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Cultural characteristics of endophytic fungal PGPMs associated with chickpea

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Abstract

Chickpea is third most important legume crop in India. It is one of the earliest cultivated annual legumes of the family Fabaceae. The boosting agricultural productivity relies heavily on the use of chemicals, which negative environmental impacts and also a major constraint to plant growth and yield. Therefore, to increase global agricultural production in a more economically and environmentally sustainable way, there is need to use plant growth-promoting microorganisms (PGPMs). PGPMs improve plant growth by enhancing the availability of nutrients, as phytostimulators, by regulation of phytohormones and by increasing plant tolerance against biotic stresses. The present investigation was focused on the compilation of endophytic fungal PGPMs from the chickpea plants. Forty fungal endophytic PGPMs were isolated from healthy chickpea plant samples collected from different districts of northern Karnataka and their cultural characters were observed. All the forty fungal endophytic PGPMs showed very distinct cultural characters among them.

Keywords: Chickpea, cultural characters, endophytes, legume, PGPMs

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the earliest cultivated annual legumes of the family Fabaceae (Zohary and Hopf, 2000) [9]. It's probable place of origin lies in South East Turkey. It is the third most important pulse in the world after common bean and field pea. India is the largest producer of chickpea in the world. In India, 11911.18 thousand tonnes of chickpea is cultivated under 9995.92 thousand ha of area with productivity of 1192 kg/ha (Anon., 2021) [1].

The productivity of crop relies heavily on the use of chemicals enhancing the crop growth and management of diseases, which are economically unavailable to many farmers throughout the world and can cause negative environmental impacts. In addition, environmental stresses may also be a major constraint to plant growth and yield, causing low crop productivity, affecting global food security (Lopes *et al.*, 2021) [5]. Therefore, to increase global agricultural production in a more economically and environmentally sustainable way, there is need to use lesser chemicals and increase plant tolerance to biotic stresses. The use of plant growth-promoting microorganisms (PGPMs) is potentially advantageous for improving crop productivity, food quality and security in more sustainable and eco-friendly manner (Etesami, 2020) [2].

The rhizosphere and endophytic fungal and bacterial community can harbor beneficial organisms known as PGPMs. Based on the interaction of roots with plants, PGPMs includes organisms present in the soil *i.e.*, Plant Growth Promoting Rhizobacteria (PGPR) as well as Plant Growth Promoting Fungi (PGPF) and also organisms present inside the plant *i.e.*, endophytes (Mitra *et al*., 2019) [6].

Endophytes are those microorganisms which live inside the plant tissues for part of their life cycle or for their entire lifespan without showing their presence as the plants do not express any visible symptoms. An endophyte can be fungi, bacteria or an actinomycete. Numerous studies suggested that endophytic PGPMs colonization may result in enhanced phosphorus, improved drought tolerance, improved plant growth and provides tolerance against various biotic and abiotic stresses (Suman *et al.*, 2013) [8]. Therefore, they can be used as bio-regulators to induce resistance against diseases and as biological control agents against certain pathogens as well as weeds. Most reported endophytes are *Pseudomonas* sp., *Bacillus* sp., *Burkholderia* sp. *Streptomyces* sp., *Actinoplanes* sp., *Alternaria* sp., *Trichoderma* sp., *Fusarium* sp., *etc*., (Mitra *et al*., 2019) [6].

However, despite the importance of the PGPMs-plant relationship, the knowledge on the interactions between PGPMs and crops under hostile environmental conditions is still rather limited in case of chickpea. Hence, there is a need to explore PGPMs for the purpose of improving plant growth and as well as management of chickpea diseases. Keeping this in view, it is essential to collect and isolate the PGPMs from different geographic regions for better understanding of their characters.

2. Materials and Methods

2.1 Collection, isolation, identification of PGPMs and their antagonistic potential against *Rhizoctonia bataticola*

2.1.1 Collection of PGPMs

Plant parts of healthy chickpea plants were collected during Rabi, 2020 for collection of isolates of endophytes. The isolates were collected from 11 different districts of northern Karnataka *viz*., Bagalkot, Bellary, Bidar, Dharwad, Gadag, Haveri, Kalaburagi, Koppal, Raichur, Vijayapur and Yadgir wherever chickpea is grown.

2.1.2 Isolation of endophytic PGPMs

Isolation of 40 fungal endophytic PGPMs from plant samples were carried out by randomly excising different parts (Leaf, shoot and root of 0.5 cm length each) using sterile scissors. The surface sterilization of selected plant tissues was done for by dipping in 1% sodium hypochlorite for 1 min. and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hypochlorite. After that, the pieces were then transferred by using forceps on to 70 per cent alcohol for few seconds followed by rinsing in sterile double distilled water and later tissues were dried in laminar air flow before placing it on nutrient medium. The sterilized plant tissues were transferred by using forceps on to the PDA nutrient medium which was previously added with 1% streptomycin to suppress bacterial growth. Later, Petri dishes were incubated at 25 ± 2 °C to obtain mycelial growth and required cultures were purified in PDA media for further studies. The mycelial growth for each isolate and cultural characters such as colony pigmentation, colony morphology, colony surface, margin, sectoring and zonations were observed after the incubation.

