Potato Research 38 (1995) 61 – 68

Greenhouse production of potato (*Solanum tuberosum* L. cv. Désirée) seed tubers using in vitro plantlets and rooted cuttings in large propagation beds

REGAN D. ROY, V. SOUZA MACHADO, S.M.M. ALAM AND A. ALI

Department of Horticultural Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Accepted for publication: 20 October 1994

Additional keywords: plant density, cutting removal, ground cover, propagules

Summary

Seed tuber production from *in vitro* potato plantlets and cuttings was conducted in large propagation beds in a greenhouse. Propagules of whole *in vitro* plantlets (WIP), plantlets with apical (ACR) and lateral (LCR) cuttings removed, the rooted apical cuttings (RAC) and rooted lateral cuttings (RLC) were planted at three densities of 25, 49 and 100 plants per m^2. The plantlets from which cuttings were removed (ACR and LCR) produced more tubers than the rooted cuttings (RAC and RLC); however, the most tubers were produced by WIP. RAC produced highest tuber yields followed by WIP and RLC. Intact WIP and plantlets with cuttings removed (ACR and LCR) produced twice as many tubers <40 g as those from rooted cuttings (RAC and RLC). The yield (kg/m^2) as well as the number of tubers per m^2 increased with the increasing planting density within the densities tested.

Introduction

Propagation of potato seed stocks by stem cuttings was developed in the 1960s as a means of eliminating bacterial and fungal pathogens normally carried over by tuber propagation (Jones, 1991). The development and application of *in vitro* plant culture technology to potato propagation enabled rapid multiplication of disease-pathogen free potato plantlets (Dodds, 1988; Knutson, 1988). These plantlets can be planted directly into the field (Bryan, 1988; Levy, 1988, Wattimena et al., 1983; Uyen & Vander Zaag, 1985) or used for production of clean potato tuber seed stocks (Wiersema et al., 1987). According to Jones (1991) most seed potato programmes involve transplanting micropropagated plantlets to pots or beds in greenhouses. The present study examined tuber production of the *in vitro* potato plantlets established in deep propagation beds in a greenhouse, in relation to planting density and the removal of one or more shoot cuttings from the established plantlets. In addition to evaluating the effects of planting density on tuber yields, an objective of this study also involved comparing tuber yields of whole in vitro plantlets, the plantlets from which the cuttings were removed and rooted cuttings.

Materials and methods

In vitro multiplication of potato plantlets. Disease indexed plantlets of cv. Désirée were obtained from Agriculture Canada Research Station at Vancouver, B.C. and New Brunswick Department of Agriculture Plant Propagation Laboratory in Fredericton, N.B. Using the procedure developed at CIP, Lima, Peru by Dodds & co-workers (Espinoza et al., 1984), these plantlets were micropropagated in the plant tissue culture facility at the Department of Horticultural Science, University of Guelph, Ontario, Canada. The plantlets were sectioned into single node cuttings which were placed, two cuttings per culture tube, on MS salts (Murashige & Skoog, 1962) based agar solidified medium containing 0.8% agar, 3% sucrose, 0.25 ppm GA_3 plus other chemicals modified for the propagation of nodal cuttings (Espinoza et al., 1984). The medium was adjusted to pH 5.7. The cultured nodal cuttings were maintained in a clean growth room at a temperature regime of 24 °C and 17 °C during day and night periods respectively. Cool white fluorescent tube lights (Philips, USA F20T12) provided light intensity of 130 μmol m^{-2} s^{-1} during a 16 hour light and 8 hour dark cycle. The procedure was repeated every 5 weeks, following regrowth of the nodal axillary shoots until the desired number of plantlets were obtained.

