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Improving Maize Growth and Productivity through Endophytic Symbiosis under Field Condition

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ABSTRACT: In this research study, a field experiment was conducted to assess the effects of three fungal endophytes, namely LAS-6 (*Chaetomium* sp), K-23 (*Fusarium* sp.), and P-37 (*Ulocladium* sp.) on plant growth and development. The experiment involved monitoring the plants at 30, 45, and 65 days after sowing (DAS) to evaluate various growth parameters. The results indicated that the application of these endophytes significantly enhanced plant height, stem girth, leaf area, shoot biomass, and SPAD chlorophyll meter reading (SCMR) compared to the control group. Moreover, the stomatal conductance, transpiration rate, and assimilation rates were notably higher in plants treated with endophytes, suggesting an improved physiological performance under field conditions at 30 DAS. Additionally, the endophyte-treated plants exhibited a shorter duration for anthesis and silking, indicating accelerated reproductive development. Furthermore, these plants recorded increased cob length, cob weight, number of kernels per row, and kernel row length, all of which are key components contributing to higher yield. These findings underscore the potential of fungal endophytes in enhancing crop productivity by positively influencing both vegetative and reproductive growth parameters. This research contributes valuable insights into the application of endophytic fungi for sustainable agricultural practices, offering a promising avenue for future studies in the field of plant-microbe interactions and crop improvement strategies.

Keywords: Maize, endophytes, field condition, growth and yield parameters.

INTRODUCTION

The plant is a complex ecosystem with a versatile communal life because they are heavily populated by pro-and eukaryotic microorganisms and represent a biological system. This system exists as an information processing entity with complex processes of communication, occurring throughout the individual plant. The plant cellular information processing network constitutes the foundation for processes like growth, defense, and adaptation to the environment. The associated microorganisms and their investment in information conditioning are often ignored. Endophytes as plant partners are an indispensable integrative part of the plant system. The endophytes are noteworthy for their ability to colonize without apparent symptoms and provide habitat-adapted fitness advantages to genetically distant hosts, both monocots and eudicots (Rodriguez *et al*., 2008). Endophytes also can transfer habitat-specific stress tolerance to plants through a process known as Habitat Adapted Symbiosis (Rodriguez *et al*., 2004). Plants in symbiosis with endophytic fungi can improve their growth and physiological status (Gamalero *et al*., 2009; Shantharaja *et al*., 2022) under stressful environments. It has been hypothesized that host fungal endophytes provide plant fitness to extreme habitats through intergenomic epigenetic mechanisms to allow the plant to tolerate specific stresses (Rodriguez *et al*., 2008). According to some reports, plants adapted to harsh habitats typically harbor endophytes that are highly tolerant to extreme conditions through mutualistic association (Manasa *et al*., 2020; Sangamesh *et al*., 2018; Roopashree and Prasad, 2021; Neekshitha *et al*., 2023).

Due to an early plant-fungus association that has occurred during evolution, endophytes have been trended as an eco-friendly biotic approach for promoting plant growth. Waqas *et al*. (2012) found that endophytic fungal isolates (*Chrysosporium pseudomerdarium*, *Aspergillus fumigatus*, and *Paecilomyces* sp.) improved seed germination, vigor index, shoot and root length by breaking down cellulose and providing carbon to seedlings. Murphy *et al*. (2017) found that using endophytic fungus to treat barley and oat seeds boosted seed germination and subsequent seedling growth. Under normal and stressful conditions, seed priming with beneficial fungi and bacteria can significantly improve seed germination and emergence, seedling establishment, crop growth, and yield parameters (Prasad *et al*., 2020; Ayesha *et al*., 2021).

The combination of the endophytic fungus *P. indica* with a nitrogen fixer and a phosphorous solubilizer significantly increased finger millet growth, yield, and quality (Arunkumar and Shivaprakash 2017). Inoculation of endophytic strain *P. indica* on *Brassica campestris* spp. *chinensis* L. increased plant growth, carotenoids, antioxidant pathways, phenolic acid, and flavonoids (Khalid *et al*., 2017). *Trichoderma harzianum* increased yield, chlorophyll content, starch content, nucleic acid content, total protein content, phytohormone content in maize plants (Akladious and Abbas 2014) and showed a significant increase in the plant height, photosynthetic rate, chlorophyll content, stomatal conductance, and also on the tiller and panicle number in rice (Doni *et al*., 2018). *P. liquidambari* forms a symbiotic relationship with rice, assisting in nitrogen accumulation and uptake, as well as significantly increasing the growth and yield of rice (Yang *et al*., 2014).

