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Effect of *Piriformospora indica* on vegetative growth of summer rice

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Abstract

The study entitled 'Mitigating water stress in summer rice using beneficial root endophytic fungus *Piriformospora indica*' was undertaken at College of Agriculture, Vellayani during 2019 - 2021. The objective of the study was to assess the performance of *P. indica* colonized rice under different levels of moisture stress during summer. The treatments included colonizing with *P. indica* [p_1- *P. indica* colonized rice and p_2- non-colonized rice (control)], three irrigation intervals [i_1- 30 mm CPE (cumulative pan evaporation), i_2- 35 mm CPE and i_3- 40 mm CPE] and two irrigation depths (d_1- to a depth of 1.5 cm and d_2- to a depth of 3 cm). Colonization with *P. indica* significantly influenced the number of tillers m^{-2} with 10.64, 16 and 15.69 per cent increase in tiller production at 30, 45 and 60 DAT respectively, compared to non-colonized plants. Colonized plants irrigated at 30 mm CPE to a depth of 3 cm evinced the maximum leaf area index (4.54) at 60 DAT and dry matter production (4559.26 kg ha^{-1}) at harvest.

Keywords: *P. indica*, water stress, summer rice, cumulative pan evaporation, tillers

Introduction

Rice is one of the most important staple foods for more than half of the world population, the majority of which are located in Asia. It has been estimated that for every one billion people added to the world's population, 100 million more tonnes of rice (paddy) need to be produced annually (RICE, 2020) [18]. In India, rice occupies 23.3 per cent of gross cropped area and 121.46 million tonnes was estimated at record in rice grain production playing an important role in national food supply (GOI, 2020) [16]. Rice is grown mostly during *Kharif and Rabi* ensuring adequate water supply. However, for meeting the additional domestic requirement of food grains and feeding livestock, rice cultivation needs to be extended to summer season.

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* 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) [15]. It was first reported as a novel endophyte promoting root development. Later, it was reported that plants colonized with *P. indica* were found to be promoted in biomass accumulation, nutrient uptake and abiotic stress tolerance especially drought stress (Sahay and Varma, 1999) [14]. This beneficial root endophytic fungus could colonize the roots of large number of plant species like rice, maize, wheat, etc., and was found responsible for the plant growth promotion in unfavourable conditions like water stress, salt stress, nutritional stress, etc. 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 natural resources.

In this scenario, the present study was conducted to assess the performance of *P. indica* colonized rice under different levels of moisture stress during summer.

Material and Methods

The experiment was laid out in the reclaimed low land areas in College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The site is located at $8^{o}\ 43^{'}$ N latitude, $76^{o}\ 98^{'}$ E longitude and at an altitude of 20.0 meters above mean sea level. *Prathyasa* (MO 21), released from Rice Research Station (RRS) Moncompu of Kerala Agricultural University was used for the study. It is a non-lodging, photo insensitive and semi-tall variety with a duration of 105-110 days. The treatments were colonizing with *P. indica* [p_1- *P. indica* colonized rice colonized plants and p_2- non-colonized rice (control)] and irrigation treatments include three irrigation intervals i1- 30 mm CPE (Cumulative Pan Evaporation), i2- 35 mm CPE, i3- 40 mm CPE and two irrigation depths d1- at 1.5 cm and d2- at 3 cm depth.

Culture of beneficial root endophyte *Piriformospora indica* was collected from the Department of Plant Pathology, College of Agriculture, Vellayani. In order to maintain the viability of the fungus, monthly subculturing of *P. indica* in sterile Potato dextrose agar (PDA) medium in petri plates was done. The broth was made with PDA solution in conical flask by addition of *P. indica* culture bits (2-3) and allowed to grow for three weeks. Rooting medium comprising 500 g coir pith compost, 500 g cowdung powder and 20 g basin flour in polypropylene covers was sterilized in autoclave at 121 °C for 21 minutes (min.). 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 five days for white fungal mat formation. Seeds were sterilized by soaking in 0.1 per cent HgCl solution for one 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 from five days after sowing (DAS) (Johnson *et al*., 2011) [13]. Simultaneously control / non-colonized seeds sterilized and soaked in distilled water for 12 hours were sown in rooting media trays without fungus. Root colonization was observed by taking ten random root bits from the co cultivated rice seedlings (Fig 1). The collected roots were dipped in 10 per cent KOH overnight, followed by five consecutive washing with water. Later the washed root bits were treated with 1 per cent HCl for four minutes. The treated roots were mounted on a sterile glass slide using the stain lactophenol cotton blue (Johnson *et al*., 2011) [13]. The seeds kept for germination in *P. indica* culturing trays for colonisation of the fungus were treated as colonized seedlings and seeds kept in the same media without the fungus were treated as non-colonized seedlings. After 14 days, both the set of seedlings were transplanted to the field as colonized and non-colonized plants under a spacing of 15 cm x 10 cm.

