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Marker assisted selection of Aerobic × Basmati segregating lines for physio-morphological and aroma trait

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Abstract
Experiments were conducted to gauge segregating Pusa 1121 x MAS26 (aerobic) F_2 populations for various physio-morphological, root traits, allelic diversity for *BAD2* (aroma) gene and microsatellite markers linked to the traits promoting aerobic adaptation. Vast variation was noted for plant height, number of productive tillers per plant, grain length/breadth ratio, root thickness, root length, fresh and dry root weight, panicle length, 1000 grain weight, and yield per plant in all the three populations. In these populations, remarkable positive correlation was found between yield per plant with 1000 grain weight, plant height, number of productive tillers per plant, length/breadth ratio, root biomass and root length biomass. At *BAD2* aroma locus in 52 plants of this populations, 36 plants had Basmati specific allele in homozygous condition, 7 plants had *indica* allele (homozygous condition) and 9 plants had both Basmati and *indica* allele (heterozygous condition). The NTSYS-pc dendrogram and 2D PCA were prepared and F_2 population was inclined towards MAS26. A number of promising F_2 plants have been selected, which had Basmati specific allele at *BAD2* locus (either in homozygous or heterozygous condition) and higher grain yield, root length and biomass greater than MAS26 for further progeny analysis. In microsatellite markers analysis, most of these selected plants owned the desired allele for the markers reported earlier to be linked with the aerobic adaptation traits.

Keywords: Aerobic rice, Basmati, *BAD2*, SSR, genetic diversity, selection of plants

Introduction
Rice (*Oryza sativa* L.) is the primary food of over 3 billion people worldwide. From agricultural perspective, rice cultivation is major venture, with nearly 90% of it being produced in Asia (Gill *et al*., 2014). Rice supplies more than 50% of calories consumed by world's population. It is an annual grass of family *Poaceae* (*Gramineae*) and belongs to genus *Oryza*. The genus *Oryza* that has two cultivated species, *O. sativa* (Asian) and *O. glaberrima* (African) and 25 wild species, is widely distributed in tropical and subtropical regions. Globally rice is grown over an area of about 167 Mha with an annual production of 782 MT (FAOSTAT 2018). The majority of the world's rice is being produced under flooded lowland conditions. Out of 147 million ha of rice land, 79 million ha are categorized as irrigated lowland, 36 million ha for rain-fed lowland, and 13 million ha as flood-prone. Rice is exclusively a major user of fresh water and consumes more than 50 per cent of the water used for irrigation in Asia. For the production of one kg of rice, it takes 3000- 5000 liters of water which is 2-3 times additional than needed for other cereals such as wheat and maize (Singh *et al.*, 2002) [1].

Increasing paucity of water has menaced agricultural productivity in many segments of the world including India. Water scarcity is a result of an imbalance between the supply and demand of water sources in a geographical area. Climatic extremies are the major problem to crop production and sustainable food security for growing population (Lesk *et al*., 2016) [19]. Crop failures due to abiotic stresses affect the economic growth of farmers (Mottaleb *et al*., 2015) [25]. An approximate 34−43% of the total world's irrigation water is received by rice. In water meager conditions, traditional flooded rice crop may not be suitable for many environments. Groundwater tables have declined on mean by 0.5 - 1.0 m y^{-1} in states of India particularly Haryana and Punjab due to over-abstraction of ground water (Bouman and Tuong, 2001) [7]. In Asia it is expected that large portion of irrigated dry season rice and irrigated wet season rice will undego "physical water scarcity," and "economic water scarcity" by 2025 (Tuong and Bouman, 2002) [6]. For reducing water usage for rice cultivation, various strategies are being used for instance alternate wetting and drying, saturated soil culture on raised beds,

(Bouman Tuong, 2001) [7], system of rice intensification, ground cover system, (Stoop *et al.*, 2002) [43] and aerobic rice cultivation (Bouman *et al.*, 2006) [8]. Out of these strategies, in terms of water-saving, aerobic rice is reflected to be one of the most favorable strategies (merging the drought-resistant traits of these upland varieties with the high-yielding traits of lowland varieties) (Tuong and Bouman, 2002) [6]. Aerobic rice production system use well-drained, non-saturated and non-puddled soils. Owing to this soil is thus "aerobic" or with oxygen in entire growing season, as contrast to traditional flooded fields, which are "anaerobic." Aerobic rice requires less quantity of seed, less labour, and saves up to 70% less water.

