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Individual and combined effects of beneficial fungal root endophytes *Piriformospora indica* and *Glomus fasciculatum* on growth, nutrient uptake and IAA production in small cardamom

M. Aishwarya, M.K. Dhanya[1]*, Joy Michal Johnson[2], M. Murugan[3], R. Beena and Ambily Paul

College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram- 695 522 , Kerala, India
[1] Regional Agricultural Research Station, Kerala Agricultural University, Kumarakom, Kottayam- 686 563, Kerala, India
[2] Farming Systems Research Station, Kerala Agricultural University, Sadanandapuram, Kollam- 691 531, Kerala, India
[3]Cardamom Research Station, Kerala Agricultural University, Pampadumpara, Idukki-685 556, Kerala, India

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Elettaria cardamomum (L.) Maton is one of the highly-priced spice crops due to its pleasant scent. Propagation of cardamom is mainly accomplished through two methods, *viz.*, seedlings and suckers. The proper establishment of seedlings in the field determines the succeeding yield. Cardamom is prone to drought, floods and other biotic or abiotic stresses; and has a deleterious effect on its establishment in the field. Therefore, the production and development of superior seedlings with a well-established root system that can withstand the hostile field conditions during establishment is the need of the hour.

Endophytic fungi, *viz*., *Piriformospora indica* and *Glomus fasciculatum,* colonising within the roots of many crops asymptomatically is well known to promote plant growth, elicit defence responses against pathogens and ameliorate abiotic stresses such as drought and salinity and biotic stress caused by fungi, bacteria and viruses (Thomas *et al.,* 1989; Serfling *et al.,* 2007; Johnson *et al.,* 2013; Vijayan *et al*., 2018; Chandran *et al*., 2021). Growth promotion in cardamom seedlings in response to *Gigapora margarita* and *Glomus monosporum* reported by Sreeramulu and Bagyaraj (1998); and biocontrol potential of *G. fasciculatum* against cardamom damping-off and root-knot infestation in cardamom reported by Latha *et al*. (1994) and Thomas *et al*. (1989) respectively confirm the beneficial interaction of arbuscular mycorrhizal fungi with cardamom plants. This study primarily demonstrates the individual and combined colonisation of two beneficial root endophytes, *P. indica* and *G. fasciculatum,* in cardamom seedlings and their potential to enhance crop growth.

Seedlings of cardamom var. *Njallani* were raised in a nursery using a sterile potting mixture (soil:sand:cow dung = 1:1:1). Seedlings of 2-3 leaf stages were used for root colonisation studies. *P. indica* (No. INBA3202001787) was mass-multiplied in coir-pith-cow dung medium amended with two per cent gram flour as per the protocol of Jojy *et al.* (2020). Vermiculite based inoculum of *G. fasciculatum* (KAU strain) was colonised in cardamom seedlings by placing in the root zone at the rate of two per cent w/w. The endophyte colonisation in cardamom roots was confirmed by the presence of chlamydospores of *P. indica* and

**Corresponding Author: dhanya.mk@kau.in*

arbuscules and vesicles of *G. fasciculatum* as per Johnson *et al.* (2013) and Philips and Hayman (1970), respectively. After colonisation, plant height, leaf breadth, and the number of leaves were recorded for up to six months (just before transplanting into the field). Fresh and dry weights of the shoot and root were taken at the end of the experiment (six months after transferring to the polybag nursery).

The P and K analyses were done as per the protocol of Jackson (1973) and Black (1965), respectively and the changes in endogenous auxin levels were carried out as per the protocol of Gordon and Weber (1951) upon endophyte(s) inoculation in the roots.

The experimental design was a completely randomised design (CRD) with ten replications per treatment, and five plants were kept under each replication. When the effects were found to be significant, the critical difference (CD) value was calculated for each observation using 't' values at a five per cent level of significance. For biometric characters, factorial analysis was carried out with different growth intervals as an additional factor. All the data were analysed using GRAPES 1.0.0. Software (Gopinath *et al.,* 2020).

