Enhancing Input Use Efficiency in Direct-Seeded Rice with Classical and Molecular Breeding

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SUMMARY

The dwindling freshwater resource and rising cost of rice cultivation have lead to the debate of ways to enhance net return from rice cultivation for ever increasing food demand for growing population. A resource use efficient system of rice cultivation with water use efficient varieties is very much needed to improve the water productivity in agriculture. Dry direct-seeded/aerobic rice system is a strategy that ensures water use efficiency in rice crop to cope with the looming problem of water scarcity. To achieve high yield, a suitable variety for this condition needs to have tolerance for moisture stress, input use efficiency, weed competitiveness, and location-specific pest and disease resistance. Recent developments in the field of dry direct seeded/aerobic rice, yield component and nutrient use efficient QTLs and breeding strategies are discussed in this chapter to improve grain yield.

1. INTRODUCTION

Puddled transplanted rice (TPR) system with stagnant water irrigation system is reported to increase the productivity more than other systems of cultivation, wherein restrained weed growth, trouble-free seedling establishment and increased nutrient availability (e.g. iron, zinc, and phosphorus) are advantages of the TPR. However, the hefty sum of water, more input, energy and time are required for TPR and these factors make rice cultivation more expensive and less profitable. Thus, improving the crop productivity and economic security to farmers with an alternate system of rice production is very much necessary. Therefore, shifting of rice cultivation from TPR to dry direct-seeded rice (DSR) is necessary to make income from rice cultivation sustainable. Additionally, water has become a precious commodity in the era of modern agriculture, but groundwater tables are started falling, and wells are going dry. Even though, the green revolution met the food demand for our country, there is a demand to extract water for irrigation has also started simultaneously. Further, millions of farmers started to drill deeper irrigation bore wells to expand their harvests and resulting in running down of groundwater table and wells in 20 countries, including three countries that together produce half the world's grain viz., India, China, and the United States (Somanathan 2010). Consequently, the increase in production of cereal grain came from irrigated land that has 40 percent share. But this inflated production from irrigated land will burst when the aquifer gets depleted. Therefore, the decline in the supply of irrigation water is of great concern. In India, three fifths of grain harvest come from irrigated land (Dick and Rosegrant 2009), where over-pumping of water for irrigation is predominant. The states such as Punjab and Haryana witnessed falling water table and green revolution achieved in India was based on water mining. In Punjab, the number of tube wells has been increased from 1.9 lakhs in 1970 to 14 lakhs in 2010, indicates that India's food production is water based and may burst (Garg and Hassan 2007) any time.

The rural-urban competition for water resource is gradually intensifying in India and was evident that water needs of Chennai depend on popular tank-truck industry. This stiff competition for water in urban areas of India does not favours the farmers, as producing 1 kg of rice requires ~3,500 L of water. The greatest impact on water consumption for urban needs is likely to continue well into the middle of the twenty-first century (FAO 2006). Consequently, in a point, water scarcity would translate into food scarcity. Thus, to reduce the level of water dependency and to improve the water productivity in rice cultivation, water use efficient rice genotypes are one of the viable options. Aerobic rice is one such extensive water-saving technology for rice cultivation/production compared to other production methods. Aerobic rice systems use less water than conventional flooded rice, by using suitable rice varieties capable of responding well to reduced water inputs in non-puddled and non-saturated soils (Atlin et al. 2006). Additionally, continuous standing water in paddy field favours more nitrogen leaching loss than either in field capacity (aerobic) or alternate wetting and drying. Moreover, application of recommended and/or excessive fertilizer is not fully utilized by the plant. The unutilized portion escapes into environment through runoff, leaching, ammonia volatilization, and N_2O into atmosphere and water systems (Zhu and Chen 2002). On the other hand, around 80–90% of applied P is also not easily available to plant and transforms into other forms and eventually lost into the water body causing eutrophication (Schindler et al. 2016). This detrimental impact needs to be addressed by increasing the Nutrient Use Efficiency (NUE) of rice in concert with other management practices. It is therefore critical that strategies for genetic improvement of NUE needs to be undertaken for cultivating rice with less water and higher NUE to maximize biomass and yield. Reducing the cost of inputs would be one of the prime solutions to increase farm income in rice cultivation. Additionally, the genetic potential of yield could be achieved through proper nutrient and weed management. This chapter addresses the major avenues for increasing the productivity and profitability of farmers by increasing the NUE under DSR, where labor workforce shortage is highly prevalent in eastern India.

