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Mini-tuber Production as Affected by Planting Bed Composition and Node Position in Tissue Cultured Plantlet in Two Potato Cultivars

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ABSTRACT

The virus-free plantlets resulted from the meristem culture of two potato (*Solanum tuberosum* L.) cultivars i.e., Marphona and Desiree were grown and their shoot tips and roots were cut off. Stems were sectioned to three upper, middle and lower segments and transferred onto four planting beds: (a) peat mass: perlite (3:1), (b) turf: perlite (3:1), (c) Leaf mould: perlite (3:1) and (d) rice hull: turf (1:1). The mini-tubers were harvested and biometrically analyzed for their number, total weight, average weight, average numbers of eyes and the average diameter of the mini-tubers generated by a single plant. It was revealed that rice hull: turf (1:1) was superior to other beds. The effect of stem position node on the total weight of mini-tubers, except an interaction between node position and planting-bed was non-significant. Desiree was only superior in total weight of the generated mini-tubers compared to Marphona.

Key Words: Minituber production; Node position; Planting-bed; Potato; Tissue culture

INTRODUCTION

Potato (*Solanum tuberosum* L.) ranks fourth after wheat, barley and sugar beet in Iran. The average yield under irrigated conditions was estimated as 19.72 metric tones ha^{-1} in 2003 (FAO, 2003). Considering that potato is propagated by tubers and the virus infestation increases inside the generated tubers annually, seed tubers decline as the result of viral infections and therefore, after few years, they will be of no quantitative or qualitative value (Rezaii & Soltani, 1996). Therefore, it is tried to prepare healthy pathogen-free tubers and put them available for farmers. As it is estimated, the application of healthy potato tubers will lead to at least 30% yield increase (Zarghami, 2001). In recent years, the *in vitro* production of healthy plants through meristemic tissue culture has been discerned as the best strategy for viral disease control.

To produce tubers from plantlets resulted from tissue cultures, they can be transferred to laboratories, where micro-tubers are produced in tuber induction medium; or to green-houses, where mini-tubers are produced (Fazeli Sabzevar, 2001). Because of the reasons like lower production costs, rapid propagation, high viability and high level of growth tenacity and suitability for direct field planting, the mini-tuber production is discerned superior to micro-tuber generation (Boyd, 2000), therefore mini-tubers are extensively produced and adopted in the programs for potato seed production and propagation. Ahloowalia (1994) concluded that mini-tuber production through planting of micro-propagated plantlets in soil can be regarded as a quick and effective approach for potato seed tuber production (Ahloowalia, 1994). To more economize the method, it is possible to propagate the plantlets through cuttings prepared and planted before or after transfer to soil, so that a propagation stage is provided without tissue culture and further costs. Ahmed *et al*. (1995) compared the mini-tubers produced from rooted virus-free plantlets with those from the cuttings of one or several nodes. They found that rooted plantlets and the cuttings with multiple nodes generated larger mini-tubers than those cuttings with only one individual node.

There are many factors that affect the transferred plantlets under green-house conditions, like planting-beds and illumination etc., among them planting-beds are of considerable importance (Jami Moeini *et al*., 2001). Tukaki and Mahler (1989) reported the advantage of vermiculite/ sand medium against other potting-mixtures and indicated that a potting-mixture of 80% vermiculite and 20% silica sands led to the maximum tuber yield under green-house conditions (Tukaki & Mahler, 1989). Solis (1998) demonstrated the favorability of a soil mixture consisting of two parts of forest litter and a part of soil for mini-tuber production. Jami Moeini *et al*. (2001) found that the bed of peat mass:sand (4:1) was a proper medium for mini-tuber production while field soil was unfit and should not be added to the planting-bed composition (Jami Moeini *et al*., 2001). With a view to high importance of planting-bed composition and node position on the mini-tuber production yield, we focused to prove the following hypotheses: (a) bed has great effects on mini-tuber production, (b) the interactive effects of node position and planting bed have a significant role in production of mini-tuber and (c) the responses of different potato varieties to the interactive effects of planting-beds and node positions are different.

