International Journal of Agriculture Innovations and Research
Volume 3, Issue 1, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 04/08/2014 | Accepted on : 19/08/2014 | Published : 26/08/2014

Variability among CMS and Maintainer Lines of Rice (*Oryza sativa* L.) under Aerobic and Irrigated Conditions

K. R. Kamalnath Reddy, K. Nagendra, Devendra. K. Payasi, C. Mohan Kumar Varma, M. S. Anantha, V. Shenoy, H. E. Shashidhar

Abstract – **Twenty CMS/Maintainer lines were evaluated for root morphology, as well as grain yield under aerobic condition. These lines were evaluated for various attributes related to morphological and physiological traits. The aim was to determine the extent of variability between and among CMS/maintainer lines and the relationship existing between yield and root related traits for effective utilization under aerobic rice hybrid breeding program. Combined ANOVA for maintainer lines related to morphological and grain yield related traits revealed significant variation for years, moisture regime and genotypes and their interactions under different moisture regimes, whereas combined ANOVA for root related traits revealed significant variation for genotypes but there was mixed results for year and type (A/B). Test of significance for root and shoot related traits revealed that out of 20 genotypes, 15 genotypes showed no significant difference between CMS lines and their respective maintainer lines. Correlation studies showed highly significant positive correlation between single plant yield and root related traits *viz.,* maximum root length and root volume under both the moisture regimes. Molecular diversity analysis of twenty CMS/maintainer lines and seven commercial checks revealed three major clusters and cluster II consists of four out of five checks.**

Keywords – **Aerobic Rice, ANOVA, CMS Lines, Correlation, Maintainer Lines, Root Morphology, Simple Sequence Repeat Markers, Test of Significance, Wetland Condition.**

I. INTRODUCTION

Food and water are the most important necessities for survival, but with an increasing demand for food and a looming water crisis, shortage of both may be on the horizon unless innovative technologies are developed. Water, is fast becoming a precious commodity. Scientists are now taking on the challenging task of developing rice production systems that can cope with water scarcity without loss in productivity. Worldwide, more than 75% of the rice production comes from 79 million ha of irrigated lowland. Over 17 million ha of Asia's irrigated rice may experience "physical water scarcity" and 22 million ha may experience "economic water scarcity" by 2025[1]. In Asia, upland rice is aerobically grown with minimal inputs and it is usually planted as a low yielding subsistence crop in the adverse upland conditions. Aerobic rice gives hope to farmers who do not have access to enough water to grow flooded lowland rice [2]. Rice (*Oryza sativa* L.) is the major food crop of India which holds globally second position in production after China. In India, rice occupies about one-quarter of cropped area and contributes 40 to 43% of total food grain production and 46% of total cereal production [3]. However, the growing domestic population of 1.3% per year urges to increase the rice productivity of double the quantity from the current status of 133 million tons [4].

The plants which cannot produce fertile pollen grains are considered as male sterile. The role of cytoplasm in causing male sterility was first reported in 1954 [5 and 6]. Cytoplasmic male sterile (CMS) lines are being exploited for commercial purpose should have a stable male sterility, adaptability to tropical rice growing conditions, good out crossing potential and should be excellent source for hybrid seed production [7]. The cytoplasmic male sterility (CMS) is controlled by the interaction of cytoplasmic and nuclear genes. Among the various sources of CMS systems, Wild Abortive (WA) system has been the most commonly used source [8]. Among different WA-CMS sources IR58025A has been one of the popular and most extensively used CMS line [9]. Knowledge of genetic diversity among perspective parental lines is important for the success of a hybrid rice breeding program as it determines the magnitude of heterosis in F_1 hybrids [10]. To improve the hybrid seed production, it is essential to increase the hybrid seed yield by improving the out crossing capacity of CMS lines [11and 12].

Hybrid vigor does not make rice hybrids more or less tolerant to biotic stress than parental lines [13]. Therefore, in order to increase the efficiency of hybrid rice breeding program, it is essential to identify the drought tolerant parental lines that confer resistance to drought stress. Yield improvement under drought stress can be achieved by selecting secondary traits contributing to drought tolerance in breeding programs [14 and 15]. Strong root system enables hybrid rice to improve its access to water. Root system helps the plant in different ways like anchorage and water uptake from deep layers of the soil. The difference between shallow and deep rooted varieties will depend on the penetration of roots i.e., more than 30cm from the soil surface [16]. Among the root traits studied, total root length is strongly related to drought tolerance under rainfed upland condition [17]. Rice roots (total dry matter, maximum root depth and root length density) will have maximum growth till flowering stage and decrease towards maturity stage [16]. Several putative traits contributing to drought stress have been reported in rice [18].

Molecular marker technology serves as a tool for selecting complex traits and allows breeders to track genetic loci controlling drought resistance traits, without going for phenotypic evaluation, thus reducing the need for extensive field testing over space and time [19]. RM212 was linked to root depth, penetrated root thickness, deep root to shoot ratio, deep root dry weight, deep root per tiller and deep root in CT9993/IR62266 DH lines [18]. Molecular markers can be used to identify

linkage to quantitative trait loci (QTL) for rooting ability and these can be selected more easily in a breeding programme than the traits themselves [20]. The target segment on chromosome 9 (RM242-RM201) significantly increased root length under both irrigated and drought stress treatments, confirming that root length QTL from Azucena functions in a *indica* genetic background [20].

II. Material and Methods

Twenty CMS lines and maintainer lines mostly derived from wild abortive cytoplasm, were used for the present study (Table 1). The study was conducted at Barwale Foundation Research Farm, Hyderabad, located at the latitude of $17^0$24'20''N and longitude of $78^0$13'31"E and altitude of 536 meters above mean sea level. The soil of the experimental conditions were vertisol/clay loam having pH more than 7. All the 20 maintainer lines were evaluated under irrigated and aerobic conditions in two consecutive *Kharif* seasons (2008 and 2009) for their agronomical traits. The twenty CMS and their corresponding maintainer lines were evaluated for roots to know the performance of root related traits during *Rabi* seasons 2009 and 2010.

