Effect of Vesicular-Arbuscular Mycorrhizae on Tissue Culture-derived Plantlets of Strawberry

MC.G. Chávez and R. Ferrera-Cerrato

Sección de Microbiología, Centro de Edafología, Colegio de Postgraduados, Montecillos 56230, Mexico

Additional index words. *Fragaria* × *ananassa*, vesicular-arbuscular endomycorrhizae, micropropagation

Abstract. The responses of four micropropagated strawberry (*Fragaria* × *ananassa* Duch.) cultivars ('Douglas', 'Tioga', 'Aiko', and 'Pajaro') to colonization by three vesicular-arbuscular mycorrhizal (VAM) fungi were determined under nursery conditions. Species of VAM endophytes were *Glomus* sp. CPH-23, *Glomus macrocarpum* Tul. & Tul., and *Glomus versiforme* Berth & Trappe. Yield in VAM plants tended to exceed that of nonVAM plants during the latter part of the harvest, but VAM effects differed widely with host-endophyte combinations. Cultivar-endophyte combinations producing the best yield were 'Douglas'-*Glomus* sp. CPH-23, 'Tioga'-*G. macrocarpum*, and 'Aiko'-*G. versiforme*. The number of strawberries per plant differed significantly ($P < 0.01$) for 'Tioga', depending on the endophytes used. Root colonization by the endophytes varied from 25% to 75%. Yield was not related to colonization.

Strawberry is a crop of great socioeconomic importance in Mexico, since its cultivation is labor-intensive, its postharvest care requires sophisticated techniques, and because of its high, quality-dependent cash yield as an export commodity (Juhrez, 1986). Tissue culture propagation was first described for strawberry in 1962 (Belkengre and Miller, 1962) and has been used since then to obtain large numbers of plants. Morandi et al. (1979) have pointed out the necessity of providing micropropagated plants with vesicular-arbuscular mycorrhizal (VAM) fungi, because of the important role these symbionts play in the establishment and growth of most plants (Granger et al., 1983; Mosse, 1973; Pons et al., 1983).

Plantlets were grown for 30 days from the time of in vitro differentiation on Murashige and Skoog culture medium (1962) before transferring them to pots containing 250 g of a steam-sterilized growth medium (2 forest soil : 1 river sand, v/v). Inocula of one of three species of VAM fungi were placed 5 cm below the surface of the growth medium before transplanting. The inocula (10 g) consisted of spores of *Glomus macrocarpum*, *Glomus versiforme*, an unidentified species (*Glomus* sp. CPH-23), and of colonized (65%) root fragments mixed with the soils of the cultures of these fungi grown on *Phaseolus vulgaris* L. as the host plant. At planting, there were 16 treatments (combinations of

the four strawberry cultivars with one of the three VAM fungal species or the nonVAM control) replicated four times, for a total of 64 experimental units.

Polyethylene bags were placed over the pots after having been watered to field capacity with distilled water. The bags were kept in place for 3 weeks to minimize transpiration. The plants were maintained at greenhouse conditions (20C, 70% RH). After 90 days in individual pots, plants and their VAM soil were transferred to large (40 × 80 × 40 cm) containers. Two separate containers were used for plants colonized by each one of the three isolates of VAM fungi and the nonVAM controls, with each container holding two replicates of the four cultivars.

The eight plants in each container were randomized.

The berries were harvested weekly after 30 days of growth in the large containers. Fresh weight (yield), total soluble solids content (SSC, determined by refractometry), and the number of fruit were determined. Root and shoot dry weights and fungal colonization (Phillips and Hayman, 1970) were measured after the final harvest. Differences between the weekly yields of the plant-fungus combinations for each cultivar and differences in total yield were tested by least significant difference. Fruit number was evaluated by Tukey's range test.

The cultivars responded differently to VAM fungal colonization. Most VAM treatments produced low yields at first, compared with the nonVAM controls, but tended to exceed the latter as the harvest progressed (Fig. 1a-c). 'Tioga' was an exception, since the control plants did not flower during the experiment. This cultivar, when grown with *Glomus* sp. CPH-23, flowered later than the other VAM plants and produced very small fruits (Fig. 1d).

