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Drought Tolerant Endophytic Fungi for Enhancing Early Seedling Growth under Stressful Conditions in Selected Crops

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Abstract

Plants have adapted a successful functional symbiosis mechanism to overcome extreme environment and for continuation of life cycle. Endophyticism is one such symbiosis which is now gaining tremendous pace in sustainable agricultural research strategy as an alternative for chemical free agriculture. An attempt was made to evaluate the potential of endophytes in alleviating drought stress and promoting early seedling growth in green gram, soybean and brinjal. Initially, endophytic fungal isolates were assayed for their ability to grow on matric modified media (PEG-8000) at ca. 0 MPa, -0.47 MPa, -1.45 MPa, -2.93 MPa, -4.92 MPa, -7.41 MPa. The nature of the response was revealed to be positive, as biomass(g) of endophytes was in proportional to induced drought stress. Then, the seedling growth of all crop systems was gauged in above mentioned PEG-8000 concentrations and LC_{50} (reduction in seedling length) was computed to be -2.57 MPa, -1.28 MPa, -3.38 MPa of PEG-8000 for green gram (BGS 9), soybean (KBS 23) and brinjal (Arka Harshitha), respectively. By reckoning LC_{50}, fungal endophytes were screened for growth promotion and drought stress alleviation in above-mentioned crop systems. Green gram (BGS 9) enriched with *Fusarium* sp. (SF-5, K-26, V4-J), *Chaetomium* sp. (LAS 6) and soybean (KBS 23) enriched with *Fusarium* sp. (V4-J) significantly enhanced seedling growth under normal as well as PEG-8000 induced drought stress condition. In brinjal (Arka Harshitha), there was no significant difference between endophyte treated and untreated seedlings. Our results suggest that host responses to colonization were variable and depended on the host ecotype which is controlled by variability in the host and endophyte genotypes.

Keywords : Endophytes, PEG-8000, Drought stress, Seedling growth

Plant-microbe interactions are among the vital processes that are not only essential for the survival of both the partners but also important in the functioning of the agricultural system. Among the plant interacting microbes, endophytic fungi have gained marvelous attention of plant biologists due to their mutualistic interactions with the host crops (Miransari, 2011). Whereas, drought stress is among the most destructive abiotic stresses that increased in intensity over the past decades and is expected to cause serious problems in more than 50 per cent of the arable lands by 2050 (Kasim *et al*., 2013). Plants perceive and respond to stress. Along with plants, the endophytes also perceive signals and transduce, which later leads to the expression of genes encoding proteins that are involved in providing drought tolerance. Several mechanisms have been proposed for endophytes-mediated drought stress tolerance in plants (Estrada *et al*., 2013), some of them include phytohormonal activity, synthesis of volatile compounds, antioxidant defense, accumulation of osmolytes and regulation of diverse types of genes and alteration in root morphology (Fig. 1). The term Induced Systemic Tolerance (IST) has been coined to accommodate the microbial-induced physical and chemical changes in plants, which result in enhanced tolerance to abiotic stresses (Yang *et al*., 2009). An amalgamation of seed priming with the application of plant beneficial fungi and bacteria can significantly improve seed germination and emergence, seedling establishment, crop growth and yield parameters under normal and stress conditions (Prasad *et al*.,

The Mysore Journal of Agricultural Sciences

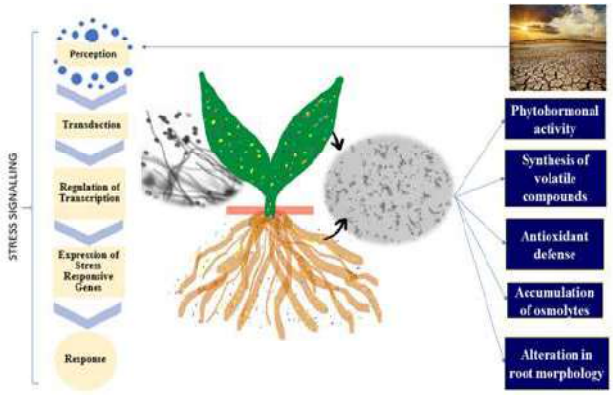

Fig. 1: Portraying potential mechanisms of endophytes in drought stress mitigation

2020). In this framework, the study was aimed to assess whether drought-tolerant endophytes promote seedling growth under normal and drought stress conditions in diverse crop systems like soybean, green gram and brinjal.

