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Original Research Paper

Root colonization by *Piriformospora indica* promotes growth, induces earliness in flowering, enhances yield and citrulline content in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)

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ABSTRACT

Piriformospora indica is a unique and versatile fungal root endophyte that colonizes a wide range of plant species, and promotes growth, early flowering and yield in addition to enhanced tolerance to abiotic and biotic stresses. The performance of *P. indica*-colonization in water melon was evaluated in two improved, seeded watermelon varieties *viz.,* Sugarbaby, and Durgapur Lal and two seedless F_1 hybrids *viz.,* Swarna and Shonima. The seedlings of the varieties colonized with *P. indica* increased root and shoot growth biomass compared to the control. Early initiation of female flowers was also observed due to the fungal colonization. Indole acetic acid (IAA) and Gibberellic acid (GA) contents of watermelon seedlings were enhanced in *P. indica*-colonized plants compared to non-inoculated control plants. The endophytic association in watermelon also increased the fruit yield in terms of the number and size of fruits, the number of seeds per fruit in seeded varieties, and citrulline, lycopene, and beta-carotene contents. Interestingly, the severity of bud necrosis was significantly reduced in *P. indica*-colonized plants compared to the uninoculated control. This is the first report on *P. indica* root colonization in watermelon which positively influences growth, yield and quality of seedless F_1 hybrids and seeded varieties of watermelon. This study also indicated the potential of *P. indica* to overcome the limitations of seedless watermelons in growth and yield compared to the seeded varieties. Therefore, there is tremendous scope for improving watermelon through *P. indica*-colonization including the seedless F_1 hybrids.

Keywords: Citrulline, gibberellic acid, indole acetic acid, *Piriformospora indica*, seedless watermelon

INTRODUCTION

Piriformospora indica, is a novel and habitat-adapted versatile fungal root endophyte (Class: Basidiomycete; Order: Sebacinales; Family: Sebacinaceae), isolated from the roots of xerophytic woody shrubs in the Thar desert of India. This fungus forms beneficial symbiotic relationships with various host plants and can be grown in axenic cultures (Varma et al., 2012). *P. indica* enhances plant growth in terms of seed germination, seedling vigour, root and shoot biomass, early flowering, fruiting, yield and secondary metabolites (Johnson et al., 2014). It also acts as a bioprotector against fungal and viral diseases (Sinijadas et al., 2024). Root colonization by *P.indica* induces auxins and cytokinins, promoting root proliferation, better nutrient acquisition and increased crop productivity. Additionally, it confers local and systemic resistance to diseases and enhances resilience to biotic and abiotic stress through the stimulation of antioxidant defense systems and stress related genes (Oelmuller et al., 2009; Sinijadas et al., 2024).

Watermelon (*Citrullus lanatus* L.), a crucial crop in the Cucurbitaceae family originated from Africa, is rich source of vitamins, minerals, and nutritional compounds. Watermelon yield and productivity are adversely affected by various biotic and abiotic stresses. Arbuscular mycorrhization in watermelon improves tolerance to drought, salinity, fusarium wilt, and bacterial wilt (Mo et al., 2016). *P. indica*, with its ability to grow in axenic cultures and a broad host range, has an edge over arbuscular mycorrhizal fungus. *P. indica* association improves drought tolerance in watermelon (Jyothymol et al., 2024). This study demonstrates positive effects of *P. indica* colonization on seed germination, seedling vigour, flowering, fruit set, yield and quality of fruits, along with reducing bud necrosis disease severity.

MATERIALS AND METHODS

Plant material and fungal culture

Seedlings of watermelon varieties Swarna and Shonima (seedless F_1 hybrids), and Sugarbaby and Durgapur Lal (seeded improved varieties) were used. The *P. indica* culture, maintained on potato dextrose agar at the Department of Plant Pathology, COA, Vellayani, was sub-cultured monthly and grown in potato dextrose broth (PDB) at 28°C and 60 rpm in an orbital shaker. The fungus grown in PDB was filtered, macerated, and the concentration of chlamydospores was checked using a hemocytometer. Watermelon seeds were soaked in 10 ml fungal solution for 2 hours as per Liu et al. (2019) and sown in trays filled with a 3:1:1 coir pith, vermiculite, and perlite medium. An additional 10 ml of the fungal solution (1 × 10^6 CFU per ml) was poured into the trays. After germination, 10-day-old seedlings of *P. indica*-colonized and uninoculated control were transplanted to the field 2 m apart.

