Interactive Effect of AM Fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on Growth and Yield of Broccoli

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Abstract

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Most of the vegetable crops are known to depend upon arbuscular mycorrhizal fungal (AM) symbiosis for growth and development, since AM fungi provide nutrients and water in exchange for photosynthates. The influences of AM fungi (*Glomus intraradices* (G) and *Acaulospora laevis* (A)) with *Trichoderma viride* (T) and *Pseudomonas fluorescens* (P) alone and in combinations on growth, mycorrhization, chlorophyll content, nutrient uptakes, and yield of broccoli plants were studied in pot culture under glasshouse conditions. The obtained results demonstrated that the single inoculation of broccoli plants with *T. viride* significantly increased the above ground fresh weight, root length, chlorophyll *b*, head diameter, root phosphorus, and shoot nitrogen in comparison to uninoculated control plants. On the other hand, consortium of G+A+T+P significantly increased plant height, above ground dry weight, root fresh weight, chlorophyll *a*, head fresh weight, and root nitrogen content. Similarly, G+T showed maximum leaf area, and P alone showed maximum uptake of shoot phosphorus. Whereas when P was supplied along with T, early flowering was recorded. AM fungal colonisation was negligible and only root tips were found infected in G or A treated plants which confirms low dependency of broccoli on AM fungi.

Keywords: *Acaulospora laevis*; *Brassica oleracea* L. var. *italica*; brassicaceae; *Glomus intraradices*; nutrient uptake; vegetable crop

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is an Italian vegetable and a completely new introduction in India (Brahma *et al*. 2010; Saha *et al*. 2010). In India, commercial cultivation of broccoli is gaining importance in the last few years due to its higher nutritive value, palatability, short growing duration, high productivity, and good market potential (Brahma *et al*. 2010). Its tender green bud, thick fleshy floral stalk, and the secondary heads are eaten raw as salad or steam cooked. It is low in calories, fat free, low in sodium, it has high amount of vitamins (A, B_1, B_2, B_5, B_6, C, and E) and minerals (Ca, Mg, Zn, and Fe) (Beecher 1994; Decoteau 2000). It also has antioxidant substances identified as glucosinolates that are potent cancer chemoprevention agents (Farnham *et al*. 2004).

In Haryana, broccoli cultivation area is very limited and the productivity of the crop is very low, which is particularly due to the lack of knowledge. At the same time there is an increase in the demand for broccoli in India as well as in the international market. Hence, there is a need for research in improving the yield of broccoli and for sustainable production; some biological agents should be implemented either alone or in combination with synthetic fertilisers to increase its cultivation and production. Among various currently used biological approaches, use of microbial inoculants at the seedling stage could prove as a promising approach. Several symbionts like arbuscular mycorrhizal (AM) fungi, *Trichoderma viride*, a known biocontrol agent, and phosphorus solubilising bacteria (PSB) like *Pseudomonas fluorescens* can

be implemented for broccoli cultivation. There are several reports of *Trichoderma* and *Pseudomonas* mediated growth promotion and development of seedlings of several vegetable crops, namely tomato, lettuce, cabbage, and chilli (Rabeendran *et al*. 2000; Bal & Altintas 2006; Joshi *et al*. 2010; Ordookhani *et al*. 2010; Tanwar *et al*. 2010). Apart from improving nutritional status and growth of various vegetable crops, there are several reports of their use as biocontrol agent against a wide range of soil borne fungal pathogens of crucifers (Cheah *et al*. 2000; Rabeendran *et al*. 2005; El-Mohamedy & El-Mougy 2009; El-Mohamedy 2012).

Arbuscular mycorrhizas are the mutualistic symbiosis between fungi in the phylum glomeromycota and most terrestrial plant roots (Smith & Read 2008). This association increases the supply of mineral nutrients to the plant, particularly those whose ionic forms have a poor mobility rate or those which are present in low concentration in the soil and thus promote plant growth (Ercolin & Reinhardt 2011). AM fungi also have the potential in plant disease control that has been demonstrated by various workers (Akhtar & Siddiqui 2008a; Kobra *et al*. 2009; Singh *et al*. 2010; Mwangi *et al*. 2011). Several mechanisms are involved in disease control by AM fungi such as sink competition with infection sites, morphological changes in roots and root tissues, and changes in chemical constituents in plant tissue (Linderman 2000). In the natural field, broccoli, a member of brassicaceae family, is considered as a non mycorrhizal crop and thus is not dependent on AM fungi for growth. Further, limited information is available on the role of plant growth promoting microbes in influencing the growth and nutrients uptake in broccoli. Knowing this fact an attempt was made to see (*i*) if other bioinoculants such as *T. viride* and *P. fluorescens* have any impact to make broccoli roots susceptible to AM fungal infection, (*ii*) AM fungal inoculum, when added into the soil, has any influence on soil mineralization, and (*iii*) the effect of AM fungi (*Glomus intraradices* and *Acaulospora laevis*), *T. viride* and *P. fluorescens* alone and in combination on the growth, nutrient uptake, and yield of broccoli.

