The Pharma Innovation Journal 2022; 11(7): 724-727

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 724-727
© 2022 TPI
www.thepharmajournal.com
Received: 12-04-2022
Accepted: 18-06-2022

Ritu Khasa
Department of Molecular Biology and Biotechnology, CCS HAU, Hisar, Haryana, India

Samriti Sharma
Assistant Professor, Department of Biotechnology, Chandigarh group of Colleges, Landran, Mohali, Punjab, India

Sushil kumar
Department of Food Science and Technology, CCS HAU, Hisar, Haryana, India

Rajendra Kumar Jain
Department of Molecular Biology and Biotechnology, CCS HAU, Hisar, Haryana, India

Corresponding Author:
Samriti Sharma
Assistant Professor, Department of Biotechnology, Chandigarh group of Colleges, Landran, Mohali, Punjab, India

Physio-morphological traits and simple sequence repeat marker based genetic diversity assessment amongst Basmati × Aerobic populations

Ritu Khasa, Samriti Sharma, Sushil Kumar and Rajendra Kumar Jain

Abstract
Experiments were conducted at Chaudhary Charan Singh Haryana Agricultural University, Hisar, India during the *Kharif* season to evaluate segregating Pusa 1121 **(**Basmati) **x** MAS25 (aerobic) F_5 and MASARB25 (aerobic) x Pusa Basmati 1460 (Basmati) F_4 populations for various physio-morphological and/or root traits and allelic diversity for *BAD2* (aroma) gene and microsatellite markers linked to the traits promoting aerobic adaptation. In both populations, enormous variation was observed for plant height, panicle length, and effective number of tillers per plant, root length, root thickness, fresh and dry root weight, 1000 grain weight, grain length/breadth ratio and yield per plant. A number of promising F_4 and F_5 plants have been selected, which had higher grain yield, root length and biomass greater than MAS25 and MASARB25 and Basmati specific allele at *BAD2* locus (either in homozygous or heterozygous condition) for further progeny analysis.

Keywords: Aerobic rice, basmati rice, SSR, aroma

Introduction
Increasing scarcity of water has threatened agricultural productivity in many parts 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. In Asia, almost 84 percent of the water withdrawal is used for agricultural purposes, compared to 71 percent in the world. Irrigated rice receives an estimated 34-43% of the total world's irrigation water. Overexploitation of groundwater has caused serious problems in many parts of India including Haryana and Punjab; groundwater tables have dropped on average by 0.5 - 1.0 m y^{-1} in states of India (Bouman and Tuong, 2001) [5]. Reasons for water scarcity are diverse and location-specific, but include decreasing water quality (chemical pollution, salinization) and resources (e.g., falling groundwater tables, silting of reservoirs), and increased competition from other sectors such as urban and industrial users. Therefore, farmers and researchers are looking for ways to decrease water use in rice production and increase its use efficiency (Bouman and Tuong, 2001) [5].

Rice (*Oryza sativa* L.) is the staple food of over 3 billion people worldwide. Rice cultivation is one of the most important agricultural activities on earth, with nearly 90% of it being produced in Asia. 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 (Vaughan, 1989) [17].

Several strategies are being used for reducing water consumption for rice cultivation, such as saturated soil culture on raised beds (Borrell *et al.*, 1997) [3], alternate wetting and drying (Bouman and Tuong, 2001) [5], ground cover system, system of rice intensification (Stoop *et al.*, 2002) and aerobic rice cultivation (Bouman *et al.*, 2006) [6]. Of these strategies, aerobic rice is considered to be one of the most promising strategies in terms of water-saving (Tuong and Bouman, 2002) [16]. Upland varieties have deeper roots than lowland varieties. Plant improvement towards deeper root system would be most effective for better adaptation to occasional or intermittent soil water deficit between irrigation events in rice (Gowda *et al*., 2011) [10]. Recently, water efficient 'aerobic rice' varieties have been developed by combining the drought-resistant characteristics of these upland varieties with the high-yielding traits of lowland varieties (Belder *et al*., 2005) [1]. Aerobic rice is a production system in which specially developed varieties are grown in well-drained, non-puddled and non-saturated soils. The soil is therefore "aerobic" or with oxygen throughout the growing season, as compared to

