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Research Communication

Root endophyte *Piriformospora indica* significantly affects mechanisms involved in mitigating drought stress in rice (*Oryza sativa*)

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ABSTRACT

An experiment was conducted during the summer season of 2020 at College of Agriculture, Kerala Agricultural University, Vellayani to study the potential of a beneficial root endophyte *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer G. Kost & P. Franken in mitigating drought stress in rice (*Oryza sativa* L.). The field experiment was undertaken in the lowland paddy fields with sandy clay loam texture in randomized block design. Treatments were *Piriformospora indica* colonized and non-colonized rice seedlings under 3 irrigation intervals of 30 mm, 35 mm and 40 mm cumulative pan evaporation and 2 irrigation depths of 1.5 cm and 3 cm. Plants colonized with *P. indica* significantly enhanced the yield attributes and root parameters. This symbiotic association alleviated the negative effects of drought stress by maintaining higher proline content (71.8 µmole/g) and relative leaf water content (74%) irrespective of interval and depth of irrigation. Colonization boosted the uptake of phosphorus and maintained higher chlorophyll stability index (79.20%). Yield attributes of *P. indica* colonized plants subjected to severe stress, were observed to be equivalent to those of non-colonized plants under moderate stress and association with the endophyte resulted in saving of 30 mm water and could help rice to combat drought stress. Higher phosphorus uptake enhanced the root volume (29.1cm^3) under severe water stress facilitated improved nutrient uptake (10.6 kg/ha) from deeper layers of soil for colonized plants compared to the control plants and acted as a mitigating mechanism against drought stress.

Key words: Climate-resilient agriculture, Drought, Moisture stress, Root endophyte, Summer rice

Climate change has multifaceted interactions with crop production and research should focus on mitigating the climate-change effects like drought, heavy rainfall and rise in temperature, so that the yield prospect will not be reduced with changing climate. In Kerala, rice (*Oryza sativa* L.) is the major foodgrain produced and intensive efforts are being made by the state government for augmenting the domestic production. However, rice cultivation has been declining over the years and the land is either kept fallow or getting converted to other upland crops especially during summer. Growing rice during the summer should be a practical option for enhancing production levels. Drought or water stress is the prominent reason for drop in paddy acreage as well as yield during the summer season (Mahapatra *et al*., 2021). Use of microorganisms that can enhance stress tolerance by plants provide an alternate and ecologically sound way of protecting plants against stress conditions. Endophytic beneficial root fungi are organisms that live in the intercellular or intracellular space of plant tissue resulting in a symbiotic association with the host plant. *Piriformospora indica* Sav. Verma, Aj. Varma, Rexer G. Kost, & P. Franken is a beneficial root endophytic fungus identified from the root zone area of xerophytic plants in the Thar Desert in Rajasthan, India (Verma *et al*., 1998). It was first reported as a novel endophyte promoting root development. This beneficial root endophytic fungus could colonize the roots of large number of plant species like rice, maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), etc., and was found responsible for the plant growth promotion in unfavourable conditions like water stress (Tsai *et al.,* 2020). It is known to mitigate the biotic and abiotic stress on the plant growth and development in a sustainable way without causing any harm to the environment and

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natural resources. The present study was undertaken to assess the potential of *P. indica* to mitigate drought stress in rice.

The field experiment was undertaken at College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram in the lowland paddy fields in randomized block design with 2 × 3 × 2 treatments during summer season (February to May) of 2020, replicated thrice, using rice variety 'Prathyasa'. The soil of the experimental site belonged to the Oxisols with sandy clay loam texture. The mean maximum temperature ranged between 30.5 and 34.3°C and mean minimum temperature 20.4 and 26.4°C, mean maximum relative humidity was 87.30–96%, and mean minimum relative humidity 65–88.1%. The total annual rainfall received during cropping season was 860.7 mm. The treatments included colonizing with *P. indica* [P_1, *P. indica* colonized rice and P_2, non-colonized rice/control]; 3 irrigation intervals [I_1, 30 mm cumulative pan evaporation (CPE); I_2, 35 mm CPE and I_3, 40 mm CPE]; 2 irrigation depths (D_1, to a depth of 1.5 cm and D_2, to a depth of 3 cm).