The balanced interactions of endophytes with their host plants during the entire symbiotic phase allow for improved adaptation to environmental changes (Rosenberg and Zilber-Rosenberg 2018). Despite the immense potential of endophytes in plant growth promotion, field-rational tests are few. As a result, more study is needed to address these constraints and characterize how plant-endophyte interactions function in the field. The present study aims to explore the potential ability of diverse fungal endophytes in seed priming to achieve the goals of sustainable agriculture and to overcome limitations associated with field applications.

MATERIALS AND METHODS

The main goal of the experiment was to examine the role of a few selected endophytic fungi in modulating plant growth and yield under field conditions.

Source of endophytic fungal isolates. Endophytic fungi from habitat-adapted plants have been collected and preserved at School of Ecology and Conservation Laboratory, Department of Crop Physiology, UASB, GKVK, Bengaluru. A few fungal endophytes from the library were selected and revived on fresh potato dextrose agar medium. The endophytes (LAS-6, K-23, and P-37) were isolated from plant species that grew in diverse parts of India like Rajasthan, Kargil (J&K) andPangong Tso.

Experimental details. Selected three endophytes *i.e*., LAS-6 (*Chaetomium* sp), K-23 (*Fusarium* sp.), and P-37 (*Ulocladium* sp.) were evaluated in maize hybrid (MAH 14-5) under field conditions in NSP, UAS, GKVK, Bengaluru. The location is situated between 13° 05" North latitude and 77° 34" East longitude at an altitude of 924 m above mean sea level. The annual rainfall ranges from 528 mm to 1374.4 mm with a mean of 915.8 mm and the location comes under agro-climatic zone V (Eastern dry zone) of Karnataka. The soil of the experimental site was red sandy clay loam with slightly alkaline (pH 6.3) and the electrical conductivity was normal (0.322 dSm^{-1} at 25°C).

Assessing the performance of the best crop-endophyte combination in the field conditions. Maize seeds were surface sterilized (70% ethanol for 2 min, 1% perchloric acid for 30 s) and kept for pre-germination. Five-day-old fungal cultures of K-23, LAS-6, and P-37 were employed to prepare inoculum by washing the mycelial mat with sterile water. Pre-germinated seeds were treated for 3 hours with the mycelia solution (2×10^6 cfu/ml) and a control (sterile water). After that, the treated pre-germinated seeds were sown in two experimental plots, each having four treatments with three replications in a randomized block design. The experimental plot was brought to a fine tilth by deep ploughing and repeated harrowings. The required number of plots (24 plots) were laid out with small bunds. The seed rows were marked and drawn at a distance of 60 cm apart in each plot. The individual plot size is $9m^2$having 10 lines with 5 plants each. Plots received all recommended agricultural practices as irrigation and fertilization in the field. The pre-germinated seeds were sown by manual dibbling at the congenial field condition. The seeds were hand-dibbled at five cm deep in the earlier marked rows at 30 cm intra-row spacing.

The necessary aftercare operations such as hand weeding, thinning, inter-cultivation, and plant protection measures were carried out as and when required to maintain a good and healthy seed crop. The crop was also given protective irrigation depending upon the soil moisture condition. Timely plant protection measures were taken to control pests and diseases as and when required. The crop was harvested when cobs started drying, and the color of the leaves turned pale yellow. The seeds were separated from cobs, then cleaned and dried in the shade until the seed moisture content reduced up to 11-12 percent.

Measurement of biometric and physiological parameters

— Plant height (cm): Plant height was measured at four stages *viz*., 30, 45, and 65 days after sowing (DAS) and at maturity. Plant height was measured from the soil surface to the apex of the topmost young leaf of the plant and expressed in centimeters.

— Stem girth (cm): Stem girth (cm) was measured using the Vernier Caliper, at 30, 45, and 65 days after sowing; it was measured at 10 cm above the ground level.