Results

P. indica colonized irrigated at 35 mm CPE to a depth of 3 cm resulted in significantly superior plant height of 103.7 and 105.76 at 60 DAT and harvest followed by $p_2i_3d_2$ (96.3 and 98.92) at 60 DAT and harvest. At 45 DAT, $p_1i_2d_2$ observed superior plant height (80.1) on par with $p_1i_1d_2$ (77.56) (Table 1).

P. indica colonized rice plants irrigated at 30 mm CPE to a depth of 3 cm registered significantly superior number of tillers m^{-2} (191.66, 267, 310 and 316) at 30, 45, 60 DAT and harvest respectively followed by $p_2i_1d_2$ (171.66, 251.66, 291.33 and 296.33) (Table 2). The lowest number of tillers m^{-2} was recorded with $p_2i_3d_1$ (70.66, 118.33, 130 and 138) at all stages of observation except at 15 DAT.

P. indica colonized plants irrigated at 30 mm CPE to a depth of 3 cm ($p_1i_1d_2$) led to the maximum LAI of 1.80, 3.54 and 4.54 at 30, 45 and 60 DAT, which were significantly superior to the rest. At the same irrigation interval and depth, non-colonized plants ($p_2i_1d_2$) produced LAI of 4.1 at 60 DAT, which was 10.73 per cent lower than colonized plants. The lowest LAI of 0.90, 2.17 and 2.87 were recorded with $p_2i_3d_1$ (non-colonized plants irrigated at 40 mm CPE to a depth of 1.5 cm) at 30, 45 and 60 DAT respectively (Table 3). At the same interval and depth, colonized plants ($p_1i_3d_1$) recorded LAI of 3.13 at 60 DAT, which was 9.05 per cent higher than non-colonized plants under severe stress situation.

Significant variation was exhibited in DMP with $p_1i_1d_2$ resulting in significantly superior value (4559.26 kg ha^{-1}), followed by $p_2i_1d_2$ (3960.21 kg ha^{-1}) which was on par with $p_1i_2d_2$ (Table 4). There was 15.12 per cent enhancement in DMP in *P. indica* colonized plants irrigated at 30 mm CPE to a depth of 3 cm over non-colonized plants at the same irrigation frequency. Other treatments, $p_1i_2d_2$ (3852.17), $p_2i_2d_2$ (3693.07), $p_1i_3d_2$ (3691.31), $p_1i_1d_1$ (3621.86), $p_2i_3d_2$ (3524.46), $p_1i_2d_1$ (3173.09), $p_2i_1d_1$ (3014.95), $p_1i_3d_1$ (2887.30) and $p_2i_2d_1$ (2570.56) were superior to $p_2i_3d_1$ (2206.08).

Table 1: Effects of P×I×D interaction on plant height, cm

Treatment combinations	15 DAT	30 DAT	45 DAT	60 DAT	At harvest
$p_1i_1d_1$	33.77	51.50	65.76	86.83	90.06
$p_1i_2d_1$	35.60	50.23	53.70	76.53	81.03
$p_1 i_3d_1$	34.70	50.66	52.06	74.10	77.60
$p_1 i_1d_2$	34.64	62.30	77.56	93.50	96.10
$p_1 i_2d_2$	35.00	59.76	80.10	103.70	105.76
$p_1 i_3d_2$	34.13	58.93	72.93	91.83	94.90
$p_2 i_1d_1$	32.70	51.10	54.96	80.50	83.90
$p_2 i_2d_1$	33.60	48.63	51.10	73.36	77.03
$p_2 i_3d_1$	32.03	46.40	48.70	68.73	71.63
$p_2 i_1d_2$	32.50	58.06	76.00	86.76	90.53
$p_2 i_2d_2$	32.60	56.56	71.76	89.43	91.96
$p_2 i_3d_2$	32.80	54.06	76.73	96.30	98.92
SEm (±)	0.05	0.73	1.52	1.86	1.80
CD (0.05)	0.164	NS	4.468	5.481	5.293

