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Effect of endophytes on seedling growth of finger millet (*Eleusine coracana* L.) under non-stress and osmotic stress conditions

AKSHATA*, NANJA REDDY, Y.A. AND NATARAJA K. N.
Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India
akshatahiremath1720@gmail.com

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Abstract

Finger millet (*Eleusine coracana* L.) is mainly cultivated as a rainfed crop in arid and semi-arid regions, where moisture stress is a major abiotic constraint limiting growth and productivity. Developing strategies for improving moisture stress adaptation is therefore essential. Endophytes, being eco-friendly and sustainable biological agents, provide a promising approach to enhance crop growth and stress resilience. In this study, the effect of endophytes in seedling growth was examined by suppressing native endophytes using fungicide, bactericide, and their combination. The elimination of native endophytes resulted in reduced seedling growth, indicating their positive influence on early development. To further evaluate their functional role, two externally introduced endophytes K-23 (*Fusarium incarnatum*) and SF-5 (*Fusarium equiseti*) were tested with adequately wetted germination paper. Additionally, the effect of K-23 was investigated under osmotic stress. The findings revealed that endophytes contribute significantly to seedling establishment by promoting growth, even under non-stress conditions. More importantly, the habitat-adapted endophyte K-23 enhanced seedling tolerance to osmotic stress, suggesting its role in imparting stress-adaptive traits. Overall, the results highlight that endophytes not only support early seedling development but also confer moisture stress resilience, making them a potential tool for improving moisture stress adaptation in finger millet cultivation.

INTRODUCTION

Drought stress is one of the most critical abiotic constraints limiting crop productivity worldwide (Fahad *et al.*, 2017). With climate change, increasing the frequency and severity of drought episodes (Jalihal *et al.,* 2019), the yields of many crops, including finger millet, are declining (Nanja-Reddy *et al.,* 2021). This highlights the need for sustainable strategies to improve plant adaptation to water-deficit environments (Lesk *et al.,* 2016). C_4 millets are generally recognized for their resilience to drought, and finger millet is particularly valued for its ability to thrive in arid and semi-arid regions of India and Africa. Nevertheless, under rainfed conditions, inadequate soil moisture

during germination and early seedling establishment reduces crop stand and productivity (Fahad *et al.,* 2017). Strengthening seedling establishment under limited moisture conditions is therefore a critical target for improving yield stability. Although genetically modified (GM) crops and advanced technologies are being developed, their acceptance remains limited due to regulatory, ethical, and social concerns. In this context, the use of endophytes offers a cost-effective, eco-friendly, and sustainable strategy for improving crop performance under stress conditions. By promoting early growth and enhancing stress tolerance, endophytes have the potential to improve finger millet establishment and resilience to drought (Moghaddam *et al.,* 2021).

Endophytes benefit host plants by enhancing nutrient uptake, promoting growth, and improving tolerance to both biotic and abiotic stresses (Lata *et al.,* 2018). Under drought stress, fungal endophytes facilitate a range of physiological and biochemical adjustments, including improved water-use efficiency, osmolyte accumulation such as proline and trehalose, modulation of stress-related phytohormones like abscisic acid and indole acetic acid, and activation of antioxidant defense systems (He *et al.,* 2022). These mechanisms collectively contribute to plant stress adaptation. Native endophytes, having co-evolved with their host plants, are often well adapted to local environments and may provide complementary advantages under stress. Similarly, the ecological origin and compatibility of introduced endophytes are critical determinants of their ability to confer stress tolerance, with some evidence suggesting that such traits can be transmitted across plant generations (Suryanarayanan *et al.,* 2018). While symbiotic associations with microorganisms are widespread in terrestrial plants (Smith and Read, 1997), the functional role of fungal endophytes in finger millet remains underexplored (Hemapriya *et al.,* 2024). Establishing their reliability

under controlled as well as moisture stress conditions is therefore essential to assess their potential for enhancing seedling establishment under drought. In this context, the present study investigates the role of native and introduced habitat adapted fungal endophytes in finger millet seedling growth under both well-watered and osmotic stress conditions.

