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Plant Growth Promoting Endophytic Fungal Association from *Cajanus cajan*, Linn. Plant which Can Improve Plant Growth Promotion

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Abstract: ***Endophytic microorganisms are little known for exogenous secretion of Plant growth regulator (PGR) and mitigation of salinity stress, which is a major limiting factor for agriculture production worldwide. Endophytic mycoflora are that dwell within robust plant tissues by having a symbiotic association. They are ubiquitously associated with all plants studied till date. Some endophytes are commonly found belonging to the genera Aspergillus sp., Drechlsera sp., Colletotrichum sp., Phomopsis sp., Gliomastix sp., Cladosporium sp., Trichoderma sp., and so forth. Endophytic population is greatly affected by climatic conditions in which they grows. They produce a wide range of compounds useful for plants for their growth. They are also found to have some important role in nutrient cycling, biodegradation, and bioremediation. In this review, we have tried to design the isolate phytohormone producing endophytic fungus from the roots, stems and leaves of Cajanus cajan, Linn., plant and identify its role in plant growth promotion. This study also suggests that these endophytic mycoflora can be used as bio - fertilizer in the agricultural fields. Because extensive use of artificial fertilizers, cause deterioration of environment and have hazardous effects for plants. Artificial fertilizers are very costly and must be supplied continuously to the fields while endophytic fungi stay in the field for years and safe to the plant and environment.***

Keywords: Endophytic microorganisms, PGR, IAA production, GA production, Endophytic population, etc

1. Introduction

Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intercellularly or intracellularly without causing any harmful effects. They are ubiquitous, colonize in all plants. They have been isolated from almost all plants examined till date. Endophytes are obligate or facultative parasites. They causes no harm to the host plants. They exhibit complex interactions with their hosts which involves mutualism and antagonism.

The plant growth regulation potential of fungal endophytes is due to the' production of phytohormones by endophytes such as indole-3-acetic acid (IAA), cytokinins, and other plant growth promoting substances. The endophytic fungi benefits to host plants, including tolerance to herbivory, heat, salt, disease, and drought. *Penicillium citrinum* and *Aspergillus fumigates* have been isolated as fungal endophytes that promoted plant growth by secreting gibberellins (GAs) and Indole -3- Acetic acid (IAA) in the plant parts of their hosts .

GAs are diterpenoid plant hormones, first detected in the 1920s from culture filtrate (CF) of *G. fujikuroi*, a known pathogen of rice plants. GAs appear to be involved in every aspect of plant growth and development, but their most typical property involves the enhancement of stem growth . GAs may modify the sex expression of flowers; induce the parthenocarpic development of the fruit, and delay senescence. Auxin is an important plant growth hormone. It was discovered in 1928 by Frits Went. Auxin controls many developmental processes and growth of plants such as cell division, cell elongation and differentiation, phototropic and geotropic responses, formation of flowers, fruit ripening, process of senescence and apical dominance. Auxin induce some variations in genes expression (Guilfoyle et al., 1998). IAA is produced by *Aspergillus niger*, *Herbaspirillum seropedicae*, *Acetobacter diazotrophicus*, *species of Erwinia, Rhizobium, Pseudomonas, Rhizopus, Azospirillum and Bacillus* (Ahmad et al., 2008). The current study aims to investigate biodiversity of isolates which produce more plant growth promoting phytohormones (IAA and GAs) and to reduce the excessive use of artificial fertilizers in future, because they are very costly, deteriorate environment and has negative impacts on plants.

2. Materials and Methods

2.1 Isolation of endophytic mycoflora from *Cajanus cajan*, Linn roots, stems and leaves

The water-cleaned plant samples were suspended in Tween 80 solution (2-3 drops in 50 ml autoclaved distilled water) and kept in shaking incubator set at 120 revolutions per minute (rpm) for 5 minutes at room temperature (Seena and Sridhar, 2004). Samples were surface-sterilized by suspending in 70% ethanol for 30 seconds, 3 % Sodium Hypo Chloride (NaOCl) for 5 minutes and again in 70% ethanol for 30 seconds. The root samples were dried between sterilized filter papers and cut into 0.25 cm pieces. Cork borer was used to cut leaves into 3–4mm diameter discs with and without midrib (Hallmann et al., (2007). Surface sterilized roots , stems and leaves pieces were placed on Czapek's dox agar medium plates (10 pieces/plate) containing antibiotic streptomycin (80 ppm) and incubated at 25ºC till the emergence of fungi (Khan et al., 2009a). To obtain pure cultures and for longer storage, PDA medium plates and slants were inoculated by the fungal isolates and fully-grown pure cultures were stored at 4ºC in refrigerator. For GA and IAA production, Czapek's broth medium (pH 7.3 ± 0.2) was used, incubated in a shaking incubator set at 30ºC with 120 rpm for 7 days (Hamayun et al., 2009; Khan et al., 2009).

