In Vitro Screening of Several Potato Genotypes for Water Stress Using High Agar Levels in the Medium

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Received: *22/4/2015*

Abstract: *In vitro* screening of several potato genotypes (27 cultivars and breeding lines) for agar-induced water stress was conducted. Single node explants were evaluated on the basis of their plantlet growth and microtuberization under different concentrations of agar (7-10 g/l). Increasing agar in the tissue culture medium resulted in reduction in plantlet growth, rooting and tuberization potential in varying degrees, depending on the cultivar. Under agar-induced water stress, Safran and Universa were generally among the ranked tolerant cvs; Nicola, Triomph and Agria as moderate; Diamant and Bolista as sensitive to drought. Photosynthetic pigment contents under agar-induced water stress were markedly decreased in most cvs tested. However, the cv. Universa showed larger decline in total chlorophyll than Diamant or Bolista. Biochemical analysis of potato plantlet indicated increase in free amino acids, proline and catalase (CAT) activities under water stress, while superoxide dismutase (SOD) was less than the control. Results also indicated that the free amino acids and CAT activity were positively correlated with the tolerance mechanism to water stress.

Keywords*: Solanum tuberosum* L., abiotic stress, tissue culture, drought.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in Egypt for local consumption and exportation. The total area devoted for production in the year 2013 in Egypt was 300,662 Faddan, with total production of about 4,500,000 tons (average 14,96 ton/fad.). Worldwide, potato is the fourth most important crop, with an annual production of 325 million tons, (FAO state; 2012). Abiotic stresses, such as extreme temperature, drought and high salinity often result in significant losses to the yields of economically important crops such as potato (Ahmed and Rashid, 1990). Plants constantly exposed to capricious conditions have adapted at the molecular, cellular, physiological and biochemical levels, enabling them to survive and cope with adverse environmental stresses.

Examining the field performance of potato genotypes under drought stress is the usual method for evaluation, but, the results are often inconclusive. Field trial is normally associated with, non-uniform moisture availability and temperature fluctuations during the growing season. This method involves considerable space, time, labor, equipment and planting material resources (Arvin and Donnelly, 2008). Therefore, *in vitro* screening of the new currently grown genotypes represents valuable tool as alternative to field trials. Furthermore, a highly significant correlation was found between *in vitro* growth parameters and field performance of ten potato clones studied by Morpurgo (1991).

Water stress also severally affects potato yield as reported by Singh (1969) and preparing sufficient water is very important for increasing potato quality and quantity. Because of its shallow root system, potatoes are classified as sensitive to drought stress (Yuan *et al.*, 2003). *In vitro* screening of potato for water stress was carried out with different water stress agents, including PEG (Hassanpanah, 2009; Daneshmand *et al*., 2010; Pino *et al*., 2013); sorbitol (Gopal and Kazuto, 2007) and mannitol (Sabbah and Moshe, 1990), using agar levels higher than normal (7.0g/l) was utilized in the study of Gopal *et al*. (2008). In three potato genotypes, the agar- induced water stress (at 10 g/l) adversely affected plantlet growth and rooting, and the response varied with genotypes. Nistor *et al*. (2014) used agar in MS medium at 9.0 to 20 g/l. Leaf and node numbers as well as plantlet length decreased with increasing agar level, depending on the cultivar. Plantlet height and nodes numbers were severely affected and represent about 40% of the control. Similar results were also found by Shafqatulla *et al*. (2007). The later reports were only confined to the *in vitro* response of agar-induced water stress on plantlet growth bioassay but no information about *in vitro* microtuberization potentials under such condition were available in the literatures. Therefore, the objectives of the present study were to screen large number of potato genotypes for tolerance to water stress using plantlet growth and microtuberization bioassays. Biochemical changes associated with drought tolerance were also determined.

MATERIALS AND METHODS

The current investigation was conducted at the Plant Tissue Culture Laboratory of the Horticulture Department, Suez Canal University, Ismailia, during the period of 2012 to 2014.

