Biotechnological Communication

Molecular Characterization of Phosphate Solubilizing Endophytic Fungi and its Effect on Growth of the Maize, *Zea mays*

Uzma Choudhary[1]*, Harish Vyas[2], Chandra Prakash Patidar[3] and Alka Vyas[4]
[1]*School of Studies in Microbiology Vikram University, Ujjain, Madhya Pradesh, India*
[2]*Department of Botany, Government Kalidas Girls P.G. College, Ujjain, Madhya Pradesh, India*
[3]*Soil Testing Lab, Ujjain, Madhya Pradesh, India*
[4]*School of Studies in Microbiology, Vikram University, Ujjain, Madhya Pradesh, India*

ABSTRACT

In most of the agricultural soils phosphorus is present in considerable amounts but its availability to plants is limited because it is fixed in soil as insoluble phosphate. The over application of chemical phosphorus fertilizers does not increase the bioavailability of phosphorus but the leads to adverse effects on soil fertility. Phosphate solubilizing microorganisms (PSMs) can hydrolyse phosphorus compound into soluble form and make them available to plants. There is need of identifying such microbes which can be used as bioinoculants in agricultural soils to increase plant growth and productivity. In this study 64 endophytic fungi were isolated from maize plants by inoculating surface sterilized parts on potato dextrose agar medium. The phosphate solubilization index of fungal cultures was determined by measuring the hole zone formed around cultures on Pikovskaya's agar medium and the morphological identification of cultures was based on study of colony morphology and microscopic characters. It was seen that about 41% of the cultures had phosphate solubilization potential. The morphological characterization of cultures revealed that 23 cultures were of *Aspergillus niger*, 2 cultures were of *Penicillium oxalicum* and 1 culture was of *Curvularia* sp. aff. *C. Verruculosa* Tandon & Bilgrami ex M. B. Ellis. *Penicillium oxalicum* (E 137) and two culture of *Aspergillus niger* (E 204 and E 215) having high PSI were identified using molecular techniques and the effect of addition of spore suspension of these cultures on seed germination, seedling growth and plant growth was determined. The results of the study indicate that the addition of fungal spores significantly increases the seedling growth and plant growth in pot culture experiments suggesting that they may serve as potential biofertilizer/ bioinoculants.

KEY WORDS: ENDOPHYTIC FUNGI, PHOSPHATE SOLUBILIZING MICROORGANISMS, PLANT GROWTH, SEED GERMINATION, ZEA MAYS.

INTRODUCTION

Agriculture is the utmost important area of Indian economy and it accounts for 18% of GDP (Gross domestic product). About 50% of Indian population depends upon agriculture for their livelihood and increasing agricultural production is crucial for feeding current and future population of humans (Madhusudhan 2015). Phosphate is one of the major limiting factors for proper plant growth due to its low availability in agricultural fields. Phosphorus forms an essential constituent of many cellular molecules including nucleic acids, phospholipids, ATP's and enzymes. It is needed in almost every aspect of plant growth like growth of roots, shoots, formation of flowers, seeds, energy production, nitrogen fixation, etc. and it accounts for 0.2-0.8% of dry weights of plants (El-Hamshary et al., 2019; Kalayu 2019).

Most of the soils contain considerable amount of phosphate but its availability becomes limited because a large portion of phosphorus is fixed as insoluble phosphate of iron, aluminium and calcium. Organic

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matter present in soil is a major source of immobilized phosphate and 20-80% of phosphate present in soil is in this form. The soluble phosphate is highly reactive with other elements andin acidic soils phosphate is complexed with iron and aluminium compounds while in calcareous soils calcium phosphate is predominately formed (Rashmi et al., 2018).The acidic soils found in tropical and subtropical regions are often phosphorus deficient because they have high phosphate fixation capacity (Rashmi et al., 2018). Most of the former use chemical overcome phosphorus deficiency (Chouyia et al., 2020; Mazrou et al., 2020).

The addition of chemical fertilizers to overcome the phosphate availability in soil results not only in increase in agricultural cost but also causes adverse effect on soil health. The addition of phosphorus fertilizers in soils does not increase its bioavailability as 75-90% of phosphate fertilizers are precipitated by formation of metal ion complexes and result in fixation in soil and only 5% of added phosphorus is available to plants (Pande et al. 2017). The repeated non judicial application of phosphate containing chemical fertilizers result in loss of soil fertility by decreasing the microbial diversity. It has been suggested that accumulated phosphate in agricultural soils can sustain crop yields over 100 years (Gizaw et al. 2017). Phosphate solubilizing microorganisms are a group of microorganisms which help in solubilizing the phosphate present in soil and make it available for plant growth (Rashmi et al. 2018). The addition of PSMs to soil is an eco-friendly alternative which can make the phosphorus available to plant and can decrease the agricultural cost and toxic effect of indiscriminate use of chemical fertilizers (Qarni et al., 2021; Raymond et al., 2021).