2. PROBLEMS OF CONVENTIONAL PUDDLED TRANSPLANTED RICE

The anticipated climate change, shortage of water and natural resources and shortage of man power are likely to be major researchable issues in future. Under such circumstances, direct-seeded rice system can mitigate the adverse situation for rice cultivation and minimizes the negative impacts of TPR by reducing the labor requirement and, increasing water and NUE. In Asia, the practice of dry seeding is popular and extensively adopted in rainfed lowlands, uplands, and flood-prone areas with an area of 26% in south Asia and 28% in India. Globally, 23% of rice is direct seeded, as wet seeding is being the common practice and predominantly practiced in irrigated areas of Australia, Brazil, Chile, Cuba, France, Italy, Japan, Korea, Malaysia, the Philippines, Thailand, Russia, Sri Lanka, Vietnam, and in some parts of Iran, due to shortage of skilled labour, high labour cost and availability of mechanised system (Mahender et al. 2015). Thus, DSR system of cultivation should be encouraged, as agricultural labor workforce is reducing relative to the total workforce in India. It is reported that ~30 million agricultural labor workforces in India was reduced as compared to 2004-05 (FICCI report). The states of Uttar Pradesh (28%), Odisha (14%), Bihar (12%) and West Bengal (12%) contributed on an average of 16.5% reduction in agricultural workforce in India.

DSR system showed substantial water saving. Trials conducted in Haryana by adopting zero or reduced till system resulted in good grain yield comparable with TPR under less water with more water productivity and greater net profit. Moreover, it increases net return, efficiency in water and fertilizer use (Anandan et al. 2015). The varieties released during and after green revolution has ability to utilize less than 50% of applied fertilizer. These varieties combined with intensive agricultural practices, effects environment through methane emission and eutrophication of water bodies. Therefore, DSR, helps farmers to earn more carbon credits than TPR by mitigating methane emission and provides higher economic returns, saves water and reduces labour requirement. However, no specific varieties possessing all suitable traits of DSR have been developed with nutrient and water use efficiency. Varieties that are necessary for DSR should possess good mechanical strength in the coleoptiles to make easy emergence of the seedlings under crust conditions, weed competitiveness with early seedling vigour, efficient root system to tap soil moisture and nutrients (Fig. 1 and 2), early maturing, photoperiod-insensitive with better drought tolerance and yield stability. Specifically, improving NUE is very much needed in our rice-wheat cropping system in India. Moreover, the consumption of N fertilizer has been increased from 0.06 Mt in 1950-1951 to 10.8 Mt in 2000-01 and P fertilizer has steeply increased from 0.01 Mt to 1.8 Mt in the last 50 years (FAI 2000-2007). Correspondingly, N and P fertilizer contributed around 64% and 78%, respectively in Indian agriculture (Pathak et al. 2010). Further, Pathak et al. (2010) highlighted that states like Tamil Nadu, Gujarat, Haryana, Punjab and West Bengal showed 50% N use efficiency and less than 50% N use efficiency was observed in the states like Bihar, Odisha and Uttar Pradesh. Also, Odisha and Bihar showed minimum P balance (Pathak et al. 2010). Thus, there is a need to increase the N use efficiency and PUE of rice in eastern India to reduce the cost of cultivation in sustainable way and increase the per capita food availability. Developing rice cultivars with improved NUE becomes a prerequisite in protecting the environment by reducing the rate of nutrient loss into the ecosystems. It also reduces input cost and improves rice yield in a sustainable manner, while maintaining soil and groundwater quality. Nutrient use efficient varieties can also be raised in marginal lands where nutrient availability is limited. Therefore, the present breeding program should be prioritized to develop rice varieties with high grain yield under

Fig.1. Variability in rice root system.

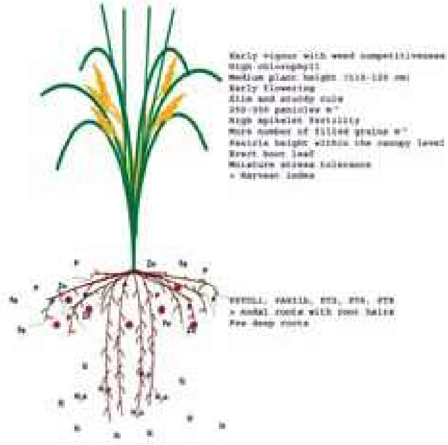

Fig. 2. Direct seeded rice genotypes should possess good numbers of lateral and deep roots for absorbing nutrient and water in symbiotic association with AMF and bacteria efficiently from soil.

low-nutrient conditions (Vinod and Heuer 2012). Significant genotypic differences in nitrogen (Singh et al. 1998) and phosphorus (Wissuwa and Ae 2001) use efficiency exist in rice with several mechanisms and morpho-physiological traits to sustain their growth. Among the several rice accessions, it is observed that landraces are being superior in nutrient uptake. It is reported that P concentration varied from 0.6 to 12.9 mg P plant^{-1} (Wissuwa and Ae 2001). Therefore, the variability exists in the form of morpho-physiological traits related tolerance to P deficiency could effectively be exploited through systematic breeding program to develop cultivars with high NUE and water use efficiency (Ali et al. 2012).