Experimental Design

The effect of *P. indica* on seed germination and seedling growth was studied in a completely randomised design (CRD) using colonized and control plants of Swarna, Shonima, Sugarbaby and Durgapur Lal. Split-plot design was used in the field to assess the influence of *P. indica* on yield and quality, with colonized and control plants in the main plot and four varieties in the subplots, creating eight treatments. Each experiment had three replications, with 5 plants per replication.

Microscopic observation of fungal structures in the root

The staining procedure was done as per Sinijadas et al. (2024) to observe the fungal structures inside the roots under a microscope at 400X magnification and photographed.

Growth and phytohormones content of watermelon seedlings

Germination percentage, days taken to germination, root and shoot length, fresh and dry weight of root and shoot and phytohormones (IAA and GA) were recorded after ten days of germination. IAA was estimated following Parthasarathy et al. (1970), and GA was estimated using a modified method by Sunderberg (1990).

Reproductive and yield related parameters of field crop

Days to the first female and male flower opening, the node number of the first female flower, days to fruit maturity, number of fruits per plant, number of seeds per fruit and total yield per plant were recorded.

Quality parameters

Total soluble solids (TSS), lycopene, beta carotene, potassium and L-citrulline contents were estimated. Lycopene, beta carotene and potassium were estimated according to Sadasivam and Manickam (1992), while L-citrulline content was assessed using gradient HPLC following Ridwan et al. (2018).

Bud necrosis virus

Virus-infected plants were scored based on severity rating scales (0-3) by Sugiyama et al. (2009) and disease severity was determined by Bos (1982).

Statistical analysis

Experimental data were statistically analyzed using GRAPES and R software developed by Kerala Agricultural University and the R Core Team, respectively. Level of significance is 5 per cent and the error bars represent SEs.

RESULTS AND DISCUSSION

P indica-mediated growth promotion in watermelon plants is correlated to increased auxin production

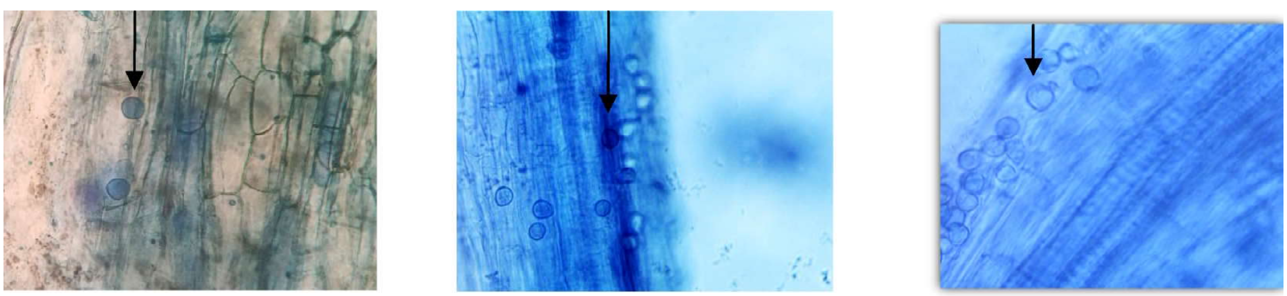
The microscopic observation of ten-day-old seedlings revealed *P. indica* colonization in the cortical cells of root tissues, with visible mycelium and chlamydospores (Fig. 1). Uninoculated control plants showed no fungal structures. Colonized seedlings exhibited significantly higher root and shoot lengths, fresh and dry weights compared to the control (Fig. 2). The colonized Swarna showed maximum increase in shoot length (10.63 cm) whereas the colonized Shonima recorded the longest roots (19.53 cm) (Fig. 3A), the highest fresh root weight (0.12 g) (Fig. 3C) and the highest dried root weight (0.007 g) (Fig. 3E). *P. indica*-colonized Sugarbaby had the

Table 1 : Effect of *P. indica* on days to germination and germination percentage

