Agronomie (1992) 12, 847-850

Vesicular–arbuscular mycorrhizal inoculation of kiwifruit (*Actinidia deliciosa*) micropropagated plants

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(COST Meeting, 21–23 May 1992, Dijon, France)

Summary — The effects of inoculation with a vesicular–arbuscular mycorrhizal (VAM) fungus (*Glomus* sp strain E_3) and fertilization with a complex (NPK) fertilizer on growth of micropropagated kiwifruit were evaluated. Plantlets were inoculated with VAM and fertilizer added when transplanting to a peat-based medium. Root fungal colonization was present in roots from all treatments 80 d after inoculation. Fertilization did not affect percent root colonization, which was increased by increasing the amount of inoculum. Plant growth was assessed in terms of leaf area and plant weight. Plant growth was low in the absence of fertilization. Increasing fertilization rates increased the growth of inoculated and non inoculated plants. VAM inoculation induced larger growth in plants receiving intermediate fertilizer rates, but reduced growth, in respect to non inoculated plants, at the highest fertilization rates.

VA mycorrhiza / *Glomus* / kiwifruit / micropropagation / fertilization

Résumé — Inoculation avec un champignon mycorhizien à vesicules et arbuscules de vitroplants de kiwi *(Actinidia deliciosa).* *L'effet de l'inoculation avec un champignon mycorhizien à vésicules et arbuscules (VA) (*Glomus *sp souche E_3) et d'un engrais complexe (NPK) a été étudié sur des vitroplantes de kiwi. Les plantes ont été inoculées et fertilisées lors du transplant dans un substrat à base de tourbe et perlite. Après 80 j les racines de toutes les plantes inoculées étaient infectées par le champignon. La fumure n'a pas eu d'influence sur l'infection racinaire, tandis que celle-ci était plus élevée en presence de quantités plus hautes d'inoculum. La croissance des plantes, mesurée d'après surface foliaire et poids frais, a été augmentée par la fumure, dans les plantes inoculées et non inoculées. L'inoculation a accru significativement la croissance des plantes, par comparaison aux plantes non inoculées, au niveau moyen de fertilisation. Au contraire les plantes inoculées, aux niveaux plus élevés de fumure, ont montré une croissance plus faible des plantes non inoculées.*

mycorhize VA* / Glomus / *kiwi* / *micropropagation* / *fertilisation

INTRODUCTION

Kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson) was introduced into Italy ≈ 20 yr ago. It is now a leading fruit crop in this country, and is traditionally propagated by cuttings (Costa and Baraldi, 1983). Several tissue culture techniques have been developed for this plant (Harada, 1975), and micropropagation is now widely applied to obtain kiwifruit plants in nurseries.

Vesicular arbuscular mycorrhizal (VAM) fungi are symbiontic organisms which colonize the roots of most higher plants and enhance their growth, taking up phosphate and other nutrients from the soil and translocating them to the host plant (Smith and Gianinazzi-Pearson, 1988). Kiwifruit is normally infected by VAM fungi in the field, and growth enhancements can be obtained by inoculating VAM fungi on kiwifruit seedlings (Schubert *et al,* 1987). VAM fungi can be inoculated onto micropropagated plants both during the *in vitro* stage and after transplant to peat-based, sterile substrates (Ravolanirina *et al,* 1989a): in both cases roots are colonized by the fungus, and inoculated plants often grow better throughout the acclimatization stage.

The aim of this work was to assess the effect of inoculation with VAM fungi on the growth of micropropagated kiwifruit, in the presence of different fertilization and inoculum rates.

MATERIALS AND METHODS

Kiwifruit plants (*Actinidia deliciosa* (A Chev) Liang et Ferguson) cv Hayward, micropropagated from axillary buds, were obtained from Zanzivivai, Ferrara (Italy). Rooted plantlets were transplanted from the agar medium into pots containing 0.8 l of acid peat, perlite and sandy soil (47.5:47.5:5 in vol) substrate. The soil component of the substrate was steam-sterilized and after sterilization had pH 7, and 8 mg kg^{-1} available (Olsen) P. Plants were inoculated at transplanting into pots. Inoculum was obtained from a 3-month-old culture of *Glomus* sp strain E_3 on *Trifolium pratense* L: soil (a sandy loam) containing spores and infected roots was used as inoculum, and mixed with the potting substrate. Inoculum potential of the inoculum (Porter, 1979) was 35 propagules g^{-1} (soil with 10% water content). Before transplant different rates of a complex fertilizer containing 15% N, 9% P_2O_5 and 15% K_2O (Nitrophoska Gold, BASF) were mixed with the potting substrate.