MATERIAL AND METHODS

Study site and soil characteristics. The experiment was carried out at a temperature of 20 ± 5°C and humidity of 50–70% in glasshouse at Botany Department, Kurukshetra University, Kurukshetra, Haryana during mid October, 2011 to February, 2012. Light was provided by cool white fluorescent lamps (8000 lx) under a 16-h photoperiod. The glasshouse also received sunlight. The soil characteristics are: sand 64.2%, silt 21.81%, clay 3.90%, pH 6.8 ± 0, EC 0.25 dS/m, total N 0.042%, available P 0.017%, and organic carbon 0.06%.

Inoculum preparation. Two dominant AM fungi, i.e. *Glomus intraradices* (Schenck and Smith) and *Acaulospora laevis* (Gerd. and Trappe), were isolated from the rhizosphere soil of field grown broccoli plants by wet sieving and decanting technique of Gerdemann and Nicolson (1963). The starter inoculum of each selected dominant AM fungus was raised by funnel technique of Menge and Timmer (1982) using lemon grass as host for three months. Likewise, *Trichoderma viride* was isolated from soil and mass-produced on wheat bran:saw dust:water (3:1:4) medium. The inoculum of *P. fluorescens* (MTCC No. 103) was obtained from Institute of Microbial Technology, Chandigarh, India and cultured in a nutrient broth medium incubated at 32°C for 48 h to obtain a concentration of 1×10^9 colony forming units (CFU) per ml. Control pots without microbial inoculation were also maintained.

Experimental setup. Soil from experimental site was sieved through a 2 mm sieve, mixed with sand:soil (1:3), and autoclaved at 121°C for 2 hours. Earthen pots (25.4 × 25 cm) were selected having capacity of 2 kg soil. Chopped AM colonised root pieces of maize plant along with the soil having AM spores (400–450/100 g inoculum) was used as AM inoculum. To each pot 10% (w/w) inoculum of AM fungi, *T. viride,* and *P. fluorescens* alone and in combinations were added. The experiment had sixteen treatments with combinations of single inoculation (*G. intraradices*, *A. laevis*, *T. viride*, *P. fluorescens*), double inoculations (*G. intraradices + A. laevis*, *G. intraradices + T. viride*, *G. intraradices + P. fluorescens*, *A. laevis + T. viride*, *A. laevis + P. fluorescens*, *T. viride+ P. fluorescens*), triple inoculations (*G. intraradices + A. laevis + T. viride*, *G. intraradices + A. laevis + P. fluorescens*, *G. intraradices + T. viride + P. fluorescens*, *A. laevis + T. viride+ P. fluorescens*), and lastly consortium of all the bioinoclulants together (*G. intraradices + A. laevis + T. viride + P. fluorescens*). In control set no inoculum was added. The seeds of broccoli were surface sterilised with 0.5% (v/v)

sodium hypochloride for 10 min, subsequently washed with sterilised deionised water, and germinated using a shallow tray containing sterilised soil:sand (3:1). Twenty days after seedlings emergence, single seedling was transplanted to each pot. Plants were watered daily and 100 ml per pot of Hoagland nutrient solution (without KH_2PO_4) was also added to each plant after regular intervals of 15 days. Each treatment was replicated five times.

Measurement and harvest. Plants were harvested 90 days after transplantation (DAT) and the effect of various bioinoculants on various growth parameters was recorded. Changes in plant height (cm), leaf area (cm^2) by using leaf area meter (Systronics 211; Systronics India Ltd., Ahmedabad, India), and chlorophyll content (mg/g FM) (Arnon 1949) were recorded in the standing crop. After that the plants were harvested, separated into roots and shoots, weighted separately for their fresh weight (g), and oven dried at 70°C until a constant weight was obtained to determine the dry weight (g). Similarly, head diameter (cm), petiole length (cm), and fresh head weight (g) were measured. Percentage mycorrhizal root colonisation was studied using Phillips and Hayman (1970) technique. The AM spore quantification was also done by following the procedure of Gerdemann and Nicolson (1963). Phosphorus content of shoot and root was determined by vanadomolybdate phosphoric yellow colour method (Jackson 1973). Total nitrogen was calculated by Kjeldahl method (Kelplus nitrogen estimation system, supra-LX; Pelican Equipments, Chennai**,** India).