Watermelon varieties	Days to germination		Germination %	
	Control	+*P. indica*	Control	+*P. indica*
Swarna	3.33[a]	3.00[a]	75.55[d]	77.77[d]
Shonima	3.00[a]	3.00[a]	79.99[cd]	82.22[bcd]
Sugarbaby	2.00[b]	1.66[b]	86.66[bc]	88.88[b]
Durgapur Lal	2.00[b]	2.00[b]	97.77[a]	97.77[a]
Mean	2.5		85.83	
CD	0.5		8.16	

highest fresh and dry shoot weights (0.96 g and 0.06 g, respectively) (Fig. 3D & Fig. 3F). The enhanced shoot and root biomass suggest that the fungus stimulates nutrient acquisition, photosynthesis efficiency and phytohormone synthesis in plants (Oelmuller et al., 2009; Varma et al., 2012; Johnson et al., 2014). When the endosymbioses are established, the fungus can access photo-assimilates and other plant nutrients. On the other hand, the fungus mobilises, transports and translocates minerals and nutrients in plants, which further encourages colonization and proliferation of the fungus in roots; thus promoting the plant growth (Oelmuller et al., 2009).

To study the role of auxins in *P. indica*-mediated growth promotion in watermelon plants, IAA contents were estimated in the roots and shoots. Interestingly, the colonized plants had higher levels of IAA in leaves and roots and also GA in leaves (Fig. 4A & Fig. 4B). *P. indica*-colonized Shonima had the highest levels of IAA and GA (22.53 µg/g and 0.19 µg/g, respectively). Thus, *P. indica* regulates auxin homeostasis, the most efficient strategy for promoting growth (Johnson et al., 2013). Activating auxin biosynthesis and signaling in the roots might cause the *P. indica*-mediated growth phenotype in watermelon (Xu et al., 2018). There was no significant difference between the colonized and control plants in days to germination and germination percentage (Table 1).



A B C

Fig. 1 : Chlamydospores of *P. indica* within the root cortex cells of watermelon plants (400X)
A & B. Roots after 10 days of germination. C. Roots of field crop at the time of harvest

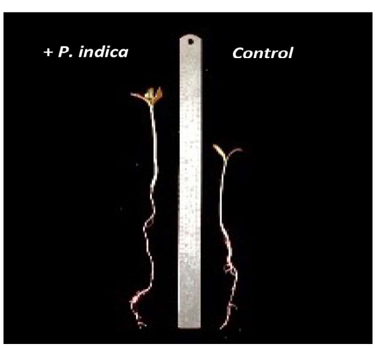


Fig. 2 : Enhanced root and shoot growth in *P. indica*-colonized Shonima plants compared to the uninoculated control plants (10 days after germination)