2.2 Screening bioassay of fungal culture filtrates on Mung bean seedlings

Different strains were selected based on growth pattern and colonial morphology. 30 strains were selected out of 127 for screening bioassay experiment. 7 strains were isolated from the *Cajanus cajan.* Linn. (Tur) leaves, 8 from stems and 21 from roots. The fungal culture filtrates were filtered by using sterilized filter paper and then centrifuged at 5000xg at 4ºC for 15 min, for the separation of pellet and supernatant. These were kept at -70ºC in refrigerator for lyophilization. Supernatants were diluted in 1ml autoclaved distilled water which was applied on mung bean seedlings (Hamayun et al., 2009; Khan et al., 2009). Mature, healthy and uniform sized seeds were chosen by physical appearance.

Mung bean seeds were first washed with Tween 80 detergent and then rinsed 3 times with sterilized distilled water to remove the detergent. Clorox (5.25% sodium hypochlorite) and 70% ethanol were used for surface sterilization of seeds. Then the seeds were shifted to sterilized container, containing water. Kept in the culture room in the dark at room temperature for 5 days for the emergence of radicle and plumule. The germinated seeds, having uniform sized radicle and plumule were shifted to the flasks containing 30ml of 0.8% water-agar medium. These flasks were then kept in growth chamber containing day/night cycle: 14 h—28 °C ± 0.3; 10 h—25 °C ± 0.3; relative humidity 70%; 6 plants per treatment for 10 days. At two leaf stage of seedlings, with the help of micropipette, 100µl of fungal supernatants were applied at the seedling tips. Two other control treatments were also used, one with autoclaved distilled water and the other with Czapek broth medium. Root and shoot lengths were recorded after 5 days of application of fungal supernatants .

2.3 Data analysis

The data were subjected to Duncan Multiple Range Test by using IBM SPSS software version 21.0 (SPSS Inc, Chicago, USA).

3. Results and Discussion

Endophytic fungi isolated from the *Cajanus cajan*, Linn. A total of 37 different endophytic fungal strains were isolated from the roots (20), stems (9) and leaves (8) of selected plant. These fungal isolates were different from each other on the basis of external morphology, color, texture, reproductive structures (sporangium, spores) mycelium depth in the medium etc. The culture filtrates of all these endophytic fungal strains were screened for plant growth-promoting hormones by applying them on mung bean seedlings.

Fungal filtrates were applied on mung bean seedlings for the growth promoting and inhibiting activities of these isolates. After 5 days application of fungal cultural filtrates, shoot and root lengths were noted. Out of 20 isolates isolated from roots, 16 were found to be growth promoters, 3 neutral and no one growth inhibitor. Among them, only 5 were noted to be best growth promoter strains (enhance more than 80% shoot length). Out of 8 fungal isolates isolated from leaves, 5 were found as slightly growth promoters, 1 (CC -1-2-1) as growth inhibitor while 2 as growth neutrals. Two negative controls (one Czapek medium and one Distilled Water) were also taken and compared with fungal filtrates.

Table: Screening bioassay of fungal cultural filtrates obtained from the roots of *Cajanus cajan*, Linn. seedlings

