The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution

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Abstract

Potato microplants were inoculated with three commercial arbuscular mycorrhizal inoculants, Vaminoc (MicroBio, Hemel Hempstead, HP2 7SU, UK), Endorize IV and *Glomus intraradices* (both produced by Societe Biorize, 21000 Dijon, France) at establishment in the glasshouse. Monitoring of percentage root length colonization confirmed the persistence of all the mycorrhizal fungi during the trial. Colonization with Vaminoc and Endorize IV levelled-off at approximately 8 weeks and with *G. intraradices* at 12 weeks. Image analysis of leaf shape parameters at 4 weeks in the field indicated that the control uninoculated microplants and the microplants from the Vaminoc treatment were significantly the most mature of the microplant-derived populations. Microplants from the Endorize IV treatment were significantly less mature than from the Vaminoc treatment and those from the *G. intraradices* treatment significantly the least mature. The *G. intraradices* treatment was late flowering. The Vaminoc and Endorize treatments promoted flowering, 80 and 76%, respectively, at 2 months after planting, relative to the microplant control (60% at 2 months), the *G. intraradices* treatment reduced flowering, 14% at 2 months. Mycorrhizal dependency determined at harvest showed that Vaminoc and Endorize IV promoted growth whereas *G. intraradices* reduced growth. Average tuber yield for a seed-tuber-derived control was 1.2 kg per plant, and the average yield per plant for the microplant control was significantly lower at 0.9 kg. The Vaminoc treatment was not significantly different from the microplant control. The Endorize IV and *G. intraradices* yields were significantly lower at 0.64 and 0.41 kg per plant, respectively. Since the objective was to evaluate the potential of mycorrhizal fungi to improve yield of seed grade tubers from microplants, tuber size distribution was analysed. The average number of seed grade tubers for the respective treatments were: 1.2 for the seed-tuber crop, 3.8 for the control microplants, 3.8 for the *G. intraradices*, 6 for Vaminoc and 8.5 for Endorize IV crops, respectively. It has been shown here that mycorrhizal inoculation can influence the yield quality of potato microplants. These results support previous findings that mycorrhizal fungi can increase or decrease yield depending on the mycorrhizal isolate and host genotype. Leaf image analysis has been shown to have predictive value in evaluating the potential of mycorrhizal treatments. The determinants of tuber yield and tuber size distribution in potato

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are complex and results, such as those obtained here, would require to be repeated in multiple sites and successive years to confirm the stability of the interactions reported. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Arbuscular mycorrhiza; Micropropagation; Potato seed-tuber production

**Abbreviations:** AM, arbuscular mycorrhizal; %rlc, percentage root length colonization

1. **Introduction**

In general, microplants offer a novel application niche for mycorrhizal fungi in that they are vulnerable to weaning stress and damping-off diseases associated with poor physiological quality and juvenility (Preece and Sutter, 1991; Lovato et al., 1996). Biotization of microplants in vitro or in vivo, i.e. inoculation with beneficial fungi and, or bacteria (Cassells et al., 1996; Duffy et al., 1999) may also result in growth promotion. In potato seed production based on microplants, there is potential also for the exploitation of mycorrhizal fungi to protect in the field against abiotic stresses and biotic stresses caused, e.g. by nematodes and soil-borne fungi such as *Rhizoctonia solani*.

The commercial exploitation of mycorrhizal inoculants in any crop will depend on technical feasibility and on cost–benefit analysis. The technical and economic aspects which have to be addressed are the selection of appropriate mycorrhizal isolates for the crop cultivar, the production of stable inoculum at an economic price and confirmation that inoculation confers benefit, usually defined in agriculture as a fourfold increase in return over treatment cost. It is recognized that AM isolates may show host genotype specificity (Mark and Cassells, 1996). Furthermore, commercial availability of formulated AM inoculum is limited with respect to isolates and is relatively expensive (approximately 15 US cents per plant). Finally, there have been few efficacy trials to date, especially with microplants (Nowak, 1998).

Previously, it was shown that arbuscular mycorrhization at potato microplant establishment improved microplant growth and significantly increased the yield of saleable potato minitubers in protected cropping (Duffy et al., 1999). Here, the objective was to determine the effects of AM inoculants on the field performance, yield and tuber size distribution of potato microplants. Bearing in mind the difficulties in large-scale production of AM inoculum, three commercially available inoculants were evaluated. Elsewhere, it has been reported that image analysis of potato leaf shape can be used to compare the developmental maturity of potato plants (Cassells et al., 1999). This approach was investigated here with a view to developing an early screening system for the evaluation of mycorrhizal inoculants.