The *G. versiforme* and nonVAM combinations of 'Aiko' had the best total yields of all treatments. *Glomus macrocarpum* produced the best results when colonizing 'Tioga', and *Glomus* sp. CPH-23 when colonizing 'Douglas'. In 'Pajaro', nonVAM and *G. macrocarpum* plants had the highest yields (Table 1). No significant differences were observed among the total number of fruit produced, except in 'Tioga', where numbers for the *G. macrocarpum* and *G. versiforme* combinations were significantly ($P < 0.01$) higher than for *Glomus* sp. CPH-23. There were no significant differences ($P > 0.05$) in SSC among any of the treatments (results not shown). Colonization by *Glomus* sp. CPH-23 enhanced plant dry-matter production, while *G. macrocarpum* and *G. versiforme*

Table 1. Effect of endomycorrhizal inoculation on total yield and number of fruits of four strawberry cultivars raised from in vitro culture.

Cultivar	Mycorrhiza	Total yield/plant (g) ^a	Total no. fruit/plant ^b
Aiko	None	95.1 a	10.2 a
	<i>Glomus</i> sp. CPH-23	71.2 b	7.7 a
	<i>G. macrocarpum</i>	68.0 b	7.2 a
	<i>G. versiforme</i>	96.8 a	8.7 a
Douglas	None	56.7 b	5.2 a
	<i>Glomus</i> sp. CPH-23	78.7 a	6.0 a
	<i>G. macrocarpum</i>	26.6 c	5.5 a
	<i>G. versiforme</i>	46.7 b	6.0 a
Pajaro	None	84.9 a	9.2 a
	<i>Glomus</i> sp. CPH-23	41.8 b	5.0 a
	<i>G. macrocarpum</i>	63.0 a	8.0 a
	<i>G. versiforme</i>	55.4 b	7.7 a
Tioga	None	None	None
	<i>Glomus</i> sp. CPH-23	7.4 c	0.5 b
	<i>G. macrocarpum</i>	80.9 a	9.2 a
	<i>G. versiforme</i>	54.8 b	6.7 a

^aData are means of four replications and are not significantly different ($P > 0.01$) when followed by the same letter by LSD.

^bStatistical analysis by cultivar according to Tukey's range test $P < 0.01$.

Received for publication 17 Aug. 1987. The work reported here was funded partially by a grant from CONACYT PVT/AG/NAL-81/1981. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

Fig. 1. Response of four strawberry cultivars to colonization by VAM fungi in days after fruit set. (a) 'Aiko', (b) 'Douglas', (c) 'Pajaro', (d) 'Tioga'. Vertical bars indicate LSD ($P < 0.01$).

Table 2. Response of strawberry plants to endomycorrhizal inoculation under nursery conditions.

Mycorrhiza	Dry matter (g)			No. stolons	Colonization (%)
	Root	Shoot	Total		
None	9.1	18.4	27.5	4.0	0.0
<i>Glomus</i> sp. CPH-23	13.9	15.2	29.1	6.0	74.6
<i>G. macrocarpum</i>	9.3	13.3	22.6	3.1	24.7
<i>G. versiforme</i>	9.1	15.6	24.7	3.9	61.7

depressed it (Table 2). Plants colonized by *Glomus* sp. CPH-23 also produced the most stolons, while those colonized by *G. macrocarpum* produced the fewest, even though these plants were the most vigorous. *Glomus* sp. CPH-23 was the most effective root colonizer, and *G. macrocarpum* was the least effective (Table 2). Since the root systems were intermingled in the containers and could not be separated according to replications, statistical analyses were not possible. The data are presented as a total of all replicates for qualitative comparison. The response of strawberry cultivars to colonization by diverse VAM fungal species varied and depended on the host-endophyte combination. Such responses, including both growth enhancement and depression, were noted by others (Daft and El-giahmi, 1975). When VAM fungal biomass is large or when conditions for photosynthesis are less than optimal, the endophytes may become parasites

that inhibit host plant growth (Bethlenfalvay et al., 1982). Such phenomena probably also depend on variations in host preference and soil adaptation (Hayman, 1982).

