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Stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. *capitata*

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Abstract

Cabbage (*Brassica oleracea*, var. *capitata*, cv. Hercules) seedlings were inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi *Glomus fasciculatum*, *G. aggregatum*,. and *G. mosseae*. Differential efficiency in mycorrhizal colonization and the specificity of fungal symbiont to stimulate the growth and nutrient uptake of the host were observed. In addition, there was an increase in phenol, protein, reducing sugar contents, and peroxidase activity in the VAM inoculated seedlings. Since these compounds are known to confer resistance against fungal pathogens, the use of VAM as a biological control agent to protect cabbage against several root diseases is suggested.

Additional key words: amino acids, cabbage, *Glomus aggregatum*, *Glomus fasciculatum*, *Glomus mosseae*, peroxidase, sugars.

Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi are obligate symbiotic microorganisms that form associations with plant roots in a host-nonspecific manner (Shaul *et al.* 1999). These fungi have been shown to promote plant growth mainly by enhancing nutrient acquisition (Harley and Smith 1983), producing growth hormones, improving rhizospheric conditions (Linderman 1994), and altering host physiological and biochemical properties (Smith and Read 1995). Mycorrhizal fungi not only play an important role in plant nutrition, they can also increase resistance of the plants to pathogens (Singh 1996).

Cabbage (*Brassica oleracea* var. *capitata)* is an important agricultural crop cultivated by subsistence farmers in the KwaZulu Natal (KZN) region, South Africa. Members of cruciferae were thought to be non-mycorrhizal, but recent studies in cabbage, have shown that they do form mycorrhiza (Nelson and Achar, in press). Since cabbage cultivation is done by poor farmers in P-deficient soils, the VAM symbiosis may be of particular significance in coping up with the P-deficiency in such systems. Efficiency of integration of symbiosis varies with different host-fungus combinations and environmental conditions (Gianinazzi-Pearson 1991). In the present study efficiency of different VAM species was tested for maximum biomass production in order to select the elite strain. The nutrient uptake stimulated by VAM in cabbage was also analyzed and the possible role of VAM as biocontrol agent against root diseases of cabbage seedlings is discussed.

Materials and methods

The vesicular-arbuscular mycorrhizal fungi, *Glomus fasciculatum* and *G. aggregatum* were obtained from D.J. Bagyaraj, Agricultural University, GKVK campus, Bangalore, India and *G. mosseae*, from S. Senthil Kumar, Department of Biotechnology, J.J. College of Arts and Science, Pudukottai, India. All the VAM fungi were maintained on onion (*Allium cepa* L.) to prepare pot cultures followed by Hwang *et al.* (1992). Two months after inoculation, the fibrous onion roots were collected, chopped (2 - 3 mm in length), and mixed with steam-sterilized sand and loam soil. This mixture of soil, chlamydospores, and segmented colonized roots was

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air-dried, packed in plastic bags, stored at 4 °C and used whenever required.

Cabbage (*Brassica oleracea* var. *capitata* cv. Hercules) seeds were surface-sterilized in 70 % ethanol for 2 min, followed by 2 min in 0.6 % mercuric chloride, rinsed three times in sterile distilled water, and sown in plastic pots (13 cm diameter) containing potting mix and vermiculite (2:1). One week after germination, the seedlings were transplanted to 13 cm diameter plastic pots. Each pot contained 1 g of mycorrhizal inoculum (500 spores per 1 g soil) of either *G. aggregatum, G. fasciculatum*, or *G. mosseae*. Inoculum was distributed in one layer, 5 cm, below the soil surface. The seedlings (24 per pot) were placed 2.5 cm above the mycorrhizal inoculum. Seedlings in soil without mycorrhizal inoculum served as controls. All pots (five replicates per treatment) were arranged randomly in growth chambers under irradiance of 1.6 μmol $m^{-2} s^{-1}$ (16-h photoperiod) provided by cool-white fluorescent tubes and day/night temperature of 16/14 °C. Seedlings were watered with distilled water and fertilized with 50 cm^3 $week^{-1}$ of 10 % Hoagland's nutrient solution (Hoagland and Arnon 1950), without phosphorus, beginning 30 d after transplanting.