The knowledge about phosphate solubilizing microorganisms has gradually increased over the last few years and a large number of bacteria and fungi have been identified which have high phosphate solubilizing activity. Fungi can travel long distances and are more important and they form about 0.1-0.5% of the total fungal soil population in nature (Mahadeva murthy et al., 2016; Zhu et al., 2017; Mazrou et al. 2020).Bioprospection of phosphate solubilizing endophytic microbes has become extremely relevant as they can colonize plants without inducing any apparent disease symptoms and benefit the host plant by providing protection against stress and pathogens (Zheng et al. 2016; Mazrou et al. 2020). They produce various secondary metabolites and help in different process of plant including nitrogen fixation, phosphate solubilization etc. (Santoyo et al. 2016). Phosphate solubilizing endophytic fungi are very competitive and aggressive colonizers. The common phosphate solubilizing endophytic fungal genera are *Aspergillus, Penicillium, Curvularia* and *Piriformospora*, where as the common phosphate solubilizing endophytic bacterial genera are *Bacillus, Pseudomonas* and *Rhizobium* (Matos et al. 2017; Singh et al., 2020; Abawari et al. 2021; Fouda et al., 2021).

An important constraint in use of biological organisms in agricultural fields is their inability to grow and adapt to different habitats. Hence, it is necessary that indigenous microorganisms may be isolated and identified which canbe used for inoculation in agricultural fields. The indigenous microorganisms are native to the particular environment as they areadapted for growth at particular climatic condition. The application of indigenous microorganisms as biofertilizers/ bioinoculants/ biocontrol agent is crucial for sustainable agriculture (Kumar and Gopal 2015; Jan et al. 2020; Fouda et al. 2021).

In view of above it was of interest to isolate and identify indigenous fungal genera having high phosphate solubilization potential, so they may be used in agricultural fields as bioinoculants for promoting sustainable agriculture in local fields. In the present study we have isolated and identified 26 end ophytic fungi having phosphate solubilizing activity from maize (*Zea mays*) plants growing agricultural fields of Ratadiya Village near local fields. Three cultures showing high phosphate solubilizing index, namely E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E137 (*Penicillium oxalicum*) were characterized at molecular level and their effects as bioinoculants on seedling growth and plant growth were studied.

MATERIAL AND METHODS

Plant material was collected in July, 2017 from the agricultural fields of Ratadiya village near Ujjain (M.P.). Randomly growing sixteen completely healthy and mature maize plants along with roots were uprooted, packed in sterilized polythene bags and brought to the laboratory. The plant samples were stored at room temperature and processed within 24 hours. Different parts of plant i.e., roots and leaves were used for isolation of endophytic fungi by following the modified method of Han et al., (2013).

The plant material was thoroughly washed with tap water and cut into 1-1.5 cm pieces and surface sterilization was done using 70% ethanol and 0.1% $HgCl_2$ for 2 minutes and then washed twice with sterilized distilled water. The plant pieces were placed on potato dextrose agar (PDA) medium containing chloramphenicol (40µg/ml) and incubated at 28 ± 2°C for 5 to 7 days. The hyphal tips which emerged from the plant material were aseptically cut and transferred to fresh PDA medium slants and incubated at 28 ± 2°C to obtained fungal cultures. Sub culturing of cultures was done to obtain pure cultures of endophytic fungi.

Morphological identification of fungal cultures was done based on study of colony characteristics and arrangement of spores using fungal keys. A small amount of culture was used for preparing wet mount on sterile glass slide. The culture was stained with lactophenol cotton blue (LCB) and the slide was examined under light microscope at different magnifications (10X, 45X and 100X). The identification was confirmed by National Fungal Culture Collection of India (NFCCI), Pune, India. The fungal

cultures were preserved in PDA slants and stored at -20°C (Visagie et al. 2014; Nyongsa et al. 2015). Molecular characterization of three cultures was done. Genomic DNA was extracted by using 5 to 7 days old fungal cultures grown on PDA medium. The DNA was amplified using primers ITS4(R-5'TCCTCCGCTTATTGATATGC3') and ITS 5(F-5'GGAAGTAAAAGTCGTAACAAGG3') and the PCR product were purified and sequenced. The resulting sequencing wasmatched in BLAST analysis software at NCBI (https://blast.ncbi.nlm.nih.gov) and phylogenetic tree were constructed using MEGA X software.