Plant material:

Twenty seven potato cultivars and breeding lines from different maturity groups, including six early genotypes (Safran, Margod, Universa, Alaska, Spunta and Elodi), six mid-early genotypes (Triomph, Lady Rosetta, Nicola, Fridor, Naga, and Diamant), one late maturing (Agria), in addition to seven potato lines under test (94f-8101, 96f-25-25, 95k-94, 97-980, 94f and 97F-267), German varieties (Jelly, Presto and Marabel) and new locally-grown cultivars (Picasso, Proventa, Arinda,

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Bolista and Sante) were used for the study. These potato genotype collections were continuously micro-propagated and kept as plantlet grown in test tubes under 20±2ºC in our tissue culture facility at the Department of Horticulture, Faculty of Agriculture, Suez Canal University.

Experimental protocol:

Under sterile conditions, 10 nodes (1cm long) were cultured per jar containing 30 ml MS medium (Murashige and Skoog, 1962) amended with the agar treatments (7, 8, 9 and 10 g/l). After 6 weeks, 5 plantlets were taken from each cultivar x agar treatment and data were taken on plantlet length and root number/plantlet.

To test *in vitro* microtuberization under the previous agar treatments, 30 ml sterilized liquid MS medium amended with high sucrose level (80 g/l) were added to each jar containing the growing plantlets after removal of the jar cap in a laminar air-flow hood. Cultures were incubated in the dark at 18-20ºC for 2 months. Microtubers produced from each treatment were harvested and data were taken on number and weight (yield) of microtuber/jar and the average single microtuber weight were calculated by dividing weight/number. Percent of tuber formation under the highest agar level (10 g/l) was also calculated as relative to the control.

Biochemical analysis of potato plant grown under *in vitro* water stress conditions.

Based on the morphological characters (plantlet height) only seven potato genotypes were chosen for biochemical analysis as representing, 1) tolerant genotypes (Universa and Safran). 2) moderately tolerant genotypes (cvs. Triomph, Nicola and Agria), and 3) sensitive genotypes (Diamant and Bolista). The biochemical analysis was made only in plantlets exposed to highest agar concentration (10 g/l) in comparison with control plantlets.

1-*Chlorophyll and carotenoid contents* were determined according to the method of Lichtenthaler (1987).

2- *Free total amino acids* were colorimetrically assayed by ninhydrin reagent at 570 nm according to the method described by Lee and Takabashi (1966).

3-*Proline* was estimated using the method described by Sadasivam and Manickam (1991).

4- *Enzymes:* The plantlet samples (weight 0.1-0.4g) were prepared for enzyme activity as described by Ni *et al.* (2001).

4-a. *Superoxide dismutase (SOD) activity* was determined by spectrophotometriclly at 560 nm (U\V Spectrophotometer spectronic 1201, Milton Roy, U.S.A) as described by the method of Nishikimi *et al.* (1972).

4-b. *Catalase (CAT) activity* was measured spectrophotometriclly at 510 nm as described by the method of Aebi (1984).

Statistical analysis:

The experimental design was a randomized complete block design in factorial fashion. Data were combined and subjected to ANOVA using CoStat program and the means were separated by DMRT test at 5% level.

RESULTS

In this experiment, 27 potato genotypes were screened for their shoot and root growth and microtuberization *in vitro* under different agar concentrations (7.0 g/l as control, 8.0, 9.0 and 10.0 g/l) in the tissue culture medium specific for shoot growth or microtuber formation.

a. Effect of agar and genotypes on plantlet growth:

a. 1. Main effects of agar:

Results revealed that the differences among agar concentrations as averaged over genotypes, were significant. Moderate (8.0 g/l) agar level in the medium recorded the highest plantlet length (Table 1) over the control and agar at 9.0g/l which were not significantly different. Plantlet length at the highest agar (10.0 g/l) was significantly the least (ave. 2.93 cm), representing 48.3% of the control. Root number per plantlet was significantly affected by agar concentration (Table 2). Averaged over all genotypes, root number at 8.0g/l agar recorded the highest value (ave. 4.86 roots) compared to the control (ave. 2.97 roots) and agar at 9.0g/l (ave. 2.64 roots), while the least average root number was found in plantlet grown at 10.0 g/l agar (ave. 0.52 root) representing only 17.5% of the control and 10.7% of the root number recorded at 8.0 g/l agar. Therefore, rooting of potato plantlet was more affected by agar – induced water stress than plantlet length.

a. 2. Main effects of genotypes:

As an average over the tested agar concentrations, potato genotypes were found to be significantly different in shoot and root characters. Plantlet length in cvs. Marabel, 95k-94 and Diamant recorded the highest values (ave. 7.1-8.2 cm) followed by cvs. Agria, Presto and Proventa. The least plantlet height was observed on cvs. Alaska, 97-980 and Arinda (about 3.3 cm) as shown in Table (1).