2. **Materials and methods**

2.1. **Potato stock plants and cultures**

Potato cv. Golden Wonder certified virus-free seed was obtained from Munster Seeds, Cork, Ireland. Aseptic potato cultures were established as described previously (Curry and Cassells, 1999). Microplants were produced from nodal cuttings proliferated on 2.206 g l\(^{-1}\) Murashige and Skoog (1962) medium with 0.1 mg l\(^{-1}\) kinetin and 0.2 mg l\(^{-1}\) gibberellic acid, 15 g l\(^{-1}\) sucrose, adjusted to pH 5.8. Polyurethane foam (Cassells and Walsh, 1998) was used to support the explants (Hortifoam, Plant Biotechnology (UCC), Cork, Ireland). Fifty millilitres of the medium was poured onto the foam in Magenta culture vessels (Sigma, Dublin, Ireland) prior to autoclaving. The lids of the culture vessels were replaced with Suncaps (Sigma, Dublin, Ireland) to facilitate gaseous exchange. Cultures (10 nodes per vessel) were incubated under growth room conditions of 16 h photoperiod, PAR light intensity of 50 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), temperature of 22±1°C.

2.2. **Glasshouse weaning**

Explants were grown for 3 weeks in vitro before the microplants were transferred to the glasshouse for acclimatization. Individual microplants were carefully removed from the foam substrate with particular attention being paid to maintaining the root system intact.
The growth substrate used to acclimatize microplants was the PVS substrate (peat:vermiculite:sand, 8:1:1) of Vestberg (1992).

2.3. In vivo mycorrhization

The commercial inoculants evaluated were Vaminoc (a mixture of isolates, MicroBio, Hemel Hempstead HP2 7SU, UK); Endorize IV (a mixture of isolates) and Glomus intraradices (Societe Biorize, 210000 Dijon, France). Inoculum was added at a rate of approximately 1 g per plant directly into the planting hole at the time of microplant weaning (Mark and Cassells, 1996). Each treatment consisted of 400 plants (2 blocks of 200 microplants). Control microplants received no inoculation. Plants were grown under a propagator lid for 5 days, watered when required, the bases of the planting trays were enclosed in order to prevent cross-contamination between treatments. After 3 weeks growth in the glasshouse, the plants were transferred outdoors during the day for 1 week’s hardening-off prior to planting out. Measurements of plant height, node number and chlorophyll content of leaves were taken weekly while in the glasshouse for 4 weeks.

A control of tuber-grown cv. Golden Wonder plants was also used (non-inoculated) to compare microplant-derived crop with a conventional seed-tuber-derived crop. To confirm mycorrhizal establishment and survival and to assess % root length colonized by the fungi, samples of roots (5 g per plant) were removed at 2 weeks intervals. Ten plants were sampled at 2, 4, 8, 12 and 16 weeks after inoculation and at final harvest which was at 20 weeks after weaning. Subsamples were cleared, stained with 0.05% trypan blue using the method described by Phillips and Hayman (1970). Mycorrhizal colonization was observed and measured using the magnified hairline intersect method (McGorgle et al., 1990).

Microplants were transferred to the field site approximately 7 weeks after initiation from nodal explants. Microplants were transferred by hand into planting holes in the drills ensuring that adhering PVS and inoculum was retained. Weeding was by hand initially until the microplants were well-established and unaffected by herbicide sprays. Routine spraying for Phytophthora infestans (late potato blight) was as recommended by Dowley and O’Sullivan (1995). Insecticides, at the manufacturer’s recommended doses, were used to control aphids.

2.4. Image analysis

Image analysis was carried out using DIAS system (Delta -T Instruments, Cambridge, UK) as defined in Curry and Cassells (1999). The following system-generated parameters were used: circularity (square root of the actual area to the area of a circle with the same circumscribed diameter), elongation (ratio of length to width) and shape factor (ratio of the actual perimeter to that of a circle with the same area). The leaflet taken for image analysis, 1 month after planting in the field, was on the fourth node down from the apical meristem. The data are for populations of terminal leaflets from 100 randomly selected leaves in each treatment and each leaflet was from a different plant.

2.5. Flowering and tuberization dates

Treatments were monitored twice weekly in order to record the number of days to flowering. Days to 100% flowering (within treatments) were also recorded. As plants were being destructively harvested to record % rlc by mycorrhizal fungi, observations at this stage included recording the length of time to when the first tubers were observed in each treatment.

2.6. Calculation of mycorrhizal dependency

Plants harvested for %rlc data were also used to determine the mycorrhizal dependency of the plants within each treatment. After removing a small amount of root tissue for analysis (approximately 5 g per plant), the remaining roots were washed free of adhering soil. Plants were placed in brown paper bags and dried in a drying oven at 53°C until a constant weight resulted. Mycorrhizal dependency (MD: Vestberg, 1992) was calculated as the dry weight (g) of the mycorrhizal plant divided by the dry weight (g) of the non-mycorrhizal plant multiplied by 100.