Fungal cultures were deposited at NFCCI (National Fungal Culture Collection of India), Pune, India, for obtaining accession numbers (Singh et al., 2020). The phosphate solubilizing potential of endophytic fungal cultures was determined by using modified method of Mazrou et al., (2020). The cultures were grown in Petri dishes containing Pikovskaya's agar medium at28 ± 2°C for 5 daysand the halozone formed around the fungal colony was measured. The phosphate solubilization index (PSI) was calculated by using the following formula:

$$PSI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

The spore suspension of fungal cultures was prepared in sterile distilled water. The fungal cultures of *Aspergillus niger* (E 204 and E 215) and *Penicillium oxalicum* (E 137)were grown in 250 ml conical flask containing 50 ml PDA medium for 10 days at 28 ± 2°C and the spores were harvested using 50 ml sterile distilled water. Concentration of spores per ml was determined using Haemocytometer and a final spore suspension 10^5-10^6 spores per ml was made. The spore suspensionwas used for bioinoculation on same day on which it was prepared (Mahadevamurthy et al., 2016). The seeds of *Zea mays* variety MRM 3777 were purchased from local market and stored in air tight containers at room temperature.

The maize seeds were surface sterilized using 70% ethanol and 0.1% $HgCl_2$ and washed twice with sterile distilled water. The seeds were soaked in spore suspension for 24 hours at room temperature and washed once with sterile distilled water. Three seeds were placed in sterilized Petri dishes containing sterilized cotton layer. The Petri dishes were incubated at 28 ± 2°C for 5 days. The experiments were done in triplicate and seeds treated with distilled water were used as control. After 5 days of growth the germination percentage and the length of root and shoot was measured. The plant parts were kept at 80°C for 2 hours after which their dry weights were determined. The vigor index was calculated using the mathematical expression described below (Mahadevamurthy et al. 2016).

Vigor index = Seed germination (%) x [Mean Root Length + Mean Shoot Length]

Soil from agricultural field of Ratadiya village was used for performing pot culture experiments. The air dried and sieved soil samples were used for determining pH, available potash, available phosphorus, available nitrogen and organic carbon (Wagh et al., 2013; Das et al., 2017). The soil used in experiments was sterilized three times with an interval of 24 hours between each sterilization cycle. This was done to remove microbes present in soil so that only the effect of inoculated fungal cultures could be observed. The experiments were performed in 1L poly propylene autoclavable beakers having capacity of containing 1kg soil. In each pot (beaker) 6 seeds were placed 1 cm below the top layer and 5 ml spore suspension (10^5-10^6 spores per ml) was added on the top of each seed then the seeds were covered with soil layer.

The pots were kept at room temperature and exposed to natural conditions throughout the day. The soil in pots was kept moist by adding 50-100 ml water every day according to weather conditions. After 7 days the plants were removed from the soil and the plant height, root length and area of largest leaf were determined. The plants parts were wrapped in aluminum foil and kept at 80°C for 24 hours and dry weights were determined to measure the biomass formed. The pot culture experiments were performed in the month of October and November (Singh et al., 2018). All the experiments were performed in triplicates and statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The mean values of samples and standard deviation were calculated. The values at $P \leq 0.01$ and $P \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Isolation and identification of phosphate solubilizing endophytic fungi: In the present study 64 endophytic fungal cultures were isolated from different parts of maize. Out of these cultures 20 (31.25%) were isolated from roots and 44 (68.75%) were isolated from leaves (Table 1). The phosphate solubilizing potential of fungal cultures was determined by measuring the halo zone formed around the cultures. It was seen that 26 (40.62%) fungal cultures had phosphate solubilizing activity (Table 1). The morphological identification of these cultures showed that 23 (35.93%) cultures belonged to *Aspergillus niger*, two (3.12%) cultures belonged to *Penicillium oxalicum* and one (1.56%) belonged to *Curvularia* sp. aff. *C. Verruculosa* Tandon & Bilgrami ex M. B. Ellis (Table 1). E137 (*Penicillium oxalicum*) showed highest phosphate solubilization index followed by E 215 (*Aspergillus niger*) and E 204 (*Aspergillus niger*) (Figure1 and Table 1). Molecular characterization two cultures of *Aspergillus niger* (E 204, E 215) and one culture of *Penicillium oxalicum* (E 137) was done using 18S rDNA sequencing and phylogenetic trees constructed using MEGA X software are shown in Figure 2, Figure 3 and Figure 4.

