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Response of Strawberry plant (*Fragaria ananassa* Duch.) to inoculation with arbuscular mycorrhizal fungi and *Trichoderma viride*

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Abstract: The present paper represents the positive role of Arbuscular Mycorrhizal (AM) fungi as biofertilizers in strawberry. Experiments were carried out to assess the effectiveness of *Trichoderma viride* and AM fungi (*Glomus mosseae* and *Acaulospora laevis*) alone or in combination, on the growth and biomass production of strawberry. After 120 days, dual inoculation of *A. laevis* + *T. viride* showed maximum increase in plant height (30.5±0.3), fresh shoot weight (10.16±0.20), dry shoot weight (2.82±0.02), fresh root weight (6.70±0.10), total chlorophyll (0.841±0.05) and phosphorus content in root (1.13±0.02) as compared to control. However root colonization and AM spore number were maximum in *G. mosseae* + *A. lavies* (90.76±1.32) and in *G. mosseae* (211.16±2.56) respectively as compared to uninoculated plants. Triple inoculation of *G. mosseae* + *A. laevis* + *T. viride* (12.33± 057) was effective in increasing the leaf area.

Keywords: Strawberry, *Glomus mosseae, Acaulospora laevis, Trichoderma viride*

INTRODUCTION

The roots of living plants harbours a complex of microbes including AM fungi which create a special habitat that influences growth and survival of plants. Arbuscular Mycorrhizae (AM) are the most common endosymbionts of herbaceous plants including horticultural plants almost in every habitat. Mycorrhizal plants are known to have altered nutritional status, increased photosynthetic rates, altered levels of growth regulating substances and altered patterns of root exudation due to change in membrane permeability (Karthikeyan *et al.*, 2009), (Manoharachary *et al.*, 2009), (Atul-Nayyar *et al.*, 2009) Further, AM fungi play significant role in fruit production by transporting slowly mobile nutrient especially P, Mn, Zn, Fe and Cu from bulk of soil beyond the depletion zone surrounding active roots (Parkash *et al.*, 2009 and Farahani *et al.*, 2009).Though, worldwide distribution of AM fungi has been reported but only little information is available on indigenous AM fungal association with strawberry.
Therefore, in the present investigation, the effect of two mycorrhizal fungi i.e., *G. mosseae* and *A. laevis* alone and in combination with *T. viride* was carried on different growth parameters of *Fragaria ananassa* i.e., shoot height, shoot weight, root weight, root length, leaf area, AM spore count, AM root colonization, chlorophyll content, phosphorus content in roots and shoots after 75 and 120 days of inoculation.

MATERIALS AND METHODS

Collection of soil samples: Composite soil sample from rhizospheric soil of strawberry was collected.

Isolation of AM spores: Isolation of mycorrhizal spores was done alone by using Wet sieving and decanting technique of Gerdemann and Nicolson (1963). Spores were picked by hypodermic needle under stereobionocular microscope.

Mycorrhizal root colonization: Mycorrhizal root colonization was studied by rapid clearing and staining merthod of Phillips and Hayman (1970). Percent mycorrhizal root colonization was calculated by using the following formula:

Percent root colonization = Total number of root segments colonized /Total number of root segments examined x100

Identification of AM fungi: Identification was done by using keys of manual of Walker (1986), Schenck and Perez (1990), Morton and Benny (1990), Tanwar *et al.* (2008), Kumar *et al.* (2009). The following morphological characters of VAM spores were taken into consideration for identification of VAM spore structure, i.e., hyphal characters, vesicles, auxillary cells, subtending hyphae, spore germination, spore position, germinating shields, sporocarp, spore wall, ornamentation.

Mass multiplication of AM spores: Dominant spores i.e. *G. mosseae* and *A. laevis* isolated from the rhizosphere of strawberry were mass produced by using maize as a host plant. After 75 days, soil containing spores and colonized roots were used to inoculate nursery seedlings.

Mass production of *T. viride*: Firstly *T. viride* was isolated from the soil and then further mass produced by using wheat bran and saw dust medium which was prepared by using wheat bran, saw dust and distilled water in 3: 1: 4 ratio.