Statistical analysis. Data were subjected to analysis of variance and means separated with Least Significant Difference test using the Statistical Package for Social Sciences (Version 11.5; SPSS, Chicago, USA).

RESULTS

Plant growth response

As depicted in Table 1, maximum increase in plant height was recorded in consortium of all the bioinoculants together, i.e. in G+A+T+P

Table 1. Growth response of broccoli plants on soil inoculation with AM fungi, *P. fluorescens*, and *T. viride*

Sample No.	Treatment	Change in plant height (cm)	Above ground (g)		Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Leaf area (cm^2)
			fresh weight	dry weight				
1	Control	37.60 ± 1.10^{d}	21.74 ± 2.45^{f}	2.24 ± 0.67^{f}	16.86 ± 1.68^{f}	1.80 ± 0.11^{e}	0.73 ± 0.093^{f}	82.03 ± 2.60^{f}
2	G	44.18 ± 1.47^{b}	40.22 ± 2.59^{c}	4.78 ± 0.48^{d}	26.76 ± 1.26^{ab}	3.87 ± 0.26^{c}	1.07 ± 0.286^{d}	108.22 ± 2.61^{c}
3	A	41.64 ± 1.90^{c}	32.28 ± 1.98^{d}	3.27 ± 0.61^{e}	17.98 ± 1.13^{e}	2.70 ± 0.13^{d}	1.15 ± 0.177^{d}	05.64 ± 2.63^{c}
4	T	52.26 ± 2.84^{a}	59.20 ± 2.49^{a}	5.54 ± 0.49^{bc}	32.74 ± 1.62^{a}	5.56 ± 0.17^{a}	1.62 ± 1.871^{bc}	18.24 ± 1.09^{b}
5	P	40.64 ± 2.06^{b}	25.96 ± 2.94^{e}	3.70 ± 0.37^{e}	23.68 ± 2.32^{b}	5.93 ± 0.39^{a}	2.51 ± 0.233^{a}	97.00 ± 1.16^{d}
6	G+A	40.36 ± 2.31^{c}	22.76 ± 1.35^{f}	2.43 ± 0.33^{f}	19.40 ± 1.38^{d}	3.60 ± 0.66^{c}	1.10 ± 0.270^{d}	103.9 ± 1.39^{c}
7	G+T	45.22 ± 2.36^{b}	38.24 ± 1.20^{c}	4.91 ± 0.22^{d}	23.82 ± 1.27^{b}	2.77 ± 0.38^{d}	1.00 ± 0.177^{d}	175.44 ± 1.76^{a}
8	G+P	41.30 ± 1.61^{c}	43.20 ± 1.04^{bc}	5.04 ± 0.21^{c}	21.16 ± 1.66^{c}	3.42 ± 0.28^{c}	1.44 ± 0.309^{c}	109.24 ± 0.78^{c}
9	A+T	37.68 ± 1.66^{d}	42.24 ± 1.65^{bc}	5.81 ± 0.31^{b}	21.68 ± 1.92^{c}	5.92 ± 0.21^{a}	1.94 ± 0.282^{b}	97.62 ± 0.91^{d}
10	A+P	42.08 ± 3.04^{c}	41.08 ± 1.53^{c}	5.09 ± 0.26^{c}	23.82 ± 1.46^{b}	6.03 ± 0.24^{a}	1.53 ± 0.210^{c}	107.84 ± 1.14^{c}
11	T+P	40.38 ± 1.76^{c}	39.68 ± 2.08^{c}	4.58 ± 0.29^{d}	22.46 ± 2.47^{c}	4.58 ± 0.39^{b}	1.37 ± 0.215^{c}	86.4 ± 0.92^{e}
12	G+A+T	33.94 ± 1.93^{e}	27.98 ± 2.12^{e}	3.83 ± 0.18^{e}	21.18 ± 1.61^{c}	4.80 ± 0.53^{b}	1.34 ± 0.266^{c}	137.72 ± 1.94^{ab}
13	G+A+P	15.08 ± 1.65^{f}	41.18 ± 1.75^{c}	5.85 ± 0.24^{b}	24.64 ± 1.84^{b}	3.48 ± 0.30^{c}	0.87 ± 0.188^{e}	102.04 ± 0.73^{c}
14	G+T+P	35.80 ± 1.86^{e}	39.72 ± 1.45^{c}	5.29 ± 0.29^{c}	20.80 ± 1.90^{d}	3.32 ± 0.45^{c}	1.28 ± 0.178^{c}	92.98 ± 1.03^{d}
15	A+T+P	49.96 ± 0.93^{ab}	46.36 ± 2.08^{b}	6.24 ± 0.36^{ab}	24.00 ± 1.47^{b}	2.84 ± 0.25^{d}	0.85 ± 0.289e	111.78 ± 0.75^{b}
16	G+A+T+P	53.86 ± 2.30^{a}	54.14 ± 3.10^{ab}	7.35 ± 0.79^{a}	29.99 ± 1.79^{ab}	6.29 ± 0.38a	1.96 ± 0.280^{ab}	111.2 ± 0.90^{b}
LSD ($P \leq 0.05$)		2.5265	2.678	0.4858	2.1642	0.4453	0.2966	1.9556
ANOVA (F 15,32)		45.699	135.907	63.917	31.971	85.104	76.627	1273.288