The accession numbers of cultures obtained from NFCCI, Pune are NFCCI 4851 (E 137: *Penicilliumoxalicum)*, NFCCI 4852 (E 204: *Aspergillus niger*) and NFCCI 4853 (E

215: *Aspergillus niger*). Earlier study of rhizospheric soil samples from different plants in Jimma town and Manna district farmlands in Ethiopia revealed that *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. had phosphate solubilization activities (Elias et al., 2016). Cultures of *Aspergillus niger* having high phosphate solubilization potential were also isolated from rhizospheric soil of *Cucumis, ocimum and* Eruca *plants* in Jeddah, Saudi Arabia (El-Hamshary et al., 2019).

Phosphate solubilizing endophytic fungi belonging to *Penicillium oxalicum*, *P. citrinums* and *Aspergillus* sp. Were also reported from sea weeds collected from Rameswarem coastal regions in India (Noorjahan et al., 2019). Culturable endophytes belonging to species of *Aspergillus niger* and *Penicillium oxalicum* having phosphate solubilizing potential have been reported from *T. wallichiana* (Adhikari and Pandey 2018).

Figure 1: Morphological characterization and phosphate solubilization potential of fungal cultures. A. Halo zone around colony of *Aspergillus niger* (E 204); B. Conidiospores of *Aspergillus niger* (E 204); C. Halo zone around colony of *Aspergillus niger* (E 215); D. Conidiospores of *Aspergillus niger* (E 215); E. Halo zone around colony of *Penicillium E. Halo zone around colony of Penicillium oxalicum (E 137); F. Conidiospores of Penicillium oxalicum (E 137)*

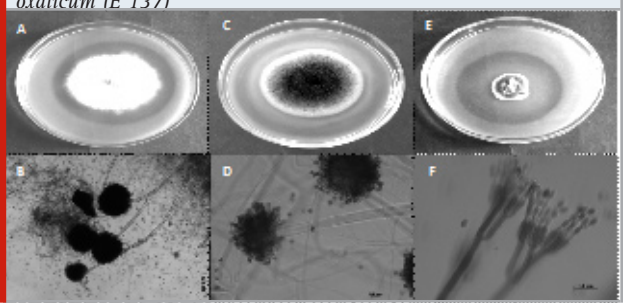


Table 1. Phosphate solubilization index of endophytic fungi isolated from different parts of maize

S. No.	Culture ID	Plant part used for isolation	Name of fungi	Phosphate Solubilization Index (PSI) on 5th Day
1	E 198	Root	*Aspergillus niger*	1.32±0.02
2	E 204	Root	*Aspergillus niger*	1.42±0.06
3	E 205	Root	*Aspergillus niger*	1.35±0.01
4	E 209	Root	*Aspergillus niger*	1.14±0.02
5	E 210	Root	*Aspergillus niger*	1.13±0.02
6	E 215	Root	*Aspergillus niger*	1.67±0.08
7	E 216	Root	*Aspergillus niger*	1.08±0.03
8	E 218	Root	*Penicillium oxalicum*	1.06±0.03
9	E 224	Root	*Aspergillus niger*	1.08±0.03
10	E 102	Leaf	*Aspergillus niger*	1.17±0.02
11	E 105	Leaf	*Aspergillus niger*	1.07±0.03
12	E 114	Leaf	*Aspergillus niger*	1.10±0.02
13	E 115	Leaf	*Curvulariasp.* aff. *C. verruculosa* Tandon & Bilgrami ex M. B. Ellis	1.02±0.02
14	E 118	Leaf	*Aspergillus niger*	1.04±0.03
15	E 122	Leaf	*Aspergillus niger*	1.27±0.03
16	E 123	Leaf	*Aspergillus niger*	1.23±0.04
17	E 124	Leaf	*Aspergillus niger*	1.10±0.02
18	E 125	Leaf	*Aspergillus niger*	1.37±0.04
19	E 128	Leaf	*Aspergillus niger*	1.12±0.04
20	E 135	Leaf	*Aspergillus niger*	1.10±0.02
21	E 137	Leaf	*Penicillium oxalicum*	2.93±0.05
22	E 139	Leaf	*Aspergillus niger*	1.04±0.03
23	E 142	Leaf	*Aspergillus niger*	1.35±0.01
24	E 147	Leaf	*Aspergillus niger*	1.08±0.03
25	E 158	Leaf	*Aspergillus niger*	1.08±0.03
26	E 164	Leaf	*Aspergillus niger*	1.16±0.04