Root colonization was done as per the procedure of Johnson *et al*., (2011) with culture of *P. indica* collected from the Department of Plant Pathology, College of Agriculture, Vellayani. The broth was made with potato-dextrose agar solution in conical flask by addition of *P. indica* culture bits (2–3) and allowed to grow for 3 weeks. Rooting medium, comprising coir-pith, cowdung powder and basin flour in polypropylene covers, was sterilized in autoclave at 121°C for 21 minutes. It was then transferred to clean, dried and sterilized plastic trays. Fungal broth (40 mL) was added to these trays filled with medium under laminar air flow and kept in incubator for 5 days for white fungal mat formation. Seeds were sterilized by soaking in 0.1% HgCl solution for 1 min. and later soaked in distilled water for 12 hours. Sterilized and pre-soaked paddy seeds were spread on the surface of the rooting media with sufficient white mycelial formation. The roots were examined for colonization under microscope 5 days after sowing (DAS). Root colonization was observed by taking 10 random root bits from the co-cultivated rice seedlings. The collected roots were dipped in 10% KOH overnight, followed by 5 consecutive washing with water. Later the washed root bits were treated with 1% HCl for 4 minutes. The treated roots were mounted on a sterile glass slide using the stain lactophenol cotton blue (Johnson *et al*., 2011). Detailed examination of the colonized roots was done under the microscope (Fig. 1). Simultaneously, control/non-colonized seeds sterilized and soaked in distilled water for 12 h were sown in rooting media trays without fungus. *Piriformospora indica* colonized/ non-colonized rice seedlings raised in trays were transplanted 14 days after sowing at 15 cm × 10 cm and uniformly irrigated till 10 days after transplanting (DAT). The crop was raised as per the KAU Package of Practices recommended for short-duration rice (KAU, 2016) and observed for the yield attributes, root and physiological characters.

Roots were cleaned and immersed in a measuring cylinder containing known volume of water and quantity of water displaced was expressed in cm^3/plant. Relative leaf water content (RLWC) was estimated using the following formula. It was expressed in percentage.

$$\text{RLWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Chlorophyll stability index (CSI) was calculated by taking fresh leaf samples of each treatment in 2 glass tubes. One was taken as control without any heat treatment and another one as stress condition subjected to heat at 56°C for 30 min. Chlorophyll content was taken at both conditions in spectrophotometer at 660 nm wavelength.

$$\text{CSI} = \frac{\text{Total chlorophyll under stress}}{\text{Total chlorophyll under control}} \times 100$$

The number of panicles and number of filled grains were counted from 1 m^2 from net plot area at harvesting. From the net plot area, rice plants in 1 m^2 area were harvested, threshed and winnowed separately and expressed as yield. After harvesting plant samples were collected, oven-dried at 70 ± 5°C, powdered and digested for analysing the P content. Nutrient uptake was calculated as:

Nutrient uptake = Nutrient content (%) × total dry-matter production (kg/ha)

Root volume

Drought is the situation of prolonged lack of water that affects plant growth and survival. Generally, roots are the foremost plant organ devoted to the uptake of water and tolerance of crop to water stress relies on the extent of root volume it has developed. Root volume is an important trait deciding the extent of water and nutrient absorption. Significantly superior root volume at 30, 45, 60 DAT and harvesting respectively were recorded in colonized plants irrigated at 30 mm CPE to a depth of 3 cm (Table 1). Higher root volume in colonized plants provided greater absorption area for water uptake and maintained plant water status which in turn might have helped to keep higher relative leaf water content even under stress situation alleviating the drought effect on plant. Hyphae of the mycorrhizal fungi are able to deeply explore the rhizosphere and transport water and minerals to plant roots and keep roots moist even when there is less water availability (Varma *et al.,* 2013). The study indicated the more beneficial effect of *P. indica* under severe stress than mild-stress condition.