Each value is a mean of five replicates; ± standard deviation; G – *Glomus mosseae*; A – *Acaulospora laevis*; P – *Pseudomonas fluorescens*; T – *Trichoderma viride*; means followed by the same letter(s) within a column are not significantly different over one another (Duncan's Multiple Range test, $P \leq 0.05$)

(53.86 ± 2.30), followed by T alone (52.26 ± 2.84). Significantly higher above ground fresh and dry weight were recorded in all the inoculated treatments. T alone showed the highest fresh weight (59.20 ± 2.49), followed by G+A+T+P (54.14 ± 3.10) as compared to uninoculated control (23.74 ± 2.45), whereas maximum above ground dry weight was recorded in G+A+T+P (7.35 ± 0.79) followed by A+T+P (6.24 ± 0.36). Similarly, root length increased from 16.86 ± 1.68 (control) to 32.74 ± 1.62 in T alone treatment. While comparing root fresh and dry weight, six-fold increase in root fresh weight was found in G+A+T+P (6.29 ± 0.38) as compared to control (1.80 ± 0.11), whereas maximum root dry weight was recorded in T alone (2.51 ± 0.233). Significantly higher leaf area was recorded in G+T (175.44 ± 1.76). It was also found that in plants supplemented with AM fungal inoculum an increase in all the growth parameters was observed but this increase was not statistically superior.

Mycorrhisation

The status of AM fungal spores and the degree of colonisation were also studied (Table 2). AM spores were observed in all the AM treated plants, whereas no positive interaction was observed for root colonisation. Only root tips of some treatments were found colonised and most of the broccoli roots remained uninfected. Maximum number of AM spores as well as the highest colonisation were recorded in the A alone treated plants (62.8 ± 6.06, 7.6 ± 5.2). The second highest spore number was observed in G (52.0 ± 8.60) followed by G+A+T+P (46.7 ± 4.47) and G+A+T (45.4 ± 4.04), whereas G+A+T+P had the second highest colonization percentage.

Leaf photosynthetic and chlorophyll content. It is clear from Table 2 that plant photosynthetic activity increased significantly in all the treatments as compared to uninoculated control. Maximum chlorophyll *a* was found in the G+A+T+P (0.056 ± 0.005) followed by T (0.052 ± 0.004) and G+A+T

Table 2. Chlorophyll content and mycorrhization of broccoli plants on soil inoculation with AM fungi, *P. fluorescens*, and *T. viride*