Figure 2: Phylogenetic tree of the fungal culture E 204 (NFCCI 4852) using ITS region of rDNA. The tree was constructed using Maximum Likelihood method and Tamura-Nei model and the highest log likelihood (-4618.01) is shown in the figure. In this analysis 24 nucleotide sequences were used and evolutionary analysis was conducted in MEGA X

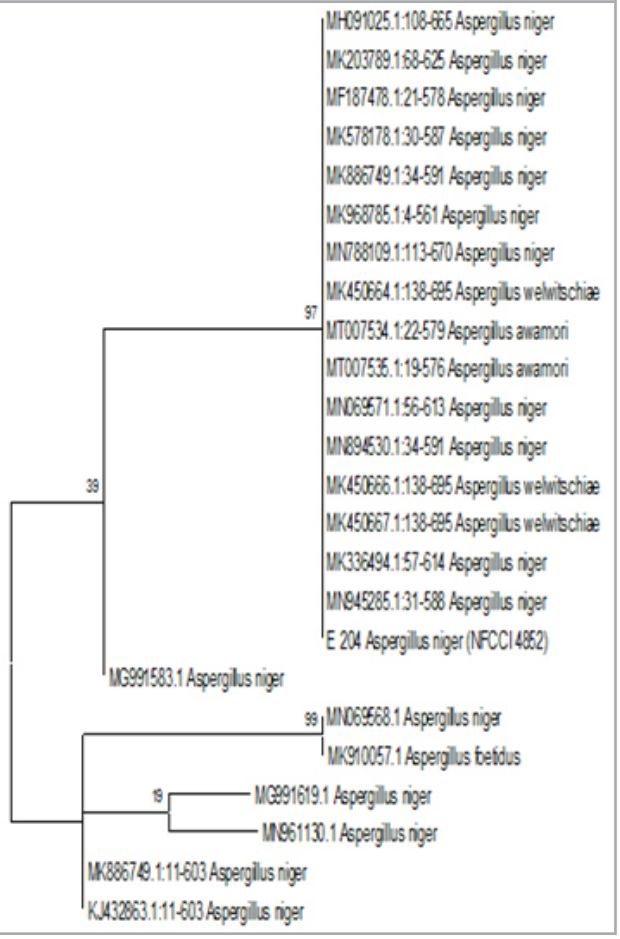
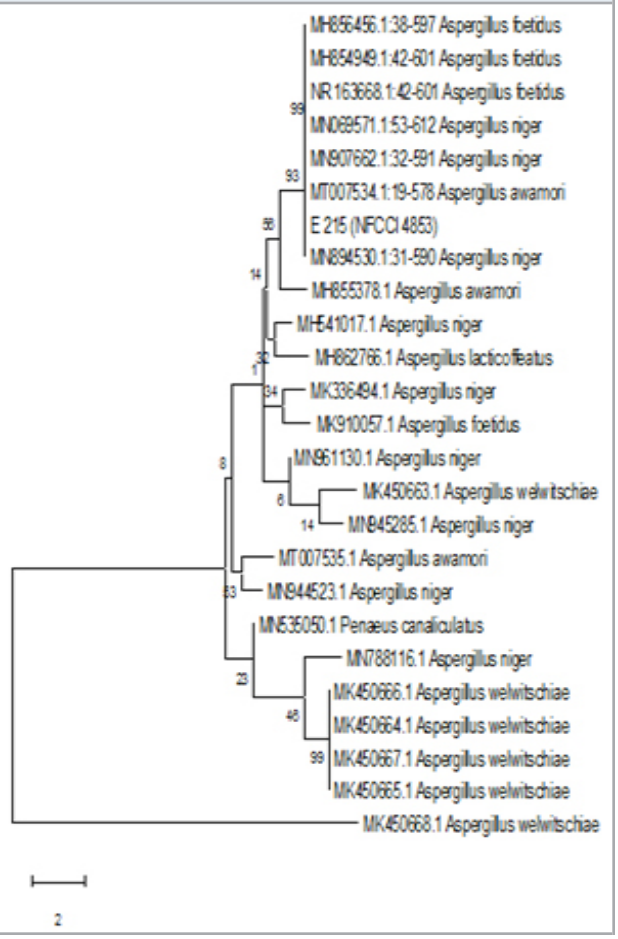