MATERIALS AND METHODS

Experiment –1: Relevance of endophytes on seedling growth of finger millet genotypes

Seeds of finger millet genotypes, KMR-630, GE-845, GE-1309, GE-4976 were surface sterilized, soaked in solutions as per the treatments, (1) control (water), (2) fungicide (bavistin, 0.2%), (3) bactericide (streptomycin, 100 ppm), and (4) in combination for 12 hours (Nelson, 2018) in three replications in completely randomized design. The soaked seeds were washed thoroughly, and 30 seeds were placed on moist germination paper, rolled, and incubated at 27 ± 1°C, 65% relative humidity (RH) with a low photoperiod (100–200 µmol m^{-2} s^{-1}) in culture room for 7 days, and then root and shoot length were measured in 10 randomly selected seedlings. The data was analyzed for ANOVA and significance between treatments in each genotype was arrived using Duncan multiple range test (DMRT) in OPSTAT software (Sheoran *et al.,* 1998).

Experiment – 2: Functional validation of habitat adapted endophytes

To investigate the potential growth promoting effect of fungal endophytes, SF-5 and K-23 and in combination along with control was conducted using popular varieties, GPU-28 and KMR-630. The purified fungal isolates were collected from endophyte library, Department of Crop Physiology, UAS, GKVK, Bangalore, and sub-cultured on PDA for 15 days under culture room under diffused light (100-200 lux), 26-27°C, and RH of 60-65%, and then 1.0 g mycelia of each endophyte was dissolved in 100 mL distilled water (~10^6 spores/mL). The pre-

germinated seeds of each genotype were soaked in endophyte solutions individually for 3 hours under sterile conditions. Following the inoculation period, the seeds were washed to remove the surface adhered endophyte and transferred onto germination papers moistened with distilled water. The setup was incubated at 27 ± 1°C, 65% RH for 7 days under aseptic conditions in the culture room with 12-hour photoperiod (100–200 µmol m^{-2} s^{-1}). At the end of the incubation period, root length and shoot length were measured, and total seedling length was calculated. The treatments were compared using 't' test.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(S_1)^2/n_1 + (S_2^2)/n_2}}$$

Where, X is the mean, S is the standard deviation and 'n' is the number of samples, when calculated 't' value was more than the table 't' value at 3,44 (t-1 and n-1-(t-1) respective degrees of freedom.

Further, for the confirmation of the colonization of habitat adopted endophytes in the seedlings, the 7 days old seedlings were cut into segments of 1-2 cm of shoot and root separately, placed on PDA individually in petri-plate of 10 cm diameter, and kept in culture room for 10 days for emergence of fungal colonies which were similar to their mother culture.

Experiment 3: Evaluation of habitat adopted fungal endophyte (K-23) for osmotic stress tolerance

To assess the effect of habitat adapted endophyte (K-23, *Fusarium incarnatum*) in inducing the osmotic stress tolerance, an experiment was conducted using four genotypes with four treatments (Control, Endophyte, PEG (-6 bars), PEG + endophyte) in three replications. The pre-germinated seeds were inoculated with K-23 for 3 hours (~10^6 spores/mL), washed and placed on the germination paper soaked with water or PEG with and without endophyte, and were incubated at 27 ± 1°C, 65% relative humidity with a photoperiod of 12-hour (100–200 µmol m^{-2} s^{-1}) in culture room for 7 days. After 7 days, shoot and root lengths were measured to evaluate the effects of endophyte treatments.

The graph was drawn using GraphPad Prisom, and comparison of treatments were made using DMRT of OPSTAT statistical software (Sheoran *et al.,* 1998)

