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Evaluation of SRL and different Root Contributing Traits of Rice (*Oryza sativa* L.) under Water Stress

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ABSTRACT: A pot experiment was conducted to evaluate the root growth and distribution of four earlier screened rice genotypes i.e. Parijata, IC 516008, Lalat and Prasad. Water stress (WS) was imposed 15 days before flowering by withdrawing irrigation and allowed to grow with controlled irrigation. Our results showed that specific root length (SRL) was positively associated with the total root length (TRL), root surface area (RSA), root volume, specific root area (SRA), and root length density (RLD) however; SRL was negatively associated with root average diameter (RAD). Under WS, Parijata and IC 516008 possessed greater survival ability by enhancing SRL and were considered tolerant genotypes compared to Lalat (moderately tolerant) and Prasad (susceptible genotypes). With higher SRL, the tolerant genotypes are associated with improving water potential and photosynthetic rate. SRL can be considered a crucial root trait for the genetic improvement of genotypes to improve yield under WS.

Keywords: Genetic improvement, root diameter, root volume, SRL, RAD.

INTRODUCTION

The relation between plant and atmosphere is an ongoing process in which water plays a key role. With the commencement of climate change extreme abiotic stress mainly WS is a major issue for inhibiting the global rice production today (Danakumara *et al.,* 2021). Rice has to face challenges of WS associated with lower rainfall in order to respond to the increase in demand (Darmadi *et al.,* 2021; Yang *et al*., 2019). To address these new challenges various strategies need to be developed. The design and distribution of root system can be seen as key factors for efficient water uptake from deeper part of soil and thus managing the performance of rice under stress (Muthu *et al*., 2020).

Roots play a key role in anchoring the plant and absorb water and nutrients from the soil to control productivity (Zhao *et al*., 2021). However, upland cultivars have a deeper root system compared to lowland cultivars that allow for tolerance under WS (Dash *et al.,* 2017; Guo *et al.,* 2020). Some root traits such as TRL, RSA, SRA, SRL, and RLD are found to be key parameters associated with increasing crop yield under WS (Kim *et al.,* 2020; Fitter 2002). SRL is a complex parameter and important indicator for tissue thickness means higher SRL significantly to thinner roots which look at more soil volume for fast water uptake during stress situation (Agustin *et al*., 2021; Kadam *et al.,* 2015). Thinner roots also enable for soil penetration ability than thicker roots and access the deeper soil water by resulting maximizes total root length (Wasaya *et al.,* 2018). However, root volume, RAD and RSA are associated significantly with greater ability towards hard soil to access deep water (Karlova *et al*., 2021)**.** Guimaraes *et al.* (2020) reported that fine root shared 80% of total root system in the plant with diameter less than 1 mm, which is responsible for transportation. According to Kitomi *et al*., (2020) a number of root traits is liable for improving the resistance limit in tolerant genotypes under WS condition. The results showed complete information of root related traits at flowering stage under both WW and WS condition in rice. The main objective of this experiment is to characterize and evaluate the appropriate root parameters and to assess the variation of SRL and, it's related components which could be provide a new approach as targeted survival traits in rice plants under WS conditions.

MATERIALS AND METHODS

A. Crop establishment and experimental site

The experiment was performed during the dry season - 2019 at ICAR-National Rice Research Institute, Cuttack (Odisha), India, was at 20˙45'N latitude and 85˙93'E longitude. Four rice (*Oryza sativa* L.) genotypes i.e. Parijata, IC 516008, Lalat and Prasad selected earlier from vegetative stage WS screening experiment were taken for the establishment of experiment. Pot experiment was performed containing 4 Kg mixture of dried dust farm soil with farm yard manure (3:1 ratio) in a completely randomized design taking four replications. Thinning was done to maintain single plant per pot. Recommended doses of fertilizer (N, P &K) was maintained, pesticides was applied whenever necessary and standard agronomic practices were followed for the growing of healthy plants. WS was imposed before 10 days of flowering by withdrawing irrigation and maintaining at 50% field capacity. Another set was under WW condition for maintaining 100% field capacity. Sampling was done at flowering stage.

B.Instrumentation system

Root traits. Roots without disturbing were taken from the both treatments in replication wise manner and washed by tap water repeatedly over a sieve. After washing the live roots were placed in culture dishes containing an iced water bath and were immediately scanned using root scanner system (Regent instrument Inc, LA 2400 scanner, EPSON). The scanned images were captured and analyzed with WinRHIZO software supplied with the instrument. TRL, RSA, root volume and RAD were analyzed from scanned images.