2.7. Final harvesting

Approximately 16 weeks after planting at the trial site, the final tuber harvest was taken. Tubers from
30% of the plants in each treatment were randomly sampled (no plants were taken from the two outermost rows in any treatment). Records were made of the number of tubers per plant, weight (g) of tubers per plant and size (mm) of tubers per plant, namely, number of tubers falling into each size grade ranging from <0.2 cm up to 20 cm in 1 cm increments.

2.8. Data analysis

Analysis of variance and Kruskal–Wallis tests were carried out using Data Desk 5.0 (Data Description, New York, USA).

3. Results

3.1. General observations

All microplants were successfully acclimatized in the glasshouse in PVS substrate. No significant differences were recorded in the parameters of plant height and node number or chlorophyll content, in the 4 weeks period of glasshouse growth, between the Vaminoc, Endorize IV, and control microplants (microplants without AM treatments). Microplants in the Glomus intraradices treatment, however, did show phenotypic differences. The established microplants in this treatment appeared mildly chlorotic and less vigorous when compared to the dark green foliage and stronger growth habit of microplants in the other treatments. Once the established microplants were transferred to the field, these differences disappeared and there were no marked morphological differences until the onset of flowering (see below). Tuber-grown plants had been planted approximately 4 weeks prior to the microplants in standard drills and received the same fungicide and insecticide applications.

3.2. Confirmation of mycorrhizal colonization

Mycorrhizal colonization was confirmed in all inoculated treatments based on the presence of arbuscules in the infected roots which were absent from the uninoculated controls. All plants were colonized by resident soil-inhabiting fungi including Pythium spp. The inoculants used were commercial formulations and were used under non-sterile conditions following the manufacturer’s recommendations. It is possible that they contained spores other than those of the AM fungi but this was not investigated. Root colonization reached the maximum level for the Vaminoc and Endorize IV treatments approximately 8 weeks after initial inoculation and for the G. intraradices treatment at 12 weeks.

3.3. Image analysis of leaf shape

The numerical value of leaf shape factor increases with maturation in potato; conversely, the numerical values for circularity and elongation decrease with maturation (Cassells et al., 1999). The relative values of these parameters have been used to predict differences in flowering date and are related to tuber yield and size distribution in potato (Cassells et al., 1999; Kowalski and Cassells, 1999). The present results (Table 1) indicated that the tuber-grown plants, control microplants and Vaminoc-inoculated plants have similar maturity at 4 weeks post-field planting. Endorize IV-inoculated plants are less mature with G. intraradices plants being, relatively, the most immature.

3.4. Flowering dates

Tuber-grown plants flowered 8 weeks after emergence. Control microplants began to flower after approximately 6 weeks in the field. Vaminoc and Endorize IV treatments also flowered after 6 weeks, while the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shape factor</th>
<th>Circularity</th>
<th>Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuber-grown plants</td>
<td>1.14840a</td>
<td>0.786535a</td>
<td>0.692718a</td>
</tr>
<tr>
<td>Controls (no VAM)</td>
<td>1.14928ab</td>
<td>0.778307a</td>
<td>0.689989a</td>
</tr>
<tr>
<td>Vaminoc</td>
<td>1.14070b</td>
<td>0.77993a</td>
<td>0.693308a</td>
</tr>
<tr>
<td>Endorize IV</td>
<td>1.13049c</td>
<td>0.853340b</td>
<td>0.800968b</td>
</tr>
<tr>
<td>G. intraradices</td>
<td>1.13491c</td>
<td>0.808821b</td>
<td>0.075232c</td>
</tr>
</tbody>
</table>

*Any two values sharing a similar letter are not significantly different at the 95% level. (Parameters defined in Section 2.) Readings were taken when the microplants had been in the field for 4 weeks.
G. intraradices treatment flowered 1 week later. When the tuber-grown plants had reached the 100% flowering stage, the following observations were recorded. The Vaminoc and Endorize IV treatments were 80 and 76% flowering, respectively. The control microplants had reached 60% flowering, while the G. intraradices plants had only reached 14% flowering.

3.5. Days to first tuber production

Tuberization was observed in the control microplants, Vaminoc and Endorize IV treatments on the third sampling date for %rlc sampling, i.e. 8 weeks after weaning. Detection of tuberization in the G. intraradices treatment was delayed until the fourth %rlc sampling. Tuberization was not monitored in the seed-tuber-derived crop which was not monitored for mycorrhizal colonization.

3.6. Mycorrhizal dependency ratio

Both Vaminoc and Endorize IV gave increased mycorrhizal dependency values, i.e. gave higher plant dry weights than the uninoculated control microplants. G. intraradices gave a decreased mycorrhizal dependency value. The MD values for control, Vaminoc, Endorize and G. intraradices treatments were 1.0, 1.2, 1.09 and 0.86, respectively. The Vaminoc and G. intraradices results were significantly higher and lower than the control, respectively (p<0.05). The results for Endorize IV were not significantly different to the control.