Samples were harvested 60 d after inoculation and the dry mass of shoots and roots were recorded after drying them in a hot air oven at 60 °C for 48 h. The collected root samples were gently washed under tap water and suitably processed (Phillips and Hayman 1970) to calculate percentage of root colonization (Giovanetti and Morse 1980). Phosphorus content of the roots and shoots was determined colorimetrically by vanadomolybdate method (Jackson 1971) and total nitrogen according to Bremmer (1960). Total soluble sugars, reducing sugars, starch, soluble proteins, and phenols were estimated according to Yemm and Willis (1954), Nelson (1944), Somogyi (1952), Lowry *et al.* (1951), and Mahadevan (1991), respectively. Total chlorophyll and carotenoid contents of the leaves were estimated according to Lichtenthaler (1987). The extraction and peroxidase assay of the roots and leaves of inoculated and control seedlings was determined according to Reuveni and Reuveni (1995).

Extraction of free amino acids was performed by homogenization of leaves and roots with blender in 80 % ethanol, treated with chloroform and concentrated under vacuum. The residue was dissolved with picric acid and then absorbed on a *Dowex 50 X 80*, 100 - 200 mesh. The fraction was eluted with 4 M NH_4OH, dried under vacuum, esterified, and then acylated with heptafluorobutyric anhydride. Amino acid composition was determined using a *Carlo Erba Fractovap 2200* (*Merck*, S. Africa) gas chromatograph equipped with a flame ionization detector. The column (2 mm × 2 m) was packed with 3 % SE 30 on *Chromosorb HP* 80 - 100 mesh. Oven temperature was maintained at 100 °C for 6 min and programmed up to 260 °C at 2 °C min^{-1}.

Data were subjected to analysis of variance and means were compared using Duncan's multiple range test with *SAS* software (*SAS Institute*, USA 1985).

Results

The overall growth and nutrient contents of the mycorrhizal seedlings were higher as compared to the controls. The host responded differently to the different VAM species used. *G. fasciculatum* caused more than two fold increase in shoot dry mass and nitrogen content and a four fold increase in P-uptake and root biomass (Table 1). Increase in biomass of the root was higher than that of the shoot.

Inoculation with *G. fasciculatum* resulted in three fold increase in total sugars and four fold increase in starch contents of host, while *G. aggregatum* inoculated plants responded least effectively. A similar trend was also observed in total chlorophyll, carotenoids, and soluble protein, phenol and amino acid contents of the host shoots (Table 1). There was also an increase in the amino acids (arginine, serine, lysine and phenylalanine) contents in the roots. There was a four fold increase in arginine and a two fold increase in phenylalanine, serine and lysine contents in inoculated seedlings (Fig. 1).

When compared to the uninoculated controls, the activity of peroxidase was higher in roots and leaves of VAM inoculated plants. There was a two fold increase in peroxidase activity in roots of *G. fasiculatum* inoculated plants than in the controls (Fig. 2). *G. fasiculatum* resulted in maximum increase in all the growth parameters (shoot and root biomass) and also colonized roots of the host at a faster rate, followed by *G. mosseae*, while *G. aggregatum* was found to be least effective.

Discussion

In the present study, VAM inoculated seedlings showed better growth compared to the controls. However, there was difference in the growth promoting efficiency of the different VAM fungi. *G. fasciculatum* and *G. mosseae* were found to be more effective than *G. aggregatum* in increasing the total biomass production and phosphorus uptake. These differences may be attributed either to the mechanism of mycorrhizal infection and development in the host tissues (Sanders *et al.* 1977) or the physiological differences in rate of nutrient uptake (Mosse 1973). Increase in shoot and root length can be attributed to the improvement in phosphate uptake as a result of VAM infection (Jeffries 1987). Though VAM inoculation in the present study resulted in significant increase in both root and shoot dry masses, there was relatively higher increase in root dry mass than shoot ones. The former may be also attributed to increase in biomass of VAM fungi within the roots.