In order to increase the NUE, traits involved in nutrient absorption, transport, utilization and mobilization should be identified and in combination with best management practices it could provide sustainable rice cultivation. On the other hand, role of microorganisms in enhancing the availability of N and P needs to be recognized. Several reports have proved that inoculations of the microbial consortium have been shown to enhance NUE particularly phosphorus, nitrogen, and carbon in many crop plants. Rice differs from most of the crops, since it is typically cultivated in flooded soil, resulting in aerobic and anaerobic zones within the rice rhizosphere, and preferred by specific physiological groups of microorganisms i.e. aerobic, anaerobic, or facultative (Brune et al. 2000). Hence, it is essential to understand the soil microbes and plant interaction for better input resource management for sustainable rice cultivation. Also, presence of variability in microbial association with plants (Hardoim et al. 2011) provides the possibility of breeding cultivars for specific microbial community (Mahender et al. 2017) to gain NUE. The objectives of this chapter are i) to review the water and nutrient (N and P) use efficiency scenario in rice cultivation of India, and ii) to understand how to improve water and NUE in rice through classical and molecular breeding approaches.

3. DRY DIRECT SEEDED/AEROBIC RICE ON WATER USE EFFICIENCY

Dry direct seeded rice refers to a cultivation system in which rice is dry direct seeded in well-tilled levelled fields with the uniform slope under unpuddled conditions. When the crop is cultivated with no standing water throughout the season under a well-aerated condition at field capacity is termed as aerobic rice, occasional water stagnation may occur under rainfed low land condition. Thus, the high proportion of water savings associated with this method compared with conventional rice growing practices has made this method increasingly popular in irrigated areas, where the problem of water shortage occurs (Kumar and Ladha 2011). The affinity of the rice crop with water is universally known. Rice cultivation in puddled fields is well known, technologies such as dry/aerobic and wet direct seeding and alternate wetting and drying (AWD) could be aviable option to produce rice in both irrigated and rainfed rice ecosystems. Aerobic rice is one such extensive water-saving technology for rice, reducing labor requirements, mitigating greenhouse gas emissions, and adapting to climatic risks; and the yield can be compared with that of transplanted rice if the crop is properly managed (Kumar and Ladha 2011).

In Brazil and northern China, aerobic rice is grown commercially in 140,000 ha. In China, temperate aerobic rice cultivars under supplementary irrigation exhibited grain yield of 6 t/ha (Bouman et al. 2005). These varieties need 60% less water than that required for lowland rice and their total water productivity was 1.6-1.9 times higher (Guang-hui et al. 2008). In temperate zone country like the United States, lowland rice varieties were tested under aerobic condition and observed 20-30% yield reduction in high yielding cultivars (7-8 t/ha). The decline in yield under aerobic was due to the reduction in panicles per meter square, spikelets per meter square, poor grain filling, and harvest index. Comparatively, flooded rice had 20% more panicles per meter square, 15% more spikelets per meter square and 13% higher grain filling than aerobic rice (Visperas et al. 2002). Under scanty water supply of 450-650 mm, 4-6 t/ha of grain yield was observed in much drier soil condition against 1300-1500 mm of water used in lowland situation. Other than the merit of efficient use of water, aerobic rice demonstrated better nitrogen use efficiency (George et al. 2001).

The rice that is being grown extensively in the upland ecosystem (direct seeding), showed wide genetic variation for aerobic adaptation in rice germplasms. Several quantitative trait loci (QTL) for grain yield have been reported for both favorable irrigated and unfavorable upland ecology (Venuprasad et al. 2009). However, very few reports have reported the genomic regions responsible for increased aerobic adaptation of rice. Few encouraging reports are available regarding grain yield QTLs found under aerobic condition from International Rice Research Institute (IRRI), Philippines. Venuprasad et al. (2012) reported two closely linked rice microsatellite (RM) markers RM510 and RM19367 located on chromosome 6 were found to be associated with grain yield under aerobic soil conditions, consistently in three genetic backgrounds. The QTL linked to this marker, *qDTY6.1*, was mapped to a 2.2 cM region between RM19367 and RM3805 at a peak LOD score of 32 in the Apo/2*Swarna population. The effect of *qDTY6.1* was tested in a total of 20 hydrological environments over a period of five seasons and in five populations in the three genetic backgrounds (Apo/2*Swarna, Apo/IR72, and Vandana/IR72). In the Apo/2*Swarna population, *qDTY6.1* had a large effect on grain yield under favorable aerobic (R^2 d” 66%) and irrigated lowland (R^2 d” 39%) conditions but not under drought stress. Further, they conclude that *qDTY6.1* is a large-effect QTL for rice grain yield under aerobic environment and could potentially be used in the molecular breeding of rice for the aerobic environment. So far, no variety has been developed that possesses traits specifically needed to produce high yield under aerobic conditions, particularly for rainfed systems that may be prone to low fertility (Sandhu et al. 2015). Kato et al. (2009) suggested that aerobic rice varieties should possess large numbers of spikelets and sufficient adaptation to aerobic conditions will consistently achieve yields comparable to the potential yield of flooded rice.

