RESEARCH ARTICLE

REVISED Variation in rice root traits assessed by phenotyping under drip irrigation [version 2; peer review: 1 approved, 2 not approved]

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Abstract

Background: Roots are the key elements in water saving rice cultivation. So, the response of rice roots are to be phenotyped under varied drip irrigation treatments. **Methods:** This study describes an investigation on rice root phenotyping under drip irrigation treatments in split-split plot design. Two lateral spacing levels (0.8 and 1.2m), laid at two depths of sub surface irrigation (5-10 and 15-20 cm) by solar powered and well operated irrigation were tested using TNRH 180, JKRH 3333 and ADT(R)45 rice genotypes during the summer season (2013 & 2014) in Coimbatore, India. Conventional aerobic irrigation was considered as control. **Results and Discussion:** An increased root length, root density (length and weight), root Adenosine Tri Phosphatase enzyme activity, root volume and filled grain percentage were favored in aerobic rice under the conditions of 0.8m lateral distance with 5-10cm depth of sub surface drip irrigation (SDI). Improved root characteristics were observed in JKRH 3333 rice hybrid, and root density and thickness favored the filled grains and yield increment in rice by drip irrigation. The 0.8m lateral distance laid out at 5-10cm depth SDI with solar system proliferated more roots at subsurface soil layer with significant yield increment in rice.

Keywords

Drip, rice, phenotyping, root, yield

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REVISED Amendments from Version 1

The new version of this paper includes detailed experimental results on treatment interactions. Also, few additional information such as the field, drip layout and drip components are given in [Supplementary File 2](#).

See referee reports

Introduction

Aerobic rice refers to rice grown under non-water stagnation. The growth of the root system in rice is restricted under aerobic conditions, which is the reason for poor yield ([Kato *et al.*, 2010](#)). In aerobic soil conditions, the soil's interaction with rice is primarily focused on the root system. Therefore, the root system is the first barrier to face stress found in aerobic soil conditions. Water management of rice crops is a sensitive tool in aerobic rice cultivation practice, which has been demonstrated by alteration in rice root anatomy ([Mostajeran & Rahimi-Eichi, 2008](#)).

A deeper root system of rice eases water stress and improves the uptake of nutrients and water in deep soil layers ([Lilley & Fukai, 1994](#)). Rice cultivars with deep rooting and higher root density are much more favorable to aerobic conditions than lowland conditions ([Matsuo *et al.*, 2010](#)).

Regulation of root traits to aerobic conditions could be attained by phenotyping for root traits and rhizosphere management by application of water and nutrients to the root zone ([Zhang *et al.*, 2010](#)). Drip irrigation stimulates fibrous root production with specific changes in root system architecture ([Raj *et al.*, 2013](#)). Similarly, drip irrigation with humigation plants shows favorable root growth with grain yield in rice ([Vanitha & Mohandass, 2013](#)).

This study focuses on phenotyping of root traits, such as root length, density, and distribution, under various drip treatments, related to the root response of rice genotypes. Consequently, the phenotyping of root traits and grain yield under drip environment was analyzed by the present study.

Methods

Experimental set up

A root phenotyping experiment was conducted during the summer season of 2013 and 2014 at Tamil Nadu Agricultural University (Coimbatore, Tamil Nadu, India). Seeds were manually sown in the field at 20×10cm spacing. The open pan evaporation (PE) values (125% pan evaporation) were used to calibrate irrigation scheduling, and drip irrigation was supplied via pipe at 40mm outside diameter (OD) by 7.5HP motor with a pressure of 1.5kg/cm² from a bore well. Solar powered and well-operated drip irrigation sources as main plot treatments (S: Source), test varieties JKRH 3333, TNRH 180 and ADT(R)45 were sown in subplots (V: Variety), drip treatments such as 0.8 and 1.2m lateral distances, 5–10 and 15–20cm depth sub-surface drip (SDI) and conventional aerobic treatments were adopted as sub-sub plots (T: Treatment) at field level. The conventional aerobic practice was scheduled at 1.25 irrigation water (IW)/cumulative pan evaporation (CPE) ratio to 3.0cm. It was named as conventional aerobic rice. The recommended dose of 150:50:50 kg:ha⁻¹: NPK water soluble fertilizers

were used to fertigate the crops using the Venturi flume at weekly intervals. Three replications were maintained in the field at a dimension of 2.4 × 7.0m plot size in split-split plot experimental design. Further information on genotypes, experimental set up, fertigation schedule, drip layout and drip components are given in [Supplementary File 1](#) and [Supplementary File 2](#).