3. Results and Discussion

3.1Collection, isolation, identification of PGPMs and their antagonistic potential against *Rhizoctonia bataticola*

3.1.1 Collection of PGPMs

Plant parts of healthy chickpea plants were collected during rabi, 2020 from eleven districts of northern Karnataka *viz.*, Bagalkot Bellary, Bidar, Dharwad, Gadag, Haveri, Kalaburagi, Koppal, Raichur, Vijayapur and Yadgir wherever chickpea is grown.

3.1.2 Isolation of endophytic fungal PGPMs

Totally 40 fungal endophytic PGPMs were isolated from the samples collected from eleven districts of north Karnataka by tissue isolation method as described in 'Material and Methods'. Forty isolates were designated with the isolate codes such as FEPGPM-1 to FEPGPM-40. This indicated that the plant growth promoting microorganisms co-evolve with the host.

3.1.3 Identification of endophytic fungal PGPMs

Variability is the universal phenomenon. Every isolate differ in their cultural and morphological characters. The knowledge on these key characteristics of the microorganisms will enable the identification of unknown PGPMs isolates. Forty fungal isolates were observed for their cultural characters on PDA medium (Table 1).

The results on cultural characters of fungal endophytes in the present investigation indicated that the isolates varied with respect to colony diameter (30.05- 90.00 mm) (Table 1a), colony pigmentation (white, black, gray, maroon, green, golden brown, golden yellow, grayish black, grayish white, light maroon, brownish black) (Table 1b), colony margin (regular and irregular) (Table 1c), mycelial growth (uniform pluffy, uniform flat, uniform granular, uniform light pluffy, uniform flat granular, centered pluffy, centered granular and irregular pluffy) (Table 1d), sectoring (Table 1e), zonation and colony surface (smooth and coarse) (Table 1f) (Fig. 1).

Similar kind of variation in cultural characters was reported by Shirasangi and Hegde (2018) [7], Chen *et al.* (2012) [10] and Hamzah *et al.* (2018) [3].The studies conducted by Keerthi (2019) [4] on cultural characters of eighteen fungal stem endophytes from sunflower showed that 6 were light grey, 5 white, 4 creamish white and remaining 3 dark grey as observed in present investigation. With respect to growth rate, 6 were slow (3.0-5.0 cm) and 5 were fast growing (7.1-9.0 cm). In the present investigation also the isolates showed fast, moderate and slow growth. The colony margin also varied in 40 isolates and similar results on margin observed by Keerthi (2019) [4] where in 8 were regular and 10 were irregular. However, with respect to texture, 7 were smooth and 11 were coarse. The elevation of colony indicated 12 were flat and 6 were raised.

Table 1: Cultural characters of endophytic fungal PGPMs on potato dextrose agar

Sl. No.	Isolate code	Colony colour		Colony margin	Mycelial growth	Sectoring	Zonation	Colony surface	Colony diameter (mm)
		Front side of the plate	Reverse side of the plate						
1	FEPGPM- 1	Black	Black	Irregular	Uniform flat	Absent	Absent	Smooth	89.0
2	FEPGPM- 2	White	White	Regular	Uniform light pluffy	Present	Absent	Smooth	80.50
3	FEPGPM- 3	Creamish white	White	Regular	Uniform pluffy	Present	Absent	Smooth	79.02
4	FEPGPM- 4	Greyish black	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	44.00
5	FEPGPM- 5	White	White	Regular	Uniform pluffy	Absent	Absent	Smooth	81.02
6	FEPGPM- 6	Gray	Greyish black	Regular	Uniform light pluffy	Absent	Absent	Smooth	59.32
7	FEPGPM- 7	Black	Black	Regular	Uniform flat	Present	Absent	Smooth	90.00
8	FEPGPM- 8	Brown and black	Brown	Regular	Uniform pluffy	Absent	Absent	Smooth	63.50
9	FEPGPM- 9	Gray	Greyish black	Regular	Uniform light pluffy	Absent	Absent	Smooth	60.50
10	FEPGPM- 10	Black	Grey	Irregular	Uniform flat granular	Absent	Absent	Coarse	90.00
11	FEPGPM- 11	White mycelial growth with dark green centre	Light green	Irregular	Centered pluffy	Absent	Absent	Smooth	74.23
12	FEPGPM- 12	Green	Light green	Irregular	Uniform granular	Absent	Absent	Coarse	29.00

