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Chrysanthemum Growth Gains from Beneficial Microbial Interactions and Fertility Improvements in Soil Under Protected Cultivation

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Abstract

An investigation was undertaken to analyse the influence of microbial inoculants on growth and enzyme activities elicited, and soil microbiome of two varieties of *Chrysanthemum morifolium* Ramat, which were grown under protected mode of cultivation. Rhizosphere soil sampling at 45 and 90 DAT (days after transplanting of cuttings) revealed up to four- to five-fold enhancement in the activity of defence-, and pathogenesis-related, and antioxidant enzymes, relative to the uninoculated control. Plant growth and soil microbial parameters, especially soil microbial biomass carbon and potential nitrification exhibited significant increases over control. Available soil nitrogen concentrations showed 40%–44% increment in inoculated treatments. Scanning electron microscopy of the root tissues revealed biofilm-like aggregates and individual short bits of cyanobacterial filaments. Analyses of DGGE profiles of archaeal and bacterial communities did not show temporal variations (between 45 and 90 DAT). However, distinct influences on the number and abundance of phylotypes due to microbial inoculants were recorded. The inoculants — Cyanobacterial consortium (BF1- 4) and *Anabaena* sp.–*Trichoderma* sp. biofilm (An-Tr) were particularly promising in terms of the plant and soil related parameters, and remained distinct in the DGGE profiles generated. The effect of *Trichoderma viride–Azotobacter* biofilm on soil bacterial and archaeal communities was unique and distinct as a separate cluster. This study highlights that microbial inoculants exert positive effects, which are specific even to the rhizosphere soil microbiome of chrysanthemum varieties tested. Such inoculants can serve as soil fertility enhancing options in protected floriculture.

Keywords: microbial interaction; biofilm; cyanobacteria; DGGE; floriculture; soil fertility

1. Introduction

Protected cultivation of crops has emerged as a promising option globally in the last decade. The major advantage of protected mode of cultivation is the significant reduction in losses, due to extremes of temperature, pests and disease incidence. However, due to intensive cultivation year round and a closed environment, a rapid decline in organic matter and nutrient levels is observed along with deterioration in physical properties of soil. Improved physical, chemical and biological properties of these soils demand the application of management practices such as the application of organic materials (Saha et al., 2008). The sustainability of soils, especially in relation to the quality and time dependent changes of the soil, deserves immediate attention in the protected mode of cultivation (Karlen et al., 1997).

Biofertilizers are a low cost environment friendly sustainable agronomic option as they can contribute to mobilization, mineralization and recycling of nutrients in an effective manner (Chaudhary, 2010). Among biofertilizers, cyanobacteria are commonly deployed in rice and more recently in other crops including wheat, cotton, legumes and vegetables (Prasanna et al., 2014, 2015). They produce a wide range of bioactive molecules known to be necessary for plant growth (Obana et al., 2007; Maqubela et al., 2009). Cyanobacteria are also known to enhance the production of secondary metabolites in plants, including essential oils in *Mentha piperita* L. and antioxidants in *Lilium alexandrae*

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(Shariatmadari et al., 2015). Several bacteria, including cyanobacteria, as well as fungi mediate such processes which results in better growth and nutrient mobilization (Pieterse et al., 2014; Prasanna et al., 2014; Triveni et al., 2015). The deployed microbial consortia and biofilmed inoculants in this investigation have been used extensively in various crops and positive impacts on growth, yields an soil nutrient mobilization have been recorded (Prasanna et al., 2013, 2014, 2015; Triveni et al., 2015; Manjunath et al., 2016). Although a number of reports are published on biofertilizer-mediated improvement in flowering and quality attributes in gladiolus, tuberose and jasmine (Dalve et al., 2009; Srivastava et al., 2013), their role in improving the fertility of soil or elicitation of plant innate immunity, particularly in such a protected mode of cultivation is a less investigated area.

Chrysanthemum displays a wide range of variability among the economic floral traits and has excellent keeping quality among its cultivars due to a wide range of colours, size, and forms. It is an annual plant that requires a long day for vegetative growth and a short day for flowering, with availability throughout the year.

Soil microbial community is highly complex and dynamic with variations in the composition both spatially and temporally. Inoculation through enrichment of soil or seed bacterization can lead to changes in the structure of the indigenous microbial communities. Viewing the microbiota from an ecological perspective can provide new insights into how to promote soil health and plant productivity. The proposed research work is aimed towards understanding the following: (i) Can microbial inoculants improve the growth and improve the fertility of soil in the chrysanthemum crop under protected mode of cultivation, (ii) Does this inoculation influence the bacterial and archaeal microbial communities in the rhizosphere, and (iii) Are there any relationships between the PCR-DGGE (denaturing gradient gel electrophoresis) analyses and the performance of inoculants, in terms of changes brought on plant growth or soil nutrient parameters under protected cultivation of chrysanthemum.