Several aerobic rice genotypes were developed using conventional breeding and marker-assisted selection techniques in combination in early 1980 using the available upland paddy and high yielding germplasm in Bangalore (Girish *et al*., 2006; Toorchi *et al*., 2007) [13, 45]. Molecular markers provide a valuable tool for genetic analysis and plant breeding (Sharma *et al*., 2019) [35]. Linkage mapping and marker assisted selection of the target traits can greatly improve the selection efficiency and precision in a Basmati rice breeding program. Molecular marker technology can help us in the identification of suitable parental lines, diversity and pedigree analyses and to introgress desirable traits without altering the genetic background. Molecular markers particularly the microsatellite markers (SSRs) have been used to know about the genetic structure of complex traits in rice based on traditional quantitative trait locus (QTL) linkage (Sharma *et al*., 2017; Sharma *et al*., 2019; Sharma *et al*., 2020) [34-36]. SSRs are valuable as genetic markers because they detect high level of allelic diversity, are co-dominant, are simply and inexpensively assayed by PCR and are easily automated. The SSRs are superior for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies on account of their technical efficiency and multiplex potential (Samriti *et al*., 2017; Sharma *et al*., 2020) [31, 35]. QTL pyramiding was used to develop drought and submergence tolerant rice varieties with increased yield (Sandhu *et al*., 2019) [15]. Rice genome sequence shows the presence of one SSR marker every 157 bp. Over 10,000 microsatellites markers have already been mapped and developed in rice (McCouch *et al.*, 2002; Temnykh *et al.*, 2001) [22, 44].

Root traits are usually governed by quantitative trait loci because they are complex and monitored by many genes, each one with a little genetic effect (QTL; Sharma *et al*., 2011). Identifying QTL for root traits and genetic variation can impart our knowledge for their role in plant growth under direct seeded conditions. The mapping and tagging of genes with molecular markers is the foundation for marker assisted selection (MAS) in crop plants. The objective of present study was to evaluate Pusa1121 x MAS26 F_2 population, for yield, yield attributes and root morphology under aerobic conditions along with molecular marker analysis of selected plants obtained from the above crosses using markers specific for aroma and root traits. The results of this research will increase knowledge about yield, yield attributes, aroma and root morphology characters of rice as well as the identification of potential cross to be used in breeding programs.

Material and Method

Plant material

The experimental plant material comprised of seeds harvested from Pusa1121 x MAS26 F_1. MAS26 is the aerobic rice variety developed at University of Agricultural Sciences, Bangalore. Pusa 1121 Basmati rice variety developed at IARI, New Delhi. All the seeds were raised, during kharif season, in pots in the net house of Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University, Hisar and in field at CCS HAU Rice Research Station, Kaul (Kaithal). Pusa1121 x MAS26 F_2 plants were evaluated for variation in physio-morphological traits in the field/net house. The F_2 plants were also evaluated for molecular diversity linked to the traits promoting aerobic adaption and *BAD2* aroma-specific markers.

Raising of crop

The crop was raised through kharif season in the net house of the Department of Molecular Biology and Biotechnology, CCS Haryana Agricultural University and in the field of CCS HAU Rice Research Station (RRS), Kaul (Kaithal). Seeds harvested from Pusa1121 x MAS26 F_1 plants were sown by direct seeded method under aerobic conditions in pots as well as in field. Yoshida nutrient solution (Supplementary Table 1) was given to the plants growing in pots in the net house. Yoshida nutrient solution was given to the plants growing in pots in the net house after 17 days from the sowing date. All recommended agronomic practices were followed for raising a good crop.