Transplanting of in vitro plantlets into propagation beds. One meter wide propagation beds of 50 cm high wood panels were erected on clean concrete floor of a greenhouse. An 18 cm deep layer of soilless propagation medium consisting of peat:perlite (5:1) containing starter phosphate fertilizer was placed in the beds. The beds were thoroughly moistened with water. The *in vitro* plantlets were removed from culture tubes and, after carefully washing agar medium from the roots, were gently transplanted in the beds and covered with propagation medium except for 2–3 cm shoot remaining above the medium. Three different planting densities of 25, 49 and 100 plantlets per m^2 were used for the *in vitro* transplants. After a 15 day growth period, 2–3 cms long apical cuttings were taken from two-third of the transplants. These cuttings were rooted for 10 days in mist beds followed by hardening for 4 days and were then transplanted into the 18 cms deep propagation beds at 25, 49 and 100 cuttings per m^2 planting densities. The *in vitro* transplants from which apical cuttings were removed resulted in growth of the axillary meristems to produce lateral shoots. From half of these plants lateral shoot cuttings were taken after 8 days of growth following the initial removal of apical cuttings. The lateral cuttings were rooted in mist beds as described above, hardened and transplanted into the 18 cm deep propagation beds at planting densities of 25, 49 and 100 lateral cuttings per m^2.

The propagules were hilled twice during the first 30 days of transplanting which resulted in adding a 6 cm layer to the initial 18 cm depth of the propagation medium. To prevent early tuberization by the transplants the photoperiod was extended to 14 hours using HPS-PL780 lamps dispersed 2.5 m above the beds at a density of one lamp per 12 m^2 area. This arrangement provided between 25–35 μmol m^{-2} s^{-1} of supplementary irradiance. The day and night temperatures in the greenhouse were 22±2 °C and 18±1 °C respectively. The plants were fertilized with a solution of

fertilizer (100 ppm N) supplied as 14:14:14 (N:P:K) until four weeks before harvest. All these propagules i.e. whole *in vitro* plantlets (WIP), plantlets from which one apical cutting was removed (ACR), plantlets from which two cuttings (apical and subsequently lateral cuttings) were removed (LCR), rooted apical cuttings (RAC) and rooted lateral cuttings (RLC) had a growth and tuberization period of 121 days, at which time the tops were removed and tubers were harvested.

Per cent ground cover. Per cent ground cover was assessed every 5 days following planting by slide photographs taken directly above the plots at a height of 2.5 m. They were later projected on to a grid pattern and the percentage of the plot covered by foliage was estimated by counting the number of squares covered by green foliage.

Biomass of foliage and tuber. The foliage and tubers per plot were harvested 121 days after planting, dried in a forced air oven at 80 °C and weighed.

Experimental design. The split-plot design was used with propagule type as the main plot factor and planting density as the sub-plots. The sub-plot size was 1 m^2. Two and four guard rows of transplants were used between each sub-plot and the main plot respectively. The three levels of planting density were randomized within each level of main plots and the five main plots were randomized within each block. There were four replicate beds planted, and each bed represented a complete block.

Statistical analyses. Yield and related parameters such as tuber weight, tuber number, total dry matter, tuber dry matter, harvest index and plant survival were recorded. The data collected was examined by analysis of variance and Duncan's New Multiple Range Test was used to separate the treatment means (Steel & Torrie, 1980).

Results

The data obtained for different parameters indicated no interaction between main plot and sub-plot factors in this study.

Potato seed tuber production from various types of propagules
Yield of tubers. The WIP produced the highest yield per plant (Table 1) and RAC highest yield per m^2 among all the propagules tested (Fig. 1). No significant difference was observed between the yield of tubers from WIP and RLC (Fig. 1). The plantlets with one or two cuttings removed (ACR & LCR) produced the lowest yield of tubers compared to other propagules (Fig. 1). A classification based on the tuber weight (size) ranges showed that the rooted cuttings produced a higher weight of tubers in the size range of >40–80 g and >80 g, whereas a lower weight of tubers in the size ranges of >20–40, >5–20 and 1–5 g was observed (Fig. 1).

Number of tubers. Rooted cuttings (RAC and RLC) produced similar but significantly less tubers per plant and per m^2 than other propagules tested (Table 1:

Table 1. Potato seed tuber yield, harvest index, plant survival and total biomass in five types of propagule averaged over three planting densities.