Material and Methods

The study was conducted at the Department of Seed Science and Technology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka.

Source of Endophytic Fungi

In a previous study, endophytes-maintained in School of Ecology and Conservation Laboratory, Department of Crop Physiology, University of Agricultural Sciences (UAS-B), Gandhi Krishi Vignana Kendra (GKVK), Bengaluru have been partially characterized for their ability to impart abiotic stress tolerance. Eight fungal endophytes isolated (Table 1) were selected for the study and rejuvenated on fresh Potato dextrose agar medium.

Pure Culture Response of Fungal Endophytes to Matric induced Water Stress

The experiment was used as the first confor-mational test in order to verify that the selected endophytes are able to grow in drought stress condition. PDA agar plug to PD amended broth is defined as the primary experiment. The combination reduces the impact of osmotic potential by utilizing chemically defined media and provides time for fungi to adjust to the environment. One 5 mm agar plugs containing actively growing mycelium were placed into an autoclaved 250 ml Erlenmeyer flask that contained 100 ml of Potato Dextrose Broth and the targeted amount of Polyethylene glycol (PEG-8000) according to the protocol of Michel (1983): $\Psi = 1.22 [PEG]^2 T - 134 [PEG]^2 - 4.4 [PEG]$. Each isolate was assayed at control (PDB without PEG), -0.47 MPa, -1.45 MPa, -2.93 MPa, -4.92 MPa, -7.41 MPa**.** The Erlenmeyer flask was covered with sterilized tin foil, sealed with Parafilm, placed in a shaker chamber (90 rpm at 27 °C) for seven days. Whatman filter paper was weighed to four decimal places and mycelium was filtered from the broth. The filter paper was rinsed with distilled water to remove any residual broth. The washed filter paper with mycelium was then placed in an oven for 12 hours, prior to obtaining the final weight. Reported fungal growth weight is the final weight of the mycelium plus filter paper minus the original weight of the filter paper. Growth types were assessed as outlined in Hutton *et al.* (1996).

In Vitro Evaluation of Green gram, Soybean and Brinjal Genotypes for Drought Stress

The seeds of drought-sensitive green gram [*Vigna radiata* (L.) Wilczek] genotype (BGS 9), soybean [*Glycine max* (L.) Merril] genotype (KBS 23) and brinjal (*Solanum melongena* L.) genotype (Arka Harshitha) were collected and surface-sterilized by following the standard protocol (Arnold *et al*., 2000). The method followed for standardization was the paper towel method with four replications for each treatment. Different levels of osmotic stress (-0.47 MPa, -1.45 MPa, -2.93 MPa, -4.92 MPa, -7.41 MPa) was created using different concentrations of polyethylene glycol (PEG-8000). Paper towel was soaked in the solution, excess solution was removed before exposing the seeds. Twenty-five seeds of each genotype were placed on the paper towel and incubated at 27 °C in the growth chamber. Seedling root and shoot length were recorded at the final count of the respective crop (Muddarsu and Manivannan, 2017). LC_{50} value was calculated by Probit analysis using statistical software

IBM SPSS statistics 20 (https://www.ibm.com/in-en/analytics/spss-statistics-software).

Screening of Fungal Endophytes for Drought Stress Tolerance under *in-vitro* Conditions

Seeds of drought-sensitive genotypes, BGS 9, KBS 23, Arka Harshitha of green gram, soybean and brinjal, respectively were surface sterilized as described previously and pre-germinated on sterile moist blotters. Five-day-old fungal colony culture was used for inoculum preparation and uniform pre-germinated seeds were treated with mycelial suspension (10^6 spores / ml) for 3 hrs (Zhang *et al.*, 2014). The concentration of mycelial suspension was calculated using hemocytometer. The corresponding control was treated with sterile distilled water. Then pre-germinated seeds were subjected to drought stress by placing them on sterile paper towel amended with PEG-8000 (at LC_{50} dose) with the corresponding control. The seedlings were incubated at 27 ºC in the growth chamber. Four replications for each treatment was maintained and each replication comprised of twenty-five seedlings. Root and shoot lengths were recorded on the final count after incubation (Sangamesh *et al*., 2017). The colonization ability of endophytes in treated seedlings was determined by re-isolation of the endophytes by preparing small segments of the shoot and root tissue using the standardized protocol (Schulz *et al*., 1993). The segments were surface sterilized as described above and inoculated on PDA and incubated at 28 ± 2 ºC for five days. The fungal colonies emerging out of the tissue segments were identified based on the morphological characteristics of the mother culture. The treatment details are, T_1 (Control), T_2 (Endophytes), T_3 (PEG-8000) and T_4 (PEG-8000 + Endophytes).