— Leaf area (cm^2): Three replications or plants from each treatment were randomly selected at 30, 45, and 65 DAS and used to measure leaf area, root length, root volume, and plant dry weight. Here, the leaves were separated from the plant and were passed through the leaf area meter immediately (WD3 Win DIAS Leaf Image Analysis System, Delta-T services, UK) to compute the total leaf area of a plant.

— SPAD chlorophyll meter reading (SCMR): The relative leaf chlorophyll content of individual plants was measured using the hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). The observations were recorded from a fully expanded fourth leaf from the top of the plants at three stages (30, 45, and 65 DAS). Three readings were recorded at each stage from each plant and averaged.

— Measurement of gas exchange parameters using IRGA: Gas exchange parameters such as CO_2 Assimilation rate A ($\mu mol\ m^{-2}s^{-1}$), stomatal conductance gs (mmol $m^{-2}s^{-1}$), and Transpiration T (mol $m^{-2}s^{-1}$) were measured in control and endophytes treated plants at 30 days after sowing, using a portable photosynthetic system, Infrared gas analyzer-IRGA (LI-COR-Inc. Lincoln, Nebraska, USA). Flag leaf was clamped to the leaf chamber, observations were recorded after A, gs, and T values reached a steady-state level (Nataraja and Jacob 1999). The measurements were made at an ambient CO_2 concentration of 360 µmol mol^{-1} and PPFD of 1400 µmol $m^{-2}s^{-1}$, Licor light source, and a chamber temperature of 28 °C (Morgan and Lecain 1991).

Measurement of yield parameters of plants

— **Days to silking:** Days to silking were measured as the number of days taken from the date of sowing to the date when silks were emerging from the ear of the individual plant.

— **Days to anthesis:** The number of days taken from the date of sowing to the date of the first pollen shed in each plant was recorded as days to anthesis.

— **Cob length (cm):** After harvesting, the cob was dehusked and the length of the cob was measured from the base to the tip of the cob in centimeters.

— **Cob diameter (cm):** After dehusking and drying the main cob, the cob diameter was measured in the middle of the main cob and expressed in centimeters.

— **Cob weight (g):** After harvesting, the cob was dehusked and the weight of the dried main cob of an individual plant was taken and recorded in grams.

— **Number of kernel rows per cob:** The total number of kernels bearing rows in the main cob was counted and recorded as a number of kernel rows per cob.

— **Number of kernels per row:** The number of kernels on the longest row in the main cob was counted and recorded as the number of kernels per row.

— **Kernel row length (cm):** The kernel row length of each cob of the individual plant was measured in centimeters from the top to the base of the longest kernel row.

— **Test weight (g):** The weight of randomly selected one hundred well-filled kernels from each plant was recorded as test weight and expressed in grams. If the number of kernels present in a plant was less than 100, the weight of all the kernels was recorded and converted to test weight.

— **Grain yield per plant (g):** The weight of the total number of kernels produced from all the cobs of the individual plant was recorded as grain yield per plant and expressed in grams.

Statistical analysis. The experiments were carried out in a randomized complete block design in field conditions. Standard errors were calculated for each parameter in each treatment. Most statistical analyses were performed in the R statistical environment and Phyton. Statistical comparisons were done by one-way ANOVA tests followed by a post-hoc Tukey analysis; the same letters above the graph are statistically indistinguishable at 95 % confidence.

RESULTS AND DISCUSSION

A field assessment of selected endophytes to bridge a technological gap. Plants face different stresses under field conditions due to the drastic and rapid global climate changes, which can alter the morphological, physiological, and molecular responses that disturb normal plant productivity. Plants attain various common and different physiological and molecular protective approaches for tolerance under field stresses. In this study, the possible cross-talk between plants and endophytes to increase yield was studied under field conditions. The increase in growth parameters like plant height, leaf area, stem girth, and dry biomass was observed at 30, 45 and 65 DAS, which is directly related to yield and yield components.

