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Association between ABA- and drought-mediated regulation of root traits and identification of potential SNPs in genes for root development in rice

G. K. Krishna, Chandrapal Vishwakarma, Paulson Thomas[1], J. Aravind[2], Sitaram Kushwaha and Viswanathan Chinnusamy*

Division of Plant Physiology, [1]Division of Agricultural Physics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012; [2]Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012

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Abstract

Genetic improvement in root traits is necessary to enhance and stabilize yield of rice crop under rainfed ecosystem. Drought stress induces abscisic acid (ABA) accumulation, which in turn regulates root growth under drought. Hence, screening of genotypes based of ABA-responsiveness of root traits in simple hydroponics system can help to identify genotypes which may have better root traits under field drought stress conditions. Towards this objective, a set of 32 rice genotypes were phenotyped for root traits in 500 nM ABA at seedling stage in hydroponics, and then evaluated under drought stress (soil matric potential -70 kPa) at anthesis stage in field conditions. When the 32 genotypes were classified based on stress susceptibility index (SSI), 11 genotypes showed superior performance in both ABA stress and yield under drought stress in field, while 12 genotypes performed poor under both conditions. The SSI based classification of genotypes under ABA response as well as drought matched with yield for 7 good performing and 7 poor performing genotypes. Thus rice genotypes, CR2624, Ching Moiramsbhi, IC526266, Moroberekan, Nerica-L26, Nerica-L42 and Sahbhagi Dhan, which exhibited enhancement or stability in root length in response to ABA, also showed stability in both root length and yield under drought stress in field conditions. Single Nucleotide Polymorphism (SNP) analysis led to the identification of 20 non-synonymous SNPs in 12 genes involved in root traits. Clustering based on these SNPs could differentiate the genotypes with better root traits from that with poor root traits. This study shows that screening for ABA-responsiveness in root traits is a potential surrogate to identify donors for better root traits under drought stress in field conditions.

Key words: Abscisic acid, drought tolerance, root phenotyping, stress susceptibility index, water use efficiency

Introduction

Rice yield is highly sensitive to even moderate deficit in soil moisture. Out of total 164 mha under rice (www.fao.org/economic/RMM), 27 mha (~16%) grown under uplands are exposed to drought stress (Zu et al. 2017). In India, rainfed lowland and upland rice is cultivated in about 45% of the rice grown area but contribute to only about 33% of total rice production due to intermittent periods of drought. Under these conditions, root traits that allow access to water and nutrients from larger and deeper soil layers is one of the most potential traits for improving drought resistance and water-use efficiency. Sufficient root length and surface area in deep soil layer is currently the most accepted target trait for improving drought tolerance in rice (Gowda et al. 2011). However due to the inherent difficulties in phenotyping of root traits it is less exploited in breeding.

Root system architecture (RSA), the spatial configuration of different types and ages of roots of a plant, is regulated by several soil conditions of which soil moisture is a major determinant. Drought perceived by roots lead to accumulation of ABA, which is transported to leaf through xylem causing stomatal closure and minimize transpiration loss (Davies and Zhang 1991). Besides its role in long distance signalling, ABA also regulates root growth (Watts et al. 1981; Biddington and Dearman 1982; Zhao et al. 2014) and root hydraulic conductivity through enhanced expression and activity of aquaporins (Sharipova et

*Corresponding author's e-mail: viswa_iari@hotmail.com; Viswanathan@iari.res.in
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al. 2016) under drought. Drought and ABA mediated modification determines the soil volume mined by roots for water. ABA positively regulates primary root elongation at low soil water potential by restricting ethylene production in maize (Spollen et al. 2000; Sharp and LeNoble 2002). Molecular genetic analysis showed that ABA promotes expression of *LONG HYPOCOTYL 5* (*HY5*) transcription factor which in turn induces the expression of *ETHYLENE RESPONSE FACTOR 11* (*ERF11*). The *ERF11* inhibits 1-aminocyclopropane-1-carboxylate synthase (*ACS5*) gene for ethylene biosynthesis and promote root elongation in Arabidopsis (Li et al. 2011). ABA also regulates *MIZU-KUSSEI 1* which promotes root hydrotropism in Arabidopsis (Moriwaki et al. 2012). Further, root growth promotion by ABA is also regulated by its cross-talk with auxin. ABA promotes basipetal auxin transport and proton secretion in the root tip cells under osmotic stress in rice and Arabidopsis (Xu et al. 2013; Lekshmy et al. 2017). On the other hand, ABA inhibits lateral root (LR) development in Arabidopsis (Signora et al. 2001; De Smet et al. 2003). The inhibition of root growth is attributed to the premature differentiation of root apical meristem in Arabidopsis (Ji and Li 2014). Taken together, ABA can act both as positive and negative regulator of root growth depending on the genotype, dosage and duration of treatment. Therefore, we hypothesized that screening of genotypes based on ABA-sensitivity of root traits in simple hydroponic screening system may help identify genotypic variation for root traits under drought stress in field conditions. To address this, genotypic variation in ABA-mediated alteration in root traits under hydroponic system and its association with root traits under field drought stress conditions was studied.

Materials and methods

Optimization of ABA dosage for root growth in hydroponics

A set of 32 rice genotypes including both *indica* and *japonica* cultivars were used in this study (Table 1).

Based on our previous study, a drought tolerant rice genotype, CR2624 and drought sensitive rice cv. IR64 were selected and used for standardization of ABA dose to screen rice genotypes under hydroponics conditions. The seeds of rice genotypes were germinated in germination paper by using a "*germination paper strip method*" developed in this study. In this method, good quality seeds were pasted on a germination paper strip with a cello tape. Care was taken to arrange the seeds in the same orientation so that when the paper strip is inserted vertically in 2 mm wide slots made in thermocol sheets (2.5 cm thick), and the embryo face downward (Supplementary Fig. 1). This set-up was placed in tray cotaining 0.5% Bavistin®DF solution prepared in de-ionised water (2-

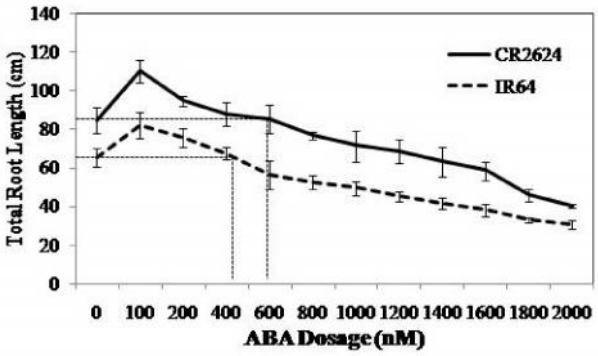

Fig. 1. Optimisation of ABA dosage for root phenotyping in rice genotypes, CR2624 (drought tolerant) and IR64 (drought susceptible)

2.5 cm height). Strips containing germinated seedlings with embryonic seminal root length of 2-5 cm were transferred to treatment solutions in Magenta™ vessel (Merck, USA). New thermocol sheets that fit the vessel were made to hold the strips. For treatment in hydroponics, ¼ strength Yoshida solution containing various concentrations of ABA ranging from 0 to 2000 nM were used. The seedlings were allowed to grow for 14 days in a culture room. The hydroponic solution was changed once in 7 days. At the end of the treatment period, the roots were scanned in Epson11000XL scanner (Epson America, Inc.) and the image obtained was analyzed using WinRHIZO™ Pro software (Regent Instruments Inc., Canada).

