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Evaluation of Potato Advanced Cultivars Against Water Deficit Stress Under *in vitro* and *in vivo* Condition

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Abstract: This experiment performed according to the factorial design and on the base of completely randomized design in three replications in 2007. Factor A included four levels of osmotic pressure (0, -1, -2 and -3 bar) and factor B included seven advanced potato cultivars (Agria, Savalan (397007-9), Satina, Caesar, Kennebec, Marfona and Sante). The PEG 6000 was used for exerting the water deficiency stress on the plantlets. The attributes such as germinating ability and root producing was measured. In this stage the cultivar that produced the roots, had moved to the greenhouse. The plantlets were planted in soil bed with mixed of Punce and Biolan with 1:1 v/v. The applied experimental design was factorial on the base of completely randomized design in three replications. Factor A has four levels of osmotic pressure and factor B included seven advanced potato cultivars. After two months, was harvested the mini-tubers and the attributes was measured such as mini-tuber average size, mini-tubers weight and number per plant. The variance analysis results showed that significant difference between osmotic pressure levels, cultivars and their interaction as attributes mini-tubers number and weight per plant and mini-tubers average size in 1% probability. The Caesar and Savalan cultivars had the most number and weight of mini-tuber per plant in -1 bar and Caesar and Kennebec cultivars in -2 bar osmotic pressures. The MP, GMP, STI and MSTI selected Caesar as better cultivar in normal and stress conditions. Results of cluster analysis showed that cultivars grouped in three clusters. The first cluster included Agria, Sante and Marfona (susceptible cultivars), the second cluster Satina, Savalan and Kennebec (moderately tolerance cultivars) and the third cluster Caesar (tolerance cultivar).

Key words: PEG, water stress, potato, mini-tuber, *in vitr*, *in vivo*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is grown and eaten in many countries more than some other crops (Stephen, 1999). It is a crop that grows mainly in climates with cool temperate and 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 regions temperate (Tarn *et al*., 1992). Among the most important crops in the world (Fernie and Willmitzer, 2001) and Iran, 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).

Water deficit is a common stress in potato production, which leads to the tuber quality and yield reduction. Because of potato susceptibility to drought (Hassanpanah *et al*., 2008) preparing sufficient water is very important for increasing potato quality and quantity. It is very necessary to study about tolerance of different potato cultivars against water deficit stress and determination of potato water consumption in Ardabil. There is water deficit problem in this region. Climatic changes were occurred in Ardabil region at the recent years. These changes caused differences in precipitation dispersion, river flowing and wells water. Therefore, we have to identify agronomic characters and water need of new potato cultivars and more improvement of their quality and quantity.

Water deficit decreased number of leaves, plant water potentials (Frensch, 1997), leaf area, plant height, ground coverage, tuber number, growth and yield, canopy radiation interception and only to a lesser extent by effects on radiation use efficiency, harvest index and tuber dry matter concentration (Schittenhelma *et al*., 2006) and plant height, root and leaf weight, root number and root dry weight (Donnelly *et al*., 2003; Tourneux *et al*., 2003) and tuber yield (Hassanpanah, 2009a).

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights. Lagrorof showed in 1961 that PEG can be used to the nutrient culture solutions osmotic potential change and to make the water deficiency in plant in compare to the controlled condition. During the 1970 and 1980 decades PEG with 4000 and 8000 molecular mass was used to make drought stress under controlled condition in nutrient solution culture in physiologic experiments (Misra *et al*., 2002; Blum, 2008).

Carpita *et al*. (1979), Bansol and Nagarjan (1987), Money (1989), Verslues *et al*. (1998), Zeid and El-Semary (2001), Kaboli and Sadeghi (2002), Li *et al*. (2005), Bermingham *et al*. (2006), Nasirzadeh and Shookoh (2006), Hassanpanah (2009b) and Imanparast and Hassanpanah (2009) evaluated the effect of water stress on different plants by use polyethylene glycol (PEG). To make the stress condition in lab was used of PEG. This material has more application because of the conditions similar to the natural environmental stress conditions (Hardgree and Emmerich, 1990).

The objective of this study, is the evaluation of different potato cultivars response to water deficit stress under *in vitro* condition to release the cultivar for the regions with water deficit.

