trueF₁s from one single cross was hybridized with that of the other single crosses to produce the first inter-crossed

hybrids (IC₁F₁s) during *Kharif*-2016. The three IC₁F₁s were then crossed in all possible combinations during *Rabi*-2017. The true IC₁F₁ genotypes carrying more than 6 QTLs have been selected for further convergence through hybridization during *Kharif*-2017. The IC₁F₁s were genotyped using QTL specific polymorphic markers and flanking markers for corresponding genes/QTLs. Further, the individuals carried higher number of genes/ QTLs (≥3) were identified and were crossed to generate the IC₂F₁ generation. The IC₂F₁ genotypes carrying more than six genes/QTLs were selected for further crossing and convergence using marker assisted genotyping. Thus, the stacking of multiple QTLs/gene in single background for nutrient use efficiency, lodging resistance, seedling vigour, anaerobic germination, yield related QTLs could assist in development of dry direct seeded rice varieties with greater productivity. Also, the input cost will be drastically reduced due to nutrient efficient rice lines which will ultimately leads to greater economic returns to the farmers.

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