3.7. Harvest data

Harvesting of the crop took place approximately 4 months after planting in the field. Tubers were lifted by hand, washed, weighed and measured for final analysis. Median yield per plant in each treatment is shown in Fig. 1. The yield of the tuber-grown crop was significantly higher than any of the treatments and was approximately 34% greater than that of the microplant control. From these data, Vaminoc was the best of the inoculants in relation to yield. However, the aim of the study was to optimize the yield of seed grade tubers and consequently, it was necessary to quantify the number of cv. Golden Wonder seed grade sized (25–35 mm) tubers per plant in each treatment. Fig. 2 shows the median number of tubers within the optimum range. The percentages of seed size tubers were 27% of Endorize tubers, 24% of Vaminoc tubers, 21% of G. intraradices, 20% of control microplants.
and 7% of tuber-grown plants, respectively. Fig. 3 shows the size distribution for the tubers produced in the Endorize IV treatment. Fig. 4 shows the tuber size distribution in the certified seed-grown crop. The vast majority of the latter were ware grade.

4. Discussion

The availability of adequate quantities AM inoculum for field trials is a limiting factor in such studies. Here, it was decided to use commercial inoculants
which consisted of either species mixtures (Vaminoc and Endorize IV) or single species (G. intraradices) while recognising that optimization of the interaction might depend on selection of appropriate AM isolates for specific crop cultivars. Observations and assessments of microplants after mycorrhizal inoculation in the glasshouse showed that the plants inoculated with G. intraradices were showing chlorosis and looked less vigorous, although no quantitative differences were recorded. On subsequent transfer to the field, the latter plants became indistinguishable from the other populations.

Early application of image analysis of leaf shape, after 4 weeks in the field, indicated differences in maturity between the populations and compared with a seed-tuber-derived reference (Table 1). The uninoculated control microplants and the Vaminoc plants showed the same maturity as the seed-tuber crop, the Endorize IV crop was relatively less mature and the G. intraradices least mature. These differences were taken as having a predictive value. Microplant crops being relatively more mature would be expected to flower earlier, give higher tuber yields with larger tubers, relatively juvenile crops would be expected to flower later and have lower yields and smaller tubers. These predictions were confirmed by the flowering data. The early poor appearance of the G. intraradices crop was reflected in negative mycorrhizal dependency in contrast with the other inoculants which showed higher mycorrhizal dependency; Vaminoc treatment showing the highest mycorrhizal dependency.

The predictive value of the image analysis and mycorrhizal dependency data was confirmed by tuber yield and size distribution analysis. Vaminoc microplants which showed the highest mycorrhizal dependency and gave the best flowering data, gave the highest yield but not significantly higher than the control (Fig. 1). The intermediate maturity Endorize IV treatment gave significantly lower yield and the relatively juvenile, low mycorrhizal dependency G. intraradices crop gave yet significantly lower yield. As mentioned above, however, high yield may be related to increased tuber fill, i.e. larger tubers and this was confirmed by analysis of the tuber size distribution for the respective crops. The tubers from the seed-tuber crop were ware grade, those from the G. intraradices crop were not significantly different from the microplant controls while the tubers from the Endorize IV and Vaminoc treatments were significantly greater; with the Endorize IV treatment being significantly greater than Vaminoc (Fig. 2). Thus, while the total yield was highest for Vaminoc treatment, but not significantly greater than the control, the higher economic value of seed grade tubers meant that the highest economic yield was from the Endorize IV treatment.

As a shade plant, potato shows a variable morphology. Further, micropropagation is known to increase juvenility. Thus, microplants tend to have weak biotic and abiotic stress resistance as young plants. Juvenility results in less late blight susceptibility in the field (young foliage is more resistant) as observed in this.
and other studies (Kowalski and Cassells, 1999). The juvenility of microplants also results in delayed tuberization and lower tuber fill, i.e. they produce less and smaller tubers. The latter is not a disadvantage in certified seed production where the produce has higher value. Here, and previously in potato minituber production in protected cropping, it has been shown that mycorrhization may have the potential to produce a higher value microplant propagule. Given that disease susceptibility may be affected by mycorrhization, this aspect also merits investigation.

In conclusion, mycorrhization should be viewed in the context of an holistic strategy to improve economic yield quality in potato micropropagation. The present studies have confirmed the specificity of the relationship of the mycorrhizal isolate to the host potato cultivar used. This needs investigation for different potato cultivars. It is also essential, bearing in mind the strong environmental genotype interaction in potato tuber production, that multiannual, multilocation trials be carried out to confirm mycorrhization has a role in sustainable seed-tuber production.

References


