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MOLECULAR MARKER ANALYSIS OF SELECTED RICE LINES FOR AEROBIC TRAITS UNDER WATER LIMITED CONDITIONS

KULDEEP KUMAR[1], KANIKA RANI[2], RAHUL KUMAR MEENA[2], MAHAVIR[2], RAJINDER KUMAR JAIN[2] AND SUNITA JAIN[2]

[1]NRCPB, Indian Agricultural Research Institute, New Delhi -110 012
[2]CCS Haryana Agricultural University, Hisar, Haryana - 125 004
e-mail: kuldeepdbc@gmail.com

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***Corresponding author**

ABSTRACT

In present scenario it is necessary to be a step ahead conferring with water scarcity. Aerobic rice is a newly developed water efficient rice system which flourishes under unsaturated and non-flooded condition. High yield in aerobic method of cultivation were ascribed to genotypes tolerance to moisture. In this context to assess the influence of water use efficiency on grain yields in rice genotypes, field experiments were conducted. Segregating aerobic x lowland progenies [MAS25/HKR47 (F_3 & F_4); MASARB25/HKR47 (F_3 & F_4) and [MASARB25/HKR47)/ HKR47 (BC_1F_3)] were evaluated for various physio-morphological and/or root traits and SSR markers linked to the traits promoting aerobic adaptation. In all the populations, enormous variation was observed for various physio-morphological and root traits. In these populations, significant positive correlation was observed between yield per plant with plant height, effective number of tillers per plant, grain length/breadth ratio, 100 grain weight, root length and/or root biomass. A total of 40 SSR markers linked to the QTL promoting aerobic adaptation (as found by others) were used for the parental screening. Presence of these traits was ascertained using single marker analysis. A total of 3-7 polymorphic microsatellite markers linked to the traits promoting aerobic adaptation were used to analyze the segregation in the selected F_4 and BC_1F_3 plants derived from the five crosses. In SSR marker analysis most of the selected plants were found to posses desirable alleles for the markers reported earlier to be linked with the aerobic adaptation traits.

INTRODUCTION

Rice is one of the most important food grain crops in the world and hence forms the staple diet of more than 70% of the world's population. Further it is estimated that nearly 5000 liters of water is needed to produce 1 kg of Rice (Bouman *et al*., 2005). A future challenge of food security depends on economizing rice cultivation and to explore alternate methods of growing low land rice (Kavitha *et al*., 2015). Sustainability of the irrigated rice production system and hence the food security is threatening due to increasing water scarcity. Answer of this major riddle can be the concept of aerobic rice (Kumari M. *et al*., 2015). Aerobic rice is extensive water saving, potentially high yielding and fertilizer responsive system in which rice is grown in non puddle condition under uniform slope (Wang *et al*., 2002; Bouman *et al*., 2005). Varieties suitable for this type of cultivation also possess ability to withstand intermittent drought spells with minimum yield loss.

Many SSR markers have been reported to be linked to QTL promoting aerobic adaptation in rice such as yield under drought (Venuprasad *et al*., 2009a; Vikram *et al*., 2011), maximum root length (Steele *et al*., 2007), basal root thickness (Qu *et al*., 2008) and root dry weight (Kanbar and Shashidhar, 2004). Sandhu *et al*. (2013) reported that QTL-qDRW$_{8.1}$ RM (152-310) flanks to dry root weight, qRL$_{8.2}$ RM (310-547) and qRL$_{9.1}$ RM(524-257) flank to root length and qRT$_{1.1}$ RM (488-237) flanks to root thickness. Dixit *et al*. (2014) identified three QTL-qDTY$_{3.1}$ (RM168-RM468), qDTY$_{6.1}$ (RM586-RM217), and qDTY$_{6.2}$ (RM121-RM541)-for grain yield under drought. QTL, qDTY$_{3.1}$ and qDTY$_{6.1}$, showed consistent effect across seasons under lowland drought-stress conditions.