Table 2: Effects of P×I×D interaction on tiller number m^{-2}

Treatment combinations	15 DAT	30 DAT	45 DAT	60 DAT	At harvest
$p_1i_1d_1$	75.00	116.67	186.67	240.00	247.33
$p_1i_2d_1$	73.33	108.33	173.33	233.33	240.00
$p_1i_3d_1$	66.67	94.00	156.67	190.00	194.66
$p_1i_1d_2$	73.33	191.67	267.00	310.00	316.00
$p_1i_2d_2$	71.67	168.33	240.00	286.66	291.66
$p_1i_3d_2$	70.00	163.33	226.67	266.66	272.66
$p_2i_1d_1$	75.00	101.67	168.33	203.33	208.33
$p_2i_2d_1$	66.67	85.00	150.00	181.33	187.33
$p_2i_3d_1$	65.00	70.67	118.33	130.00	138.00
$p_2i_1d_2$	71.67	171.67	251.67	291.33	296.33
$p_2i_2d_2$	71.67	145.00	225.00	260.00	264.66
$p_2i_3d_2$	68.33	133.33	216.67	250.00	255.66
SEm (±)	4.19	4.71	4.10	5.07	5.01
CD (0.05)	NS	13.804	12.023	14.892	14.701

Table 3: Effects of P×I×D interaction on leaf area index

Treatment combinations	15 DAT	30 DAT	45 DAT	60 DAT	At harvest
$p_1i_1d_1$	0.92	1.32	2.78	3.44	2.24
$p_1i_2d_1$	0.91	1.22	2.59	3.27	2.04
$p_1i_3d_1$	0.87	1.09	2.39	3.13	2.17
$p_1i_1d_2$	0.95	1.80	3.54	4.54	2.07
$p_1i_2d_2$	0.91	1.58	3.16	4.35	2.36
$p_1i_3d_2$	0.90	1.55	3.13	4.24	2.24
$p_2i_1d_1$	0.84	1.02	2.37	3.23	1.70
$p_2i_2d_1$	0.81	0.97	2.29	3.09	1.84
$p_2i_3d_1$	0.80	0.90	2.17	2.87	1.95
$p_2i_1d_2$	0.90	1.45	3.24	4.10	2.12
$p_2i_2d_2$	0.88	1.24	3.00	3.63	2.34
$p_2i_3d_2$	0.85	1.19	2.93	3.57	2.23
SEm (±)	0.03	0.02	0.03	0.03	0.10
CD (0.05)	NS	0.067	0.096	0.102	0.298

Table 4: Effect of P x I x D interaction on dry matter production

Treatment combinations	Dry matter production (kg ha^{-1})
$p_1i_1d_1$	3621.86
$p_1i_2d_1$	3173.09
$p_1i_3d_1$	2887.30
$p_1i_1d_2$	4559.26
$p_1i_2d_2$	3852.17
$p_1i_3d_2$	3691.31
$p_2i_1d_1$	3014.95
$p_2i_2d_1$	2570.56
$p_2i_3d_1$	2206.08
$p_2i_1d_2$	3960.21
$p_2i_2d_2$	3693.07
$p_2i_3d_2$	3524.56
SEm (±)	40.38
CD (0.05)	118.476

Images

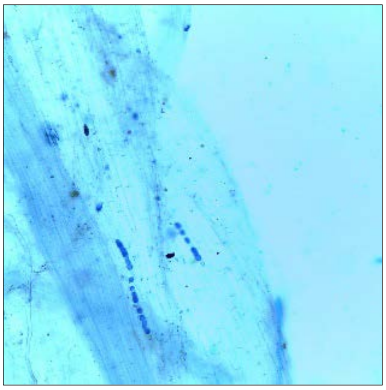

Fig 1: Root colonization of *P. indica* in rice

Discussion

Growth and growth attributes of rice plants were found to be significantly influenced by *P. indica* colonization under field conditions. Plant height, tiller number m^{-2}, leaf area index and dry matter production enhanced with *P. indica* colonization even under water stress situation.

Of the different levels of moisture stress, *P. indica* colonized plants irrigated at 35 mm CPE to a depth of 3 cm resulted in taller plants (105.76 cm). However, *P. indica* colonized plants both under ideal non stressed condition (irrigation interval at 30 mm CPE to a depth 3 cm) and severe stress condition (irrigation interval at 40 mm CPE to a depth 1.5 cm) resulted in taller plants than non-colonized/ control plants at the same degree of stress (Fig. 4). This indicated that the detrimental effect of moisture stress on plant height was counteracted by *P. indica* colonization. Rice plant requires higher amount of water (1200 mm) for its potential growth and development. Being an endophyte, presence of *P. indica* could have benefitted the plant through enhanced availability and maintenance of water within the system and in turn nutrients for the efficient growth of plants. Oelmuller *et al.* (2009) [12] stated that *P. indica* colonization improved water uptake that resulted in enhanced volume and turgor of plant cell, leading to cell elongation and plant height. Production of phytohormones like auxins by *P. indica* had also been reported which could lead to acidification and softening of cell wall responsible for cell elongation. These findings were in agreement with the reports of Hussain *et al.* (2018) [11] in rice. It was observed that colonization of *P. indica* promoted the production of ethylene, which is responsible for plant growth promotion (Barazani *et al.*, 2005) [10]. The lowest plant height (71.63 cm) was registered in control plants under severe water stress (irrigated at 40 mm CPE to a depth 1.5 cm). This could be the result of lack of water at the critical stages. Water stress alone resulted in reduced plant height due to blocking of translocation of assimilates, water and nutrients through xylem and phloem vessels (Nagarajan and Nagarajan, 2010) [9].

