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Evaluation of potato mini-tubers dormancy breaking affected by various chemicals, genotype and mini-tuber Size

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Abstract

This work was done in order to evaluation the effect of gibberellic acid to potato mini-tubers dormancy breaking at Seed and Plant Certification and Registration Institute of Karaj (SPCRI) in form of factorial based on randomized completely design in three replication at 2011. Experimental factors were considered chemicals as the first factor in four levels (control, 50 g/lit gibberellic acid, 25 mlit/m^3 carbon disulfide, 3% thiourea), potato cultivars as the second factor in two levels (agria and born) and potato mini-tuber size as the third one in four levels (12-20 millimeter, 20-25 millimeter, 25-30 millimeter and 30-35 millimeter). In this experiment characteristics such as sprout length, days to sprouting and days to breaking dormancy were studied. The results of this experiment showed that the main and interactions effects of three factors were affected statistically significant on days to sprouting with probability level of 99%. Sprout length affected by main and interaction effects of experimental factors too. Also dormancy period length showed significant differences about application of chemicals treatments, different seed cultivars and different sizes of potatoes seed noted in 99% probability level. In general it can be stated that application of gibberellic acid left greatest effects among the used chemicals. Also Dormancy period length had greater reduction than other chemicals by application of gibberellic acid. Difference seed cultivars and sizes showed significant differences in their response due to genetic and physiological characteristics. In fact, based on these test's results can be declared that application of gibberellic acid lead to reduce the time between planting and germination of tubers, to increase sprout length and to reduce the potato seed dormancy period length.

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Introduction

Potato crop is very sensitive to plant diseases including the viruses which lead to tuber yield reduction due to vegetative propagation. One way in which this problem can be overcome by is to use healthy seed. The appropriate size of seed tubers is 50-60 grams tuber weight. Mini-tubers are the small potatoes tubers which generated by the seedlings that have been reproduced in vitro in the greenhouse (Emilson 1999). Mini-tubers can be used to pre-basic and basic seed production by direct sowing in the field (Anonymous 2005). If planting is possible only once a year on the farm, mini-tubers should be kept in the garner. Mini-tuber dormancy period is long and usually between 2 to 4 months (Rezaei and Soltani, 1997). Therefore should be kept longer in the garner. But in the case of excessive storage, they will produce more stems which are weak and cannot survive. It is relatively easy and approved to maintenance of mini-tubers for 200 days or more. To be much less Mini-tubers weight lead to longer dormancy period. Mini-tubers obtained from the first harvest have longer dormancy period than the next harvest one. Until the normal dormancy breaking of Mini-tubers should be kept in the garner. But if maintenance done in the garner by very low temperatures for a long time (about 300 days), they will have very low vigor. It must be understood that the mini-tubers have longer dormancy period than macro-tubers (Mirlohi and Khayyam Nekouee, 2005; Lommen and Struik, 1992a).

In Iran, a large volume of mini-tubers are harvested between March and April. Mini- tubers normal planting date is in the field or in greenhouses is in May or early June. So to break dormancy naturally, there is not enough time between harvest and planting. This requires a reliable chemical method to break the dormancy of these small tubers.

Thiourea is a catalyse inhibitor, which triggers potato tubers germination and healing tubers injuries. Thiourea solution in an appropriate concentrate not only facilitates germination, but also produces more than one sprout in each eyes of potato, so that, thiourea dominates over inhibiting effects of major sprout on minor ones in each eye, and neutralizes terminal buds capacity to stop lower buds growth in seeding tuber. It is reported that using thiourea treatment and/or applying H2O2 enables one to remove tubers dormancy (Bajji *et al.*,2007).

Generally on a commercial scale, to break seed dormancy of potato tubers, materials are used such as rindite (Rehman *et al.*, 2001), bromoethane (Coleman 1983; Rezaee and Soltani, 1997), CS_2 (Meijers 1972; inquiring 2002), and GA_3 (Alexopoulos *et al.*, 2008; Rezaee and Soltani, 1997).

Gibberellins are able to breaking dormancy in potato tubers (Herrera *et al.*, 1991). Application of gibberellic acid on potato, either by dipping or by spraying the tuber cause to break dormancy and increasing and elongation sprouts in potatoes (Dyson, 1965; Lorreta *et al.*, 1995; Marinus and Bodleander, 1987; Rappaport *et al.*, 1957). By use of gibberellic acid in potato greater number of buds per unit area occur because gibberellin with reduced apical dominance, increase number of stems or Stolones and thereby creates greater number of tuber (Mikitzel, 1993; Salcow, 1991).

Also, gibberellic acid hormone in a potato plant decreases tuber weight (Gronzal, 1988). Gibberellins increase sprouting potatoes tubers.