Types of Propagules	Tuber yield per plant		Harvest index (%)	Plant survival (%)	Total Biomass (kg m^{-2})
	Weight (g)	Number			
WIP	151.9a[z]	11.7a	68.0b	81.8b	1.8a
ACR	132.2c	10.2b	66.0b	72.4c	1.4b
LCR	121.7d	10.6b	66.0b	74.4c	1.4b
RAC	144.5b	5.5c	73.0a	91.5a	1.8a
RLC	123.5d	5.0c	69.0b	96.2a	1.7a

[z] Means are separated using Duncan's new multiple range tests P<0.05.

Fig. 1). Rooted cuttings produced significantly more tubers of size >40 g. However, WIP produced approximately twice the number of tubers of size <20 g than that of the cuttings (Fig. 1).

Planting densities and tuber production

Yield of tubers. The yield of tubers per plant decreased (Table 2) whereas the total tuber yield per m^2 increased significantly with higher planting density (Fig. 2a). A classification based on tuber size indicated that tuber yield in the size ranges of >5–20 g

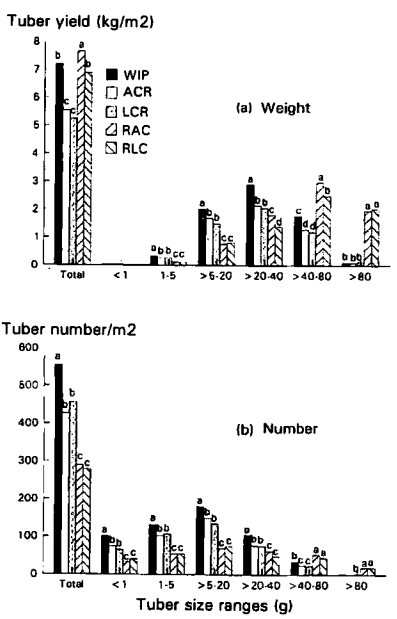

Fig. 1. Tuber yield (a) weight and (b) number from five types of propagules averaged over three planting densities.

Table 2. Potato seed tuber yield, harvest index and plant survival at three planting densities.

Planting density per m^2	Tuber yield per plant		Harvest index (%)	Plant survival (%)
	Weight (g)	Number		
25	248.2a[z]	11.1a	70.0a	95.2a
49	134.5b	7.5b	68.0a	87.2a
100	71.3c	4.8c	69.0a	73.0b

[z] Means are separated by Duncan's new multiple range tests P<0.05.

and >20–40 g increased significantly with an increase in planting density (Fig. 2a). The yield of tubers of size up to and including 5 g were similar at three planting densities. However, the yield of larger size tubers of >80 g decreased significantly with higher planting densities.

Number of tubers. The number of tubers produced per plant decreased (Table 2) whereas the total number of tubers per m^2 increased significantly with an increase in planting density (Fig. 2b). The number of tubers in all size ranges up to 40 g, increased significantly at higher planting density (Fig.2b).

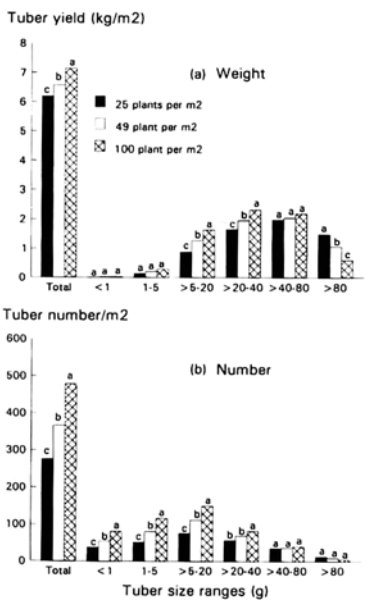

Fig. 2. Tuber yield (a) weight and (b) number from all propagules at three planting densities.