Screening of genotypes based on root traits by hydroponics

The 32 genotypes were raised by the *germination paper strip method* (Supplementary Fig. 1). The strips having seedlings that reached 2-5 cm seminal root length only were transferred to treatment solutions. The treatment solution contained ABA concentration of 0 or 500 nM prepared in ¼ strength Yoshida solution. Blue plastic trays of 15 cm depth were used in the hydroponic set-up. The seedlings were kept under treatment for 14 days and the solution was changed once in 7 days. At the end of the treatment period, the roots were analyzed by using WinRHIZO™ Pro software (Regent

Table 1. Classification of rice genotypes based on stress susceptibility index (SSI) for root traits and grain yield.

S.No.	Genotype	SSI for root traits under ABA				SSI for root traits under drought				SSI for yield
		≤1	>1	≤1	>1	≤1	>1	≤1	>1	
		Root length		Root surface area		Root length		Root surface area		
1	CR2624	–1.38		–0.28		0.84		0.97		0.39
2	Ching Moiramsbhi	–9.65		–2.79		0.47		0.54		0.47
3	IC526266	–0.80		–0.48		0.94		0.83		0.95
4	Moroberekan	–5.63		–1.07		0.90		0.83		0.89
5	Nerica-L26	–7.98		–2.82		0.67		0.60		0.68
6	Nerica-L42	–2.04		–0.50		0.77		0.67		0.72
7	Sahbhagi Dhan	–2.10		0.02		0.74		0.62		0.75
8	Pusa44	–2.09		–1.40		0.92			1.05	0.65
9	Way Rarem	–0.25		–0.54			1.04	0.99		0.75
10	Pusa Sugandh 2	–6.40		–3.78			1.71		1.83	0.93
11	IC369769	–4.49		–0.37			1.15		1.12	0.50
12	Vandana	–4.62		–0.86			1.09	0.48		1.03
13	Pusa Sugandh 5	–1.66		–0.33			1.39		1.30	1.26
14	Nerica-L44	–3.84		–2.14			1.24		1.17	1.38
15	Bakal		6.02		3.11		1.22		1.18	1.01
16	CR143-2-2		4.51		3.25		1.21		1.41	1.37
17	IC458319		5.87		3.49		1.33		1.43	1.03
18	IC0132765		5.05		2.10		1.08		1.17	1.35
19	IC346818		3.03		1.74		1.31		1.26	1.39
20	IC36753		3.90		2.43		1.10		1.07	1.38
21	IR64		4.53		2.50		1.41		1.53	1.20
22	Nagina22		1.32		1.86	0.90		0.94		1.07
23	Vanaprava		6.89		3.51	0.56		0.75		1.13
24	MTU1010		5.25		3.33	0.70		0.44		1.26
25	Abhishek		4.55		3.03	0.65		0.60		1.33
26	Pusa Basmati 6		10.40		4.69	0.75		0.89		1.36
27	IC305692		1.13	0.69		0.92		0.78		1.28
28	IC258219		1.08	0.90			1.15		1.33	1.42
29	IC371949		3.73		2.51	0.92		0.95		0.66
30	Pusa Basmati 1		5.55		2.02	0.87		0.89		0.77
31	Apo		3.90		3.30		1.08	0.99		0.72
32	Rasi		5.29		2.19	0.73		0.45		0.95

The SSI values obtained for each genotype were sorted into two classes (≤1, tolerant and >1 susceptible to a treatment)

Instruments Inc., Canada). Total root length (cm), root surface area (cm^2) and root diameter (mm) data were recorded.

Screening of genotypes for root traits and yield in field conditions

The 32 genotypes were transplanted in puddled field during *kharif* 2014 at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi. The

weather conditions during the field experiment is presented in Supplementary Fig. 2. Plants were grown under well watered conditions, and one set of plants were imposed with drought stress treatment of about –70 kPa soil matric potential (SMP) at booting stage. The SMP was measured using tensiometer at a soil depth of 20 cm. In irrigated plots, SMP was maintained between 0 to –10 kPa. The stress was given as cycles of drought and recovery i.e., when SMP reached –70 kPa, plots were irrigated. Root sampling was when the SMP reached –70 kPa at physiological maturity. Prior to sampling, both the control and drought plots were saturated in 5 cm standing water. A rectangular block of about 30 cm width and 20 cm depth, covering three uniform plants in a row was dug out from field for each genotype. It was immediately put in the irrigation channels and washed carefully with minimal loss of roots. Then, the roots were placed in plastic mesh trays and carefully cleaned with good quality water. Washed roots were scanned by using WinRHIZO^{TM} Pro software (Regent Instruments Inc., Canada) as described above. Grain yield per m^2 was recorded at maturity.

SNP variations in genes for root development

SNP genotyping for 28 genotypes were done to understand the allelic variation in genes involved in root development. SNP genotyping was done using single copy (SC) gene based 50K rice SNP chip (Singh et al. 2015). Various genes regulating root traits were selected from literature. The SNP variations for genes were found from the corresponding SNP Chip probes, and then narrowed down those SNPs which caused change in amino acid. For this, the nucleotide sequence from OryGenesDB (http://orygenesdb. cirad.fr/tools.html), deduced amino acid sequence from ExPASy Translate (http://web.expasy. org/translate/) and sequence information from RGAP (http:// rice.plantbiology.msu.edu/index.shtml) were compared. Only the functional SNPs which lead to change in amino acid among genotypes were selected for constructing the dendrogram. A total of 20 functional SNPs from 12 genes involved in root development were chosen (Table 2). In RStudio 1.0.151 (RStudio Team 2015) using the 'adegenet' package (Jombart 2008), the distance matrix was derived by Euclidean distance calculation based on SNP allele frequency within genotypes. Then this matrix was used for construction of dendrogram using R package 'ape' by Neighbour-Joining algorithm (Paradis et al. 2004).