Growth parameters. Biometric parameters like plant height, stem girth, leaf area, and shoot biomass were recorded at 30, 45, and 65 DAS (Fig. 1, 2 & 7). At 30 DAS, plant height (cm) varied between 78.71 to 86.47 cm/plant among treatments, with highest in P-37 (86.47 cm), LAS-6 (86.34 cm) and K-23 (85.75 cm) compared to control (78.71 cm). Stem girth varied between 1.66 to 1.95 cm/plant, with significant increase in P-37 (1.99 cm), K-23 (1.98 cm) and LAS-6 (1.95 cm) compared to control (1.66 cm). Leaf area varied between 2838.27 cm^2 to 3438.51 cm^2/plant with significant increase in K-23 (3438.51 cm^2), P-37 (3398.88 cm^2) followed by LAS-6 (3359.82 cm^2) compared to control (2838 cm^2). Shoot biomass varied between 18.59 to 22.32 g/plant with a significant increase in K-23 (22.32 g) followed by LAS-6 (21.73 g) and P-37 (21.17 g) compared to control (18.59 g).

At 45 DAS, plant height (cm) varied between 117.80 to 130.58 cm/plant among treatments, with highest in LAS-6 (130.58 cm), P-37 (129.18 cm) and K-23 (128.73 cm) compared to control (117.80 cm). Stem girth varied between 2.22 to 2.49 cm/plant, with a significant increase in LAS-6 (2.49 cm), P-37 (2.46 cm), and K-23 (2.45 cm) compared to control (2.22 cm). Leaf area varied between 4989.00 cm^2 to 5492.82 cm^2/plant with significant increase in P-37 (5492.82 cm^2), K-23 (5415.88 cm^2) followed by LAS-6 (5357.62 cm^2) compared to control (4989.00 cm^2). Shoot biomass varied between 52.16 to 61.66 g/plant with a significant increase in LAS-6 (61.66 g) followed by K-23 (61.06 g) and P-37 (58.83 g) compared to control (52.16 g).

At 65 DAS, plant height (cm) varied between 164.35 to 174.13 cm/plant among treatments, with highest in LAS-6 (174.13 cm), P-37 (173.72 cm) and K-23 (172.59 cm) compared to control (164.35 cm). Stem girth varied between 2.25 to 2.78 cm/plant, with a significant increase in LAS-6 (2.78 cm), K-23 (2.76 cm), and P-37 (2.72 cm) compared to control (2.55 cm). Leaf area varied between 6605.24 cm^2 to 7091.25 cm^2/plant with significant increase in LAS-6 (7091.25 cm^2), K-23 (7054.56 cm^2) followed by P-37 (7010.65 cm^2) compared to control (6605.24 cm^2). Shoot biomass varied between 83.17 to 93.77 g/plant with a significant increase in K-23 (93.77 g) followed by LAS-6 (92.63 g) and P-37 (92.59 g) compared to control (83.17 g).

The growth promotion may be attributed to the increase in photosynthetic efficiency upon endophytes treatment (Fig. 3). However, in recent studies, it was revealed that plant growth promotion due to the secretion of phytohormones, such as indole-3-acetic acid (IAA), gibberellins, cytokinin, and secondary metabolites by the endophytic fungi (Waqas *et al*., 2015; Ismail *et al*., 2021) and partly owing to the fact that endophytes could have enhanced the host's uptake of nutritional elements such as nitrogen and phosphorus from the soil (García-Latorre *et al*., 2021), and also endophytes might have genes that assist to produce some signaling molecule for growth promotion of plants (Ali *et al*., 2014).

A similar effect on plant growth due to endophytic treatment has been reported previously in various plant species under *in-vivo* conditions. The positive plant growth promotion is also evidenced by hormone-producing fungal isolates like *Penicillium chrysogenum*, *P. crustosum*, *Phoma glomerata, Pestalotiopsis neglecta, Williopsis saturnus* in different crop systems (Fu *et al*., 2015; Fouda *et al*., 2015; Hassan, 2017). The symbiotically induced growth response was observed in tomato and cucumber plants when treated with *Trichoderma harzianum*, *Penicillium janthinellum*, *Exophiala* sp. (Mastouri *et al*., 2010; Khan *et al*., 2013).

Physiological parameters. Physiological parameters like SPAD chlorophyll meter reading (SCMR) were taken at 30, 45, and 65 DAS (Fig. 1), and photosynthetic rate, stomatal conductance, and transpiration rate were recorded at 30 DAS (Fig. 3). At 30, 45, and 65 DAS, the SCMR was significantly increased in endophyte-treated plants compared to non-inoculated plants.