Measurements

Root length was estimated during the flowering phase (80 days after sowing) from core samples ([Kato *et al.*, 2006](#)). Rice roots were removed carefully from the soil by root auger without damaging the roots. After the samples were oven-dried at 80°C for 72h, root lengths and weights were measured. Root length (m hill⁻¹) = sample root length (cm) × total root weight (g)/sample root weight (g). Root dry weight was expressed as g/hill. The specific root length was estimated as a ratio by root length to root dry weight. Four soil cores (50mm diameter, 35cm depth) per plot were taken next to the plant and between the rows (20cm) with a soil sampler. Cores of soil were separated into 0–15 and 15–35cm, then washed using water and sieved by 0.5mm mesh sieve. The root length (RLD), as well as root mass density (RMD), was determined using the formula of [Pantuwan *et al.* \(1997\)](#), and the values were expressed as cm/cm³ and mg/cm³ of the soil, for RLD and RMD respectively. Root volume was recorded using the water displacement technique ([Bridgit & Potty, 2002](#)) and expressed as cm³/hill.

Core sampled roots were washed thoroughly and dehydrated using 80, 90 and 100% alcohol. Dehydrated roots were embedded in slides using paraffin. Slides were kept for imaging the root system using camera (Sony 12.1 megapixel) mounted Leica D1000 microscope at 10X magnification ([Guo *et al.*, 2008](#)).

Adenosine triphosphatase (ATPase) activity of the root was assayed according to [Wayne \(1955\)](#) at 32°C using ATP (sodium salt) as a substrate, and the reactions were terminated by the addition of 2.0 mL cold 10% trichloroacetic acid. The ATPase enzyme activity was expressed as µg Pi g⁻¹ h⁻¹.

Harvesting of the crop (grain) was performed from the net plot level (2.4×7.0m) at 120 days after sowing. The yield of rice grain was calculated to hectares at 14% moisture level and expressed as kg ha⁻¹. The filled grain percentage (%) was calculated by the ratio of total filled grain with total spikelet numbers in panicles. The dry weight of grain and total grain dry weight per hill ratio was used to measure the harvest index (HI; %) at harvest stage of the crop ([Yoshida *et al.*, 1971](#)).

Statistical analysis

The recorded mean data were analyzed with AgRes software (version 7.01) ANOVA (Analysis of Variance) package for researchers 1994, Pascal Intl software solutions. Significance was assessed at 95% (p<0.05) and 99% (p<0.01) confidence level ([Gomez & Gomez, 1984](#)). F values were calculated using the method as described in <http://www.biokin.com/tools/fcrit.html>.

Results

Total root length (TRL) is the size of the total root system, which is the major determinant for water and nutrient uptake.

The drip irrigation system used in the present study in aerobic rice showed significant variation among root traits. Regarding the TRL for the different rice genotypes, a longer length was observed in with JKRH 3333 (6.2m/hill), followed by TNRH 180 (50.7m/hill) and ADT(R) 45 (38.6m/hill) (Table 1b). A significant increase in the total root length observed in 0.8 m lateral distance (LD) laid at 5–10 cm depth subsurface drip irrigation treatment (66.6m/hill).

Among the genotypes, a significantly higher root volume was observed in JKRH 3333 (66cm³/hill), followed by TNRH 180 (61.7cm³/hill) and ADT(R)45 (53.2cm³/hill). From the main plot treatment, the solar drip irrigation recorded an increased root volume of 43.4% compared with the well-operated drip irrigation treatment (Table 1b.). The 0.8 m lateral distance in SDI laid out at 5–10 cm soil depth improved the root volume of JKRH 3333 rice with greater significance (Table 1a).

Table 1a. Phenotyping of root length (m/hill), volume (cc/hill) and ATPase activity ($\mu\text{g Pi/g/h}$) of rice under different drip irrigation treatment. LD: lateral distance; S.Ed: standard error difference; CD: critical difference. *significance level at 0.05%; *significance at 0.01%; NS: not significant.

Source	Genotypes	Treatments	Total root length (m/hill)	Root volume (cc/hill)	ATPase activity ($\mu\text{g Pi/g/h}$)
Solar	TNRH 180	0.8 m LD + 5–10 cm depth	56.3	64.9	45.9
		0.8 m LD + 15–20 cm depth	54.7	65.9	43.8
		1.2 m LD + 5–10 cm depth	49.1	62.8	41.8
		1.2 m LD + 15–20 cm depth	45.2	58.6	40.7
		Conventional	51.5	61.6	42.5
	JKRH 3333	0.8 m LD + 5–10 cm depth	66.6	69.9	51.6
		0.8 m LD + 15–20 cm depth	63.9	70.8	51.0
		1.2 m LD + 5–10 cm depth	58.2	67.6	49.0
		1.2 m LD + 15–20 cm depth	52.7	63.0	47.3
		Conventional	60.1	65.6	50.2
	ADT(R)45	0.8 m LD + 5–10 cm depth	43.1	56.4	40.0
		0.8 m LD + 15–20 cm depth	41.7	58.1	35.7
		1.2 m LD + 5–10 cm depth	36.2	53.9	32.0
		1.2 m LD + 15–20 cm depth	35.6	49.8	30.8
		Conventional	39.2	53.0	33.9
Well powered	TNRH 180	0.8 m LD + 5–10 cm depth	54.5	62.7	46.0
		0.8 m LD + 15–20 cm depth	53.1	63.7	44.0
		1.2 m LD + 5–10 cm depth	46.4	61.2	42.3
		1.2 m LD + 15–20 cm depth	45.2	56.7	42.2
		Conventional	50.7	59.4	42.5
	JKRH 3333	0.8 m LD + 5–10 cm depth	65.0	67.3	52.1
		0.8 m LD + 15–20 cm depth	63.5	65.3	50.5
		1.2 m LD + 5–10 cm depth	58.2	64.8	47.5
		1.2 m LD + 15–20 cm depth	53.8	61.9	47.0
		Conventional	59.7	63.8	49.8
	ADT(R)45	0.8 m LD + 5–10 cm depth	42.4	55.2	38.7
		0.8 m LD + 15–20 cm depth	41.1	53.6	36.9
		1.2 m LD + 5–10 cm depth	34.6	52.5	31.6
		1.2 m LD + 15–20 cm depth	33.4	48.6	31.0
		Conventional	38.2	51.1	34.3
		Mean	49.8	60.3	42.4

