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The Effects of Size and Microtuber Dormancy on Production of Potato Minitubers

Ahmad Reza Bolandi, Hassan Hamidi and Raheleh Ahmadzadeh Ghavidel

Department of Tissue Culture,
Agricultural and Natural Resource Research Center of Khorasan, Mashhad, Iran

Abstract: In this study, sprouting potential and functions of microtubers gained from two commercial potato cultivars Agria and Marfona were investigated which categorize in three size groups <5mm, 5-10mm and >10mm which all had gone to dormancy for 3-5 months. Sprouting, nonsprouting microtubers percentage along with weight, number and diameter of minitubers were recorded. A significant and positive correlation between minitubers diameter and their sprouting percentage was observed. Microtubers higher size show more efficient functionality than others with thin diameters. Results showed that Marfona with sprouting percentage 56.37% has better functionality in comparison to Agria with 48.87%, while Agria has better functionality index. Among studied cultivar, greater diameter microtubers which spent most times in dormancy in comparison to thin diameter microtubers with less time in dormancy and showed superiority in weight, number and minituber diameter.

Key words: Potato · *Solanum tuberosum* · Minituber · Dormancy

INTRODUCTION

Potato (*Solanum tuberosum* L.) plant is one of leading agricultural products in the world with 325.3 million ton glands in year stands in fourth place after wheat, rice and corn [1]. Potato plant generally propagates by gland which is low in reproduction process. In tropical and semitropical regions without any cold winters, pathogen life cycle ceases and consequently by accumulation of pathogenic factors, potato production encounters with problems. In these regions, to produce potato they have to provide glands from regions which have inappropriate conditions for pathogens growth and due to high costs for virus test, more than 50% of production cost devotes to this issue [2]. Cultivating plant texture to produce a healthy plant without any pathogenic factors with purpose of producing initial culture seed has been examined by many researchers for regions which are not able to produce healthy seeds [3, 4, 5, 6]. Then as appropriate and healthy seeds grew, reproduction process begins in vitro condition inside glass compartment. As plantlets growth reaches to appropriate point (about 4 weeks after culture), they are directly transferred to greenhouse [3] or kept inside glass compartment for more time length (12 to 16 weeks) to produce microtubers, then their culture begins in greenhouse to produce initial culture seed minituber [7, 8, 9, 6]. The advantage of using microtuber in comparison to plantlet for producing minituber is their damage in transferring to greenhouse, high culture speed, no need to compatibility, easy move from one place to others and possibility to save microtubers [5, 6]. However to produce minitubers from microtubers it takes more time than plantlet, since microtubers must pass dormancy before cultivation, also they lose easily their water as high proportion of leaf surface to mass and might demolish in bad conditions [8, 9, 10]. Resulting minitubers as first generation of glass cultured plants are something between glass cultured plants and natural cultured plants on farms [11]. This seed class is main source of basic seed for potato, [3]. The function of minitubers resulting from microtubers is affected by many factors like sort of specie, microtuber size and length of itsdormancy. Kawakami *et al.* [7] indicated that the growth and function of potato plants resulted from microtuber culture in farm condition. They used microtubers cultivar Norin 1 with 0.5-1g and 1-3 g weight and glands with 50 g weight and show that there is potential to use microtubers for

Corresponding Author: Raheleh Ahmadzadeh Ghavidel, Department of Tissue Culture, Agricultural and Natural Resource Research Center of Khorasan, Mashhad, Iran.
Tel: +98-511-8458782, Mob: +989364398682, E-mail: ahmadzadeh_ra@yahoo.com.