Under field conditions, LAS-6, K-23, and P-37 showed a significant increase (Fig. 3) in photosynthetic assimilation rate, stomatal conductance, and transpiration rate compared to non-inoculated plants. SCMR obtained at 30 DAS also showed a similar trend with respect to photosynthetic parameters in plants inoculated with endophytes. In total, endophyte-treated plants had high photosynthetic assimilation rates due to higher stomatal conductance, transpiration rate, and higher chlorophyll content maintained by endophyte-inoculated plants.

The increase in photosynthetic efficiency of plants obtained in this study is in agreement with the increased photosynthetic rate, chlorophyll content, stomatal conductance of maize plants treated with endophyte *Trichoderma harzianum* (Akladious and Abbas 2014; Doni *et al*., 2019). Furthermore, Morse *et al*. (2007) showed that in *Festuca*, the net photosynthetic rate, stomatal conductance, and transpiration rate of E+ plants were considerably higher than those of E- plants. Xu *et al*., 2021 demonstrated that endophyte infection improved all photosynthetic indicators of *F. sinensis* under drought stress, except for intercellular carbon dioxide concentration. These findings suggest that endophytes can improve plant growth by improving photosynthesis, which is consistent with the finding that endophytes can alter the host plant's strategy and enhance metabolism (Xia *et al*., 2018).

Yield parameters. Yield and yield-related parameters like days to anthesis, days to silking, cob length, cob diameter, cob weight, number of kernel rows per cob, number of kernels per row, kernel row length, test weight, and grain yield per plant were recorded in endophyte inoculated and non-inoculated plants grown under field conditions.

The plants that were not inoculated with endophytes took more days for anthesis (58.75 days) and silking (59.88 days) under field conditions. Whereas the plants inoculated with endophytes, K-23 (55.60, 56.73 days), P-37 (55.78, 56.97 days), and LAS-6 (55.88, 56.95 days) took fewer days for anthesis and silking respectively (Fig. 4). The cob length of plants that were inoculated with endophytes K-23 (20.01 cm), LAS-6 (19.52 cm), and P-37 (19.33 cm) showed a significant increase compared to non-inoculated plants (17.30 cm). Endophyte inoculation did not change the cob diameter significantly (Fig. 4). The cob weight of plants that were inoculated with endophytes K-23 (277.18 g), P-37 (274.87 g), and LAS-6 (267.85 g) showed a significant increase compared to non-inoculated plants (241.88 g) and given in Fig. 5. The number of kernel rows per cob did not differ due to endophyte inoculation. Whereas the number of kernels per row was significantly increased in the endophytes K-23 (38.53), LAS-6 (38.45), and P-37 (37.78) treated plants compared to the control (36.95). The kernel row length was highest in K-23 (18.24 cm), LAS-6 (18.14 cm), and P-37 (17.98 cm) treated plants and lowest in non-inoculated plants (16.74 cm), which is represented in Fig. 5.

All these attributes correspond to the yield of the plant. The plants inoculated with endophytes K-23 (42.95 g), LAS-6 (42.76 g), and P-37 (41.73 g) showed an increase in the test weight of seeds than the non-inoculated plants (38.85 g) under field conditions. Grain yield per plant was significantly high from endophytes K-23 (188.85 g), LAS-6 (185.15 g), and P-37 (187.59 g) treated plants compared to untreated plants (177.50 g) in field conditions (Fig. 6). Thus, an increase in seed yield upon endophyte treatment could be due to an increase in net photosynthetic rate (Fig. 3) and the rate of photoassimilate remobilization from source to sink. This study provides proof of concept that endophyte inoculation can improve plant growth and yield under field conditions in a habitat-specific manner through symbiotic association with plants.