Sandhu et al. (2015) identified several promising QTLs that showed large and consistent effects from two mapping populations derived from crosses of Aus276, a drought tolerant *aus* variety, with MTU1010 and IR64, high-yielding *indica* mega-varieties. They have reported that QTLs *qGY1.1*, *qGY6.1*, and *qGY10.1* were found to be effective in both populations under multiple conditions. On the other hand, several of the QTLs identified for grain yield in their study (*qGY1.1, qGY6.1, qGY8.1, qGY9.1,* and *qGY10.1*) were found to be previously reported and consistent across different mapping populations, under different drought severities.

In 2012, Ye et al. (2012) have identified two major QTLs *qHTSF1.1* ($R^2 = 12.6\%$) and *qHTSF4.1* ($R^2 = 17.6\%$), were detected on chromosome 1 and 4, respectively, in BC_1F_1 and F_2 progeny generated from the cross IR64 x N22. Later in 2015, Ye et al. through fine-mapping validated the effect of *qHTSF4.1* with PCR-based SNP markers. They found that the sequence in the QTL region is highly conserved and large numbers of genes in the same gene family were observed to be clustered in the region. The QTL *qHTSF4.1* consistently increased spikelet fertility in all of the backcross populations (BC_2F_2, BC_3F_2, BC_3F_3, and BC_5F_2) and this was confirmed again in 24 rice varieties. Most of the rice varieties with this QTL showed a certain degree of increase in spikelet fertility. In a BC_5F_2 population with the clean background of IR64, QTL *qHTSF4.1* increased spikelet fertility by about 15%. Therefore, it could be an important source for enhancing spikelet fertility in rice at the flowering stage. PCR-based SNP markers developed from their study would be useful for QTL introgression and for pyramiding with other agronomically important QTLs/genes through marker-assisted selection.

Several research institutes/universities across India started developing rice varieties that consume less water for rice cultivation with more water use efficiency. ICAR-NRRI, too involved in aerobic rice research and developed 9 aerobic (water use efficient) rice varieties and released through Central Sub-Committee on Seed Standards, Notification and Release (CVRC) and State Variety Release Committee (SVRC). Three aerobic rice varieties Anagha (ARB 6), MAS 26 and MAS 946-1 were released from the University of Agricultural Sciences (UAS), GKVK, Bangalore for the state of Karnataka. Performance of these varieties was found to be well under aerobic with fair degree of drought tolerance. These genotypes need to be irrigated at the intervals of 5 to 7 days and irrigation can be skipped in the event of rainfall. The grain yield potential of these lines was 7.0 t/ha in station trials at UAS, Bangalore, while in farmers' field it recorded with an average yield from 3.0 to 5.0 t/ha (Shashidhar 2012).

Efforts have been taken to identify QTLs responsible for grain yield under aerobic/ dry direct seeded rice in India. However, limited reports are available in relation to QTLs. Recently, Sandhu et al. (2013) have mapped 35 QTLs associated with 14 traits on chromosomes 1, 2, 5, 6, 8, 9, and 11 in MAS ARB25 x Pusa Basmati 1460 and 14 QTLs associated with 9 traits were mapped on chromosomes 1, 2, 8, 9, 10, 11, and 12 in HKR47 × MAS26 from CCS Haryana Agricultural University, Hisar. The QTLs, *qGY8.1* (R^2 value of 34.0%) and *qGY2.1* (R^2 value of 22.8%) of MAS ARB25 × Pusa Basmati 1460 population and QTL *qGY2.2* (R^2 value of 43.2%) of HKR47 × MAS26 population were found promising for grain yield under aerobic condition. Among the three yield QTLs, *qGY8.1* showed an increased stable effect over two different years and combined over two years with 26.6% yield improvement. Further, the authors highlighted the QTL hotspot region at 25.1cM segment between RM589 and RM314 on chromosome 6 affects different root (RV, RT, and FRW) and shoot (FSW and DSW) traits under aerobic conditions of two mapping populations (MAS ARB25 x Pusa Basmati 1460 and HKR47 × MAS26). This region is found to be co-localized with *qDTY6.1* region reported by Venuprasad et al. (2011). It was found to be associated with grain yield in the aerobic environment, in total of 20 hydrological environments over a period of five seasons and in five populations in three genetic backgrounds using bulk-segregant analysis (Venuprasad et al. 2011).