Number of roots/plantlet was significantly different among the potato genotypes in this experiment (Table 2). As tested over agar levels, the cv. Alaska recorded the highest number of roots/plantlet (ave. 6.4 roots), followed by cvs. Universa (ave. 5.95 roots), cv. Safran (ave. 5.45 roots), 95k-94 and Diamant (ave. 5.2 roots/plantlet).

a. 3. Effect of Agar x genotype interaction:

Results indicated that shoot length was significantly affected by the agar x genotype interactions (Table 1). At 7.0g/l, the cvs Marabel, Presto and Diamant, followed by 95k-94, 96F-25-25 and Safran recorded the highest plantlet length (8.2-10.33cm), while at 8.0g/l, the cvs. Agria and Marbel had the highest shoot length among all agar x genotype combinations (>10.0 cm), and was followed by cvs. 95k-94, Presto and Proventa. On the other hand, the cvs.Safran and Marabel had the highest shoot length at 9.0g/l agar. None of the tested genotypes had shoot length higher than the control when grown on medium with high agar level (10.0g/l). The interaction of agar x genotype was found significant for number of roots/plantlet (Table 2). While the highest root number was found on cv. Diamant under 7.0 g/l agar (ave. 10.0 roots/plantlet), the highest rooting of

cvs. Marabel and Picasso was at 8.0g/l agar (ave. 9.0-9.3 roots). Most cvs. (22 cvs.) did not form roots at the highest agar-induced water stress level (10.0g/l).

b. Effects of agar levels and genotypes on microtuberization:

Results revealed significant main effects of agar and potato genotypes, as well as their interaction on several aspects of potato microtuberization.

b.1. Main effects of agar concentrations on microtuberization:

Agar levels on the medium significantly affected thenumber of microtuber produced/jar (Table 3). As an average over all genotypes, there were no significant differences in microtuber number between 7.0 and 8.0g/l (ave. 5.3 and 4.9 microtuber/jar). At 9.0 and 10.0g/l agar, microtuber number decreased (ave 3.6 and 1.97, respectively). At 10.0g/l agar, the number of microtuber was only 36.8% of the control. The average microtuber weight was also affected by the agar concentrations, which was decreased dramatically with the increase in agar level in the medium (Table 4). The highest average microtuber weight was recorded at the control agar treatment (7.0g/l) reaching ave. 519 mg/tuber, followed by agar at 8.0g/l (432 mg), 9.0g/l (284 mg), and was the least at the highest agar level (ave. 72.0 mg), representing only 13.8% of the control at 7.0g/l agar.

Table (1). Effect of agar concentration on the *in vitro* shoot (plantlet) length in 27 potato genotypes.