In the present study, nonVAM control plants tended to have better growth and productivity than some VAM plants. The negative responses during the early growth phase of the VAM plants may have been related to source-sink effects within the symbiotic association (Bethlenfalvay et al., 1983). The phenomenon of delayed flower development in 'Tioga' in the absence of VAM fungi needs further investigation. Phosphorus availability is known to affect floral differentiation and growth (Blatt, 1972; Bould, 1974), and the additional P presumably provided to these cultivars by VAM fungi may have led to flowering in VAM plants; however, other possibilities cannot be excluded. These possibilities include changes in hormonal and nutritional conditions as a result of VAM

fungal colonization. The lack of relationship between VAM fungal colonization and host growth response has been noted by others (Graham et al., 1982) and can be ascribed to as yet unelucidated relationships between the extraradical and intraradical components of the fungal mycelium and their respective functions as sources and sinks within the association. It has been demonstrated that VAM fungal colonization of strawberry plants propagated by tissue culture affects the growth and productivity of these plants. Further work is needed to determine optimal host-endophyte combinations under given edaphic and environmental conditions.

Literature Cited

- Belkengre, R.C. and P.W. Miller. 1962. Culture of apical meristem of *Fragaria vesca* strawberry plants as a method of excluding latent virus. *Plant Dis. Rptr.* 46:119-121.
- Bethlenfalvay, G.J., H.G. Bayne, and R.S. Pacovsky. 1983. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: The effect of phosphorus on host plant-endophyte interactions. *Physiol. Plant.* 57:543-548.
- Bethlenfalvay, G.J., M.S. Brown, and R.S. Pacovsky. 1982. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: Development of the host plant. *Phytopathology* 72:889-893.
- Blatt, C. 1972. Effect of phosphorus applications on strawberry cultivar Acadia. *J. Hort. Sci.* 47:93-97.
- Bould, C. 1974. Mineral nutrition on fruit plants. *Proc. XIX Intl. Hort. Congr.* 111:26-32.
- Daft, M.S. and A.A. El-giahmi. 1975. Effect of *Glomus* infection on three legumes, p. 581-592. In: F.E. Sanders, B. Mosse, and P.B. Tinker. (eds.). *Endomycorrhizas*. Academic, New York.
- Graham, J.H., R.G. Linderman, and J.A. Menge. 1982. Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of Troyer Citrange. *New Phytol.* 91:183-189.
- Granger, R.L., C. Plenchette, and J.A. Fortin. 1983. Effect of vesicular-arbuscular endomycorrhizal fungus *Glomus epigaeum* on the growth and leaf mineral content of two apple clones propagated *in vitro*. *Can. J. Plant Sci.* 63:551-555.
- Hayman, D.S. 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* 72:1119-1125.
- JuBrez, M.J. 1986. Desarrollo del cultivo en fresa (*Fragaria ananassa* Duch.) en un medio de cultivo hidropónico partiendo de plantas propagadas *in vitro*. MS Thesis, Colegio de Postgraduados, Chapingo, Mexico.
- Morandi, D., S. Gianinazzi, and V. Gianinazzi-Pearson. 1979. Intérêt de l'endomycorhization dans la reprise et la croissance du Framboisier issu de multiplication végétative *in vitro*. *Ann. Amélior. Plantes* 29:623-630.
- Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annu. Rev. Phytopathol.* 11:171-196.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473-497.
- Phillips, J.M. and D.S. Hayman. 1970. Improved

procedures for clearing root and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit.

Micol. Soc. 55:158-160.
Pons, F.V., V. Gianinazzi-Pearson, S. Gianinazzi, and J.C. Navatel. 1983. Studies of VAM

mycorrhizae *in vitro*; mycorrhizal synthesis of axenically propagated wild cherry (*Prunus avium* L.) plants. Plant & Soil 71:217-221.