4. NUTRIENT USE EFFICIENCY

Nitrogen is an imperative element for improving higher grain yield, root development for uptake of water and other nutrient elements from the soil, regulation of flowering time, and grain quality. Several QTLs have been identified for N use efficiency in rice. In a mapping population of Nipponbare and Kasalath, 6-7 QTLs have been identified for glutamine synthase and NADH glutamate synthetase involved in nitrogen uptake pathway (Obara et al. 2001). Similarly, qNUEP-6, pneu9 and QTL for low N tolerance have been reported in rice (Zhou et al. 2017). Over expression of one of the nitrate transporter gene OsNRT3.2b has been reported to increase the yield of rice. Interestingly, a heterotrimeric G protein was identified to regulate N use efficiency in rice (Sun et al. 2014). This gene was previously identified as dense and erect panicle 1 (*DEP1*) and reported to alter the panicle architecture. In addition, natural variation of DEP1 locus was shown to increase the yield of rice (Huang et al. 2009). Thus, this gene could be used for simultaneously increasing the yield and NUE of rice. The genetic loci associated with N use efficiency were mapped in ASD16 x Basmati 370 population (Senthilvel et al. 2004). In another report, seven QTLs were identified for nitrogen use in the mapping population of IR64 x Azucena (Senthilvel et al. 2008).

Next to nitrogen, phosphorus is considered as one of the major nutrient for rice and essential for better root development (Bovillet al. 2013). The available form of phosphorus in the soil is limited due to its fixation nature in the soil. Several reports have suggested the possibility of improving the genetic potential of rice towards efficient utilization of phosphorus. The major QTL for low phosphorus tolerance 'phosphorus uptake 1' (*Pup1*) was identified in the *aus* genotype Kasalath and the causal gene (*PSTOL1*) was found to be a protein kinase (Gamuyao et al. 2012). It increases the root biomass of rice crop under low P condition. On the other hand, *OsPHO1* gene was identified to play an important role in transfer of P from roots to shoots (Secco et al. 2010). Several phosphorus transporter genes have been identified to play an important role in P uptake and remobilization in rice. The expression studies of phosphorus transporter genes have identified *PT2* and *PT6* gene as high affinity phosphorus transporter gene in rice and *PT8* as gene responsible to transport of P from source to sink organs in rice (Li et al. 2015). Till date, several QTLs have been reported for phosphorus use efficiency (PUE) in rice. Further, Mahender et al. (2017) has done a comprehensive review on QTLs and recent advances on PUE. They reported that to date around 133 P associated QTLs of morpho-physiological traits were available and found to be distributed on all 12 chromosomes and the majority of them were localized on chromosome 1, 2 and 12. A high density SNP mapping of RIL population has identified 26 QTLs for eight PUE traits (Wang et al. 2014). Apart from PUE traits, genetic variation of root traits has been studied to understand the P uptake and low P tolerance in rice (Vejchasarn et al. 2016). Recently, Mehra et al. (2017) characterized purple acid phosphatase (*PAP21b*) gene from Dular genotype that confers low phosphorus tolerance through enhancing the availability of P present in organic sources in the soil. On the other hand, Yugandhar et al. (2017) screened six N22 mutants in three conditions, normal, low P and AWD and estimated the genetic diversity. They found that *Pup1* gene-specific marker, K-1 was associated with tiller number under low P conditions.

Phosphorus use efficiency can also be improved by mycorrhization of the rice plant with Arbuscular Mycorrhizal Fungi (AMF) that causes significant mobilization of insoluble P in substrate and plant uptake. Arbuscular mycorrhizal fungi inoculated rice through direct or indirect mechanism facilitates uptake of P from poorly soluble P (Panneerselvam et al. 2016a; 2016b), Zn, Cu etc. (Harley and Smith 1983; Wellings et al. 1991). The AMF colonization in roots helps the plants to uptake fixed soil P by rhizosphere modification through multiple mechanisms, by secretion of organic acids, phosphatase enzyme and metabolites like siderophores (Shenoy and Kalagudi 2005; Panneerselvam and Saritha 2017). The mycorrhizal plant also could absorb P from poorly soluble P source like aluminum phosphates, iron and rock phosphate (Shenoy and Kalagudi 2005). Reports suggests that AMF association in plants also causes changes in pH and root exudation profile (Li et al. 2001; Sowarnalisha et al. 2017), which may alter the rhizosphere microbial community and in turn, enhances P solubilization mechanism. However, it was observed that the plant growth performance and nutrient uptake will vary from species to species; hence there is a need for selection of suitable/efficient AMF for better plant growth and development (Bagyaraj et al. 1989).