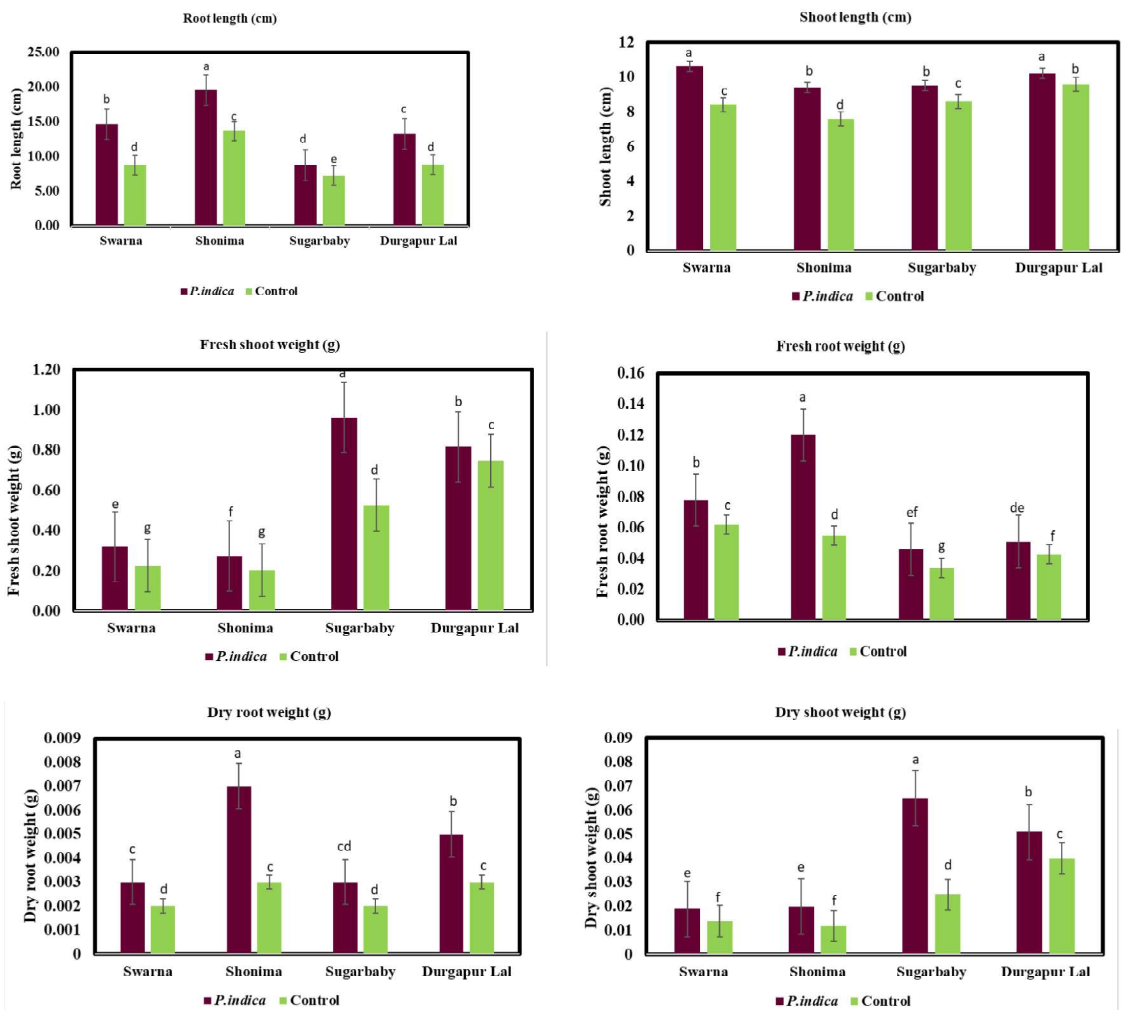


Fig. 3 : Influence of *P. indica* on root length, shoot length, fresh root weight, fresh shoot weight, dry root weight and dry shoot weight of ten days old watermelon seedlings as compared to un-inoculated control plants

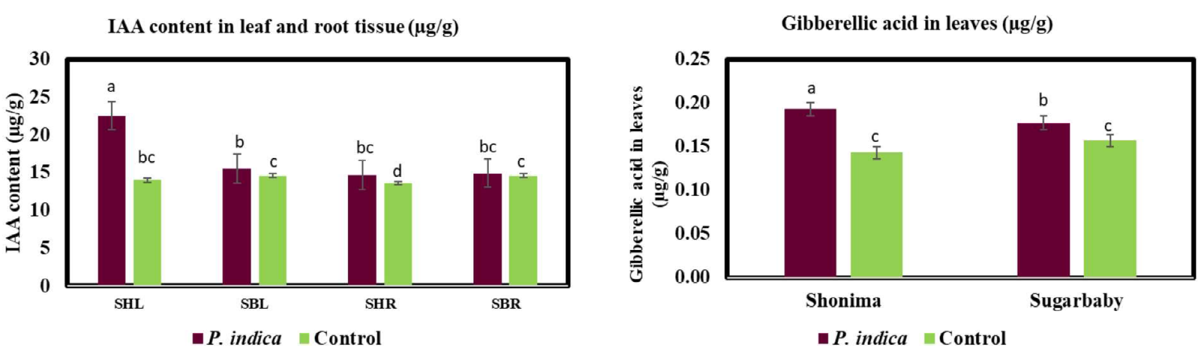


Fig. 4 : IAA and GA contents of *P. indica*-colonized and uninoculated watermelon plant tissues 10 days after germination. 'SHL'- Shonima leaf, 'SBL'- Sugarbaby leaf, 'SHR'- Shonima root, 'SBR'- Sugarbaby root