Figure 3: Phylogenetic tree of the fungal culture E 215 (NFCCI 4853) using ITS region of rDNA. The tree was constructed using Maximum Likelihood method and Tamura-Nei model and the highest log likelihood (-13958.58) is shown in this figure. In this analysis 24 nucleotide sequences were used and evolutionary analysis was conducted in MEGA X



In the present study also phosphate solubilizing potential was seen in endophytic fungal cultures belonging to *Aspergillus niger*, *Penicillium oxalicum* and *Curvularia* sp. aff. *C. verruculosa* Tandon & Bilgrami ex M. B. Ellis. Thus, the results of present study corroborate with the findings of earlier worker and strengthen the fact that cultures of *Aspergillus* spp. and *Penicillium* spp. have high phosphate solubilizing potential. It is suggested that the application of these fungi as bioinoculants in agricultural fields can act as alternatives of chemical fertilizers and help in promoting sustainable agriculture practices (Baron et al., 2018; Noorjahan et al., 2019). The potential of phosphate solubilizing microorganisms as biofertilizers / bioinoculants was also been suggested by other workers (Mazrou et al., 2020; Abawari et al., 2021).

Effect of fungal treatment on seed germination, seedling growth and plant growth: The effect of E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E 137 (*Penicillium oxalicum*), as bioinoculants on seed germination and seedling growth was studied in Petri dishes (Figure 5). It was seen that when spore suspension containing 10^5-10^6 spores per ml was used then percentage of germination in control (seeds treated with sterile distilled water) and seeds treated with fungal spores remained 100% indicating that these fungi do not have negative effect on germination of seeds when added in this concentration.

However, the application of spore suspension above this concentration resulted in damage to seeds. The effect of fungal treatment on root length and shoot length is shown in Figure 6. The analysis of results reveal that these fungal cultures significantly enhance the root length and shoot length. The mean dry weights of roots in control seeds were0.087±0.003 mg and the mean dry weights of roots of seeds treated with E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E 137 (*Penicillium oxalicum*) was 0.147±0.004 mg, 0.183±0.003 mg and 0.135±0.003 mg respectively. Similarly, the mean dry weights of shoots in control seeds were 0.673±0.004

mg and the mean dry weights of shoots of seeds treated with E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E 137 (*Penicillium oxalicum*) was 0.762±0.003mg, 0.883±0.004 mg and 0.889±0.004 mg respectively.

The seedling vigor also increased as a result of fungal treatment. The vigor index of control seeds was 1886 and the vigor index increased on treatment with fungal spores. The highest vigor index was seen by treatment with E 204 (3603) followed by E 137 (2856) and E 215 (2759). These results indicate that treatment of maize seeds with fungal spores significantly improves seedling growth and vigor of seedlings.

Figure 4: Phylogenetic tree of the fungal culture E 137 (NFCCI 4851) using ITS region of rDNA. The tree was constructed using Neighbor-Joining method. The sum of branch length = 0.27637271 is shown in tree. In this analysis 38 nucleotide sequences were used and evolutionary analysis was conducted in MEGA X

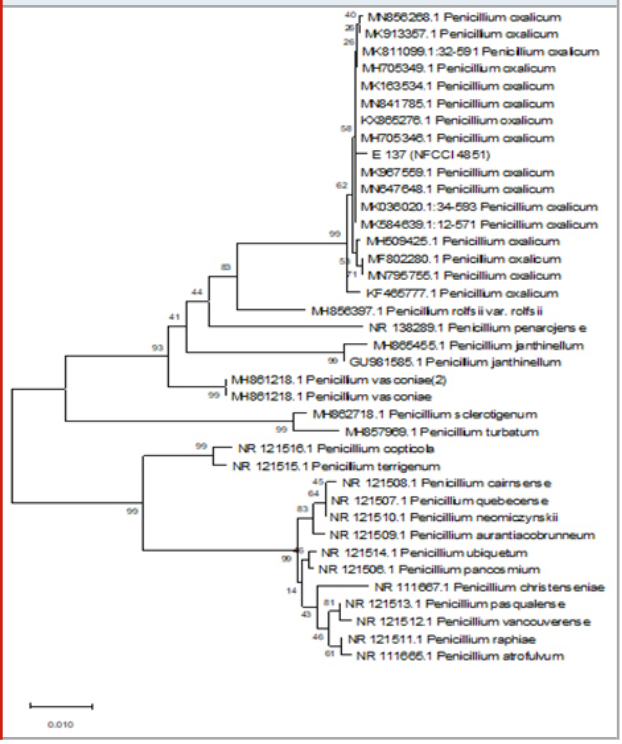
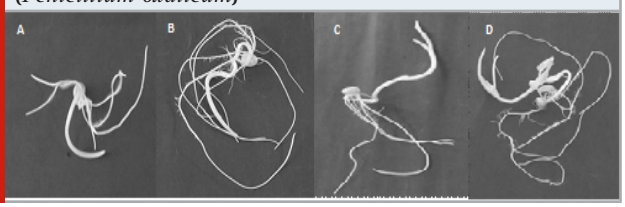