In the field, seeds were dry seeded at 1-2 cm depth with a row interspacing of 20 cm and plant to plant distance of 15 cm. Plants were irrigated at an interlude of 5 days up to panicle emergence and after panicle emergence at an interlude of 3 days. The level of water was kept 790-1430 mm/ha.

Physio-morphological traits characterization:

For every plant, observations were recorded on the following characters. The data was next analyzed to determine the variability.

1. Plant height (cm)

The plant height of fully mature plant was recorded on centimeter scale.

2. Panicle length (cm)

The panicle length of fully mature plant was recorded on centimeter scale.

3. Effective no. of tillers per plant

Effective no of tillers per plant were recorded in numbers.

4. Root length (cm)

The root length of fully mature plant was recorded on centimeter scale.

5. Root thickness (mm)

The root thickness of fully grown plants was recorded in mm.

6. Fresh and dry root weight (g)

The fresh and dry root weight of fully grown plants was recorded in grams.

7. Grain yield per plant (g)

The total average grain yields of all the plants were weighed in grams.

8. Length-Breadth ratio
The length – breadth of five seeds from each plant was recorded using digital Vernier Caliper.

9. 1000 Grain weight (g)
The 1000 grain weight of all the plants was weighed in grams.

Statistical Methods
Mean and standard error were calculated by using standard procedure. Correlation coefficient was calculated by Pearson Correlation Matrix.

A. Parameters of variability
(i) Mean: The mean value of each length-breadth ratio was worked out by dividing the totals by corresponding number of observation:

$$x = \frac{\sum Xi}{N}$$

Where, Xi - any observation in i^{th} treatment, N - Total number of observations

(ii) Standard Deviation: The positive square root of mean of squared deviations from arithmetic mean, so called root mean square deviation. The standard deviation is a measure of how widely values are dispersed from the average value (the mean).
Standard deviation uses the following formula:

$$\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$$

Where, x - Sum of all values of the variable, $\overline{X}$ - Mean of values, N - Sample size

Genotyping
Genomic DNA was isolated using CTAB method from young leaf tissues of the F_2 plants (4M NaCl; 10% CTAB; 0.5 M ethylene diamine tetra acetic acid (EDTA); 1 M Tris HCl pH 8.0; 0.2% b-mercaptoethanol and 2% poly vinyl pyrrolidone (PVP) (Doyle and Doyle 1987). The quality and wholeness of nucleic acid were checked on agarose gel having composition of 1% by electrophoresis (Singh *et al*., 2019) [42]. Nano drop spectrophotometry was used to determine the concentration of DNA at 260 nm (Thermo scientific, California- USA).

Microsatellite marker analysis
A total of five molecular markers were used for preparation of DNA fingerprint database of selected Pusa 1121 x MAS26 F_2 plants. These primers have been identified to be linked with the root traits (Shen *et al.*, 2001; Kanagaraj *et al.*, 2010) [40, 17] (supplementary file Table 2). Specific primers for betaine aldehyde dehydrogenase 2 (*BAD2*) gene (Bradbury *et al.*, 2005) [9] were used for assessing the diversity between Basmati and non-Basmati rice F_2 plants (supplementary file Table 3). PCR amplifications were performed using PTC-100™ 96V thermocycler (MJ Research, Inc., Watertown, MA, USA) and Taq DNA polymerase. The PCR reaction was prepared in a reaction volume of 20 µl comprising 1X PCR buffer, 1mM dNTPs, 0.4 µM of each primer, 1 unit Taq DNA polymerase and 20-40 ng template DNA. PCR amplification was performed with initial denaturation at 94 ^{0}C for 5 min succeeded by 35 cycles of 94 ^{0}C for 30 sec, 55 ^{0}C for 50 sec, 72 ^{0}C for 1 min and final extension at 72 ^{0}C for 8 min. Amplified products were resolved on 3% agarose gel at a constant voltage of 5 V/cm for about 2h in submerged conditions (Sharma *et al.*, 2019) [36]. The products were visualized by gel documentation system and ABI gene mapper software v.4.1 was used for analysis (Applied Biosystem). Primers used for the analysis of fragrance (Bradbury *et al.*, 2005) [9] are given in the supplementary file Table 3.