Genotype	Agar concentration (g/l)								Mean Genotype		% of control**
	7		8		9		10				
	Plantlet length (cm)										
Safran	3.450*	E-M	7.950	e-i	9.400	a-d	2.550	K-S	5.838	efg	73.913
94f-8101	8.200	d-h	7.300	g-n	2.900	H-Q	5.250	s-A	5.913	ef	64.024
Margod	3.500	E-M	3.620	D-L	5.200	s-A	3.100	H-P	3.855	klm	88.571
Universa	5.200	s-A	8.700	c-f	3.800	B-K	3.550	F-N	5.250	gh	68.269
Alaska	2.850	H-Q	5.250	s-A	3.850	C-J	1.800	Q-U	3.438	mn	63.158
Spunta	6.850	i-q	6.600	j-r	6.250	l-t	3.200	G-O	5.725	efg	46.715
Elodi	3.850	B-J	7.550	f-k	4.850	v-D	1.950	O-U	4.550	ij	50.649
96f-25-25	8.700	c-f	7.150	g-o	5.800	p-x	1.540	R-U	5.798	efg	17.701
Triomph	4.050	A-I	6.900	i-p	5.070	t-B	2.100	N-U	4.530	ij	51.852
Lady Rosetta	4.900	u-C	3.850	B-J	4.600	x-E	1.300	STU	3.663	lm	26.531
95k-94	8.950	b-e	8.950	b-e	8.400	d-g	3.050	H-Q	7.338	A	34.078
Nicola	6.300	k-t	6.450	k-s	5.150	t-A	3.200	G-O	5.275	fgh	50.794
Fridor	5.750	p-x	6.600	j-r	5.950	o-w	4.720	w-E	5.755	efg	82.087
Naga	5.700	p-y	6.900	i-p	5.960	o-w	1.250	TU	4.953	hi	21.930
Diamant	9.700	abc	7.450	f-l	7.300	g-n	4.050	A-I	7.125	ab	41.753
Agria	7.400	g-m	10.350	a	5.500	r-z	3.750	C-K	6.750	abc	50.676
97-980	3.300	F-N	4.450	y-G	4.090	A-H	2.100	N-U	3.485	mn	63.636
94f	7.850	e-j	7.750	e-j	4.400	z-G	3.500	E-M	5.875	efg	44.586
Jelly	5.050	t-B	6.100	n-v	3.100	H-P	3.250	G-N	4.375	ijk	64.356
Presto	9.700	abc	8.350	d-g	7.400	g-m	1.000	U	6.613	bcd	10.309
Marabel	10.330	a	10.050	ab	9.700	abc	2.800	I-R	8.220	cde	27.106
97f-267	5.250	s-A	6.950	h-p	1.850	P-U	1.550	R-U	3.900	n	29.524
Picasso	7.300	g-n	5.600	q-z	5.750	p-x	4.550	x-F	5.800	efg	62.329
Proventa	4.070	A-I	6.900	i-p	7.850	e-j	5.900	p-w	6.180	cde	144.963
Arinda	3.300	F-N	5.300	s-A	2.400	L-T	2.330	M-T	3.333	mn	70.606
Bolista	7.850	e-j	6.150	m-u	7.550	f-k	2.700	J-R	6.063	de	34.395
Sante	5.450	r-z	5.550	r-z	2.900	H-Q	3.300	F-N	4.300	jkl	60.550
Mean Agar	6.088	a	6.841	a	5.443	b	2.938	c			

* Means with the same letters are not significantly different at $p \leq 5\%$.
** Value for each cv. at 10.0 g/l agar as relative to the control.

Table (2). Effect of agar concentration on root number per plantlets in 27 potato genotypes.

Genotype	Agar concentration (g/l) 7		8		9		10		Mean Genotype		% of control**
	Root no. per plantlets										
Safran	8.300*	b-e	8.000	c-f	3.600	p-u	1.900	x-D	5.450	bc	22.89
94f-8101	1.000	D-G	2.200	w-C	2.700	u-A	0.000	G	1.475	ijkl	0.000
Margod	1.600	A-E	7.600	d-g	3.900	o-t	0.000	G	3.275	e	0.000
Universa	2.400	v-B	8.500	bcd	6.300	h-k	6.600	g-j	5.950	ab	275.0
Alaska	8.800	bc	8.000	c-f	8.800	bc	0.000	G	6.400	a	0.000
Spunta	3.100	s-w	1.700	z-E	3.300	r-w	0.000	G	2.025	fghi	0.000
Elodi	1.000	D-G	8.000	c-f	1.400	B-E	0.000	G	2.600	fgh	0.000
96f-25-25	1.300	B-F	1.900	x-D	0.000	G	0.000	G	0.800	mn	0.000
Triomph	1.700	z-E	5.900	i-l	0.000	G	0.000	G	1.900	ghi	0.000
Lady Rosetta	2.900	s-y	4.300	n-r	2.300	w-C	0.000	G	2.375	fgh	0.000
95k-94	5.500	j-m	6.800	ghi	8.800	bc	0.000	G	5.275	c	0.000
Nicola	1.800	y-D	5.400	k-n	2.900	s-y	0.000	G	2.525	fg	0.000
Fridor	5.200	k-n	2.400	v-B	2.900	s-y	0.000	G	2.625	f	0.000
Naga	1.900	x-D	4.600	m-q	1.800	y-D	0.000	G	2.075	fghi	0.000
Diamant	10.000	a	6.600	g-j	4.000	o-s	0.200	FG	5.200	c	2.000
Agria	0.900	D-G	3.800	o-u	2.400	v-B	0.000	G	1.775	hij	0.000
97-980	1.000	D-G	4.700	m-p	0.000	G	0.000	G	1.425	jklm	0.000
94f	3.700	o-u	2.400	v-B	0.000	G	0.000	G	1.525	ijk	0.000
Jelly	1.200	C-F	2.300	w-C	0.000	G	0.000	G	0.875	lmn	0.000
Presto	7.000	f-i	0.000	G	0.000	G	0.000	G	1.750	hij	0.000
Marabel	1.000	D-G	9.000	abc	4.800	l-o	0.000	G	3.700	de	0.000
97f-267	1.000	D-G	4.800	l-o	0.000	G	0.000	G	1.450	jklm	0.000
Picasso	1.600	A-E	9.300	ab	7.000	f-i	2.400	v-B	5.075	c	150.0
Proventa	3.500	q-v	7.200	e-h	2.200	w-C	3.000	s-x	3.975	d	85.71
Arinda	1.000	D-G	1.000	D-G	0.000	G	0.000	G	0.667	n	0.000
Bolista	0.600	EFG	2.800	t-z	2.400	v-B	0.000	G	1.450	ijkl	0.000
Sante	1.700	z-E	1.900	x-D	0.000	G	0.000	G	0.900	klm	0.000
Mean Agar	2.982	b	4.856	a	2.648	bcd	0.522	c			