traditional flooded fields, which are "anaerobic."
Aerobic rice requires less quantity of seed, less labour, and saves up to 70% less water. Aerobic rice shows efficient fertilizer utilization, less incidences of pests/diseases, reduced methane emission, profuse rooting and high tillering, less lodging and high grain and fodder yield, retention of soil structure and quality, etc. Water is further saved because of the absence of continuous seepage, percolation and water evaporation from the ponded water. Thus, aerobic cultivation is not only environment-friendly but also saves time, labor money and water (that can be used for energy (hydropower), fisheries, mining, environment and recreation).
Molecular markers provide a valuable tool for genetic analysis and plant breeding (Tanksley, 1989) [15]. 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 markers particularly the microsatellite markers (SSRs) have been used to know about the genetic architecture of complex traits in rice based on traditional quantitative trait locus (QTL) linkage (Flowers *et al.*, 2000) [9]. The objective of present study was to evaluate the progenies of selected MASARB25 x Pusa1460 and MAS25 x Pusa1121 F_3 and F_4 plants, respectively, 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 MAS25 x Pusa 1121 F_4 and MASARB25 × Pusa Basmati 1460 F_3 plants. MAS25 and MAS26 are aerobic rice varieties developed at University of Agricultural Sciences, Bangalore. MASARB25 is a drought tolerant rice genotype developed from IR64/-Azucena/ xx IR64 crosses at IRRI. Pusa 1121 and Pusa Basmati 1460 Basmati rice variety developed at IARI, New Delhi.

Chemicals

Chemicals used for preparing Yoshida medium, DNA extraction and PCR amplification were obtained from Sigma Chemicals Co. USA, Life Technologies (India) Pvt. Ltd., Imperial Bio Medics (Chandigarh, India). All other chemicals used in this study were of molecular biology grade or analytical grade and procured from Sigma Chemicals Co., Promega Inc., Gibco BRL Inc. (Gaitherburg, MD, USA) and E. Merck Ltd. (Worli, Mumbai-400018, India).

Molecular markers

A total of 37 microsatellite markers linked with the root traits (Shen *et al.*, 2001; Kanagaraj *et al.*, 2010) [14, 12] were used for assessing the molecular diversity in $F_2/F_4/F_5$ plants. The rice microsatellite primer pairs were obtained from Sigma Chemicals Co. USA. The original source, repeat motifs, primer sequences and chromosomal position of these markers can be found in the Rice Genes database (http://www.gramene. Org/micrsat/RMprimers.htm1). Specific primers for betaine aldehyde dehydrogenase 2 (*BAD2*) gene (Bradbury *et al.*, 2005) [7] were used for assessing the diversity between Basmati and non-Basmati rice varieties. These primers were got synthesized from Integrated DNA Technologies, Inc. USA.

Raising of crop in field and net house

For field experiments, seeds of selected F_3/F_4 plants and their parents were sown in the replicate of three at the Rice Research Station, Kaul, India, during kharif season 2014, by direct-seeded aerobic cultivation practices. Aerobic fields were irrigated for about one week with a 2-3 cm water layer to facilitate crop establishment; thereafter, the fields were re-irrigated once at a 10-days interval. Grain yield per plant was recorded after harvesting, threshing and drying to moisture content adjusted to 14%.
For net house experiment, each selected line and their parents were sown in the replication of five, with one plant per pot during kharif season 2014. The standard agronomic practices were followed to raise the crop. The pots were irrigated with one liter of water for the first 15 days, and then with one liter after every third day up to panicle emergence. After twenty and forty days the pots were supplemented with Yoshida solution. Five best plants of each line were harvested at maturity and the data were recorded on agronomic traits like plant height, number of effective tillers per plant, panicle length, number of panicles per plant, number of grains per panicle, 1000 grain weight, grain length/breadth ratio and grain yield per plant. Also, data on root morphological traits i.e. root length, root thickness, fresh and dry root weight were recorded and analyzed in net house plants.

Statistical Analysis

Mean and standard deviations were used as the parameter of variability and phenotypic correlation coefficient was calculated using OPSTAT statistical tool.

Preparation of DNA fingerprint database of genotypes using SSR markers

Genomic DNA was isolated using CTAB method of Saghai-Maroof *et al.* (1984) [13] from young leaf tissues of the F_4 and F_5 plants. RNA contamination was removed by adding 1 µl of 10 mg/ml RNase (RNase-A, Sigma chemical Co. USA, No.R-5503) in the DNA samples which were dissolved in 100 µl T.E. buffer. Completely dissolved DNA was checked for its quality and concentration by running DNA samples on 0.8% agarose gel electrophoresis using a standard containing 100 ng/µl genomic λ DNA.