culture on farm. Results indicate that in beginning those plants resulted from microtubers culture has less leaf surface than gland cultured plants, but in next 40 days after sprouting, leaf structure index is same in all plants. In this research, gland production in plants produced by microtuber culture begins 7 days later than plants produced by gland culture. Pruski *et al.* [13] reported that species sort and plantlet conditions *in vitro* growth period affects on minitubers on farm and in order to achieve proper production, optimization of specie sort and conditions is essential for any certain genotype. In this research three genotypes were studied which one of them is not capable to produce microtuber and there is a significant difference between two other sorts of genotypes in number and weight of produced minitubers. Ahmed *et al.* [1] pointed that the function of minitubers produced by six genotypes plantlets culture in greenhouse and report significant difference in weight, number and size of minituber among genotypes. The also reported that the number of produced minitubers from plantlets is 600 to 1500 and their weight from 0.89 to 2.61 kg/m^2. Struik *et al.* [10] reported the the microtubers of six cultivars, a significant difference in sprouting speed. They also indicated that microtubers with 3 g weight sprout after 10 days, while microtubers with 375 and 750 mg sprout after 15 and 13 days respectively. Other study reported by Park *et al.* [9] recommend using microtuber for culture *in vitro* or directly on farm and argue that the size of chosen microtubers has notable influence on product condition as most important indexes to determine buds number. Donnelly *et al.* [12] reported that microtuber size is important and bigger glands have more functionality. Struik and Lommen [10] reported that produced microtubers in glass culture condition generally have longer dormancy and need to be kept in low temperature for 4 months to sprout. They also recommend pre sprouted microtubers which have completed their dormancy to increase the number of active buds inside gland and sprouting speed. Other study, Pruski *et al.* [6] reported that when dormancy of microtubers is not completed, less number plant is produced. The technology for producing minitubers is possible in recent years domestically, but more studies in relation to pretreatment of mother microtubers with purpose to increase minitubers functionality is needed. Therefore, this study aimed to investigate the effects of size and microtuber dormancy on resulting minitubers traits on two commercial genotypes in region, so it can be choose the best treatment to produce maximum minitubers with premium quality in greenhouse condition.

MATERIALS AND METHODS

Microtubers were produced from non-virus plantlets of two potato cultivars Agria and Marfona with 4 months age which grew in glass compartment. In order to have microtubers which have passed their proper dormancy period at culture, in three different times (3, 4 and 5 months before culture), then microtubers were harvested. Then microtubers transferred to petry dish with 4°C and kept in darkness. In cultivation time, microtubers related to each dormancy period categorized into three groups based on their diameter <5, 5-10 and >10mm. Microtubers were cultured inside vases with 20cm diameter and height containing leaf mold, fertilizer and culture soil equally, each microtuber inside one vase irrigation in initial stages once a day and in later stages based on plant needs in appropriate times. The amount of sprouted microtubers 10 and 20 days post cultivation were counted. 40 days post cultivation recounting process implemented and the amount of microtubers which were not able to sprout or their sprouts were too weak to continue lives were gathered for each treatment. As growth period ends and after minitubers harvest, total weight and number of minituber within each plant along with average weight and diameter of minituber calculated. Experimental design was factorial on the basis of complete randomized block design (CRBD) in three replication was comprised of 10 vases. Data were analyzed using the software [13]. The effect of each treatment was quantified and mean values were compared using Duncan's Multiple Range Test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Variance analysis results show that there is a significant difference between used cultivars and microtuber size. In this experiment, witness significant difference between various levels of dormancy in terms of microtubers sprouting percentage after 10 days and weight of produced minituber by each plant as well as average weight of minitubers. This difference for sprouting percentage is 5% and for two other traits is 1% (Table 1).

In this study, after transferring microtubers into soil, inharmonic germination and sprouting rate based on microtuber size and other treatments is seen. Difference among various cultivarsin terms of sprouting speed is more in initial culture stages, in a way that 10 days post culture, 44.86 % of Marfona microtubers sprouted, while Agria microtubers sprouting percentage during

Table 1: Analysis of variance for potato minituber production in the greenhouse

Mean Squares (MS) (S.O.V.)	d.f	Sprouting micotubers after 10 days (%)	Sprouting microtubers after 20 days (%)	Non-Sprouting microtubers after 40 days (%)	Total weight of minitubers per plant (g)	Total number oe minitubers per plant	Avarage minituber weight (g)	Avarage minituber diameter (mm)
Replication	2	261.64ns	219.3ns	195.27ns	73.38ns	0.29ns	0.04ns	0.64ns
Cultivar (a)	1	2251.7**	758.62*	918.43*	461.77**	0.96*	8.7**	21.47**
Microtuber size (b)	2	9030.08**	14710.15**	14504.03**	1730.34**	11.49**	13.9**	25.44**
Dormancy (c)	2	880.88*	87.82ns	106.88ns	162.65**	0.27ns	9.36**	5.24ns
$a \times b$	2	54.76ns	198.15ns	133.86ns	36.33ns	1.17*	1.13ns	0.12ns
$a \times c$	2	6.62ns	62.44ns	62.71ns	29.05ns	2.65**	1.32ns	0.88ns
$b \times c$	4	185.93ns	375.79ns	366.55ns	38.87ns	1.7**	5.95**	7.11*
$a \times b \times c$	4	199.92ns	216.16ns	237.44ns	256.56**	0.87*	7.64**	9.18**
Error	34	203.93	170.13	170.36	17.49	0.23	0.81	2.14