5. RESEARCH ON DIRECT SEEDED RICE AT ICAR-NATIONAL RICE RESEARCH INSTITUTE

The aerobic rice breeding at ICAR-NRRI was initiated with the support of the Asian Development Bank (ADB) by hybridizing high yielding irrigated rice varieties with drought tolerant lines, aerobic rice germplasm and other exotic donors from International Rice Research Institute (IRRI), Philippines. Under this project, large variability of genotypes for the aerobic condition was generated and promising genotypes were selected by adopting the pedigree breeding method. On the other hand, several segregating populations and fixed lines were introduced from IRRI, Philippines, to select superior lines under Cuttack condition. In 2012, a promising variety Apo was identified from AICRIP Varietal Improvement Programme. Apo is one of the popular aerobic variety of Philippines was found suitable in Odisha, and it was 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. At NRRI, promising aerobic lines nominations were started in 2007 to the national AICRIP trials for evaluation of materials at various target locations in different states of the country. Subsequently, nine aerobic varieties were released through CVRC and SVRC (Table 1).

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

Upland rice is known to have significant uptake ability of P under deficient condition. Therefore, 70 upland rice genotypes and wild species (*O.nivara* and *O. rufipogon*) were screened under P deficient soil (6 ppm) with two tolerant checks (Kasalath and Dular) and one susceptible check (IR 36). Among them, eight upland genotypes and four wild species exhibited superiority over the positive checks. They were genotyped to assess the presence of *Pup1* allele. Genotypes AC100062, AC100117, Dular, Sekri, Brown gora and AC 100117 had similar amplification pattern with Kasalath (NRRI annual report 2014-15). Similarly, Elssa Pandit et al. (2016) genotyped 96 upland cultivars and landraces for better P-uptake. Among them, 46 possessed the Pup1 locus that accounts for 47.92% of the total genotypes considered. 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

6. KNOWLEDGE GAPS

Direct seeded rice is a viable alternative to TPR and suitable for targeted areas suffering from water availability and labor scarcity. Further, DSR/aerobic rice is found most suitable for rainfed lowland areas where insufficient precipitation occurs, delta regions where there is no availability of water in-time or delay in water release from reservoir (Anandan et al. 2015), limited water during early stage of crop growth but later crop faces flood, pumping from deep bore well and favorable upland has access to supplementary irrigation (Bouman 2001) are the most suitable location to adapt DSR/aerobic rice. Accordingly, Chhattisgarh, parts of Bihar, Jharkhand, Karnataka, Odisha, Tamil Nadu, and eastern Uttar Pradesh are the projected areas, where there is an uneven distribution of rainfall and frequent occurrence of soil moisture limitation. Further, the maintenance of physical soil structure in DSR helps to have timely sowing of succeeding crop.

The success of DSR system depends on availability of suitable variety and crop management practices. However, non-availability of suitable breeding program and limited knowledge on the genetics of donors are the major drawback of this system to achieve adaptable and maximum yield potential under DSR. The ICAR-NRRI, Cuttack has released several rice varieties (Table 1) suitable for DSR/aerobic condition and the grain yield of those genotypes yield 4.5t/ha (Anandan et al. 2015) under continuous aerobic condition. To improve the grain yield further, several traits adaptable for DSR need to be addressed.

The phenotypic traits directly relevant to DSR genotypes should have the increased ability to germinate from deeper soil depth with longer coleoptile and mesocotyl length and uniform germination ability to have uniform plant population with early seedling vigour (Anandan et al. 2016a; 2016b). As weed has the major impact on DSR, uniform plant population with uniform height facilitates timely intercultural operation to manage weeds. Increased numbers of nodal roots, root length density, lateral root length and branching with more root hairs for proper nutrient uptake under dry conditions are necessary traits for DSR (Fig. 2) (Kumar et al. 2017). Anaerobic germination is another important trait that improves crop establishment in uneven field or flash flood after rain. Roots of DSR variety should have sufficient plasticity to function under both aerobic and water logging condition. During early stage of crop establishment, the seedling should possess early vigour with good canopy cover to smoother the weed growth. The later stage of crop should have low specific leaf area, high chlorophyll, medium plant height (110-120 cm), early flowering, slim and sturdy culm, 250-300 panicles per meter square, more number of filled grains per meter square, retaining panicle height within the canopy level, erect boot leaf and high harvest index (Fig. 2). Deep rooting plants would be more advantageous, as they could mine water from deeper layer (Fig. 1). On the other hand, switching over the rice crop from flooded condition to aerobic/dry rice, soil can reduce the indigenous supply of P, Fe (Fan et al. 2012) and Zn. Continues cultivation of rice over the years under dry condition, would result in soil sickness. Moreover, soil Fe fertilization increased concentration in shoot and dry weight at tillering, but failed to increase the same during physiological maturity (Fan et al. 2012). Therefore, the traits starting from germination to maturity needs to be addressed to achieve yield on par with TPR.