Table 1: List of CMS and maintainer lines used for morphological trait evaluation for root, agronomical traits and for screening the molecular markers related to root traits

S.No.	Barwale Foundation Code	CMS/Maintainer Lines	Parentage
1	25A & B	IR58025A&B	IR48483A/PUSA 167-120-3-2
2	6A & B	IR68885A&B	IR62829BMI/IR62829BMO
3	7A & B	IR68888A&B	IR62829A/IR62844-15-6-1-10-3-4
4	9A & B	IR68897A&B	IR62829A/IR62856-15-3-1-1-7-5-10-3
5	11A & B	IR68902A&B	IR62829A/IR62856-162-3-4-7-6-1-3
6	16A & B	IR70369A&B	IR62829A/IR62849-110-13-8-7-4-5-6
7	17A & B	IR70372A&B	IR62829A/PUSA BASMATI
8	18A & B	IR70959A&B	IR62829A/IR62852-2-4-3-1-5-2-6-3-1
9	19A & B	IR69624A&B	IR62829A/IR62849-110-1-4-8-5-3-10
10	23A & B	IR72081A&B	IR58025A/IR65493-67-3-2-2-4-10-9-1
11	26A & B	IR73321A&B	IR68895A/IR68952-7-3-3-5-3-9-1
12	30A & B	IR73793A&B	IR68895A/IR68950-6-1-10-8-3-2-1-1
13	31A & B	IR75596A&B	D297A/IR68897B
14	36A & B	IR68886A&B	IR62829A/IR62832-58-7-8-1-8-12-4
15	39A & B	IR79128A&B	IR68897A/IR71567-69-2-1
16	40A & B	IR79156A&B	IR68897A/IR72798-42-1-2
17	41A & B	IR80151A&B	IR68897A/IR72800-39-3-2
18	43A & B	IR80155A&B	IR75608A/IR73330-16-3-2
19	44A & B	IR80156A&B	IR75608A/IR71591-9-3-2
20	46A & B	IR80559A&B	IR73328A/IR73330-25-2-2

Source: IRRI (International Rice Research Institute, Philippines)

Phenotyping:

To identify the drought tolerant maintainer lines with good yield related traits under water limited condition, 20 sets of CMS/maintainer lines were evaluated for two consecutive *Kharif* seasons in 2008 and 2009. The experiment was conducted using randomized complete block design (RCBD) with two replications under two different conditions, i.e. Irrigated condition and aerobic condition. Under irrigated condition, 21 days seedlings were transplanted by using 20 X 20 cm spacing and one plant per hill and allowed to grow, whereas under aerobic condition sowing was done using direct seeding method by using 30 X 15 cm spacing. Observations were recorded for days to fifty percent flowering, plant height, tiller number, panicle number, panicle length, spikelet fertility, 100 seed weight, single plant yield, straw weight and harvest index. Various root screening methodologies were used to identify the root trait association with the drought tolerance in rice. Root length and root dry weight traits are the best indicators to predict the yield in rice [21 and 22]. Conventional breeding for drought resistance in rice for root related traits was conducted using farmer-participatory plant breeding approaches by [23]. Most root research for drought resistance has focused on characterizing root architecture, especially root growth at depth, in response to drought in different agro ecological systems [24]. For root phenotyping, the experiment was carried out in complete randomized design (CRD) with three replications. The experiment was taken up in PVC cylindrical tube model, fabricated with 1 mm thick acrylic sheet folded in a cylindrical shape with dimensions of 1.2 m length and 25 cm diameter. Each pipe was filled with 80 kg of air dried fine soil mixture which was comprising of 4:1:1 (Black soil, vermicompost and farm yard manure) in three stages. Soil compacting was done by using the circular metal plate attached with a rod to achieve uniformity throughout the pipe. Top dressing was done by using the 4:1:1 soil mixture along with basal dose of

fertilizer [25 and 26]. 3-4 seeds per pipe were directly sown, and after 15-20 days of germination, the thinning was done and allowed only single plant to grow in each pipe and irrigation was given on daily basis. After 100 days, the sampling was done by separating shoot part from the root to record shoot observations like shoot length (SL) in cm, Tiller No (TN) and shoot dry weight (SDW) in grams. Sampling was done as described by [27] with care taken to retain roots, root hairs and root branches. Pipes were submerged in water for soaking to loosen the soil for about 4-5 hours, then the pipes were opened up for washing with 1 HP water jet motor by controlling water pressure to remove the soil intact with the roots and fine wash was done to remove the fine soil particles. Then the roots were stored in a polythene bags for recording observations. The observations recorded on root traits were maximum root length (MRL) which was measured in cm from collar region to longest root, root dry weight (RDW) measured in grams once roots are oven dried at 65^0 C for about 72 hours, Root volume (RV) in cm^3 measured by using Archimedes principle (the additional amount of ethanol outflow from the measuring jar was recorded when whole roots was dipped in a ethanol filled measuring jar). Other traits such as root to shoot length ratio (R: S ratio), total plant length (TPL) shoot and root observations were also recorded.

Genotyping:

Fifty one SSR markers related to root traits were used for the molecular diversity. The genomic DNA was extracted from the young seedlings of 20 CMS and their corresponding maintainer lines by using Dellaporta method [28]. PCR reaction was carried out in total of 15 µl reaction volume consisting of 1X PCR buffer (10 mM Tris-Cl; 50 mM KCl), 2.5 mM dNTPs 5 pM each of forward and reverse primers, 0.5 U of *Taq* Polymerase enzyme (Merck Specialties Pvt. Ltd.), and 50 ng/ µl of DNA template. PCR was carried out in eppendorf EP master by programming initial denaturation of 95^0 C for 5 min., followed by 35 cycles of denaturation at 94^0 C for 15 sec., primer annealing at 60^0 C for 45 seconds, primer extension for 45 sec. and final extension of 72^0 C for 5 min.The amplified products along with the 100 bp ladder marker were resolved using 4% polyacrylamide gel electrophoresis, followed by silver staining procedure [29]. The genotypic dataset was generated using the PCR amplification product size scored as different alleles based on the base pair (bp) size of the amplified product.

Data Collection and Statistical analysis:

Replicated data were recorded on three plants from each entry by excluding the border plants. Data were collected in two consecutive *Kharif* seasons for the agronomical traits *viz.,* days to 50% flowering (DFF), plant height (PH), tiller number (TN), panicle number (PN), panicle length (PL), average filled grains (AFG), spikelet number (SN), spikelet fertility (SF), test weight (TW), single plant yield (SPY), biomass (BM) and harvest index (HI). Replicated data were recorded on three plants from each entry by excluding the border plants. Data were collected in two consecutive *Rabi* seasons for the physiological and morphological traits *viz.,* shoot length (SL), tiller number (TN), maximum root length (MRL), root volume (RV), root dry weight (RDW), shoot dry weight (SDW), total plant length (TPL), total dry weight (TDW), root to shoot length ratio (RSLR), root to shoot dry weight (RSDW), root growth rate (RGR), shoot growth rate (SGR), plant length growth rate (PLGR) and plant weight growth rate (PWGR).

Data was analysed for analysis of variance (ANOVA) using Crop Stat Version 7.2. 2007.3. Fischer's protected Least Significant Difference (LSD) at 5% probability was used to compare means where the F was significant. For root related traits each CMS/ maintainer line was checked for significance of difference in their means by Fischer's protected Least Significant Difference (LSD) at 5% probability where 't' test was significant.