Ns: not significant (p>0.05), *and**: significant at p ≤ 0.05 and 0.01, respectively

Table 2: Main effects of various factors on sprouting microtuber and minituber production in the greenhouse

Treatments		Sprouting micotubers after 10 days (%)	Sprouting microtubers after 20 days (%)	Non-Sprouting microtubers after 40 days (%)	Total weight of minitubers per plant (g)	Total number oe minitubers per plant	Avarage minituber weight (g)	Avarage minituber diameter (mm)
Cultivars	Agria	31.95b	48.87b	54.26a	42.4a	5.87a	7.22a	17.77a
	Marfona	44.86a	56.37a	46.01b	36.55b	5.61a	6.51b	16.51b
Microtuber size	<5mm	15.44c	23.25c	79.07a	29.48c	4.92c	5.99c	16.06b
	5-10mm	39.58b	54.24b	49.01b	39.85b	5.78b	6.89b	16.96b
	>10mm	60.19a	80.36a	22.33c	49.08a	6.52a	7.53a	18.41a
Dormancy	3 months	34.36b	50.44a	52.26a	40.93a	5.84a	7.01a	17.63a
	4 months	34.37b	52.56a	50.68a	36.01b	5.77a	6.24b	16.56b
	5months	46.48a	54.86a	47.48a	41.47a	5.6a	7.4a	17.24ab

Means within the same column and treatment followed by the same letter are not significantly different according to Duncan (p ≤ 0.05)

this period is only 31.95%. This difference in next notation 20 days post culture descends to 7.5% and from 56.37% in Marfona to 48.87% in Agria. The percentage of non sprouting microtubers or their plantlets which demolished in initial growth stages varies based on genotype sorts, 54.26 % for Agria and 46.01 % for Marfona (Table 2).

In the case of functionality traits, in this research except for minitubers number in each plant, for other traits Agria cultivar shows better functionality than Marfona in a way that in terms of total weight of produced minitubers by each plant, Agria with 42.4 g shows priority in comparison to Marfona with 36.55 g. Average weight and diameter of minitubers in Marfona is 6.64 g and 16.51 mm respectively, while this amount for Agria increases to 7.44 g and 17.77 mm respectively (Table 2). Rolot *et al.* [11] used six-variance microtubers with density 59 microtubers per square meter to produce minitubers. The results indicate that minitubers functionality in examined cultivars varies from 224 to 779 minitubers with over 10 mm diameter per square meter based on genotype. Gopal *et al.* [14] studied numbers of 22 morphological and culture traits of two microtuber sorts white and green on 16 potato genotypes and witness significant difference among genotypes, microtubers types and their counter effects for various traits. In addition to genotype effect, microtuber status and its production condition affects on functionality potential of produced minitubers [15, 12, 14, 5, 9]. Data in the present study indicated that, thick diameter microtubers react better to sprouting and plantlet production than thin diameter microtubers in a way that there is a significant positive between microtuber diameter and sprouting percentage. The percentage of sprouting microtubers after 20 days which have >10 mm diameter is 3.46% more than those with <5mm diameter. In contrast, the percentage of death among >10mm length microtubers is 56.74% less than microtubers with <5mm length which it was decreased from 79.07% to 22.33% (Table 2). Moreover, the results indicated a direct and significant relationship between used microtuber size and weight and number of produced minitubers in each plant, so that total weight of produced minituber by each plant which is produced from microtubers <5mm is 29.48 g and for microtubers >10mm is 49.08 g. In addition, the comparison of average level of diameter and weight

Table 3: Effects of size and microtuber dormancy on minituber production in two potato cultivars