Results and Discussion

Pure Culture Response of Fungal Endophytes to Matric Induced Water Stress

Several methods utilizing PEG exist for evaluating the response of fungi to matric-induced water stress. Polyethylene glycol has been reported to be nontoxic and not metabolized by fungi (Mexal & Reid, 1973). All fungal endophytes (Table 1) increased biomass(g) at -7.41 MPa compared to control (without PEG-8000) which demonstrates a Type III growth response and significant change was in the increase of over-all growth, as determined by weight (Fig. 2). To overcome water stress, most fungi produce compatible solutes as compounds that are able to change in concentration in the cell in response to a change in external water potential, thus maintaining turgidity while having no significant effect on enzyme activity (Jennings and Burke, 1990). Hutton *et al.* (1996) examined PEG-induced, matric water stress tolerance (-0.16 MPa to -2.96 MPa) for ericoid endophytes of Epacridaceae hosts and described three growth response types: Type I: overall minimal growth after five weeks, Type II: maximum growth at

Table 1

Sites all over India from where plants were sampled for endophytic fungal isolation

Sample Code	Location	Latitude (°N)	Longitude (°E)	Altitude (MSL)	Plant part/ Host
K-23	Kargil (J&K)	34°34'22"	76°7' 57"	2750	Leaf
K-26	Kargil (J&K)	34°34'22"	76°7' 57"	2750	Leaf
P-10	Pangong Tso	33°43'2.74"	78°53' 29.08"	4250	Stem
P-37	Pangong Tso	33°43' 2.74"	78°53' 29.08"	4250	-
SF-5	Tamil Nadu	11.1271° N	78.6569° E	-	*Suaeda filiformis*
PJ-9	Karnataka	15.3173° N	75.7139° E	-	*Protis juliflora*
V4-J	Pokkali	9.9667°N	76.3168° E	-	Paddy (*Vytilla-4)*
LAS-6	Thar desert	27.4695° N	70.6217° E	-	*Lasiurus scindicus*

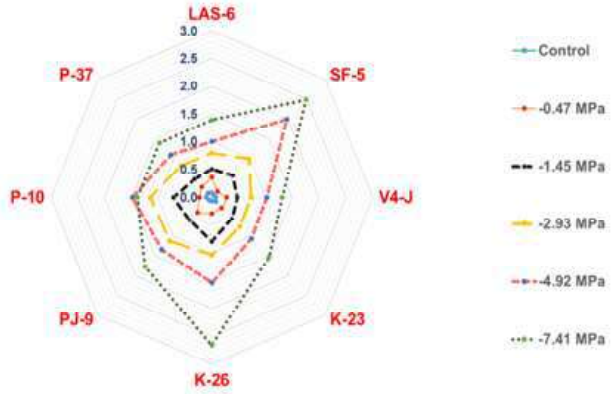

Fig. 2: Growth response of fungal endophytes to different matric induced water stress (PEG-8000) at 7 days on shake culture. Inoculum on PDA agar transferred to broth.

control with decreased growth as matric water stress increased and Type III: maximum growth under least degree of matric water stress. Chen *et al.* (2003) examined ericoid mycorrhizal (ERM) fungi for PEG induced water stress tolerance (-0.05 MPa to -2.24 MPa) and concluded that ERM endophytes function under water stress conditions. In axenic culture, the endophytic fungi from four saline habitat adapted plants showed tolerance to NaCl concentrations as high as 1.0 M. These results suggest that endophytes may also acquire tolerance to abiotic stresses, just as expressed by their respective host plants (Manasa *et al*., 2015).

