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Research Article

Two dimensional *in-vitro* phenotyping of root system architecture using Poly Ethylene Glycol in backcross inbred lines harboring drought tolerant QTLs of rice (*Oryza sativa* L.)

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Abstract

The present investigation was made to validate the effect and expression of multiple drought QTLs from Apo in the background of high yielding drought susceptible variety IR 64 under two dimensional *in-vitro* root architecture studies. The genotype CB 229 ($DTY_{2.2}$, $DTY_{3.1}$ and $DTY_{8.1}$) recorded a higher network bushiness, network width, specific root length and network distribution of roots under PEG treatment (stress) and in network area CB 193-3 outperformed than CB 229. This clearly shows that introgression of multiple drought QTLs in various combination in the same genetic background enhances different level of expression of QTLs and tolerance to varied intensity of drought. Under field screening for drought, CB 229 out performed in both spikelet fertility percentage and yield than the remaining BILs. The traits *viz.,* major ellipse axis, maximum number of roots, network bushiness, specific root length, network convex area and network width are positively correlated with spikelet fertility percentage and single plant yield under drought experiment. Principal component analysis accounted for 78.47 per cent of variability among root traits in PC1 and was loaded with all the traits observed under *in-vitro* condition. Hence, an understanding on complex nature of QTL expression associated with root system architecture under *in-vitro* stress in rice may help the breeder to develop new cultivars with enhanced level of tolerance against water limited situations in near future.

Keywords

Drought, Poly ethylene glycol, QTL, Rice, Root system architecture

INTRODUCTION

Rice is the staple food for more than 3 billion people in Asia, where more than 90% of the world's rice is produced and consumed (Li and Xu, 2007). In order to provide adequate food in the future, rice yields in Asia need to increase by 60% by 2050. So each rice-producing hectare that currently feeds 27 people will need to provide food for 43 people in future (Sheehy *et al.,* 2008). It is estimated that 50% of the world rice production is affected by drought (Bouman *et al.,* 2005). Drought, the most complex and toughest stress can be overcome by genetic improvement of rice. But, the progress in genetic improvement of rice for water-limiting environments has been slow and limited (Evenson and Gollin, 2003) due to poor understanding of the inheritance of tolerance and lack of efficient techniques for screening breeding materials for drought tolerance (Khush, 2001).

Water stress due to drought is one of the most significant abiotic factors that limit the seed germination, seeding growth, plants growth and yield (Van den Berg and Zeng, 2006). Several novel and effective methods have been developed to screen drought tolerant germplasm in plant species. Among the screening techniques, the usage of PEG is effective in inducing water stress which in turn helps in the selection of drought tolerant plants (Kaur *et al*, 1998). Roots are primarily effected plant part under drought conditions than any other parts (Ghafoor, 2013). The measured traits in root phenotyping were those that were most easily observable, such as maximum root length, root thickness, root number, mass of roots at different depths and root to shoot ratio. Transfer of primary or secondary traits, such as those associated with root growth to desirable backgrounds to enhance grain yield is complicated by a lack of clear understanding of the genetics of component traits and their interactions, and the lack of tightly linked markers. The ability to develop the root system in response to a changing environment, such as changes in soil moisture, reflects phenotypic plasticity (Wang and Yamauchi, 2006; Kano *et al*., 2011). Deeper root penetration is potentially an important component of drought resistance (Reynolds *et al*., 2007; Wasson *et al*., 2012).

The roots perform an essential functions including the uptake of water and nutrients for plant growth, serve as storage organs, anchor the plants in the soil and are the site of interactions with pathogenic and beneficial organisms in the rhizosphere. Controlling plant root architecture is a fundamental part of plant development and evolution, enabling a plant to respond to changing environmental conditions and allowing plants to survive in different ecological niches. A modest evaluation suggests that nearly 90% of global rural land area is affected by abiotic stress factors at some point throughout the growing period (Cramer *et al*, 2011). Generally plants tolerant to particular abiotic stress establish a metabolic homeostasis and carry on their growth without suffering stress induced injuries. Various methods have been employed from time to time to identify drought tolerant genotypes and have been used often as abiotic stress inducer in many studies to screen drought tolerant germplasm (Ahamd *et al*., 2013; Jatoi *et al*., 2014). PEG is a polymer and considered as a better chemical than others to induce water stress artificially (Kaur *et al*., 1998), which induces osmotic stress to decrease cell water potential (Govindaraj *et al*., 2010). Several reports have shown that *in vitro* screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study water stress on plant germination indices (Sakthivelu *et al*, 2008).

The root system architecture (RSA) is determined by both endogenous factors and environmental constraints such as nutrient availability (Kellermeier *et al*., 2014). Therefore, the understanding of the mechanisms regulating RSA is important for future crop improvement. The common root parameters include maximum root depth, the total root length, root surface area, root volume, root diameter, root length density, root distribution pattern in the soil column, root to shoot ratio, root branching, root hydraulic conductance, root anatomy, root elongation rate, total plant length, and hardpan penetrability (Wang *et al.,* 2006). A deep root system able to extract water at depth and respond to evaporative demand, provided there is water in the profile, is the most consensual of the traits contributing to drought avoidance in upland conditions (Nguyen *et al*. 1997; Price *et al*. 2002). Marker trait association especially with root studies could be an alternative approach for improving rice grain yield under drought situations. Genomic regions responsible for improved root phenomics under drought have been already explored (Vishnu *et al*., 2017). There are reported traditional accessions which are resistant to drought because of a long history of natural selection in the target environments (Vikram *et al*, 2012; Vengatesh and Govindarasu, 2017).

More recently, the progress on high-throughput root phenotyping and 3D image reconstruction has led to a renewed interest in QTL detection in mapping populations, focusing on original traits (e.g., the root network bushiness or its convex hull volume), in addition to more classical traits, such as specific root length or specific root area (Topp *et al.* 2013). Phenotypic selection for root morphological traits in conventional breeding programs is unfeasible. The use of molecular markers could provide a useful tool to support phenotypic selection. With that point of view, the present study was undertaken to investigate the root system architecture under *in vitro* condition using a set of backcross inbred lines of IR64 introgressed with various QTL combinations associated with drought tolerance from Apo. Studying the effects of these QTLs responsible for root growth will help to improve the knowledge on crop adaptation to drought stress.