III. RESULTS

ANOVA results for different morphological and yield related traits under different moisture regimes:

The ANOVA (Table 2) revealed that there was significant difference across year, moisture regime and genotype for all the traits *viz.* days to 50% flowering (DFF), plant height (PH), tiller number (TN), panicle number (PN), average panicle length (APL), average filled grains (AFG), spikelet number (SN), spikelet fertility (SF), test weight (TW), single plant yield (SPY), biomass (BM), harvest index (HI). The interaction Y*M showed significant difference for all the interactions except for three traits i.e., DFF, SF and TW. The interaction M*G also showed significant difference for all the interactions. The interaction Y*G revealed that significant differences existed for all the interactions. The interaction Y*M*G showed that there was significant difference for all the interactions except DFF and HI. The range for all the traits has been showed in the Table 3.

Table 2: ANOVA for morphological and grain yield related traits under different moisture regimes

Source of Variation	DFF	PH	TN	PN	APL	AFG	SN	SF	TW	SPY	BM	HI
Year (Y)	**	*	**	**	**	**	**	**	**	**	**	**
Moisture regime (M)	**	**	**	**	**	**	**	**	**	**	**	**
Genotype (G)	**	**	**	**	**	**	**	**	**	**	**	**
Y*T	ns	**	**	**	**	**	ns	**	ns	**	**	*
T*G	**	**	**	**	**	**	**	**	**	**	**	**
Y*G	**	**	*	*	**	**	**	**	**	**	**	**
Y*T*G	ns	**	*	**	**	**	**	*	**	*	*	ns

ns: not significant; * significant at P<0.05% ; ** significant at P<0.01% level by F test

International Journal of Agriculture Innovations and Research
Volume 3, Issue 1, ISSN (Online) 2319-1473

Table 3: Morphological and grain yield related traits range under different moisture regimes

Trait*	Moisture regime	*Kharif* 2008		*Kharif* 2009	
		Minimum	Maximum	Minimum	Maximum
DFF	Aerobic	84.00	105.50	86.50	109.00
	Control	83.50	101.00	81.50	103.50
PH (cm)	Aerobic	62.17	87.33	64.63	88.50
	Control	84.00	106.67	77.25	110.25
TN	Aerobic	13.33	16.83	11.75	18.63
	Control	12.17	17.17	11.33	18.50
PN	Aerobic	11.84	16.17	11.67	16.17
	Control	11.75	18.63	9.83	18.00
APL	Aerobic	21.33	26.19	21.13	27.00
	Control	22.08	26.92	22.48	26.32
AFG	Aerobic	91.33	134.83	86.19	137.38
	Control	106.17	155.25	107.83	151.92
SN	Aerobic	129.00	181.08	141.25	181.50
	Control	125.44	181.13	142.29	194.26
SF (%)	Aerobic	63.02	85.75	65.33	85.26
	Control	73.92	87.83	74.29	86.63
TW (g)	Aerobic	1.81	2.21	1.75	2.23
	Control	2.00	2.40	1.96	2.38
SPY (g)	Aerobic	11.24	19.12	11.67	23.06
	Control	15.51	20.66	15.66	25.95
BM (g)	Aerobic	35.00	47.63	35.21	50.07
	Control	41.04	52.95	43.37	58.08
HI	Aerobic	0.31	0.44	0.32	0.46
	Control	0.32	0.45	0.35	0.48

*DFF: days to 50% flowering, PH: plant height, TN: tiller number, PN: panicle number, APL: average panicle length, AFG: average filled grains, SN: spikelet number, SF: spikelet fertility, TW: test weight, SPY: single plant yield, BM: biomass, HI: harvest index

Combined ANOVA for root and Shoot related traits:

The ANOVA (Table 4) revealed that there was significant difference for genotype for all the traits *viz.* shoot length (SL), tiller number (TN), maximum root length (MRL), root volume (RV), root dry weight (RDW), shoot dry weight (SDW), total plant length (TPL), total dry weight (TDW), root to shoot length ratio (RSLR), root to shoot dry weight (RSDW), root growth rate (RGR), shoot growth rate (SGR), total plant length growth rate per day (PLGR), total plant weight growth rate (PWGR) whereas for the year there was significant difference for four traits i.e. MRL, RV, RDW, TDW, PWGR and the other traits such as SL, TN, SDW, TPL, RSLR, RSDW, RGR, SGR and PLGR had shown no significant difference. The type comparison (A/B) i.e. to know the difference between CMS and maintainer lines the ANOVA showed there was significant difference in all the traits except RSLR and RSDW.

The ANOVA revealed that the interactions Y*T and Y*G*T showed no significant differences for all the traits where as for Y*G it has shown no significant difference for SL, TN, MRL, TPL, RSLR, SGR and PLGR. Also it showed significant difference for RV, RDW, SDW, TDW, RSDW, RGR and PWGR. The interaction G*T showed no significant difference in most of the traits except RSLR and RSDW. The ranges for all the traits are depicted in the graphical representation Figure 1.

Table 4: ANOVA for root morphological traits under well watered condition

Source of Variation	SL	TN	MRL	RV	RDW	SDW	TPL	TDW	RSLR	RSDW	RGR	SGR	PLGR	PWGR
Year (Y)	ns	ns	**	**	**	ns	ns	*	ns	ns	ns	ns	ns	*
Genotype (G)	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Type (A/B)	**	**	*	**	**	**	**	**	ns	ns	**	**	**	**
Y*G	ns	ns	ns	**	**	**	ns	**	ns	**	*	ns	ns	**
G*T	ns	ns	ns	ns	ns	ns	ns	ns	**	*	ns	ns	ns	ns
Y*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*G*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: not significant; * significant at P<0.05% ; ** significant at P<0.01% level by F test

