RESEARCH ARTICLE

Variation in rice root traits assessed by phenotyping under drip irrigation [version 1; peer review: 1 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), two depths of 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 Adinosine Tri Phosphotase 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 proliferated more roots at subsurface soil layer with significant yield increment in rice.

Keywords

Drip, rice, phenotyping, root, yield

This article is included in the Agriculture, Food and Nutrition gateway.

Open Peer Review

Reviewer Status ✗ ✓ ✗

	Invited Reviewers		
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version 2 (revision) 28 Jun 2017		✓ report	✗ report
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Any reports and responses or comments on the article can be found at the end of the article.

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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 *et al.*, 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, using JKRH 3333, TNRH 180 and ADT(R)45 as the test rice varieties 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/cm^{-2} from a bore well. Solar powered and well-operated drip irrigation sources, 0.8 and 1.2m lateral distances, 5–10 and 15–20cm depth sub-surface drip (SDI) were the treatments adopted 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. Further information on genotypes, experimental set up and fertigation schedule are given in Supplementary File 1.

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/cm^3 and mg/cm^3 of the soil, for RLD and RMD respectively. Root volume was recorded using the water displacement technique (Bridgit & Potty, 2002) and expressed as cm^3/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 1).

Among the genotypes, a significantly higher root volume was observed in JKRH 3333 (66cm^3/hill), followed by TNRH 180 (61.7cm^3/hill) and ADT(R)45 (53.2cm^3/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.

The various genotypes of rice had varying RLD values: 1.513cm/cm^3 (JKRH 3333); 1.267 cm/cm^3 (TNRH 180); and 1.077cm/cm^3 [ADT(R)45]. Conventional aerobic rice observed a decreased RMD value of 1.133cm/cm^3. Among the genotypes, JKRH 3333 had a higher RMD value (1.214mg/cm^3) with statistical significance over TNRH 180 (1.109 mg/cm^3) and ADT(R)45 (0.996 mg/cm^3). The root density changed in the drip system, which was higher for the JKRH 3333 genotype than the other genotypes (Figure 1). The root dry weight (2.56 g/hill) and specific root length (0.160) was found higher in JKRH 3333 over the rest (Figure 2). 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).

Table 1. Phenotyping of root length (g hill^{-1}) and volume (cc hill^{-1}) of rice. LD: lateral distance; S.Ed: standard error difference; CD: critical difference. *significance level at 0.05%; *significance at 0.01%; NS: not significant.

Root trait	Source of drip irrigation		Rice varieties			Drip treatments				
	Solar	Well	TNRH 180	JK RH 3333	ADT (R) 45	0.8m LD	1.2m LD	5–10 cm	15–20 cm	Conventional
length (m/hill)	50.3	49.3	50.7	60.2	38.6	54.6	47.1	53	44.3	49.9
volume (cc/hill)	61.5	59.2	61.7	66.0	53.2	62.7	62.9	60.5	56.4	59.1
ATPase activity (µg Pi /g/h)	42.4	42.1	43.2	49.6	34.5	45.7	42.2	43.6	39.8	40.7

Root trait	Source of drip irrigation		Varieties		Drip treatments	
	S.Ed.	CD <0.05	S.Ed.	CD <0.05	S.Ed.	CD <0.05
Length	0.191	NS	0.106	0.245**	0.024	0.048**
Volume	0.134	0.576**	0.078	0.180**	0.019	0.038**
ATPase activity	0.068	NS	0.105	0.243**	0.020	0.041**