MATERIALS AND METHODS

The biological material for this study was developed from the cross combination of IR64 and Apo. Apo, a drought tolerant upland variety served as an important source for mining drought tolerant QTLs. IR64, high yielding variety but susceptible to drought was used as recipient parent. Backcross Inbred lines (BILs - BC_2F_4) carried three mega QTL in different combination of classes namely *DTY 2.2, DTY 3.1* and *DTY 8.1*. About 252 progenies of BC_2F_4 were genotyped using foreground markers and resulted in the identification of 67 positive progenies with target drought QTLs in different combinations under homozygous condition. Out of 67 BC_2F_4 progenies, four different BILs *viz*., CB 229, CB 193-1, CB 193-2 and CB 193-3 were selected based on the recurrent parent genome recovery (above 80 per cent). The four BIL lines of CB 229 ($qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{8.1}$), CB 193-1 ($qDTY_{2.2}$ and $qDTY_{3.1}$), CB 193-2 ($qDTY_{2.2}$ and $qDTY_{8.1}$) and CB 193-3 ($qDTY_{3.1}$ and $qDTY_{8.1}$) were used for *in-vitro* screening of root architecture using gelzan medium (transparent medium) with PEG (stress induced treatment) and without PEG (Control) in four replicates.

A comparative root study of the parents and the BILs were analysed under *in vitro* to know the effect of the QTL on root growth and development. The medium used is half

MS medium with 0.3% gelzan and 0.01% polyethylene glycol. The seeds were kept for incubation at 37^{0}C for 72 hours and dehusking was done with the help of fine forceps. The seeds were sterilized with ethanol (70%) for 1 minute followed by sodium hypochloride (0.05%) for 3 to 4 minutes. Then they were washed with sterile water three times to remove the chemical residues. It was dried for five minutes on sterilized tissue paper and later the sterilized seeds were planted approximately at 1 cm below the surface of the gel. To impart drought, PEG (dehydration) was added to the media and the same set of seeds were grown under non-stress condition. Plants were grown in 38 mm x 200 mm size culture tubes filled with 100 ml half MS medium with 0.3 per cent Gelzan at ambient temperature. Images were taken at 14th day after inoculation and quantitative features of root namely Maximum number of roots, Network length, Special root length, Network area, Average root width, Network depth, Network length distribution and Network width to depth ratio were estimated using General Image Analysis of Roots (GiA roots).

The seeds of BILs along with parents were sown (direct sowing) in irrigated and rain out shelter (to avoid rain intervention during experimental period) during *Rabi* 2014-2015. Seeds were sown with the spacing of 20 x 20 cm and an experimental plot size of 0.48 m^2 was maintained for each genotype. The design followed was randomized block design with four replications. Two weeks after sowing, thinning was done and maintained single seedling per hill. Regular irrigation was given up to 48 days of crop from the date of sowing; later crop

Table 1. *Per se* performance of various root traits related to drought tolerance under *in-vitro* screening system

Genotype Trait	Treatment	IR 64	Apo	CB 229	CB 193-1	CB 193-2	CB 193-3	Mean	CD@5%	CD@ 1%
Major Ellipse Axis (cm)	C	8.25**	8.72**	8.07**	6.49	5.23	8.64**	7.57	0.27	0.37
	S	4.59	9.45**	9.94**	9.47**	7.86	6.91	8.04	0.18	0.24
Maximum number of roots	C	12	12	15.00**	16.00**	15.00**	14	14	0.4	0.55
	S	7	12	18.00**	17.00**	19.00**	12	14.17	0.4	0.54
Network length (cm)	C	133.66**	97.07	172.01**	117.03	95.12	107.72	120.43	2.95	4.03
	S	38.38	134.59	140.01	224.71**	245.34**	121.89	150.82	4.46	6.11
Network Bushiness (cm)	C	1.71	1.5	1.67	2.00**	1.88	2.33**	1.85	0.04	0.06
	S	1.4	1.71*	1.71*	1.70*	1.73**	1.64	1.65	0.06	0.08
Network width (cm)	C	2.31	2.82	2.92	2.54	3.24**	3.59**	2.9	0.1	0.14
	S	2.27	3.29	4.63**	3.83**	4.17**	2.97	3.52	0.07	0.1
Specific root Length (cm)	C	1490.70**	946.79	1328.72	1134.86	1032.04	955.61	1326.29	16.61	22.76
	S	830.00	1647.52**	1961.64**	1640.43**	1264.03	1323.89	1266.42	31.68	1.23
Network area (cm2)	C	2.55*	1.77	3.48**	2.66**	2.26	2.2	2.49	0.05	0.07
	S	0.98	3.35*	2.64	5.45**	5.01**	1.99	3.24	0.1	0.13
Network convex area (cm2)	C	15.11	15.16	18.86**	16.2	20.53**	10.9	16.13	0.43	0.59
	S	6.44	21.47*	29.35**	27.43**	26.47**	14.06	20.87	0.63	0.86
Average root width (cm)	C	0.025	0.024	0.026	0.028*	0.030**	0.027	0.027	0.0011	0.0016
	S	0.022	0.031**	0.034**	0.031**	0.027	0.024	0.028	0.001	0.0014
Ellipse axis ratio	C	0.265	0.272	0.426	0.535**	0.646**	0.440 *	0.431	0.0141	0.0193
	S	0.613**	0.372	0.422	0.445	0.484	0.594**	0.489	0.0119	0.0162
Medium number of roots	C	8.246**	8.720**	8.068**	6.493	5.234	8.643**	7.568	0.2697	0.3696
	S	4.594	9.449**	9.943**	9.472**	7.86	6.909	8.038	0.1736	0.2379
Minor ellipse axis	C	7	8.00**	9.00 **	8.00**	8.00**	6	7.667	0.207	0.283
	S	5	7	11.00**	10.00**	11.00**	7	8.524	0.0712	0.0975
No of connected components	C	8.013**	7.358	7.728*	7.086	7.377	6.848	7.402	0.2207	0.3024
	S	4.384	8.285	10.815**	9.397**	8.04	7.868	8.131	0.2433	0.3334
Network depth (cm)	C	8.013**	7.358	7.728*	7.086	7.377	6.848	7.402	0.147	0.2014
	S	4.384	8.285	10.815**	9.397**	7.868	8.040	8.131	0.2433	0.3334
Network length distribution	C	0.239*	0.159	0.312**	0.073	0.211	0.567**	0.225	0.0081	0.011
	S	0.678**	0.276	0.14	0.232	0.524**	0.19	0.34	0.0107	0.0146
Network width to depth ratio	C	0.288	0.384	0.378	0.358	0.439**	0.524**	0.395	0.0079	0.0109
	S	0.517	0.397	0.369	0.407	0.428	0.529**	0.441	0.0119	0.0163

* Significant at 5% level and ** significant at 1% level

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was denied irrigation for the rest of its duration in stress experiment whereas in irrigated experiment, irrigation was provided till the end of physiological maturity. Crop was harvested on single plant basis, when the grains reached physiological maturity stage in both irrigated and stress plot. The traits namely Maximum number of roots, Network length, Special root length, Network area, Average root width, Network depth, Network length distribution and Network width to depth ratio means and critical difference at 5% and 1% of the confidence interval for the BILs along with the parents were estimated. Principal component analysis (PCA) analysis was performed on the characters showing variability using xl-stat.