In-vitro Evaluation of Green gram, Soybean and Brinjal Genotypes under PEG-Induced Stress

Polyethylene glycol, a non-toxic osmotic agent, is widely used to induce osmotic stress which reduces water potential. In the present study, in all three crops tested, the seedling growth was significantly affected as PEG-8000 concentration increased (Fig. 3). Results were expressed as absolute growth under different levels of drought stress along with control. The analysis indicated that osmotic potential of -2.57 MPa, -1.28 MPa and -3.38 MPa was the most differentiating concentration for assessing the tolerance levels in green gram (BGS 9), soybean (KBS 23) and brinjal (Arka Harshitha), respectively. The stress level was considered as sub-lethal concentrations to evaluate seedlings treated with different fungal isolates for stress tolerance. PEG induces artificial water deficit which affects water uptake, decreases turgor pressure and reduces cell division that eventually causes reduction in shoot and root growth (Khakwani *et al*., 2011).

Evaluation of Endophytic Fungal Isolates for Early Seedling Growth under Normal and Drought Stress Conditions in Crop System

All eight fungal isolates obtained from the culture collections were used to evaluate their ability to modulate early seedling growth in normal and drought stress conditions in crop systems such as green gram (BGS 9), soybean (KBS 23) and brinjal (Arka Harshitha). Green gram enriched with *Fusarium* sp. (SF-5, K-26, V4-J), *Chaetomium* sp. (LAS-6) and soybean enriched with *Fusarium* sp. (V4-J) significantly enhanced seedling growth under normal as well as PEG-8000 induced drought stress conditions. Yet in brinjal, there was no significant difference between endophytes treated and untreated seedlings (Table 2 and 3). Each endophytic isolate behaved differently in each crop system studied. This

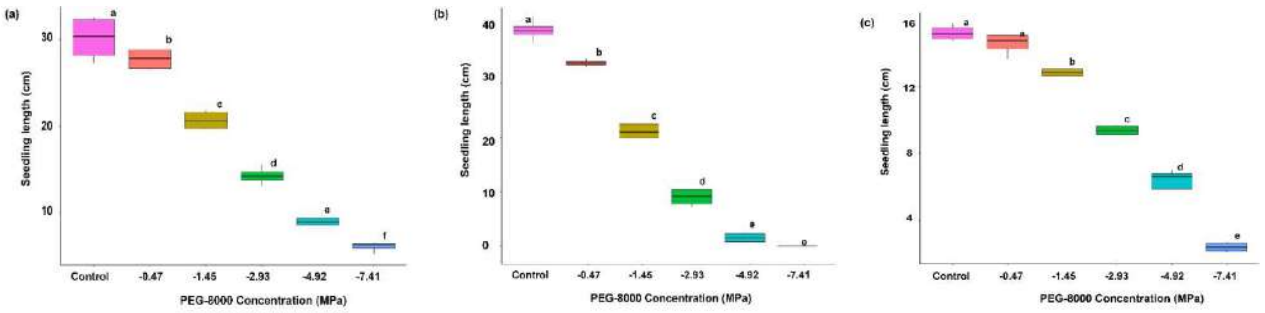

Fig. 3: Effect of PEG-8000 concentration on seedling length (cm) of (a) Green gram (BGS 9), (b) Soybean (KBS 23), (c) Brinjal (Arka Harshitha). Different letters indicate significantly different values (P<0.05)

TABLE 2

Effect of endophytic fungi on seedling length of green gram, soybean and brinjal under normal condition

Treatments	Normal condition [Seedling length (cm)]		
	Green gram (BGS 9)	Soybean (KBS 23)	Brinjal (Arka Harshitha)
Control	22.86 ± 0.26 ^{c}	34.01 ± 0.27 ^{b}	11.56 ± 0.13
LAS-6	25.57 ± 0.28 ^{a}	32.23 ± 0.31 ^{c}	11.49 ± 0.12
SF-5	25.59 ± 0.31 ^{a}	34.22 ± 0.30 ^{b}	11.41 ± 0.14
K-26	25.48 ± 0.37 ^{a}	34.62 ± 0.39 ^{b}	11.66 ± 0.16
PJ-9	22.33 ± 0.30 ^{c}	30.13 ± 0.31 ^{d}	11.73 ± 0.14
V4-J	26.06 ± 0.29 ^{a}	35.92 ± 0.31 ^{a}	11.83 ± 0.16
K-23	22.54 ± 0.31 ^{c}	34.59 ± 0.31 ^{b}	11.89 ± 0.18
P-10	23.88 ± 0.32 ^{b}	28.99 ± 0.31 ^{e}	11.71 ± 0.13
P-37	22.93 ± 0.28 ^{c}	34.79 ± 0.38 ^{b}	11.88 ± 0.18
F-Test (p=0.05)	*	*	NS
C.D (0.05)	0.84	0.89	-
S.Em±	0.3	0.32	0.15

Note: Means with same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Values represented are mean ± SE (n=40).