Progress has been made in detecting large effect QTL conferring drought tolerance in lowland and irrigated rice [Price *et al*. (1997) and Serraj *et al*. (2011)]. Several QTL for grain yield under drought stress have been reported for both upland and lowland rice [Bernier *et al*. (2007) and Venuprasad *et al*. (2009b)].

Attempts have been made to transfer the QTL's responsible for aerobic adaptation in the aerobic rice varieties (MAS25 and MASARB25) to a high yielding rice variety (HKR47) so that the water requirement of the high yielding variety can be reduced. In present study attempts have been made to dissect the presence of these QTLs using SSR marker linked to them in the populations listed in material and methods.

MATERIALS AND METHODS

Planting material includes HKR47 (lowland high-yielding rice cultivars), MAS25, MASARB25 (both are aerobic rice genotypes) and progenies derived from them. Progenies of two crosses i.e. HKR47/MAS25, HKR47/MASARB25 were grown for two generation (F_3 and F_4 generation) while BC_1F_3 of (MAS25/HKR47)/HKR47 was grown for one year in the replicate of three at the Rice Research Station, Karnal, India during *kharif* season 2014 and 2015, respectively. Row to

row and plant to plant spacing were maintained at 20 cm × 15 cm. Standard package of practices were followed to raise good stand of crop. Surface irrigation was given whenever it's needed. Five best plants among the three replicate of each line were harvested at maturity and the data were recorded for agronomic traits like plant height, number of effective tillers per plant, panicle length, number of panicles per plant, number of grains per panicle, 100 grain weight, grain length/ breadth ratio and grain yield per plant.

The F_2 and F_3 of HKR47/MAS25, HKR47/MASARB25 populations were also sown in the net house in the replication of five, with one plant per pot. The data on root morphological traits i.e. root length, root thickness, fresh and dry root weight from five plants from each line at maturity were recorded and analyzed. Mean and standard deviations were used as the parameter of variability and phenotypic correlation coefficient was calculated using OPSTAT statistical tool.

Promising plants from respective crosses under both field and net house conditions were selected and used for genotyping. Performances of plant for yield and root traits were the main criteria for selection in field and net house respectively. Genomic DNA was isolated from young leaves using the CTAB method (Saghai Maroof *et al*., 1984). DNA quantity was estimated by ethidium bromide staining on 0.8% agarose gels using a standard containing 100 ng/µl *ë* genomic DNA. PCR amplification was essentially carried out as described earlier by Jain *et al*. (2006).

A total of 40 SSR were screened for polymorphism between the parents. A total of 5 markers showed polymorphism in both MAS25/HKR47 and MASARB25/HKR47 F_3 populations and were run on 10 and 18 selected plants respectively while 7, 6 and 6 markers showed polymorphism on 14, 19 and 16 selected plants from (MASARB25/HKR47)/HKR47 BC_1F_3, MAS25/HKR47 F_4 and MASARB25/HKR47 F_4 progenies, respectively.

RESULTS AND DISCUSSION

The net house experiments clearly indicated that root length, root thickness and root biomass in MAS25 and MASARB25 were significantly higher as compared to HKR47. Under water-limited conditions in net house, most of the plants of both crosses showed transgressive segregation for root traits and grain yield. F_3 and F_4 plants had greater root length, dry root weight, grain yield per plant and grain length-breadth ratio than the respective aerobic rice parents. Almost all of the promising plants selected after evaluation of root traits showed significantly better performance than parents for all the root traits analyzed (see figure 1, table 2,3,4 and 5).

A number of markers used in this analysis have been found linked to various traits promoting aerobic adaptation in different varieties of lowland/upland rice (see table 1), marker RM259 has been reported to be linked with *qDTY*1.2 controlling grain yield (Sandhu *et al.,* 2014), RM217 to *qDTY*6.1 controlling grain yield (Dixit *et al.,* 2014), RM6 to *qGE*2-2 for grain elongation (Cheng *et al.,* 2014), RM310 to *qRL*8.2, *qDRW*8.1, *qTN1.2,* and *qNPP*8.1 controlling root length, dry root weight, tiller number and number of panicles per plant respectively (Sandhu *et al.,* 2014) and RM231 was found to be linked with region controlling dry root weight (Venuprasad *et al.,* 2009). The molecular result signifies that some of the plant in all the