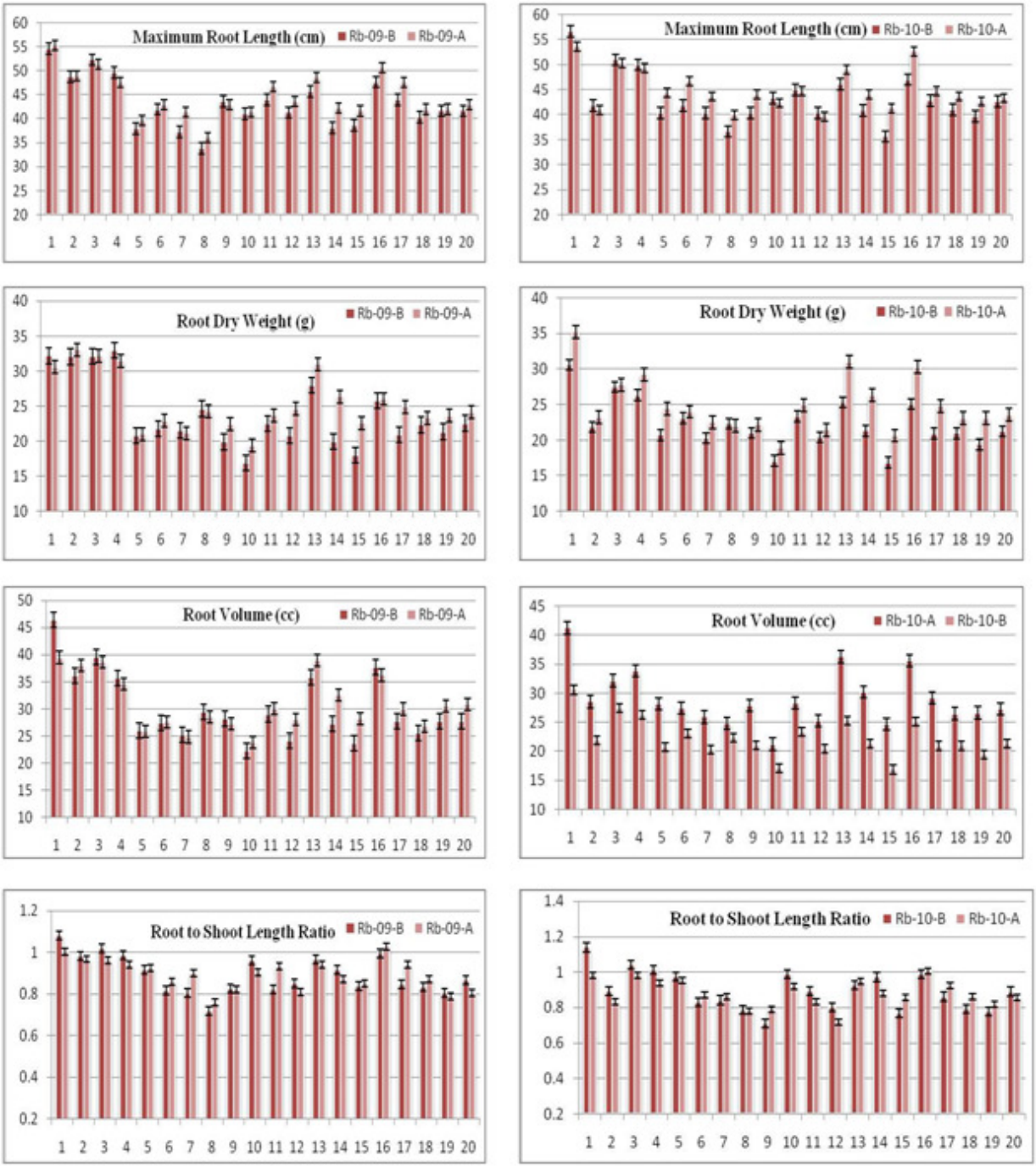

Fig.1. Graphical representations of different root morphological traits in two *Rabi* (Rb) seasons

Note: Entries from 1 to 20 are as mentioned in the Table 1

Percentage decrease in yield and related traits under different moisture regimes:

Spikelet fertility: In spikelet fertility, the percentage decrease between aerobic and irrigated conditions for the year 2008 was in the range of 2.37% to 16.46%, whereas, in the year 2009 the percentage reduction for SF was in the range of 1.46% to 12.61% (Table 5).

Single plant yield: In single plant yield, the percentage reduction in the year 2008 between aerobic and irrigated conditions was in the range of -0.93% to 33.20%, whereas, in 2009 the percentage decrease for SPY was in the range of 3.83% to 41.21% (Table 5).

Biomass: In Biomass, the percentage decrease between aerobic and irrigated condition for the year 2008 was in the range of -2.18% to 19.37%, whereas, in the year 2009 the percentage reduction for the BM was in the range of -4.21% to 21.29% (Table 5).

Harvest Index: In harvest index, the percentage decrease between aerobic and irrigated condition for the year 2008 was in the range of -0.23% to 19.64% and in the year 2009 the percentage reduction for the HI was in the range of 0.96% to 17.26% (Table 5).

International Journal of Agriculture Innovations and Research
Volume 3, Issue 1, ISSN (Online) 2319-1473

Table 5: Combined means of twenty maintainer lines for different attributes with % decrease under different moisture regimes