Potato tubers contain gibberellin-like substances (Okazawa, 1959). Gibberellin levels vary in different organs during development stage. Generally in the rapid growth stage, gibberellin activity is at the highest level and tuber during dormancy period is in the least amounts (Mikitzel, 1993). Gibberellic acid hormone by to overcome the apical dominance and increasing the number of sprouts consequently lead to increasing the number of produced tubers stolones into smaller. In the research took placed in the Netherlands on seed potato varieties Alpha, Spunta Jaerla and Ostar, gibberellic acid at concentrations of 5 and 10 ppm used on the plant foliage at different stages of plant growth leading to elongation of stem

and stolones and reducing tuber produce before beginning the producing, but this hormone increased tubers by 27 to 45 mm sizes(Bodleander and Waderzag, 1989). Sharma *et al* (1998) with gibberellic acid spraying before potato plant in full bloom founded that GA treatment increases stolones and stems growth and dry matter.

Additionally, some potato varieties are react strongly against gibberellic acid treatment. Also treating mini-tubers with gibberellic acid cause to sprouts increasingly elongation which lead to creating thin, weak and breakable sprouts during the research (Suttle 2008; Salimi *et al*., 2010).

The experiment was conducted to select the best chemicals and minituber size to decrease the Dormancy period length .

Materials and methods

This study was done in the greenhouse of SPCR Institute (Seed and Plant Certification and Registration research Institute) (35 degrees 45 minutes north latitude - 51 degrees, 6 east longitude and 1313 m above sea level) at 2010-2011. This research carried out in form of factorial based on randomized completely design in three replication. Experimental factors were considered chemicals as the first factor in four levels (control, 50 g/lit gibberellic acid, 25 mlit/m^3 carbon disulfide, 3% thiourea), potato cultivars as the second factor in two levels (agria and born) and potato mini-tuber size as the third one in four levels (12-20 millimeter, 20-25 millimeter, 25-30 millimeter and 30-35 millimeter).

In Vitro Seedlings Production

Virus-free seedlings were produced using combination method of heat treatment and meristem isolated and cultured in MS liquid medium onto paper bridges and then transferred cultures in suitable growth condition in the growth chamber. Obtained seedlings using single-node cuttings on solidified MS medium multiply with agar and were transmitted to a growth chamber with a temperature of 24 degrees Celsius, light for 16 hours and light intensity of 4500 lux and were kept there about 4 weeks to grow and become the new seedlings (Hassanpanah *et al*, 2006).

Minituber Production in Greenhouse

After obtaining the required number of seedlings from each cultivar, seedlings 25-30-day lifetime having 7 to 9 leaves were selected for transfer to greenhouse. In the greenhouse, seedlings were brought out of the pots and the roots were washed with water to remove medium residual matter. Cleaned seedlings transferred carefully into the bed including a mixture of soil disinfected by fungicides and insecticides (Sevyn and captain) and perlite and peat moss in the ratio of 1: 2: 1 and for a few days plastic cap was placed on them to adapting seedlings with greenhouse environment and preventing damage to seedlings. Seedlings were growth in growth chamber with day and night temperatures of 18 and 12 ° Celsius respectively, 12 hours day length and 85% relative humidity. After passing about 100 days, the mini-tubers were harvested and transferred to laboratory for sprouting (Garmchy, 2010).

After selecting mini-tubers but prior the applying chemicals and Growth-stimulating hormone mini-tubers were washed with water and placed in a tray.

Gibberellic Acid Treatment

Sample containing 240 mini-tuber of each cultivar were immersed for three hours by 50 ppm gibberellic acid treatment solution in groups (12-20 millimeter, 20-25 millimeter, 25-30 millimeter and 30-35 millimeter) and then samples were placed into plastic tray with room temperature for germination.

Thiourea Treatment

Sample containing 240 mini-tuber of each cultivar were immersed by thiourea 3% solution for one hour in groups (12-20 millimeter, 20-25 millimeter, 25-30 millimeter and 30-35 millimeter) and then samples were placed into plastic tray in garner with 90 relative humidity, absolute darkness and temperature of 18 ° C for germination.

Carbon Disulfide Treatment

For the carbon disulphide, minitubers were put in 15×19×32 plastic containers with tightly fitting lids at room temperature for 72 h. Sufficient CS2 was supplied in liquid form in 25 ml beakers to give the required concentration in the container volume. 60 micro-tubers from each cultivar was placed immediately after harvest and washing with distilled water in standard storage conditions as a control treatment without the application of hormones and chemicals.

The time interval between the applications of chemicals till to sprout emergence in micro-tubers was recorded. Emersion of sprout about 2 mm from tubers is an appropriate criterion for dormancy period ending and whenever 80 percent of mini-tubers show that symptoms it consider as dormancy break time. During the experiment, tubers having sprout were counted and were separated every 10 days. Average of 2 mm sprouts of each micro-tuber was recorded one week after the end of dormancy.