Data analysis
On agarose gel, the presence of DNA band was taken as one and absence of band was taken as zero. The similarity genetic distance was calculated by 0/1 matrix using 'simqual' sub-program of software NTSYS–pc (Rohlf, 1993) [39]. The distance matrix by the unweighted pair-group method with arithmetic average (UPGMA) NTSYS-pc sub programme was used to construct dendrogram. The 'PCA' NTSYS-pc sub programme was used for principle component analysis (PCA).

Results and Discussion
In this investigation, experiments were conducted to evaluate Pusa1121 x MAS26 F_2 population for variation in root morphology, physio-morphological traits, molecular profile for *BAD2* specific locus for aroma and SSR markers linked to traits promoting aerobic adaptation.

Field Evaluation Pusa 1121 x MAS26 F_2 population (50 plants) grown under aerobic conditions
Aerobic rice is an new rising production system with less utilization of water than conventional flooded rice. In aerobic rice cultivation, fields remain unsaturated in the entire season and increase production with less water (Bouman, 2002) [2]. In aerobic rice, combined amount of rainfall and irrigation water from sowing to harvest varied from 470 to 650 mm, compared with 1200-1300 mm in lowland rice (Martin *et al*., 2007) [21]. Water uptake in rice depends on root system (Nguyen *et al*., 1997) [27]; consequently, studying the root system is one of the crucial aspect for understanding mechanism underlying aerobic adaptation and water stress tolerance in rice.
Huge variation was observed under field condition among 50 Pusa 1121 x MAS26 F_2 plants and for plant height (92-155 cm, Pusa 1121 - 95.8 cm and MAS26 – 99.9 cm), panicle length (19.8-28.7 cm, Pusa 1121 -26.1 cm and MAS26-23.7 cm), effective numbers of tillers per plant (4-17, Pusa 1121 – 9.2 and MAS26 – 13.6), length/breadth ratio of grain (dehusked) (3.42-5.26, Pusa 1121-5.53 and MAS26 -3.99), grain yield per plant (4.65-36.6 g, Pusa 1121 -10.4 g and MAS26 – 11.2 g), 1000 grain weight (20.6-32.9 g, Pusa 1121 -22.4 g and MAS26 – 24.8 g) (Table 3). This large variation could be due to segregation of genes and QTLs for promoting aerobic adaptation in rice. The above results were supported by (Xiaoguang *et al*., 2005) [49] which showed that the aerobic varieties HD502 and HD297 yielded higher than the lowland variety JD305 under aerobic conditions. Total rice production can be maximized by growing aerobic rice in all the areas where water is comparatively limited than land. Under flooded conditions, the lowland varieties had stronger performance but under aerobic conditions the aerobic varieties had higher yields (Yang *et al*., 2005) [50]. The approximate yield potential of aerobic rice varieties is 6-7 t ha $^{-1}$ has been produced by breeders in China (Wang *et al*., 2002)

[48]. Out of segregating plants derived from the three crosses, revealed a positive correlation between grain yield per plant with number of productive tillers per plant, panicle length, plant height, and 1000 grain weight.

Net house Evaluation of Pusa 1121 x MAS26 F_2 population (50 plants) grown under aerobic conditions

Amudha *et al*. (2009) [2] reported that a cultivar having more root number, high fresh and dry root can explore more soil volume for effective absorption of water. Root development plays a predominant function in water uptake and water use efficiency under aerobic cultivation (Corales *et al*., 2020) [24]. Results also revealed that deep roots are associated with water stress tolerance in aerobic rice. Root system is examined as one of the important physiological parameter to fight against the water scarce conditions because at molecular level, number of differential expressed transcripts involves in sugar and nutrient uptake were higher in roots than shoots (Phule *et al.*, 2019) [28]. Greatest root thickness and root length were observed to be linked with drought resistance in upland conditions amid the root morphological traits.