RESULTS AND DISCUSSION

1. Relevance of endophytes on seedling growth of finger millet genotypes

The influence of native endophytes on seedling growth was evaluated by eliminating endophytes through fungicide, bactericide, and combined treatments. Significant differences were observed among treatments in all genotypes (Fig. 1 and 2). Under the control condition (water), all genotypes exhibited greater shoot length, root length, and total seedling length. The total seedling length was 8.52 cm, 8.77 cm, 10.62 cm, and 5.80 cm, in KMR-630, GE-845, GE-4976 and GE-1309, respectively, reflecting normal growth in the presence of native endophytes (Fig. 1). The phenotypic expression has been presented in Fig. 2. In contrast, treatments with either Bavistin (fungicide) or streptomycin (bactericide) resulted in a significant reduction in root, shoot and total seedling length. The combined Bavistin + Streptomycin treatment producing the strongest inhibition of total seedling length to 3.44, 2.35, 2.21, and 1.84 cm in KMR-630, GE-845, GE-4976 and GE-1309, respectively. The percent reduction in total seedling length due combined treatment ranged from 59.6 (KMR-630), 68.3 (GE-1309), 73.2 (GE-845) and 79.2% in GE-4976 as compared to the control (Fig. 1 and 2). This depicts that the elimination of internal microbial communities results in a significant reduction in seedling growth, indicating the necessity of endophytes in seedling growth promotion. Similar findings were reported by Vasanthakumari *et al.* (2019), who showed that systemic fungicides negatively influence seedling establishment by disrupting beneficial endophytic associations with the plant. Ayesha *et al.* (2021) further emphasized that indiscriminate seed treatment with fungicides can destabilize natural microbial consortia which is critical

for maintaining plant vigor. The inhibitory effects of antibacterial treatments observed in the present study demonstrated that plant-associated microorganisms play essential roles in supporting seedling growth, nutrient acquisition, and soil–plant interactions, and are also consistent with earlier reports (Olsson and Alstrom, 1996; Tasho *et al.*, 2020; Huang *et al.*, 2022). Furthermore, the root, shoot and total seedling length did not differ significantly between bavistin, streptomycin and combined treatments (Fig. 1), suggesting that both endophytic bacteria as well fungi are essential for normal seedling growth. Collectively, these results highlight the integral role of native microbial communities in sustaining early seedling growth. The disruption of these associations compromises the plant vigor, underscoring the ecological significance of endophytes as natural partners in crop establishment.

2) Functional validation of habitat adapted endophytes

Following the observation that native endophytes are essential for seedling growth, the present study further investigated the effect of habitat-adapted endophytes, isolated from extreme environments. Two well-characterized fungal endophytes, K-23 (*Fusarium incarnatum*) collected from dry habitat of Kargil region, India, and SF-5 (*Fusarium equiseti*) from marine regions of Tamil Nadu, India (Manasa, 2019), were inoculated into popular finger millet varieties, KMR-630 and GPU-28. The results showed that both the strains significantly enhanced the root, shoot and total seedling length compared to the uninoculated control. However, the seedling length did not differ significantly between the habit adapted endophytes (Table 1). Notably, the SF-5 or the combined inoculation of K-23 and SF-5 resulted in a greater increase in total seedling length of 5.64 and 6.08 cm, respectively as compared to the control (4.14 cm) in the variety, GPU-28. Similarly, in KMR-630 it was 5.29 and 5.33, respectively against the control (3.63 cm) (Table 1). The phenotypic expression is presented

in Fig. 3. These findings demonstrate that habitat-adapted endophytes are capable of establishing the finger millet, and these endophytes could be present in the finger millet seedlings. In this regard, it is verified that both the strains are present both in the root and shoot system of both finger millet varieties (Fig. 4). These results suggest that the external supply of additional habitat adapted endophytes could be added advantage for robust seedling growth of finger millet. Further, the synergistic effect suggests that multiple habitat-adapted endophytes can interact positively with host plants to stimulate growth-related traits. Similar findings have been reported in other crop systems (Ayesha *et al.*, 2021; Pallavi and Nataraja, 2022; Pallavi *et al.*, 2024; Pawar and Nataraja, 2024). The consistency of these effects underscores the broad adaptability and functional relevance of habitat-adapted endophytes or the native endophytes in promoting the seedling growth.

3) Relevance of habitat adopted fungal endophyte (K-23) for osmotic stress tolerance

Building on the observed growth-promoting effects of habitat-adapted endophytes, the potential strain (K-23) of *Fusarium incarnatum* to osmotic stress tolerance in finger millet genotypes was further investigated. The results demonstrated that K-23 colonized seedlings maintained significantly superior root, shoot and seedling length compared to their respective controls in genotypes, KMR-630 and GPU-28 (Fig. 5). Furthermore, endophyte (K-23) + PEG treatment significantly increased the root, shoot and total seedling length in all the genotypes compared to the PEG stress alone (Fig. 5 and Fig. 6). Similarly, a strong growth-promoting effect of K-23 under osmotic stress was observed in earlier studies (Roopashree and Prasad, 2021; Pawar and Nataraja, 2024; Akshata *et al.*, 2025). These findings suggest that the endophyte not only promotes early growth under optimal conditions but also confers resilience to abiotic stress like osmotic stress, likely through mechanisms that

enhance water-use efficiency and stress adaptation. Such traits make endophyte (K-23) a promising candidate for improving seedling establishment and drought tolerance in finger millet under rainfed cultivation systems.