* Means with the same letters are not significantly different at $p \leq 5\%$.
** Value for each cv. at 10.0 g/l agar as relative to the control.

b.2. Main effect of genotypes on microtuberization:

Significant differences among potato genotypes were observed on number of microtuber formed/jar (Table 3). The cvs. Safran and Presto had the highest average microtuber number/jar as an average over the tested agar levels, reaching an average of 8.0microtubers/jar, followed by cvs. Fridor and Naga (ave. 7.3 microtuber/jar), 95k-94 and 94f-8101 (6.5 microtubers). Genotypes were significantly different in microtuber weight (Table 4). The cvs. Safran and 95k-94 recorded the highest ave. microtuber weight (893 mg/tuber and 853 mg/tuber), followed by cv. Presto (733mg), cv. Nicola (694mg) and cv. 94f.8101 (622.0mg). The least microtuber weight was detected in cvs. Picasso, 97f-267, Sante, 97-980, Agria and 96f-25-25.

b.3. Effect of agar x genotype interaction on microtuberization:

Number of microtuber per jar was significantly affected by the interaction of agar x genotype (Table 3).

The cv. Safran recorded the highest number of microtuber produced per jar (ave. 12.67 microtubers) on medium supplemented with 8.0g/l agar. Also, the cvs 95k-94 and Safran had the highest microtuber number (ave. 10.0 microtubers/jar) at 7.0g/l. At higher agar level (9.0g/l) the cv. Presto recorded higher microtuber number (ave. 12 microtubers) among the rest of cvs. under this agar level. At the highest agar level, the cvs. Naga and Fridor were the highest in microtuberization under water stress.

The average microtuber weight was significantly affected by the interaction of agar x genotype (Table 4). On medium with 7.0g/l agar, the cv. Safran and 95k-94 recorded the highest microtuber weight (>1.8g/tuber). The cv. Safran also had higher microtuber weight at 8.0g/l agar, while the cv. Presto recorded the highest microtuber weight at 9.0g/l agar (ave. 1.48g/tuber). At the highest agar (10.0g/l) treatment, the cv. Fridor had more microtuber weight (624mg/tuber) than all cvs under this agar level.

Table (3). Effect of agar concentration on the *in vitro* average microtuber number/jar in 27 potato genotypes.