Pusa 1121 **x** MAS26 F_2 population (34 plants) showed enormous variation under net condition for plant height (47.0-104.0 cm, Pusa 1121 – 51.2 cm, MAS26 – 61.5 cm), panicle length (16.0-24.7 cm, Pusa 1121 -23.9 cm and MAS26-20.3 cm), effective numbers of tillers per plant (1.0-16.0, Pusa 1121 - 4 and MAS26 – 4.5), length/breadth ratio of grain (dehusked) (3.39-5.88, Pusa 1121-5.10 and MAS26 -3.99), grain yield per plant (0.85-20.9 g, Pusa 1121 -5.3 g and MAS26 – 5.98 g), 1000 grain weight per plant (9.0 -23.8 g, Pusa 1121 -24.8 g and MAS26 – 18.4 g), root length (16-59 cm, Pusa 1121 -25.9 cm and MAS26 – 27.8 cm), root thickness (3.81-27.0 mm, Pusa 1121 -9.8 mm and MAS26 – 11.5 mm), fresh root weight (1.64-27.2 g, Pusa 1121 -2.5 g and MAS26 – 3.59 g), dry root weight (0.61-7.37 g, Pusa 1121 -1.0 g and MAS26 – 2.6 g) (Table 9). Phenotypic correlation coefficient analysis of Pusa 1121 x MAS26 F_2 population, evaluated in field under aerobic condition revealed a positive correlation between grain yield per plant with effective number of tillers per plant (0.547, p=0.01), panicle length (0.552, p=0.01). A positive correlation was also obtained between panicle length with plant height (0.329, p=0.05) and effective no. of tillers per plant (0.300, p=0.05) (Table 10). Phenotypic correlation coefficient analysis of Pusa 1121 x MAS26 F_2 population, evaluated under net house condition revealed a positive correlation between grain yield/plant with plant height (0.733, p=0.01), effective number of tillers/plant (0.769, p=0.01), panicle length (0.442, p=0.01), root length (0.355,p=0.05) and fresh root weight (0.535, p=0.01). A positive correlation was also found between plant height with panicle length (0.654, p=0.01), effective no. of tillers/plant (0.433, p=0.01), root length (0.405, p=0.01) and fresh root weight (0.524, p=0.01) (Table 11). But no significant correlation with basal root thickness and dry root weight were observed. In limited water conditions, ameliorated root systems have central role in nutrient acquisition and increased water uptake (Kharb *et al*., 2015; Meister *et al*., 2014) [18, 23].

Deeply embedded root system is advantageous for the plant in various aspects like increase stomatal conductance, to reduce lower canopy temperature, increase water uptake, and finally increase grain yield under water limited conditions. Under aerobic conditions, some plants with greater root length and root biomass had higher grain yield compared to parents.

In Pusa 1121 x MAS26 F_2 plants revealed a positive correlation between grain yield per plant with plant height, effective number of tillers per plant, panicle length, root length and fresh root weight. A positive correlation was also found between plant height with panicle length, effective no. of tillers per plant, root length and fresh root weight. But no significant correlation with basal root thickness and dry root weight were observed. Genotypic and phenotypic correlations were observed for grain yield and its components. The results revealed that the traits like number of productive tillers per plant, dry root weight, panicle length and relative water content they showed significant and positive correlation with single plant yield and also positive inter correlations among themselves (Sathya and Jebaraj, 2013). Root traits are important for improving rice performance under an aerobic rice system since a positive correlation between root traits and water uptake (Ngugen *et al*., 2015).

The association between root morphological characters and yield-related traits distinctly revealed the significance of root dry weight and root length. Thus, for the grain yield, the contribution of root characters is more important under aerobic condition than well-watered condition.