Genotype	Year	Spikelet fertility			Single plant yield			Biomass			Harvest index		
		Wetland	Aerobic	% DEC	Wetland	Aerobic	% DEC	Wetland	Aerobic	% DEC	Wetland	Aerobic	% DEC
IR58025B	2008	87.83	85.75	2.37	20.09	19.12	4.81	44.63	43.30	2.98	0.45	0.44	1.90
	2009	86.83	85.26	1.82	25.95	23.06	11.12	55.46	50.07	9.73	0.47	0.46	1.55
IR68885B	2008	83.07	76.41	8.02	17.61	12.54	28.81	45.36	36.58	19.37	0.39	0.34	11.78
	2009	78.07	74.06	5.14	19.85	11.67	41.21	49.65	35.21	29.09	0.40	0.33	17.26
IR68888B	2008	81.06	79.09	2.44	17.81	17.97	-0.93	42.55	43.48	-2.18	0.42	0.41	1.27
	2009	85.67	79.89	6.75	20.73	19.37	6.55	45.62	45.24	0.82	0.45	0.43	5.78
IR68897B	2008	84.25	81.13	3.71	18.84	17.69	6.11	44.85	42.02	6.33	0.42	0.42	-0.23
	2009	85.36	80.09	6.18	21.86	19.91	8.93	49.16	45.90	6.63	0.44	0.43	2.53
IR68902B	2008	80.65	73.88	8.40	16.83	11.24	33.20	42.11	35.00	16.89	0.40	0.32	19.64
	2009	77.06	72.61	5.77	15.66	11.91	23.96	43.79	37.03	15.43	0.36	0.32	10.06
IR70369B	2008	82.92	74.48	10.18	16.78	14.96	10.82	52.95	47.63	10.03	0.32	0.31	0.79
	2009	79.23	75.18	5.11	20.40	16.57	18.75	58.08	49.44	14.88	0.35	0.33	4.56
IR70372B	2008	79.22	76.03	4.03	17.18	16.27	5.33	48.26	46.03	4.62	0.36	0.35	0.69
	2009	78.08	73.04	6.46	19.86	16.44	17.22	54.25	46.63	14.06	0.37	0.35	3.72
IR70959B	2008	75.50	67.08	11.15	15.55	15.19	2.30	43.33	42.85	1.11	0.36	0.35	1.24
	2009	75.23	68.54	8.90	19.13	16.63	13.06	44.30	41.60	6.09	0.43	0.40	7.56
IR69624B	2008	80.24	72.72	9.37	17.33	16.39	5.46	42.21	41.10	2.64	0.41	0.40	2.96
	2009	79.24	78.09	1.46	22.73	18.29	19.51	54.55	44.96	17.57	0.42	0.41	2.35
IR72081B	2008	75.43	63.02	16.46	15.51	14.88	4.07	43.53	41.98	3.56	0.36	0.35	0.48
	2009	74.98	65.53	12.61	22.19	18.55	16.42	49.43	44.20	10.59	0.45	0.42	6.62
IR73321B	2008	73.92	63.78	13.72	17.54	14.73	16.03	43.58	38.66	11.28	0.40	0.38	5.41
	2009	77.66	73.26	5.66	18.01	15.70	12.83	43.72	40.54	7.27	0.41	0.39	6.10
IR73793B	2008	75.20	70.80	5.85	15.76	13.72	12.90	44.38	38.79	12.60	0.36	0.35	0.49
	2009	75.82	68.71	9.38	19.13	14.04	26.60	51.53	40.21	21.98	0.37	0.35	5.98
IR75596B	2008	82.89	79.21	4.44	18.10	16.38	9.48	43.06	41.00	4.79	0.42	0.40	4.92
	2009	80.57	78.28	2.83	21.77	20.04	7.92	49.96	47.33	5.26	0.44	0.42	2.74
IR68886B	2008	84.38	77.02	8.72	19.39	17.12	11.71	45.09	41.51	7.95	0.43	0.41	4.08
	2009	82.59	80.74	2.24	20.71	19.91	3.83	43.37	45.19	-4.21	0.48	0.44	7.74
IR79128B	2008	74.00	68.73	7.13	15.82	15.22	3.79	41.04	39.56	3.59	0.39	0.38	0.17
	2009	74.29	71.34	3.98	17.94	15.14	15.61	45.20	39.26	13.15	0.40	0.39	2.78
IR79156B	2008	85.85	80.06	6.74	20.66	18.52	10.37	46.49	43.86	5.67	0.44	0.42	4.96
	2009	86.54	84.30	2.58	24.22	22.57	6.82	51.36	48.63	5.32	0.47	0.46	1.56
IR80151B	2008	77.90	74.19	4.76	17.58	16.86	4.12	43.83	42.68	2.61	0.40	0.39	1.57
	2009	78.79	76.26	3.21	21.76	17.26	20.66	52.01	45.90	11.75	0.42	0.38	10.13
IR80155B	2008	79.15	73.87	6.68	17.36	16.19	6.77	44.06	42.33	3.93	0.39	0.38	2.95
	2009	79.45	75.85	4.53	20.76	19.13	7.83	48.73	46.65	4.26	0.43	0.41	3.95
IR80156B	2008	73.95	68.60	7.24	16.16	15.53	3.93	42.39	41.40	2.32	0.38	0.37	1.71
	2009	80.00	74.49	6.89	18.44	17.59	4.61	46.11	44.39	3.75	0.40	0.40	0.96
IR80559B	2008	74.27	68.80	7.37	17.97	14.80	17.67	43.93	39.93	9.10	0.41	0.37	9.40
	2009	76.11	74.88	1.62	21.89	18.62	14.94	48.29	42.90	11.16	0.45	0.43	4.30
LSD (5%)		3.02			1.79			3.39			0.02		
C.V. %		7.00			8.50			6.8			5.6		

Test of significance: The t test results for seasonal mean of all the 20 CMS/maintainer lines revealed significant difference for all the root traits except RSLR and RSDW. Similar results were reported by [30] in their study that there was significance difference for maximum root length and root volume between CMS and maintainer lines.

Individual types (A & B lines) were subjected to t test to know the differences among CMS and maintainer lines. Among twenty set of CMS/maintainer lines, 't' test revealed that fifteen lines did not show significant differences for the six traits *viz.,* maximum root length, root volume, root dry weight, shoot dry weight, total plant length and total dry weight. IR68886A/B showed

significant difference for all the six traits as depicted in (Table 6).

Table 6: Comparison of different attributes between CMS and maintainer lines

ENTRY	MRL	RV	RDW	SDW	TPL	TDW
25A&B	ns	ns	ns	ns	ns	ns
6A&B	ns	ns	ns	ns	ns	ns
7A&B	ns	ns	ns	ns	ns	ns
9A&B	ns	ns	ns	ns	ns	ns
11A&B	*	ns	ns	*	ns	ns
16A&B	ns	ns	ns	ns	ns	ns
17A&B	ns	ns	ns	ns	*	*
18A&B	ns	ns	ns	ns	ns	ns
19A&B	ns	ns	ns	ns	ns	ns
23A&B	ns	ns	*	**	ns	*
26A&B	ns	ns	ns	ns	ns	ns
30A&B	ns	ns	ns	ns	ns	ns
31A&B	ns	ns	ns	ns	ns	ns
36A&B	*	**	*	*	*	**
39A&B	**	ns	ns	ns	ns	ns
40A&B	ns	ns	ns	ns	ns	ns
41A&B	ns	ns	ns	ns	ns	ns
43A&B	ns	ns	ns	ns	ns	ns
44A&B	ns	**	**	ns	ns	*
46A&B	ns	ns	ns	ns	ns	ns

*, ** represents significance difference at 5% and 1% level of probability by 't' test

Maximum root length showed no significant difference in seventeen lines while three showed significant difference. Highest significant difference was found between IR79128A&B. Root volume difference was non significant in eighteen lines and significant differences were found only in two lines. Highest significant difference was found in IR68886A&B and IR80156A&B. For root dry weight, seventeen lines were non significantly different and three lines were significantly different. The highest significant difference was found in IR80156A&B. Regarding shoot dry weight, seventeen lines showed non significant difference, three lines were significant by different and highest significant difference was found in IR72081A&B. For total plant length, eighteen lines showed no significant difference and two lines were significantly different. Total dry weight showed non significant differences in sixteen lines and four showed significant difference and highest significant difference was seen in IR68886A&B (Table 6).

Correlations: As presented in Table 7 maximum root length (MRL) showed significant positive correlation with the root volume (r = 0.881) and root dry weight (r = 0.835). Root volume showed significant correlation with root dry weight (r = 0.939) in well watered condition.

Maximum root length showed significant correlation with single plant yield (r = 0.565) and there was no significant correlation with biomass (r = 0.317). Root volume showed significant correlation with single plant yield (r = 0.589) and there was no significant correlation with biomass (r = 0.327). Root dry weight had no correlation with single plant yield (r = 0.439) and biomass (r = 0.237). Single plant yield showed significant correlation with the biomass (r = 0.787) in mean season of aerobic condition.