Genotype	Agar concentration (g/l)								Mean Genotype		% of control**
	7		8		9		10				
	Average microtuber no./jar										
Safran	10.000*	bcd	12.670	a	4.000	l-q	5.667	i-m	8.083	a	56.667
94f-8101	9.667	cde	5.670	i-m	8.330	d-g	2.333	p-u	6.500	bc	24.138
Margod	7.667	e-i	6.670	g-j	2.670	p-t	2.333	p-u	4.833	defg	30.435
Universa	4.000	l-q	8.330	d-g	7.670	e-i	0.000	v	5.000	def	0.000
Alaska	5.333	j-n	1.000	tuv	4.000	l-q	1.000	tuv	2.833	ijkl	18.750
Spunta	4.000	l-q	9.000	def	7.000	f-j	2.667	p-t	5.667	cde	66.667
Elodi	6.333	g-k	1.670	r-v	4.000	l-q	1.000	tuv	3.250	hijk	15.789
96f-25-25	3.667	m-r	2.330	p-u	1.000	tuv	1.000	tuv	2.000	klmn	27.273
Triomph	4.333	k-p	8.330	d-g	1.000	tuv	0.000	v	3.417	ghijk	0.000
Lady Rosetta	5.000	J-o	4.000	l-q	3.330	n-s	1.000	tuv	3.333	hijk	20.000
95k-94	10.000	bcd	5.670	i-m	8.000	d-h	2.333	p-u	6.500	bc	23.333
Nicola	5.333	j-n	2.000	q-v	6.000	h-l	0.333	uv	3.417	ghijk	6.250
Fridor	9.667	cde	10.000	bcd	1.670	r-v	8.000	d-h	7.333	ab	82.759
Naga	9.333	de	8.000	d-h	3.330	n-s	8.333	abc	7.250	bcd	89.286
Diamant	3.333	n-s	3.000	o-t	2.000	q-v	1.000	tuv	2.333	jklm	30.000
Agria	3.000	tuv	1.000	tuv	1.000	tuv	1.000	o-t	1.500	lmn	33.333
97-980	1.333	s-v	0.000	v	1.000	tuv	0.000	v	0.583	n	0.000
94f	7.000	s-v	3.670	m-r	2.330	p-u	1.333	f-j	3.583	fghij	19.048
Jelly	1.000	tuv	1.000	tuv	1.670	r-v	1.000	tuv	1.167	mn	100.000
Presto	7.667	e-i	9.330	de	12.000	ab	3.667	m-r	8.167	a	47.826
Marabel	3.667	m-r	5.670	i-m	3.000	o-t	4.000	l-q	4.083	fghi	109.091
97f-267	5.667	i-m	3.000	o-t	4.000	l-q	1.667	r-v	3.583	fghij	29.412
Picasso	1.333	s-v	1.330	s-v	1.000	tuv	0.000	v	0.917	mn	0.000
Proventa	8.000	d-h	6.670	g-j	2.000	q-v	1.000	tuv	4.417	efgh	12.500
Arinda	3.000	o-t	4.000	l-q	2.000	q-v	1.667	r-v	2.667	ijkl	55.556
Bolista	4.000	l-q	8.000	d-h	3.330	n-s	1.000	tuv	4.083	fghi	25.000
Sante	1.667	r-v	1.000	tuv	1.000	tuv	0.000	v	0.917	mn	0.000
Mean Agar	5.370	a	4.926	a	3.642	b	1.975	c			

* Means with the same letters are not significantly different at $p \leq 5\%$.
** Value for each cv. at 10.0 g/l agar as relative to the control**.**

c. Screening results of potato genotypes for *in vitro* water stress using high agar concentration in the tissue culture medium:

The main effects of agar-induced water stress indicated its significant effect on plantlet growth, rooting and microtuber induction and development *in vitro*. Classification of the 27 potato cultivars and new breeding lines into tolerant, moderate and sensitive groups were made taking the measure of growth and development values under the highest used agar level (10.0g/l) as percent of control. Based on plantlet growth at 10g/l agar as % of control (Table 1), potato genotypes are ranked as follow: Proventa>Margod>Fridor>Safran>Arinda>Universa>94F-8101>Jelly>99-981>Alaska>97-980>Picasso >Sante>Triomph>Elodi = Agria = Nicola >Spunta> 94F >Diamant>95K-94 >Bolista> 97F-267 >Marabel> Lady Rosetta >Naga >96F-25- 25 > Presto.