CONCLUSION

The present study highlights the critical role of endophytes in finger millet seedling growth and stress resilience. Elimination of native microbial communities significantly reduced the root, shoot, and total seedling length, underscoring their importance in early establishment. Inoculation with habitat-adapted fungal endophytes K-23 (*Fusarium incarnatum*) and SF-5 (*Fusarium equiseti*) enhanced seedling vigor, reflected the presence of endophytes for seedling growth of finger millet. Endophyte, not only supported growth under optimal conditions but also conferred improved tolerance to PEG-induced osmotic stress across the genotypes. Together with previous evidence from other crops, these findings demonstrate that both native and habitat-adapted endophytes play complementary roles in promoting growth and stress adaptation. Harnessing such endophyte–plant associations represent a promising, eco-friendly strategy to improve finger millet establishment and resilience under drought-prone, rainfed systems.

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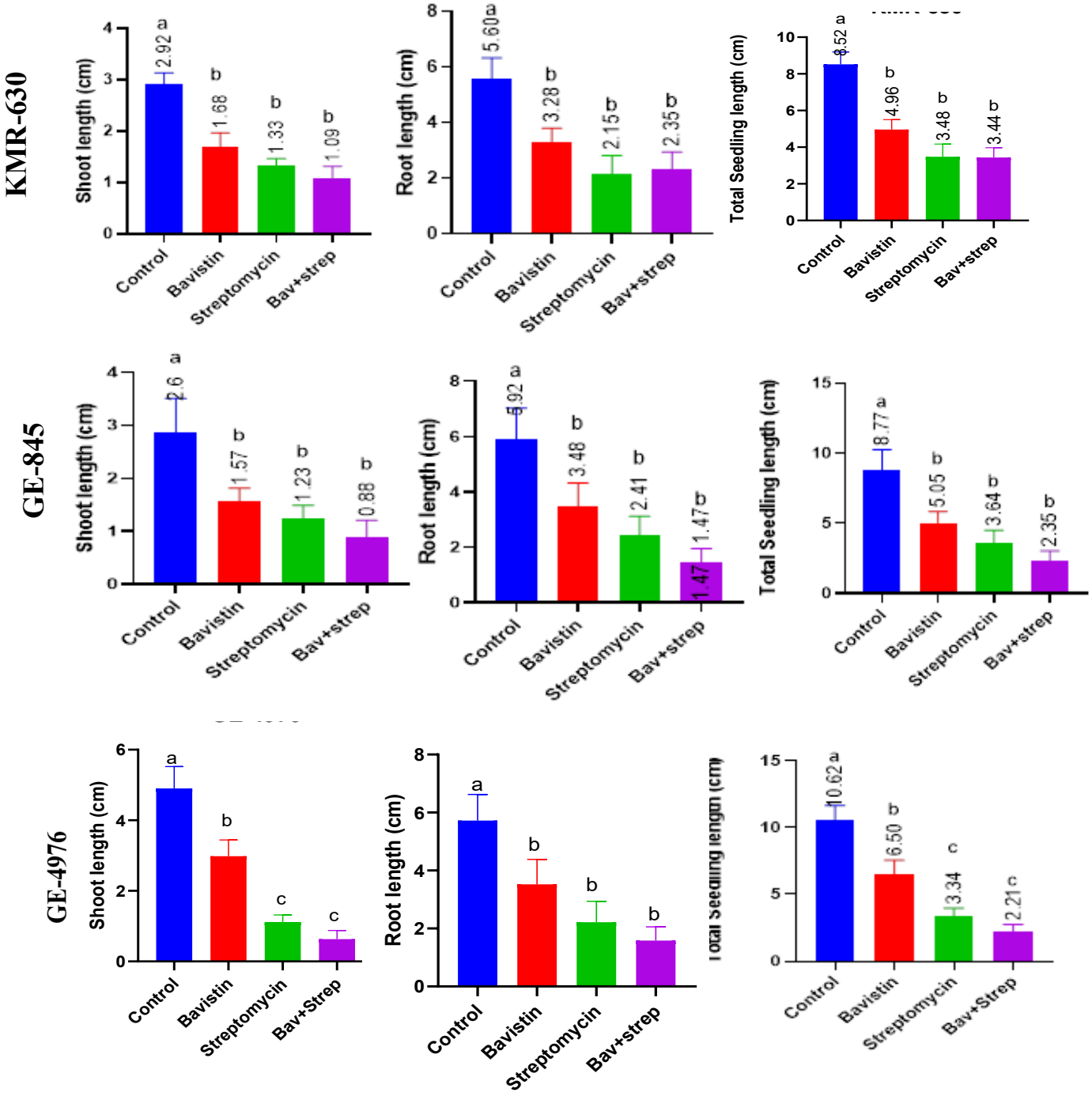
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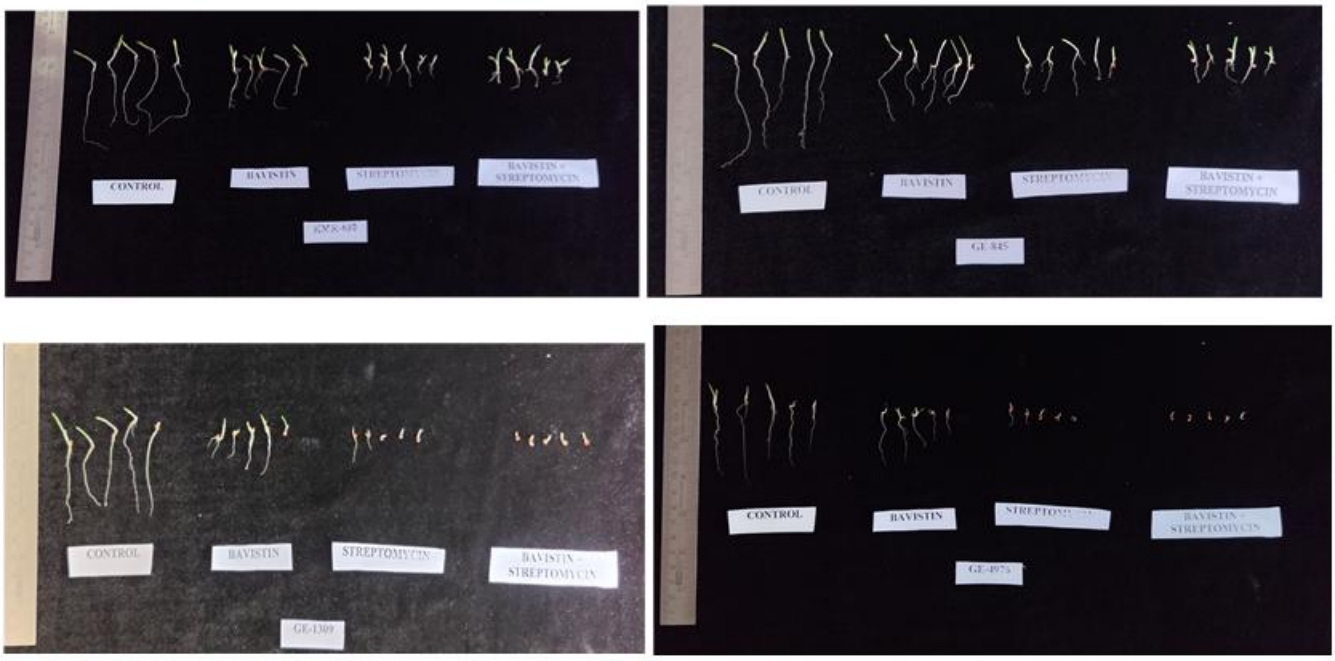


Fig. 2. Phenotypic expression of seedling growth of finger millet genotypes due to removal of endophytes treated with fungicide, bactericide and in combination (Genotypes used: KMR-630, GE-845, GE-4976 and GE-1309).

incarnatum) on seedling growth of finger millet genotypes (KMR-630 and GPU-28).