Sample No.	Treatment	Chlorophyll *a* (mg/g FM)	Chlorophyll *b* (mg/g FM)	AMF spore number per 10 g of soil	AMF root colonisation (%)
1	control	0.019 ± 0.005^{f}	0.123 ± 0.008^{f}	0	0
2	G	0.027 ± 0.004^{d}	0.158 ± 0.004^{de}	52.0 ± 8.60^{ab}	2.5 ± 3.4^{c}
3	A	0.022 ± 0.004^{e}	0.136 ± 0.004^{e}	62.8 ± 6.06^{a}	7.6 ± 5.2^{a}
4	T	0.052 ± 0.004^{a}	0.284 ± 0.005^{a}	0	0
5	P	0.028 ± 0.004^{d}	0.216 ± 0.005^{b}	0	0
6	G+A	0.022 ± 0.004^{e}	0.132 ± 0.004^{e}	29.0 ± 3.08^{d}	1.25 ± 2.8^{d}
7	G+T	0.030 ± 0^{cd}	0.178 ± 0.008^{cd}	20.2 ± 2.86^{e}	2.50 ± 3.4^{c}
8	G+P	0.026 ± 0.005^{d}	0.188 ± 0.004^{c}	34.4 ± 5.37^{cd}	2.66 ± 3.64^{c}
9	A+T	0.026 ± 0.004^{d}	0.134 ± 0.005^{e}	38.6 ± 5.08^{c}	3.83 ± 5.64^{b}
10	A+P	0.032 ± 0.004^{c}	0.166 ± 0.005^{d}	26.4 ± 3.78^{de}	2.58 ± 3.54^{c}
11	T+P	0.040 ± 0.0^{b}	0.154 ± 0.005^{d}	0	0
12	G+A+T	0.044 ± 0.005^{b}	0.166 ± 0.005^{d}	45.4 ± 4.04^{b}	1.25 ± 2.80^{d}
13	G+A+P	0.032 ± 0.004^{c}	0.182 ± 0.004^{c}	29.0 ± 3.67^{d}	2.49 ± 5.56^{c}
14	G+T+P	0.036 ± 0.005^{c}	0.128 ± 0.005^{f}	30.6 ± 4.34^{d}	0
15	A+T+P	0.034 ± 0.005^{c}	0.214 ± 0.005^{b}	37.8 ± 2.86^{c}	1.29 ± 2.88^{d}
16	G+A+T+P	0.056 ± 0.005^{a}	0.226 ± 0.005^{ab}	46.7 ± 4.47^{b}	6.21 ± 6.20^{ab}
LSD ($P \leq 0.05$)		0.0059	0.0071	5.25	4.57
ANOVA (F 15,32)		25.81	272.306	129.618	1.815

Each value is a mean of five replicates; ± – standard deviation; G – *Glomus mosseae*; A – *Acaulospora laevis*; P – *Pseudomonas fluorescens*; T – *Trichoderma viride*; FM – fresh matter; means followed by the same letter(s) within a column are not significantly different over one another (Duncan's Multiple Range test, $P \leq 0.05$)

Table 3. Broccoli yield as affected by soil inoculation with AM fungi, *P. fluorescens*, and *T. viride*

Sample No.	Treatment	Days of flowering	Head diameter (cm)	Head fresh weight (cm)	Petiole length (cm)
1	Control	87.2 ± 1.92^{e}	0.52 ± 0.083^{f}	0.88 ± 0.038^{f}	0.92 ± 0.13^{f}
2	G	72.6 ± 2.97^{c}	2.06 ± 0.086^{c}	2.12 ± 0.104^{d}	2.90 ± 0.16^{b}
3	A	73.6 ± 2.07^{c}	2.18 ± 0.158^{bc}	3.05 ± 0.166c	4.76 ± 0.18^{a}
4	T	62.4 ± 1.82^{b}	3.68 ± 0.130^{a}	5.58 ± 0.052^{ab}	4.24 ± 0.11^{ab}
5	P	66.0 ± 1.58^{b}	1.62 ± 0.084^{d}	2.02 ± 0.151^{d}	2.24 ± 0.11^{c}
6	G+A	72.8 ± 2.39^{c}	0.72 ± 0.083^{e}	1.04 ± 0.096^{ef}	1.32 ± 0.23^{de}
7	G+T	87.2 ± 1.64^{e}	1.98 ± 0.130^{d}	0.97 ± 0.074^{ef}	1.60 ± 0.10^{d}
8	G+P	79.6 ± 2.30^{d}	2.10 ± 0.10^{c}	1.38 ± 0.086^{e}	1.80 ± 0.14^{d}
9	A+T	66.4 ± 1.82^{b}	2.24 ± 0.089^{b}	1.15 ± 0.107^{ef}	2.20 ± 0.12c
10	A+P	81.0 ± 2.24^{d}	2.42 ± 0.084^{b}	1.73 ± 0.108^{e}	2.66 ± 0.18^{bc}
11	T+P	53.8 ± 1.64^{a}	2.82 ± 0.084^{ab}	3.37 ± 0.131^{b}	3.10 ± 0.10^{b}
12	G+A+T	66.0 ± 3.08^{b}	2.26 ± 0.114^{b}	2.95 ± 0.130^{c}	2.00 ± 0.16^{c}
13	G+A+P	85.8 ± 1.92^{e}	2.36 ± 0.114^{b}	0.96 ± 0.082^{ef}	0.98 ± 0.08^{f}
14	G+T+P	59.2 ± 1.64^{ab}	2.80 ± 0.071^{ab}	3.04 ± 0.163^{c}	2.34 ± 0.11^{c}
15	A+T+P	76.0 ± 2.55^{d}	0.56 ± 0.055^{f}	1.39 ± 0.117^{e}	1.10 ± 0.10^{e}
16	G+A+T+P	58.2 ± 2.39^{ab}	2.96 ± 0.114^{ab}	6.76 ± 0.098^{a}	4.86 ± 0.17^{a}
LSD ($P \leq 0.05$)		2.743	0.1289	0.1418	0.1798
ANOVA (F 15,32)		132.172	389.428	1095.42	396.424