Sr. No.	Fungal isolates	Plant height (cm)	Shoot length (cm)	Root length (cm)	Growth status
1.	Control (Czk)	12.87 ± 2.75	08.62 ± 2.1 ab	4.25 ± 0.75	NA
2.	Control (DW)	12.75 ± 0.59	07.80 ± 0.53	4.95 ± 0.89	NA
3.	CCR 1-3-4	19.57 ± 2.31	10.90 ± 1.24	8.67 ± 1.23	Promoted
4.	CC R2-4-1	18.37 ± 2.3	12.22 ± 3.2	6.15 ± 1.5	Promoted
5.	CC R1-3-1	23.87 ± 2.85	14.77 ± 1.3	9.10 ± 1.78	Promoted
6.	CC R1-3-2	18.57 ± 1.56	10.80 ± 0.97	7.77 ± 0.94	Promoted
7.	CC R2-4-5	21.35 ± 1.27	13.25 ± 0.58	8.10 ± 1.56	Promoted
8.	CC R1-1-10	13.95 ± 1.20	09.42 ± 0.53	4.52 ± 0.78	Promoted
9.	CC R1-2-3	15.90 ± 1.32	08.77 ± 0.95	5.87 ± 0.69	Promoted
10.	CC R1-3-5	20.75 ± 0.25	13.25 ± 0.75	7.50 ± 1	Promoted
11.	CC R1-3-6	19.27 ± 1.05	10.90 ± 1.02	8.37 ± 0.97	Promoted
12.	CC R1-5-1	17.50 ± 0.43	08.85 ± 0.2	8.65 ± 0.57	Promoted
13.	CC R2-4-5	21.35 ± 1.27	13.25 ± 0.58	8.10 ± 1.56	Promoted
14.	CC R2-1-1	19.00 ± 1	11.52 ± 0.71	7.47 ± 0.62	Promoted
15.	CCR2-3-4	21.47 ± 1.78	13.50 ± 1.3	7.97 ± 0.65	Promoted
16.	CCR 2-4-6	19.12 ± 1.69	11.30 ± 1.04	7.82 ± 0.73	Promoted
17.	CCR 1-4-2	21.62 ± 1.89	12.97 ± 0.8	8.65 ± 1.09	Promoted
18.	CCR 1-2-2	16.15 ± 1.9	07.85 ± 1.06	5.80 ± 1.02	Promoted
19.	CC R1-1	19.50 ± 2.52	12.42 ± 1.1	6.82 ± 1.44	Promoted

[**Note:** (cm) = Centimeters, Czk= Czapek Medium, DW = Distilled Water. Values with different letters in the same column in that group are significantly different at the 5% level by DMRT (Duncan's Multiple Range Test). Values within the table refers to the mean ± SE (n = 4)].

Majority of endophytic fungi form a mutualistic relationship with their host is very advantageous for both partners (Tejesvi, 2007). During mutualistic relationship, host plants get benefit in the form of enhanced growth and development. It also give immunity against abiotic and biotic stresses such as draught, heat, pathogens, enhancement of phosphorus absorption, nitrogen fixation, phosphate solubilization . It promotes production of Phytohormones (auxin, gibberellins, abscisic acid and cytokinin) which help in the plant growth and development (Firakova et al., 2007).

Using fungal culture filtrates, enhanced growth of mung bean seedlings, indicates the presence of plant growth promoting hormones (GA and IAA). They were also confirmed by using Perkin Elmer Lambda 25 spectrophotometer (Hamayun, 2008; Choi et al., 2005). Fungal culture filtrates had been known and will continue, to be a rich source of biologically active secondary metabolites (Tejesvi, 2007). Isolates were screened by the method used for the determination and identification of novel and biologically active microbial secondary metabolites is a simple (Higgs et al., 2001; Kumar et al., 2005). A similar procedure was used by Rim et al. for the detection, identification and quantification of plant growth promoting secondary metabolites in the *Fusarium proliferatum* (Rim et al., 2006). Results showed that out of 37 fungal strains, 18 were found to be growth promoters of mung bean seedlings,

only 6 were found to be growth inhibitor and the rest of strains were neutral or slightly growth promoters.

4. Conclusion

From all this study we can conclude that endophytic fungi are cosmopolitan. They are diverse group of living organisms. Endophytic mycoflora found in most parts of the plant body (root, stem and leaves) and play very important role for supporting their host plants. They are responsible for the synthesis of secondary metabolites such as GA_3 and IAA. These can be used as bio-fertilizer because extensive use of artificial fertilizers in agricultural fields cause deterioration of environment which have hazardous effects for plants. They are very costly and must be supplied continuously to the fields. Hence, use of endophytic fungi is suggested as bio-fertilizers in the future because they are safe to the plant and environment, and stay in the field for years.

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