Physiological parameters

Among the physiological parameters studied, proline content declined in the order of decreasing CPE, 40 mm > 35 mm > 30 mm. The *P. indica*-colonized plants irrigated at 40 mm CPE to a depth of 1.5 cm produced significantly greater proline (115.33 and 106.00 µmole g per g at panicle initiation and flowering respectively) (Table 1). Proline is highly soluble and zwitterionic in nature, so its higher accumulation does not damage plants. Instead, it stabilizes cellular structures, acts as water substitute through hydrophilic interactions and hydrogen bonding. Co-cultivation of *P. indica* enhanced the reactive oxygen species (ROS) scavenging/signaling which reduced ROS thereby reducing the oxidative damage to cells directed to drought tolerance (Tsai *et al.,* 2020). It also induces drought tolerance by scavenging ROS and by being utilized as energy source after the release of stress. Colonized rice plants irrigated at 30 mm CPE to a depth of 3 cm showed the maximum relative leaf water content and chlorophyll-stability index at flowering respectively.

Phosphorus uptake

The P uptake in *P. indica*-colonized plants was higher even under severe drought stress. It could be deducted that, colonization enhanced the uptake of P from soil to plant which in turn resulted in the development of a profuse root-system in terms of higher volume. The *P. indica*-enhanced high P uptake is responsible for maintaining optimum leaf relative water contents. Colonized plants irrigated at 40 mm CPE to a depth of 1.5 cm resulted in significantly superior available P (179.93 kg/ha). Under severe stress condition, high available P (179.93 kg/ha) was recorded in soils with colonized plants (Fig. 1). It might be possible owing to production of acid and alkaline phosphatases by *P. indica* which dissolves the unavailable phosphorus to available form (Tripathy *et al*., 2021). Higher P uptake enabled root growth under severe water stress in colonized plants facilitated improved water and nutrient uptake from deeper layers of soil compared to the control plants and acted as an adaptive and tolerance mechanism to alleviate drought stress and better plant performance under stress condition.

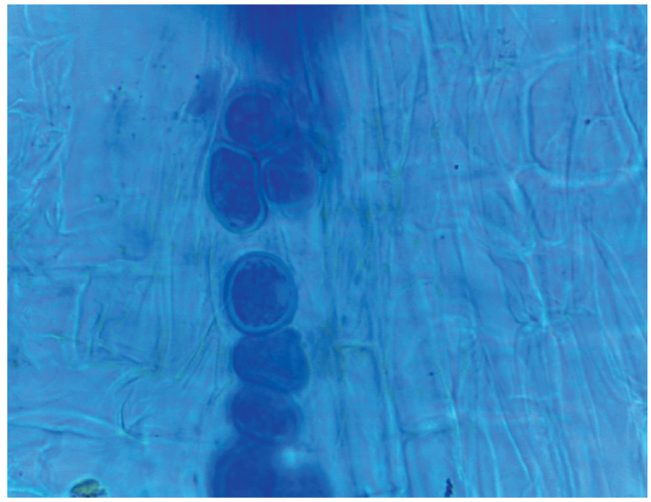

Fig. 1. *Piriformospora indica* colonization in rice root with chlamydospores

Yield and yield attributes

In the current study, *P. indica* colonization resulted in higher yield attributes namely productive tillers and filled grains/panicle. Colonized plants irrigated at 30 mm CPE to a depth of 3 cm showed superior yield attributes to the control plants irrigated at the same frequency. Plants under severe moisture stress (irrigation at 40 mm CPE to a depth