Each value is a mean of five replicates ± standard deviation; G – *Glomus mosseae*; A – *Acaulospora laevis*; P – *Pseudomonas fluorescens*; T – *Trichoderma viride*; AMF – arbuscular mycorrhizal fungi; means followed by the same letter(s) within a column are not significantly different over one another (Duncan's Multiple Range test, $P \leq 0.05$)

(0.044 ± 0.005). An increased chlorophyll *b* was recorded in plants with single inoculation of T (0.284 ± 0.005), whereas G+A+T+P and P showed an increase in chlorophyll *b* content (0.226 ± 0.005 and 0.216 ± 0.005), respectively, compared to uninoculated control (0.123 ± 0.008).

Broccoli yield. Soil amendment by different microbes enhanced all the yield parameters (head diameter, head fresh weight, and petiole length) studied in comparison to uninoculated control (Table 3). Flower appeared in all the treatments but T+P (53.8 ± 1.64) and G+A+T+P (58.2 ± 2.39) showed early flowering as compared to control (87.2 ± 1.92). Maximum head diameter was observed in T (3.68 ± 0.130) inoculated plants in comparison to control. However, maximum head weight showed G+A+T+P (6.76 ± 0.098), followed by T (5.58 ± 0.052) and T+P (3.37 ± 0.131), respectively. Contrary to this, control plant showed the least yield. Moreover, the longest petiole was also recorded in G+A+T+P (4.86 ± 0.17) followed by A (4.76 ± 0.18) and T (4.24 ± 0.11), respectively.

Plant nutrient uptake. We screened different plant growth promoting microbes to access their efficacy on broccoli nutrition (Table 4). Microbial inoculation consistently accumulated more quantities of phosphorus in the root than shoot and nitrogen in shoot than root. Phosphorus content in shoot was found significantly higher in plants inoculated with the P alone (0.132 ± 0.004) as compared to control (0.058 ± 0.0025), whereas root P was found best in T alone (0.186 ± 0.005) compared to uninoculated control (0.060 ± 0.004). As for shoot nitrogen content, maximum was found in plants with T (2.28 ± 0.084) followed by G+T+P (1.90 ± 0.084) and G+A+T+P (1.90 ± 0.080), whereas consortium of all the bioinoculants together (G+A+T+P) showed maximum root nitrogen content (1.85 ± 0.056) followed by T (1.60 ± 0.120). None of the treatments showed P and N content lower than the control treatment but for shoot phosphorus content, although all the inoculated plants showed an increment in phosphorus content as compared to control, but in G+T and G+A the increase was marginal.

Table 4. Effect of soil inoculation with AM fungi, *P. fluorescens*, and *T. viride* on phosphorus and nitrogen content of broccoli plants