Inoculation: Seedlings of strawberry were grown in earthen pots under polyhouse conditions. To each pot ten percent of inoculum of AM fungi was added alone and in combination with *T. viride* and then the effect of *G. mosseae* and *A. laevis* was recorded on different growth parameters of strawberry after 75 and 120 days of inoculation. Each treatment was replicated thrice.

Root and shoot biomass: Roots and shoots were harvested after 75 days and 120 days from each treated pots. These were then oven dried at 70^0C for dry weight.

Root length: Root length of freshly harvested roots were measured with the measurements tape.

Leaf area: Leaf area of different treatments were studied separately after 75 days and 120 days using Leaf area meter (Systronics 211).

Mycorrhizal spore count estimation: Mycorrhizal spore count of strawberry after 75 days and 120 days was studied by 'wet sieving and decanting technique' (Gerdemann and Nicolson, 1963) and the quantification of AM spores was done by 'Grid line intersect method' (Adholeya and Gaur, 1994).

Estimation of AM root colonization: Root coloniztion was studied by 'Rapid clearing and staining technique' (Phillips and Hayman, 1970).

Chlorophyll content: Chlorophyll content was determined by using Arnon's method (1949) after 75 and 120 days.

Estimation of Phosphorus content of plant roots and shoots:

Phosphorus estimation: The phosphorus after digestion was estimated by 'Phospho-vanadomolybdate yellow colour method' (Jackson, 1973).

RESULTS AND DISCUSSION

Growth response: It is evident from the Tables (I and II) that the results of various growth parameters observed after 75 and 120 days depicted an increase in all parameters in treated plants as compared to control. After 75 days, change in shoot height was found to be maximum in single combination of *G. mosseae* (9.1±0.6). After 120 days, AM inoculated plants i.e., (*A. laevis* + *T. viride*) showed almost seven times increase in plant height (30.5±0.3), as compared to control (4.0±0.2).

Glomus fasciculatum significantly increased shoot length, internode number, number of leaves, stem diameter, root length, and root number of cashew (Ananthakrishnan *et al*., 2003). The most effective fungus for promoting growth of citrus seedlings was *Glomus fasciculatum* compared to *G. mosseae* as reported by Sharma *et al.* (2009). Micropropagated plants of two pineapple clones were inoculated with *Glomus* sp. or with *G. intraradices* increased growth as measured by leaf number, fresh and dry weights of aerial parts or roots, and leaf area, and the effect was greater in plants not given P and in those grown in acidic soil (Guillemin *et al*., 1991).

Shoot biomass: After 75 days fresh shoot weight was found to be highest in double treatment of *A. laevis* + *T. viride* (3.5±0.1).Similarly the dry weight was found to be maximum with *G. mosseae* + *T. viride* (1.64±0.03).After 120 days fresh shoot weight was found to be highest in double treatments of *A. laevis* + *T. viride* (10.16±0.20). Similarly the dry weight was found to be maximum with *A. laevis* + *T. viride* (2.82± 0.02). This can be due to maximum branched shoots of this plant. In pear seedlings height, stem diameter and dry weight of shoot increased significantly with AM inoculation. A greater increase in shoot compared with root production in VAM inoculated plants of pineapple was reported by Guillemin *et al.* (1992). Mycorrhizal grapevine plants showed a three fold increase in shoot growth compared with uninoculated controls (Lovato *et al*., 1992).

Root biomass: After 75 days of inoculation fresh root biomass was found to be highest in double treatment of *A. laevis* + *T. viride* (5.42 ±0.06). Similarly, dry root weight was found to be maximum in double treatment by *A. laevis* + *T. viride* (1.95±0.03). Similarly after 120 days of inoculation fresh root weight was found to be maximum in double treatment of *A. laevis* + *T. viride* (6.70±0.10). The dry root weight was found maximum in double treatment of *G. mosseae* + *T. viride* (3.52±0.01). *G. fasciculatum* was responsible for increasing seedling biomass and uptake of N, P, K, Ca and Mg than control non – inoculated seedlings of *Zizyphus mauritiana* (Mathur and Vyas, 1996). Similarly increase in biomass in AM inoculated plants of *Citrus aurantium* was reported by Nemec and Vu (1990).