Microsatellite marker analysis

A total of five, five and twelve molecular markers were used for preparation of DNA fingerprint database of selected Pusa 1121 x MAS26 F_2 plants, MAS25 x Pusa 1121 F_5 plants and MASARB25 x Pusa Basmati 1460 F_4 plants respectively. These primers have been reported to be linked with the root traits (Shen *et al.*, 2001; Kanagaraj *et al.*, 2010) [14, 12]. Specific primers for betaine aldehyde dehydrogenase 2 (*BAD2*) gene (Bradbury *et al.*, 2005) [7] were used for assessing the diversity between Basmati and non-Basmati rice $F_2/F_4/F_5$ plants (Table 3.2). PCR amplifications were performed using PTC-100™ 96V thermo cycler (MJ Research, Inc., Watertown, MA, USA) and Taq DNA polymerase. The PCR reaction was conducted in a reaction volume of 20 µl containing 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 followed by 35 cycles of 94 ^{0}C for 1 min, 55 ^{0}C for 1 min, 72 ^{0}C for 2 min and final extension at 72^0 C for 10 min before cooling at 4 ^{0}C. Amplified products were stored at -20 ^{0}C till further use.

Result and Discussion

Physio-morphological studies

In field evaluation of Pusa 1121 x MAS25 F_5 Enormous variation was observed among 75 Pusa 1121 x MAS25 F_5 plants for plant height (76-141 cm, MAS25 - 92.2 cm and Pusa 1121 - 95.8 cm), panicle length (18.8-29.5 cm, MAS25 - 21.7 cm and Pusa 1121-26.1 cm), effective numbers of tillers per plant (3-14, MAS25 - 8.2 and Pusa 1121 - 9.2), length/breadth ratio of grain (dehusked) (4.01-6.04, MAS25-3.87 and Pusa 1121 - 5.53), grain yield per plant (2.9-21.7 g, MAS25 - 10.9 g and Pusa 1121 - 10.4 g), 1000 grain weight (16.8-28.6 g, MAS25 -23.7 g and Pusa 1121 – 24.4 g) (Table 1).

Table 1: Correlation analysis between different morphological traits in Pusa 1121 x MAS25 F_5 population in field under aerobic conditions

Traits	Plant height (cm)	Effective no. of tillers per plant	Panicle length(cm)	1000 grain weight (g)	Grain yield per plant (g)	Grain length/breadth ratio (dehusked)
Plant height (cm)	1.000					
Effective no. of tillers per plant	-0.052^{NS}	1.000				
Panicle length (cm)	0.387**	-0.313**	1.000			
1000 grain weight (g)	0.178^{NS}	-0.079^{NS}	0.185^{NS}	1.000		
Grain yield per plant (g)	0.246*	0.579**	0.193^{NS}	0.152^{NS}	1.000	
Grain length/breadth ratio (dehusked)	0.086^{NS}	-0.222^{NS}	0.434**	-0.207^{NS}	-0.296**	1.000

Net house evaluation of Pusa 1121 x MAS25 F_5 population large variation was observed among 23 Pusa 1121 x MAS25 F_5 plants and parental rice genotypes for plant height (45-108 cm, MAS25 – 59 cm and Pusa 1121 - 51.2 cm), panicle length (15-27.5 cm, MAS25 - 16.8 cm and Pusa 1121 - 23.91 cm), effective numbers of tillers per plant (3-12, MAS25 - 11.01 and Pusa 1121 - 9.22), length/breadth ratio of grain (dehusked) (4.23-5.55, MAS25 - 3.79 and Pusa 1121 - 5.10), grain yield per plant (1.4-24.65 g, MAS25 - 6.61 g and Pusa1121 - 5.33g), 1000 grain weight (4-19.5 g, MAS25 - 16.9 g and Pusa1121 - 24.8 g), root length (17-51 cm, MAS25 - 34 cm and Pusa 1121 - 25.9 cm), root thickness (7.2-29.6 mm, MAS25 - 17.4 mm and Pusa 1121 - 9.8 mm), fresh root weight (1.72-33.4 g, MAS25 - 13.3 g and Pusa 1121 - 2.50 g), dry root weight (0.49-9.88 g, MAS25 - 3.55 g and Pusa 1121 - 1.00 g).

