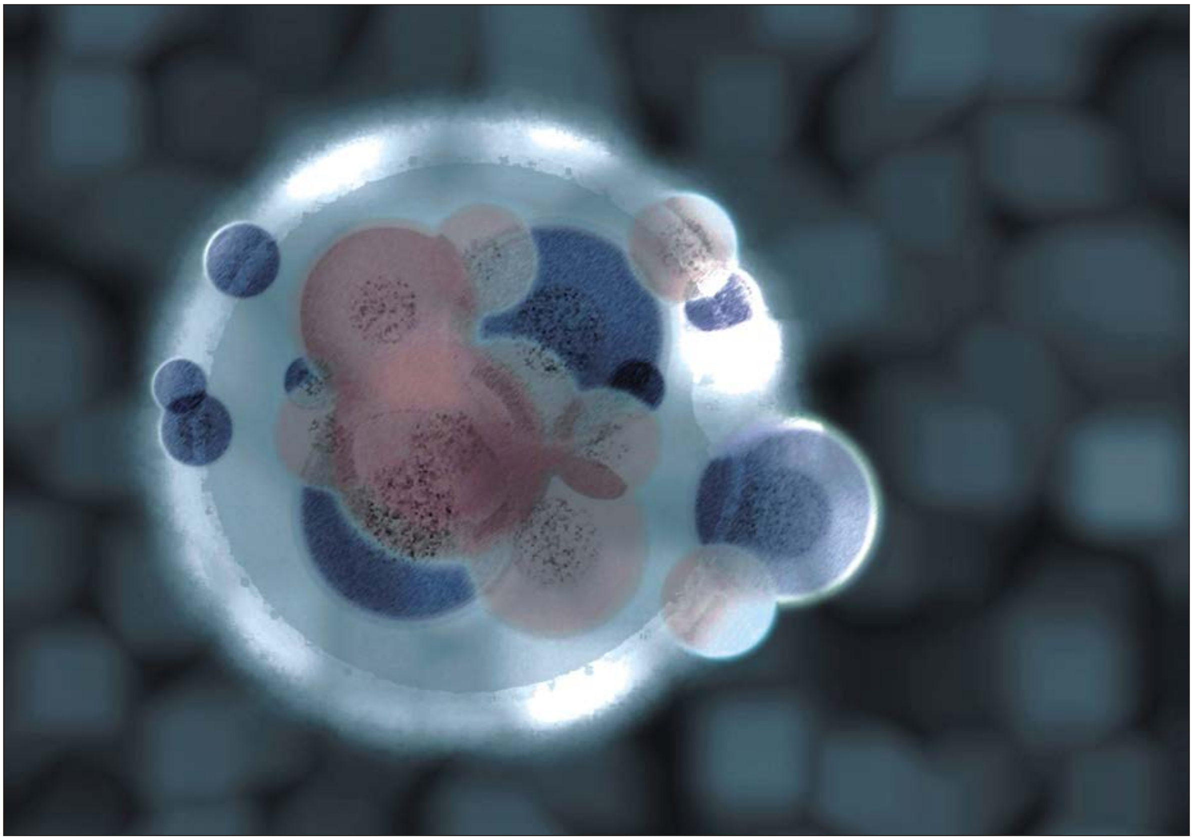

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Study the Plantlet Age Effect and Planting Beds on Agria Potato Mini-Tuber Production under *in vivo* Condition