Statistical Analysis

To analyze this study's obtained data applied ANOVA analysis procedure of SAS software. For downing the mean comparisons LSD test was used by a probability level of 5%.

Results and discussion

Days to Sprouting

According to Table 1 results of analysis of variance laboratory traits shows that all of experimental factors main and interaction effects was significant (P<0/01) on days to sprout emergence. But tripartite effect did not the same significant differences. The highest days to sprouting were observed by average of 25/24 days in control and the lowest days obtained in gibberellic acid treatment by average of 13/99 days. Based on achieved results application of all three growth regulators hormones used in this experiment, reduces the number of days until the appearance of the sprout. It seems that application of growth regulators and gibberellic acid most of all, causes to postponement of sprouting which is in consistent by Helsinki *et al* (2002) and Bamberg, (1999) results.

Table 1. Analysis of variance for laboratories traits.

SOV	Degree of Freedom	Days to Sprouting	Sprout Length	Days to Dormancy Breaking
Growth stimulants (A)	3	519.29**	20.49**	2220.59**
Variety (B)	1	37.76**	2.29**	319.01**
Mini-tuber size (C)	3	18.08**	2.92**	204.84**
A * B	3	35.83**	1.12**	26.68*
A * C	9	2.20**	0.63**	37.51*
B * C	3	8.65**	1.55**	20.98 ns
A * B * C	9	1.08 ns	1.25**	14.50 ns
Error	62	0.69	0.206	9.33
CV	-------	4.35	13.51	7.53

*, ** and ns: Significant at the 5% and 1% level of probability and non-significant, respectively.

It is worth noting that the number of days to sprouting showed different results in two varieties that could be stated due to different physiological characteristics of different varieties.

It was also observed that the number of days to sprouting, depending on seed size showed different results. So that just 30 to 35 mm with an average of 17/81 days significantly affected the number of days to sprouting compare to the other sizes. Results of Khorshidi and Hassanpanah (2009) also showed that increasing the seed size increases monotonically on the size of the sprout, but it can be linked to the most

vigorous seeds of larger seed for creating bigger sprouts.

Sprout Length

The results (Table 1) showed that the application of growth regulators and seed tubers varieties and different sizes have a significant effect on the mean of sprout length.

Table 2. Mean comparison of main effects.

Experimental Treatment		Days to Sprouting(day)		Sprout Length (mm)		Days to Dormancy Breaking (day)	
Growth stimulants (A)	Control (wash by water) (a_1)	25.24	d	2.46	d	54.75	c
	gibberellic acid (a_2)	13.99	a	4.62	a	33.50	a
	carbon disulfide (a_3)	18.04	b	2.97	c	37.58	b
	Thiourea (a_4)	19.13	c	3.37	b	36.75	b
Variety (B)	agria (b_1)	19.73	b	3.51	a	36.85	b
	born (b_2)	18.47	a	3.20	b	33.23	a
Minituber size (C)	12-20 (c_1)	19.60	b	3.04	c	45.54	b
	20-25 (c_2)	19.36	b	3.19	bc	42.54	b
	25-30 (c_3)	19.62	b	3.35	b	38.45	a
	30-35 (c_4)	17.81	a	3.85	a	37.66	a

Mean in each column, followed by similar letter (s) not significantly different at 5% probability level, using LSD test.

The results of this experiment can be stated that the use of chemicals used in these experiments was increased sprout length of potato. In this study, compare to control chemicals treatments increased sprout length, that among the all, highest rates in sprout length belongs to the application of gibberellic acid. Also according to reports (Khorshidi and Hassanpanah, 2009) sprout length in the case of non-Application gibberellic acid showed a significant increase compare to the application case.

The results of these experiments is consistent with the results of the Lommen and Struik (Lommen and Struik, 1992a).

It should be noted that the average length of the sprout in Agria cultivar 3/51 was higher than the born cultivar which were also observed in Aung and Peterson experiments (Aung and Peterson, 1974). These differences can occur due to various physiological habits.

Also difference in seed size as well causes a difference in the length of the sprout. So that the amount of sprout increased whatever as well as seed tuber size increased. This indicates the importance of nutrient available storage in the seed tubers. Results of this experiment corresponded with the results of Hassanpanah *et al* (2008).

Days to Dormancy Breaking

Achieved results of these experiments revealed that the application of plant growth regulator hormones causes significant differences in the number of days to break dormancy in compare to control. It is worth noting that the difference created by the gibberellic acid in turn was different from the other two hormones. The lowest Day to break dormancy was observed in gibberellic acid hormone application by the rate of 33/50 days which in comparison to highest rate observed in control plots (54/75), the difference is significant and unavoidably.