Root length: After 75 days maximum root length was found highest in case of double treatment in *G. mosseae* + *A. laevis* (12.46±0.057). After 120 days maximum root length was found in case of *G. mosseae* (17.83±0.55). More branched and increased root length allowed maximum surface area available for nutrient absorption. The inoculation with *G. macrocarpum* and phosphorus fertilizer, increased plant height, root length, number of leaves and dry matter of peach seedlings (Awad, 1999). Endomycorrhiza formation caused increase in lateral root number and total root length in *Vitis vinifera* as reported by Schellenbaum *et al.* (1991).

Leaf area: Leaf area of all the treated plants increased after 75 and 120 days of inoculation. Highest leaf area after 75 days was observed in double treatment of *A. laevis* + *T. viride* (11.15±0.21). Similarly after 120 days the highest leaf area was found in triple treatment of *G. mosseae* + *A. laevis* +*T. viride* (12.33±0.57). The plants inoculated with AM fungi had greener and larger leaves, greater bunch weight and number of fruits per bunch in banana plant treated with 500 g of AM inoculums per plant than in untreated plant or non- inoculated plant

Table 1. Growth response of strawberry fruit plant after 75 days with single, double and triple inoculation of AM fungi and *Trichoderma viride.*

Sr. No.	Treatments	Plant height in (cm)	Fresh shoot weight (gm)	Dry shoot weight (gm)	Root length (cm)	Fresh root weight (gm)	Dry root weight (gm)	Leaf area (Sq.cm)	AM spore count/ 50 gm soil	AM root colonization (%)
1.	Control	*3.0± 0.1	0.94± 0.38	0.73± 0.11	5.6± 0.45	1.06± 0.20	0.74± 0.04	5.33± 0.66	101.33± 2.30	31.11± 1.92
2.	*Trichoderma viride*	5.93± 1.0	2.23± 0.15	1.05± 0.02	8.26± 0.15	4.11± 0.10	1.74± 0.01	9.13± 0.15	138.33± 0.57	51.85± 3.20
3.	*Glomus mosseae*	9.1± 0.6	2.1± 0.10	0.95± 0.02	11.16± 0.15	2.17± 0.06	0.78± 0.005	10.2± 0.1	145± 1.0	71.49± 1.58
4.	*Acaulospora laevis*	3.6± 0.1	1.1± 0.10	0.86± 0.03	8.33± 0.32	2.48± 0.10	0.96± 0.02	7.16± 0.02	128± 2.0	71.11± 1.92
5.	*G.mosseae+ A. laevis*	7.4± 0.2	2.34± 0.13	0.84± 0.02	12.46± 0.057	1.39± 0.01	0.87±0.01	7.3± 0.1	161.75± 1.5	93.87± 0.20
6.	*G.mosseae+ T. viride*	4.0± 0.1	3.1± 0.10	1.64± 0.03	11.93± 0.32	5.12± 0.01	1.73± 0.03	9.26± 0.37	171.33± 0.57	90.04± 3.24
7.	*A. laevis + T. viride*	5.1± 0.1	3.5± 0.10	0.93± 0.02	11.06± 0.11	5.42± 0.06	1.95± 0.03	11.15± 0.21	163.33± 2.08	65.65± 1.74
8.	*G.mosseae+ A.laevis+ T. viride*	4.2± 0.2	3.07±0.005	1.27± 0.02	10.43± 0.057	2.78± 0.16	1.07± 0.01	8.2± 0.26	115.66± 0.57	81.59± 1.58

According to one way analysis of variance (ANOVA), the mean value is significant at 95% level. *= Mean of three replicates ± = Standard deviation.

Table 2. Growth response of strawberry fruit plant after 120 days with single, double and triple inoculation of AM fungi and *Trichoderma viride.*