P. indica triggers early flowering through enhanced GA production

P. indica-colonized plants produced female flowers early at lower node than control. *P. indica*-colonized Durgapur Lal took minimum days to the first female flower (24.46 days), whereas *P. indica*-colonized Swarna was the earliest in the node number of the first female flower (5.26). Significant interaction between

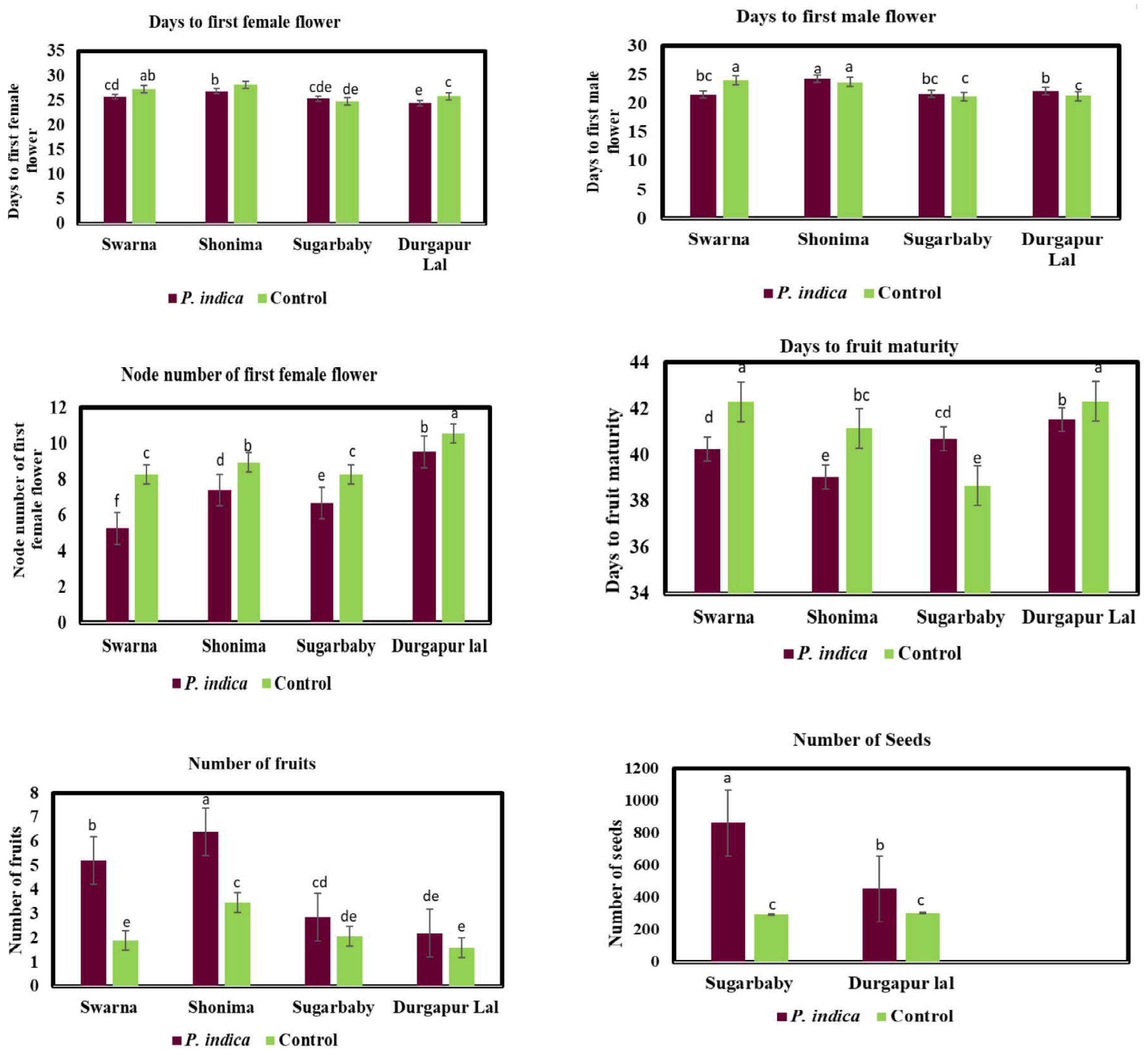


Fig. 5 : Influence of *Piriformospora indica* on days to the first female flower, days to first male flower, node number of female flower, days to fruit maturity, number of fruits and number of seeds

the fungal colonization and varieties was seen in days to the first female and male flowers (Fig. 5) and node number of the first female flower. The interaction of macro-micronutrients and phytohormones regulates early flowering (Varma et al., 2012). Our data indicate that an induction of GA may mediate early flowering. The previous studies also showed that gibberellic acid biosynthesis genes were up-regulated in *P. indica*-colonized plants for its early flowering (Kim et al., 2017).