Interestingly, there is a vast amount of research on beneficial microbes for plants that may be utilized to guide the screening process, develop best practices for validation, and uncover some problems that may prevent benefits from being transferred from greenhouse to field. Numerous publications support the beneficial effects of fungal endophytes on plant development and performance in adverse conditions (Gundel *et al*., 2013). In the United States, Australia, and New Zealand, *Epichloe* (Class 1) endophyte strains have been employed to boost the productivity of forage grasses in the field and the robustness of turf grasses (Young *et al*., 2014; Kauppinen *et al*., 2016). Endophyte-mediated plant trait improvement provided roughly $200 million per year to the New Zealand economy (Johnson *et al*., 2013). *Trichoderma* sp. which lives on the roots of stressed plants improves yields by activating metabolic processes that convert the hazardous reactive oxygen species produced during stress into less toxic chemicals (Harman *et al*., 2021). *Fusarium equiseti* increased *T. subterraneum* herbage yield in the greenhouse, while *B. spectabilis* improved *T. subterraneum* forage quality by reducing fiber content and *P. pratensis* fodder quality by increasing crude protein. *S. intermedia* increased Ca, Cu, Mn, Pb, Tl, and Zn mineral uptake in subclover, while *M. hiemalis* increased K and Sr uptake in Kentucky bluegrass, demonstrating the potential of fungal endophytes to improve herbage productivity and nutritional value of fodder (Garcia-Latorre *et al*., 2021).

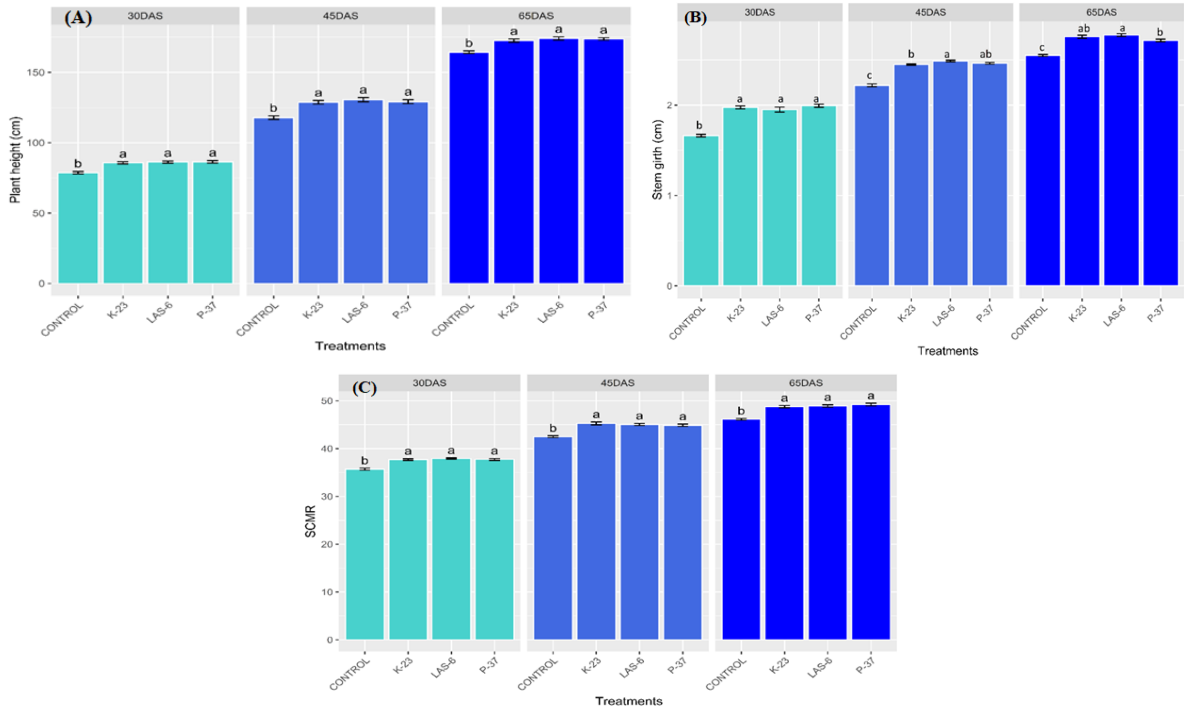

Fig. 1. Effect of selected endophytic fungi on growth parameters of maize under field conditions (A) Plant height, (B) Stem girth, and (C) SCMR. Values represented are mean±SE (n=60) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