Table 1. Effect of *Piriformospora indica* colonization on yield attributes, root and physiology of rice

Treatment	Productive tillers/m^2	Filled grains/ panicle	Root volume at 60 DAT (cm^3)	Relative leaf water content at flowering (%)	Proline at flowering (µmole/g)	Chlorophyll stability index at flowering (%)	Phosphorus uptake (kg/ha)
Colonization (P)							
P_1 (*P. indica* colonized rice)	222.8	81.1	29.1	74.0	71.8	70.01	10.6
P_2 (non-colonized rice)	149.2	69.7	20.5	70.3	60.4	62.98	8.8
SEm±	2.97	0.60	0.16	0.15	0.40	0.18	0.08
CD (P=0.05)	8.72	1.77	0.47	0.44	1.19	0.535	0.23
Irrigation interval (I)							
I_1 (30 mm CPE)	210.1	84.6	31.8	76.5	56.9	74.15	11.0
I_2 (35 mm CPE)	191.3	73.8	23.6	71.7	65.6	65.45	9.5
I_3 (40 mm CPE)	156.7	67.8	19.1	68.4	75.9	59.88	8.7
SEm±	3.64	0.74	0.19	0.18	0.49	0.22	0.10
CD (P=0.05)	10.68	2.17	0.57	0.54	1.45	0.655	0.29
Depth of irrigation (D)							
D_1 (1.5 cm)	148.1	60.9	12.1	66.4	83.4	54.60	8.2
D_2 (3 cm)	223.9	89.9	37.6	78.0	48.9	78.39	11.2
SEm±	2.97	0.60	0.16	0.15	0.40	0.18	0.08
CD (P=0.05)	8.72	1.77	0.47	0.44	1.19	0.535	0.23

CPE, Cumulative pan evaporation; DAT, days after transplating of 1.5 cm) on colonization exhibited 73.88 and 18.80% improvement in number of productive tillers and filled grains/panicle over non-inoculated plants experiencing the same stress. Panicle initiation (PI) is a critical stage of water requirement, deciding the number of productive tillers per unit area. In our study, maintaining a higher relative leaf water content at PI stage by colonized plants might have resulted in higher productive tillers. Colonized plants, with improved root volume could have enhanced water and nutrient uptake avoiding pollen abortion. The *P. indica* colonization improved the water and nutrient uptake even from stressed soil by higher absorbing surface area and rooting depth and length (Ahmadvand and Hajinia, 2018). Water stress at vegetative stage can cause an inhibition at reproductive stage as reproductive organs are covered by vegetative tissues.

Yield is an integration of productive tillers and filled grains/panicle. The *P. indica*-colonized plants irrigated at 30 mm CPE to a depth of 3 cm recorded superior grain yield to non-colonized/ control plants irrigated at the same frequency. Under severe stress condition (40 mm CPE, 1.5 cm depth), colonization resulted in enhancement in filled grains/panicle by 23.24% over the uncolonized plants. Water-stress condition induces lower water status in plant cells and chlorophyll stability which were not tolerant to drought leading to damage to the cell-membrane, chlorophyll apparatus and low photosynthates to plant for yield. Taghinasab *et al.,* (2018) observed higher yield with enhanced P uptake in *P. indica*-colonized wheat plants. The *P. indica* colonization improved the physiological factors, viz. proline and relative leaf water content, enhancing tolerance to drought situation without breakdown of cell-membrane and helped to maintain the process of photosynthesis for accumulation and translocation of assimilates to grain. All these favourable conditions provided by *P. indica* helped to produce more number of productive tillers and filled grains/panicle and contributed to higher yield.

The study highlighted the positive interaction of endophyte with plant under drought stress and concluded that, *P. indica*-induced drought tolerance in rice seedlings by significantly improving plant physiological parameters, root growth and P uptake. The symbiotic association with endophyte can help to maintain grain production under water-stress conditions which is uncertain in the era of climate change.

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