Cultivar	Microtuber size (mm)	Dormany (month)	Sprouting micotubers after 10 days (%)	Sprouting microtubers after 20 days (%)	Non-Sprouting microtubers after 40 days (%)	Total weight of minitubers in each plant (g)	Total number of minitubers per plant	Mean weight of minituber (g)	Mean diameter of minituber (mm)
Agria	<5	3	10.43ef	18.6g	84.73a	37.7fgh	5.63cdef	6.7bc	18.2abc
		4	6.77f	12.13g	89.87a	29.16ijk	4.77fgh	6.11cdef	16.81bcd
		5	7.33f	17.33g	83.67a	25.49jk	4.28gh	5.96cdef	15.24de
	5-10	3	26.37cdef	35.3efg	68.7abc	42.99def	6.75ab	6.37cde	16.85bcd
		4	27.93cdef	58.3cde	64.7cd	41.41defg	5.77cde	7.18bcd	17.5bcd
		5	51.07bc	67.23abcd	36.43de	45.79cde	4.81fgh	9.52a	18.15abc
	>10	3	45.67bcd	88.3a	15.23e	55.25ab	6.5bc	8.5a	20.34a
		4	48.2bc	69.67abcd	33.67de	44.53cdef	7.49a	5.94def	17.36bcd
		5	63.77ab	72.97abcd	29.37de	59.24a	6.84ab	8.66ab	19.51ab
Marfona	< 5	3	18.63def	28.9fg	72.73ab	23.64k	5.1efg	4.63f	13.69e
		4	20.8def	30.53fg	73.47ab	22.66k	4.1h	5.53def	15.45cde
		5	28.7cdef	32.03fg	69.97abc	38.26efgh	5.65cdef	6.77cde	16.95bcd
	5-10	3	34.83cde	48.7def	52.97bcd	34.91ghi	5.46def	6.39bcd	16.46cd
		4	48.77bc	62.97bcd	38.2de	31.37hij	5.79cde	5.42ef	15.61cde
		5	48.5bc	52.93def	51.07bcd	42.62defg	6.07bcd	7.02cde	17.2bcd
	>10	3	70.23ab	82.83abc	19.2e	51.11bc	5.61cdef	9.11a	20.23a
		4	53.77bc	81.77abc	22.17e	46.96cd	6.71ab	7bcde	16.65cd
		5	7953a	86.63ab	14.37e	37.41fgh	5.96bcde	6.28cdef	16.38cde

Mean within the same column and treatments followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$)

among produced microtubers showed significant difference based on the size of used microtubers. Most mean for both traits belongs to microtubers >10 mm which is 7.9 g and 18.4 mm for weight and diameter. These figures for micrometers <5mm are 6.14g and 16.06mm, respectively. Lakhoua and Ellouze [5] used micrometers in three different sizes <3mm, 3-5mm and > 5mm to produce minituber. They also indicated that there is a direct relation between diameter and sprouting percentage of microtubers. In this study only 46% of microtubers with <3mm diameter are able to sprout, while this amount for microtubers with 3-5mm and >5mm diameter is 75% and 98% respectively. Also these results show that larger microtubers produce more minitubers than smaller microtubers. Moreover, minitubers functionality was increased by microtubers size and reaches to 20, 27 and 40 tons/ha for microtubers with <3mm, 3-5mm and >5mm diameter. Alsadon *et al.* [15] reported that functionality of plants glands produced by small microtubers is less than larger microtubers. Georgakis *et al.* [16] studied four different minitubers size (<10, 10-15, 15-20 and >20mm) and witness significant difference between various treatment levels in terms of size, number and weight of produced glands. The dormancy of microtubers as a main factor in this study that affects sprouting percentage of microtubers, as well as, total weight of produced minituber by each plant. The results also showed that sprouting rate among microtubers in which dormancy in longer is faster than microtubers with less dormancy time in a way that 10 days post culture 46.48% of microtubers with 5 months dormancy sprouted, while this amount for microtubers with 3 months dormancy time is 34.36%. Data also indicated that microtubers with 5 months dormancy produced 41.47g minitubers by each plant which has priority in comparison to microtubers with 4 months dormancy 36.01%. Mulet [17] reported that microtubers over 7mm, the amount of produced minitubers was increased by microtuber dormancy. In this study the amount of produced minitubers from microtubers with 7 months dormancy is 500 microtuber per square meter, while this amount for microtubers with 3 months dormancy is 200 microtuber. Ranalli *et al.* [18] showed that dormancy has converse relation with microtuber size. Therefore, microtubers with small size 90 to 120mg due to inability to be conserved for long time and consequently incomplete dormancy, have less ability to sprouting. Encounter effects of treatments show a significant difference based on studied traits. For weight trait of produced minituber, highest value belongs to Agria cultivar for microtubers >10mm with 5 months dormancy 59.24g, while in Marfona cultivar the highest value relates to microtubers >10mm with 3 months dormancy 51.11g. By studying weight of those minitubers gained