RESULTS AND DISCUSSION

In-vitro phenotyping for Root System Architecture under normal and drought simulated conditions revealed that all the four BILs were found to possess similar RSA compared to the parents under normal conditions. Growth of roots in IR64 was found to be affected by PEG treatment whereas the PEG treatment had a growth promotional effect on the root growth of BILs. Among the genotypes, CB 229 recorded a significantly higher major ellipse axis (9.94 cm) when compared to IR64 (4.59 cm) and the other genotypes exhibited major ellipse axis ranging from 9.47 cm to 6.91 cm with an average value of 8.04 cm under dehydrated condition using PEG. Growth of roots in IR64 was found to be affected by PEG treatment whereas PEG treatment had growth promotional effect on the root growth of BILs. Under stress condition, maximum number of roots were found to be significantly higher in CB 193-2 (19.00) whereas lower in IR 64 (7.00) (**Table 1**). The other genotypes ranged from 12 to 18 with an average of 14.17 roots. IR 64 recorded the lowest specific root length (830 cm) under stress condition. Among the genotypes CB 193-1 had a significantly higher network area of 5.45 cm^2 followed by CB 193-2 (5.01 cm^2), the least value was exhibited by IR 64 (0.98 cm^2). CB 229 had a highest network convex area of 29.35 cm^2, an increased network bushiness, network width, specific root length, network depth under stress condition (Fig 1) than other BILs, the lowest value was recorded in IR64 (6.44 cm^2). This was followed by CB 193-1 and CB 193-2 which showed root growth on par with CB 229 under both normal and stress conditions. The BIL CB 193-3 which was lacking *qDTY*$_{2.2}$ recorded root growth which was on par with IR 64 in most of the traits. It is interesting to see that *qDTY*$_{2.2}$ showed its effect only under severe stress conditions. The lines with *qDTY*$_{2.2}$ showed an increase in root length and overall root network showing that this QTL had an effect on root growth (Fig 2). Similarly, QTLs for root length within the *qDTY*$_{2.2}$ region (MacMillan *et al.* 2006; Kamoshita *et al.* 2002) and for root thickness (Champoux *et al.* 1995) adjacent to the *qDTY*$_{2.2}$ region have been reported.

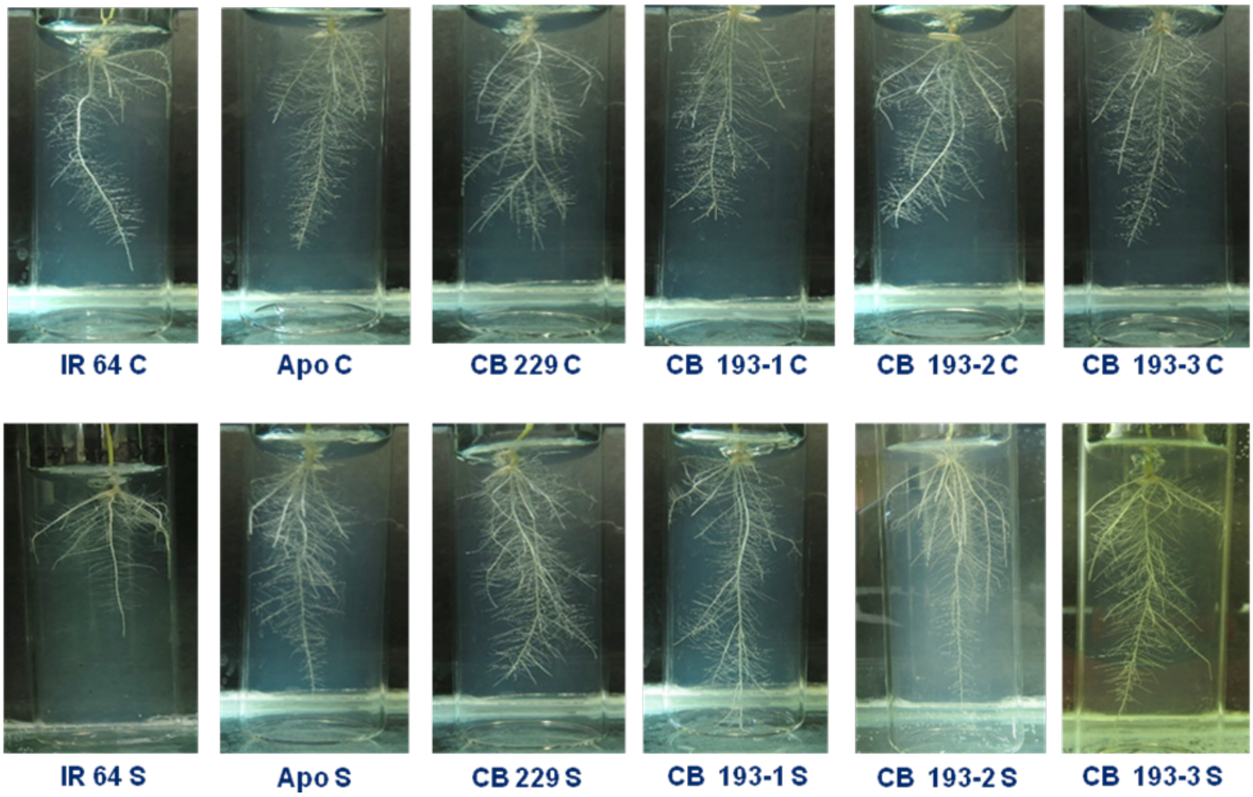

Fig 1. Variation of traits related to root system architecture among BILs and parents under *in- vitro* condition (C- Control (without PEG) and S – Stress (with PEG))