Table 1: Details of the markers used

Crosses	Makers found polymorphic
MAS25/HKR47 F_3	RM200; RM441; RM453; RM276
	RM217 [linked to *qDTY6.1*, Dixit *et al*. (2014)];
MASARB25/HKR47 F3	RM200; RM276;RM475
	RM175 [linked to *rn3*, Li *et al*. (2005)];
	RM217 [linked to *qDTY6.1*, Dixit *et al*. (2014)]
MASARB25/KHR47)/	RM12; RM222
HKR47 BC_1F_3	RM6 [linked to *bm2.1*, Swamy *et al* (2014)]
	RM211 [linked to *qDTY2.2*, Sandhu *et al*. (2014)]
	RM162 [linked to *ph6.1*, Thomson *et al*. (2013)];
	RM336 [linked to *dth7.1*, Thomson *et al*. (2013)];
	RM220 [linked to *gpl1.1* Septiningsih *et al*. (2003)]
MAS25/HKR47 F_4	RM582; RM512
	RM231 [linked to *qgy3.1*, Thomson *et al*. (2013)];
	RM217 [linked to *qDTY6.1*, Dixit *et al*. (2014)];
	RM336 [linked to *dth7.1*, Thomson *et al*. (2013)];
	RM526 [linked to $qGY_{2.2,}$ Zou *et al*. (2005)];
MASARB25/HKR47 F_4	RM12; RM304;
	RM259 [linked to *qDTY1.2,* Li *et al.,* 2005];
	RM410 [linked to $qPH_{9.1}$, Sandhu *et al*. (2013)];
	RM310 [linked to $qRL_{8.2,}$ Li *et al*. (2005)]
	RM161 [linked to *q* rn5, Li *et al*. (2005)];
Other markers used in the screening	RM281, RM275, RM224, RM8, RM10,
but not found polymorphic	RM19, RM226, RM258, RM269, and RM431, RM475, RM528, RM285
	RM17 [linked to *gw12.1*, Thomson *et al*. (2013)];
	RM282 [linked to *gpp3.1,* Septiningsih *et al*. (2003), *dth3.2* and *sh3.2*, Thomson *et al*. (2013)];
	RM306 [linked to *rfw1c*, Li *et al*. (2005)];
	RM287 [linked to *rn11* and *brt11c*, Li *et al*. (2005)]
	RM13 [linked to *QRbm5*, Yue *et al*. (2006)]

Figure 1 : Variation for different root trait in (a) MASARB25/HKR47 F_4 and (b) MAS25/HKR47 F_4 population respectively,where A and B stands for aerobic and high yeilding parents respectively while C, D, E and F stands for their respective progenies.

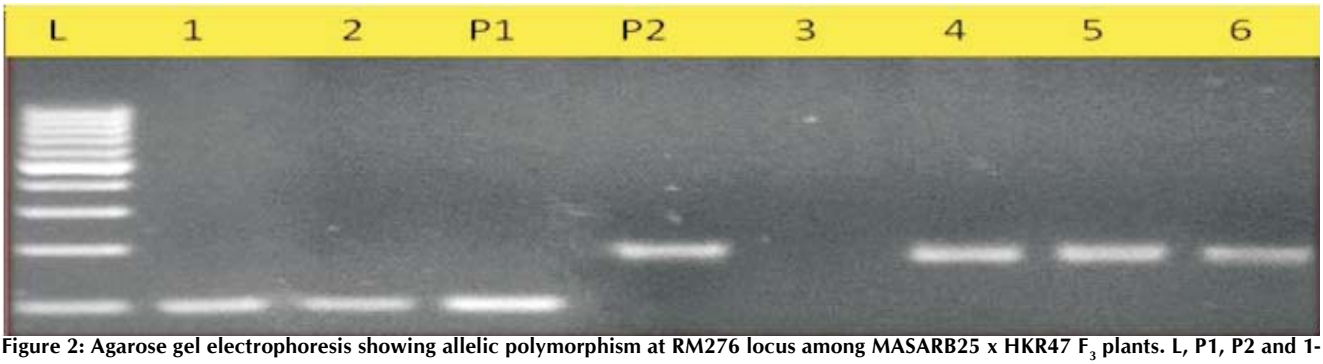

Figure 2: Agarose gel electrophoresis showing allelic polymorphism at RM276 locus among MASARB25 x HKR47 F_3 plants. L, P1, P2 and 1-6 stands for 100bp ladder, HKR47, MASARB25 and the selected F_3 plants respectively