After transplant, plants were kept in a greenhouse with natural light supplemented when necessary with 250 µmol m^{-2} s^{-1} photon flux density to reach a photoperiod of 15 h. Pots were watered daily with tap water.

Total plant leaf area was assessed by measuring leaf width and calculating leaf area with reference to a standard width/area curve calculated from data measured on 100 leaves. Shoot and root fresh weight were measured at the end of the experiment after washing roots free of soil and blotting on paper.

Root infection was measured at the end of the experiment. Roots were washed free of soil, cleared in KOH 100 g l^{-1} at 80°C for 60 min, treated with HCl 0.1 N for 10 min, then stained 10 min at 80 °C with trypan blue 0.5 g l^{-1}. Percent root colonization was assessed by the grid intersect method (Giovannetti and Mosse, 1980).

Each treatment consisted of 12 plants. Results were analyzed by ANOVA and averages were separated with the Duncan test.

RESULTS

At the end of the experiment (80 d after transplant) all inoculated plants were infected by mycorrhizal fungi (fig 1), while non inoculated plants had no root infection. Percent root colonization was affected by the amount of inoculum used: 10 g/pot inoculum resulted in a maximum of 32.5% root colonization, while the use of 20 g/pot significantly increased percent colonization rate (maximum value 82%). A further increase to 30 g/pot did not influence root colonization. Fertilization did not significantly affect (P = 0.05) percent root colonization.

As could be expected, fertilization affected growth of the plants, as expressed by plant leaf

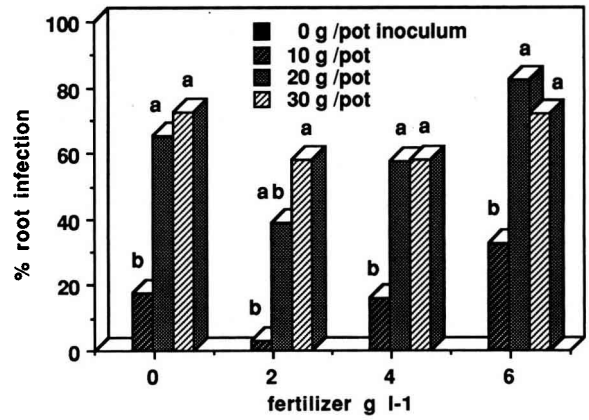

Fig 1. Percent root colonization of micropropagated *A deliciosa* plants cv Hayward measured 80 d after inoculation. Plants were inoculated with different amounts of inoculum of *Glomus* sp strain E_3, and grown in a potted substrate containing different amounts of a complex fertilizer. Within each fertilization rate, values followed by a common letter do not differ significantly at P = 0.05.

area, when fertilization rates higher than 2 g l^{-1} were applied (table I).

Plant leaf area did not increase significantly due to inoculation when no fertilizer was added (table I). However, when 2 g l^{-1} fertilizer were added, leaf areas of inoculated plants (20 and 30 g inoculum/pot) were significantly higher than leaf areas of non inoculated ones. With further increase in fertilizer the leaf area enhancement due to inoculation disappeared, and, at the highest level of fertilization, inoculated plants had smaller leaf areas than non inoculated plants.

Fresh weight of roots and shoots confirmed the effects of inoculation and fertilization on plant growth. In non inoculated plants, again, fertilization at the rates 4 and 6 g l^{-1} significantly in-

Table I. Total plant leaf area (dm^2) of micropropagated *A deliciosa* plants cv Hayward, measured 80 d after inoculation. Plants were inoculated with different amounts of inoculum of *Glomus* sp strain E_3, and grown in a potted substrate containing different amounts of a complex fertilizer. Values followed by a common letter do not differ significantly at P = 0.05.