Calculation of Stress Susceptibility Index (SSI)

Root data and yield data were subjected to the SSI analysis as given by Fischer and Maurer (1978) as described below. Genotypes with SSI ≤ 1 are considered tolerant to stress.

Step 1: Mean stress intensity, $\overline{\mathrm{StI}} = 1 - \frac{\overline{s}}{\overline{c}}$

Where, $\overline{\mathrm{C}}$ is the mean value of a trait in all genotypes in control, $\overline{c} = \frac{1}{n}\sum_{i=1}^{n} c_i$

and $\overline{\mathrm{S}}$ is mean value of the trait in all genotypes in stress, $\overline{\mathrm{S}} = \frac{1}{n}\sum_{i=1}^{n} S_i$

Step 2: Stress intensity of each genotype, $\mathrm{StI_i} = 1 - \frac{s_i}{c_i}$

Where, Ci is the mean value (of replications) of a trait in a genotype in control;

Si is mean value (of replications) of the trait in a genotype under stress

Step 3: Stress susceptibility index of each genotype, $\mathrm{SSI_i} = \frac{StI_i}{StI}$

In silico gene expression profiling and statistical analysis

The *in silico* gene expression analysis of selected genes was done by using Genevestigator^{TM} database (Hruz et al. 2008) and eFP browser (Winter et al. 2007). The data on root traits and yield was subjected to ANOVA by F-test at P ≤ 0.05 level of significance.

Results and discussion

Optimization of ABA dosage

Selection of appropriate ABA concentration for phenotyping under hydroponics is critical to distinguish tolerant and sensitive genotypes. Hence, the effect of different doses of ABA on total root length on a drought tolerant rice genotype, CR2624 and drought sensitive rice cv. IR64 was analyzed in hydroponics. The results showed that ABA causes promotion in root growth at low doses, and then inhibits root growth at higher concentration (Fig. 1). In both the genotypes, the root

Table 2. Genes coding for root development with functional SNPs.

S.No.	Locus ID(MSU)	Gene name	Probeset ID	SNP	Amino acid change	Reference
1	*Os03g02800*	RICE DWARF VIRUS MULTIPLICATION 1 (RIM1/ONAC054)	AX-95935876	[A/G]AG	K582E	Yoshii et al. 2010
2	*Os03g19280*	ROOT ELONGATION DEFECT (RED1/ ASL1.1)	AX-95949414	A[A/G]A	K470R	Xia et al. 2014
3	*Os04g36070*	RESPONSE REGULATOR (RR1)	AX-95951364	AA[C/G]	N132K	Kitomi et al. 2011
4	*Os05g15630*	BRASSINOLIDE ENHANCED 3 (BLE3)	AX-95952606	G[C/G]G	A139G	Yang et al. 2006
5	*Os06g03710*	DWARF AND LOW TILLERING (DLT1/GRAS32/D62)	AX-95915744	[G/A]TC	V367I	Tong et al. 2009
6	*Os06g10880*	ABA-RESPONSIVE ELEMENT (ABRE) BINDING FACTOR (ABF2/ABL1/bZIP46)	AX-95928603	AA[G/T]	K296N	Tang et al. 2012
7	*Os06g10880*	-do-	AX-95955121	G[A/G]G	E32G	
8	*Os06g34180*	FON2-LIKE CLE PROTEIN 2 (FCP2)	AX-95927263	AT[G/T]	M1I	Chu et al. 2013
9	*Os07g06970*	HUA ENHANCER 1 (HEN1/WAF1)	AX-95929823	AA[T/G]	N638K	Abe et al. 2010
10	*Os07g06970*	-do-	AX-95957523	T[G/T]T	C795F	
11	*Os07g31450*	CROWN ROOT LESS 6 (CRL6/CHR4/CHR729)	AX-95929531	A[T/C]C	I2074T	Wang et al, 2016
12	*Os07g31450*	-do-	AX-95957672	GA[C/A]	D1988E	
13	*Os07g45570*	BRASSINOLIDE ENHANCED 2 (BLE2)	AX-95937533	A[C/A]C	T422N	Yang et al. 2006
14	*Os07g45570*	-do-	AX-95937581	[C/T]CA	P385S	
15	*Os07g45570*	-do-	AX-95937597	AT[G/T]	M437I	
16	*Os07g45570*	-do-	AX-95940092	AT[G/T]	M395I	
17	*Os07g45570*	-do-	AX-95957137	CA[T/A]	H232Q	
18	*Os07g45570*	-do-	AX-95957575	[C/T]AC	H882Y	
19	*Os09g16510*	WRKY74	AX-95959299	GA[C/A]	D213E	Dai et al. 2016
20	*Os10g25780*	DIMINUTO (DIM/DWF1/BRD2)	AX-95938337	A[A/G]G	K5R	Hong et al. 2005

length was higher at 100 and 200 nM ABA as compared to the control (0 nM ABA). At 400 to 600 nM ABA, the root growth reduced significantly below that of control level in drought susceptible IR64, but the root length was maintained at control level in drought tolerant CR2624 at 400-600 nM ABA (Fig. 1). This results were in consistent with the previous study (Shi et al. 2015), where it was reported that that genotype accumulating a low level of ABA (~300 ng/g DW) in roots showed a higher root elongation as compared to hyper accumulating (~600 ng/g DW) genotype. Hence, a concentration of 500 nM ABA concentration was selected to differentiate tolerant genotypes and sensitive genotypes.

ABA-mediated changes in root traits of rice genotypes in hydroponics

The screening of rice genotypes under 500 nM ABA showed a genotype dependent response. The mean reduction in total root length of all 32 genotypes under ABA treatment was about 8%. Rice cv. Pusa Basmati 6 showed 62% reduction, while Ching Moiramsbhi

showed 57% increase in root length under ABA as compared with control. Of the 32, 10 and 16 genotypes showed statistically significant increase and decrease in root length, respectively, in ABA treatment as compared with control, while seven genotypes namely Pusa Sugandh 5, CR2624, IC526266, Way Rarem, IC258219, IC305692 and Nagina22 maintained root length (Fig. 2a). In root surface area, 6 genotypes showed significant increase in surface area, while 17 genotypes showed significant decrease in surface area in ABA treatment as compared with control. Among them, Pusa Sugandh 2 showed 53% increase, while Pusa Bamati 6 showed 66% reduction in surface area under ABA as compared with control (Fig. 2c). Rice genotypes, Ching Moiramsbhi, Nerica-L26, Pusa Sugandh 2, Moroberekan, Vandana, IC369769, Nerica-L44, Pusa 44 and Nerica-L42 showed significant increase in both RL and SA in response to ABA as compared with control. The mean root diameter of all rice genotype showed about 10% reduction under ABA treatment conditions. Only four rice genotypes Pusa Sugandh 2, Way Rarem, Pusa Basmati 1, Nerica-L 44 and Pusa 44 showed significant increase in root diameter, while 26 genotypes showed reduction in root diameter in response to ABA (Supplementary Fig. 3a). Only limited efforts have been made earlier to analyze the genotypic variation in root traits in response to ABA in rice. Xiao et al. (2013) found 500 nM ABA induced reduction in primary root length in *japonica* cv. Zhonghua 11 but Nipponbare maintained the root length. In another study, 500 nM ABA was found to reduce root length significantly in *japonica* rice cv. Tainung 67 (Tseng et al. 2013).