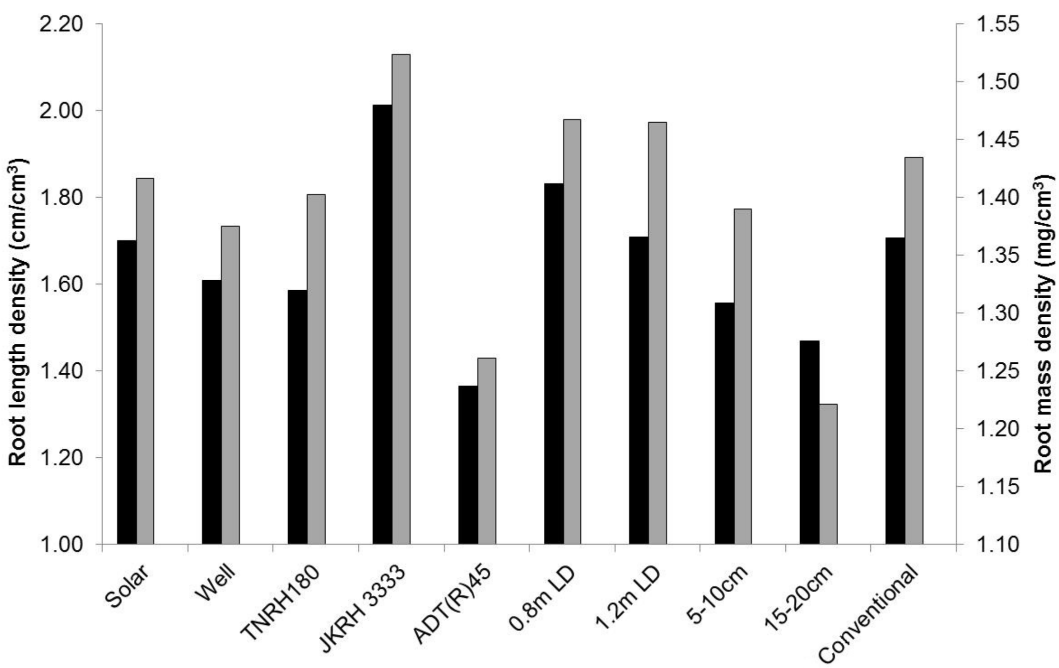

Figure 1. Response of root length (black bar) and mass density (gray bar) of rice.

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). 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).

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%). 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%).

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 2). Among the performance of genotypes under drip irrigated aerobic rice, JKRH 3333 was statistical superior in mean grain yield (4831kg/ha) followed by TNRH 180 (4639kg/ha) and ADT(R)45 (4224kg/ha).

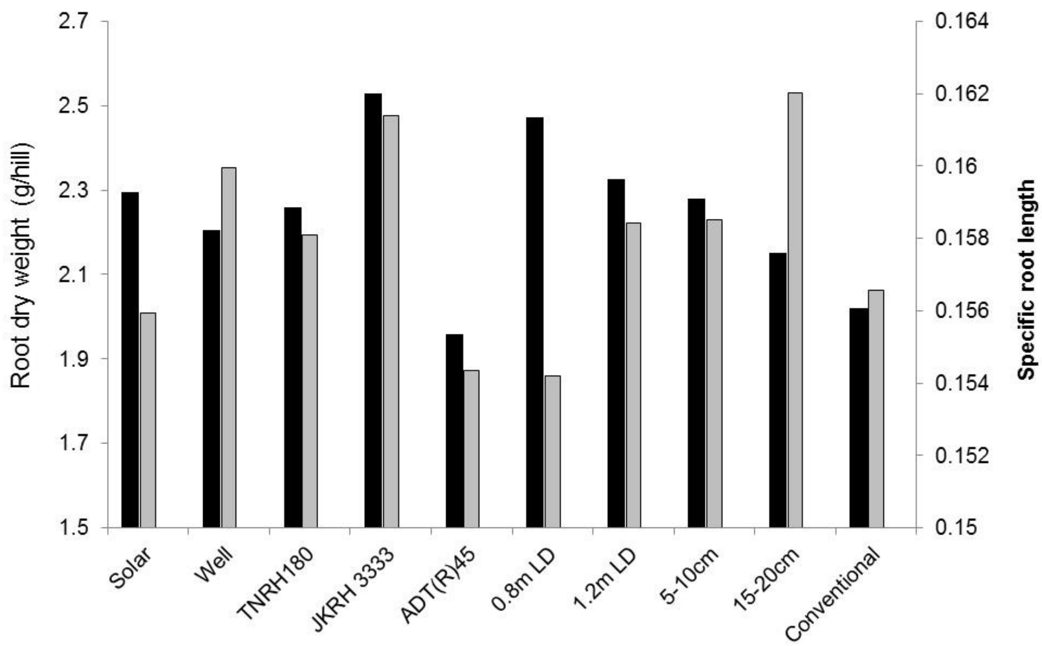

Figure 2. Responses of root dry weight (black bar) and specific root length (gray bar) of rice.

Table 2. Effect of grain yield 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.

Grain yield (kg/ha)	Source of drip irrigation		Varieties			Drip treatments				
	Solar	Well	TNRH 180	JK RH 3333	ADT (R) 45	0.8m LD	1.2m LD	5–10 cm	15–20 cm	Conventional
	4817	4313	4639	4831	4224	5121	4874	4302	4106	4421

Grain yield	Source of drip irrigation		Varieties		Drip treatments	
	S.Ed.	CD <0.05	S.Ed.	CD <0.05	S.Ed.	CD <0.05
	8.54	36.75**	20.83	47.90**	35.34	71.03**

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

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Source of irrigation: S_1, solar powered; S_2, well operated. Drip treatments: T_1, 0.8m LD; T_2, 1.2m LD; T_3, 5–10cm; T_4, 15–20cm; T_5, conventional aerobic rice. Genotypes: V_1, TNRH 180; V_2, JKRH 3333; V_3, ADT(R)45.

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 120 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.

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, 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. 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 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.