Genotype	Treatments	Root length (cm)	Shoot length (cm)	Total length (cm)
GPU-28	Control	2.43^{a}	1.72^{a}	4.14^{a}
	SF-5	3.56^{b}	2.08^{a}	5.64^{b}
	K-23	3.03^{ab}	1.75^{a}	4.78^{ab}
	SF-5 + K-23	4.12^{b}	1.96^{a}	6.08^{b}
KMR-630	Control	2.18^{a}	1.44^{a}	3.63^{a}
	SF-5	3.22^{b}	2.08^{b}	5.29^{b}
	K-23	3.46^{b}	2.01^{b}	5.47^{b}
	SF-5 + K23	3.26^{b}	2.08^{b}	5.33^{b}

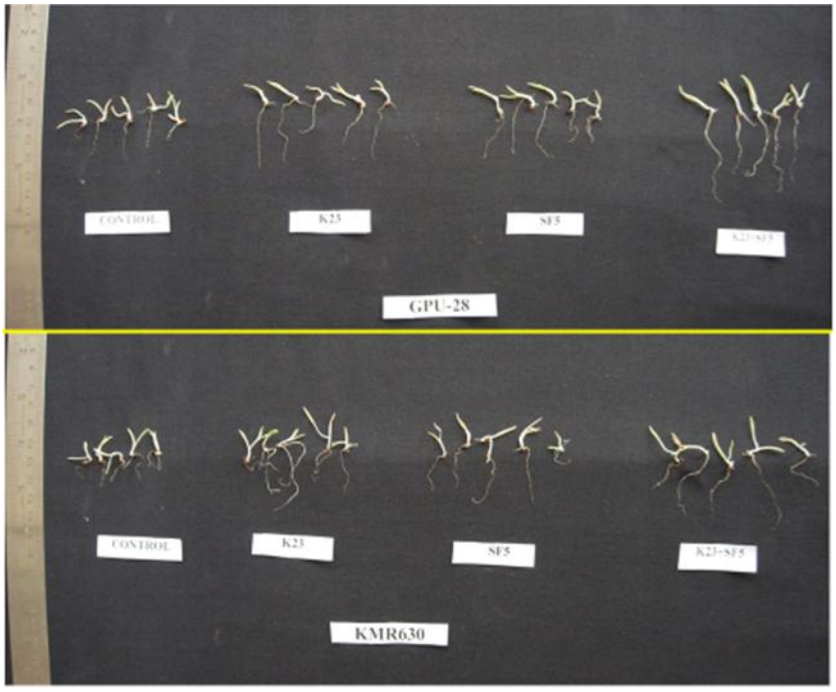


Fig. 3. Phenotypic expression of influence of endophytes on seedling length of popular finger millet genotypes (KMR-630 and GPU-28)

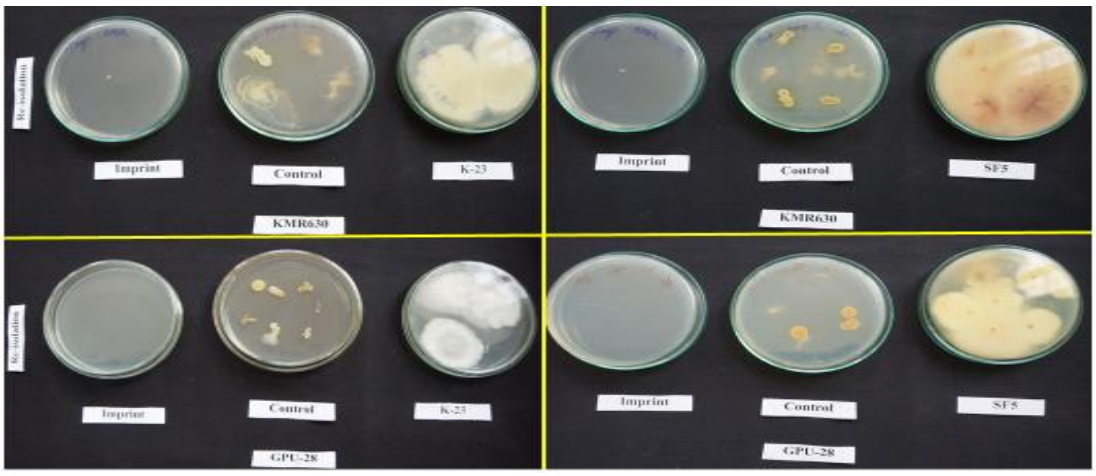


Fig. 4. Phenotypic expression of confirmation of colonization of endophytes (K-23 and SF-5; in the endophytes isolated from the root and shoot tissues of finger millet genotypes inoculated with habitat adapted endophytes)

