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Arbuscular mycorrhizal inoculation of micropropagated strawberry and field observations in Finland

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Summary — Micropropagated plantlets of strawberry (*Fragaria* x *ananassa* Duch Senga Sengana) were inoculated at the beginning of the weaning stage with a *Glomus mosseae* strain from Rothamsted Experimental Station (UK) or with 8 Finnish *Glomus* isolates, including 1 *G mosseae* isolate, 4 *G intraradix* isolates and 3 isolates of an unidentified *Glomus* species. After 8 wk (July 1990), inoculated plants were planted in the field: the most efficient fungi, *ie G mosseae* Rothamsted, *Glomus* sp V_3 and *Glomus* sp V_4, increased shoot growth several-fold during the weaning stage. Growth responses persisted throughout the 1st yr in the field, and partly throughout the 2nd yr. Root colonization by different fungi was from near 0–60% during the weaning stage. In the 2nd yr, after overwintering, root colonization of inoculated plants ranged from 8–35% as compared to 4% for the control plants. The need for more precise studies of the influence of winter on the biological efficiency of introduced VAM fungi to strawberry roots is discussed.

strawberry / arbuscular mycorrhizal fungi / microplant weaning stage

Résumé — Inoculation de mycorhizes arbusculaires à des fraisiers micropropagés et observation en champ en Finlande. *Des plantes de fraisier (*Fragaria + ananassae *Duch, Senga Sengana) ont été inoculées au début du sevrage avec une souche de* Glomus mosseae *provenant de Rothamsted Experiment Station, ou avec 1 des 8 isolats finnois de* Glomus *dont 1 de* G mosseae *4 de G intraradix et 3 isolats d'une espèce non identifiée de* Glomus. *Après 8 semaines, en juillet 1990, les plantes inoculées ont été plantées au champ. Les champignons les plus efficaces, c'est-à-dire* G mosseae *Rothamsted,* G *sp, V_3 et* G *sp V_4 ont multiplié la croissance des tiges de plusieurs fois pendant la période de sevrage. La réponse de croissance persista pendant toute la première année au champ, et partiellement pendant la deuxième année. La colonisation des racines par les différents champignons a été de presque 0 à 60% pendant le sevrage. La deuxième année, après passage de l'hiver, la colonisation variait de 8 à 35%, contre 4% pour les témoins. Le besoin d'études plus précises sur l'influence de l'hiver sur l'efficacité biologique des VAM introduites dans les racines de fraisier est discuté.*

fraisier / champignon mycorhizien arbusculaire / microplant / sevrage

INTRODUCTION

Cultivated strawberry was originally claimed to benefit from arbuscular mycorrhizal (AM) inoculation only when soil phosphorus is limiting for plant growth (Holevas, 1966; Daft and Okusaya, 1973). Dunne and Fitter (1989), however, found that strawberry has a very high phosphorus demand, especially during the reproductive phase. They therefore concluded that the presence of a functional symbiosis can be important even under commercial cultural conditions with high fertilizer applications. The general use of micropropagation for the production of elite stock of strawberry has opened up even more possibilities of obtaining benefits from AM inoculation (Robertson *et al*, 1988; Chavez and Ferrara-Cerrato, 1990; Niemi and Vestberg, 1992; Vestberg, 1992).

At the Laukaa Research and Elite Plant Unit of the Agricultural Research Centre of Finland, strawberry microplants inoculated with AM fungi at the beginning of the weaning stage have shown high growth responses in a number of experiments of different types (Vestberg, unpublished observations). Based on this experience, a

field experiment was begun in 1990, the objective of which was to observe how such AM-inoculated microplants manage after outplanting in the field and how they are affected by overwintering. Eight Finnish isolates of *Glomus*, screened for use with strawberry, were used together with one isolate of *Glomus* from Rothamsted Experimental Station, UK.

MATERIALS AND METHODS

At the beginning of the weaning stage (May 1990), micropropagated plantlets of strawberry (*Fragaria* x *ananassa* Duch cv Senga Sengana were transplanted into Vefi pots (0.25 l) containing sterilized sand fertilized with bone meal. The inocula of different fungal isolates were placed at the bottom of the planting hole before transplanting. The AM treatments were: 1) none; 2) *G mosseae* Rothamsted; 3) G sp V_3 Finland; 4) *G* sp V_4 Finland; 5) *G intraradix* $V_{50/87}$ Finland; 6) *G* sp $V_{21/88}$ Finland; 7) *G mosseae* V_{11b} Finland; 8) *G intraradix* V_{18} Finland; 9) *G intraradix* V_{20} Finland; and 10 *G intraradix* V_{31} Finland. Plants were grown in a greenhouse.