Sr. No.	Treatments	Plant height in (cm)	Fresh shoot weight (gm)	Dry shoot weight (gm)	Root length (cm)	Fresh root weight (gm)	Dry root weight (gm)	Leaf area (Sq.cm)	AM spore count/ 50 gm soil	AM root colonization (%)
1.	Control	*4.0± 0.2	2.63± 0.51	0.753± 0.06	0.753± 0.81	0.52± 0.24	0.513± 0.04	4.5± 3.53	151.33± 1.52	37.77± 3.85
2.	*Trichoderma viride*	9.0± 0.1	3.13± 0.15	1.33± 0.02	10.2± 0.26	1.70± 0.10	0.97± 0.01	7.33± 1.52	196.66± 0.57	44.27± 3.70
3.	*Glomus mosseae*	18.3± 0.4	4.43± 0.37	1.62± 0.02	17.83± 0.55	2.30± 0.09	1.43± 0.01	8± 2.0	211.16± 0.98	64.15± 2.56
4.	*Acaulospora laevis*	10.9± 0.6	4.6± 0.43	1.73± 0.01	9.56± 0.32	1.51± 0.12	0.83± 0.03	6± 2.6	180.66± 1.15	56.86± 3.39
5.	*G .mosseae +A. laevis*	24.2± 0.3	4± 0.1	1.32± 0.005	12.13± 0.15	1.13± 0.06	0.55± 0.02	10.33± 2.08	177.33± 1.15	90.76± 1.32
6.	*G. mosseae+ T. viride*	15.3± 0.3	3.6± 0.2	136± 0.03	14.23± 0.25	6.05± 0.05	3.52± 0.01	11± 1.0	161.66± 0.57	42.5± 2.16
7.	*A. laevis + T. viride*	30.5± 0.3	10.16± 0.20	2.82± 0.02	15.36± 0.37	6.70± 0.10	3.14± 0.02	11.66± 1.52	164.66± 0.57	45.5± 2.16
8.	*G.mosseae+ A.laevis+ T. viride*	20.1± 0.3	8.16± 0.20	2.63± 0.01	15.46± 0.057	3.37± 0.06	1.86±0.01	12.33± 0.57	157.33± 1.15	49± 1

According to one way analysis of variance (ANOVA), the mean value is significant at 95% level. * = Mean of three replicates, ± = Standard deviation

Table 3. Chlorophyll content and phosphorus content of strawberry fruit plant after 75 days with single, double and triple inoculation of AM fungi and *Trichoderma viride***.**

Sr. No.	Treatments	Chlorophyll content (mg/ ml)			Percent Phosphorus	
		Chl a	Chl b	Total Chl	Root	Shoot
1.	Control	*1.099± 0.15	0.282± 0.17	1.300± 0.37	0.04± 0.02	0.016± 0.01
2.	*Trichoderma viride*	1.485± 0.1	0.348± 0.16	1.890± 0.24	0.126± 0.02	0.32± 0.02
3.	*Glomus mosseae*	1.299± 0.1	0.382± 0.15	2.034± 0.15	0.36± 0.25	0.24± 0.02
4.	*Acaulospora laevis*	1.259± 0.11	0.346± 0.1	1.677± 0.25	0.22± 0.02	0.63± 0.01
5.	*G. mosseae+A. laevis*	1.300± 0.1	0.378± 0.15	171.2± 0.11	0.143± 0.01	0.03± 0.01
6.	*G. mosseae+ T. viride*	1.304± 0.05	0.495± 0.002	1.633± 0.14	0.08± 0.005	0.49± 0.06
7.	*A. laevis+ T. viride*	1.209± 0.1	0.460± 0.15	1.7702± 2.08	0.07± 0.005	0.31± 0.01
8.	*G. mosseae+ A. laevis+ T. viride*	1.204± 0.15	0.442± 0.05	1.780± 0.05	0.06± 0.02	0.34± 0.02

According to one way analysis of variance (ANOVA), the mean value is significant at 95% level. * = Mean of three replicates, ± = Standard deviation

(Eswarappa *et al.*, 2002). The VA mycorrhizae also increased stomatal resistance, thereby reducing the rate of transpiration of *Ziziphus mauritiana* (Mathur and Vyas, 1995).

Percent root colonization and spore number: AM treated plants showed higher percent root colonization and spore number over control plants (Tables I and II). After 75 days of inoculation, percent root colonization was maximum in double treatment of plants i.e., in *G. mosseae +A. laevis* (93.87 ±0.20). *G.. mosseae + T. viride* showed highest spore number (171.33±0.57). Similarly after 120 days, percent root colonization was maximum in double treatment of *G. mosseae +A. laevis* (90.76±1.32). *G. mosseae* (211.16 ±2.56) showed highest spore number. The improved vegetative parameters and rootstock growth observed in the field and pot investigations can be attributed to increased AM fungal association in terms of percent root colonization and spore count leading surface area for absorption and uptake of nutrients in the rootstocks as observed by Patil and Patil (2007). Apple showed significant level of AM colonization and higher number of mycorrhizal spores as a result of AM treatment (Karagiannidis and Velimis, 2000).