MATERIALS AND METHODS

Tubers from Marphona and Desiree potato cultivars were planted in the greenhouse. When the plants were reached to the height of 15 - 20 cm, their upper ends were cut off and incubated for four weeks under thermotherapic conditions of 37ºC for 16 h and 30ºC for 8 h. Also, a continuous illumination of the intensity of 5000 lux and a relative humidity of 80 ± 5% were provided. After four weeks, the developed lateral branches were cut off and taken to the laboratory, where they were superficially sterilized meristems were then isolated using a needle together with one or two primordia and placed on a paper bridge put on a medium containing MS salts, glycine 2 mg L^{-1}, nicotinic acid 0.5 mg L^{-1}, pyridoxine 0.5 mg L^{-1}, thiamine 0.4 mg L^{-1}, gibberellic acid 0.1 mg L^{-1}, kinetin 0.04 mg L^{-1} and sucrose 2.5 g L^{-1}. The set was transferred to a growth chamber at 22 ± 2ºC and 100 lux illumination. After the growth of meristems and the generation of plantlets of multiple nodes, they were tested following the usual double antibody sandwich enzyme-linked immun-osorbant assay (DAS-ELISA) (Adams & Clark, 1977) to assure that the resulted plantlets were virus-free. The tested virus-free plantlets were then extensively propagated in MS medium without phytohormones.

The propagated plantlets of both cultivars were used to study the bed effects in a completely randomized design (CRD) in factorial arrangement with five replications. The factors were as: (a) cultivar: Marphona and Desiree, (b) node position on the stem: 1st position (lower part of the stem), 2nd position (middle part of the stem) and 3rd position (top part of the stem) and (c) planting beds: peat and perlite (3:1), turf and perlite (3:1), leaf mould and perlite (3:1) and rice hull and turf (1:1).

Plantlets were transferred to the green-house. The shoot tips and roots of the plantlets were removed and the stems were sectioned to three equal-sized segments, transferred to culture trays containing four planting-beds. To maintain their vitality for longer period of time, the trays were covered with plastic covers to retain higher humidity around the cuttings. When the lateral buds on the cuttings began to grow and the roots developed, the plantlets were transferred from the trays to the pots with the same type of the respective beds mentioned above. 112 days after bud growth, the generated mini-tubers were harvested, cleaned, put in plastic bags and coded with the same code given to the relative plant. The plastic bags were taken to the laboratory and the data related to the traits as the number of mini-tubers, total weight of mini-tubers per pot, average weight of the mini-tubers, average number of the eyes per mini-tuber and the average diameters of the mini-tubers were recorded and analyzed.

RESULTS

Results from DAS-ELISA with plantlets originated from meristem cultures indicated that all the plantlets were virus-free and suitable for mini-tuber production.

No significant differences were found between both cultivars, except that total weight of mini-tubers was higher with Desiree (28.68 g) than that of Marphona (26.18 g). Also, it was notable that the average weight of a mini-tuber was not statistically different. Node position had no effect on the traits investigated. Planting-beds significantly affected all the attributes tested (Table I) in total mini-tuber weight produced by a single plant and average weight of a produced mini-tuber traits, the planting bed, rice hull:turf, was of the most highest productivity, respectively followed by culture beds turf: perlite, peat mass: perlite and leaf mould: perlite. In the average number of eyes per mini-tuber and average diameter of the produced mini-tubers traits, the beds rice hull: turf and turf: perlite were of the most abundant average number of eyes and most greatest average of mini-tuber diameter, while planting beds peat mass: perlite and leaf mould: perlite, respectively were placed in the following ranks (Table II).