Figure 5: Effect of fungal spore suspension on seedling growth of maize. A. Control (Distilled water); B. E 204 (*Aspergillus niger*); C. E 215 (*Aspergillus niger*); D. E 137 (*Penicillium oxalicum*)



The effect of fungal treatment on plant growth was studied in pot culture experiments (Figure 7). The maize plants were grown in sterilized soil having pH 7.61, organic carbon 0.86%, available nitrogen 291 kg/ha, available phosphorus 36.33kg/ha and available potassium 723.33 kg/ha. This indicates that the agricultural field soil contains high level of phosphorus and other nutrients which could be due to continuous addition of chemical fertilizers in the agricultural fields. The plant height, root length, area of largest leaf and dry weights of roots and shoots were compared in control plants and in plant grown in soil supplemented with fungal spores of E 204, E 215 and E 137 (Yin et al., 2015).

Figure 6: Effect of fungal spore treatment on root length and shoot length of maize seeds

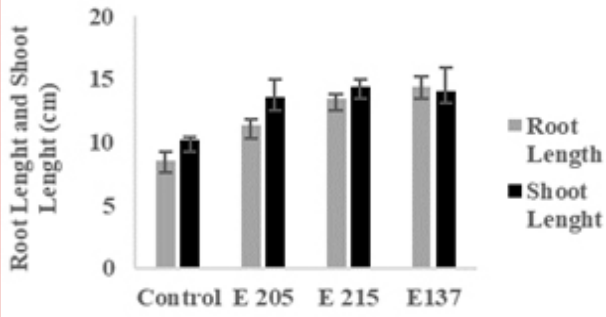
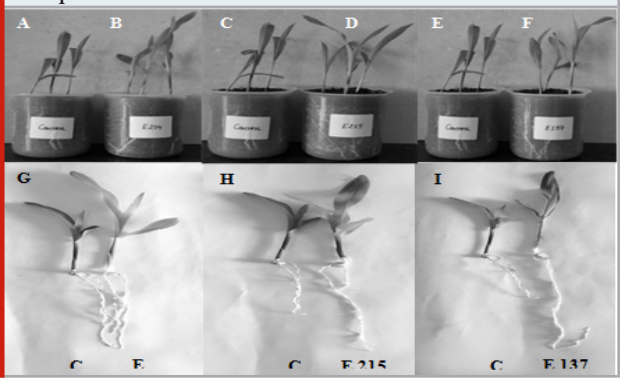


Figure 7: Effect of fungal treatment on growth of maize plants. A, C and E: Control; B, D and F: Treated with E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E 137 (*Penicillium oxalicum*) respectively. G, H and I: Comparison

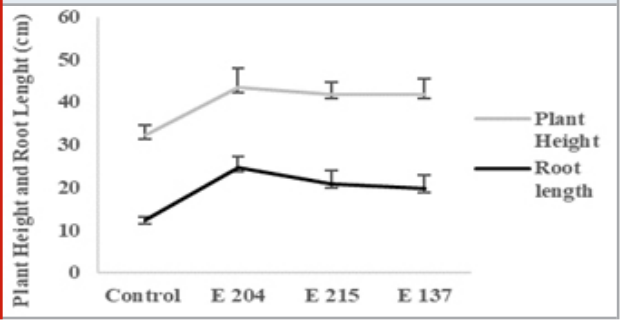


It was seen that root length and shoot length significantly increased inall the fungal treatmentsin comparison to control (Figure 7 and Figure 8). E 204 (*Aspergillus niger*) culture showed best results and there was 1.34 folds increase in plant height and 1.58 folds increase in root length when treated with E 204 (*Aspergillus niger*). There was 1.29 folds increase in plant height when treated with E 215 (*Aspergillus niger*) and 1.29 folds increase in plant height when treated with E137(*Penicillium oxalicum*). The root length increased 1.66 folds when treated with E 215 (*Aspergillus niger*) and 1.97 folds whentreated with E137(*Penicillium oxalicum*). Further, the mean dry weight of control plants was 0.117± 0.004 mg and the mean dry weight plants grown in soil containing E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E137(*Penicillium oxalicum*) was found to 0.123± 0.005

mg, 0.132± 0.004 mg and 0.123± 0.005 mg respectively (Jan et al. 2020).