Table 1b. Phenotyping of root length (m/hill), volume (cc/hill) and ATPase activity ($\mu\text{g Pi/g/h}$) of rice under different drip irrigation treatment.

	Treatments	Total root length (m/hill)	Root volume (cc/hill)	ATPase activity ($\mu\text{g Pi/g/h}$)
Source (S)	Solar	50.3	61.5	42.4
	Well operated	49.3	59.2	42.4
Variety (V)	TNRH 180	50.7	61.7	43.2
	JKRH 3333	60.2	66.0	49.6
	ADT(R)45	38.6	53.2	34.5
Treatment (T)	0.8 m LD + 5–10 cm depth	54.6	62.7	45.7
	0.8 m LD + 15–20 cm depth	53.0	62.9	43.6
	1.2 m LD + 5–10 cm depth	47.1	60.5	40.7
	1.2 m LD + 15–20 cm depth	44.3	56.4	39.8
	Conventional	49.9	59.1	42.2
	Mean	49.8	60.3	42.4
S	S.Ed.	0.191	0.134	0.068
	C.D. (<0.05)	NS	0.576**	NS
V	S.Ed.	0.106	0.078	0.105
	C.D. (<0.05)	0.245**	0.180**	0.243**
T	S.Ed.	0.024	0.019	0.02
	C.D. (<0.05)	0.048**	0.038**	0.041**
S at V	S.Ed.	0.227	0.161	0.140
	C.D. (<0.05)	0.845**	0.594**	0.444**
S at T	S.Ed.	0.194	0.136	0.073
	C.D. (<0.05)	0.822**	0.575**	0.313**
V at S	S.Ed.	0.150	0.110	0.149
	C.D. (<0.05)	0.346**	0.255**	0.344**
V at T	S.Ed.	0.112	0.084	0.110
	C.D. (<0.05)	0.255**	0.190**	0.254**
T at S	S.Ed.	0.034	0.027	0.029
	C.D. (<0.05)	0.068**	0.054**	0.058**
T at V	S.Ed.	0.042	0.033	0.035
	C.D. (<0.05)	0.083**	0.066**	0.070**
T at SV	S.Ed.	0.059	0.047	0.050
	C.D. (<0.05)	0.118**	0.094**	0.100**
V at ST	S.Ed.	0.159	0.118	0.156
	C.D. (<0.05)	0.361**	0.268**	0.359**
S at VT	S.Ed.	0.133	0.099	0.146
	C.D. (<0.05)	0.301**	0.224**	0.338**

The various genotypes of rice had varying RLD values: 1.513cm/cm³ (JKRH 3333); 1.267 cm/cm³ (TNRH 180); and 1.077cm/cm³ [ADT(R)45]. Conventional aerobic rice observed a decreased RMD value of 1.133cm/cm³. Among the genotypes, JKRH 3333 had a higher RMD value (1.214mg/cm³) with statistical significance

over TNRH 180 (1.109 mg/cm³) and ADT(R)45 (0.996 mg/cm³). The root density changed in the drip system, which was higher for the JKRH 3333 genotype than the other genotypes (Figure 1). Comparing the treatment interactions, solar operated drip system (0.8 m LD laid out at 5–10 cm SDI) treatment significantly improve

Figure 1. Response of root length (black bar) and mass density (gray bar) of rice.

the root density in JKRH 3333 (RLD: 2.36, RMD: 1.59) over the control (Table 2a.) (Figure 2.). The root dry weight (2.56 g/hill) and specific root length (0.160) was found higher in JKRH 3333 over the rest (Figure 3). The drip treatment 0.8 m LD + 5–10 cm depth SDI showed significant increment in root dry weight by comparing the genotypes and on par by comparing the irrigation sources (Table 2a.). Comparing the drip treatments, increased root dry weight observed in 0.8 m LD (2.5g/hill) laid out at 5–10 cm SDI over conventional rice (1.9g/hill). The specific root length was superior in solar operated drip irrigation system (i.e. 0.8 m LD laid out at 5–10 cm depth SDI) than the well-operated drip irrigation system.