The analyzed roots were dried under oven at 60°C for 72 hours (Sandhu *et al*., 2017) and mean root dry weight was determined. Derived parameters were obtained by using Excel sheet from the measured parameters. The following are the important derived parameters based on computed data.

Specific root length (SRL) - Root length/ root dry mass

Specific root area (SRA) - Root surface area/ root dry mass

Root length density (RLD) - Total length of root/ soil volume

However, the same plant was used for water potential by taking second leaf from top of the plants. Meanwhile, analyzed shoots were dried under oven at 60°C for 72 hours (Sandhu *et al.,* 2017) and mean shoot dry weights were determine.

Leaf water potential (LWP). Instantaneously second fresh leaves of plant were taken for measurement of LWP by using the water potential system (PSYPRO, Wescor). The sampling was done at midday (12 pm-2 pm) and data was taken in quadruplicate using the protocol of Barrs and Weatherly, (1962).

Photosynthetic rate. The photosynthetic rate (P_n) was recorded with the help of LI-6400 (LI-COR, Lincoln, Nebraska, USA) under bright sunny days between 8.00 AM to 12 PM. The second leaf was placed in the leaf chamber at a PFD of 1000 µmol $m^{-2} s^{-1}$, leaf temperature was 28°C, ambient CO_2 concentration was 410 µmol CO_2 mol^{-1} air and vapour pressure deficit was 2.0 kPa.

C. Statistical analysis

The mean data were collected and ANOVA for root traits from each treatment were performed using Crop Stat 7.2 software (IRRI, 2009) least significant difference (LSD, $p<0.05$). Simple scatter graph (x, y pair) regression analysis between different traits was realized using Excel sheet.

RESULTS AND DISCUSSION

Induction of WS at flowering stage on rice plants caused significant ($p<0.05$) variation in root traits among the genotypes and the results of TRL, RSA, root volume, SRL, SRA, RLD, and RAD was clearly distinguished between tolerant and susceptible genotypes.

Root architectural traits. Different root parameters were observed under WW and WS condition at the flowering stage (Fig. 1). Under WS, root dry weight/shoot dry weight (RDW/SDW) was significantly ($p<0.05$) increased in Parijata, IC 51600 and Lalat while, significant reduction was studied in Prasad compared to WW condition (Fig. 1a). With consistence performance the genotype Parijata (0.252) recorded higher value of RDW/SDW followed by IC 516008 (0.239) and Lalat (0.221) while a sharp reduction in RDW/SDW was recorded in Prasad (0.121). The results suggesting that plant transport more assimilates from source organ to sink organ. In this study we found that, the genotypes Parijata and IC 516008 was able to maintain higher RDW/SDW compared to Prasad and considered as tolerant genotypes under WS condition, and our findings corroborate with the findings of (Xu *et al.,* 2015).

A significant variation ($p<0.05$) in TRL was observed in all studied genotypes under WS over WW condition (Fig. 1b). The genotype IC 516008 (36843.25cm) able to increase in TRL under WS followed by Parijata (36456.70cm) and Lalat (26125.33cm) whereas, sharp reduction of TRL was studied in the genotypes Prasad (15227.15cm). Under WS, root system grows to deeper part of soil for searching of water source, hence more assimilates are diverted towards root system and tolerant genotypes possessed higher TRL for sustain under WS conditions (Nada and Abogadallah, 2018).

The value of RSA was significantly ($p<0.05$) increased in the genotype Parijata ($6289.67 cm^2$) and observed maximum RSA followed by IC516008 ($5617.35 cm^2$) and, Lalat ($4633.55 cm^2$) whereas, the value was significantly decreased in Prasad ($1827.91 cm^2$) (Fig. 1c). However, root volume was reduced significantly ($p<0.05$) among all the genotypes under WS condition compared to WW (Fig. 1d). The ANOVA analysis of root volume was clearly discriminated between the genotypes under WS condition ($p<0.05$). The genotype IC 516008 ($95.50 cm^3$) observed higher value of root volume followed by Parijata ($79.95 cm^3$) and Lalat ($71.70 cm^3$) whereas, the sharp reduction of root volume was noticed in Prasad ($42.24 cm^3$) compared to WW. Higher value of RSA and root volume assists the plant to acquire deeper water by enhancing efficiently root hydraulic conductance. More RSA get in touch with soil water and ability to build up deeper rooting system by increasing penetrability capacity (Guimaraes *et al.*, 2020).

