Endophytes for the Enhanced Growth of *Coleus forskohlii* and Enhanced Production of Forskolin

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Abstract

Plant–fungi interaction renders enhanced production of plant secondary metabolites as most of these compounds are produced due to activation of defense-related biosynthetic pathways.

Fungal endophytes of *Coleus forskohlii* like *Piriformospora indica, Fusarium redolens*, *Phialemoniopsis cornearis*, and *Macrophomina pseudophaseolina* were used for in planta enhancement of forskolin.

Keywords

Coleus forskohlii · Forskolin · Endophytes *Fusarium redolens* · *Phialemoniopsis cornearis* · *Macrophomina pseudophaseolina* · *Piriformospora indica*

9.1 Introduction

Coleus forskohlii is a perennial medicinal shrub cultivated mainly for its forskolin content. The plant has been used since ancient times in ayurvedic traditional medicines for the treatment of hypertension, glaucoma, asthma, congestive heart failure, obesity, and cancer. The growth rhythm of the medicinal plant is slow and the alkaloid accumulation pattern is highly influenced by environmental and/or geographical conditions. Also, the quantity of the main secondary metabolite forskolin in natural conditions is found to be usually very low which restrains its commercial value. Plant–fungi interaction renders enhanced production of plant secondary metabolites as most of these compounds are produced due to activation of defense-related biosynthetic pathways. The use of endophytic microorganisms presents a special interest in the development of value-added bioactive compounds through agriculture. Limited investigations have been undertaken on in planta enhancement of forskolin content using endophytic fungus in sustainable agriculture.

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Endophytes are an endosymbiotic group of microorganisms that reside in various tissues of plants without triggering any visible external sign of infection. Endophytes provide benefits to host plants and the environment. Interestingly, medicinal plants have been identified as a good host for a variety of endophytic microorganisms including fungi which synthesize secondary metabolites with biological activity (Pullaiah and Anuradha 2020). Plant–endophyte interactions can interfere with plant growth, development, and resistance against various stresses. Plant–fungi interaction renders enhanced production of plant secondary metabolites as most of these compounds are produced due to activation of defense-related biosynthetic pathways.

9.2 Endophytes and Forskolin Production

C. forskohlii being succulent in nature responds well to in vitro propagation and thus various explants viz., nodal segments, shoot tip, and leaf, are effectively used. Hence, symbiotic interaction and in vitro propagation in combination provide a promising alternative strategy to enhance accumulation of phytochemicals. *Piriformospora indica*, an endophytic root colonizing basidiomycete fungus, mimics Arbuscular Mycorrhizal Fungi (AMF) in many morphological, functional, and growth promotional aspects.

Piriformospora indica can colonize monocot as well as dicot plants and it acts as a bioregulator, biofertilizer, and bioprotector against root pathogens; it overcomes water stress (dehydration), acidity, desiccation, and heavy metal toxicity; it protects from pests, delays the wilting of the leaves, prolongs aging of callus tissues, it enhances secondary metabolites production, and also increases the nutritional value of the plant (Das et al. 2012). In contrast to AMF, *P. indica* can be easily grown on synthetic media and thus therefore can be very useful in sustainable agriculture for crop improvement. Das et al. (2012) investigated the influence of plant probiotic fungus *Piriformospora indica* on the medicinal plant *C. forskohlii*. Interaction of the *C. forskohlii* with the root endophyte *P. indica* under field conditions resulted in an overall increase in aerial biomass, chlorophyll contents, and phosphorus acquisition. Increases of 41% in length of the branches and of 44% in the number of leaves were observed in *P. indica* colonized plants as compared with the non-colonized plants. *P. indica* not only induced a faster development of the aerial part of the plant but also caused early maturation with respect to flowering. The faster development of *P. indica* colonized roots compared with the non-colonized plants during all stages of growth could be due to the earlier expression of developmentally regulated genes. The fungus also promoted inflorescence development, consequently, the amount of *p*-cymene in the inflorescence increased. Growth of the root thickness was reduced in *P. indica* treated plants as they became fibrous, but developed more lateral roots. Because of the smaller root biomass, the content of forskolin was decreased. The symbiotic interaction of *C. forskohlii* with *P. indica* under field conditions promoted biomass production of the aerial parts of the plant including flower development (Fig. 9.1). The plant's aerial parts are important source of metabolites for medicinal