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Abstract: This experiment was done about suitable composition of planting bed and plantlet age in greenhouse for increasing mini-tubers number and weight per plant. This research was conducted in Ardabil in 2007 and 2008. Plantlets of potato Agria cultivar were grown under *in vitro* condition. Then plantlets were transferred to greenhouse in four ages of plantlets (20, 30, 40 and 50 days) on a randomized complete blocks design with four replications in greenhouse. During growth period in greenhouse, were measured the traits as plant height, main stem per plant, mini-tuber number, weight and average weight per plant and uniformity per plantlets. The results of combine analysis of variance showed that there were significant differences between years as mini-tubers weight per plant, mini-tubers average weight per plant, between plantlet age as mini-tubers number and weight per plant, mini-tubers average weight per plant and stem number per plant. Interaction of years and plantlet age were significant on mini-tubers weight per plant. Plantlets, which transferred to greenhouse after 20 and 30 days, showed higher mini-tubers number and weight per plant in compare with other ages. There was positive significant correlation between mini-tubers number with mini-tubers weight per plant and mini-tubers average weight per plant. After, the better plantlet age selected in greenhouse and plantlets age of 30 days cultured in 12 different planting beds prepared from Iran and Finland reigns on the basis of ten replications completely randomized blocks. Statistical analysis showed that the maximum number and average weight of mini-tuber per plant produced by use of three planting beds of Iran (Ardabil Neogen soil and forest peat mass with large Zeolite) and Finland (Biolan peat mass with large Zeolite).

Key words: Plantlet age, planting bed, mini-tuber, potato, zeolite

INTRODUCTION

Potato (*Solanum tuberosum* L.) is grown and eaten in greater countries more than some other crops (Jackson, 1999). It is a crop that grows mainly in climates with cool temperate with full sunlight, moderate daily temperatures and cool nights. Short days generally induce tubers in potatoes, although many modern cultivars can initiate tuberization in the long days of north temperate regions (Tarn *et al*., 1992). Among the most important crops in the world (Fernie and Willmitzer, 2001) and Iran (FAO, 2008), potato is ranked in fourth grade in annual production after the cereal species rice, wheat and barley. Iran is the world's 12th potato producer and the third biggest producer in Asia, after China and India's mentioned above (FAO, 2008).

In compared with other species, potato is very sensitive to water-stress for that its shallower root system (Iwama and Yamaguchi, 2006). Potato is highly amenable to tissue culture, micro-propagation and micro-tuberization have become established methods of rapidly multiplying cultivars for seed production as well as for germplasm conservation (Gopal *et al*., 2005; Donnelly *et al*., 2003). The most effective of these techniques has also been studied to facilitate the selection for tuber characters including yield (Donnelly *et al*., 2003), maturity (Lentini and Earle, 1991) and disease resistance (Platt, 1992). *In vitro* propagation and micro-tuberization by nodal cuttings has become an established method of rapid multiplication in potato (Ranalli *et al*., 1994).

Mini-tubers are small seed potato tubers produced after acclimatization from plants propagated *in vitro* and planted at high density in the glasshouse in seed beds or in containers using different substrate mixtures. Mini-tubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting (Lommen, 1999; Ritter *et al*.,

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2001). The size of mini-tubers may range from 5-25 mm although in current systems larger mini-tubers also have become common. This size range coincides with a weight range of 0.1-10 g or more (Struik, 2007). The quality of the transplant is very important for the production of mini-tubers (the original *in vitro* plantlet) (Struik, 2007; Jami Moein *et al*., 2001).

There are many factors that affect the transferred plantlets under greenhouse conditions, like planting bed, plantlet age and illumination etc., among them planting-beds are of considerable importance (Jami Moeini *et al*., 2001).

Ahmed *et al.* (1995) compared the produced mini-tubers from rooted plantlets that are free of virus with those that produced 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. Belletti *et al.* (1990) studied the day length and temperature on *in vitro* mini-tuber initiation and resulted that, day length increases mini-tuber weight and low temperatures increase mini-tuber number. Ahmad and Alam (1995) comprised mini-tuber productive potential of different plantlets that are free of virus which produced via *in vitro*, multi node scions and single node plantlets and resulted that rooted plantlets and multi node scions produced mini-tubers are bigger than single node plantlets. Roots of *in vitro* produced plantlets were vulnerable and damaged rapidly after transferring to *in vivo* condition and must replanted. Undeveloped root system encounters with problems *in vivo* condition, especially in high rate transpiration condition (Bagheri and Saffari, 1997).