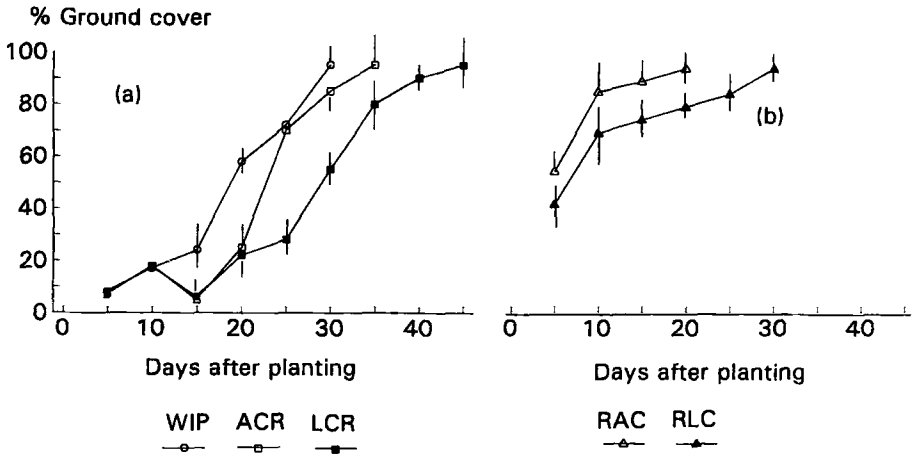

Fig. 3. Percent ground cover of (a) in vitro plantlets (WIP, ACR and LCR) and (b) rooted cuttings (RAC and RLC) planted in greenhouse beds. Vertical bars at each point indicate standard errors of means.

Other characteristics. The results also showed that RAC had the highest harvest index and that RLC and RAC had the highest plant survival (Table 1). In both rooted cuttings (RAC and RLC) tuber initiation occurred in 4 weeks after transplanting as compared to 6 weeks for the *in vitro* plantlets (WIP, ACR and LCR). Compared to the *in vitro* plantlets, WIP, ACR and LCR which completed 95% ground cover in 4, 5 and 6 weeks respectively after transplanting, the cuttings (RAC and RLC) took 4 weeks or less to reach the same ground cover (Fig. 3). The biomass per m^2 was lower in both ACR and LCR, as compared to WIP, RAC and RLC (Table 1). A significant correlation was observed between yield per m^2 with total dry matter, tuber dry weight for all of the treatment tested (data not shown).

Discussion

Although all types of propagules were harvested 121 days after transplanting, the foliage of *in vitro* plantlets was removed on May 31 whereas with the rooted cuttings it was removed on June 25 (RAC) or later on July 5 (RLC). Therefore the cuttings had 4 weeks (RAC) to more than 4 weeks (RLC) of higher light intensities and longer periods of sunlight during June which allowed more photosynthate to be produced, translocated and stored into the developing tubers. Furthermore, fewer tubers per plant or per m^2 were produced by the rooted cuttings and therefore the tuber yield by weight (per plant; per m^2) was higher or equal to the *in vitro* plantlets (Table 1; Fig. 1), indicating a higher mean tuber weight. The relatively few tubers could also have been because fewer nodes of the rooted cuttings were within the production medium. However in the rooted cuttings, tuber initiation and 95% ground cover occurred in four weeks after transplanting as compared to 6 weeks and

7 weeks respectively for the *in vitro* plantlets. This rapid growth and enhanced tuber initiation response of the rooted cuttings could be attributed to an increased light interception as a result of increasing photoperiod and light intensities. This correlation seems valid in light of the previous reports of a positive correlation between per cent ground cover and radiation interception (Haverkort et al., 1991), as well as dry matter accumulation and radiation intercepted by potato foliage (Haverkort & Harris, 1987). This study showed that the removal of cuttings (ACR and LCR) from established single stem whole *in vitro* plantlets (WIP) significantly reduced the number as well as weight of tubers per plant (Table 1). Furthermore, the total dry matter yield per m^2 was reduced in both ACR and LCR, as compared to WIP, RAC and RLC (Table 1), indicating a set-back of plant growth following the removal of actively growing shoots.