TABLE 3

Effect of endophytic fungi on seedling growth of green gram, soybean and brinjal under drought stress condition

Treatments	Drought stress condition [Seedling length (cm)]		
	Green gram (BGS 9)	Soybean (KBS 23)	Brinjal (Arka Harshitha)
PEG-8000	11.89 ± 0.16 ^{d}	14.08 ± 0.11 ^{c}	6.12 ± 0.08
PEG-8000 + LAS-6	13.85 ± 0.17 ^{b}	14.94 ± 0.19 ^{b}	6.10 ± 0.09
PEG-8000 + SF-5	13.81 ± 0.15 ^{b}	14.33 ± 0.13 ^{c}	5.98 ± 0.07
PEG-8000 + K-26	13.01 ± 0.17 ^{c}	14.22 ± 0.07 ^{c}	6.17 ± 0.08
PEG-8000 + PJ-9	10.80 ± 0.14 ^{e}	14.01 ± 0.10 ^{c}	6.19 ± 0.08
PEG-8000 + V4-J	15.60 ± 0.16 ^{a}	16.05 ± 0.10 ^{a}	6.01 ± 0.09
PEG-8000 + K-23	11.08 ± 0.15 ^{e}	16.03 ± 0.08 ^{a}	6.07 ± 0.09
PEG-8000 + P-10	12.08 ± 0.15 ^{d}	13.44 ± 0.14 ^{d}	6.09 ± 0.09
PEG-8000 + P-37	11.96 ± 0.15 ^{d}	15.09 ± 0.10 ^{b}	6.08 ± 0.09
F-Test (p=0.05)	*	*	NS
C.D (0.05)	0.43	0.33	-
S.Em±	0.16	0.11	0.08

Note: Means with same superscript in a column do not differ significantly as per Duncan Multiple Range Test (DMRT). Values represented are mean ± SE (n=40)

The Mysore Journal of Agricultural Sciences

could be due to the differences in plant-endophyte interactions and each host system may have unique signaling molecules or mechanisms. In some cases, endophytic fungal enrichment resulted in a lower magnitude of growth. Reduction in growth in response to endophytic colonization can be seen as weak parasitism (Mandyam *et al*., 2013) or as induction of host resistance resulting in the allocation of carbon to the production of expensive defense compounds rather than to vegetative growth (Aime *et al*., 2013). Positive effects could be due to the production of plant growth hormones (You *et al*., 2013) by the endophytes. Obledo *et al.* (2003) isolated *Fusarium oxysporum* from *Agave- victoria reginae* Moore. *In vitro* raised *Agave- victoria reginae* inoculated with the *F. oxysporum* showed a higher photosynthetic efficiency compared to uninoculated plants. Khan *et al*. (2015) isolated endophytic fungi *Fusarium tricinctum* RSF-4L from leaves of *Solanum nigrum*. The culture filtrate of the above fungus enhanced the growth attributes of Dongjin rice plants. Subsequently, gas chromatography / mass spectrometry analyses revealed that RSF-4L produced indole acetic acid. This indicates that endophytes synthesize bioactive compounds that could play important roles in promoting plant growth. Recent studies have demonstrated that certain endophytes promote host plant growth through the synthesis of phytohormones such as indole-3-acetic acid, gibberellins and cytokinins (You *et al*., 2013 and Khan *et al*., 2017).

The study demonstrates the importance of endophytes from plants that are adapted to extreme habitats and their importance towards the improvement of crop growth in stressed conditions. It would be interesting to examine the underlying physiological and molecular basis of such cross-adaptations to make the endophyte-based strategy more robust as an alternative to conventional crop improvement programs.

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