Zeolite is an inorganic porous material that has a highly regular structure of pores and chambers that allows some molecules to pass through and causes others to be either excluded or broken down. Zeolites have many useful purposes. They can perform ion exchange, filtering, odor removal, chemical sieve and gas absorption tasks. The most well known use for Zeolites is in water filtration applications (Anonymous, 2008). Pumice is opportune in rooting media if is mixed with either media of plant culture or alone to aerate and drain.

Obradovic and Sukha (1993) showed that planting bed with 80% of vermiculite and 20% of sand was found to be best for plantlet growth that resulted in the production of many mini-tubers in the greenhouse.

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. 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.

Tukaki and Mahler (1989) reported the advantage of vermiculite/s and 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.

Solis (1998) resulted that the best soil mixture is two parts of forest litter and one part of soil to produce mini-tuber.

Modarres Sanavy and Jami Moeini (2003) resulted the best mixture is peat mass and sand with 4:1 ratio for plantlets growth.

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.

Ozkaynak and Samanci (2005) results the percentage of >4 g tuber weight was obtained for approximately 80% in greenhouse and field plantlets and between 45 to 55% in seed bed plantlets.

Fazeli *et al.* (2007) studied four planting beds [peat mass: perlite (3:1), turf: perlite (3:1), (c) leaf mould: perlite (3:1) and rice hull: turf (1:1)] and results that rice hull: turf (1:1) was superior to other beds.

Vanaei *et al.* (2008) studied substrate combination including three planting bed soil, sand with perlite (1:1:1), turb with perlite (1:1) and collected from 20-25 days old greenhouse grown plants turb/rice hull with perlite (1:1:1) and results turb with perlite in large pots has the highest value for minituber total weight (95 g per plant) and number (15 mini-tuber per plant).

This experiment was conducted to select the suitable plantlets age and planting bed to produce potato mini-tubers.

MATERIALS AND METHODS

Plantlet age: This study was conducted in biotechnology laboratory and greenhouse of Villkej Company in Ardabil Province, Iran in 2007 and 2008. Plantlet of Agria was raised using single node cuttings under optimum light intensity and temperature for two months. Then, produced plantlets of meristem culture of Agria cultivar propagated by single node cuttings. Produced plantlets were planted in four plantlet ages (transferring of plantlets after 20, 30, 40 and 50 days to greenhouse) as a randomized complete blocks experimental design with four replications in greenhouse and in pots which they included peat mass in 2007 and 2008. Ten plantlets of Agria cultivar with each age in each replication cultured in planting bed in a greenhouse. The plantlets were planted with 10 cm distances between rows and 10 cm between plantlets. The plantlets were irrigated after planting by normal water. Macro and micro nutrients were used to provide nutrition for plantlets. All of practices such as irrigation and control of weeds, pests and diseases were done regularly during growth period. Control the pests and fungus diseases were done, respectively by use of 250 cc ha^{-1} Confidor and 400 g ha^{-1} Equation-pro. Mini-tubers harvested after two months. The traits such as mini-tuber number, weight and average per plant, main stem number per plant and plant height were measured.

Planting bed: After this, the suitable plantlet age selected in greenhouse. The effects of 12 planting beds were studied on plantlet growth in the greenhouse. Planting beds were prepared using Ardabil forest peat mass with punce, large Zeolite and fine Zeolite; Finland Biolan peat mass with punce, large Zeolite and fine Zeolite; Ardabil Neogen soil with large Zeolite; Latrit soil with large Zeolite; Morass soil with Vermicompost; Vermicompost; Ardabil Aladezga peat mass with large Zeolite and Meadowy soil with large Zeolite from Iran and Finland reigns on the basis of ten replications completely randomized blocks. Plastic pots of 15 cm diameter were used. One plantlet was transferred to each pot. Observations were recorded after two months for mini-tuber number and average weight per plant. We prepared these planting beds in a 1:1 voluminal form. The traits of mini-tuber number and average weight per plantlet were measured. All of plantlets planted into the pots and then irrigated water. Macro and micro nutrients were used for nutrition of potato plantlets. Analysis of variance and the means comparisons with LSD test was done by MSTATC software. Linear correlation coefficients between different traits were done by SPSS software. Cluster analysis was done for the mini-tuber number and average weight per plant by use Ward method.