Sample No.	Treatment	Shoot phosphorus (%)	Root phosphorus (%)	Shoot nitrogen (%)	Root nitrogen (%)
1	Control	0.058 ± 0.0025^{f}	0.060 ± 0.004^{f}	0.48 ± 0.072^{f}	0.32 ± 0.035^{f}
2	G	0.078 ± 0.0045^{de}	0.086 ± 0.002^{ef}	0.58 ± 0.079^{f}	0.89 ± 0.060^{de}
3	A	0.092 ± 0.004^{cd}	0.092 ± 0.005^{e}	0.97 ± 0.073^{d}	1.16 ± 0.097^{c}
4	T	0.122 ± 0.004^{ab}	0.186 ± 0.005^{a}	2.28 ± 0.084^{a}	1.60 ± 0.120^{ab}
5	P	0.132 ± 0.004^{a}	0.158 ± 0.004^{ab}	1.61 ± 0.071^{b}	1.15 ± 0.051^{c}
6	G+A	0.066 ± 0.005^{e}	0.104 ± 0.005^{d}	0.78 ± 0.074^{e}	0.62 ± 0.037^{ef}
7	G+T	0.060 ± 0^{e}	0.104 ± 0.005^{d}	0.60 ± 0.076^{f}	0.38 ± 0.068^{f}
8	G+P	0.084 ± 0.005^{d}	0.132 ± 0.004^{c}	1.00 ± 0.112^{d}	0.60 ± 0.063^{ef}
9	A+T	0.092 ± 0.004^{cd}	0.130 ± 0^{c}	1.17 ± 0.080^{c}	0.71 ± 0.055^{e}
10	A+P	0.106 ± 0.005^{c}	0.142 ± 0.005^{b}	1.45 ± 0.119^{b}	1.10 ± 0.063^{c}
11	T+P	0.116 ± 0.005^{b}	0.148 ± 0.004^{b}	1.86 ± 0.081^{ab}	1.24 ± 0.085^{b}
12	G+A+T	0.096 ± 0.005^{cd}	0.108 ± 0.004^{d}	1.27 ± 0.116^{c}	1.00 ± 0.067^{d}
13	G+A+P	0.092 ± 0.004^{cd}	0.106 ± 0.005^{d}	1.09 ± 0.084^{d}	0.74 ± 0.082^{e}
14	G+T+P	0.116 ± 0.005^{b}	0.162 ± 0.004^{ab}	1.90 ± 0.084^{ab}	1.31 ± 0.073^{b}
15	A+T+P	0.100 ± 0.012^{c}	0.138 ± 0.004^{c}	1.20 ± 0.079^{c}	0.81 ± 0.026^{de}
16	G+A+T+P	0.128 ± 0.004^{ab}	0.162 ± 0.008^{ab}	1.90 ± 0.080^{ab}	1.85 ± 0.056^{a}
LSD ($P \leq 0.05$)		0.0069	0.145	0.0112	0.0871
ANOVA (F 15,32)		88.047	5.278	184.147	205.724

Each value is a mean of five replicates; ± – standard deviation; G – *Glomus mosseae*; A – *Acaulospora laevis*; P – *Pseudomonas fluorescens*; T – *Trichoderma viride*; means followed by the same letter(s) within a column are not significantly different over one another (Duncan's Multiple Range test, $P \leq 0.05$)

DISCUSSION

The need to preserve soil fertility and protect the environment from detrimental agronomic technique has brought about a revision of productive systems in agriculture. Results of the present study revealed that inoculation of *T. viride* alone brought about substantial increase in most of the growth parameters and combination of all the bioinoculants together, i.e. G+A+T+P, further improved plant growth, thereby showing the synergistic beneficial activity of all the microbes in better plant growth. Perhaps this could be due to the synergistic effect of *T. viride* with *P. fluorescens* which can solubilise more P in the soil by producing organic acids (Avis *et al*. 2008). *Trichoderma* species are also known to produce a large number of antibiotics like trichodermin, trichodermol, polyketides, peptaiboils, sesquterpenes, and steroids, all these compounds are known to promote plant growth besides having biocontrol activity (Harman *et al*. 2004). Similarly *T. viride* or *P. fluorescence* or their combined effect on growth improvement was also reported by other workers (Shanmugaiah *et al*. 2009; El-Mohamedy *et al*. 2011; Mishra *et al*. 2011). All growth parameters were increased by bioinoculants, except for plant height, which was not satisfactory in some AM fungi inoculated plants (G+A+T, G+A+P, G+T+P) when compared with uninoculated control. This might be due to the non-establishment of the inoculants in the rhizosphere or due to competition for space and nutrition between the inoculants.