Successful root colonisation was observed in *P. indica* and *G. fasciculatum* treated roots six and seven days after inoculation. Chlamydospores of *P. indica* were observed in cortical and epidermal layers. *G. fasciculatum* colonisation was confirmed by the presence of arbuscules and vesicles inside the roots (Fig 1). Aishwarya *et al.* (2021) reported *P. indica* colonisation and dual colonisation by *P. indica* and *G. fasciculatum* in small cardamom roots for the first time. Both the endophytes, individually and in combination, colonised inside roots and promoted growth, shoot and root biomass, and the number and area of cardamom leaves (Table 1). The seedlings dually inoculated with *P. indica* and *G. fasciculatum* were superior at all stages of the study with maximum height, number of leaves and leaf length, *i.e.*, 70.28, 10.20 and 39.24 cm, respectively, at 180 days after the fungal endophyte inoculation (just before transplanting to the field). The factorial analysis of vegetative parameters at different intervals as additional factors revealed their interactive effect. It was observed that the plant height of dually-colonised plants at the 5^{th} and 6^{th} months and that of *P. indica*-colonized plants at the 6^{th} month were superior and statistically on par. The highest plant height of dually-colonised plants in the 5^{th} month enables them to be transplanted in the field one month earlier. The number of leaves was also high in the dually-colonised plants at the 5^{th} and 6^{th} month growth stages and was on par.

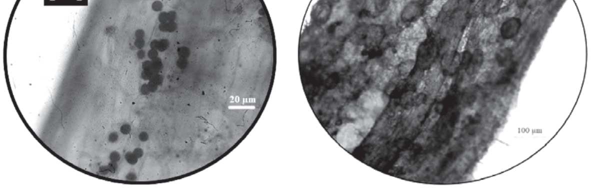

Fig. 1. Micropscopic view of root colonisation of endophytes in cardamom seedlings
a) Chlamydospores of *P. indica* inside root cortex
b) Vesicles of *G. fasciculatum* inside cardamom root

The endophytes colonisation had remarkable positive effects, both individually and in combination, on the growth and development of roots compared to the control (Figs. 2 and 3). The root length and number of secondary roots at six months after the endophytes colonisation were superior and on par compared to control. The root volume was significantly highest for *P. indica*-colonized plants (27.60 cm^3), followed by the dually colonised (23.20 cm^3) and *G. fasciculatum*-colonized (15.40 cm^3) seedlings compared to uninoculated plants (7.40 cm^3). Both fresh and dry weights were more in the fungal root endophytes-colonised plants (Table 2, Fig. 4). *P. indica*-colonized plants recorded the highest root weight (43.00 g $plant^{-1}$ fresh and 4.95 g $plant^{-1}$ dry), whereas the combination of the endophytes contributed the highest shoot weight (95.62 g $plant^{-1}$ fresh and 13.26 g $plant^{-1}$ dry). The root dry weight of combinatorial treatment was on par with *P. indica*-colonized plants though there was a significant difference in their fresh root weight. These findings are in accordance with Yaghoubian *et al.* (2014) and Das *et al.* (2014), where *Glomus* sp. and *P. indica* promoted the growth of wheat and rice plants, respectively, and enhanced root and shoot biomass in the endophytes-colonised plants.

Table 1. Effect of individual and combined colonisation of the endophytes, *P. indica* and *G. fasciculatum* on different vegetative characters of cardamom seedlings at 180 days after colonisation

Treatments	Plant height (cm)	Number of leaves	Leaf length (cm)
P. indica	67.90±4.40[ab]	7.60±1.34[b]	36.72±5.02[a]
G. fasciculatum	63.64±5.57[b]	6.60±1.64[b]	34.38±4.85[ab]
P. indica + *G. fasciculatum*	70.28±2.23[a]	10.20±0.83[a]	39.24±1.80[a]
Control	48.38±4.79[c]	5.40±0.55[c]	30.38±3.69[b]
SE ± (m)	2.20	0.45	1.81
CD (0.05)	5.932	1.572	5.430
CD (0.05) Interaction	4.229	1.160	NS