13	FEPGPM- 13	Grayish black	Black	Regular	Uniform flat	Absent	Absent	Smooth	90.00
14	FEPGPM- 14	Gray color with white border	Brown	Regular	Uniform pluffy	Absent	Absent	Smooth	85.56
15	FEPGPM- 15	White mycelial growth with purple centre	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	81.00
16	FEPGPM- 16	Golden brown	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	88.20
17	FEPGPM- 17	Grayish black	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	73.00
18	FEPGPM- 18	White	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	90.00
19	FEPGPM- 19	Black	Black with zonations	Irregular	Centered pluffy	Absent	Absent	Smooth	40.30
20	FEPGPM- 20	Black centre with white border	Black	Regular	Centered pluffy	Absent	Present	Smooth	69.00
21	FEPGPM- 21	Gray	Black	Regular	Uniform pluffy	Absent	Absent	Coarse	71.00
22	FEPGPM- 22	Green	Light green	Irregular	Uniform granular	Absent	Absent	Coarse	50.23
23	FEPGPM- 23	Grayish black	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	84.13
24	FEPGPM- 24	Golden yellow	Cream	Regular	Centered pluffy	Absent	Absent	Smooth	80.00
25	FEPGPM- 25	Grayish black	Black	Irregular	Uniform pluffy	Absent	Present	Smooth	80.00
26	FEPGPM- 26	Grayish black	Black and white	Regular	Uniform pluffy	Absent	Absent	Smooth	70.35
27	FEPGPM- 27	Black	Black	Regular	Uniform flat	Absent	Absent	Smooth	90.00
28	FEPGPM- 28	Creamy golden with green at centre	Golden yellow	Regular	Uniform flat granular	Absent	Present	Coarse	80.80
29	FEPGPM- 29	Grayish black	Black and white	Regular	Uniform pluffy	Absent	Present	Smooth	90.00
30	FEPGPM- 30	Green	Light green	Regular	Uniform flat granular	Absent	Absent	Smooth	80.45
31	FEPGPM- 31	Maroon	Black and maroon with zonations	Regular	Uniform pluffy	Absent	Present	Smooth	80.98
32	FEPGPM- 32	White	Yellow	Regular	Centered pluffy	Absent	Absent	Smooth	80.05
33	FEPGPM- 33	Grayish white	Black and brown with zonations	Regular	Uniform pluffy	Absent	Present	Coarse	90.00
34	FEPGPM- 34	Green	Light green	Regular	Uniform granular	Present	Present	Coarse	90.00
35	FEPGPM- 35	Black	White	Regular	Uniform granular	Absent	Present	Smooth	70.23
36	FEPGPM- 36	Grayish black	Black	Regular	Uniform pluffy	Absent	Absent	Smooth	75.03
37	FEPGPM- 37	Gray and white	Black	Irregular	Irregular pluffy	Absent	Absent	Smooth	38.08
38	FEPGPM- 38	Light maroon	Cream	Irregular	Centered granular	Absent	Absent	Coarse	39.02
39	FEPGPM- 39	Golden yellow and green	Brown	Irregular	Irregular pluffy	Absent	Absent	Coarse	90.00
40	FEPGPM- 40	Brownish black	Black	Regular	Uniform flat	Absent	Absent	Smooth	90.00

Table 1a: Grouping of fungal endophytic PGPMs based on colony diameter

Sl. No.	Isolate code	Colony diameter	No. of isolates
1	FEPGPM-1, FEPGPM-3, FEPGPM-5, FEPGPM-7, FEPGPM-10, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-18, FEPGPM-23, EPGPM-24, FEPGPM-25,FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-33, FEPGPM-34, FEPGPM-36, FEPGPM-39, FEPGPM-40	Fast growth (75-90 mm)	23
2	FEPGPM-2, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-17, FEPGPM-20, FEPGPM-21, FEPGPM-26, FEPGPM-35	Moderate (60-75 mm)	9
3	FEPGPM-4, FEPGPM-6, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-32, FEPGPM-37, FEPGPM-38	Slow growth (30-60 mm)	8

Table 1b: Grouping of fungal endophytic PGPMs based on colony color

Sl. No.	Isolate code	Colony color	No. of isolates
1	FEPGPM-2, FEPGPM-3, FEPGPM-5, FEPGPM-11, FEPGPM-15, FEPGPM-18, FEPGPM-32	White	7
	FEPGPM-1, FEPGPM-7, FEPGPM-10, FEPGPM-19, FEPGPM-20, FEPGPM-27, FEPGPM-35	Black	7
3	FEPGPM-4, FEPGPM-13, FEPGPM-23, FEPGPM-17, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-36	Grayish black	8
4	FEPGPM-12, FEPGPM-22, FEPGPM-30, FEPGPM-34	Green	4
5	FEPGPM-6, FEPGPM-9, FEPGPM-14, FEPGPM-21, FEPGPM-33, FEPGPM-37	Grey	6
6	FEPGPM-8, FEPGPM-40	Brownish black	2
7	FEPGPM-38, FEPGPM-31	Maroon	2
8	FEPGPM-24, FEPGPM-39	Golden yellow white	2
9	FEPGPM-16	Golden brown	1
10	FEPGPM-28	Creamy	1