Broccoli belongs to *Brassicaceae* family, which is a non-mycorrhizal family, even though an experiment was performed to see if other bioinoculants alter the morphology and physiology of roots to make it susceptible to AM fungal infection or AM fungi have any potential in soil mineralisation and release of bound minerals in the presence of other microbes. As expected, the broccoli roots were poorly colonised and the percent colonisation was almost negligible. These results support the work of Purakayastha *et al*. (1998). Inhibition of AM fungal colonisation of broccoli roots may be due to secondary substances released by the

roots including glucosinolates and other allelopathic chemicals that have antifungal properties (Roberts & Anderson 2011). The tips of roots were distinctly colonised indicating the presence of intraradical as well as extraradical hyphae but the vesicles and arbuscules were not seen. Presence of AM fungal colonization in members of *Brassicaceae* family has also been reported by other workers under glasshouse condition (Kamalakannan & Manivannan 2002; Orlowska *et al*. 2002; Regvar *et al.* 2003). This inhibition is especially due to locally induced changes in the endogenous glucosinolate concentrations (Tong *et al*. 2011) by the AM fungi. The presence of growth hormone – auxin – in the root tip could be responsible for the initial AM fungal colonisation of the root tip as auxin signalling within host roots is required for early stages of AM fungal infection (Hanlon & Coenen 2011). Although the roots were colonised, the symbiotic exchange of nutrients was not clear. Other bioinoculants might have influenced the survival and initial colonisation by AM fungi in the roots of broccoli.

The present study demonstrated that single inoculation with *T. viride* and consortium of G+A+T+P increased photosynthetic rate by increasing plant chlorophyll content, both *a* and *b*. The progressive increase in photosynthetic pigment by bioinoculants could be a result of enhanced gas exchange capacity due to decreased stomatal resistances and increased transpiration fluxes. Larger leaf area also increases the number of stomata per leaf and hence better photosynthetic rate. *Trichoderma* strains colonise the plant roots, establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology and may result in the improvement of abiotic stress resistance, nitrogen fertiliser uptake, and resistance to pathogens and photosynthetic efficiency (Hermosa *et al*. 2010; Harman *et al*. 2012).

Early flowering was observed in treated plants and T+P showed flowering at 54 (DAT) as compared to control in which flowers appeared after 87 DAT. AM fungi notably enhanced broccoli yield, regardless of symbiotic association. The considerable increase in head yield with T and G+A+T+P observed in the present study could possibly be due to the increase uptake of nutrients such as phosphorus, nitrogen, potassium, and other micronutrients by the synergistic effect of both *T. viride* and *P. fluorescens.* Vinale *et al.* (2008) reported that *Trichoderma* and plant interaction might produce the secondary metabolites such as auxin-like compounds or auxin-inducing substances that resulted in improved growth and yield. A higher efficiency of coinoculation with G+A+T+P for increasing head fresh weight was recorded, despite the lower percentage of mycorrhizal colonisation by *G. intraradices* as well as *A. laevis*.

Maximum shoot P content was recorded in *P. fluorescens* alone treated plants. *P. fluorescens* has the ability to produce plant growth promoting substances and some secondary metabolites which enhance nutrient uptake and plant growth (Burr *et al*. 1978). *T. viride*, when applied alone, showed maximum root P and shoot N content. The enhanced vegetative growth of broccoli in *Trichoderma* treated plants could be due to the root colonising ability of the fungus that resulted in better nutrient absorption through increased root biomass (John *et al*. 2010). Other possibility is that *Trichoderma* might have increased the solubility of phosphates which are then absorbed and assimilated in plant roots (Altomare *et al*. 1999). Significant differences between different treatments were not observed for shoot P uptake but were higher in all the treatments in comparison with control plants. The present study is in accordance with the other findings that application of consortium of AM fungi, *Pseudomonas* resulted in increased phosphorus and nitrogen content (Akhtar & Siddique 2008b). Prominent increase in the root N content in consortium of all the bioinoculants indicates that AM fungi might have played little role in the solubilisation of soil minerals, especially phosphorus and nitrogen. These mineral nutrients are then taken up by plant roots but there is currently a lack of information on how different microbes interact with AM fungi to affect nutrient mineralisation in non-mycorrhizal crop.

CONCLUSION

The benefits of growth promoting microbes in vegetable crops production represent great opportunities for current agricultural practices. The present investigation clearly demonstrated that application of *T. viride* alone or in combination with other growth promoting microbes (AM fungi and *P. fluorescens*) proved to be a promising factor for improved growth performance, nutrition, and yield of broccoli to an acceptable level. Thus, it could be considered as a suitable substitute for chemical fertiliser in low input on agricultural systems. Further, *T. viride* strain used in the current study can be tested in field conditions that could be important for its commercial application in natural conditions.

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