Values are mean of 10 replications ± standard deviation; the values following the same letters in the superscripts are not significant at 5% level of significance

The nutrient analysis of plant samples revealed the highest uptake of phosphorus to root by *G. fasciculatum*-colonized seedlings, *i.e.,* approximately 12-fold higher than that of control. Nevertheless, the phosphorus uptake to the shoot was 11-fold higher, and leaves were 9-fold higher in dually-colonised seedlings and *P. indica*-colonized seedlings, respectively (Table 3). *P. indica* stimulates the release and availability of immobilised P to plants by higher phosphatase activity and higher expression of the gene ACP5 in *Brassica napus* (Wu *et al.,* 2018). Moreover, its ability to grow on various P sources such as inorganic, organic, and polyphosphates reflects its role as an active P-solubilizer (Johnson *et al.,* 2014). *P. indica*-phosphate transporter (PiPT), which aids in the movement of phosphates from the soil to the plant, has been identified in maize (Yadav *et al.,* 2010). Likewise, AMFs also have active Pi transporters that take Pi from the soil and transport it to the plant. On the other hand, plants also have mycorrhizal-specific Pi transporters responsible for obtaining Pi from the apoplast and transporting it to the cytoplasm (Harrison and van Buuren, 1995). Khatun (2020) also reported that the symbiotic association of *G. fasciculatum* amounts to greater

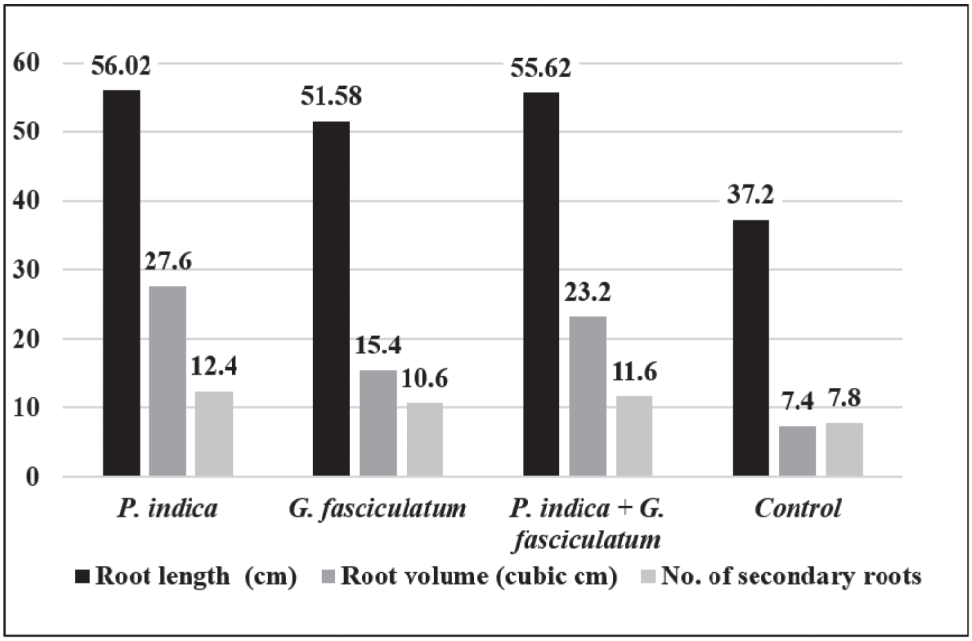

Fig. 2. Effect of individual and combined colonisation of the endophytes, *P. indica* and *G. fasciculatum* on root parameters of cardamom seedlings at 180 days after colonisation

Fig. 3. Root architecture (6 month) of endophyte colonised cardamom seedlings compared to the uninoculated control (a) *P. indica* colonised seedling compared to control seedling (b) *G. fasciculatum* colonised seedling compared to control seedling c) Dually colonised *P. indica* and *G. fasciculatum* seedling compared to control

phosphorus uptake and promoted different growth parameters like plant height, root length, number of roots, number of leaves, and fresh weight in *Coleus forskohlii*.