The root ATPase activity of JKRH 3333 (33.1µg Pi/g/h) showed was more statistically significant supremacy than TNRH 180 (29.5µg Pi/g/h) and ADT(R)45 (23.8µg Pi/g/h) (Table 2b.). Among the drip irrigation treatments, increased root activity was obvious in 0.8m LD in SDI laid at the soil depth of 5–10cm treatment (31.2µg Pi/g/h) and lesser activity was evident in conventional aerobic rice (26.4µg Pi/g/h). Also, the interactive treatment 0.8 m LD in SDI laid at 5–10 cm soil depth with the solar operated irrigation system favorably enhanced the root ATPase activity (Table 2a.) than the other treatment interactions.

Genotypic variation of rice showed an increased filled grain percentage in JKRH 3333 (88.2%) followed by TNRH 180 (85.0%) and ADT(R) 45 (76.4%). The SDI system at 0.8m lateral distance was found to be higher (85.4%). Solar operated drip system (0.8 m LD in SDI laid out at 5–10 cm) showed significant

enhancement of filled grain percentage in JKRH 3333 rice genotype (Table 3a.). Higher HI values were observed in JKRH 3333 (39.2%) followed by TNRH 180 (38.5%) and ADT(R) 45 (37.8%). The solar operated drip irrigation treatments were significantly superior with an elite value of 39.3% compared with the well-operated drip irrigation system (37.7%). The treatment combination of 0.8 m LD in SDI laid out at 5–10 cm along with solar operated system improved the HI (41.5%) of JKRH 3333 rice genotype than the rest.

JKRH 3333 genotype was statistically superior among all the genotypes in grain yield. The grain yield was observed to be significantly higher in the solar operated drip irrigation treatment (4817kg/ha) compared with well-operated drip irrigation (4313kg/ha) (Table 3a.). Among the performance of genotypes under drip irrigated aerobic rice, JKRH 3333 was statistical superior in mean grain yield (4831kg/ha) (Table 3b) followed by TNRH 180 (4639kg/ha) and ADT(R)45 (4224kg/ha). The 0.8 m LD in SDI laid out at 5–10 cm depth with the solar operated irrigation system, raise the rice grain yield in JKRH 3333 genotype (6177 kg/ha) over the control.

Dataset 1. Response of root traits phenotyped under different drip irrigation treatments

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Source of irrigation: S₁, solar powered; S₂, well operated.

Drip treatments: T₁, 0.8m LD+5–10cm; T₂, 0.8m LD+5–10cm; T₃, 1.2m LD+5–10cm; T₄, 1.2m LD+15–20cm; T₅, conventional aerobic rice.

Genotypes: V₁, TNRH 180; V₂, JKRH 3333; V₃, ADT(R)45.

Table 2a. Phenotyping of root dry weight (g/hill), specific root length (m/g), root length density (cm/cm³) and root mass density (g/cm³) of rice under different drip irrigation treatment.

Source	Genotypes	Treatments	Root dry weight (g/hill)	Specific root length (m/g)	Root length density (cm/cm ³)	Root mass density (g/cm ³)
Solar	TNRH 180	0.8 m LD + 5–10 cm depth	2.48	0.206	1.73	1.46
		0.8 m LD + 15–20 cm depth	2.31	0.189	1.65	1.41
		1.2 m LD + 5–10 cm depth	2.38	0.204	1.72	1.52
		1.2 m LD + 15–20 cm depth	2.21	0.185	1.54	1.49
		Conventional	2.08	0.192	1.44	1.33
	JKRH 3333	0.8 m LD + 5–10 cm depth	2.81	0.225	2.36	1.59
		0.8 m LD + 15–20 cm depth	2.62	0.205	2.15	1.54
		1.2 m LD + 5–10 cm depth	2.68	0.208	2.09	1.62
		1.2 m LD + 15–20 cm depth	2.48	0.230	1.96	1.59
		Conventional	2.31	0.223	1.89	1.34
	ADT(R)45	0.8 m LD + 5–10 cm depth	2.28	0.172	1.57	1.39
		0.8 m LD + 15–20 cm depth	2.00	0.173	1.36	1.28
		1.2 m LD + 5–10 cm depth	2.09	0.171	1.50	1.42
		1.2 m LD + 15–20 cm depth	1.91	0.165	1.31	1.16
		Conventional	1.78	0.185	1.22	1.10
Well powered	TNRH 180	0.8 m LD + 5–10 cm depth	2.41	0.195	1.64	1.44
		0.8 m LD + 15–20 cm depth	2.26	0.206	1.60	1.40
		1.2 m LD + 5–10 cm depth	2.32	0.195	1.61	1.39
		1.2 m LD + 15–20 cm depth	2.14	0.190	1.50	1.38
		Conventional	2.01	0.190	1.43	1.20
	JKRH 3333	0.8 m LD + 5–10 cm depth	2.77	0.212	2.32	1.56
		0.8 m LD + 15–20 cm depth	2.52	0.222	2.02	1.52
		1.2 m LD + 5–10 cm depth	2.55	0.227	1.90	1.61
		1.2 m LD + 15–20 cm depth	2.34	0.240	1.74	1.54
		Conventional	2.21	0.241	1.69	1.32
	ADT(R)45	0.8 m LD + 5–10 cm depth	2.09	0.180	1.48	1.37
		0.8 m LD + 15–20 cm depth	1.96	0.183	1.36	1.26
		1.2 m LD + 5–10 cm depth	1.94	0.183	1.42	1.41
		1.2 m LD + 15–20 cm depth	1.82	0.174	1.28	1.17
		Conventional	1.72	0.165	1.14	1.03
		Mean	2.25	0.20	1.65	1.40