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In rice, the beneficial root traits have recently been introgressed successfully into elite crop varieties. Multiple quantitative trait loci (QTL) associated with thicker and longer roots were introgressed from a *japonica* into an *indica* line via marker-assisted selection (Steele *et al*., 2007, 2013). It is generally acknowledged that a deeper, thicker and more branched root system with a high root to shoot ratio can enhance the tolerance of rice to water deficits (Fukai and Cooper, 1995; Gowda *et al.* 2011). Root systems are reported to be critical to cope with water deficit and increase yield. For example, deep and dense roots with high abilities of penetration and branching into the soil help rice plants to be drought tolerant (Pandey and Shukla, 2015). Root signaling plays an important role in water stress tolerance by reduction in leaf expansion and promotion of root growth (Singh *et al*., 2010). Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al*., 2011).

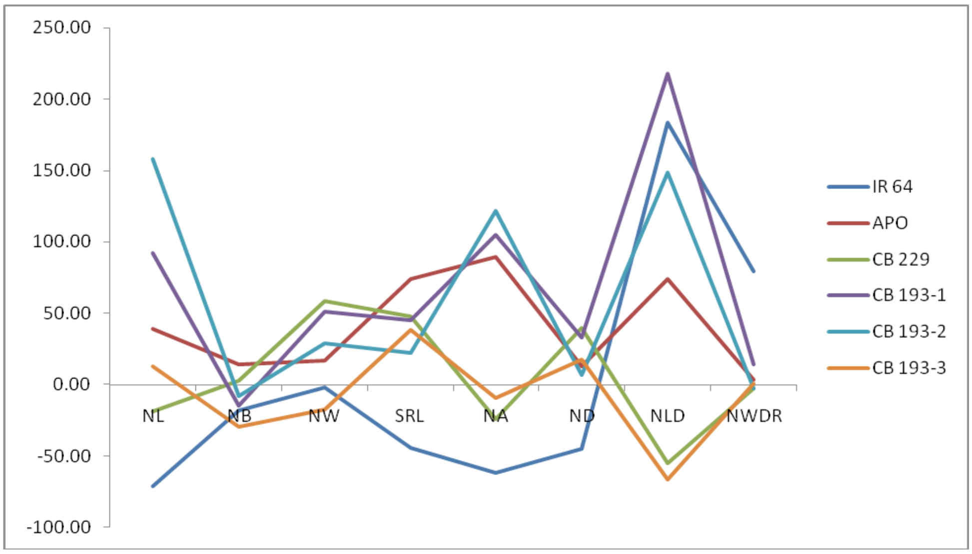

Fig. 2. Percentage reduction of root traits (*in-vitro*-PEG treatment) over control (without PEG).

Association analysis were carried out to study the inter relationship among component traits of root system architecture in rice both under control and PEG treated condition. Under stress condition, the trait Average root width (ARW) had registered a significantly negative correlation with SRL (-0.978), whilst the same trait ARW (0.975) showed a high and significant positive association with Ellipse axis ratio (EAR) and negative association with Major ellipse axis (MEA -0.886) and Specific root Length (SRL -0.974) under control condition. The trait network bushiness does not show correlation with any one of the trait under control, whereas in PEG treatment it recorded positive correlation with Network depth (ND 0.862), MEA (0.892), Network width (NW 0.821) and Network convex area (NCA 0.894) (Table 2). Under PEG treated situation, the trait NCC exhibited a significant and high positive association with NW (0.828), Minor ellipse axis (MiEA 0.828), Network area (NA 0.855), Network convex area (NCA 0.894) and NL (0.871). Whilst in control, NCC recorded a high and significant positive association with NA (0.853). Network depth (ND) had recorded significantly higher positive correlation with MEA (0.924), NW (0.862) and NCA (0.879) under stress condition induced by treating with PEG. The trait EAR had registered a significant positive correlation with NLD (0.854) and Network width to depth ratio (NWDR 0.945) under PEG treated condition whereas in control, it showed a positive relationship with Maximum number of roots (MNR 0.861) and negative correlation with MEA (-0.869) and SRL (-0.968). Under stress, NLD exhibited a positive association with NWDR (0.918), whereas in control it revealed a positive correlation with NCA (0.876). In control condition, MEA recorded a positive correlation with SRL (0.871) whereas in stress (with PEG), it had shown a significant positive correlation with NCA (0.905). Under both control and stress situations, the trait NA had a significant positive correlation with NL (0.928, 0.959).

Association studies made among the root phenotypic traits indicated that, most of the root component traits exhibited positive relationship among them. The associations were also made between the root traits under PEG simulated treatment and yield component traits observed under field stress. (Fig 3 & 4). The results revealed positive association of, the most affected traits of water stress namely spikelet fertility percentage and single plant yield with major ellipse axis, maximum number of roots, network bushiness, specific root length, network convex area, medium number of roots, number of connected components and network depth. The interrelationships clearly identified the importance of root to shoot length, weight ratios and root dry weight under well-watered condition. While, the maximum root length and root number are a better combination under low moisture stress condition (Kanbar *et al*., 2009). For the most non-root crops, including cereals and legumes, direct

Table 2. Simple correlation coefficients of various root traits related to drought tolerance under *in-vitro* screening system