by microtubers <5mm was recorded with Agria cultivar and the highest functionality was 37.7g from microtubers with 3 months dormancy, while in Marfona the highest value belongs to microtubers with 5 months dormancy (Table 3).

In general, present results showed that size and dormancy of mother microtubers has significant influence on functionality of produced minitubers. Also in studied cultivars, microtubers with thick diameter which have passed more times in dormancy have better functionality than small microtubers with less time in dormancy.

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REFERENCES

1. FAO., 2007. International year of the potato 2008. www.potato2008.org.
2. Leclerc, Y., 1994. The production and utilization of potato microtubers. Ph.D. Thesis. McGill University. Canada.
3. Ahmed, A., S. Alam and V. Souza, 1995. Potato minituber production from nodal cuttings compared to whole *in vitro* plantlets using low volume media in a greenhouse. Potato Res., 38: 69-76.
4. Bolandi, A.R. and R. Zarghai, 2004. The study of effective factors on growth of buds and microtubers in potato *In vitro* condition. Agriculture Res., 4(2): 24-32.
5. Lakhoua, L. and O. Ellouze, 1993. Utilisation des microtuber cules produits *in vitro* pour la production de semences de pomme de terre (*Solanum tuberosum* L.). Le progres genetique et l'inventaire des genes: Ed. Aupelf-Uref, John Libbey Eurotext, Paris., pp: 233-236.
6. Pruski, K., T. Astatkie, P. Duplessis and P.C. Struik, 2003. Manipulation of microtubers for direct field utilization in seed production Amer. J. Potato Res., 80: 173-181.
7. Kawakami, J., T. Iwamak, Hasegawa and Y. Jitsuyama, 2003. Growth and yield of potato plants grown from microtubers in fields. American J. Potato Res., 80: 371-378.
8. Leclerc, Y., D.J. Donnelly, W.K. Coleman and R.R King, 1995. Microtuber dormancy in 3 potato cultivars. Am. Potato J., 72(4): 215-223.
9. Park, S.W., J. Heung, S.K. Hyun and J.H. Se, 2008. The effect of size and quality of potato microtubers on quality of seed potatoes in the cultivar 'Superior' Scientia Horticulturae. Article in Press.
10. Struik, P.S. and W.J. Lommen, 1999. Improving the field performance of micro- and minitubers. Potato Res., 42: 559-568.
11. Rolot, J., H. Seutin and D. Michelant, 2002. Production de minitubercules de pomme de terre par hydroponie. Biotechnol. Agron. Soc. Environ., 6(3): 155-161.
12. Donnelly, D.J., W.K. Coleman and S.E. Coleman, 2003. Potato microtuber production and performance. Am. J. Potato Res., 80(2): 103-115.
13. SAS (Statistical Analysis System), 1990. SAS user's guide. Version 6.0. cary, NC: Statistical Analysis System Institute.
14. Gopal, J., J.L. Minocha and J.S. Sidhu, 1997. Comparative performance of potato crops raised from microtubers induced in the dark versus microtubers induced in light. Potato Res., 40: 407-412.
15. Alsadon, A.A., K.W. Knutson and J.C. Wilkinson, 1988. Relationships between microtuber and minituber production and yield characteristics of six potato cultures. American Potato J., 65: 468.
16. Georgakis, D.N., D. Karafyllidis and N.I. Stavropoulos, 1997. Effect of planting density and size of potato seed minitubers on the size of the produced potato seed tubers. Acta Horticulture, 462: 935-942.
17. Mulet, D., 1991. Uutilisation des microtubercules. Resultats experimentations dans la Region Nord. La Pomme de Terre Francaise, 463: 72-79.
18. Ranalli, P., F. Bassi, G. Ruaro and G. Mandolino, 1994. Microtuber and minituber production and field performance compared with normal tubers. Potato Res., 37: 383-391.