Discussion

Roots are the main component in the absorption of water and minerals, which are essential in plant physiological processes. Fageria (2007) observed that root length followed a significant quadratic response with the advancement of plant age from 19 to 110 days after sowing, and shows a linear increase in root length during flowering.

Favored root length under SDI at 5–10cm treatment is due to deep rooting of rice to combat water limited conditions. Genotypic variation in TNRH 180 revealed deep rooting to reduce the limited water application effect. The increased root growth and development of the root system help the rice to explore the wider area of soil and the deeper soil layers for water and nutrients. These results were corroborated with Henry *et al.* (2011) in rice under drought.

Figure 2. Imaging of root system of rice genotypes under microscope.

Figure 3. Responses of root dry weight (black bar) and specific root length (gray bar) of rice.

Table 2b. Phenotyping of root dry weight (g/hill), specific root length (m/g), root length density (cm/cm³) and root mass density (g/cm³) of rice under different drip irrigation treatment.

	Treatments	Root dry weight (g/hill)	Specific root length (m/g)	Root length density (cm/cm ³)	Root mass density (g/cm ³)
S	S.Ed.	0.0028	0.018	0.0256	0.0050
	C.D. (<0.05)	NS	0.077*	NS	0.0215**
V	S.Ed.	0.0003	0.004	0.0226	0.0017
	C.D. (<0.05)	0.0007*	0.009*	0.0521**	0.0039**
T	S.Ed.	0.0001	0.001	0.0189	0.0009
	C.D. (<0.05)	0.0001*	0.003*	0.0380**	0.0017**
S at V	S.Ed.	0.0028	0.019	0.0365	0.0054
	C.D. (<0.05)	0.0106*	0.069*	NS	0.0216**
S at T	S.Ed.	0.0036	0.023	0.0350	0.0051
	C.D. (<0.05)	0.0106*	0.067*	0.1133**	0.0214**
V at S	S.Ed.	0.0027	0.017	0.0319	0.0024
	C.D. (<0.05)	0.0052*	0.033*	NS	0.0056**
V at T	S.Ed.	0.0004	0.006	0.0370	0.0022
	C.D. (<0.05)	0.0008*	0.012*	0.0785**	0.0048**
T at S	S.Ed.	0.0032	0.020	0.0267	0.0012
	C.D. (<0.05)	0.0064*	0.040*	0.0538**	0.0025**
T at V	S.Ed.	0.0007	0.010	0.0328	0.0015
	C.D. (<0.05)	0.0014*	0.019*	0.0659**	0.0030**
T at SV	S.Ed.	0.0051	0.034	0.0463	0.0021
	C.D. (<0.05)	0.0104*	0.069*	NS	0.0043**
V at ST	S.Ed.	0.0045	0.029	0.0523	0.0031
	C.D. (<0.05)	0.0087*	0.056*	NS	0.0067**
S at VT	S.Ed.	0.0034	0.022	0.0490	0.0027
	C.D. (<0.05)	0.0067*	0.044*	NS	0.0059**

The genotype JKRH 3333 registered an increased root length and specific root length of 34.9% and 3.9% over conventional aerobic rice (Figure 2). Specific root length was an indicator for environmental changes. The genetic potential of this rice genotype for maintenance of increased root length favors lateral root branching (Figure 3). This effect was in accordance with Kato & Okami (2011) in rice.

Root volume of plants covers huge soil volumes and water uptake from the soil in water-limited conditions (Kanbar *et al.*, 2004). Altered root volumes were observed in the present study under SDI with a 5–10cm drip laid out at 0.8m LD, due to greater assimilation allocation in rice roots by drip irrigation. Similar results were observed by Parthasarathi *et al.* (2012) under drip irrigation.