Phenotyping of root traits in rice genotypes under field drought stress conditions

The mean root length of rice genotypes was 4626 (±307) and 2507 (±167) cm per plant under control and drought stress conditions, respectively. Rice genotypes Nerica-L26, Abhishek, Vanaprabha and Ching Moiramsbhi showed <30% reduction, while Pusa Sugandh 2, IC36753, IR64 and Pusa Sugandh 5 showed >60% reduction in root length under drought stress as compared with that of irrigated plants (Fig. 2b). Similarly root surface area per plant was significantly reduced in all genotypes under drought with a mean reduction root surface area of 45% in rice genotypes. Rice genotypes, MTU1010, Rasi, Vandana, Ching Moiramsbhi, Nerica-L26, Abhishek, Sahbhagi Dhan and Nerica-L42 showed <30% reduction in root surface area while IC346818, Pusa Sugandh 5, IC258219, CR143-2-2, IC458319, IR64, IC36753 and Pusa Sugandh 2 showed >50% reduction in root surface area under drought as compared to that of irrigated plants (Fig. 2d). In contrast to the reduction in root length and surface area under drought stress, mean root diameter of rice genotypes was not reduced. The mean root diameter of rice genotypes was 0.85 (±0.013) and 0.87 (±0.015) mm, under control and drought, respectively. The root diameter increased significantly in 18 genotypes, decreased significantly in 12 genotypes and maintained in two genotypes. Rice genotypes Vandana, Rasi, Pusa Sugandh 5, MTU1010, IC346818, IC305692, Nerica-L44, Apo and IC526266 showed >10% increase, while IC258219 and CR143-2-2 showed >10% decrease in root diameter under drought stress as compared with control (Supplementary Fig. 3b). Mild drought stress induces root growth but when the stress increase, the root growth is reduced in rice. In our previous study with direct seeded rice, we found that mean root length density of rice genotypes increases, when soil matric potential (SMP) decreased from –10 kPa to –20 kPa, while at –40 kPa it remained same as that of –20 kPa stress levels. However, when the stress level was increased to –40 kPa, genotype dependent increase or reduction in root growth was observed in rice (Singh et al. 2008). In another study, with rice cv. Takanari, the total root length decreased at both –30 and -80 kPa SMP stress levels (Kato and Okami 2011). Similarly, when SMP decreased below –40 kPa, reduction in root length was found in rice genotypes under field conditions (Suralta and Yamauchi 2008).

Stress susceptibility index (SSI) of rice genotypes for yield under drought

The mean yield of 32 rice genotypes under control conditions was 362 gm^{-2}, which was reduced to 118 gm^{-2} under drought with a drought stress intensity of 0.67. Grain yield significantly decreased under drought stress in all the genotypes studied (Supplementary Fig. 4). Drought susceptibility analysis showed 15 genotypes with DSI values $\leq$ 1.0, and rest of the 17 genotypes with DSI>1.0. Among the known drought tolerant cultivars Nagina22 showed relatively more susceptibility as compared with Apo and Sahbhagi Dhan. The drought qDTY donor genotypes viz., Moroberekan ($qDTY_{3.2}$ and $qDTY_{11.1}$; Kumar et al. 2014) and Way Rarem ($qDTY_{12.1}$; Bernier et al. 2007; Kumar et al. 2014) showed drought tolerance with a DSI value of $\leq$1.0 under the severe drought stress in this study (Table 1).