Trait		ARW	NB	NCC	ND	EAR	NLD	MEA	MNR	NW	MeNR	MiEA	NA	NCA	SRL	NL	NWDR
ARW	C	1	0.544	0.205	-0.397	**0.975****	-0.342	**-0.886***	0.775	0.406	0.030	0.687	0.083	-0.371	**-0.974****	-0.279	0.417
	S	1	-0.483	-0.226	-0.569	0.260	0.636	-0.271	-0.563	-0.457	-0.560	-0.255	0.037	-0.308	**-0.978****	-0.218	0.403
NB	C		1	-0.149	-0.716	0.480	0.480	-0.140	0.454	0.570	-0.671	0.740	-0.067	0.403	-0.522	-0.243	0.678
	S		1	0.735	**0.862***	-0.527	-0.647	**0.892***	0.678	**0.821***	0.579	0.717	0.730	**0.894***	0.307	0.798	-0.397
NCC	C			1	0.386	0.258	-0.322	-0.348	0.582	-0.546	0.600	0.167	**0.853***	0.014	-0.409	0.703	-0.561
	S			1	0.765	-0.155	-0.423	0.736	0.619	**0.828***	0.548	**0.828***	**0.855***	**0.894***	0.076	**0.871***	-0.140
ND	C				1	-0.445	-0.226	0.112	-0.415	-0.641	0.378	-0.665	0.411	-0.286	0.329	0.568	-0.781
	S				1	-0.555	-0.810	**0.924****	0.458	**0.862***	0.367	0.723	0.501	**0.879***	0.443	0.573	-0.544
EAR	C					1	-0.357	**-0.869***	**0.861***	0.438	0.186	0.757	0.152	-0.309	**-0.968****	-0.216	0.446
	S					1	**0.854***	-0.617	-0.223	-0.104	-0.183	0.091	-0.141	-0.265	-0.238	-0.093	**0.945****
NLD	C						1	0.733	-0.201	0.455	-0.587	0.277	0.108	**0.876***	0.334	0.277	0.468
	S						1	-0.691	-0.436	-0.416	-0.398	-0.188	-0.186	-0.472	-0.604	-0.252	**0.918****
MEA	C							1	-0.653	-0.041	-0.322	-0.344	-0.060	0.700	**0.871***	0.281	-0.032
	S							1	0.347	0.807	0.233	0.717	0.623	**0.905***	0.116	0.607	-0.490
MNR	C								1	0.248	0.347	**0.840***	0.501	0.058	**-0.874***	0.164	0.283
	S								1	0.452	**0.991****	0.369	0.682	0.499	0.460	**0.817***	-0.240
NW	C									1	-0.315	0.680	-0.223	0.253	-0.291	-0.314	**0.978****
	S									1	0.350	**0.966****	**0.626**	**0.958****	0.287	0.722	-0.048
MeNR	C										1	-0.039	0.503	-0.292	-0.135	0.426	-0.407
	S										1	0.267	0.610	0.389	0.480	0.751	-0.230
MiEA	C											1	0.317	0.378	-0.724	0.054	0.717
	S											1	0.680	**0.930****	0.077	0.741	0.176
NA	C												1	0.342	-0.281	**0.928****	-0.298
	S												1	0.778	-0.212	**0.959****	.003
NCA	C													1	0.276	0.466	0.305
	S													1	0.124	**0.811***	-0.159
SRL	C														1	0.090	-0.305
	S														1	0.041	-0.427
NL	C															1	-0.404
	S															1	0.008
NWDR	C																1
	S																1

* Significant at 5% level and ** significant at 1% level, (C- without PEG and S- with PEG)

ARW- Average root width, NB- Network Bushiness, ND- Network depth, EAR- Ellipse axis ratio, NLD- Network length distribution, MEA- Major ellipse axis, MNR- Maximum number of roots, NW- Network width, MeNR- Medium number of roots, MiEA- Minor ellipse axis, NA- Network area, NCA- Network area, SRL- Specific root Length, NL- Network length, NWDR- Network width to depth ratio

phenotypic selection of root traits has not been widely attempted. However, an indirect selection of root traits has undoubtedly supported historical yield increases (Lynch, 2007, 2011). Examples of root traits shown to correlate with improved field performance include thicker, longer roots in rice (Steele *et al*., 2013), increased root hair elongation in maize (Hochholdinger *et al*., 2008) and shallower roots under low phosphorus (P) conditions in soybean (Zhao *et al*., 2004) and common bean (Miguel *et al*., 2013). Hence, selection based on the component root traits quantifies enhanced yield under adverse drought situations. Ingram *et al*. (1994) showed that among the root traits studied, the total root length is strongly related to drought tolerance under rainfed upland conditions. Root traits found to confer drought tolerance under rainfed lowland conditions are root length density in the 10-30 cm soil layer and dynamic shedding of roots and production of root length in response to changing moisture conditions. Therefore, the variables that are having strong associations may share some underlying biological relationship, and these associations are often useful for generating hypothesis for better understanding of complex traits especially drought tolerance.

Principal Component Analysis (PCA) measures the contribution of component variables to total variance and used for the measurement of independent impact of a particular trait to the total variance whereas each coefficient

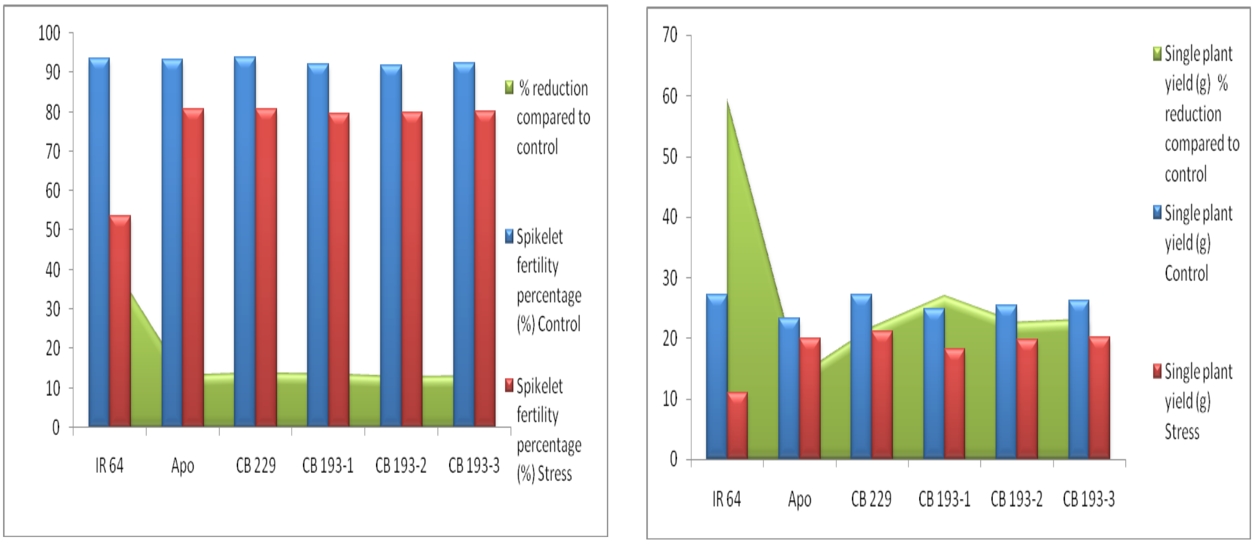

Fig. 3. Variation in spikelet fertility percentage and single plant yield in stress experiment and reduction percentage of spikelet fertility percentage and single plant yield over control