P. indica enhances yield and seed number in watermelon

P. indica-colonization enhanced the number of fruits per plant and number of seeds. The highest number of fruits was observed in *P. indica*-colonized Shonima (6.40) (Fig. 5E). The highest number of seeds was seen in *P. indica*-colonized Sugarbaby (864.2), followed by Durgapur Lal (456.8) (Fig. 5F), the seeded varieties. Similarly, the fungus had a significant influence on the total yield per plant. The highest yield was observed in Sugarbaby (8.22 kg), followed by Durgapur Lal (7.85 kg), which is on par with Shonima (7.72 kg) (Fig. 6A). The increased root growth and phytohormones contributed to early flowering and the production of more fruits per plant. Compared to the control plants, a higher yield of 65.11, 38.96, 40.67 and 40.93 per cent was observed in *P. indica*-colonized Swarna, Shonima, Sugarbaby and Durgapur Lal, respectively. Growth and yield benefits are primarily attributed to root proliferation, higher nutrient uptake and production of the phytohormones *viz.,* IAA, CK, and GA (Xu et al., 2018).

P. indica-colonization improved fruit quality in watermelon

P. indica-colonization significantly affected all quality parameters except potassium. The highest TSS was observed in *P. indica*-colonized Sugarbaby (11.77°B) (Fig. 6B). The highest lycopene and beta-carotene content was recorded in *P. indica*-colonized Shonima (5.42 mg/g; 0.65 mg/100g, respectively) (Fig. 6C & Fig. 6D). A significant difference was observed among the treatments for citrulline content and was higher in *P. indica*-colonized fruits. Citrulline content was highest in *P. indica*-colonized Swarna (2266 ppm) (Fig. 6E).The fungus positively regulates synthesis and accumulation of solutes, usually polyols, sugars, amino acids, betaines and related compounds in fruits to improve its quality (Sinijadas et al., 2024). Our findings demonstrated that *P. indica*-colonization significantly increases the sugars in watermelon fruits. Watermelon is a good source of citrulline, an amino acid that can be converted into arginine.

P. indica suppresses the natural incidence of *Watermelon bud necrosis virus*

The *P. indica*-colonized plants of all varieties performed better to natural infection of *Watermelon bud necrosis virus* (WBNV) compared to control plants. Among the varieties, uninoculated Swarna was