Table 1. Effect of *Glomus* species on biomass, nitrogen, phosphorus, sugar, phenols, pigments, proteins, and amino acids contents in *Brassica oleracea* var. *capitata*. Means of five replicates ± SE. For each variant, any two values without common letters in their superscripts are significantly different by Duncan's multiple range test ($P = 0.01$).

	Control	*Glomus aggregatum*	*G. fasciculatum*	*G. mosseae*
Root dry mass [mg plant^{-1}]	83.5 ± 3.80a	113.6 ± 2.40b	172.4 ± 4.40c	142.0 ± 7.30d
Shoot dry mass [mg plant^{-1}]	207.7 ± 4.80a	317.0 ± 2.10b	428.0 ± 3.20c	370.8 ± 1.70d
Total dry mass [mg plant^{-1}]	291.2 ± 4.40a	430.6 ± 2.20b	598.4 ± 2.70c	512.8 ± 2.10d
Nitrogen [mg plant^{-1}]	3.1 ± 0.19a	4.3 ± 0.18a	7.7 ± 0.15c	5.3 ± 0.17d
Root phosphorus [mg plant^{-1}]	9.4 ± 2.50a	12.5 ± 1.90b	23.6 ± 3.60c	22.3 ± 2.00c
Shoot phosphorus [mg plant^{-1}]	16.6 ± 2.20a	24.7 ± 1.80b	51.2 ± 4.30c	39.4 ± 3.60d
Total sugars [% (d.m.)]	2.5 ± 0.18a	5.5 ± 0.17b	8.5 ± 0.08c	7.2 ± 0.09d
Reducing sugars [%]	1.6 ± 0.28a	3.6 ± 0.16b	5.9 ± 0.25c	5.0 ± 0.13c
Non-reducing sugars [%]	0.9 ± 0.02a	1.9 ± 0.06b	2.6 ± 0.10b	2.0 ± 0.12b
Starch [%]	0.9 ± 0.03a	1.8 ± 0.20b	2.9 ± 0.32c	2.0 ± 0.43b
Carotenoids [mg g^{-1}(d.m.)]	70.7 ± 1.44a	89.6 ± 1.54b	119.5 ± 1.24c	114.8 ± 1.30c
Chlorophylls [mg g^{-1}(d.m.)]	283.7 ± 1.74a	325.1 ± 2.60b	363.4 ± 2.20c	360.3 ± 4.34c
Proteins [mg g^{-1}(d.m.)]	16.2 ± 1.30a	25.3 ± 2.12b	36.6 ± 3.62c	34.6 ± 4.14c
Amino acids [mg g^{-1}(d.m.)]	11.2 ± 1.23a	13.4 ± 1.82a	44.5 ± 2.20c	21.4 ± 2.53b
Phenols [mg g^{-1}(d.m.)]	7.4 ± 1.42a	11.2 ± 1.23b	18.6 ± 1.85c	12.3 ± 2.12b
VAM colonization [%]	0a	40.2 ± 0.57b	74.2 ± 1.10c	46.1 ± 1.3b

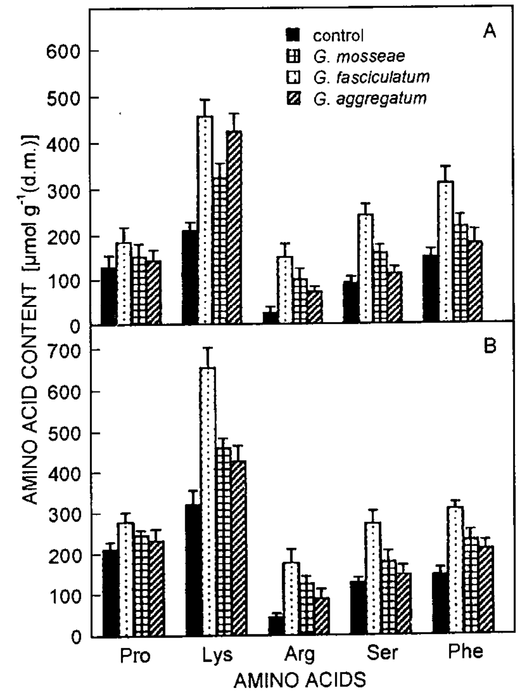

Fig. 1. Changes in proline, (Pro), lysine (Lys), arginine (Arg), serine (Ser), and phenylalanine (Phe) contents in shoots (*A*) and roots (*B*) of mycorrhizal and non-mycorrhizal cabbage seedlings. *Error bars* represent standard deviation of the means.