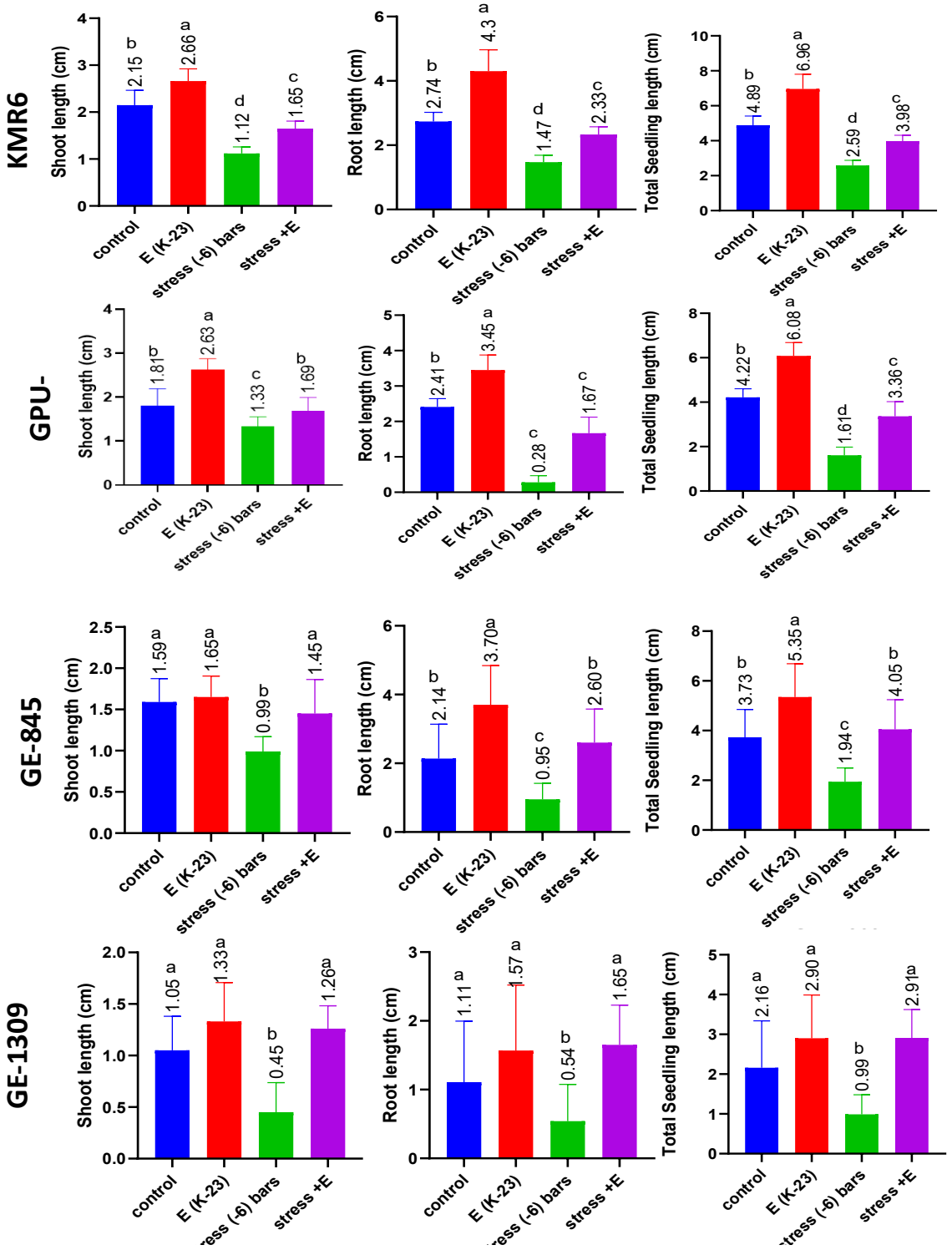


Fig. 5. Effect of K-23 fungal endophyte on seedling growth under PEG induced osmotic stress condition



Fig. 6. Influence of habitat adapted endophyte on seedling growth of finger millet genotypes under osmotic stress conditions