The root length density (RLD) is the length of roots per unit volume of soil, is an important parameter required to understand plant performance. In the present experiment, the SDI at 5–10cm depth

using JKRH 3333 increased the RLD and RMD, due to the root zone of rice exposed to frequent wetting and slight drying and nutrient accessibility (Figure 1.). Also, the drip fertigation at 5–10 cm soil depth favourably altered the root density. A similar increase in density was verified by Sharda *et al.* (2017) in drip irrigated dry seeded rice with nitrogen fertigation. The dry weight of roots was 36.8% superior in JKRH 3333 hybrid under drip irrigation. Similar variation obtained in rice was observed by Vanitha (2011) and could support the present results. This unique response of root length and mass density under drip irrigation to improve nutrient and water accessibility was due to more root proliferation at topsoil. Comparing the root images of genotypes (Figure 3) revealed that, even though the appearance of white roots was common, an increase in root numbers and density was higher in drip irrigation.

Light energy absorbed by chlorophyll is converted into stable chemical energy and drives ATP formation via ATPase in the plastids of roots. ATPase is widely present in plant tissues and

Table 3a. Effect of grain filling(%), harvest index(%) and grain yield(Kg/ha) on rice under drip irrigation. LD: lateral distance; S.Ed: standard error difference; CD: critical difference. **significance level at 0.05%; *significance at 0.01%; NS: not significant.

Source	Genotypes	Treatments	Filled Grain Percentage (%)	Harvest Index (%)	Grain Yield (Kg/ha)
Solar	TNRH 180	0.8 m LD + 5–10 cm depth	87.9	40.0	5630
		0.8 m LD + 15–20 cm depth	86.4	38.9	5084
		1.2 m LD + 5–10 cm depth	88.0	39.6	5500
		1.2 m LD + 15–20 cm depth	85.5	37.5	4775
		Conventional	81.3	36.3	4121
	JKRH 3333	0.8 m LD + 5–10 cm depth	91.8	41.5	6177
		0.8 m LD + 15–20 cm depth	90.5	39.5	5473
		1.2 m LD + 5–10 cm depth	91.2	41.2	5990
		1.2 m LD + 15–20 cm depth	88.2	39.3	5271
		Conventional	84.2	38.7	4563
	ADT(R)45	0.8 m LD + 5–10 cm depth	81.1	37.7	4730
		0.8 m LD + 15–20 cm depth	78.1	35.9	4227
		1.2 m LD + 5–10 cm depth	79.4	36.1	4409
		1.2 m LD + 15–20 cm depth	76.5	35.7	4109
		Conventional	71.1	35.6	3973
Well powered	TNRH 180	0.8 m LD + 5–10 cm depth	88.0	38.2	5090
		0.8 m LD + 15–20 cm depth	84.7	37.3	4787
		1.2 m LD + 5–10 cm depth	87.0	37.6	4929
		1.2 m LD + 15–20 cm depth	81.6	36.2	4471
		Conventional	79.6	35.5	3918
	JKRH 3333	0.8 m LD + 5–10 cm depth	89.6	41.0	5917
		0.8 m LD + 15–20 cm depth	87.9	39.3	5178
		1.2 m LD + 5–10 cm depth	88.5	40.0	5560
		1.2 m LD + 15–20 cm depth	87.0	39.1	4950
		Conventional	83.7	38.2	4238
	ADT(R)45	0.8 m LD + 5–10 cm depth	80.5	37.0	4579
		0.8 m LD + 15–20 cm depth	75.9	35.8	4135
		1.2 m LD + 5–10 cm depth	78.5	35.8	4202
		1.2 m LD + 15–20 cm depth	74.6	35.2	4030
		Conventional	68.5	35.6	3919
		Mean	83.2	37.8	4798

involved in the active transport of ions across membranes of the cell (Martínez-Ballesta *et al.*, 2003). In the present study, higher levels of ATPases were observed in SDI + 0.8m LD at 5–10cm lateral depth with the JKRH 3333 hybrid.

The grain filling percentage is an important contributory factor to grain yield. The SDI laid out at 5–10cm depth with 0.8m LD treatment registered more grain production and filling percentage. Among the genotypes, the hybrid (JKRH 3333) excelled the variety in filled grain percentage by 15.4% (Figure 4). The increase in the

water supply to the spikelets might reduce the floret abortion during flowering, and may be the reason behind higher filled grains in SDI. These results are indirectly supported by Kato *et al.* (2008) in aerobic rice.

Harvest index (HI) reflects the proportion of assimilate distribution between economic and total biomass (Donald & Hamblin, 1976). Among the genotypes, a higher in HI was recorded in JKRH 3333 with a 1.6 and 4.5% increment over the TNRH 180 and ADT(R)45 genotypes, respectively (Figure 3). This might be attributed to

Table 3b. Effect of grain filling(%), harvest index(%) and grain yield(Kg/ha) on rice under drip irrigation.