Dogonadze and *et al* (2000) reported that with respect to that in addition abscisic acid another ingredients involved in tuber natural dormancy (Lecrec *et al*., 1995), application of gibberellic acid on dormant tubers can reduce endogenous abscisic acid of tubers.

The reports also state that gibberellic acid causes breakdown of starches and accumulation of renewable sugars in potato tubers which this issue that can stimulates the germination and consequently

the dormancy breaking (Hemberg, 1985; Alexopoulos *et al.*, 2007).

Some other reports has been stated that dipping the seeds with gibberellic acid before planting for anhour, will break seed dormancy of the potato (Rahman *et al.*, 2002).

Table 3. Mean comparison of interaction effect.

Experimental Treatment		Days to Sprouting (day)		Sprout Length (mm)		Days to Dormancy Breaking (day)	
Control (wash by water) (a_1)	agria (b_1)	25.38	a	2.48	a	57.50	b
	born (b_2)	25.11	a	2.44	a	52.00	a
gibberellic acid (a_2)	agria (b_1)	16.42	b	4.55	b	36.00	b
	born (b_2)	11.56	a	4.69	a	31.00	a
carbon disulfide (a_3)	agria (b_1)	18.27	a	3.23	a	38.00	a
	born (b_2)	17.82	a	2.70	b	34.75	a
Thiourea (a_4)	agria (b_1)	18.85	a	3.78	a	37.17	a
	born (b_2)	19.41	a	2.97	b	38.00	a
Control (wash by water) (a_1)	12-20 (c_1)	25.53	b	2.21	a	59.67	c
	20-25 (c_2)	25.20	b	2.58	a	57.67	bc
	25-30 (c_3)	26.42	b	2.62	a	53.33	b
	30-35 (c_4)	23.82	a	2.43	a	48.33	a
gibberellic acid (a_2)	12-20 (c_1)	15.23	c	4.52	b	39.33	b
	20-25 (c_2)	14.52	bc	4.18	b	35.33	b
	25-30 (c_3)	13.47	ab	4.57	b	28.83	a
	30-35 (c_4)	12.76	a	5.23	a	30.50	a
carbon disulfide (a_3)	12-20 (c_1)	18.29	b	2.79	b	37.33	a
	20-25 (c_2)	18.45	b	2.72	b	39.83	a
	25-30 (c_3)	19.15	b	2.98	ab	37.17	a
	30-35 (c_4)	16.30	a	3.38	a	36.00	a
Thiourea (a_4)	12-20 (c_1)	19.38	a	2.66	c	37.83	a
	20-25 (c_2)	19.28	a	3.28	b	37.33	a
	25-30 (c_3)	19.48	a	3.22	b	34.50	a
	30-35 (c_4)	18.37	a	4.34	a	35.83	a
agria (b_1)	12-20 (c_1)	19.65	ab	2.97	b	45.42	a
	20-25 (c_2)	19.62	a	3.42	b	43.42	a
	25-30 (c_3)	20.44	b	3.83	a	39.92	a
	30-35 (c_4)	19.20	a	3.83	a	40.75	a
born (b_2)	12-20 (c_1)	19.56	b	3.12	b	41.67	a
	20-25 (c_2)	19.09	b	2.96	b	41.67	a
	25-30 (c_3)	18.81	b	2.86	b	37.00	a
	30-35 (c_4)	16.42	a	3.86	a	34.58	a

Mean in each column, followed by similar letter (s) not significantly different at 5% probability level, using LSD test.

However, we have to consider the fact that excessive use of gibberellic acid causes disorders such as stem elongation (Rezaei and Soltani, 1997; Mirlohi and Khayyam Nekouei, 2005), excessive growth of the shoots, tuber deformities (Mirlohi and the hearts of Omar Khayyam, 1383), delay in tuber angiogenesis and reduction in root formation.

Days to break dormancy in Agria was lower than the result born cultivar showed. Differences in this characteristics have been observed in different varieties in the other experiments (Bamberg, 1999). In Salimi *et al* (2011) experiments also born and Agria cultivar achieved longest and shortest dormancy period.

Mean comparison table shows that the average dormancy duration inversely with the amount of seed size. This means that whatever size of the tuber has grown bigger dormancy period fell by. It can be said

that this is probably due to the larger potato seeds contain higher levels of dormancy inhibitors. Results of this study were similar to results reported in earlier studies (Salchow, 1991).

In brief, if the intention is cultivation of potato mini-tubers, GA3 application at 50 ppm is the most suitable concentration for dormancy alleviation.

Appreciation

It is necessary to appreciation from leaders in Seed and Plant Certification and Registration research Institute of Karaj and also agronomy department of Karaj Islamic Azad University Faculty Agriculture and Natural Resources which help us to carrying out this experiment.

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