Field evaluation of MASARB25 x Pusa Basmati 1460 F_4 population Large variation was observed among 50 MASARB25 x Pusa Basmati 1460 F_4 plants for plant height (60-118 cm, MASARB25 – 91.6 cm and Pusa Basmati 1460 - 93 cm), panicle length (17.38-30.4 cm, MASARB25-19.17 cm and Pusa Basmati 1460 – 27.48 cm), effective numbers of tillers per plant (3–13, MASARB25 – 8.2 and Pusa Basmati 1460 -7), length/breadth ratio of grain (dehusked) (4.14-5.69, MASARB25 -3.96 and Pusa Basmati 1460 -4.93), grain yield per plant (1.48-17.46 g, MASARB25 – 10.02 g and Pusa Basmati 1460 -9.38 g), 1000 grain weight per plant (12.3-26.4 g, MASARB25 – 20.78 g and Pusa Basmati 1460 -20.22 g) (Table 2).

Table 2: Correlation analysis between different morphological traits in MASARB25 x Pusa Basmati 1460 F_4 population (50 plants) under aerobic conditions

Traits	Plant height (cm)	Effective no. of tillers per plant	Panicle length (cm)	1000grain weight (g)	Yield per plant (g)	Grain length/breadth ratio (dehusked)
Plant height (cm)	1.000					
Effective no. of tillers per plant	0.165^{NS}	1.000				
Panicle length (cm)	0.454**	0.055^{NS}	1.000			
1000grain weight (g)	0.306*	0.261^{NS}	0.319*	1.000		
Yield per plant (g)	0.214^{NS}	0.698**	0.308*	0.542**	1.000	
Grain length/breadth ratio (dehusked)	0.166^{NS}	0.027^{NS}	0.075^{NS}	0.138^{NS}	-0.066^{NS}	1.000

Net house evaluation of MASARB25 x Pusa Basmati 1460 F_4 population Large variation was observed among 50 MASARB25 x Pusa Basmati 1460 F_4 plants for plant height (45-67 cm, MASARB25 - 65.4 cm and Pusa Basmati 1460 - 70.2 cm), panicle length (19-24.5 cm, MASARB25 - 19.3 cm and Pusa Basmati 1460 - 23.3 cm), effective numbers of tillers per plant (4-6, MASARB25 - 3 and Pusa Basmati 1460 - 4), length/breadth ratio of grain (dehusked) (5.01-5.48, MASARB25 - 3.92 and Pusa Basmati 1460 - 4.9), grain yield per plant (5.24-7.21 g, MASARB25 - 3.67 g and Pusa Basmati 1460 - 4.96 g), 1000 grain weight per plant (18.5-21.2 g, MASARB25 - 21.8 g and Pusa Basmati 1460 -19.5 g), root length (41.2-48 cm, MASARB25 - 41.5 cm and Pusa Basmati 1460 - 33.6 cm), root thickness (5.07-21.6 mm, MASARB25 - 23.4 mm and Pusa Basmati 1460 - 21.7 mm), fresh root weight (18.9-25.7 g, MASARB25 - 24.1 g and Pusa Basmati 1460 - 17.62 g), dry root weight (5.22-7.92 g, MASARB25 - 7.3 g and Pusa Basmati 1460 - 4.1 g).

Analysis of molecular diversity linked with promoting aerobic adaptation

Rice root growth encompasses a remarkable genetic diversity in terms of growth patterns, architecture, and environmental adaptations. Root traits are generally controlled by many genes through quantitative trait loci (QTL). Since the first study by (Champoux *et al.*, 1995) [8] to locate genes controlling rice root traits with molecular markers, many QTLs related to root traits have been identified in rice (Gowda

et al., 2011) [10]. Several markers have been identified to be linked with aerobic root traits/drought tolerance in rice. Forty two Pusa 1121 x MAS25 F_5 plants and 20 MASARB25 x Pusa Basmati 1460 F_4 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*., 2006); 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) [2].

Selection of promising plants

Field evaluation of Pusa 1121 x MAS25 F_5 and MASARB25 x Pusa Basmati 1460 F_4 plants under aerobic conditions led to identification of 24 plants having higher or comparable grain yield and/or grain length/breadth ratio than the parental genotypes under aerobic conditions. Though root traits were not studied, the better growth and yield productivity in these selected $F_{4/}$ F_5 plants may be due to the presence of aerobic genes/QTLs promoting better adaptation to aerobic conditions. Several of these selected high-yielding F_3/F_4 plants were in fact close to Pusa 1121 and Pusa Basmati 1460, indicating large amount of genetic content from these parents in addition to MAS25 and MASARB25 genes/QTLs from the aerobic rice parent promoting adaptation to aerobic conditions.