DISCUSSION

In this research, except for total weight of mini-tuber, there was no significant differences were found between cultivars Desiree and Marphona in contrast to Jami Moeini *et al*. (2001) who concluded that average weight and diameters of mini-tubers produced by potato cultivar Agria were more than those of Marphona. However, no significant difference was noted between two cultivars when the number and total weight of mini-tubers were compared (Jami Moeini *et al*., 2001). Studying mini-tuber generation by two cultivars Agria and Draga, Ghahestani (1997) found that from the standpoint of the total weight of the produced mini-tubers per plant, Agria was superior to Draga, producing 20.52 g mini-tubers.

Although we found no effect of node position on the traits investigated, Ahmadian and Zarghami (1998) concluded that the upper nodes originated from *in vitro* plantlets produced micro-tubers with more length, diameters and weights. Also, Ahmed *et al*. (1995) studied the mini-tuber production by whole *in vitro* plantlets and the single

Table I. Variance analysis of mini-tuber characters

Source of Variation	df	Means of Squares: Average minituber diameter (mm)	Average minituber eye number	Average minituber weight (g)	Total minituber weight (g)
Cultivar(A)	1	7149201	2.76	3.07	186.38**
Node position (B)	2	15224343	1.43	0.33	13.58
Planting bed(C)	3	21712928**	52.08**	109.45**	7896.81**
A×B	2	18838623	0.78	0.44	5.83
A×C	3	14379737**	0.53	2.59*	45.22
B×C	6	654417	0.21	0.91	65.44*
A×B×C	6	13298566	0.95	0.49	17.43
Error	96	14022580	0.71	0.86	26.46

*, ** Significant at the 5% and 1% levels of probability, respectively

Table II. Mean comparison of mini-tuber characters

Treatment	Average minituber diameter (mm)		Average minituber eye number		Average a minituber weight (g)		Total minituber Weight (g)	
	Mean	**LSD 5%**	**Mean**	**LSD 5%**	**Mean**	**LSD5%**	**Mean**	**LSD5%**
Cultivars (A)		1.359		0.306		0.337		1.866
Marphona	23.7		4.93		4.62		26.18b	
Desiree	23.21		5.23		4.94		28.68 a	
Node position (B)		1.664		0.375		0.413		2.286
Botton	22.76		4.89		4.7		27.83	
Middle	23.69		5.27		4.76		27.7	
Top	23.92		5.09		4.87		26.7	
Planting bed (C)		1.922		0.433		0.476		2.64
Peat mass/perlite(3:1)	22.17 b		4.52 b		4.06 c		18.63 c	
Turf/ perlite(3:1)	27.4 a		5.97 a		5.79 b		34.54 b	
Leaf mould/perlite(3:1)	157.75 c		3.5 c		2.46 d		10.1 d	
Rice hull/turf(1:1)	28.51 a		6.34a		6.79 a		46.44 a	

cuttings made from whole *in vitro* plantlets from 6 potato cultivars concluding that whole *in vitro* plantlets and the terminal Node-5 cluster could produce significantly larger mini-tubers (more than 3 g) compared to single node cutting. Yiem *et al*. (1990) declared that cuttings from the middle and lower parts of *in vitro* plantlets produced more micro-tubers than upper cuttings from the same positions.

Considering the results with culture beds, it was noted that beds with turf produced better yields in all the studied traits compared with those from peat mass and leaf mould. This is in contrast to the results obtained by Jami Moeini *et al*. (2001), who introduced peat mass amended culture bed as the best out of the various planting-beds. Solis (1998) knew the bed of 2 parts of forest litter plus a part of soil as the best bed for the production of potato mini-tubers. Comparing two beds including peat mass and sand, and vermiculite and sand Obradovic and Sukha (1993) discerned the bed with vermiculite as a proper one for the longer survival of *in vitro* plantlets and the production of mini-tubers.

CONCLUSION

Planting bed had great effects on mini-tuber production and a significant effect in all of traits was observed. Node position had no significant effect on mini-tuber production except when the interactive effect of node position and planting bed on total mini-tuber weight trait was investigated. Total mini-tuber weight of Desiree was higher than Marphona and there was significant interactive of cultivar and bed on average mini-tuber weight its and diameter.

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