Fertilizer	*Inoculum (g/pot)*			
(g.l^{-1})	*0*	*10*	*20*	*30*
0	0.81^{c}	0.82^{c}	0.71^{c}	0.93^{c}
2	1.02^{c}	1.37^{bc}	1.60^{b}	1.84^{ab}
4	2.12^{a}	1.74^{ab}	1.75^{ab}	1.75^{ab}
6	1.93^{a}	1.45^{bc}	1.55^{b}	1.35^{bc}

Table II. Shoot and root fresh weight (g) of micropropagated *A deliciosa* plants cv Hayward, measured 80 d after inoculation. Plants were inoculated with different amounts of inoculum of *Glomus* sp strain E_3, and grown in a potted substrate containing different amounts of a complex fertilizer. Values followed by a common letter do not differ significantly at $P = 0.05$.

Fertilizer (g.l^{-1})	*Inoculum (g/pot)*			
	0	*10*	*20*	*30*
Shoot fresh weight				
0	2.05^{b}	2.21^{b}	1.66^{b}	2.78^{b}
2	3.10^{b}	5.69^{ab}	6.31^{ab}	6.26^{ab}
4	8.11^{a}	4.89^{ab}	4.96^{ab}	6.00^{ab}
6	6.90^{ab}	4.94^{ab}	5.78^{ab}	5.24^{ab}
Root fresh weight				
0	2.31^{b}	1.77^{b}	1.47^{b}	2.81^{b}
2	2.11^{b}	5.02^{a}	5.16^{a}	5.86^{a}
4	5.72^{a}	4.15^{ab}	4.94^{a}	4.97^{a}
6	5.31^{a}	5.17^{a}	5.29^{a}	4.05^{ab}

creased shoot and root fresh weight (table II). Inoculation with the mycorrhizal fungus enhanced root fresh weight, as compared to non inoculated plants, when 2 g l^{-1} fertilizer were added to the substrate. The amount of inoculum used did not significantly affect plant fresh weight.

DISCUSSION AND CONCLUSION

Micropropagation systems of horticultural plants may benefit from inoculation with mycorrhizal fungi. Increased growth of micropropagated plants has been obtained by inoculation with VAM fungi on cherry (Pons *et al,* 1983), grapevine (Ravolanirina *et al,* 1989b; Schubert *et al,* 1990), pistachio (Schubert and Martinelli, 1987), and several other plants (see papers in this volume). Such growth enhancements were obtained either with inoculation during the *in vitro* stage, or with inoculation at the start of the acclimatization stage; however, the latter technique is normally employed, due to its easier feasibility (Ravolanirina *et al,* 1989a).

Our results show that VAM inoculation can have similar positive effects on micropropagated kiwifruit, as was formerly observed on kiwifruit seedlings (Schubert *et al,* 1987) and cuttings (Powell and Santhanakrishnan, 1986). We have not investigated here the mechanism which allows such growth enhancement, but one can suppose that the well-proven ability of VA mycorrhizae to increase plant nutrient uptake from the soil (Smith and Gianinazzi-Pearson, 1988) may be at least partly responsible.

In this experiment we tested the effects of fertilization on inoculated and non-inoculated plants. The results follow a common pattern, *ie* mycorrhiza enhances plant growth at low soil nutrient levels, while this effect is lost with higher nutrient availability (Abbott and Robson, 1978). However, in our case this was not true when no fertilizer was added to the substrate, although roots were largely mycorrhizal. A possible interpretation is that at this fertilization level nutrient supply was so low that the carbon drain from the plant due to mycorrhiza balanced the growth enhancement induced by the improvement in phosphate uptake. Similar growth depressions have been previously reported (Buwalda and Goh, 1982).

We also tested the effect of the amount of inoculum added to the plants. Inoculum rate significantly affected root infection, but did not influence plant growth. At a fertilization level of 2 g l^{-1}, all mycorrhizal plants had higher leaf areas and root fresh weights than non inoculated plants, independently of the intensity of root fungal colonization. Thus the amount of inoculum apparently is not a key factor for plant growth response, provided a minimum amount is given (in our case 350 propagules per plant).

Mycorrhizal inoculum can be successfully applied to micropropagated kiwifruit plants during the acclimatization stage. The nutrient conditions of the substrate, however, must be adjusted in order to obtain a benefit from inoculation. The relations between the soil content of single nutrients, especially phosphate, and the activity of the fungus must be further studied. Also a better insight into the physiological events taking place in the plant during acclimatization, as growth of roots and photosynthesis, will considerably increase the possibility to apply VAM inoculation in commercial plant production.

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