WS significantly reduced ($p<0.05$) RAD in all the studied genotypes compared to WW condition (Fig. 1e). With a wide variation of RAD, the genotypes were discriminated significantly under WS condition. The genotype Parijata (0.41mm) exhibited thinner RAD followed by IC 516008 (0.44mm) while, thicker RAD was studied by the susceptible genotypes Prasad (0.50mm) and intermediate value was observed in Lalat (0.46mm). SRL encapsulates the whole effect in terms of TRL per unit dry biomass allocated in the root system. The values of SRL was significantly increased ($p<0.05$) in IC 516008 ($5493.60 cm\ g^{-1}$) and Parijata ($4875.46 cm\ g^{-1}$) whereas, no significant variation ($p>0.01$) of SRL was noticed in the genotype Prasad ($2978.87 cm\ g^{-1}$) (Fig. 1f).

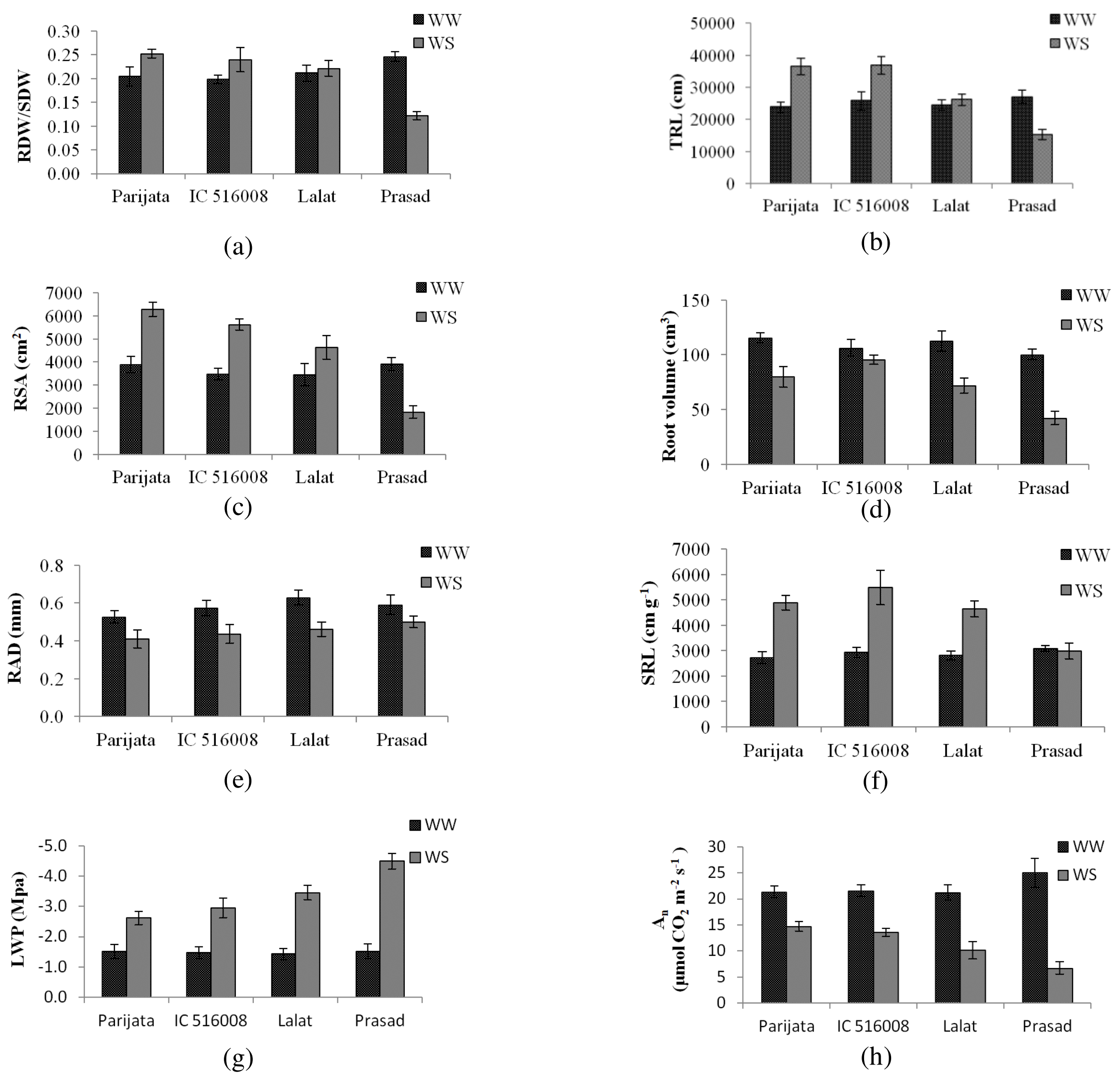

Fig. 1. Variation in (A) root dry wt/shoot dry wt (RDW/SDW), (B) total root length (TRL), (C) root surface area (RSA), (D) root volume, (E) root average diameter (RAD), (F) specific root length (SRL), (G) Leaf water potential (LWP) and (H) photosynthetic rate (P_n) of four rice genotypes under well watered (WW) and water stress (WS) condition. Values are expressed as means of four biological replications ± SE at the 5% level.

However, intermediate value of SRL was observed in Lalat (4648.67 cm g^{-1}) under WS conditions. Higher SRL with thinner RAD confers tolerance to WS (Ostonen *et al.*, 2007; Wang *et al.*, 2018). Compared with the genotype Prasad, higher values of SRL, TRL, RSA and root volume specifies that the genotypes Parijata and IC 516008 construct a healthy root system and maximize their water search ability under WS conditions. Accordingly, decrease in root diameter results mounting hydraulic conductance by declining apoplastic barrier of water, increase the RSA and penetrability ability (Wasaya *et al.*, 2018).

Under WS different rice genotypes behaved different sensitivity limit through their root performance. Our findings showed that, LWP was significantly ($p<0.05$) reduced under WS over WW condition (Fig. 1g). Under WS the genotype Parijata (-2.61 MPa) able to maintain higher value of LWP followed by IC 516008 (-2.95MPa), where as Prasad possessed lower value of LWP (-4.50MPa). The genotype Lalat (-3.46 MPa) showed intermediate value of LWP. High LWP indicates higher water status that showed the tolerance mechanism of the genotypes with dehydration avoidance and turgor pressure maintenance under WS. Our findings showed that the genotypes Parijata and IC 516008 perform better and, Lalat showed intermediate behavior of LWP under WS and our results were corroborating with the findings of Yang *et al.,* (2019). The decrease of LWP was responsible for the reduction of P_n under WS conditions. The P_n of the studied genotypes showed a significant declined ($p<0.05$) under WS condition over WW (Fig. 1h). The genotype Parijata (14.76 μmol CO_2 m^{-2} s^{-1}) exhibited higher value of P_n followed by IC 516008 (13.59 μmol CO_2 m^{-2} s^{-1}). A sharp reduction of P_n was recorded in Prasad (6.70 μmol CO_2 m^{-2} s^{-1}). The genotype Lalat (10.16 μmol CO_2 m^{-2} s^{-1}) showed intermediate value. Maintenance higher value P_n is signifies better adapted in stress period. The results might be due to tolerant genotypes showed more stability in their thylakoid membrane, than susceptible genotypes under WS condition. This results in agreement with the previous findings on various stresses (Zhao *et al.,* 2021)

SRL and root trait variation. In our experiment the outcome of regression analysis (Fig. 2) revealed that the value of SRL was positively significant correlated with TRL (R^2= 0.888, $p< 0.05$) (Fig. 2a), RSA (R^2= 0.858, $p< 0.05$) (Fig. 2b), root volume (R^2= 0.985, $p< 0.05$) (Fig. 2c), SRA (R^2= 0.894, $p< 0.05$) (Fig. 2d) and RLD (R^2= 0.888, $p< 0.05$) (Fig. 2e). In contrast, SRL was negatively significant associated with RAD (R^2= 0.981, $p< 0.05$) (Fig. 2f). SRL, TRL, RSA, root volume, SRA, RLD, and RAD were assessed to understand the distribution pattern between the tolerant and susceptible genotypes under WS condition. The genotypes possesses higher SRL (thinner roots) at flowering stage had a higher TRL, RSA, SRA, RLD, root volume, however SRL was negatively associated with RAD (Liu *et al.,* 2018). Furthermore, high SRL along with thinner root diameter under WS condition confer high WS tolerance (Karlova *et al.,* 2021: Wang *et al.*, 2018).