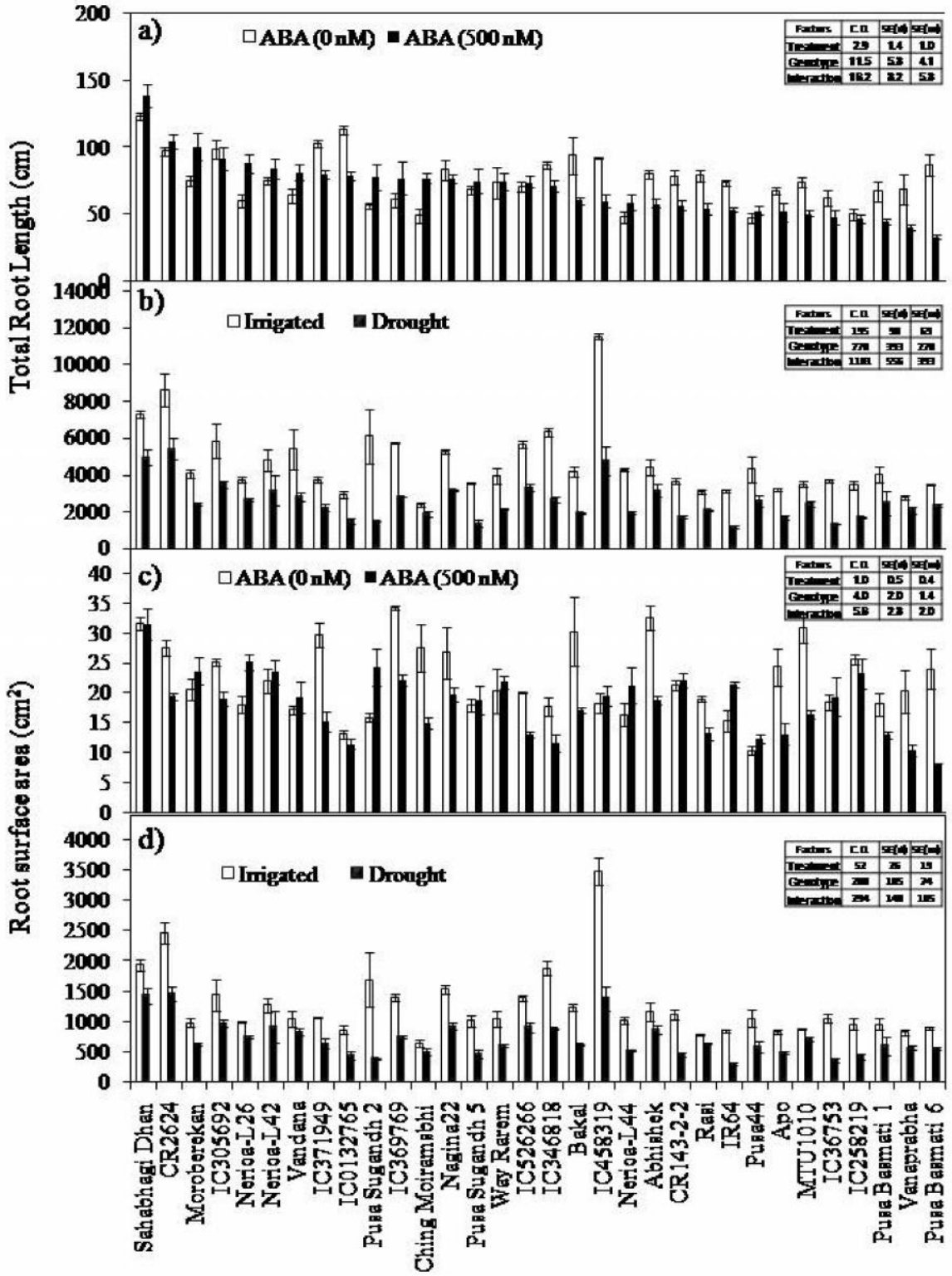

Fig. 2. Root traits of rice genotypes under ABA and drought treatments. a) Total root length (cm) under ABA (500nM) in hydroponics. b) Total root length (cm) under drought stress (-70 kPa) in field. c) Root surface area (cm^2) under ABA (500nM) in hydroponics, d) Root surface area (cm^2) under drought stress (-70 kPa) in field. Error bars indicate ±SE of mean. The critical difference (C.D.), standard error of deviation (SE(d)) and standard error of mean (SE(m)) of variables are given in the inset

Association between ABA- and drought-induced changes in root traits

The stress susceptibility index (SSI) for root traits under ABA treatment in hydroponics and root traits under field drought stress conditions were compared. The SSI value >1.0 indicates susceptibility, and ≤1.0 indicates tolerance to a treatment. We compared how the genotypes got classified based on root traits in response to ABA treatment when compared with susceptibility index of root traits and yield under field drought stress conditions. When the total root lengths were considered for comparison, a total of 14 genotypes was identified as tolerant and 18 genotypes as susceptible under ABA in hydroponics. Among the 18 susceptible genotypes for ABA, 9 genotypes were also identified as susceptible under field drought conditions. Further, 11 out of 14 genotypes identified as tolerant under ABA also were identified as superior based on SSI of yield (Table 1). Eight genotypes namely CR2624, IC526266, Ching Moiramsbhi, Moroberekan, Nerica-L26, Nerica-L44, Sahbhagi Dhan and Pusa 44 showed SSI values ≤ 1.0 for root length under ABA and drought and grain yield under drought. Further, 7 genotypes with SSI > 1.0 for root length under ABA also showed SSI >1.0 for root length and grain yield under field drought conditions (Table 1). When the root surface area was considered, 16 and 18 genotypes showed SSI ≤ 1.0 under ABA and drought, respectively. Out of 16 genotypes identified as susceptible to root surface area under ABA, 12 were identified as susceptible to drought based on SSI of yield. Seven genotypes namely Bakal, CR143-2-2, IC458319, IC0132765, IC346818, IC36753 and IR64 were identified as susceptible based on the performance of genotypes for RSA under ABA and field drought stresses in relation to yield (Table 1).

SNP variations in genes for root development

From the 50K SNP analysis of 28 rice genotypes for a large set of genes involved in root development, we found SNP variation that caused amino acid change in 12 genes (Table 2). These 12 genes were selected for construction of dendrogram (Fig. 3). The 28 genotypes were grouped in to five groups. Maximum number of genotypes were grouped in to group II (13 genotypes) and group III (8 genotypes). Among the top 3 genotypes that produced longer root length under normal and drought conditions in the field (Fig. 2), IC458319 and Sahbhagi Dhan were grouped in to group I, while CR2624 was grouped in to group III (Fig. 3). Most of the qDTY donors *viz*., Apo ($qDTY_{1.1}$, $qDTY_{2.1}$, $qDTY_{3.1}$ and $qDTY_{6.1}$), Way Rarem ($qDTY_{12.1}$),

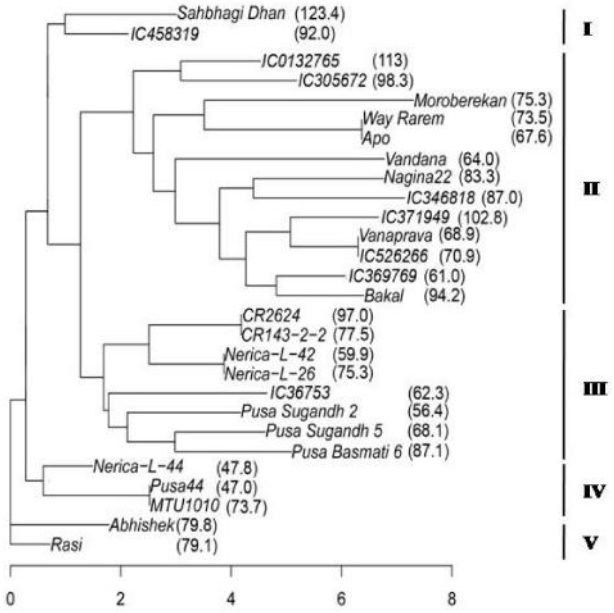

Fig. 3. Dendrogram constructed using non-synonymous SNPs in selected genes for root traits among 28 genotypes. The Euclidean distance matrix was calculated to construct dendrogram by Neighbor Joining algorithm from 500 boot strap replicates. The scale bar indicates Euclidean distance. Values in parenthesis indicates root length under control (0 nM ABA) in hydroponics

Moroberekan ($qDTY_{3.2}$ *and* $qDTY_{11.1}$), Nagina 22 ($qDTY_{1.1}$ *and* $qDTY_{3.2}$) and Vandana ($qDTY_{6.1}$) were grouped in to group II. Rice cultivars developed for transplanted irrigated cultivation such as Pusa Sugandh 2, Pusa Sugandh 5, Pusa Basmati 6 were grouped in to sub-group III, while Pusa 44 and MTU1010 were grouped into group IV (Fig. 3). Sahbhagi Dhan and IC458319 which produced higher root length both under control conditions in hydroponics and irrigated field conditions. Sahbhagi Dhan showed high stability in root traits under ABA and drought stress, while IC458319 was highly sensitive under both the conditions. These two genotypes differed on only one SNP in *FCP2* gene among the 12 genes analyzed. A mutation in Sahbhagi Dhan abolishes the original initiation codon (MAI) in *FCP2*, a negative regulator of root growth (Chu et al. 2013). Vandana which had similar SNP in *FCP2* gene also showed high stability (SSI) in root traits response to ABA. In another negative regulator of root growth, *ABF2* gene, SNP variations resulted in change of E32G and N296K in *ABF2* proteins in 8 genotypes which were classified in to group II (Fig. 3).