RESULTS AND DISCUSSION

Effects of plantlet age on mini-tuber production: Combined ANOVA plantlets age showed that there were significant differences among years as mini-tubers weight per plant, mini-tubers average weight per plant, between plantlet age as mini-tubers number and weight per plant, mini-tubers average weight per plant and main stem number per plant and among years and plantlet age interaction of the mini-tubers weight per plant (Table 1).

Plantlets which transferred to greenhouse after 20 and 30 days showed higher mini-tubers number and weight per plant in compare with other ages (Table 2).

The highest mini-tubers weight per plant produced in 20 and 30 days plantlet age in 2008. In both years, 20 and 30 days plantlet age produced higher mini-tubers number and weight per plant (Table 3).

Table 1: Attributes combined ANOVA of Agria cultivar plantlets different ages

		MS				
SOV	df	Mini-tuber No.	Mini-tuber weight	Average mini-tuber weight	Plant height	Main stem No.
Year (Y)	1	0.050	33.13**	23.76**	0.33	0.001
Ea	6	0.130	0.28	0.84	18.89	0.038
Plantlets Age (PA)	3	1.340**	28.81**	1.63^{+}	5.52	0.101*
Y×PA	3	0.081	4.38*	1.37^{+}	6.93	0.001
Eb	18	0.059	0.88	0.99	7.30	0.038
CV%		13.430	10.52	19.56	10.51	18.440

+, * and **: Significant at 10, 5 and 1% level of probability, respectively

Table 2: Attributes mean in plantlets different ages

Plantlets age (day)	Mini-tuber No.	Mini-tuber weight (g)	Average mini-tuber weight (g)	Plant height (cm)	Main stem No.
20	2.18a	10.51a	4.99ab	25.29a	1.00b
30	2.16a	10.48a	5.02ab	26.50a	1.23a
40	1.44b	8.030b	5.710a	26.30a	1.00b
50	1.47b	6.670c	4.62ab	24.75a	1.00b

Mean values with the same letters in each column does not have significant difference at the 5% level of probability to according to value of LSD

Table 3: Attributes mean of Agria cultivar plantlets different ages in two years

Year	Plantlets age (day)	Mini-tuber No.	Mini-tuber weight (g)	Average mini-tuber weight (g)	Plant height (cm)	Main stem No.
2007	20	2.35ab	8.41de	3.650d	24.83a	1.00a
	30	2.40a	9.88bc	4.210d	27.25a	1.20a
	40	1.50d	7.13ef	4.76cd	26.85a	1.00a
	50	1.50d	6.17f	4.280d	23.50a	1.00a
2008	20	2.00bc	12.60a	6.340ab	25.75a	1.00a
	30	1.92c	11.10b	5.82abc	25.75a	1.25a
	40	1.39d	8.92cd	6.6005a	25.75a	1.00a
	50	1.44d	7.15ef	4.97bcd	26.00a	1.00a

Mean values with the same letters in each column does not have significant difference at the 5% level of probability to according to value of LSD

Table 4: Correlation between attributes in plantlets different ages

Correlation coefficient	Mini-tuber No.	Mini-tuber weight	Mean mini-tuber weight	Plant height	Main stem No.
Mini-tuber No.	-				
Mini-tuber weight	0.95**	-			
Mean mini-tuber weight	0.91**	0.95**	-		
Plant height	0.23	0.46	0.26	-	
Main stem No.	0.56	0.55	0.27	0.63	-