2. Material and methods

2.1. Experimental site

The study was conducted at the Centre for Protected Cultivation and Technology (CPCT), ICAR-Indian Agricultural Research Institute (IARI), New Delhi-110012 (latitude 28°38' N, longitude 77°12'E and altitude 228.4 m) between November 2014 and March 2015. Two cultivated varieties of *Chrysanthemum morifolium* Ramat (thereafter referred to as chrysanthemum)– 'Golden Ball' and 'White Star', were planted in November 2014 in soil beds (15 m long and 1.25 m wide) at a density of 4 plants $\cdot$ m^{-2} (with three rows) in a randomized block design with 3 replications, and each replication contained 20 plants in a naturally ventilated greenhouse. The 30 d old plug plants raised in soil less media composed of a mixture coco-peat, vermiculite and perlite (3:1:1, w/w), were transplanted. The plants were maintained as mother stock and grown under long days (>13 h day length, with the light given for fixed hours during the night); thus retained at the vegetative stage as a result of low temperature. After planting, these plug plants (cuttings) were drip-fertigated on the 10 cm raised beds in a naturally ventilated greenhouse.

The average monthly temperature and humidity of the greenhouse varied from 14 °C to 22 °C and 62% to 75%, respectively. The average minimum and maximum solar radiation was found to be between 225 and 285 Watt $\cdot$ m^{-2} inside the greenhouse. The number of irrigations for chrysanthemum varied from 4 to 8 during the study period. The irrigation quantity varied from 200 to 400 litres for the chrysanthemum grown in 50 m^2 area. Fertigation for the major nutrients- N, P and K varied from 75–90, 40–60 and 60–80 μg $\cdot$ L^{-1} during the study period, with an average of 21, 13 and 9 g $\cdot$ m^{-2}, respectively. During the study period, the EC and pH were maintained at 1.3–1.5 dS $\cdot$ m^{-1} and 7.5 respectively for irrigation and 1.8–2.2 dS $\cdot$ m^{-1} and 7.0 for fertigation.

2.2. Details of microorganisms and inoculation treatments

All the strains used in this investigation are available in the germplasm of the Division of Microbiology, ICAR — Indian Agricultural Research Institute, New Delhi and their details are given in earlier investigations (Prasanna et al., 2008, 2011, 2014).

Formulations were prepared using optimized protocols with compost: vermiculite as carrier with bacterial or fungal partners maintained at 10^7–10^{10} cfu $\cdot$ g^{-1} and chlorophyll *a* content of 100 μg $\cdot$ g^{-1} carrier, as described in our earlier investigations (Prasanna et al., 2011, 2014; Triveni et al., 2012, 2015). Microbial inoculation at the rate of 1 g per cutting (plug plants) was done before placing the cutting in the holes. The treatments included: Control, no inoculation; *Anabaena* sp.–*Trichoderma* sp. biofilm (An-Tr); *Anabaena–Azotobacter* sp. biofilm (An-Az); Cyanobacterial consortium of BF1 *Anabaena torulosa*; BF2 *Nostoc carneum*; BF3 *Nostoc piscinale;* BF4 *Anabaena doliolum* (BF1-4); *Trichoderma viride–Azotobacter* biofilm (Tr-Az). The rhizosphere soil sampling was done after 45 days and 90 days after transplanting of cuttings (plug plants).

2.3. Assay of defence and hydrolytic enzyme activities in root and shoot tissues

The whole plant samples were collected after 45 and 90 days of transplanting of the cuttings. The leaf and root tissue samples were homogenized using 50 mmol $\cdot$ L^{-1} Tris-HCl buffer, Polyphenol oxidase (PPO) activity was measured using catechol, which served as the substrate (Jennings et al., 1969). The enzyme activity was determined spectrophotometrically at 546 nm and the changes in absorbance were recorded at 30 s intervals for 3 min. Phenylalanine ammonia-lyase (PAL) activity was assayed in the tissue extracts of leaves and roots by measuring the amount of trans-cinnamic acid formed from L-phenylalanine spectrophotometrically at a wavelength of 290 nm against the blank (Beaudoin-Eagan and Thorpe, 1985).

Chitosanase (EC 3.2.1.99), endoglucanase (β-1,3-glucanase and β-1,4-glucanase; EGases, EC 3.2.1.39 and EC 3.2.1.4 respectively) activities were assayed spectrophotometrically using glycol chitosan, laminarin and carboxy methyl cellulose respectively, as substrate, as given in Prasanna et al. (2013, 2015).

2.4. Analyses of soil microbial parameters and available nutrient concentrations

Soil samples from the rhizosphere of two varieties were collected at 90 DAT from 0 to 20 cm depth. By using the 6 g soil