Eight wk later plants were outplanted into a field (silty soil), prepared for strawberry production by addition of light *Sphagnum* peat and lime (6 tons/ha) to give a final pH of 6.0. No additional inoculation was performed and fertilization was as for commercial strawberry fields. The amount of fertilizers (300 kg/ha 7 N:5 P:15 K, Kemira; and 400 kg/ha 0 N:10 P:18 K, Kemira) was calculated on the basis of soil analysis. The strawberries were planted in 10 replicates on top of small ridges covered by black plastic. The distance between ridges was 2 m and between plants 33 cm.

In mid-September of 1990 and 1991, 1 plant per treatment and per replicate was removed for assessment of shoot dry weight and runner growth, and of root colonization by the gridline intersect method (Giovannetti and Mosse, 1980). Plants removed from the experiment were replaced by new plants to fill the gaps.

Experimental data were statistically analyzed by 1-way analysis of variance; treatment means were separated by Duncan's multiple range test.

RESULTS

At the end of the weaning stage, strawberry roots were colonized by different AM isolates from 0–60% (table I). *G mosseae* Rothamsted, *G* sp V_3, *G* sp V_4 and *G intraradix* V_{18} all caused high infection (50–60%). Three isolates, *ie G mosseae* V_{11b}, *G intraradix* V_{20} and V_{31} resulted in infection levels close to zero. At the end of the 1990 growing season, plants collected from the field experiment still showed 50% root colonization after inoculation with *G* sp V_4 at the weaning stage, while those inoculated with *G mosseae* Rothamsted showed only 1% colonization. At this stage, *G intraradix* V_{20}-inoculated plants had increased their infection rate from 1–16% as compared with the weaning stage. After overwintering, in 1991, root colonization of the inoculated strawberries varied from 8–35% as compared to 4% for uninoculated plants. Plants inoculated with *G intraradix*, *ie* $V_{50/87jo}$, V_{20} and V_{31} had increased colonization as compared to the previous year.

Table I. The effect of arbuscular mycorrhizal inoculation on root colonization and shoot dry weight (mother plant + runners) of strawberry at the end of the weaning stage and at the end of the 1990 and 1991 growing seasons in the field.

Treatment	*End of weaning*		*End of 1990*		*End of 1991*	
	VAM (%)	*Shoot dry weight (g)*	*VAM (%)*	*Shoot dry weight (g)*	*VAM (%)*	*Shoot dry weight (g)*
Control	0	0.21	3	2.8	4	9.5
Gm Roth	50	0.70*	1	12.5*	12	17.1*
V_3	44	0.51	15	10.4*	22	14.0
V_4	60	0.38	50	6.1*	35	16.3*
$V_{50/87jo}$	10	0.32	12	5.6*	23	11.9
V_{2188}	12	0.30	22	6.4*	10	13.0
V_{11b}	0	0.22	9	6.1*	13	12.9
V_{18}	50	0.37	4	7.8*	8	13.6
V_{20}	1	0.42	16	5.6	33	13.0
V_{31}	1	0.30	4	4.6	18	9.5

Within columns, means of shoot dry weights followed by an asterisk differ significantly from the control at $P = 0.05$.

G mosseae Rothamsted caused the highest growth increase in strawberry at the weaning stage (table I). This significant growth response persisted throughout the first and the second years in the field. The Finnish isolate V_3 also increased growth significantly during weaning. During the 1st yr in the field, the effect of inoculation performed at the weaning stage was pronounced, and *G mosseae* Rothamsted as well as all the Finnish isolates except V_{20} and V_{31} gave rise to significant growth increases. After overwintering, in 1991, however, only *G mosseae* Rothamsted and *G* sp V_3 caused significant growth responses.

The fungi which promoted the greatest growth increases in shoot growth (*G mosseae* Rothamsted, V_3, V_4 and V_{18}), also caused the greatest increase in runner production at the end of the 1990 growing season (results not presented). In 1991, however, no significant differences in runner production were observed between uninoculated and inoculated strawberries.

DISCUSSION AND CONCLUSION

Micropropagated strawberry benefits from AM inoculation (Kiernan *et al,* 1984; Robertson *et al,* 1988), a fact which was also observed in this investigation. The growth increasing effect of the most efficient isolates persisted throughout the 1st yr in the field and even after overwintering in the 2nd yr. This suggests that the introduced strains which were highly efficient were able to compete with the indigenous strains and were not destroyed during the winter. The influence of winter on the management of introduced AM fungi in roots of strawberry warrants detailed studies, in which development of both the AM fungi and roots should be considered.

Inoculation also positively influenced runner development. There was, however, no indication of the phenomenon observed by Hrselova *et al* (1989), according to whom inoculation can decrease the shoot dry weight of the mother plant and at the same time strongly increase runner plant production.

Strawberry is the most important small fruit plant in Finland. Approximately 80% of the strawberry plants sold originate from micropropagated elite plants. From this point of view, successful inoculation of strawberries at the beginning of the weaning stage may have a potential as a useful technique even in commercial strawberry production. The benefits of inoculation at the weaning stage could possibly be improved further by modifying growth substrata and using slow release fertilizers (Williams *et al,* 1992).

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