**: Significant at probability levels of 1%

Table 5: Mean comparison of attributes in different planting beds

	Planting bed											
	Ardabil forest peat mass with			Finland Biolan peat mass with								
Attributes	Punce	Large Zeolite	Fine Zeolite	Punce	Large Zeolite	Fine Zeolite	Neogen soil with large Zeolite	Latrit soil with large Zeolite	Morass soil with Vermicompost	Meadowy soil with large Zeolite	Ardabil Aladezga peat mass with large Zeolite	Vermicompost
Minituber No.	2.7cd	3.8a	3.6ab	2.9bcd	3.9a	3.6ab	4.0a	3.5abc	2.5d	3.2abcd	2.5d	2.8cd
Minituber average weight	6.5c	7.2b	7.0b	6.5c	7.5a	7.0b	7.5a	5.5d	5.0e	6.5c	5.5d	7.5a

Mean values with the same letters in each column does not have significant difference at the 5% level of probability to according to value of LSD

Table 6: Clusters of planting beds for mini-tuber number and average weight per plant

Clusters	Planting beds	Minituber No. per plant
First	1. Ardabil Neogen soil with large Zeolite	4.0
	2. Finland Biolan peat mass with large Zeolite	3.9
	4. Ardabil Forest peat mass with fine Zeolite	3.6
	5. Finland Biolan peat mass with fine Zeolite	3.6
	3. Ardabil Forest peat mass with large Zeolite	3.8
Second	7. Finland Biolan peat mass with Punce	2.9
	8. Ardabil Forest peat mass with Punce	2.7
	11. Ardabil Aladezga Meadowy soil with large Zeolite	2.5
	12. Vermicompost	2.8
Third	9. Morass soil with Vermicompost	3.2
	10. Ardabil Aladezga peat mass with large Zeolite	2.5
	6. Latrit soil with large Zeolite	3.5

There was positive significant correlation between mini-tubers number with mini-tubers weight per plant and mini-tubers average weight per plant (Table 4).

Roots of *in vitro* produced plantlets were vulnerable and damaged rapidly after transferring to *in vivo* condition and must replanted. Undeveloped root system encounters a problem *in vivo*, especially under high rate transpiration condition. Restricted water loss is an important note mean while transferring plantlets from *in vitro* to *in vivo* conditions. Root primary cells must develop well *in vitro*, which lead to be better the growth of deep roots *in vivo*. Weak vascular relation between root and stem decreased water translocation. Hetero-trophism is another important note for *in vitro* plantlets. They must change to auto-troph condition and do photosynthesis (Bagheri and Saffari, 1997).

Effects of planting bed on mini-tuber production: Results of planting beds showed that differences among different planting beds were significant for the mean of number and average weight of mini-tuber per plantlet.

The most highest mini-tuber number and average weight per plant produced in Ardabil Neogen soil, Finland biolan peat mass and Ardabil forest peat mass with large Zeolite; Ardabil forest peat mass and Finland biolan peat mass with fine Zeolite and the lowest in Ardabil Aladezga peat mass and Ardabil Aladezga Meadowy soil with large Zeolite and vermicompost (Table 5).

There was positive significant correlation between mini-tubers number with mini-tubers weight per plant and mini-tubers average weight per plant (r = 0.64*). This result is according to the result that found Vanai *et al.* (2008).

Results of cluster analysis showed that planting beds grouped in three clusters. The first cluster included planting beds of Ardabil Neogen soil with large Zeolite, Finland Biolan peat mass with large Zeolite, Ardabil forest peat mass with fine Zeolite, Finland Biolan peat mass with fine Zeolite and Ardabil forest peat mass with large Zeolite, the second cluster planting beds of Finland Biolan peat mass with punce, Ardabil forest peat mass with punce, Ardabil Aladezga Meadowy soil with large Zeolite and vermicompost and the third cluster planting beds of morass soil with vermicompost, Ardabil Aladezga peat mass with large Zeolite and latrit soil with large Zeolite. The highest mini-tuber number and average weight per plant related to first cluster (Table 6).