Maximum root length showed positive significant correlation with single plant yield (r = 0.720) and there was no significant correlation with biomass (r = 0.232). Root volume showed significant correlation with single plant yield (r = 0.718) and showed no correlation with biomass (r = 0.172). Root dry weight showed significant correlation with single plant yield (r = 0.573) and no significant correlation with biomass (r = 0.158). Single plant yield showed no positive correlation with the biomass (r = 0.417) under aerobic condition.

Table 7: Phenotypic correlation between root and yield related traits

AR/WL	MRL	RV	RDW	AR SPY	AR BM
MRL	1	0.881**	0.835**	0.565**	0.317
RV	0.881**	1	0.939**	0.589**	0.327
RDW	0.835**	0.939**	1	0.439	0.237
WL SPY	0.720**	0.718**	0.573**	1	0.787**
WL BM	0.230	0.170	0.160	0.42	1

** Significant at the 0.01 level of probability

Note: Above the diagonal for aerobic condition/ Below the diagonal for control

Clustering analysis based on molecular data:

The genotypic data with fifty one markers related to root related trait across 12 chromosomes was utilized for generating dendrogram for 20 sets of CMS/maintainer lines along with seven commercial checks, using DARwin v 5.0. programme [31]. Dendrogram was generated by UPGMA module using genetic dissimilarity and neighbor joining procedure [32]. Tree construction was performed using the vertical tree representation mode (Figure 2).

Results depict clearly that three major clusters were formed, designated as I, II and III. I major cluster was further divided into two sub clusters A1 and A2; A1 sub cluster consisting of 12 sets of CMS/maintainer lines and A2 sub cluster consisting of 3 sets of CMS/maintainer lines. II major cluster was divided in to two subclusters B1 and B2; B1 sub cluster consisting one set of CMS/maintainer lines and checks *viz.*, Rasi, ARB6, Vandana, IR64 and BPT5204. B2 sub cluster consisted of three sets of CMS/maintainer lines along with the check IR20 which has shallow root system. III major cluster consisted of one set of CMS/maintainer lines and other cluster had one check i.e., Moroberekan with deep root system.

Disimilairity index shows that maximum dissimilarity was found between IR70369A and Moroberekan and also between IR70372A and BPT5204. Minimum dissimilarity was found between IR58025A and IR58025B.

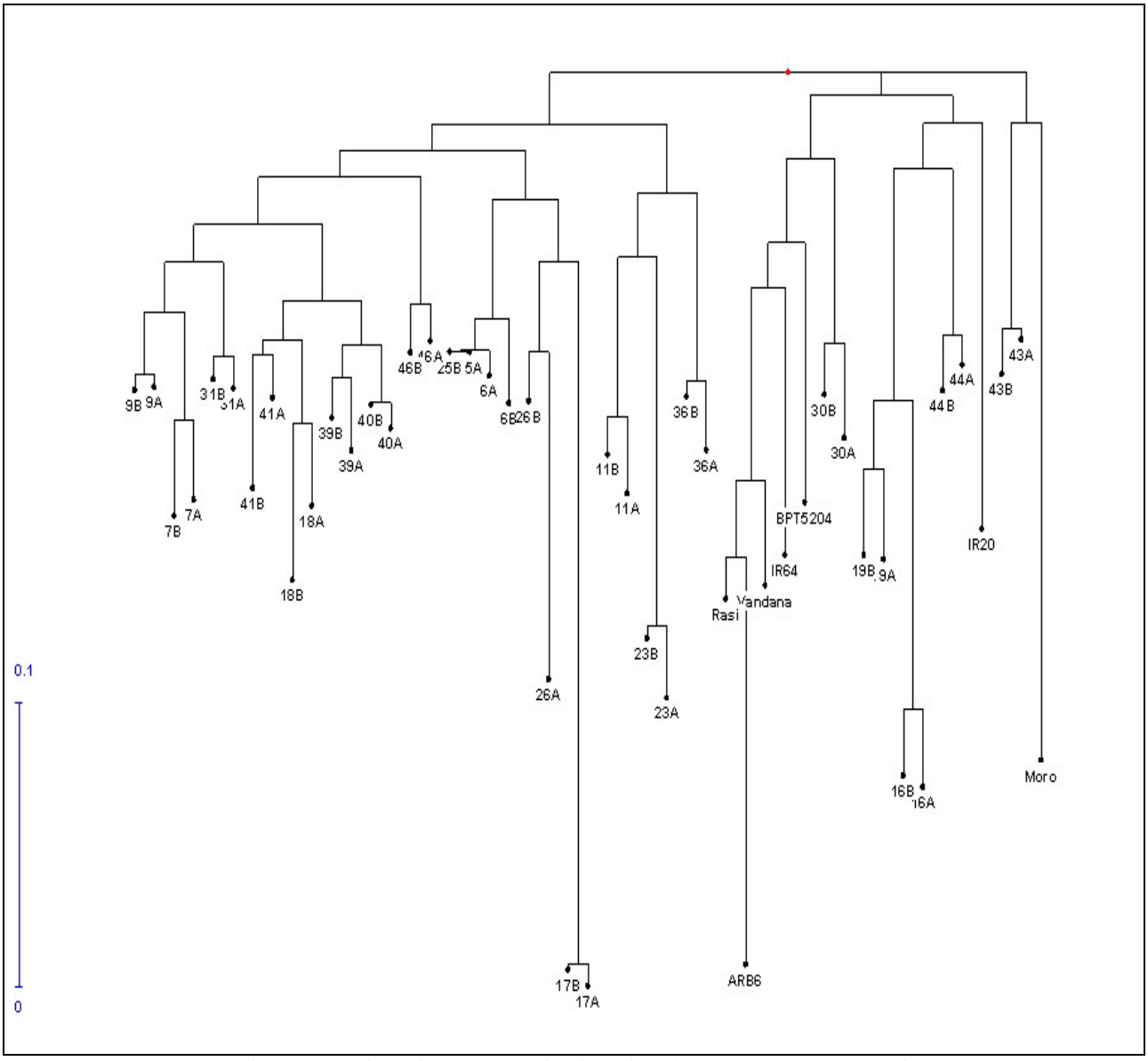

Fig.2. Clustering of 20 CMS/maintainer lines based on root related traits

25A/B: IR58025A/B; 6A/B: IR688885A/B; 7A/B: IR68888A/B; 9A/B: IR68897A/B; 11A/B: IR68029A/B; 16A/B: IR70369A/B; 17A/B: IR70372A/B; 18A/B: IR70959A/B; 19A/B: IR69624A/B; 23A/B: IR72081A/B; 26A/B: IR73321A/B; 30A/B: IR73793A/B; 31A/B: IR75596A/B; 36A/B: IR68886A/B; 39A/B: IR79128A/B; 40A/B: IR79156A/B; 41A/B: IR80151A/B; 43A/B: IR80155A/B; 44A/B: IR80156A/B; 46A/B: IR80559A/B; Moro: Moroberekan.