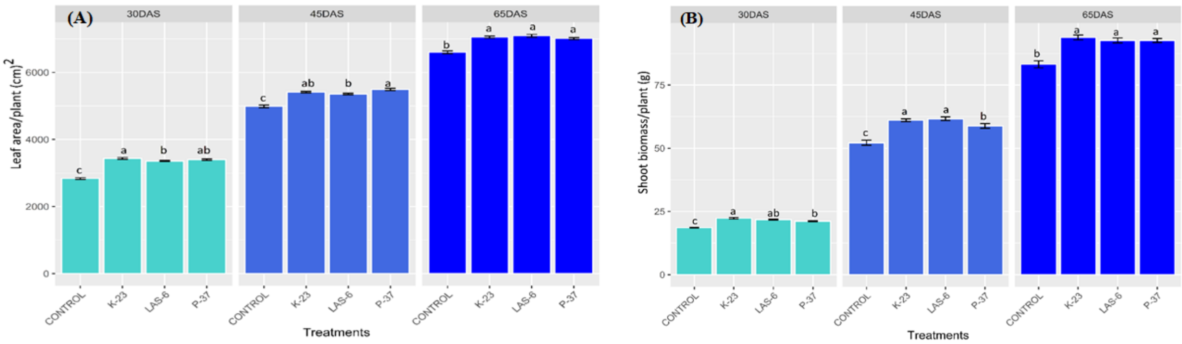

Fig. 2. Effect of selected endophytic fungi on growth parameters of maize under field conditions (A) Leaf area and (B) Shoot biomass. Values represented are mean±SE (n=18) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

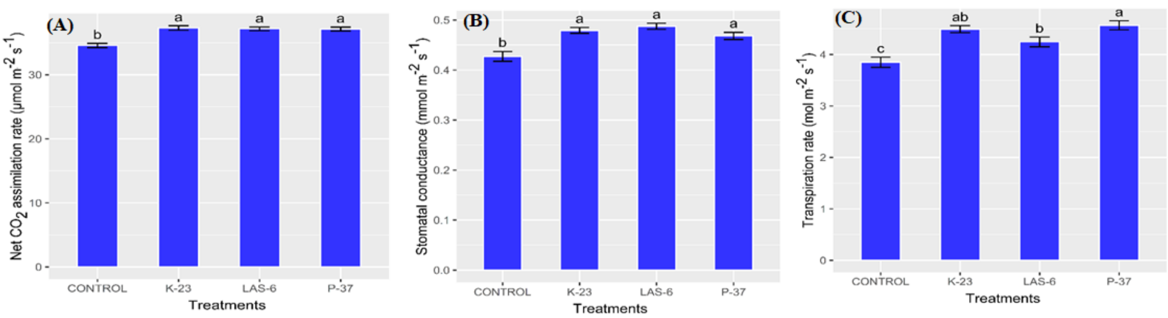

Fig. 3. Effect of selected endophytic fungi on physiological parameters of maize under field conditions (A) Photosynthetic rate, (B) Stomatal conductance, and (C) Transpiration rate. Values represented are mean±SE (n=30) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

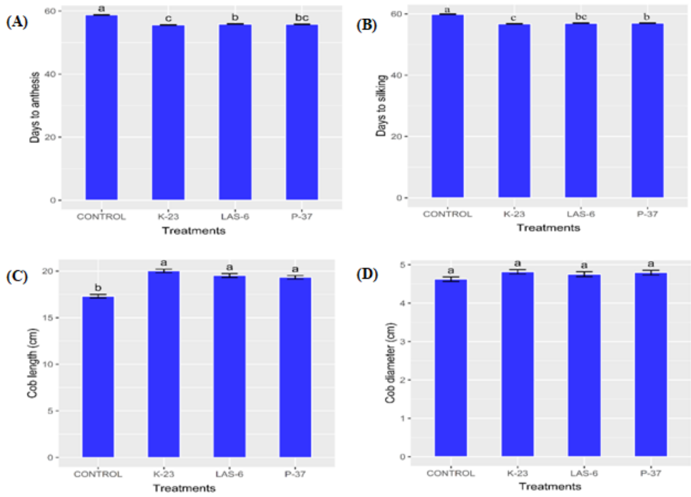

Fig. 4. Effect of selected endophytic fungi on (A) Days to anthesis, (B) Days to silking, (C) Cob length and (D) Cob diameter of maize under field conditions. Values represented are mean±SE (n=60) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