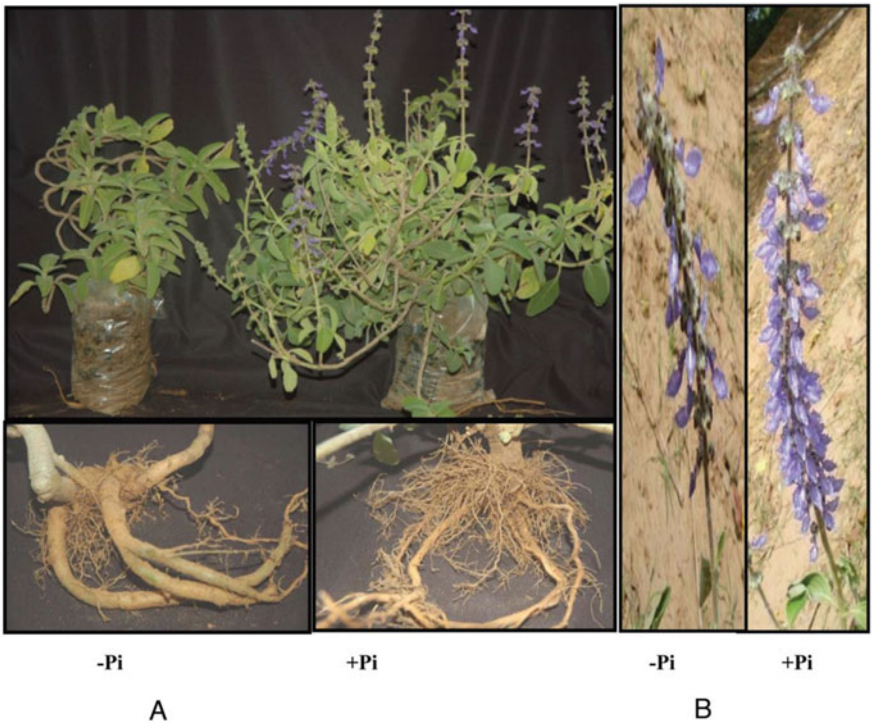

Fig. 9.1 (**a**) Influence of *Piriformospora indica* on 6 months old *C. forskohlii* under field conditions. Top panel represents plant morphology as a result of interaction between *P. indica* on *C. forskohlii* under field conditions. Each polythene bag contained 2.5 kg of unsterile sand, field soil, and compost (1:1:0.25 w/w). The fungal inoculum was 2%(w/v). Each bag contained 30 days old rooted plant cuttings. Irrigation was done on alternate days using underground water. Lower panel represents root morphology of *C. forskohlii*. (**b**) Inflorescence in *C. forskohlii*. −Pi: non-colonized plants; +Pi: colonized plants. Photographs were taken after 6 months (Source Das et al. 2012)

application. Therefore, authors suggested that the use of the root endophyte fungus *P. indica* in sustainable agriculture will enhance medicinally important chemical production.

Das et al. (2014) conducted for optimization of in vitro substrates under aseptic conditions for interaction of *Piriformospora indica* with the medicinal plant *C. forskohlii*. They tested the effects of different substrates on *P. indica* colonization as well as growth parameters of the in vitro raised *C. forskohlii*. Interaction of in vitro *C. forskohlii* with root endophyte *P. indica* under aseptic conditions resulted in increase in growth parameters in fungus colonized plants. It was observed that *P. indica* promoted the plant's growth in all irrespective of substrates used for co-culture study. The growth was found inferior in liquid compared to semisolid medium as well as there was problem of hyperhydricity in liquid medium. *P. indica* treated in vitro plantlets were better adapted for establishment under greenhouse compared to the non-treated plants due to fungal intervention.

Deployment of plant endophytes at field level is reported to make an impact on agricultural crop productivity; development and deployment of suitable crop-specific plant probiotics in a suitable delivery matrix is a value-added task. Mastan et al. (2019b) attempted to develop bioformulations of native, fungal endophytes of *Coleus forskohlii* to improve plant yield using two different carrier-based materials (talc and wheat bran). Initially, fungal endophytes *Fusarium redolens* (RF1), *Phialemoniopsis cornearis* (SF1), and *Macrophomina pseudophaseolina* (SF2), were grown on sterilized wheat bran under solid-state condition and their growth kinetics and pattern were analyzed by ergosterol content and scanning electron microscope, respectively. Ten-day grown fungal endophytic cultures were used for the development of two types of formulations (wheat bran and talc-based formulations) and tested for their efficacy on host plant, *C. forskohlii* under field conditions. Interestingly, application of wheat bran-based endophytic formulations significantly enhanced plant height (12–29%), number of branches (51–63%), root biomass (26–33%), photosynthetic pigments (32–101%), and forskolin content (35–56%) compared to talc-based formulations under field conditions. Shelf life of endophytes (RF1, SF1, and SF2) in both formulations revealed spore viability in wheat bran-based formulations for 6 months storage period as compared to talc-based formulations. Overall, this investigation envisages developing plant probiotic bioformulations of functional endophytes of *C. forskohlii* to enhance root biomass and in planta forskolin content.