Microsatellite marker analysis of Pusa 1121 x MAS26 F_2 population plants

Microsatellite marker data was generated using six molecular markers for thirty one Pusa 1121 x MAS26 F_2 plants. Out of these six, five markers were linked with the traits promoting aerobic adaption and one marker was specific for the *BAD2* aroma gene.

Variation in allelic profile at *BAD2* locus and SSR markers for promoting aerobic adaptation

Fragrance or aroma in rice is considered as a special trait with huge economic importance that determines premium price in global trade. For the acceptability and marketability of rice, fragrance is an important quality trait (Ashokkumar *et al*., 2020) [3]. Aromatic rice constitutes a small but special group of rices which are considered best in quality. More recently in order to select the aromatic rice, many simple sequence repeats (SSRs) and SNPs have been developed which are genetically associated with fragrant (Cordeiro *et al*., 2002) [11]. Among the several chemical constituents of rice aroma, 2-acetyl-1- pyrroline (AP) has been considered to be crucial part in Basmati and other fragrant rices (Lorieux *et al*., 1996) [20]. By using mapping population derived between Pusa1121 and Pusa1342, three different loci were mapped (one each on chromosome 3,4 and 8) and by fine mapping ARO8.1 loci have resulted to the identification of BADH2 (Amarawathi *et al*., 2008; Singh *et al*., 2010) [1, 41]. Bradbury *et al*. (2005) [9] reported A significant polymorphisms was observed in the coding region of fragrant rice genotypes in comparison to non-fragrant genotypes for a gene with homology to the *BADH2* gene. The oxidation of γ-amino-butyraldehyde (AB-ald; 2AP precursor) is catalyzes by BADH2 protein. The cumulation of both AB-ald and its cyclic form, 1 pyrroline due to non functional allele leads to enhanced 2AP synthesis. Bradbury *et al.* (2005) [9] reported a single tube Allele Specific Amplification (ASA) assay which use to distinguish between fragrant and non-fragrant rice varieties. The population segregating for fragrance has homozygous non-fragrant, homozygous fragrant and heterozygous non-fragrant individuals was identified using ASA. The PCR product of nearly 580 bp assists as a positive control and is present in

every sample. Fragrant individuals have The second PCR product of 257 bp in size is present in fragrant plants, while non-fragrant plants give a product of 355 bp in size, the presence of all three PCR products can also help in distinguishing heterozygotes .

The Allele Specific Amplification (ASA) assay is a simple and vigorous method for screening rice to identify its fragrance status among segregating populations using DNA isolated from rice following simple, inexpensive and rapid extraction protocol (Bradbury *et al.*, 2005) [9].

Pusa 1121 x MAS26 F $_2$ plants had a ratio of 32:4:6 for homozygous, heterozygous and non-fragrant plants. The overall size of PCR amplified products ranged between 78 bp (RM413) and 196 bp (RM336) (Table 16). At a SSR locus, all the F_2 plants had specific allele for MAS26 or Pusa 1121 or for both the parents indicating the heterozygous state (Table 16). The distribution of Pusa 1121 and MAS26 specific alleles are shown in (Table 17). On an average, 80.1 % alleles were from Pusa 1121 and 66.7 % alleles were from MAS26 in 31 F_2 plants. Molecular polymorphism at *BAD2* aroma locus is thus highly efficient, a powerful tool, in discriminating fragrant and non-fragrant rice genotypes and the trait of fragrance shows ideal co-segregation within the mapping population (Sakthivel *et al.*, 2006) [30].

Genetic relationship among Pusa 1121 x MAS26 F_2 population (31 plants)

Rice root growth encompasses a remarkable genetic diversity in terms of growth patterns, architecture, and environmental adaptations. Root traits are controlled by many complex quantitative trait loci (QTLs) (Corales *et al*., 2020) [24]. Since the first study by Using molecular markers, to locate genes controlling rice root traits (Champoux *et al*., 1995) [10] and root trait related many QTLs have been identified in rice (Gowda *et al*., 2011) [14].