The endophytes colonised plants had significantly higher uptake of K in the root, shoot, and leaves than the control (Table 3). K uptake in shoot, root and leaf of the endophytes-colonised plants were on par. A similar increase in K was reported by Kumar *et al*. (2012) in *P. indica*-colonized mung bean plants in glasshouse and field conditions and other nutrients (N and P), which remarkably contributed to the growth promotion. K is an essential element and is also found in spores, hyphae, and vesicles of AM fungi (Pallon *et al.,* 2007). Additionally, the up-regulation of a plant K^+ transporter has been reported in AMF colonised roots of *Lotus japonicus* (Guether *et al.,* 2009).

Auxin content was higher in *P. indica*-colonized seedlings (83.80 µg g^{-1}) followed by combinatorial (44.80 µg g^{-1}) and *G. fasciculatum* (43.33 µg g^{-1}) treated seedlings. *P. indica*-colonized plants had approximately twice the amount of IAA compared to the non-colonised seedlings (40.62 µg g^{-1}). The higher endogenous level of IAA in *P. indica*-colonized plants would have resulted in superior root growth compared to the dually-colonised plants, though the latter recorded superior shoot growth. Lee *et al.* (2011) and Hilbert *et al.* (2012) stated that the promotion of the growth and

Table 2. Effect of individual and combined colonisation of the endophytes, *P. indica* and *G. fasciculatum* on root and shoot biomass of cardamom seedlings at 180 days after colonisation

Treatments	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})
P. indica	43.00±1.99^{a}	4.95±0.57^{a}	86.02±10.92^{b}	12.19±1.62^{ab}
G. fasciculatum	27.14±0.51^{c}	3.98±0.52^{b}	75.86±3.34^{c}	10.76±1.79^{b}
P. indica + *G. fasciculatum*	33.26±1.42^{b}	4.62±0.93^{ab}	95.62±4.97^{a}	13.26±1.12^{a}
Control	19.46±1.15^{d}	2.93±0.16^{c}	54.24±3.60^{d}	8.61±1.59^{c}
SE (m) ±	1.77	0.27	2.90	0.69
CD (0.05)	1.844	0.941	8.692	13.850

Values are mean of 10 replications ± standard deviation; the values following the same letters in the superscripts are not significant at 5% level of significance