Mastan et al. (2019a) reported specific roles of three fungal endophytes, *Fusarium redolens* (RF1), *Phialemoniopsis cornearis* (SF1), and *Macrophomina pseudophaseolina* (SF2), functionally acting as plant probiotic fungus, regulating secondary metabolite (forskolin) biosynthesis in *C. forskohlii*. The root endophyte, RF1, and shoot endophytes, SF1 and SF2, were found to enhance forskolin content by 52–88% in pot and 60–84% in field experiments as compared to uninoculated control plants. The three endophytes also enhanced total biomass owing to plant growth-promoting properties. The expression of diterpene synthases (CfTPSs) like CfTPS1, CfTPS2, CfTPS3, and CfTPS4 were significantly upregulated in endophyte-treated *C. forskohlii* plants. Elevated expression of key diterpene synthases (CfTPS2) in the forskolin biosynthesis pathway, exclusively present in the root cork of *C. forskohlii*, was observed following SF2 endophyte treatment. Furthermore, endophyte treatments conferred a variety of antagonistic activity against nematode galls (80%) and plant pathogens like *Fusarium oxysporum, Colletotricum gloeosporioides*, and *Sclerotium rolfsii*. RF1 and SF1 fungal endophytes showed positive for IAA production; however, SF1 also indicated phosphate solubilization activity. Overall, the qualitative and quantitative improvement of in planta forskolin represents an area of high commercial interest, and hence, Mastan et al. (2019a) focused on novel insights for the application of three fungal endophytes for in planta enhancement of forskolin content for *C. forskohlii* cultivation by a sustainable approach.

Mastan et al. (2020) reported the role of plant–probiotic bacterial endophytes of *C. forskohlii*, CFLB1 and CFRB1, isolated from leaf and root, which regulate plant growth and in plant forskolin content. Native bacterial endophyte, CFRB1 (*Alcaligenes faecalis*), significantly modulated primary plant productivity and forskolin content under pot and field conditions. Under field conditions, CFRB1 endophyte application significantly enhanced photosynthetic pigments and reduced the severity of root-knot and root rot diseases. Expression analyses of functional genes involved in the forskolin biosynthesis in *C. forskohlii* plants treated with CFRB1 endophyte under field conditions revealed differential upregulation of four *C. forskohlii* diterpene synthases (CfTPSs), CfTPS1, CfTPS2, CfTPS3, and CfTPS4, along with cytochrome P450 (CfCYP76AH15) and acyltransferase (CfACT1-8) genes. CFRB1 treatment reduced the severity of nematode infection and root rot in *C. forskohlii* plants by 81 and 78%, respectively. Overall, cross-talk of plant–endophyte interaction in *C. forskohlii* is beneficial, leading to enhanced forskolin content through modulation of forskolin biosynthetic pathway genes along with increased plant yield and reduced disease incidence. Thus, endophytic isolate, *A. faecalis* (CFRB1) could be deployed as a novel bio-stimulant for enhancing in planta forskolin content during cultivation of *C. forskohlii.*

Field experiments were conducted by Mastan et al. (2021) to understand the compatibility of three native, endophytic fungi *Phialemoniopsis cornearis* (SF1), *Macrophomina pseudophaseolina* (SF2), and *Fusarium redolens* (RF1) with *Trichoderma viride* (TV1) on *Coleus forskohlii* in enhancing plant growth and forskolin content. Co-inoculation of RF1+TV1 showed significant improvement in plant growth (52%), root biomass (67%), and in planta forskolin content (94%), followed by treatment of SF2+TV1 and SF1+TV1. qRTPCR was carried out to quantify the expression of five key forskolin biosynthetic pathway genes (CfTPS2, CfTPS3, CfTPS4, CfCYP76AH15, and CfACT1-8) in RF1+TV1-treated *C. forskohlii* plants. Elevated expression of CfTPS2, CfTPS4, CfCYP76AH15, and CfACT1-8 genes was noticed with RF1+TV1 combination as compared to uninoculated *C. forskohlii* plants. Besides, RF1+TV1 treatment considerably reduced the severity of nematode infection of *C. forskohlii* plants under field conditions. Thus, congruent properties of *F. redolens* (RF1) were noticed with co-inoculation of *T. viride* (TV1) under field conditions resulted in enhanced forskolin content, root biomass, and reduced nematode infections in *C. forskohlii.*

Molecular identification of endophytic fungi associated with *C. forskohlii* was carried out by Crasta and Raveesha (2021). A total of 85 endophytic fungi were isolated from 280 leaf segments. Molecular identification revealed 34 fungal genera. Among these, species of *Cladosporium* sp., *Alternaria* sp., *Aspergillus niger, Aspergillus* sp., *Colletotrichum* sp., *Nigrospora oryzae, Penicillium* sp., and *Phyllosticta fallopiae* were found to be predominant genera. The percentage occurrence of members of Ascomycota was the highest, with 96.47% distribution and Basidiomycota members were distributed the least, with 3.53%.

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