In silico gene expression profiling

The *in silico* gene expression profiling by Genevestigator and eFP browser showed that all the selected genes were expressed in root tissues (Supplementary Fig. 5). *ABF2* gene was upregulated at tillering and panicle elongation stage, while *CRL6* was upregulated at tillering stage under drought stress in roots of IR64 (Supplementary Fig. 5). Overexpression of *ABF2* encoding an ABA induced bZIP46 transcription factor reduced the root length significantly under 3 mM ABA (Tang et al. 2012), while loss of function of *CRL6* gene which encodes a chromatin remodeling factor is defective in crown root primordia initiation with fewer crown roots (Wang et al. 2016). Here, we found that *RIM1*, *RR1*, *BLE3*, *DLT1* and *WRKY74* genes were downregulated to different levels in roots of IR64 under drought stress (Supplementary Fig. 5). The *rim1* mutants had a short seminal root in seedlings (Yoshii et al. 2010), while *rr1* mutation causes reduction in crown root number as it is essential for crown root primordia formation (Kitomi et al. 2011). *BLE3* gene silencing with antisense approach significantly reduced root length (Yang et al. 2006). Loss of function mutant of *DLT1* gene, involved in the GA-BR signaling cross-talk, exhibited a reduction in root growth (Tong et al. 2009). The *WRKY74* transcription factor, a nutrient deficiency induced gene, whose overexpression lines showed better root growth under Pi deficiency than wild type (Dai et al. 2016). The drought induced down regulation of these positive regulators of root growth viz. *RIM1*, *RR1*, *BLE3*, *DLT1* and *WRKY74* (Supplementary Fig. 5) is associated with the drastic reduction in root length (62% reduction) under drought stress in IR64 (Fig. 2).

In conclusion, the results showed that phenotyping for ABA-responsiveness in root traits under hydroponics is a potential surrogate to identify genotypes for better root traits under field drought stress conditions in rice and in other crops. Rice genotypes, Sahbhagi Dhan and CR2624 can be used as source of larger root system, while Ching Moiramsbhi with highest inductive root system in response to ABA and high stability in root traits under field drought condition can be used to understand the molecular genetic basis of inductive root system in rice. SNP analysis and *in silico* expression analysis identified potential genes for genetic improvement of root traits in rice.

Authors' contribution

Conceptualization of research and designing of the experiments (KG, VC); Root studies in field/ lab (KG, PT); Field evaluation of drought tolerance (CV, SK); Analysis of data, interpretation and preparation of manuscript (KG, AJ, VC).

Declaration

The authors declare no conflict of interest.