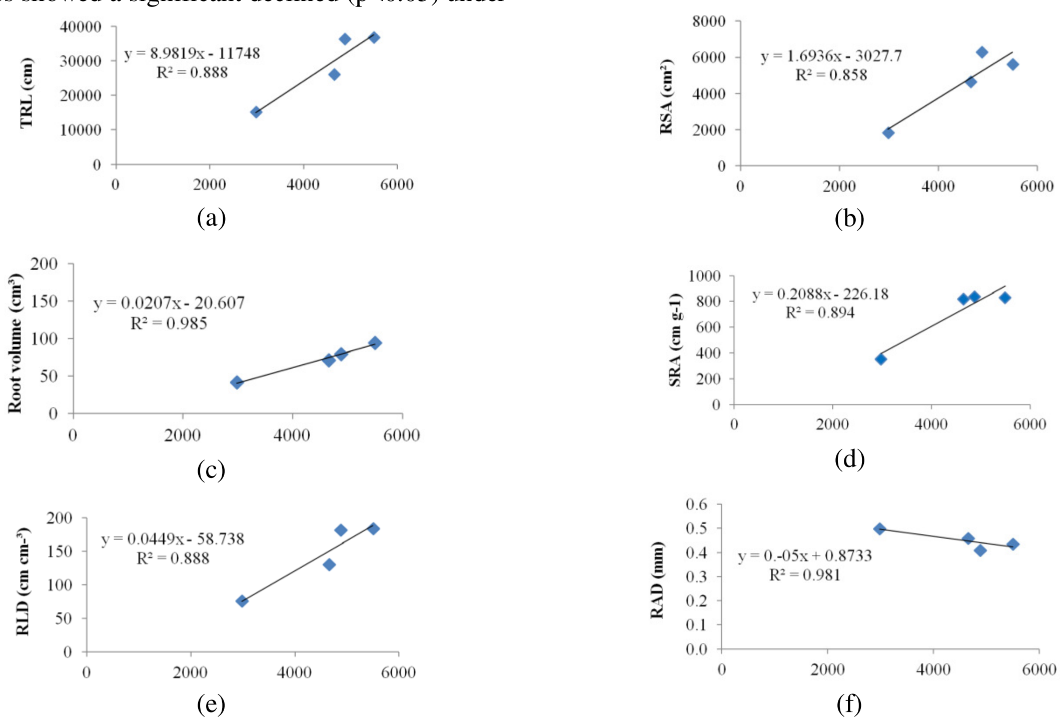

Fig. 2. The relationship of specific root length (SRL) with (A) total root length (TRL), (B) root surface area, (C) root volume, specific root area (SRA), root length density (RLD), and root average diameter (RAD) under WS condition at flowering stage of four rice genotypes. Values shown are means of four biological replications ± SE at the 5% level.

Tolerant genotype Parijata and IC 516008 could devote less biomass to construct a healthy root system with less root diameter; ensuing increase soil interface area in given soil volume (Lynch *et al*., 2014) compared to the susceptible genotype Prasad. Selection of proper and reasonable morphological root traits for identification of WS tolerant genotypes is complex and variable. Based on proper information of root traits, SRL and RAD under WS condition considered as an important root trait while selecting tolerant genotypes.

CONCLUSION

Root traits such as RDW/SDW, TRL, RSA, root volume, SRL, RAD, SRA, and RLD showed strong differences between tolerant and susceptible genotypes. In the contrast to our findings, with the higher value of TRL, RSA, root volume, SRL, SRA, RLD, and lower value of RAD, the genotypes Parijata and IC 516008 would be superior genotype as compared with Prasad. SRL provides a new approach in rice plants under WS conditions for promoting the root traits and establishing the individual genotypes. Again the trait can improve our understandings towards different root function and maintain the accuracy by minimizing the errors with which our related root parameters can be predicted. Hence, SRL is the source of adaption to evaluate the genotypes under WS conditions and needs further investigation.

FUTURE SCOPE

High throughput phenotyping techniques of the identified genotypes are required to link plant root traits to phenomes and its application to unravel the novel traits contributing to stress tolerance.

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Conflict of Interest. None.

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