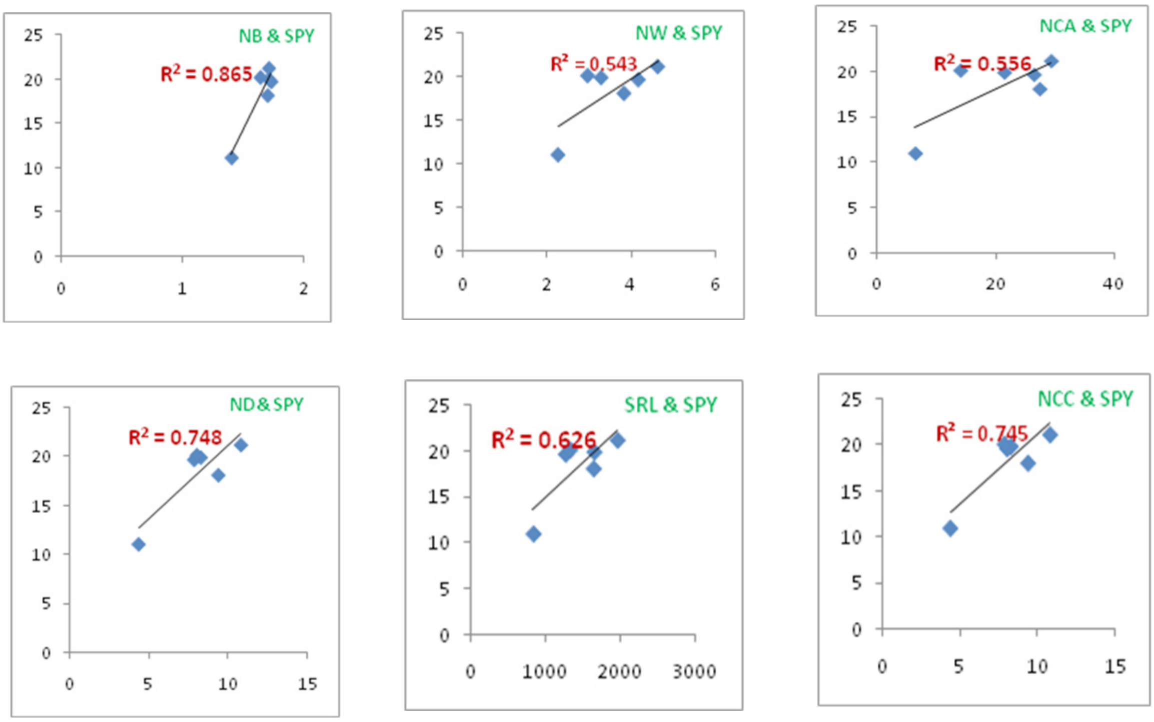

Fig 4. Relationship between root architectural traits (*in-vitro*) and single plant yield of parents and BILs under *in-vivo* stress experiment

of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated. Two principal components (PC1) and (PC2) accounted for most of the variability observed among the introgressed lines of IR64 and their parents studied. Principal component 1 (PC1) accounted for 78.47 per cent of the root trait variation in the introgressed lines and was loaded with all the traits observed under *in vitro* condition which indicated that PC1 had a higher discriminatory power of variables than other components

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(Fig 5a&5b). Mehlman (1995) pointed out that the value of the first principal component which was a comprehensive index obtained through principal component analysis and included maximum information of original indexes and not that of the comprehensive principal component should be considered the comprehensive evaluation value. In this study, the evaluation of root traits associated with drought tolerance for each line and parent was carried out using the first principal component as the comprehensive evaluation value. Characterization is a routine endeavor for plant breeders and application PCA tool, cluster and multivariate statistical analysis provide a useful means for estimating variation within and between genotypes. However, this study suggest the need for breeders to evaluate genotypes for their root parameters for drought tolerance in rice enhances the progression on their utility in the rice breeding programme in the development of improved varieties with better genetic base.

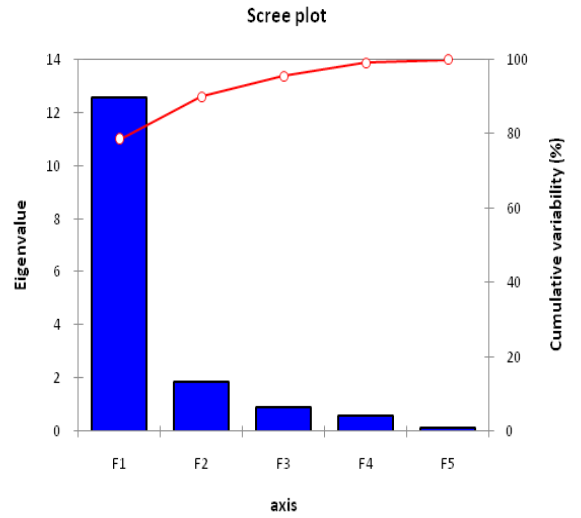

Fig 5a. Screen plot depicting the variability present in different components

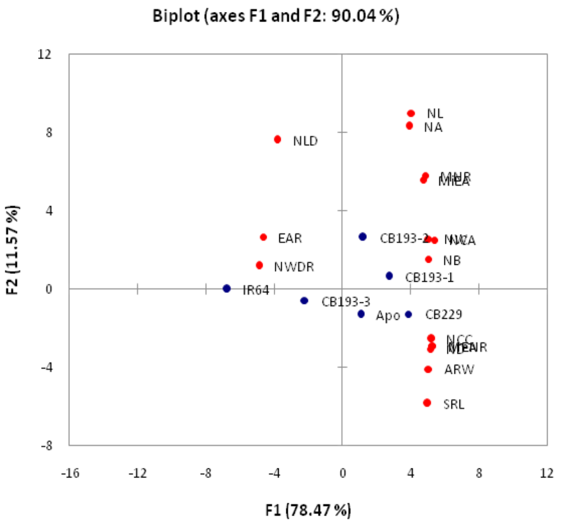

Fig 5b. PCA with phenotypic data of root traits studied under *in-vitro* condition (with PEG)

Table 3. Eigen value, Eigen vector and scores of three four factors retained from PCA of root traits under PEG treatments