Table 7: Chemical analysis of Zeolite (natural Clinoptilolite) (% w/w)

SiO_2	Al_2O_3	TiO_2	Fe_2O_3	MgO	CaO	Na_2O	K_2O	H_2O
70.08	11.72	0.14	0.67	0.71	3.18	0.55	3.50	9.45

Table 8: Analysis of planting beds selected

Planting bed	Fe (ppm)	Zn (ppm)	K (ppm)	P (ppm)	N (%)	OC (%)	TNV (%)	Mg (ppm)	SP (%)	pH	EC (dS m^{-1})
Ardabil Forest	27.8	38.9	252	32.87	0.48	5.10	2.13	72.0	86	6.33	1.58
Finland Biolan	-	-	880	91.89	0.71	7.11	4.00	62.4	17	5.98	1.79

Planting bed had great effects on mini-tuber production and a significant effect in traits was observed. In this research planting bed of Ardabil Neogen and forest peat mass and Finland Biolan with large and fine Zeolite increased to 3.6-4.0 numbers. Chemical analysis of Zeolite and selected planting beds have shown in Table 7 and 8.

Tukaki and Mahler (1989) reported that a potting-mixture of 80% vermiculite and 20% silica sands led to the maximum tuber yield under green-house conditions. Obradovic and Sukha (1993) showed that planting bed with 80% of vermiculite and 20% of sand was found to be best for plantlet growth that resulted in the production of many mini-tubers in the greenhouse. Solis (1998) resulted that the best soil mixture is two parts of forest litter and one part of soil to produce mini-tuber. Modarres Sanavy and Jami Moeini (2003) results a mixture of peat mass and sand in the ratio of 4:1 proved best for growing plantlets. 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. Silva *et al.* (2006) shown turb with perlite in large pots has the highest value for total weight and number of mini-tuber. Fazeli *et al.* (2007) resulted that rice hull: turf (1:1) was superior to other beds. Vanaei *et al.* (2008) results turb with perlite in large pots has the highest value for total weight and number of mini-tuber. The substrates with soil combination are not suggested for mini-tuber production because present results showed that they have the lowest value for mini-tuber number and total weight per plant (Vanaei *et al.*, 2008).

Ardabil Province (in Iran) with about 28,000 ha under cultivation area and production over 800,000 ton potato and pay attention to weather conditions, it is one of the potential and suitable areas for cultivation of this crop. We need to know about suitable composition of planting beds and plantlets age in the greenhouse for increasing in the number and weight of produced mini-tubers per plant. The number of produced mini-tubers is about 1-2 in Ardabil. However, in this research use of plantlets age of 20-30 days increased mini-tuber number to 2.20 and planting bed of Ardabil Neogen and forest peat mass and Finland Biolan with large and fine Zeolite increased to 3.6-4.0 numbers. Thus we would like to apply this result for improvement of mini-tuber production that will be lead to decrease in production cost, until farmers could buy cheaper mini-tubers. The price of one mini-tuber is about 0.25$ in Iran. In this study increased the number of mini-tubers per plant to 4 numbers. Therefore about 115$ increased for production of mini-tubers per square meter. We would like to apply results of this research for increasing production of mini-tuber per plant, so decrease in production cost until farmers could buy cheaper mini-tubers.

CONCLUSION

In regard to above mentioned subject, transplant age in 20 and 30 days was suitable for higher root initiation and plantlet organs firmness for complete establishment and increase mini-tubers number per plant in greenhouse.

In this research plantlets age use of 20-30 days increased mini-tuber number to 2.20 and planting bed of Ardabil Neogen and forest peat mass and Finland Biolan with large and fine Zeolite increased to 3.6-4.0 numbers.

We resulted that planting bed of Ardabil forest peat mass use is better than Biolan (originated from Finland) because Ardabil forest peat mass is local. So, the Zeolit use is better than Punce. Furthermore we can produce potato mini-tubers seed into our country by lower costs and independent.

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