IV. DISCUSSION

In hybrid rice breeding program, utilization of parental lines with considerable variability is of primary concern for exploitation of maximum level of heterosis or hybrid vigor in the F_1seed production [4]. Analysis of variance revealed that maintainer lines were significantly different for all the traits for year, genotype and treatment which shows there is considerable variation among the genotypes used in the present study.

Spikelet fertility percent is very important criteria in hybrid breeding program. Since this trait has a direct bearing on the yield and hence manifestation of heterosis in positive direction is desirable for this trait. The grain number is determined by the number of spikelets at anthesis and determines the proportion of spikelets, which produce filled grains [33]. Spikelets fertility is one of the yield contributing characters that is mainly considered for yield improvement. Under aerobic condition there was a general reduction in spikelet fertility level in most of the maintainer lines. Results clearly indicate that the seasonal mean for percentage decrease between aerobic and irrigated conditions was variable. Among all the maintainer lines IR582025B has showed highest spikelet fertility with least reduction for spikelet fertility in average of two seasons. Earlier studies also suggested that the spikelet fertility is reliable parameters for mass screening of genotypes for yield performance [34 and 35]. Single plant yield and grain weight are the two traits that decide the final grain yield. It is a multiplication and product of several basic components of yield [36]. In rice, a self pollinated crop, the commercial exploration of hybrid vigour depends on magnitude of heterosis for grain yield. Harvest index which directly influences the grain yield through controlling the mechanism of distribution of photosynthesis to economic and non-economic organs though a yield component directly, it indirectly influence grain yield. So, it is an important consideration of genetic improvement under different moisture regimes. The highest single plant yield was observed in IR58025B followed by IR79156B and also these lines had shown highest harvest index in both aerobic and irrigated conditions.

Rapid development of a deep root system is considered a drought avoidance strategy for plants as it enables absorption of water in deep soil layers [37 and 38]. IR58025A&B had shown maximum root length, root volume and root dry weight. Correlation studies revealed that there is significant positive correlation between maximum root length, root volume and root dry weight. Maximum root length showed significant correlation with single plant yield and root volume showed significant correlation with single plant yield. Similar results have also been reported by [39 and 40]. IRRI (1984) also reported a significant positive association between shoot dry weight, maximum root length, root thickness, root number and root dry weight. Single plant yield showed significant correlation with the biomass in seasonal mean of aerobic condition. Root dry weight showed significant correlation with single plant yield. Molecular diversity study revealed similarity between CMS and maintainer lines on expected lines. Further analysis using the molecular markers for tagging the root related traits can help in generating tools for marker assisted selection for trait conferring drought resistance as also opined by [41].

V. CONCLUSION

Heterosis breeding work has been extensively carried out for the above-ground traits in drought tolerance for rice. The present study clearly gives an indication that the below-ground traits i.e. root related traits have a direct/indirect relation in improving the yield under aerobic condition. Our results revealed that there is a positive correlation between root traits and single plant yield under both aerobic and wetland conditions and biomass under aerobic condition, which in turn will help to select the best CMS/maintainer lines having good root traits and yield. The results revealed that IR58025A&B are the best parental lines to develop hybrids under aerobic condition.

ACKNOWLEDGMENT

The authors are thankful to Dr. Usha B. Zehr, Director and Mr. Dinesh C. Joshi former Executive Director, Barwale Foundation for their encouragement and financial support.

REFERENCES

[1] Tuong TP and Bouman BAM. 2001. Rice production in water-scarce environments, In: Proc. Water Productivity Workshop, 12-14 November 2001, Colombo, Sri Lanka. International Water Management Institute, Colombo, Sri Lanka.

[2] Lal B, Nayak AK, Priyanka Gautam, Rahul Tripathi, Teekam Singh and Katara JL. 2013. Aerobic Rice: A Water Saving Approach for Rice Production Popular Kheti. 1(2): 1-4.

[3] Krishnaiah K, Shobha RN. 2000. New avenues for augmenting and sustaining rice exports from India. Intl. Rice Comm. Newsl. (FAO) 49: 42-51

[4] Rajendran N, Lipi Mukherjee, Kamalnath Reddy K and Shashidhar HE. 2012 DNA fingerprinting and estimation of genetic diversity among hybrid rice parental lines (*Oryza sativa* L.) using simple sequence repeats (SSR) markers. Journal of Plant Breeding and Crop Science 4(11): 169-174. DOI:10.5897/JPBCS 12.008.

[5] Weeraratne H. 1954. Hybridization technique in rice. Trop. Agri. 110: 93-97.

[6] Sampath S and Mohanty HK. 1954. Cytology of semi sterile rice hybrids. Curr. Sci. 23: 82-183.

[7] Virmani SS and Kumar I, 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. IRRN 29: 10-19.

[8] Yao FY, Xu CG, Yu SB, Li JX, Gao YJ, Li XH and Zhang QF. 1997. Mapping and genetic analysis of two fertility restorer loci in the wild abortive cytoplasmic male sterility system of rice (*Oryza sativa* L). Euphytica 98: 183-187.

[9] Xie F. 2009. Priorities of IRRI hybrid rice breeding. In: Xie F, Hardy B (eds.) Accelerating hybrid rice development, International Rice Research Institute, Los Banos, Philippines. pp: 49-61.

[10] Kaladhar K, Ramesha MS, Ramakrishna S, Ahmed MI, Viraktamath BC, Sarla N 2004. Clustering of maintainer and restorer lines of rice based on morphological and molecular diversity. Rice Genet. Newsl. 21: 27-28.