MATERIALS AND METHODS

***In vitro* condition:** Experiment was done in biotechnology laboratory and greenhouse of Villkej company in Ardabil province, Iran in 2007. In this experiment, produced plantlets from seven advanced potato cultivars meristem were propagated by single node cutting method. This experiment performed according to the factorial design and on the base of completely randomized design in three replications. A factor included four levels of osmotic pressure (0, -1, -2 and -3 bar) and B factor included seven advanced potato cultivars (Agria, Savalan (397007-9), Satina, Caesar, Kennebec, Marfona and Sante). PEG 6000 was used for exerting the water deficiency stress on the plantlets. Polyethylene glycol added by using diffusion based method (Taji *et al*., 2002). The attributes such as germinating ability and root producing was measured during two months.

***In vivo* condition:** In this stage the cultivar that produced the roots, had moved to the greenhouse. The plantlets were planted in soil bed with mixed of Punce and Biolan with 1:1 v/v. The applied experimental design was factorial on the base of completely randomized design in three replications. A factor has four levels of osmotic pressure and B factor included seven advanced potato cultivars. 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 for plantlets nutrition. All of practices such as irrigation and control of weeds, pests and diseases were done regularly during growth period. Control of pests and fungal diseases were done, respectively by use of 250^{cc} ha^{-1} Confidor and 400 g ha^{-1} Equation-Pro. After two months, was harvested the mini-tubers and the attributes was measured such as mini-tuber average size, mini-tubers weight and number per plant.

The Fischer and Maurer Stress Index (SSI), Fernandez Tolerance Index (STI), Rosielle and Hamblin tolerance index (TOL), Baron Geometric Index (GMP) and Modified Tolerance Index (MSTI), were calculated by using of the following formulae (Fischer and Maurer, 1998; Fernandez, 1992; Naderi *et al*., 1999):

Stress Susceptibility Index (SSI):

$$SSI = \frac{\left(1-\frac{Y_{Si}}{Y_{pi}}\right)}{SI}$$

$$SI = 1-\frac{Y_S}{Y_p}$$

Stress Tolerance Index (STI):

$$STI = \frac{Y_{pi} \times Y_{Si}}{(\bar{Y}_P)^2}$$

Tolerance Index (TOL):

$$TOL = Y_{Pi} - Y_{Si}$$

Geometric Mean Index (GMP):

$$GMP = \sqrt{Y_{Pi} \times Y_{Si}}$$

Mean Productivity (MP):

$$MP = \frac{Y_{Pi} + Y_{Si}}{2}$$

Modified Stress Tolerance Index (MSTI):

$$MSTI = K\left(\frac{Y_{pi} \times Y_{Si}}{(\bar{Y}_P)^2}\right)$$

Where:

$K = Y_{si}^2/Y_s^2$

Y_{si} = Yield of cultivar in stress condition

Y_{pi} = Yield of cultivar in normal condition

Y_s = Total yield mean in stress condition

Y_p = Total yield mean in normal condition

Analysis of variance was carried out through MSTATC software and the results were used to evaluate the effect of drought stress. The means were compared by LSD method and using of MSTATC software program.

RESULTS

The variance analysis showed that significant difference between osmotic pressure levels, cultivars and their interaction as attributes mini-tubers number and weight per plant and mini-tubers average size in 1% probably (Table 1).

The mini-tubers number and weight per plant and mini-tubers average size in osmotic pressure levels showed that the most amounts were in 0 bar osmotic pressure and the lowest amounts were in -2 bar osmotic pressure. In the -3 bar osmotic pressure cultivars don't have mini-tubers (Table 2).

Water deficit decreased number of leaves (Frensch, 1997), yield (Schittenhelma *et al*., 2006), plant height, root and leaf weight, root number and root dry weight (Donnelly *et al*., 2003; Tourneux *et al*., 2003).

Caesar cultivar had the most number and weight of mini-tuber per plant and mini-tubers average size in compare with the other cultivars (Table 3).

The interaction of osmotic pressure levels and cultivars showed that Caesar, Savalan and Satina cultivars had the most numbers and weight of mini-tuber per plant in compare with the other cultivars under normal condition (0 bar osmotic pressures).

The Caesar and Savalan cultivars had the most numbers and weight of mini-tuber per plant in -1 bar and Caesar and Kennebec cultivars in -2 bar osmotic pressures (Table 4).