Table 1c: Grouping of fungal endophytic PGPMs based on colony margin

Sl. No.	Isolate code	Colony margin	No. of isolates
1	FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-20, FEPGPM-21, FEPGPM-23, FEPGPM-24, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-33, FEPGPM-34, FEPGPM-35, FEPGPM-36, FEPGPM-40	Regular	30
2	FEPGPM-1, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-19, FEPGPM-22, FEPGPM-25, FEPGPM-39, FEPGPM-37, FEPGPM-38	Irregular	10

Table 1d: Grouping of fungal endophytic PGPMs based on mycelial growth

Sl. No.	Isolate code	Mycelial growth	No. of isolates
1	FEPGPM-3, FEPGPM-4, FEPGPM-8, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-21, FEPGPM-23, FEPGPM-25, FEPGPM-26, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-36	Uniform puffy	16
2	FEPGPM-1, FEPGPM-5, FEPGPM-7, FEPGPM-13, FEPGPM-27, FEPGPM-40	Uniform flat	6
3	FEPGPM-12, FEPGPM-22, FEPGPM-34	Uniform granular	3
4	FEPGPM-2, FEPGPM-9, FEPGPM-6	Uniform light pluffy	3
5	FEPGPM-10, FEPGPM- 28, FEPGPM-30	Uniform flat granular	3
6	FEPGPM- 11, FEPGPM- 19, FEPGPM- 20, FEPGPM- 24, FEPGPM-32	Centered puffy	5
7	FEPGPM- 37, FEPGPM- 39	Irregular puffy	2
8	FEPGPM- 38.	Centered granular	1

Table 1e: Grouping of fungal endophytic PGPMs based on sectoring

Sl. No.	Isolate code	Sectoring	No. of isolates
1	FEPGPM-2, FEPGPM-3, FEPGPM-7, FEPGPM-34	Present	4
2	FEPGPM-1, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-8, FEPGPM-9, FEPGPM-10, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM- 19, FEPGPM-20, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-28, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM- 32, FEPGPM-33, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40	Absent	36

Table 1f: Grouping of fungal endophytic PGPMs based on zonation

Sl. No.	Isolate code	Zonation	No of isolates
1	FEPGPM-10, FEPGPM-19, FEPGPM-26, FEPGPM-28, FEPGPM-29, FEPGPM-31, FEPGPM-33, FEPGPM-34, FEPGPM-35	Present	9
2	FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM-9, FEPGPM-11, FEPGPM-12, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-27, FEPGPM-20, FEPGPM-21, FEPGPM-22, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-30, FEPGPM-32, FEPGPM-36, FEPGPM-37, FEPGPM-38, FEPGPM-39, FEPGPM-40	Absent	31

Table 1g: Grouping of fungal endophytic PGPMs based on colony surface

Sl. No	Isolate code	Colony surface	No of isolates
1	FEPGPM-1, FEPGPM-2, FEPGPM-3, FEPGPM-4, FEPGPM-5, FEPGPM-6, FEPGPM-7, FEPGPM-8, FEPGPM- 9, FEPGPM-11, FEPGPM-13, FEPGPM-14, FEPGPM-15, FEPGPM-16, FEPGPM-17, FEPGPM-18, FEPGPM-19, FEPGPM-20, FEPGPM-23, FEPGPM-24, FEPGPM-25, FEPGPM-26, FEPGPM-27, FEPGPM-29, FEPGPM-30, FEPGPM-31, FEPGPM-32, FEPGPM-35, FEPGPM-36, FEPGPM-37, FEPGPM-40	Smooth	31
2	FEPGPM-10, FEPGPM-12, FEPGPM-21, FEPGPM-22, FEPGPM-28, FEPGPM-33, FEPGPM-34, FEPGPM-38, FEPGPM-39	Coarse	9

Fig. 1: Cultural characters of endophytic fungal PGPMs on potato dextrose agar

4. Conclusion

The study provided the information on cultural characteristics of endophytic fungal PGPMs which varied with respect to colony diameter, colony color, colony margin, colony surface, sectoring and zonation. Based on the cultural identification, we can further utilize these endophytes for testing their antagonistic potentiality against pathogens which cause diseases in chickpea and also their ability for plant growth promotion.

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