[11] Cheng SH, Zhuang JY, Fan YY, Du JH and Cao LY. 2007. Progress in research and development on hybrid rice, a super-domestic in China. Ann Bot. 100: 959-966.
[12] Yang SH, Cheng BY, Wu JL, Shen WF, Cheng SH. 2006. Review and prospects on rice breeding and extension in China. Rice Sci. 13(1): 1-8.
[13] Cohen M, Bernal C and Virmani SS. 2003. Do rice hybrids have heterosis for insect resistance? A study with *Nilaparvatalugens* (Hemiptera: Delphacidae) and *Marasmia patnalis* (Lepidoptera: Pyralidae). J. Econ. Entomol. 96(6):1935-1941.
[14] Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh SK, Palchamy A, Sadasivam S, Sarkarung S, Wade LJ, and Nguyen HT. 2003. Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. Crop Science 43: 1457-1469.
[15] Lafitte R. 2003. Managing water for controlled drought in breeding plots. In: Fischer KS, Lafitte, R, Fukai, S, Atlin, G, Hardy, B (eds.). Breeding rice for drought-prone environments. Los Baños (Philippines): International Rice Research Institute. pp. 23-26.
[16] Yoshida S, Hasegawa S. 1982. The rice root system: its development and function. In: Drought resistance in crops, with emphasis on Rice. International Rice Research Institute, Los Banos. Laguna, Philippines. pp: 97-114.
[17] Ingram KT, Bueno FO, Namuco OS, Yambao EB, Beyrouty CA. 1994. Rice root traits for drought resistance and their genetic variation. In: Kirk GJO (ed.s, Rice Roots: Nutrient and Water use. IRRI, Los Banos, Manila, Philippines, pp. 67-77.
[18] Kamoshita A, Zhang J, Siopongco J, Sarkarung S, Nguyen HT, Wade L. 2002. Effects of phenotyping environment on identification of Quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci. 42: 255-265
[19] Nguyen HT, Babu RC and Blum A. 1997. Breeding for drought resistance in rice: Physiology and molecular genertics considerations. Crop Sci. 37: 1426-1434.
[20] Steele KA, Price AH, Shashidhar HE, Witcombe JR. 2006. Marker assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theor. Appl. Genet. 112: 208-221. DOI: 10.1007/S00122-005-0110-4.
[21] Beyrouty CA. 2002. Ecophysiology of roots of aquatic plants. In the Hidden half, 3rd Edn. eds. Y. Waisel, A.Eshel and U. Kafkafi (Marcel Dekker, Inc. New York.) pp. 1007-1024.
[22] Fageria NK and Moreira A. 2011. The role of mineral nutrition on root crop growth of crop plants. Adv. Agron., 80: 63-152.
[23] Shashidhar, HE. 2008. Aerobic rice- and efficient water management strategy for rice production. In: Aswathanaryana, U. (Ed.). Food and water security. Taylor and Francis, London. UK. pp: 131-139
[24] Gowda VRP, Henry A, Yamanuch A, Shashidhar HE, and Serraj R. 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. 122: 1-13.DOI: 10.1016/J.FCR.2011.3.001.
[25] Shashidhar HE, Gowda H.S, Raveendra GM, Kundur PJ, Naveen Kumar G, Suprabha N, Preethi Upadhya, and Rakhi Sonam. 2012. PVC tubes to characterize roots and shoots to complement field plant productivity studies. In: Methodologies for root drought studies in rice. (eds.) Shashidhar HE, Henry A and Hardy B. IRRI, Los Banos, Philippines. pp.15-21.
[26] Gouda KP, Mohan Kumar Varma C, Saikumar S, Kiran B, Vinay Shenoy, Shashidhar HE. 2012. Direct selection for grain yield under moisture stress in *Oryza sativa* cv. IR58025B X *Oryza meridionalis* population. Crop Science. 52: 644-653. DOI:10.2135/CROPSCI2011.04.0206.
[27] Hemamalini GS, Shashidhar HE and Hittalmani S. 2000. Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica. 112:69-78
[28] Dellaporta SL, Wood J and Hicks JB. 1983. A plant DNA mini preparation: version II. Plant Mol. Biol. Rep., 1: 19-21.
[29] Bassam BJ, Annolles GC and Gresshoff PM. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochemistry. 196: 80-83.
[30] Anantha MS. 2011. Synthesis and evaluation of aerobic rice (*Oryza sativa* L.) hybrids and survey of molecular markers associated with drought tolerance. Ph.D. Thesis, University of Agricultural Sciences, Bengaluru, India.
[31] Perrier X and Jacquemond-Collet JP. 2006. DARwin software (http://darwin.cirad.fr/darwin/)
[32] Saitou N, and Nei M.1987. The Neighbour-Joining method- a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.
[33] Boonjung H. 1993. Modelling growth and yield of upland rice under water limiting conditions. Ph.D. Thesis, The University of Queensland. Australia.
[34] Garrity DP and O'Toole JC. 1994. Screening rice for drought resistance at the reproductive stage. Field Crops Research 39: pp. 99-110.
[35] Rajkumar S and Ibrahim SM. 2013. Aerobic Rice: Identification of suitable rice hybrids adaptability to aerobic condition in water-short areas. International Journal of Agricultural Sciences. 3 (7): 586-595.
[36] Saitou N, and Nei M. 1987. The Neighbour-Joining method- a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.
[37] Grafius JE.1959. Heterosis in barley. Agron. J. 51: 551-554.
[38] Fukai S and Cooper M. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40: 67–87.
[39] Price A and Courtois B. 1999. Mapping QTLs associated with drought resistance in rice: progress, problems and prospects. Plant Growth Regul. 29: 123–133.
[10] Toorchi M, Shashidhar HE, Hittalmani S and Gireesha TM. 2002. Rice root morphology under contrasting moisture regimes and contribution of molecular marker heterozygosity. Euphytica. 126: 251-257.
[41] Yue B, Xiong LZ, Xue WY, Xing YZ, Luo LJ, Cui K, Jin D, Xing Y and Zhang Q. 2006. Genetic basis of drought resistance at reproductive stage in Rice: Seperation of drought tolerance and drought avoidance. Genetics. 172(2): 1213-1228.
[42] Chaitra J, Vinod MS, Sharma N, Hittalamani S and Shashidhar HE. 2006. Validation of markers linked to maximum root length in rice (*Oryza sativa* L.) Current Science. 90(6): 835-838.

AUTHOR'S PROFILE:

Mr. K.R.Kamalnath Reddy
Barwale Foundation,
C/o Barwale Chambers, #3-6-666, Street No.10, Himayatnagar,
Hyderabad-500 029, Telangana, India.

Mr. K.Nagendra
Barwale Foundation, Himayatnagar, Hyderabad, Telangana, India.

Dr. Devandra Kumar Payasi
Jawaharlal Nehru Krishi Vidyalaya (JNKV), Regional Agricultural Research Station, Sagar, Madhya Pradesh, India.

Dr. C. Mohan Kumar Varma
Bayer Crop Science, Singapore.

Dr. M.S Anantha
Central Rainfed Upland Rice Research Station (CRRI), Hazaribag, Jharkhand, India

Dr. Vinay Shenoy
Barwale Foundation, Himayatnagar, Hyderabad, Telangana, India.

Dr. H.E.Shashidhar
Dept. of Biotechnology, University of Agricultural Sciences, GKVK, Bengaluru, India.