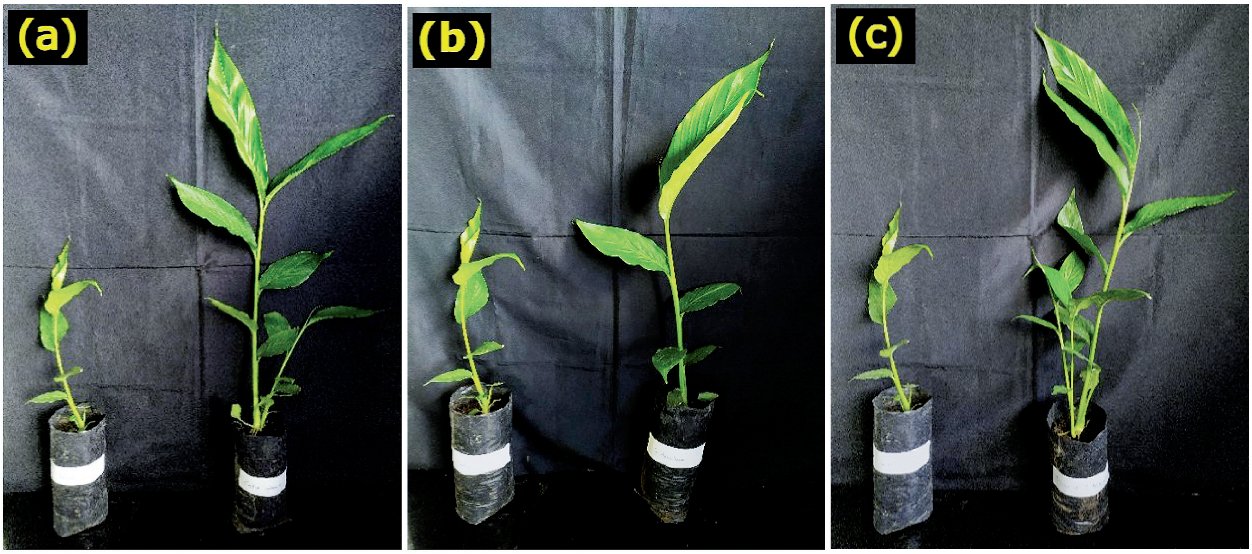

Fig. 4. Comparison of plant growth (5 month) in endophyte colonised cardamom seedlings var. *Njallani* compared to the uninoculated control a) *P. indica* colonised seedling (right) compared to control seedling (left) b) *G. fasciculatum* colonised seedling (right) compared to control seedling (left) c) Dually colonised *P. indica* and *G. fasciculatum* seedling (right) compared to control (left)

development of P. indica-colonized Chinese cabbage and barley contributed to the increased level of auxin in roots. Double-subtractive expressed sequence tag (EST) library from Chinese cabbage roots grown in the presence or absence of *P. indica* resulted in upregulation of many genes involved in auxin signalling and metabolism, thus demonstrating the positive role of auxin in *P. indica*-mediated growth promotion. Likewise, Yu *et al.* (2014) reported that AMF inoculation considerably increased the endogenous levels of auxin and cytokinin in tomato seedlings. Root transcriptome analysis revealed that the plant hormone signal transduction was substantially enriched, and gene set enrichment analysis (GSEA) found 109 genes positively linked with the AMF-inoculated plant phenotypes, including nine genes related to IAA. The above findings suggest a significant role of auxin in plant growth promotion by endophytes.

The endophytes-colonised cardamom seedlings also showed better establishment in the polybag nursery and also under field conditions. According to Ankegowda (2008), the standard healthy seedlings in a primary nursery should be four to five leaved; and at the transplantable stage (after 180 days) to the field from polybag, a plant height of 65 cm or above and nine leaves are required for

Table 3. Effect of endophyte colonisation on nutrient uptake to root, shoot and leaf in cardamom plants var. *Njallani*

Treatments	Phosphorous (%)			Potassium (%)		
	Root	Shoot	Leaf	Root	Shoot	Leaf
P. indica	0.040 ^{b}	0.028 ^{c}	0.055 ^{a}	2.143 ^{a}	4.205 ^{a}	2.492 ^{a}
G. fasciculatum	0.110 ^{a}	0.041 ^{b}	0.037 ^{bc}	2.209 ^{a}	3.664 ^{b}	2.464 ^{a}
P. indica + *G. fasciculatum*	0.050 ^{b}	0.058 ^{a}	0.041 ^{ab}	2.329 ^{a}	4.142 ^{a}	2.574 ^{a}
Control	0.009 ^{c}	0.028 ^{c}	0.026 ^{c}	1.333 ^{b}	3.002 ^{c}	2.110 ^{b}
SE (m) ±	0.003	0.005	0.004	0.07	0.13	0.08
CD (0.05)	0.010	0.011	0.013	0.211	0.399	0.258

Values are mean of 10 replications; the values following the same letters in the superscripts are not significant at 5% level of significance better establishment. In the present study, the colonised seedlings attained the above stages one month before the control plants (Table 1). The results indicate that the beneficial interaction with both the endophytes reduced the prolonged nursery period by two months (30 days each in both primary and polybag nurseries). The possible reason for the above growth promotion could be due to root elongation, production of more number of secondary roots, significantly higher uptake of P and K to shoot and leaves and higher endogenous auxin levels in dually colonised plants. The present study stipulates a better scope for establishing cardamom seedlings in the field during transplantation due to well-established root system and reduction in nursery period by two months in the *P. indica* and *G. fasciculatum* colonised seedlings.

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