The net house evaluation of Pusa 1121 x MAS25 F_5 and MASARB25 x Pusa Basmati 1460 F_4 plants also led to the identification of 13 rice genotypes on the basis of higher or comparable grain yield, root length and root biomass (comparable to respective aerobic rice parent), grain length/ breadth ratio (comparable to respective Basmati rice parent) and allelic profile at *BAD2* locus. The plants having Basmati specific allele in homozygous or heterozygous condition were selected.

All the selected lines from this study will serve as novel material for the selection of stable direct seeded varieties development of these direct seeded varieties could be significant benefit 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

Significant and positive association of physiomorphic traits indicates that selection based on these traits would ultimately improve grain yield under water-limited situations. A high positive correlation of root traits with yield components is a clear indication that thicker and deeper roots facilitate easy uptake of water from deeper layers of soil and help the plants improve their water relationship and thereby yield. The inter relationships between root morphological characters and yield-related traits clearly identified the importance of root length, fresh root weight, root thickness and root dry weight in breeding rice genotypes for water limited aerobic soils.

References

1. Belder O, Bouman BAM, Spiertz JHJ, Peng S, Castaneda AR, Visperas RM. Crop performance, nitrogen and water use in flooded and aerobic rice. Plant and Soil. 2005;273:167-182.
2. Bernier J, Kumar A, Ramaiah V, Spaner D, Atlin G. A Large-effect QTL for Grain Yield under Reproductive-Stage Drought Stress in Upland Rice. Crop Sciences. 2007;47:505-516.
3. Borell A, Garside A, Fukai S. Improving efficiency of water for irrigated rice in a semi-arid tropical environment. Field Crops Res. 1997;52:231-248.
4. Bouman BAM, Peng S, Castaneda AR, Visperas RM. Yield and water use of irrigated aerobic rice systems. Agricultural Water Management. 2005;74:87-105.
5. Bouman BAM, Tuong TP. Field water management to save water and increase its productivity in irrigated rice. Agricultural Water Management. 2001;49(1):11-30.
6. Bouman BAM, Xiaoguang Y, Huaqi W, Zhimin W, Junfang Z, Bin C. Performance of aerobic rice varieties under irrigated conditions in North China. Field Crops Research. 2006;97:53-65.
7. Bradbury LMT, Henry RJ, Jin Q, Reinke F, Waters DLE. A perfect marker for fragrance genotyping in rice. Molecular Breeding. 2005;16:279:283.
8. Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, *et al*. Locating genes associated root morphology and drought avoidance in rice via linkage to molecular markers. Theoretical Applications of Genetics. 1995;90:969-981.
9. Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR. QTL: their place in engineering tolerance of rice to salinity. Journal of Experimental Botany. 2000;51:99-106.
10. Gowda VRP, Henry A, Yamauchi A, Shashidhar HE, Serraj R. Root biology and genetic improvement for drought avoidance in rice. Field Crops Research. 2011;122:1-13.
11. Ikeda H, Kamoshita A, Manabe T. Genetic analysis of rooting ability of transplanted rice (*Oryza sativa* L.) under different water conditions. Journal of Experimental Botany. 2007;58:309-318.
12. Kanagaraj P, Prince KSJ, Sheeba JA, Biji KR, Paul SB, Senthil A, *et al*. Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). Current Science. 2010;98:836-839.
13. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proceedings of National Academic Science. 1984;81:8014-8019.
14. Shen L, Courtois B, McNally KL, Robin S, Li Z. Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theoretical Applications of Genetics. 2001;103:75-83.
15. Tanksley SD. Mapping polygenes. Annual Review of Genetics. 1989;27:205-233.
16. Tuong TP, Bouman BAM. Rice production in water-scarce environments. Proceedings of the Water Productivity Workshop, Colombo, Sri Lanka. Colombo (Sri Lanka): International Water Management Institute, 2002.
17. Vaughan DA. The Genus Oryza L. Current Status of Taxonomy. IRPS 138. IRRI, Manila, Philippines, 1989.
18. Yoshihashi T. Quantitative analysis on 2-acetyl-1-pyrroline of an aromatic rice by stable isotope dilution method and model studies on its formation during cooking. Journal of Food Sciences. 2002;67:619-622.