Table 2 : Performance of F_3 population grown in the net house under aerobic conditions

Trait	MASARB25 Mean ± S.E. (Range)	HKR47 Mean ± S.E. (Range)	MAS25 Mean ± S.E (Range)	MASARB25/HKR47 F_3 population (17 plants*) Mean ± S.E. (Range)	MAS25/HKR47 F_3 population (12 plants*) Mean ± S.E (Range)
PH (cm)	69.2 ± 0.74 (67-71)	65 ± 0.51 (64-67)	68 ± 1.21 (64-71)	64.9 ± 0.84 (47-77)	68.5 ± 1.6 (50-94)
RL (cm)	41.6 ± 0.51 (40-43)	39.2 ± 0.67 (37-41)	40 ± 0.7 (38-42)	36.3 ± 1.26 (18-48)	37.9 ± 0.76 (28-45)
FRW(g)	8.15 ± 0.5 (6.5-9.45)	5.84 ± 0.12 (5.5-6.2)	7.81 ± 0.29 (7.2-8.4)	4.97 ± 1.36 (1.96-13.5)	13.3 ± 1.46 (4.24-23.4)
RT (mm)	15.1 ± 0.47 (13.6-16.2)	10.4 ± 0.77 (10.2-14.6)	12.4 ± 1.63 (10.1-18.9)	12.2 ± 1.47 (4.45-22.6)	10.3 ± 1.83 (1.58-26.1)
DRW (g)	2.45 ± 0.55 (1.64-4.62)	1.62 ± 0.29 (1.03-2.67)	3.42 ± 0.32 (2.33-4.17)	1.66 ± 0.9 (0.7-5.22)	3.47 ± 0.98 (0.53-8.14)
ET	4.8 ± 0.2 (4-5)	4.2 ± 0.38 (3-5)	4.5 ± 0.25 (4-5)	3.99 ± 0.9 (2-10)	4.42 ± 0.85 (2-8)
PL (cm)	21.3 ± 0.54 (19.8-24.7)	20.7 ± 0.26 (20.1-21.4)	20.4 ± 0.6 (18.8-22.3)	19.4 ± 0.9 (10.9-24.4)	19.0 ± 0.69 (12.7-22.8)
GW (g)	1.9 ± 0.71 (1.67-2.06)	1.88 ± 0.91 (1.56-2.13)	2.12 ± 0.41 (2.04-2.26)	1.42 ± 0.9 (0.91-2.02)	1.52 ± 0.17 (0.5-2.22)
Y (g)	3.65 ± 0.26 (2.97-4.2)	3.3 ± 0.18 (2.9-3.7)	4.77 ± 0.13 (4.4-5.2)	2.18 ± 0.96 (0.87-5.8)	3.1 ± 1.1 (0.09-7.79)
L/B	5.15 ± 0.04 (5.01-5.26)	4.59 ± 0.12 (4.34-4.87)	5.06 ± 0.11 (4.8-5.3)	4.92 ± 0.1 (4.56-5.41)	5.1 ± 0.2 (4.28-6.1)

progenies studied showed positive results for the presence of the QTL to which the marker is linked. The net house evaluation of F_3 plants also led to the identification of 16 rice plants 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 low land *indica* rice parent). These plants shall be further analyzed in order to select stable high yielding aerobic

Table 3 : Performance of F_3 population grown in the field under aerobic conditions