Traits	PC1	PC2
MEA	0.934	0.042
MNR	0.779	0.163
NL	0.546	0.393
NB	0.846	0.011
NW	0.849	0.032
SRL	0.829	0.168
NA	0.505	0.336
NCA	0.961	0.030
ARW	0.849	0.082
EAR	0.711	0.034
MeNR	0.934	0.042
MiEA	0.746	0.151
NCC	0.913	0.032
ND	0.886	0.046
NLD	0.480	0.282
NWDR	0.786	0.007
Eigen value	12.555	1.851
Variability (%)	78.47	11.57
Cumulative %	78.467	90.036

Values in bold indicate the most relevant characters (>0.480) that contributed to the variation of the particular component

MEA- Major Ellipse Axis, MNR- Minor ellipse axis, NL- Network length,NB- Network Bushiness,NW- Network width, SRL- Specific root Length, NA- Network area,NCA-, Network convex area ,ARW- Average root width (cm), EAR- Ellipse axis ratio, MeNR- Medium number of roots,MiEA- Minor ellipse axis, NCC-, ND- Network depth,NLD- Network length distribution,NWDR- Network width to depth ratio

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Table 3 presents a summary of PCA and displays eigen values, the cumulative per cent of variance accounted for by the principal components (PCs), and the eigen vectors estimated based on the averages of each of the sixteen variables studied under *in-vitro* root screening. Different traits were plotted into different principal components based on the absolute value of each eigenvector. The greatest absolute value of the same trait lay in each factor that served as the principal component to which this trait belonged. The first two components in the PCA analysis with the Eigen values > 1 contributed the cumulative variability of 98.04 per cent. Principal component 1 (PC1) with Eigen value 12.555 contributed 78.47 per cent of total variability. PC2 with Eigen value of 1.851 contributed 11.57 per cent to total variability respectively. The first principal component accounts for the total variability, where all the root traits exhibited a positive contribution to the variability. The trait network convex area (0.961), major ellipse axis (0.934), medium number of roots (0.934) and the number of connected components (0.913) had higher and positive contribution towards the variability in the genotypes studied under field stress condition. The traits namely network area (0.505) and network length distribution (0.480) had recorded lower contribution to the existing variability (**Table 3**).

Thus from the study the genotype CB 229 ($DTY_{2.2}$, $DTY_{3.1}$ and $DTY_{8.1}$) showed a higher network bushiness, network width, specific root length and network distribution of roots under PEG treatment (stress). Similarly under field condition while screening for drought, CB 229 out performed in both spikelet fertility percentage and yield than the remaining BILs. This clearly shows that introgression of multiple drought QTLs in various combination in the same genetic background enhances different level of expression of QTLs and tolerance to varied intensity of drought.

REFERENCES

Ahamd, M., Shabbir, G., Minhas, M. N. and Shah, M.K.N. 2013. Identification of Drought Tolerant Wheat Genotype based on Seedling. Trait, J. Agric., **29**: 21-27.

Ali, S.G., Rab, A., Khan, N.U. and Nawab, K. 2011. Enhanced proline synthesis may determine resistance to salt stress in tomato cultivars. Pak. J. Bot., **43**: 2707-2710.

Bouman, B.A.M., S. Peng, A.R. Castaòeda and R.M. Visperas. 2005. Yield and water use of irrigated tropical aerobic rice systems. Agricultural Water Management, **74**: 87-105. [Cross Ref]

Champoux, M.C., G. Wang, S. Sarkarung, D.J. Mackill, J.C. O'Toole, N. Huang, *et al*., 1995. Locating genes associated for root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. **90**: 969–981. [Cross Ref]

Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M. and Shinozaki, K. 2011. Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol.,**11**: 163. [Cross Ref]

Evenson, R.E. and G. Gollin. 2003. Assessing the impact of the green revolution, 1960–2000. Sci., **300**: 758–762. [Cross Ref]

Fukai, S. and M. Cooper. 1995. Development of drought resistant cultivars using physio-morphological traits in rice. Field Crops Res., **40**: 67–86. [Cross Ref]

Ghafoor, A. 2013. Unveiling the mess of red pottage through gel electrophoresis: a robust and reliable method to identify *Vicia sativa* and *Lens culinaris* from a mixed lot of split "red dal". Pak. J. Bot., **45**: 915-919.

Govindaraj, M., Shanmugasundaram, P., Sumathi, P. and Muthiah, A.R. 2010.Simple, rapid and cost effective screening method for drought resistant breeding in pearl millet.Electron. J. Plant Breed., **1**: 590- 599.

Gowda, V.R.P., Henry, A., Yamauchi, A., Shashidhar, H.E., Serraj, R., 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. **122**, 1–13. [Cross Ref]

Hochholdinger F, Wen T-J, Zimmermann R, *et al*. 2008. The maize *Zea mays* L. root hairless3 gene encodes a putative GPI-anchored, monocot-specific, COBRA like protein that significantly affects grain yield. The Plant Journal **54**: 888–898. [Cross Ref]

Ingram, K.T., Bueno, F.D., Namuco, O.S., Yambao, E.B., Beyrouty, C.A., 1994.Rice root traits for drought resistance and their genetic variation. In: Kirk, G.J.D. (Ed.), Rice Roots: Nutrient and Water Use. International Rice Research Institute, Manila, Philippines.

Jatoi, S.A., Latif, M.M. Arif, M. Ahson, M. Khan, A. and Siddiqui, S.U. 2014. Comparative Assessment of Wheat Landraces against Polyethylene Glycol Simulated Drought Stress. Sci. Tech. and Dev., **33**: 1-6.