	Treatments	Filled Grain Percentage (%)	Harvest Index (%)	Grain Yield (Kg/ha)
Source (S)	Solar	84.1	38.2	4935
	Well operated	81.3	37.4	4660
Variety (V)	TNRH 180	84.7	37.7	4831
	JKRH 3333	88.2	39.8	5332
	ADT(R)45	76.4	36.0	4231
Treatment (T)	0.8 m LD + 5–10 cm depth	86.3	39.2	5354
	0.8 m LD + 15–20 cm depth	83.9	37.8	4814
	1.2 m LD + 5–10 cm depth	85.6	38.4	5098
	1.2 m LD + 15–20 cm depth	82.2	37.2	4601
	Conventional	78.1	36.6	4122
	Mean	83.2	37.8	4798
S	S.Ed.	0.14	0.052	8.54
	C.D. (<0.05)	0.62**	0.225**	36.75**
V	S.Ed.	0.07	0.339	20.83
	C.D. (<0.05)	0.16**	0.780**	47.90**
T	S.Ed.	0.09	0.320	35.34
	C.D. (<0.05)	0.19**	0.643**	71.03**
S at V	S.Ed.	0.16	0.395	25.52
	C.D. (<0.05)	0.63**	NS	81.21**
S at T	S.Ed.	0.19	0.408	45.51
	C.D. (<0.05)	0.63**	NS	195.82**
V at S	S.Ed.	0.10	0.480	29.46
	C.D. (<0.05)	0.23**	NS	67.92**
V at T	S.Ed.	0.16	0.600	58.57
	C.D. (<0.05)	0.33**	1.384**	135.07**
T at S	S.Ed.	0.13	0.452	49.98
	C.D. (<0.05)	0.26**	NS	100.48**
T at V	S.Ed.	0.16	0.554	61.21
	C.D. (<0.05)	0.32**	1.113**	123.06**
T at SV	S.Ed.	0.23	0.783	86.56
	C.D. (<0.05)	0.45**	1.574**	NS
V at ST	S.Ed.	0.22	0.849	82.84
	C.D. (<0.05)	0.46**	1.957**	NS
S at VT	S.Ed.	0.22	0.804	81.52
	C.D. (<0.05)	0.45**	NS	NS

Figure 4. Effect of filled grain percentage (black bar) and harvest index (gray bar) of rice.

the fact that producing a larger sink size and efficient transport of assimilates from leaves and stems ('source') into developing spikelets ('sinks'), thus resulting in the increased grain yield (Guan *et al.*, 2010).

The higher grain yield of JKRH 3333 recorded a 21.4% increase in drip over conventional aerobic rice cultivation. Comparing the depth of SDI treatment at a 5–10 cm soil depth achieved a 18.9 and 13.0% increased yield over 15–20 cm soil depth and conventional aerobic irrigation method, respectively. The 0.8 m LD in SDI treatment laid at 5–10 cm depth with solar operated drip system attained 36.7%, 36.0% and 19.0% higher grain yield in JKRH 3333, TNRH 180 and ADT(R)45 respectively over the control. The SDI system maintained equal soil wetting, reduced the evaporation with direct point application of water in root, which improves the grain yield of rice. A previous study supports this argument (Dough *et al.*, 2013).

Conclusions

This drip-irrigated aerobic rice study concluded that there is an increase in grain yield along with increased root parameters. Based on the data of lateral spacing, lateral depth and the root characters of rice under drip significantly showed that there was characteristic flexibility in the roots of the rice plant. The root length, root density, root hairs and root ATPase activity exhibited a significant

association with filled grain percentage and grain yield. Comparing the two irrigation sources, solar-powered drip favors the rice root density. The genotype JKRH 3333 showed 14.3% increased grain yield with favorable root density and root dry weight over ADT(R)45. The drip irrigation treatment 0.8 m lateral distance in subsurface drip irrigation (SDI) laid out at 5–10 cm soil depth with solar irrigation system improved the rice grain yield by 19–37% over the conventional aerobic rice. It could be recommended that 0.8 m lateral distance laid out at 5–10 cm depth SDI may proliferated more roots at subsurface soil layer with a yield increment in rice.

Data availability

Dataset 1: Response of root traits phenotyped under different drip irrigation treatments. doi, [10.5256/f1000research.9938.d151043](https://doi.org/10.5256/f1000research.9938.d151043) (Parthasarathi *et al.*, 2017).

Source of irrigation: S₁, solar powered; S₂, well operated.

Drip treatments: T₁, 0.8 m LD+5–10 cm; T₂, 0.8 m LD+5–10 cm; T₃, 1.2 m LD+5–10 cm; T₄, 1.2 m LD+15–20 cm; T₅, conventional aerobic rice.

Genotypes: V₁, TNRH 180; V₂, JKRH 3333; V₃, ADT(R)45.

Author contributions

TP, SM, EV, and KV designed the experiments. TP performed the experiments in the field. TP and VM analyzed the data using statistics. TP, VM, and KV contributed reagents/materials/analysis tools. TP wrote the manuscript. KV and VM corrected the manuscript.