Trait	MASARB25 Mean ± S.E. (Range)	HKR47 Mean ± S.E. (Range)	MAS25 Mean ± S.E. (Range)	MASARB25/HKR47 F_3 population(30* plants) Mean ± S.E.(Range)	MAS25/HKR47 F_3 population(30* plants) Mean ± S.E.(Range)
PH (cm)	90.6 ± 0.75 (88-92)	85.8 ± 0.6 (85-87)	91.5 ± 0.58 (86-99)	80.5 ± 0.54 (68-132)	83.7 ± 0.9 (64-110)
ET	9.4 ± 0.51 (8-11)	8.2 ± 0.52 (7-9)	9.5 ± 0.75 (8-12)	7.4 ± 1.09 (2-13)	7.83 ± 0.89 (4-14)
PL (cm)	23.2 ± 0.15 (22.7-23.6)	18.1 ± 0.24 (17.6-8.9)	22.4 ± 0.24 (22.8-4.2)	20.3 ± 1.5 (11.5-27.7)	19.9 ± 0.42 (15.8-24)
Y (g)	10.8 ± 0.46 (6.58-11.4)	9.97 ± 0.44 (9.1-11.7)	11.3 ± 0.56 (9.87-2.5)	9.75 ± 1.8 (2.78-18.2)	7.79 ± 1.1 (2.94-5.8)
GW (g)	2.06 ± 0.45 (1.98-2.19)	1.98 ± 0.42 (1.88-2.02)	2.03 ± 0.35 (1.97-2.14)	2.15 ± 2.45 (1.2-2.74)	2.0 ± 0.66 (1.28-2.39)
GN	55.77 ± 0.97 (48-66)	61.41 ± 0.96 (50-70)	51.72 ± 1.3 (47-62)	61.28 ± 2.8 (51-72)	49.75 ± 1.9 (38-60)
L/B	4.12 ± 0.04 (4.02-4.21)	3.58 ± 0.04 (3.34-3.98)	4.21 ± 0.03 (4.2-4.3)	3.99 ± 0.45 (3.6-4.88)	3.91 ± 0.12 (3.43-4.29)

Table 4: Performance of F_4 population grown in the net under aerobic conditions

Trait	MASARB25 Mean ± S.E. (Range)	HKR47 Mean ± S.E. (Range)	MAS25 Mean ± S.E (Range)	MASARB25/HKR47 F_4 population (22 plants*) Mean ± S.E. (Range)	MAS25/HKR47 F_4 population (32 plants*) Mean ± S.E (Range)
PH (cm)	70.8 ± 0.86 (63-81)	66.0 ± 0.81 (55-71)	100.4 ± 0.82 (87-108)	77.3 ± 1.12 (57.7-96.5)	81.89 ± 0.77 (66-97)
RL (cm)	38.6 ± 0.55 (34.7-42.2)	32.24 ± 0.86 (27.9-38.1)	34.2 ± 1.65 (20.7-44.4)	30.34 ± 1.00 (13.9-55.0)	33.57 ± 0.84 (19.4-50.4)
FRW(g)	8.05 ± 0.29 (7.23-9.34)	5.46 ± 0.34 (4.27-6.13)	8.52 ± 0.34 (7.41-9.94)	12.33 ± 0.87 (3.11-37.3)	18.12 ± 2.17 (6.1-57.1)
RT (mm)	10.66 ± 0.54 (8.32-12.78)	9.58 ± 0.22 (8.68-10.28)	11.5 ± 0.35 (10.23-13.12)	12.53 ± 1.09 (2.03-32.41)	23.56 ± 1.70 (6.1-38.1)
DRW (g)	2.4 ± 0.27 (1.99-2.97)	1.78 ± 0.12 (1.62-2.01)	3.48 ± 0.27 (2.94-4.21)	3.85 ± 0.40 (0.59-19.98)	5.91 ± 1.04 (2.11-12.26)
ET	3.00 ± 0.71 (1-4)	2.6 ± 0.71 (1-4)	2.8 ± 0.50 (2-4)	4.27 ± 0.23 (1-8)	4.83 ± 0.71 (1-8)
PL (cm)	12.6 ± 0.25 (12-14)	10.4 ± 0.35 (9-12)	12.0 ± 0.58 (11-14)	12.66 ± 0.49 (6.5-23.1)	17.26 ± 0.91 (7.5-28.2)
GW (g)	2.01 ± 0.09 (1.87-2.21)	1.81 ± 0.04 (1.75-1.90)	1.97 ± 0.14 (1.80-2.23)	1.94 ± 0.04 (0.94-2.73)	1.87 ± 0.18 (1.3-2.39)
Y (g)	2.63 ± 0.73 (0.89-3.91)	2.03 ± 0.54 (1.56-3.38)	2.43 ± 0.76 (1.59-4.47)	4.97 ± 0.47 (0.14-15.36)	4.52 ± 1.15 (0.11-9.72)
L/B	3.74 ± 0.06 (3.56-3.86)	3.86 ± 0.04 (3.72-3.95)	3.79 ± 0.09 (3.63-3.98)	3.65 ± 0.03 (3.04-4.08)	3.63 ± 0.08 (3.28-3.98)