Kamoshita, .A, Wade, L.J., Ali, M.L., Pathan, M.S., Zhang, J., Sarkarung, S., Nguyen, H.T. 2002. Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. TheorAppl Genet **104**:880–893. [Cross Ref]

Kanbar, A., Toorchi, M., Shashidhar, H.E., 2009.Relationship between yield and root morphological characters in rainfed lowland rice (*Oryza sativa* L).Cereal Res. Commun. **37**, 261–268. [Cross Ref]

Kano, M., Inukai, Y., Kitano, H., Yamauchi, A., 2011. Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. Plant Soil. [Cross Ref]

Kaur, S. Gupta, A.K. and Kaur, N. 1998. Gibberellic acid and kinetin partially reverse the effect of water stress on germination and seedling growth in chickpea. Plant Growth Regul., **25**: 29–33. [Cross Ref]

Kellermeier, F., Armengaud, P., Seditas, T.J., Danku, J., Salt, D.E., Amtmann, A. 2014. Analysis of the root system architecture of Arabidopsis provides a quantitative readout of crosstalk between nutritional signals. Plant Cell Online , **26,** 1480–1496. [Cross Ref]

Khush, G.S. 2001. Green revolution: the way forward. Nature Rev, **2**: 815–822. [Cross Ref]

Li, Z.K. and J.L. Xu. 2007. Breeding for drought and salt tolerant rice (Oryza sativa L.): progress and perspectives. In: Jenks MA *et al* (eds) Advances in molecular breeding toward drought and salt tolerant crops. Springer, USA: pp 531–564. [Cross Ref]

Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiology, **156:** 1041–1049. [Cross Ref]

Lynch, J.P., 2007. Rhizoeconomics: the roots of shoot growth limitations. Hortscience, **42**, 1107–1109. [Cross Ref]

MacMillan, K., Emrich, K., Piepho, H-P, Mullins, C.E., Price, A.H. 2006. Assessing the importance of genotype 9 environmental interaction for root traits in rice using a mapping population. II.Conventional QTL analysis.TheorAppl Genet, **113**:953–964. [Cross Ref]

Mehlman, D.W., Sheperd, U.L., Kelt, D.A. 1995. Bootstrapping principal components analysis: a comment. Ecology, **76**:640–643. [Cross Ref]

Miguel, M.A., Widrig, A., Vieira, R.F., Brown, K.M., Lynch, J.P. 2013. Basal root whorl number: a modulator of phosphorus acquisition in common bean(*Phaseolus vulgaris*). Annals of Botany, **112**: 973–982. [Cross Ref]

Nguyen, H. T., Babu, R. C., and Blum, A. 1997.Breeding for drought resistance in rice: physiology and molecular genetics considerations.Crop Sci. **37**, 1426–1434. [Cross Ref]

Pandey, V. and A. Shukla, 2015. cclimation and tolerance strategies of rice under drought stress,‖ Rice Sci., **22**, (4)14-161. [Cross Ref]

Price, A.H., Steele, K.A., Moore, B.J., Jones, R.G.W. 2002. Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: II. Mapping quantitative trait loci for root morphology and distribution.Field Crops Research **76**, 25–43. [Cross Ref]

Reynolds, M., Dreccer, F., and Trethowan, R. 2007. Droughtadaptive traits derived from wheat wild relatives and landraces. J. Exp. Bot. **58**, 177–186. [Cross Ref]

Sakthivelu, G., Devi, M.K.A., Giridhar, P., Rajasekaran, T., Ravishankar, G.A. Nedev, T. and Kosturkova, G. 2008. Drought- induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars. Genet. Appl. Plant Physiol., **34**: 103-112.

Sheehy, J.E., A.B. Ferrer and P.L. Mitchell. 2008. Harnessing photosynthesis in tomorrow's world: Humans, crop production and poverty alleviation. In: Photosynthesis. Energy from the sun. (Eds.) J.F. Allen, E. Gantt, J.H. Golbeck and B. Osmond. 14th International Congress on Photosynthesis, Springer, 1243–1248. [Cross Ref]

Singh, V., van Oesterom, E.J., Jordan, D.R., Messina, C.D., Cooper, M., Hammer, G.L. 2010 .Morphological and architectural development of root systems in sorghum and maize. Plant Soil, **333**:287–299. [Cross Ref]

Steele, K.A., Price, A.H., Witcombe, J.R., *et al*. 2013. QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. Theoretical and Applied Genetics, **126**: 101–108. [Cross Ref]

Steele, K.A., D. S. Virk, R. Kumar, S. C. Prasad and J. R. Witcombe. 2007. Field evaluationof upland rice lines selected for QTLs controlling root traits. Field Crop Res,**101**: 180–186. [Cross Ref]

Topp, C.N., Iyer-Pascuzzi, A.S., Anderson, J.T., Lee, C.R., Zurek, P.R., Symonova, O., Zheng, Y., Bucksch, A., Mileyko, Y., Galkovskyi, T., Moore, B.T., Harer, J., Edelsbrunner, H., Mitchell-Olds, T., Weitz, J.S., Benfey, P.N. 2013. 3D phenotyping and quantitative trait locus mapping identify core regions of the rice genome controlling root architecture. Proc Natl AcadSci **110**:E1695–E1704 . [Cross Ref]

Van den Berg, L. and Zeng, Y. J. 2006 Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000.Afr. J. Bot., **72**: 284-286. [Cross Ref]

Vengatesh M. and R. Govindarasu. 2017. Studies on root traits for drought tolerance in aerobic rice (Oryza sativa L.) using polythene bags. Electronic Journal of Plant Breeding, **8**(2): 708-711. [Cross Ref]

Vikram P., B. P. M. Swamy, S. Dixit, H. Ahmed, M. T. Sta Cruz, A. K. Singh, G. Ye, A. Kumar al., 2012. Bulk segregant analysis: "An effective approach for mapping consistent-effect drought grain yield QTLs in rice". Field Crops Research, **134**: 185-192. [Cross Ref]

VishnuVarthini N, Pushpam, R, Jyothsna, M, Manonmani S, Raveendran, M and Robin S. 2017. Molecular tagging of genomic regions influencing root phenomics for improving drought resistance in rice. Electronic Journal of Plant Breeding, **8**(4): 1059-1068. [Cross Ref]

Wang, H., S. Taketa, A. Miyao, H. Hirochika and M. Ichii. 2006. Isolation of a novel lateral-rootless mutant in rice (*Oryza sativa* L.) with reduced sensitivity to auxin. Plant Sci., **170**: 70–77. [Cross Ref]

Wang, H. and Yamauchi, A., 2006. Growth and function of roots under abiotic stress in soil. In: Huang, B. (Ed.), Plant-Environment Interactions (3rd). CRC Press, New York. [Cross Ref]

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, Kirkegaard JA, Christopher J, Watt M. 2012.Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops.Journal of Experimental Botany, **63**, 3485–3498. [Cross Ref]

Zhao, J., Jiabing, F., Hong, L., *et al*. 2004. Characterisation of root architecture in an applied core collection for phosphorus efficiency of soybean germplasm. Chinese Science Bulletin, **49**: 1611–1620. [Cross Ref]