Production of tillers was significantly affected with the colonization and water stress condition. *P. indica* colonized plants registered superior tiller production with an enhancement by 11.65, 6.09 and 6.4 per cent over non colonized control plants under non-stress conditions (irrigated at 30 mm CPE to a depth of 3 cm, IW/CPE is 1.0) at 30, 45 and 60 DAT. At the same level of soil moisture, *P. indica* colonized plants could produce a greater number of tillers per m^2 which could be attributed to the endophyte mediated enhanced absorption of nutrients and moisture. Availability of sufficient water and air in the crop root zone implies adequate acquisition of water and nutrients (Duvvada *et al.*, 2020) [8]. In addition to the ideal conditions for tiller production, *P. indica* colonization enhanced nutrient absorption in a better way than control plants and resulted in superior tiller production (Fig. 5). Higher root volume recorded in *P. indica* colonized plants indicated the superiority of tiller production in colonized plants for more absorption of water and nutrients from soil. Colonized plants increased tiller production by 33.03, 32.39 and 46.15 per cent over control plants at severe stressed conditions (irrigated at 40 mm CPE to a depth of 1.5 cm, IW/CPE is 0.37) at 30, 45 and 60 DAT. Lack of sufficient water to crop creates moisture deficit and cell flaccidity which eventually reduces the cell mitosis. Water stress specifically at tillering stage might have resulted in lower accumulation of assimilates, photosynthates, water and nutrient uptake and hence effected the tiller production. The findings were on agreement with the result of Hossain *et al.* (2020) [6].

P. indica colonization significantly influenced the leaf area index (LAI) at stress and non-stress situation. Colonized plants recorded superior LAI with plants irrigated at 30 mm CPE to a depth of 3cm recording the highest leaf area index upto 60 DAT. Under non-stress situation, maintenance of higher water status under colonization might have improved LAI. With increasing in drought stress, LAI was found to be decreased at all the stages of observation. These results were in agreement with Praba *et al.* (2009) [7]. *P. indica* colonized plants irrigated at 40 mm CPE to a depth of 1.5 cm enhanced leaf area index over non-colonized/ control plants irrigated at the same frequency at 30, 45 and 60 DAT. At the same irrigation interval and depth, non-colonized plants ($p_2i_1d_2$) produced LAI of 4.1 at 60 DAT, which was 10.73 per cent lower than colonized plants However, *P. indica* colonized plants which experienced severe stress (irrigated at 40 mm CPE to a depth of 1.5 cm) showed an increment of 21.34, 10.13 and 9.05 per cent in LAI over non-colonized plants at 30, 45 and 60 DAT. Researchers observed that inadequate leaf water potential reduced the leaf expansion ratio to half the level. According to Cutler *et al.* (1980) [4] growth of leaf is totally dependent on the leaf water potential, which will be limited under a situation of water stress. In the current study, colonized plants maintained superior relative leaf water content even under water stress which helped to maintain water potential in leaves. This coupled with higher chlorophyll stability might have resulted in higher LAI. Fungal colonization also enhanced nitrogen uptake which could lead to chlorophyll production thereby higher leaf area index. These results are in conformity with the findings of Hosseini *et al.* (2017) [5] where *P. indica* colonization resulted in higher LAI under stressed situation which was attributed to higher water status in leaf that led to high LAI for drought tolerance in stress situation. At harvest, LAI was found to decline in all treatments might be due to leaf mortality, senescence and translocation of assimilates to grain.

Dry matter production (DMP) of a plant explains the growth and development of plant in response to the net photosynthetic efficiency. Colonization of fungal endophyte enhanced the dry matter production by 30.87 per cent over control plants under stressed situation. There was 15.12 per cent enhancement in DMP in *P. indica* colonized plants irrigated at 30 mm CPE to a depth of 3 cm over non-colonized plants at the same irrigation frequency. Biomass production positively correlate with the production of assimilates. High water and nutrient uptake mediated by *P. indica* might have enhanced the photosynthetic efficiency and production of assimilates that resulted in a greater number of tillers and panicles leading to higher DMP. Jolly *et al.* (2019) [2] pointed out that dry matter production enhanced with high consumptive use of water, which was possible with greater number of irrigations during crop period. Several studies authenticated the role of *P. indica* in enhancing biomass accumulation as reported by Kumar *et al.* (2009) [3] in maize and Gosal *et al.* (2013) [1] in sugarcane.

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