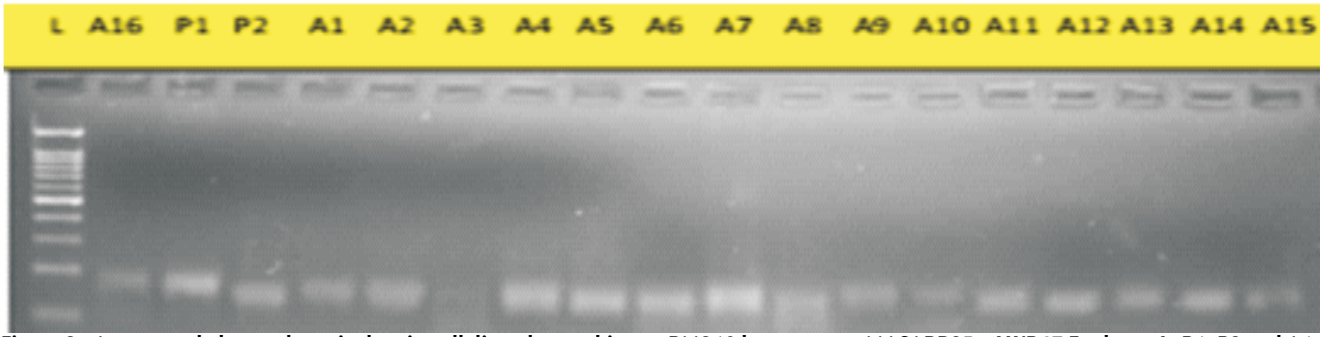

Figure 3 : Agarose gel electrophoresis showing allelic polymorphism at RM310 locus among MASARB25 x HKR47 F_4 plants. L, P1, P2 and A1-A16 stands for 100bp ladder, HKR47, MASARB25 and the selected F_4 plants respectively All MAS25 x HKR47 derived F_3 and F_4 plants had either MAS25 specific allele or HKR47 specific allele or both as shown in figure 4 and 5.

Table 5 : Performance of F_4 and BC_1F_3 population grown in the field under aerobic conditions