Competing interests

No competing interests disclosed.

Grant information

The project was funded by Netafim Irrigation Ltd., Israel.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary materials

Supplementary File 1: Detailed information on experimental set up (Table S1), genotypes (Table S2) and fertigation schedule (Table S3) of drip irrigated rice study.

[Click here to access the data.](#)

Supplementary File 2: Field and drip lay out of field experiment & drip irrigation components.

[Click here to access the data.](#)

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Raju Bheemanahalli Rangappa

Department of Agronomy, Kansas State University, Manhattan, KS, USA

This study hypothesised to assess the impact of different drip irrigation treatments at different later depth on root growth particularly at flowering stage in rice. They have shown that there is positive response of 0.8m lateral distance with 5-10cm depth drip irrigation treatment on root growth in comparison to conventional and other irrigation treatments. This is an important piece of observation from this study. However, the hypothesis of the study has not been detailed, the materials and methods have been inadequately detailed, and there are some typographical or grammatical errors. Also the approach taken for root phenotyping may not be completely justifiable.

Major issues

Introduction: the motivation to conduct this study has not been justified sufficiently. What was the reason for measuring ATPase and how is it relevant to root physiology or drip irrigation? There are many more such links missing the introduction, which the authors need to address.

Materials and methods: are these experiments conducted under field conditions? If the answer is yes, then the root extraction procedure under field conditions is questioned. Also regarding the details provided on how the root system were extracted and how many days after sowing, were all the genotypes tested flowered at the same time? Which type (primary or nodal) of portion of the roots were used for imaging and estimation of the ATPase? A number of such gaps in the MnM will need to be seriously addressed.

Results: What was the maximum root length across the genotypes tested; authors should provide maximum root length along with total root length; i find it hard to understand the total root length data - is it an average or how was it measured? It is not clear whether the authors had used winRhizo to capture root system variability? Did the authors take into account the different days on which the genotypes flowered; was the scheduled irrigation different for each genotype? I did not see soil moisture content in the study at different drip irrigation depths.

Discussion: I could see the repetition of results in the discussion section, and the logical connection seems to be missing. There is a lot more scope to improve the manuscript qualitatively.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Abiotic stress physiology (drought and heat stresses) and association mapping - root physiology, reproductive physiology, traits and markers or QTL discovery in response to abiotic stresses.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 06 October 2017

<https://doi.org/10.5256/f1000research.12965.r20815>

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M. Alagu Palamuthir Solai

ICAR-Indian Institute of Spices Research, Kodagu, India

This is a useful basic study that focuses on evaluating rice genotypes and drip irrigation method for better productivity by using root traits. The authors have followed suitable experimental design, materials and method and delivered result and discussion with appropriate references.

The authors tried to use latest references. Some carbon isotope discrimination studies need to be added in future.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 15 March 2017

<https://doi.org/10.5256/f1000research.10709.r20988>

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Kazuki Saito

Africa Rice Center (AfricaRice), Cotonou, Benin

The experimental set up was not properly written. In the abstract, the authors indicated a split-split plot design. In M&M, there were four factors including (i) lateral spacing levels (0.8 and 1.2m), (2) depth of irrigation (5-10 and 15-20 cm), (3) solar powered and well operated irrigation, and (4) variety (TNRH 180, JKRH 3333 and ADT(R)45). However, there was no info on experimental design, plot size, and no. replications used for this study.

Furthermore, it is not clear how these factors were analyzed. The authors showed the main factor only. There were no information on interaction effects among source of drip irrigation, variety, and drip treatments.

Without examining such interactions, the authors could not draw the conclusion that JKRH3333 is best suitable material under drip irrigated conditions, and 0.8m lateral distance laid out at 5–10cm depth sub-surface drip may proliferated more roots at subsurface soil layer with a yield increment in rice. There might have been interaction among variety x drip irrigation on traits measured in this study.

The authors could report the effect of each factor on traits in the results section rather than describing the results in each trait for different factors. Then, the interaction effect should be highlighted. If there is significant interaction, detailed results should be shown (e.g. if there is variety x drip treatment on yield, yield of each variety in different drip treatment should be shown).

Some of the results were not described in the results section but the discussion section (e.g. figures 2 and 3). These should be reported in the results section.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 20 Jun 2017

PARTHASARATHI THEIVASIGAMANI, Ben-Gurion University of the Negev, Israel

Dear Kazuki Saito

I accept your comments on including the interaction effect of treatments. My co-authors also accepted your view. In the version one, interactive effects of treatments are missing.

As per your comments, I have included the main, sub and sub-sub plot, interaction treatment results in the paper. I have mentioned the replications, experimental design, plot dimension in the paper. Also, please view the supplementary file for additional details such as experiment layout, drip layout.

Thank you for your peer review to made the manuscript better.

Please review the revised version (Version 2) and give your comments.

Thanking you.

Kind regards
Parthasarathi

Competing Interests: No competing interests were disclosed.

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