7. RESEARCH AND DEVELOPMENT NEEDS

Under the growing water scarcity in Indian agriculture scenario, rice cultivation is gradually progressing from continuous flooded TPR system to partial or complete aerobic condition. Therefore, a new plant system that differs from regular transplanted rice has to be developed for this condition (Fig. 2). The varieties needed for DSR should have wider adaptation to suit the aerobic rice ecosystem with maximum water use efficiency, along with less yield penalty.

7.1. Breeding for yield component traits

Grain yield is the collective phenotypic expression of yield component traits whereas, adaptability is due to buffering capacity of genotypes for which many minor genes are in additive response. Thus, the accrual of favourable genes, those expressing at critical stage of rice plant under DSR/aerobic condition has to be combined.

7.2. Seedling vigour and its associated traits for early establishment

Early and uniform crop establishment is necessity for DSR. High seedling vigour, improved field emergence, weed competitiveness, tolerance against anaerobic condition and reduced moisture stress during germination (Mahender et al. 2015), enhanced foliar growth to combat weeds at the vegetative stage (Dingkuhn et al. 1991), seedling dry weight, rapid shoot growth, shoot dry weight, mesocotyl and coleoptile length (Fujino et al. 2008; Trachsel et al. 2010), germination rate, germination index, amylase activity, root activity and chlorophyll content (Diwan et al. 2013; Dang et al. 2014; Sandhu et al. 2015) are significantly contributes to early establishment in direct seeded rice. Additionally, these traits would facilitate to overcome limited moisture condition and/or submergence during monsoon time. Therefore, genetic improvements in rice breeding program for direct seeded condition should focus on improving those traits to make rice suitable for DSR. In recent years, several QTLs were identified for early establishment of rice seedling and they were elaborated well by Mahender et al. (2015) and Kumar et al. (2017). QTLs for traits related to early seedling vigour such as shoot length, germination rate and root dry weight in 28 days old seedling (Haritha et al. 2017; Anandan et al. 2016a; Mahender et al. 2015) and qEMM1.1 for early uniform emergence were identified form direct seeded condition (Dixit et al. 2015). Successful introgression of these QTLs would provide an opportunity to the early establishment and increases the plant population, thereby smothers the weed growth. Early seedling vigour cultivars are reported to be a low-cost, durable and decrease the detrimental effect of herbicide on the environment (Anandan et al. 2016a).

7.3. Understanding the traits for vegetative and reproductive phases

The photosynthates accumulated during vegetative stage from available biomass of aerial and underground portion determines the final output of the crop. Therefore, moderate tillering of 10–12 numbers and enhanced assimilate export from leaves to stems during the late vegetative and reproductive phases are important to achieve higher grain yield by grain filling (Dingkuhn et al. 1991). Ghosh et al. (2012) from the ICAR-NRRI, Cuttack has observed that 17% yield decline under aerobic system occur due to inhibition in the structural development of roots as increase in the concentration of hydrogen peroxide (24.6%) and proline (20%) and a lower concentration of total soluble protein (20%). Therefore, the QTLs identified under DSR would be more appropriate to utilize in rice breeding for DSR than the QTLs identified form TPR condition. Introgression of such QTLs would definitely be beneficial and improves better adaptation to DSR condition with high yield potential. Successful introgression of such QTLs provides an opportunity to study their performance and interaction between them. Sandhu et al. (2015) identified QTLs for nodal root (qNR4.1 and qNR5.1) and root hair density (qRHD1.1 and RHD5.1) under direct seeded condition. Similarly, Dixit et al. (2015) reported QTLs qLDG3.1 and qLDG4.1 for lodging tolerance. Several grain yield QTLs were identified (qGY1.1, qGY2.1, qGY2.2, qGY8.1 and qGY10.1) by Sandhu et al. (2013; 2015) under aerobic condition. Therefore, the breeding program for DSR should aim to increase the yield and adaptability by introgressing such QTLs for the benefit of farmers in the target areas struggling for water and labor.