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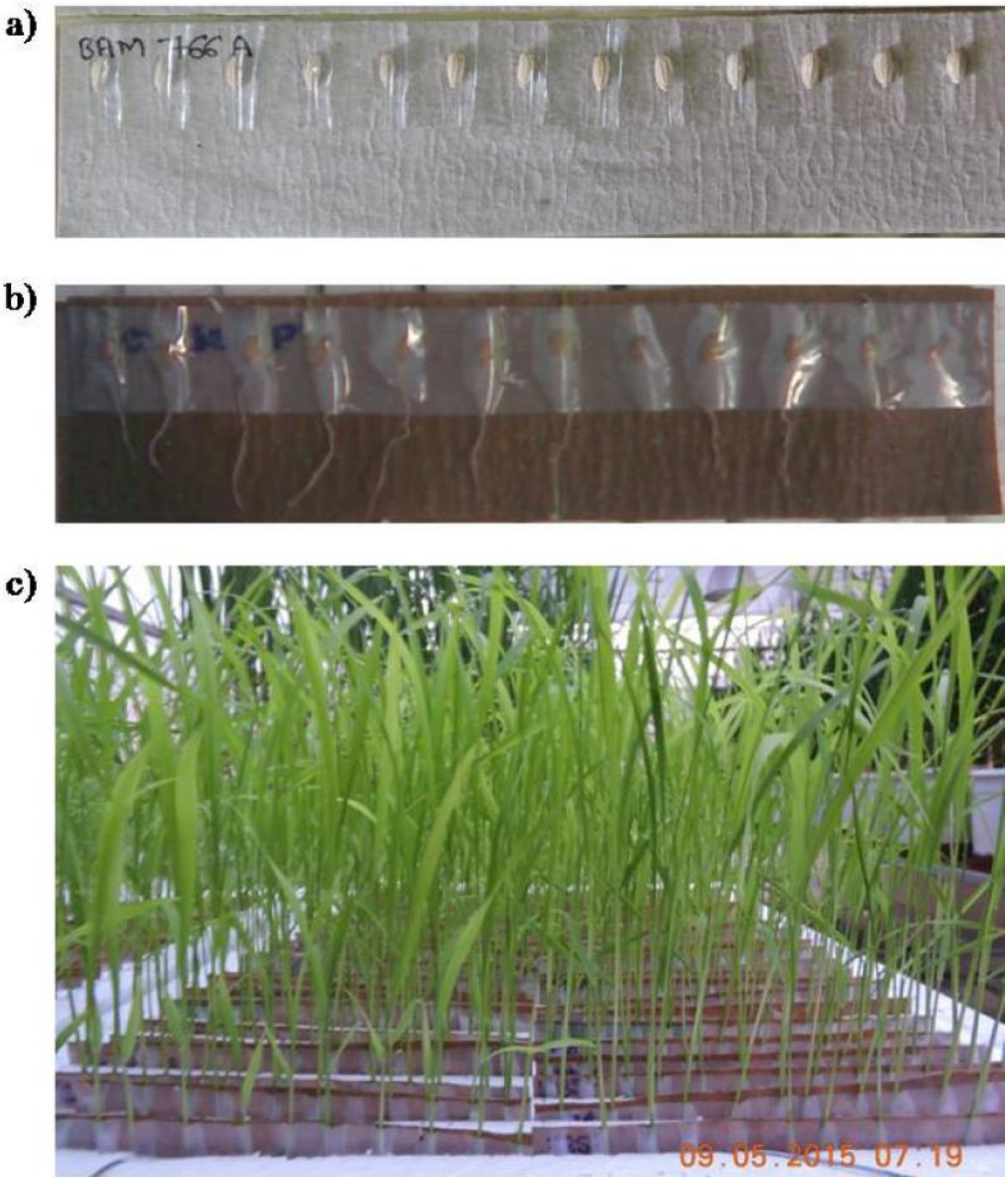
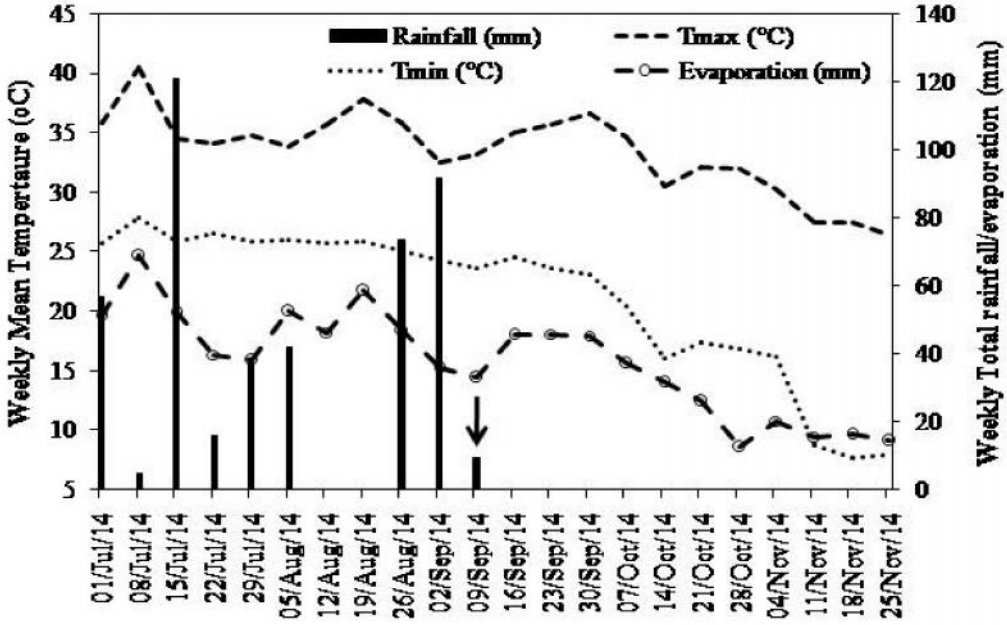
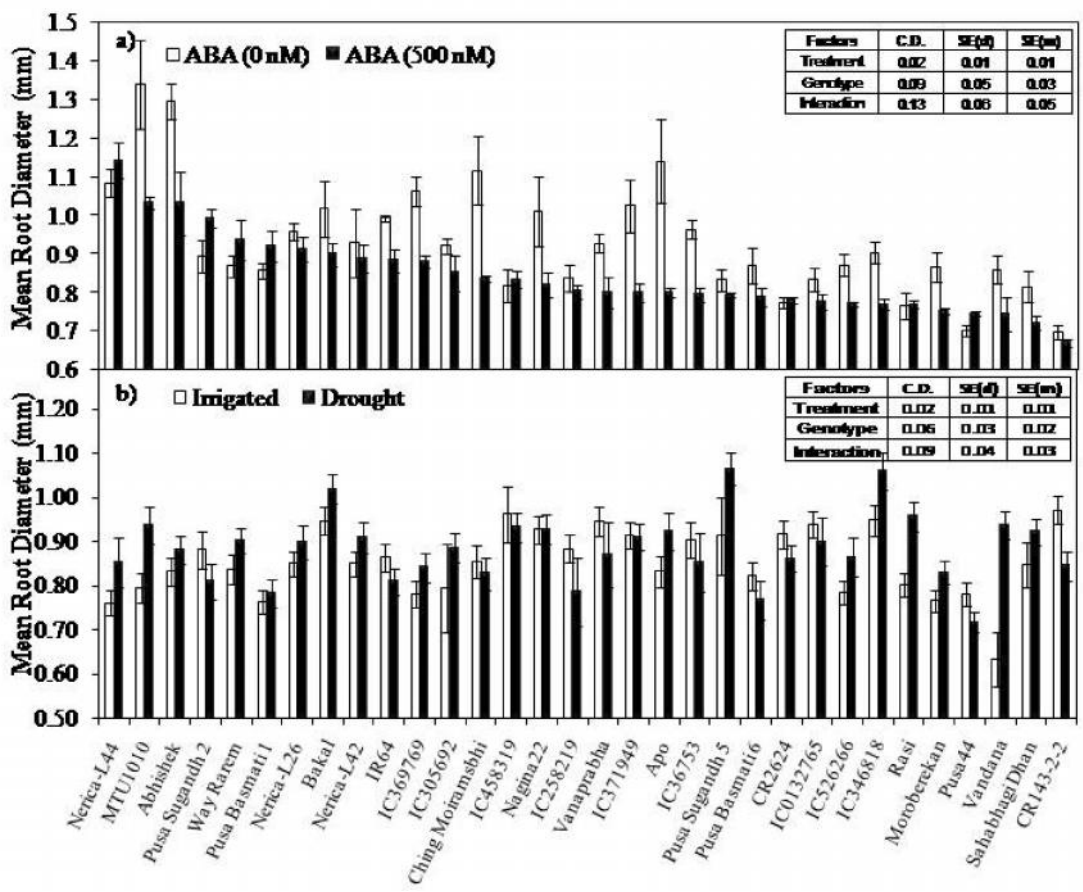