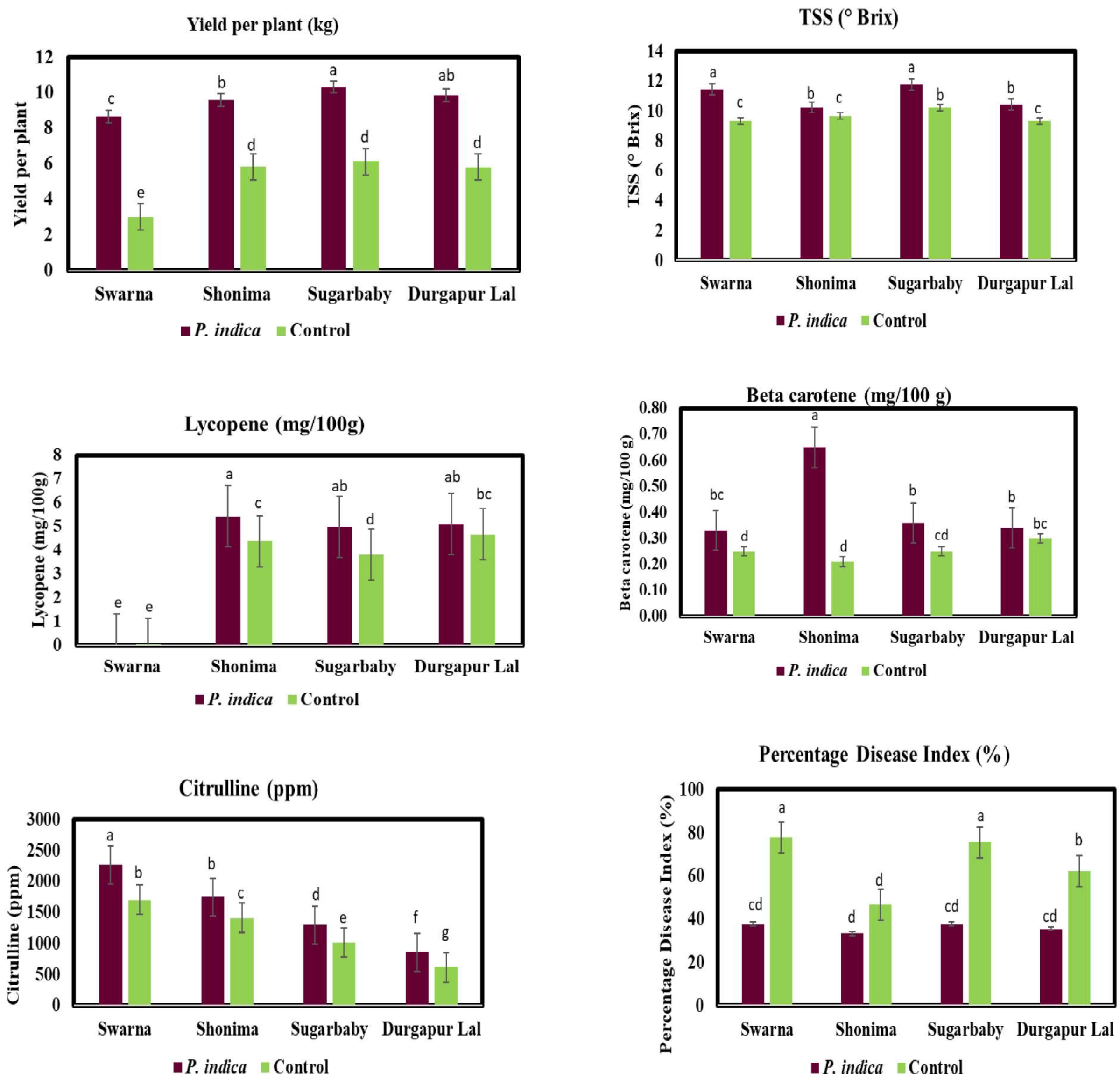


Fig. 6 : Influence of *Piriformospora indica* on (A) yield per plant, (B) TSS, (C) lycopene, (D) betacarotene, (E) Citrulline, (F) Percentage disease index

the worst affected by the virus (VI-77.77), while, colonized Shonima was the least affected with VI of 33.33 (Fig. 6F). Virus infection impairs the biosynthesis and signalling of auxins and gibberellins in plants leading to morphogenetic alterations like dwarfing, stunting, leaf deformations, mosaics and bud necrosis (Mishra et al., 2020; Gyula et al., 2022). But *P. indica*-colonization promotes biosynthesis and signalling of auxins and gibberellins in roots and shoots (Johnson et al., 2013; Kim et al., 2017) which compromised the impaired state of the growth regulators in virus infected plants to reduce the symptoms. *P. indica* also suppresses the viral disease by enhancing the antioxidants which mitigates the adverse effects of ROS produced by viruses (Sinijadas et al., 2024). After colonizing plants, *P. indica* offers systemic bio-protection for the crop by triggering signaling pathways that change the plant's transcriptomes, proteomes and metabolomes; and produce phytohormones such as auxins, gibberellins, and cytokinins (Johnson et al., 2014).

CONCLUSION

The present study is the first report on *P. indica* beneficially influencing growth, yield, and fruit quality in addition to the reduced WBNV infection in seedless F1 hybrids; Swarna and Shonima, as well as improved varieties; Sugarbaby and Durgapur Lal of watermelon. The major bottlenecks in the cultivation of seedless watermelon *viz*. low seed germination, poor fruit set, lower yield etc. could be overcome by P. *indica* colonization.

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