Table 1: Measured traits square mean for potato cultivars in osmotic pressure levels

		MS		
SOV	df	Minituber No. per plant	Minituber weight per plant (g)	Minitubers average size
Osmotic pressure levels (A)	3	34.57**	717.44**	84.61**
Cultivar (B)	6	7.64**	147.71**	12.94**
A×B	18	2.02**	40.85**	7.92**
Error	56	0.05	1.65	0.21
CV %	-	16.97	21.75	20.93

**Significant at probability levels of 1%

Table 2: Mean of measured traits in osmotic pressure levels

Osmotic pressure levels	Minitubers No. per plant	Minituber weight per plant (g)	Minitubers average size
0 bar (control)	3.00a	13.57a	4.66a
-1 bar	1.43b	6.913b	2.79b
-2 bar	0.71c	3.110c	1.27c
-3 bar	0.00d	0.000d	0.00d

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

The indices for water stress tolerance and susceptibility are given in Table 4 and 5. Stress intensity with regard to the total yield under mild condition was about 52% (Table 5). The mean mini-tuber number per plant was reduced from 3 numbers under mild condition to 1.57 numbers under severe condition (Table 2).

Caesar and Savalan cultivars produced the highest mini-tuber number per plant under the normal and mild condition in compared with Agria cultivar (control) and other cultivars (Table 3).

SSI and TOL indices of Caesar and Savalan cultivars were low and GMP, MP, STI and MSTI indices higher than Agria under mild condition (Table 5). Under severe condition, Kennebec and Caesar cultivars SSI and TOL indices were low and GMP, MP, STI and MSTI indices higher than Agria and other cultivars (Table 6). Caesar cultivar with mean mini-tuber number per plant of 3.0 number and SSI = 0.76 performed better than all other cultivars under the severe condition (Table 6).

Table 3: Mean of measured traits for potato cultivars

Cultivars	Minitubers No. per plant	Minituber weight per plant (g)	Minitubers average size
Caesar	2.67a	12.18a	3.43a
Kennebec	1.58b	7.440b	3.56a
Satina	1.33c	6.646b	2.56b
Marfona	0.63d	2.408c	0.99e
Savalan (397007-9)	1.75b	6.855b	1.96c
Agria	0.54d	3.204c	1.45d
Sante	0.50d	2.563c	1.28de

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

Table 4: Mean of measured traits for osmotic pressure levels on potato cultivars

Osmotic pressure levels	Cultivars	Minitubers No. per plant	Minituber weight per plant (g)	Minitubers average size
0 bar (control)	Caesar	4.67a	20.44a	4.39cdef
	Kennebec	2.33de	10.59de	4.65cde
	Satina	3.33c	15.77b	4.81bc
	Marfona	2.50d	9.63e	3.96ef
	Savalan	4.00b	15.52b	3.88f
	Agria	2.17de	12.82c	5.79a
	Sante	2.00e	10.25de	5.13abc
-1 bar	Caesar	3.00c	16.23e	5.41ab
	Kennebec	2.00e	9.44e	4.72bcd
	Satina	2.00e	10.82cde	5.41ab
	Marfona	0.00f	0.00f	0.00g
	Savalan	3.00f	11.90cd	3.97ef
	Agria	0.00f	0.00f	0.00g
	Sante	0.00f	0.00f	0.00g
-2 bar	Caesar	3.00c	12.04cd	4.01bc
	Kennebec	2.00e	9.73e	4.87bc
	Satina	0.00f	0.00f	0.00g
	Marfona	0.00f	0.00f	0.00g
	Savalan	0.00f	0.00f	0.00g
	Agria	0.00f	0.00f	0.00g
	Sante	0.00f	0.00f	0.00g

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

Table 5: Stress susceptibility and tolerance indices of seven potato cultivars under mild condition

Cultivars	SSI	GMP	MP	TOL	STI	MSTI
Caesar	0.68	3.74	3.84	1.67	1.56	3.77
Kennebec	0.27	2.16	2.17	4.33	0.52	0.31
Satina	0.76	2.58	2.67	5.33	0.74	0.91
Marfona	1.91	0.00	1.25	2.5	0.00	0.00
Savalan	0.48	3.46	3.5	7.00	1.33	2.37
Agria	1.91	0.00	1.09	2.17	0.00	0.00
Sante	1.91	0.00	1.00	2.00	0.00	0.00

SI = 0.52

Table 6: Stress susceptibility and tolerance indices of seven potato cultivars under severe condition

Cultivars	SSI	GMP	MP	TOL	STI	MSTI
Caesar	0.47	3.74	3.84	1.67	1.56	3.77
Kennebec	0.19	2.16	2.17	4.33	0.52	0.31
Satina	1.31	0.00	1.67	3.33	0.00	0.00
Marfona	1.31	0.00	1.25	2.50	0.00	0.00
Savalan	1.31	0.00	2.00	4.00	0.00	0.00
Agria	1.31	0.00	1.09	2.17	0.00	0.00
Sante	1.31	0.00	1.00	2.00	0.00	0.00

SI = 0.76

DISCUSSION

Fischer and Maurer (1998) index classified genotypes as tolerant or sensitive. This index can recognize genotypes as tolerant or sensitive, be regardless to their yield and have a good efficiency for finding genotypes with resistance genes.