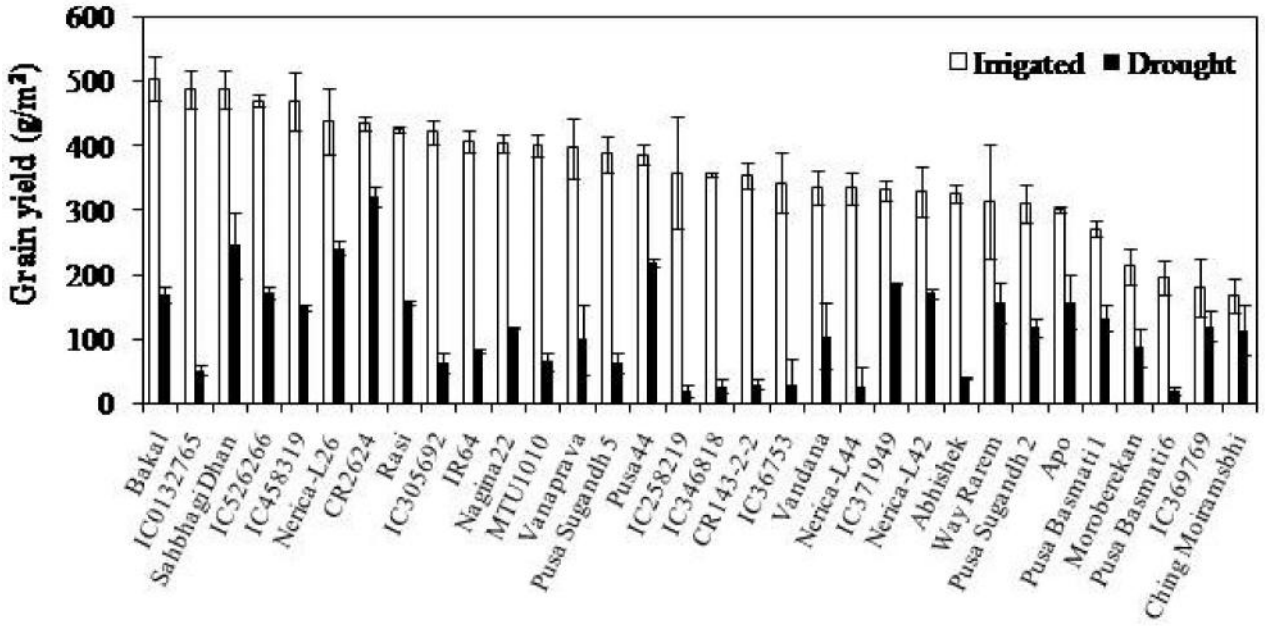
Supplementary Fig. 1. Major steps followed in germination paper strip method. a) Arrangement of seeds on germination paper strips. Note that pasting the cello tape creates a void at the top and bottom to favour seedling emergence. b) Seedlings with optimum growth for transfer to treatment solutions. c) Seedlings at the end 14 days growth period



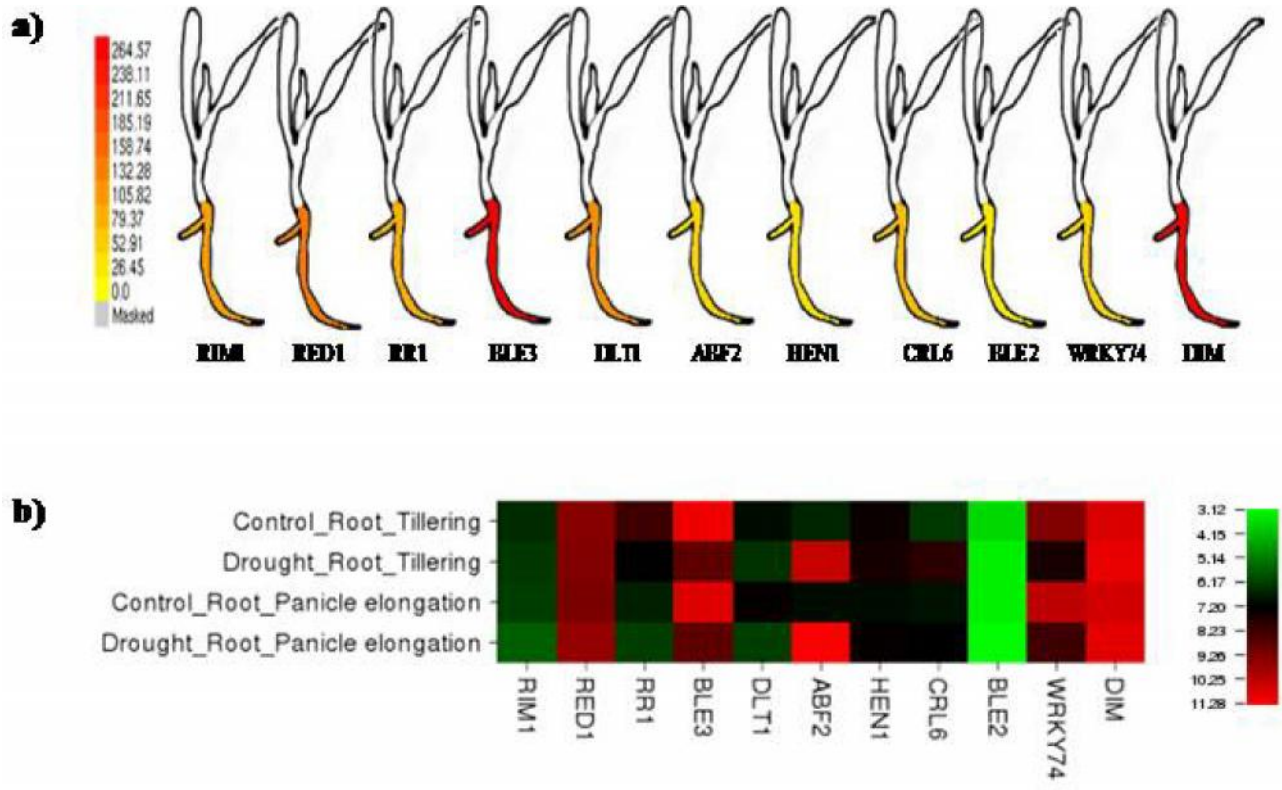
Supplementary Fig. 2. The weather parameters during the field experiment at IARI, New Delhi during *kharif* 2014. Downward arrow on rainfall bar indicates date of start of drought stress



Supplementary Fig. 3. Mean root diameter of rice genotypes under ABA and drought treatments. a) Mean root diameter (mm) under ABA (500nM) in hydroponics, b) Mean root diameter (mm) under drought stress (-70 kPa) in field. Error bars indicate ±SE of mean. The critical difference (C.D.), standard error of deviation (SE(d)) and standard error of mean (SE(m)) of variables are given in the inset



Supplementary Fig. 4. Grain yield (g/m^2) of rice genotypes under irrigated and drought stress treatments. Error bars indicate ±SE of mean



Supplementary Fig. 5. In silico expression analysis of selected genes involved in root development. a) By eFP browser for expression studies in seedling roots b) Genevestigator expression analysis of genes in roots at tillering and panicle elongation stages of rice cv. IR64 under drought stress