Chlorophyll content: Chlorophyll content was also found to be increased in all inoculated plants over control as shown in Table (III and IV). After 75 days of inoculation

Table 4. Chlorophyll content and Phosphorus content of strawberry fruit plant after 120 days with single, double and triple inoculation of AM fungi and *Trichoderma viride.*

Sr.No.	Treatments	Chlorophyll content (mg/ ml)			Percent Phosphorus	
		Chl a	Chl b	Total Chl	Root	Shoot
1.	Control	*0.164± 0.15	0.139± 0.02	0.270± 0.17	0.31± 0.02	0.12± 0.03
2.	*Trichoderma viride*	0.242± 0.17	0.270± 0.15	0.513± 0.15	0.72± 0.04	0.16± 0.01
3.	*Glomus mosseae*	0.418± 0.11	0.28± 0.15	0.705± 0.15	0.74± 0.04	0.22± 0.02
4.	*Acaulospora laevis*	0.464± 0.1	0.216± 0.1	0.781± 0.1	0.46± 0.05	0.24± 0.02
5.	*G. mosseae+ A. laevis*	0.432± 0.20	0.296± 0.16	0.769± 0.15	0.63± 0.02	0.36± 0.05
6.	*G. mosseae+ T. viride*	0.485± 0.05	0.301± 0.1	0.719± 0.11	0.51± 0.02	0.35± 0.03
7.	*A. laevis+ T. viride*	0.348± 0.15	0.2593± 0.05	0.841± 0.05	1.13± 0.02	0.31± 0.01
8.	*G. mosseae+ A. laevis+ T. viride*	0.374± 0.05	0.237± 0.1	0.645± 0.05	0.65± 0.02	0.26± 0.01

According to one way analysis of variance (ANOVA), the mean value is significant at 95% level. * = Mean of three replicates, ± = Standard deviation

Chl.a was maximum in *T. viride* (1.485±0.1), Chl.b in *G. mosseae + T. viride* (0.495± 0.002) and total Chl in *G. mosseae* (2.034±0.15) over control Chl a (1.099± 0.15), Chl b (0.282±0.17) and total Chl (1.300±0.37) respectively. Chlorophyll a (0.485±0.05), Chlorophyll b (0.301±0.1) were maximum in plants treated with *G. mosseae +T. viride.* The total chlorophyll was maximum in double treatment of *A. laevis + T. viride* (0.841±0.05) over control Chl a (0.164 ±0.15), Chl b (0.139 ±0.02), total Chl (0.270±0.17) after 120 days. The AM fungi inoculation has been reported to increase chlorophyll content of leaves in a number of fruit crops. Godara (1993) reported an increase in total chlorophyll content in peach seedlings leaves inoculated with AM fungi. Higher chlorophyll content in apple seedling was in AMF inoculated plant (Sharma *et al.*, 1998).Thaker and Fasrai (2002) observed significant increase in total chlorophyll of papaya leaves when the plants were inoculated with AM fungi.

Phosphorus content: All the inoculated seedlings depicted increased phosphorus content when compared with uninoculated control seedlings as shown in tables III and IV. The *G. mosseae* showed maximum P content after 75 days in root (0.36±0.25) and *A. laevis* showed maximum P content in shoot (0.63±0.01). After 120 days *A. laevis + T. viride* (1.13±0.02) showed maximum P content in root and *G. mosseae + A. laevis* in shoot (0.36+0.05).The growth of papaya and banana plants increased due to higher content of 'P' when plants were inoculated with AM fungi as reported by Manjunath *et al.* (2002).

Hence, the present study clearly suggests that in strawberry, double inoculation of *A. laevis + T. viride* or *G. mosseae + A. laevis or G. mosseae + T. viride* shows better vegetative growth of all the plants. Such a dual combination as mentioned above can be recommended for producing better seedlings after transplantation, better growth and yield of strawberry.

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