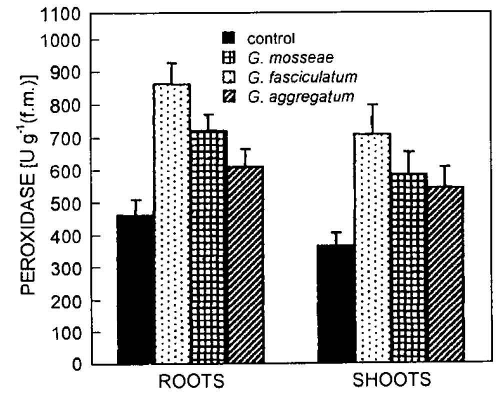

Fig. 2. Peroxidase activity in roots and shoots of mycorrhizal and non-mycorrhizal cabbage seedlings. *Errors bars* represent standard deviation of the means.

Significant increase in nitrogen content was noticed in VAM inoculated cabbage seedlings. Increased nitrogen content in mycorrhizal plants might be due to the increased nitrate reductase activity in consequence of improved P-nutrition provided by VAM symbiosis as suggested by Oliver *et al.* (1983). VAM inoculated cabbage seedlings had higher phosphate content than uninoculated seedlings. Increase in phosphorus content of VAM inoculated seedlings is due to the additional absorbing surface provided by the external hyphae of endomycorrhizal fungi, which enhances the ability of the plants to retrieve phosphates (Marschner and Dell 1994). The improved P nutrition by the mycorrhizal fungi is known to play an important role in reducing the severity of several diseases (Krishna and Bagyaraj 1983) and this may also be true for root diseases of cabbage. Leaves of VAM inoculated cabbage seedlings had higher chlorophyll content than the controls. Increase in chlorophyll content of mycorrhizated seedlings is due to the increase in the cytokinin content, which prevents chlorophyll degradation (Allen 1981).

Increase in amino acid contents was observed in roots and leaves of VAM inoculated seedlings. Moreover, increase in arginine, serine and phenylalanine contents were observed in the roots. These amino acids are known to play an important role in providing resistance to the mycorrhizated plant against pathogens (Caron 1989). That these amino acids contribute to the resistance of *Brassica* species against leaf and root diseases is to be established. Increased in protein contents in VAM inoculated plants, similar to our studies, have also been reported. This has been attributed either to the post-infectional stimulation of *de novo* protein synthesis in the host plants or to the presence of fungal proteins in mycorrhizal roots (Mathur and Vyas 1996).

The roots and leaves of mycorrhizal cabbage seedlings also had higher concentration of phenols than the controls. The increase in total phenols in VAM inoculated plants could be due to the general triggering of aromatic biosynthesis (Mahadevan 1991). Present study also revealed that the ratio of non-reducing sugars to reducing sugars and starch was relatively higher for VAM inoculated seedlings as compared to the controls. The increased amount of reducing sugar is a characteristic feature of high P-plants (McArthur and Knowles 1993). However, increase in starch may be due to the increased rate of photosynthesis mediated through the supply of P and altered hormonal level in mycorrhizal plants (Dixon *et al.* 1988).

The peroxidase activity was higher in the roots and leaves of VAM inoculated seedlings than in the controls. The enhanced peroxidase activity especially in the roots of mycorrhizated seedlings was due to the activation, solubilization or *de novo* synthesis of enzymes or their production by the invading VAM fungi (Spanu and Bonfante-Fasolo 1988).

Our results indicate that during the early stages of colonization, VAM fungi may trigger host defense responses such as production of phenolic compounds, proteins and activation of peroxidase, which may involved in the cross protection against some root pathogens in cabbage. Since these compounds are known to confer resistance, the application of selected VA mycorrhizal fungi during cabbage cultivation may benefit plant growth and development and also offers the possibility of increasing resistance against important soil-borne pathogens of cabbage.

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