7.4. Breeding for biotic stresses

Rice cultivation under aerobic favors blast (*Magnaporthe grisea*), bacterial leaf blight (BB) (*Xanthomonas oryzae* pv. oryzae) and brown spot (*Helminthosporium oryzae*). Therefore, rice varieties of aerobic should have durable resistance genes for blast and BB. This could also help to reduce resources to be incurred and environmental pollution. Brown spot often occurs in poorly managed and deficient soils under inadequate soil moisture. Therefore, balanced dose of nitrogenous fertilizer needs to be maintained with sufficient soil moisture to avoid the incidence of blast and brown spot. Stem borer and leaf folder are the major concern of aerobic rice. Cultivation of resistance varieties and summer ploughing are recommended to reduce pest incidence in aerobic. The menace of root-knot nematode *Meloidogyne graminicola* is increasing under aerobic and causes severe damage to rice plants. Therefore, raising resistance varieties are recommended to avoid yield reduction.

7.5. Breeding for nutrient use efficiency

Saturation level increases the availability of nutrients P and Fe. There are reports on soil sickness, which appears after few years of aerobic rice cultivation. Moisture at field capacity in aerobic condition may reduce the nutrient availability. Therefore, detailed study must be taken to understand the nutrient dynamics of major and micronutrient and their bioavailability. Pal et al. (2007) observed the differential responses of rice cultivars to applied Fe. In an experiment to test rice genotypes under aerobic condition for Fe, two lines (CT 6510-24-1-2 and IR 71525-19-1-1) performed better compared to the varieties IR 36 and IR 64 suitable for TPR. These deferential responses sound that inherent ability of the genotype plays important role in Fe absorption. Sandhu et al. (2015) has reported QTLs qN5.1, qP5.2, qFe5.2 for nodal root and root hair density, that plays major role in higher nutrient uptake (Al, Fe, and P), under DSR. QTLs for higher nutrient uptake, higher nutrient concentration and root hair density were also found to be co-localized in the same region. Enhancing root growth with root hair density enhances P uptake (Mahender et al. 2017). Fine mapping of *Pup1* region lead to the identification of P starvation tolerance 1 (PSTOL1) gene encoding a PSI protein kinase. Under P deficient condition, PSTOL1 enhances the early root elongation and improves the grain yield (Gamuyao et al. 2012). Recently Mehra et al. (2017) characterized purple acid phosphatase (PAP21b) gene from Dular that enhances the uptake of P present in organic form in the soil. Thus, stacking of PSTOL1, PAP21b, and QTLs of nodal root and root hair density into elite varieties by marker-assisted selection would definitely improve nutrient concentration in plants under aerobic condition. Stacking up of nutrient uptake genes/QTLs may result in improved performance by complementary effects will reduce the cost incurred by resource-poor farmers.

7.6. Molecular understanding and their role of microbes to improve nutrient use efficiency

The genomics and transcriptomics studies revealed that a successful AMF root colonization is controlled by the genotype of plants. The HAR1 gene i.e. Hypernodulation and Aberrant Root Formation1 are reported to control number of root nodules in legumes and the same gene also plays a role in the regulation of AMF symbiosis (Solaiman et al. 2000), but the mode of action of this gene is still not understood. The genetic requirements for rhizobial and AMF association in plants overlap in a common symbiosis pathway (CSP), which leads to successful root nodule and AMF symbiosis (Tirichine et al. 2006). The CSP plays an important role in accommodating root nodule and AMF symbionts, by which plant cells vigorously decompose their cell wall structures to facilitate microbial colonization (Parniske, 2000). The effects of the *Oryza sativa* calcium/calmodulin-dependent protein kinase (*OsCCaMK*) indicated that a strong expression of *OsCCaMK* was detected in rice roots, where mycorrhizal colonization is expected to occur (Parniske 2009). The other study revealed that *OsCCaMK* gene expression has the positive correlation with the diversity of root-associated bacteria and the growth of rice plants (Ikeda et al. 2011). The above information indicates that studying the plant associated gene and its expression related to the specific microbial association is very important to develop rice varieties, which have affinity towards the specific group of microbes to improve the NUE in rice (Fig. 2).

8. WAY FORWARD

The modern plant breeding has moved from classical breeding to precision breeding by adapting powerful indirect selection technique of employing molecular marker technology. During the last two decades, the modern plant breeding is progressing in faster pace; more number of popular rice varieties of favorable land to marginal land with assured yield is developed by researchers in India. Several genes were identified and characterized for their functions related to yield, nutrient deficiency tolerance, biotic and abiotic stresses. Therefore, designing of a plant variety with desired traits becomes possible for any specific ecosystem. However, more efforts are needed to identify new genes responsible for nutrient deficiency tolerance and water use efficiency. On the other hand, further understanding is required to know the genes involved for regulating the differences in use efficiency and tolerance mechanism. This would maximize their role in crop improvement program for resource use efficiency. Developing next-generation rice suitable for the direct seeded system is very much necessary to tackle increased food demand under the limited labor, land, water, and nutrient during this changing climate period. The molecular approaches along with best crop management could significantly increase the productivity in aerobic rice cultivation.

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