Trait	MASARB25 Mean ± S.E (Range)	HKR47 Mean ± S.E. (Range)	MAS25 Mean ± S.E (Range)	MASARB25/ HKR47 F_4 population (65 plants*) Mean ± S.E. (Range)	MAS25/ HKR47 F_4 population (50* plants) Mean ± S.E (Range)	(MASARB25 /HKR47)/HKR47 population (150 plants*) Mean ± S.E. (Range)
PH (cm)	93.6± 0.43 (88-98)	83.6 ± 0.36 (81-89)	91.2 ± 0.3 (88-95)	90.1 ± 1.01 (66-120)	82.2 ± 1.00 (60-100)	91.0 ± 0.89 (66-120)
ET	10.6± 0.47 (9-12)	10.0± 0.22 (9-11)	10.4 ± 0.35 (9-12)	7.1± 0.79 (3-13)	8.2± 1.00 (3-21)	9.7± 1.25 (4-24)
PL (cm)	24.4± 0.23 (23-26)	14.80±0.67 (11-18)	25.2 ± 0.47 (22-28)	21.9 ± 0.47 (16-26)	21.56 ± 0.47 (15-25)	23.5 ± 0.43 (17-28)
Y (g)	2.08± 0.24 (1.67-.62)	1.95± 0.35 (1.23-2.61)	2.01 ± 0.08 (1.89-2.17)	2.30± 0.10 (1.86-2.87)	2.22± 0.13 (1.49-2.59)	2.27± 0.14 (1.07-2.91)
GW (g)	10.9 ±0.32 (9.89-2.5)	9.46± 0.37 (9.87-12.6)	11.4 ± 0.28 (10.5-13)	14.92± 1.91 (3.48-36.79)	16.71± 2.67 (3.01-56.27)	18.66± 2.54 (2.01-78.0)
GN	67.33±0.79 (59-77)	68.46±0.33 (65-72.6)	67.9 ± 0.47 (63.3-73)	91.0±2.66 (30.67-156.0)	99.8±3.32 (32.7-195)	85.5±4.48 (30.67-194.7)
L/B	3.70 ± 0.07 (3.48-3.84)	3.67± 0.16 (3.23-4.03)	3.71 ± 0.07 (3.52-3.86)	3.74± 0.14 (3.25-4..44)	3.79± 0.14 (3.28-4.73)	3.54± 0.08 (3.07-4.18)

In Tables 2, 3, 4 and 5 * indicates number of plants survived from the selected plant when grown in replication of five. In all the tables (2, 3, 4 and 5) PH, ET, PL, GW, Y, NG and LB stands for plant height, number of effective tillers per plant, panicle length, 100-grain weight, yield per plant, number of grain per panicle and grains length/breadth ratio respectively

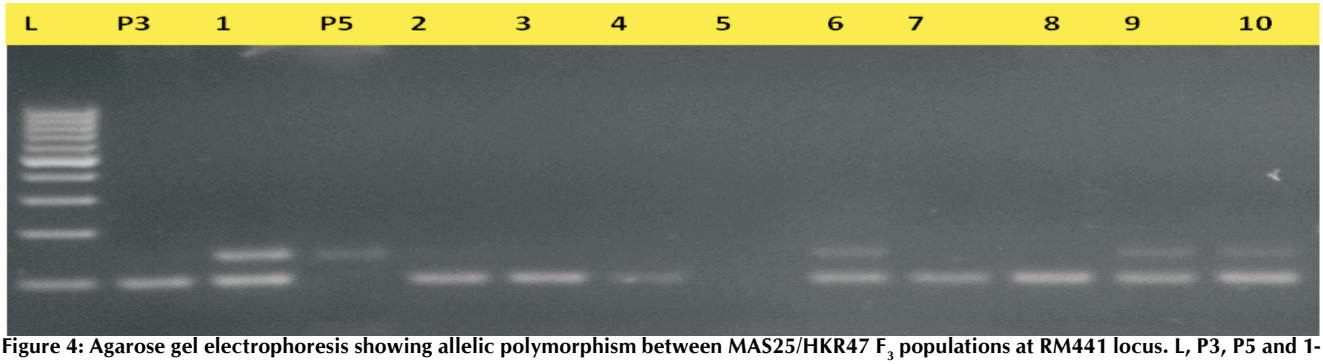

Figure 4: Agarose gel electrophoresis showing allelic polymorphism between MAS25/HKR47 F_3 populations at RM441 locus. L, P3, P5 and 1-10 stands for 100bp ladder, MAS25, HKR47 and the selected F_3 plants respectively.