Mean dry weight of roots of control plants was 0.122± 0.005 mg while the mean dry weight roots grown in soil containing E 204 (*Aspergillus niger*), E 215 (*Aspergillus niger*) and E137(*Penicillium oxalicum*) was seen to be 0.130± 0.004 mg, 0.140± 0.004 mg and 0.172± 0.004 mg respectively. The leaf area of the longest leaf of control plant was 81.9 cm^2 and it increased significantly when treated with fungal cultures.The largest leaf area was found by treatment with E 137 (170.0 cm^2) followed by E 215 (116.9 cm^2) and E 204 (109.2 cm^2). These results indicate that addition of fungal spores in soil positively influence the growth of maize plants which could be due to phosphate solubilizing ability of these fungal cultures or due to other plant growth promoting activities of these endophytic fungi (Yin et al. 2015).

Figure 8: Graph showing difference in plant height and root length of maize plants



Over application of chemical fertilizers for increasing the agricultural productivity adversely affects agricultural sustainability and causes harmful effects on environment. Hence, steps are needed to develop alternative strategies for improving plant nutrition and increasing agricultural production (Yin et al. 2015). There is a global trend for bioprospecting indigenous microorganisms which may be used for promoting sustainable agriculture practices and help in decreasing the use of chemical fertilizers in agricultural fields. It has been suggested that bioformulations of indigenous microbes having phosphate solubilizing activities may be used for promoting plant growth in view of food security scenario. The application of indigenous microbes is an ecofriendly, environmentally safe and healthy practice having potential to create better crops. It has been observed that endophytic microbes have several plant growths promoting activities including phosphate solubilization, siderophore production, IAA production, etc (Kumar and Gopal 2015; Ahkami et al., 2017; Jan et al., 2020).

The relationship of host plant with endophytic microorganisms is very special and significantly influence the formation of different metabolites in plant which provide various benefits to plants (Jia et al., 2016). In this study it has been demonstrated that bioinoculation of endophytic fungi belonging to *Aspergillus niger* and *Penicillium oxalicum* significantly improves growth of maize plants. Earlier studies have also demonstrated that inoculation of endophytic bacteria and fungi have positive influence on plant growth (Matos et al., 2017; Adhikari and Pandey 2018). It has been reported that endophytic fungi *Cladosporium cladosporiodes* positively influenced the shoot growth while *Aspergillus amstelodami* positively influenced the root length in the rice seedlings (Lalngaihawmi et al., 2018).

Study by Yin et al. (2015) reported that *Penicillium oxalicum* iscapable of promoting maize growth in calcareous soils and studies in Egypt have demonstrated that inoculation of *Penicillium crustosum* and *Penicillium chrysogenum* significantly increase root length in maize (Hassan et al., 2017). The plant growth promotion activities of various bacteria and fungi have also been reported by other (Banu et al., 2019; Aliyat et al., 2020, Chouyia et al., 2020; Turbat et al., 2020). Hence, our results are in accordance to earlier studies. Moreover, the fungal cultures isolated in this study are indigenous to this region andit appears that they may be used as bioinoculants for improving soil health and increasing plant growth. However, further studies are needed to examine the effects of these cultures in unsterilized soil and in field conditions (Turbat et al. 2020).

CONCLUSION

In this study 26 phosphate solubilizing endophytic fungi have been isolated and identified. Three fungal cultures having high PSI have been identified as *Aspergillus niger* (E 204 and E 215) and *Penicillium oxalicum* (E 137) using 18S rDNA sequencing. These three fungi positively influence the seedling growth and growth of maize plants in pot culture experiments when used as bioinoculants. The findings suggest that these fungi may be used as bioinoculants, biofertilizers for promoting sustainable agriculture. However, further studies are needed to strengthen these findings and examine the effect of these cultures in field conditions in unsterilized soils before theymay be used as bioinoculants in field conditions.

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