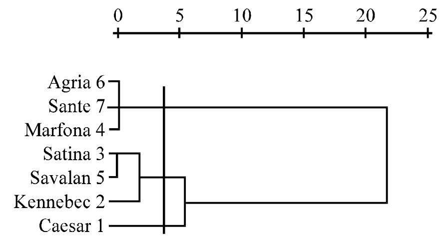
TOL has a conditional efficiency, but after classifying genotypes to equal TOL, we can select resistant genotypes with MP. Finding equal TOL in different groups is very hard. With regard to role of TOL and MP, genotypes with high MP may not be in the least TOL groups and selecting superior genotypes may be difficult. Frenandez (1992) index use stress and non-stress yield and geometric mean. There is a problem and it is geometric equation of coupling data that have natural difference.

Environmental changes in all of Iran provinces are visible. The MSTI index with calculating KSTI for suitable and unsuitable conditions is useful for selecting superior genotypes for each region. The MSTI results are very notable.

MP, GMP, STI and MSTI selected Caesar as better cultivar under normal and stress conditions. Phenologic investigation of Caesar in susceptible periods can lead to understanding strategic methods in agronomy and breeding practices.

Results of cluster analysis showed that cultivars grouped in three clusters. The first cluster included Agria, Sante and Marfona, the second cluster Satina, Savalan and Kennebec and the third cluster Caesar (Fig. 1).

In the first cluster Agria, Sante and Marfona cultivars located. They had lower mini-tuber number, MSTI, STI, GMP and MP and higher SSI and TOL and selected susceptible cultivars for water deficit stress condition.



Cluster 1	Cluster 2	Cluster 3
Agria	Satina	Caesar
Sante	Savalan	
Marfone	Kennebec	

Fig. 1: Dendrogram of measured traits mean for potato cultivars by using of the Ward method

In the second cluster Satina, Savalan and Kennebec cultivars located. They had average mini-tuber number, MSTI, STI, GMP, MP, SSI and TOL and selected moderately-tolerance cultivars for water deficit stress condition.

In the third cluster Caesar cultivar located. It had higher mini-tuber number, MSTI, STI, GMP and MP and less SSI and TOL. Therefore, this cultivar was selected to be the best cultivar for water stress condition in Ardabil region of Iran.

According to the received results, Caesar cultivar selected as more production and tolerance to the water stress for region.

Carpita *et al*. (1979), Bansol and Nagarjan (1987), Money (1989), Verslues *et al*. (1998), Zeid and El-Semary (2001), Kaboli and Sadeghi (2002), Li *et al*. (2005), Bermingham *et al*. (2006), Nasirzadeh and Shookoh (2006), Hassanpanah (2009b) and Imanparast and Hassanpanah (2009) evaluated the effect of water stress on different plant by use polyethylene glycol (PEG). They interdict genotypes for water deficit stress.

CONCLUSIONS

The mini-tubers number and weight per plant and mini-tubers average size in osmotic pressure levels showed that the most amounts were in 0 bar osmotic pressure and the lowest amounts were in -2 bar osmotic pressure. In the -3 bar osmotic pressure cultivars don't have mini-tubers. Caesar cultivar had the most number and weight of mini-tuber per plant and mini-tubers average size in compare with other cultivars. The Caesar and Savalan cultivars had the most numbers and weight of mini-tuber per plant in -1 bar and Caesar and Kennebec cultivars in -2 bar osmotic pressures. The MP, GMP, STI and MSTI selected Caesar as better cultivar in normal and stress conditions. Results of cluster analysis showed that cultivars grouped in three clusters. The first cluster included Agria, Sante and Marfona (susceptible cultivars), the second cluster Satina, Savalan and Kennebec (moderately-tolerance cultivars) and the third cluster Caesar (tolerance cultivar).

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