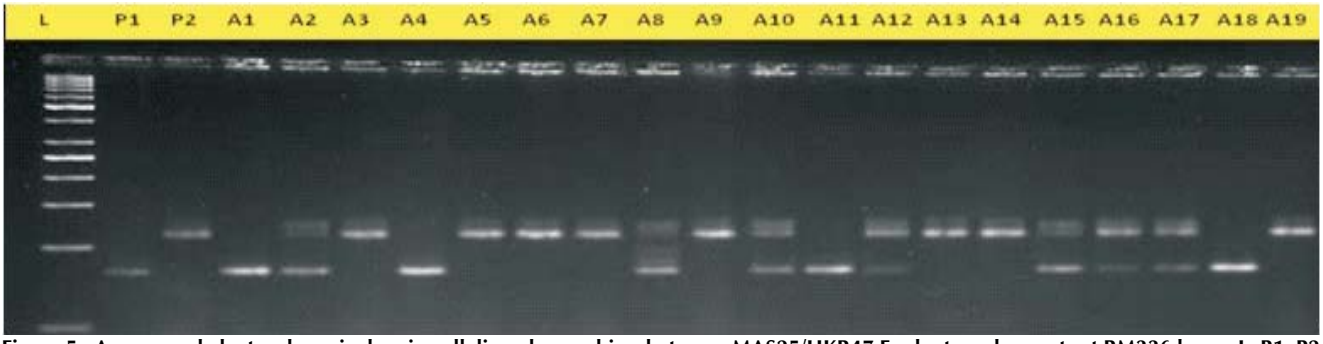

Figure 5 : Agarose gel electrophoresis showing allelic polymorphism between MAS25/HKR47 F_4 plants and parents at RM336 locus. L, P1, P2 and A1-A19 stands for 100bp ladder, HKR47, MAS25 and F_4 plants respectively.All (MASARB25/HKR47)/HKR47 derived BC_1F_3 plants had either MASARB25 specific allele or HKR47 specific allele as shown in figure 6.

rice lines. Progenies showing the positive results are good material for further generation studies.

At a SSR locus, all the F_3 and F_4 plants had either MASARB25 specific allele or HKR47 specific allele or both in MASARB25/ HKR47 progenies (Figure 2 and 3).

Besides this correlation analysis of each agronomic trait signifies that under aerobic conditions root traits such as root length, root thickness, fresh root weight and dry root weight etc, are directly correlated with yield. Significant and positive association of these traits indicates that selection based on these traits would ultimately improve grain yield under drought stress 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. Similar results were already reported by Sheeba (2005) for root length, Anbumalarmathi (2005) for dry root weight and Sinha *et al*. (2000) for root thickness. The interrelationships between root morphological characters and

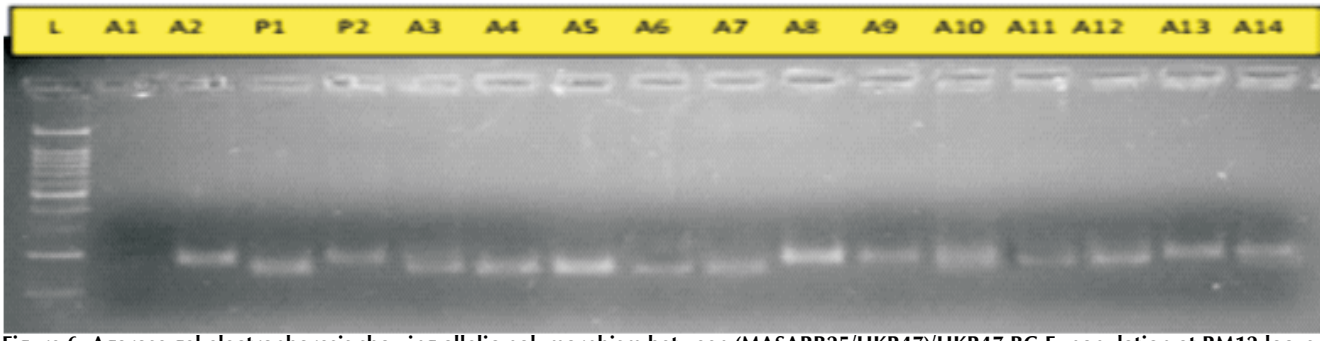

Figure 6: Agarose gel electrophoresis showing allelic polymorphism between (MASARB25/HKR47)/HKR47 BC_1F_3 population at RM12 locus. L, P1, P2 and A1-A14 stands for 100bp ladder, HKR47, MASARB25 and the selected BC_1F_3 plants in this cross respectively.

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.

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