There were no significant interactions between the various types of propagules and planting densities. In general, total tuber yield per m^2, tuber number and weight per plant increased significantly with the increasing planting density up to 100 propagules per m^2 (Fig. 2). This trend of yield increase was more evident in the tuber weight classes of 40 g and smaller. With the larger tuber weight class of >40–80 g the planting density had little effect (Fig. 2). However, the number and weight of tubers per plant, averaged over all 5 types of propagules, showed a progressive decline with increasing planting density (Table 2). At the highest planting density of 100 propagules per m^2 there was a significant loss of plants, possibly due to competition for light and space. As planting density significantly influenced the number and weight of tubers per m^2, it should be possible to optimize tuber yields by adjusting the planting density. However, very high plantlet density could result in high plant mortality and other problems due to competition and overcrowding. This study confirmed earlier work (Wiersema et al., 1987) that mass production of potato seed tubers is possible by high density planting of *in vitro* plantlets directly into large propagation beds under controlled conditions of a greenhouse. It also showed that cuttings taken from established plantlets after rooting could be transplanted directly into propagation beds to produce more tubers, and thus considerably increase yield potential of the original number of *in vitro* plantlets and reduce the cost of micropropagating these plants. Total tuber weight and dry biomass per m^2 of the cuttings (RAC and RLC) was similar to that of the whole *in vitro* plantlets. However, the number of tubers per m^2 was considerably reduced in the cuttings and could be due to a time differential between planting two groups of propagules. Removal of cuttings from single stem established whole *in vitro* plantlets significantly depressed their tuber yield. Therefore it may be economic and efficient to separately maintain disease indexed, and vigorously growing *in vitro* plantlets as sources of cuttings.

Acknowledgements

Financial assistance from the Canadian International Development Agency to the Senior Author and facilities provided by the Ontario Ministry of Agriculture and Food are gratefully acknowledged.

References

Bryan, J.E., 1988. Implementation of rapid multiplication and tissue culture methods in third world countries. *American Potato Journal* 65: 199–207.

Dodds, J.H., 1988. Tissue culture technology: practical application of sophisticated methods. *American Potato Journal* 65: 167–180.

Espinoza, N., R. Estrada, P. Tovar, J. Bryan & J.H. Dodds, 1984. Tissue culture micropropagation, conservation, and export of potato germplasm. Specialized Technology Document, International Potato Center, Lima, Peru, pp. 1–20.

Haverkort, A.J., D. Uenk, H. Veroude & M. van de Waart, 1991. Relationships between ground cover, intercepted solar radiation, leaf area index and infrared reflectance of potato crops. *Potato Research* 34: 113–121.

Haverkort, A.J. & P.M. Harris, 1987. A model for potato growth and yield under tropical highland conditions. *Agricultural and Forest Meteorology* 39: 271–282.

Jones, E.D., 1991. Progress in seed production technology. *American Potato Journal* 68: 247–248.

Knutson, K.W., 1988. Implications of new technologies for seed potato certification programs and seed growers. *American Potato Journal* 65: 229–235.

Levy, D., 1988. Propagation of potato by the transfer of transplants of in vitro proliferated shoot cuttings into the field. *Scientia Horticulturae* 36: 165–171.

Murashige, T. & F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497.

Steel, R.G.D. & J.H. Torrie, 1980. Principle and Procedures of Statistics, A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., New York, pp. 633.

Uyen Naguen van & P. Vander Zaag, 1985. Potato production using tissue culture in Vietnam: The status after four years. *American Potato Journal* 62: 237–241.

Wattimena, G., B. McCown & G. Weis, 1983. Comparative field performance of potatoes from microculture. *American Potato Journal* 60: 27–33.

Wiersema, S.G., R. Cabello, P. Tovar & J.H. Dodds, 1987. Rapid seed multiplication by planting into beds microtubers and in vitro plants. *Potato Research* 30: 117–120.