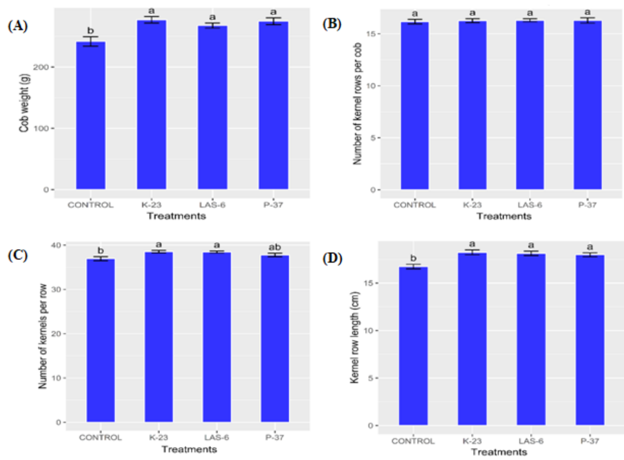

Fig. 5. Effect of selected endophytic fungi on (A) Cob weight, (B) Number of kernel rows per cob, (C) Number of kernels per row and (D) Kernel row length of maize under field conditions. Values represented are mean±SE (n=60) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

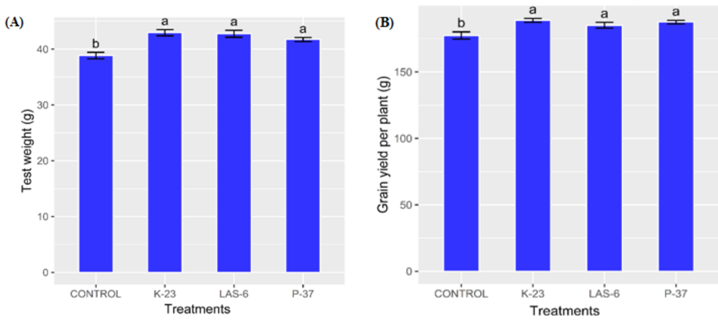

Fig. 6. Effect of selected endophytic fungi on (A) Test weight, and (B) Grain yield per plant of maize under field conditions. Values represented are mean±SE(n=60) and with dissimilar alphabets are significantly different at Tukey's p≤0.05. Lines over bar plots indicate standard error over mean.

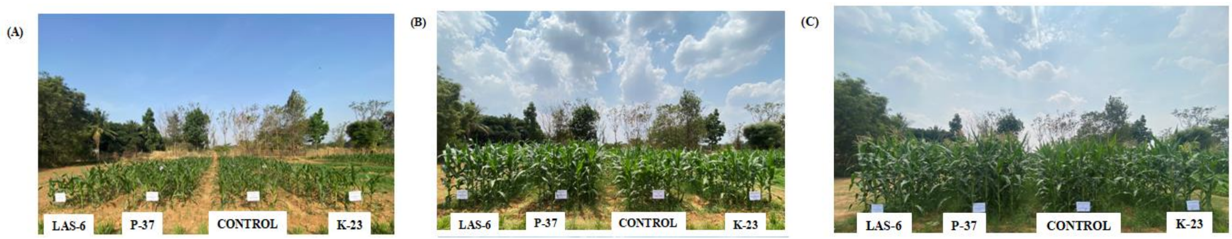

Fig. 7. Phenotype of non-inoculated and endophytes inoculated plants of maize at (A) 30 DAS, (B) 45 DAS, and (C) tasselling and silking stage under field condition.

CONCLUSIONS

In conclusion, this study explored the potential synergistic relationship between plants and endophytes as a means to enhance growth and yield in field conditions. These improvements can be attributed to the increased photosynthetic efficiency facilitated by endophyte treatment, possibly involving the secretion of phytohormones and enhanced nutrient uptake. Moreover, this positive impact on plant growth and physiology aligns with previous findings in different plant species. Furthermore, the study demonstrated that endophyte-inoculated plants exhibited a shorter time to anthesis and silking, increased cob weight, and higher test weights, resulting in an overall higher grain yield per plant.

FUTURE SCOPE

It calls for further exploration into the diversity of fungal endophytes, optimizing application methods for maximum impact, and conducting crop-specific studies to tailor their use. Additionally, assessing the long-term environmental and economic sustainability of endophyte applications and delving into genomic and mechanistic studies to better understand their influence on plant growth will be crucial for advancing the field of plant-microbe interactions and sustainable crop improvement strategies.

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Conflict of Interest. None.

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