To calculate the coefficient values, similarity coefficient data based on the five SSR markers was used for the selected 31 Pusa 1121 x MAS26 F_2 plants and parental rice genotypes and undergo UPGMA tree cluster analysis. A dendrogram (cluster tree analysis, NTSYS-pc) (Figure 5) was produce using allelic diversity to reveal the genetic relationship among 31 selected F_2 plants and the parental rice varieties. Two parental varieties, Pusa 1121 and MAS26 had low similarity coefficient and bifurcate at coefficient value of 0.55. At the similarity coefficient of 0.55, all the 31 F_2 plants clustered in two major groups. Major group I included 20 F_2 plants and MAS26 although the major group II had 11 F_2 plants and Pusa 1121. PCA analysis (NTSYS-pc) was also used to evaluate the genetic relationships among these rice genotypes. Two parental genotypes were noticeable on two dimensional PCA scaling while 31 F_2 plants were dispersed between the two parental lines with proclivity towards MAS26 (Figure 6).

Several markers have been identified to be linked with aerobic root traits/drought tolerance in rice (Qu *et al*., 2008). Thirty one Pusa 1121 x MAS26 F_2 plants were selected for analysis of molecular diversity. Allelic profile using RM 205 (165 bp and 125 bp) locus was comparable to that reported by (Ikeda *et al*., 2007) [15]; this marker has been found to be related with root length. RM205 on chromosome 9, RM547 on chromosome 8 showed significant association with root length (Bernier *et al*., 2007) [4] With the help of markers large number of QTLs have been identified qDTY12.1, qDTY3.1, qDTY2.1 which linked with grain yield under drought conditions (Dixit *et al*., 2012) [12]. It have been reported that a positive interaction of qDTY12.1 with qDTY2.2 and qDTY3.2 (Shamsudin *et al*., 2016) [33] and qDTY7.1 with qDTY4.1 and qDTY9.1 (Sandhu *et al.*, 2018) [32] shows a significant increase in yield under aerobic conditions.

Selection of promising plants

Based on field data, a total of eight promising Pusa 1121 x MAS26 F_2 plants have been selected depending on plant height, effective no. of tiller, grain L/B ratio, yield per plant and status of *BAD2* aroma allele (supplementary file Table 8). Based on nethouse data, a total of five promising Pusa 1121 x MAS26 F_2 plants have been selected depending on root morphology (length & biomass), seed L/B ratio and status of *BAD2* aroma allele (supplementary file Table 9). The plants having homozygous or heterozygous condition for Basmati specific allele were selected.

All the selected lines from this study will act as novel material for the selection of stable direct seeded varieties development of these direct seeded varieties could be significant advantage to farmers in the region who depends on upland rice for food security. The challenges ahead are the effective use of these roots and GY QTL and their combination in breeding for direct seeded rice varieties, fine mapping of QTL to facilitate precise introgression without undesirable linkage, and understanding the physiological and molecular mechanism associated with these major-effect QTL under direct seeded conditions.

Conclusion

In this investigation, field evaluation of Pusa 1121 (premium Basmati rice variety) and MAS26 (aerobic rice variety) for various physio-morphological traits such as plant height, panicle length, effective number of tillers, yield per plant, 1000 grain weight and grain length-breadth ratio was carried out. In addition, 34 Pusa 1121 x MAS26 F_2 plants were evaluated for various physio-morphological and root traits in pots in the net house under water limited conditions. Out of these total 100 plants grown in net house and field, 52 were analyzed for allelic profile at *BAD2* aroma locus and SSR markers linked to the traits fostering aerobic adaptation. Out of segregating plants derived from the three crosses, revealed a positive correlation between grain yield per plant with effective number of tillers per plant, plant height, panicle length, and 1000 grain weight. These plants could be further analyzed in order to select stable high yielding aerobic rice lines with intact Basmati rice traits.

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