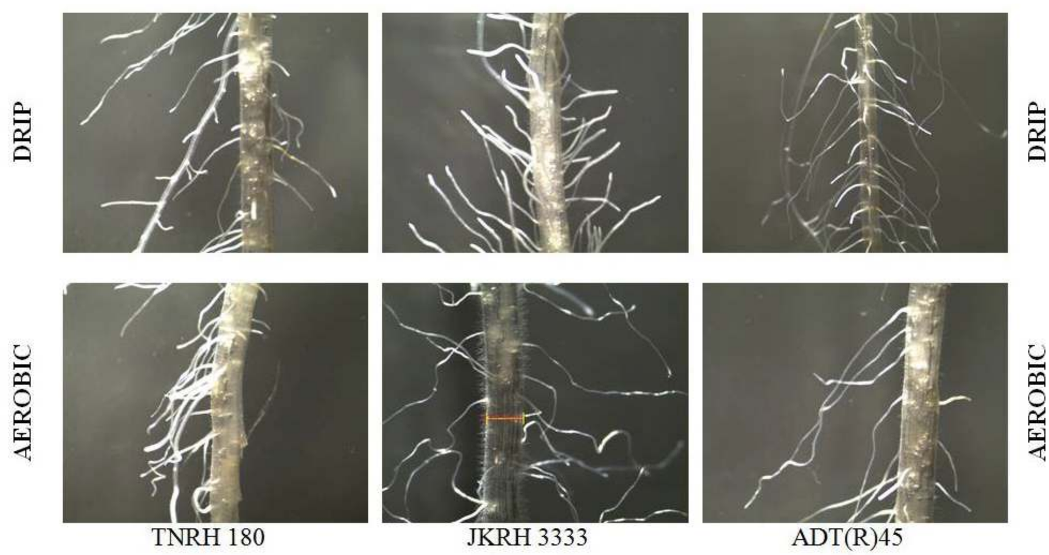

Figure 3. Imaging of root system of rice genotypes under microscope.

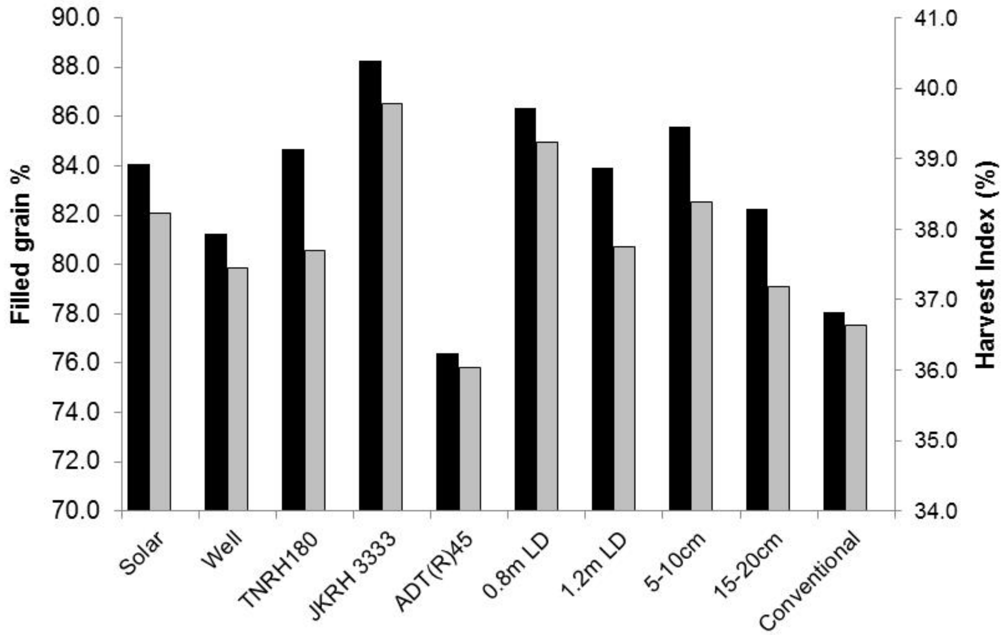

Figure 4. Effect of filled grain percentage (black bar) and harvest index (gray bar) of rice.

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 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 5–10cm soil depth achieved a 18.9 and 13.0% increased yield over 15–20cm soil depth and conventional aerobic irrigation method, respectively. 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 (Douh *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, discharge variations 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. The genotype JKRH 3333 showed 14.3% increased grain yield with favorable root density and root dry weight over ADT(R)45. It could be recommended that 0.8m lateral distance laid out at 5–10cm 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 (Parthasarathi *et al.*, 2017).

Source of irrigation: S_1, solar powered; S_2, well operated.

Drip treatments: T_1, 0.8m LD; T_2, 1.2m LD; T_3, 5–10cm; T_4, 15–20cm; T_5, conventional aerobic rice.

Genotypes: V_1, TNRH 180; V_2, JKRH 3333; V_3, 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.

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Publisher Full Text

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Version 1

Reviewer Report 15 March 2017

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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.

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.