From Table (3), based on microtuber number under high agar level as % of control, the potato genotypes could be ranked as follow: Marabel> Jelly > Naga >Fridor>Spunta>Safran>Arinda> Presto>Agria>Margod = Diamant> 97F-267 > 96F-25-25 >Bolista> 94F-8101 > 95K-94 > Lady Rosetta> 94F> Alaska >Elodi>Proventa> Nicola >Universa = Triomph = Picasso = Sante = 97-980.

Table (4). Effect of agar concentration on the *in vitro* average microtuber weight in 27 potato genotypes.

Genotype	Agar concentration (g/l)										% of control**
	7		8		9		10				
	Average microtuber weight (g)								Mean Genotype		
Safran	1.874*	a	1.230	bc	0.210	t-C	0.253	s-C	0.893	a	13.472
94f-8101	1.072	cd	0.650	f-l	0.760	e-i	0.007	C	0.622	cde	0.606
Margod	0.549	i-q	0.950	de	0.320	n-y	0.269	r-C	0.523	ef	49.013
Universa	0.369	m-x	0.400	l-v	0.170	v-C	0.000	C	0.234	hijk	0.000
Alaska	0.752	e-i	0.050	z-C	0.570	h-p	0.078	y-C	0.363	gh	10.376
Spunta	0.369	m-x	0.400	l-v	0.260	r-C	0.031	ABC	0.266	hijk	8.327
Elodi	0.775	e-i	0.860	d-g	0.610	g-m	0.004	C	0.564	def	0.478
96f-25-25	0.063	y-C	0.040	z-C	0.030	BC	0.019	BC	0.037	mn	30.466
Triomph	0.175	v-C	0.920	def	0.230	t-C	0.000	C	0.330	hi	0.000
Lady Rosetta	0.960	de	0.380	l-x	0.080	y-C	0.017	BC	0.359	gh	1.772
95k-94	1.929	a	0.510	i-s	0.940	de	0.036	z-C	0.853	ab	1.889
Nicola	1.080	cd	0.960	de	0.710	e-k	0.029	ABC	0.694	cd	2.692
Fridor	0.303	p-z	0.390	l-w	0.030	BC	0.624	g-m	0.335	hi	206.357
Naga	0.083	y-C	0.450	k-u	0.150	v-C	0.070	y-C	0.189	jkl	84.259
Diamant	0.730	e-j	0.390	l-w	0.050	z-C	0.035	z-C	0.300	hij	4.728
Agria	0.035	z-C	0.090	y-C	0.020	BC	0.007	C	0.038	mn	20.000
97-980	0.044	z-C	0.000	C	0.110	x-C	0.000	C	0.038	mn	0.000
94f	0.478	y-C	0.150	v-C	0.300	q-A	0.055	z-C	0.247	hijk	11.506
Jelly	0.193	u-C	0.020	BC	0.050	z-C	0.014	C	0.068	lmn	7.215
Presto	0.577	h-n	0.830	d-h	1.480	B	0.049	z-C	0.733	bc	8.433
Marabel	0.125	w-C	0.300	o-z	0.040	z-C	0.178	v-C	0.162	klm	142.693
97f-267	0.008	C	0.010	C	0.010	C	0.004	C	0.006	n	51.807
Picasso	0.033	z-C	0.020	BC	0.030	BC	0.000	C	0.020	n	0.000
proventa	0.534	i-r	0.960	de	0.290	q-B	0.137	v-C	0.478	fg	25.587
Arinda	0.705	e-k	0.070	y-C	0.030	BC	0.032	z-C	0.208	ijk	4.496
Bolista	0.139	v-C	0.570	h-o	0.190	u-C	0.009	C	0.229	hijk	6.715
Sante	0.065	y-C	0.080	y-C	0.010	C	0.000	C	0.039	mn	0.000
Mean Agar	0.519	a	0.432	b	0.284	c	0.072	d			

* Means with the same letters are not significantly different at $p \leq 5\%$.
** Value for each cv. at 10.0 g/l agar as relative to the control.

d. Photosynthetic (PS) pigment contents as affected by agar, genotypes and their interaction:

d.1. Main effect of agar induced water stress:

Results shown in Table (5) indicate that high agar concentration significantly affected all photosynthetic pigment accumulation. Normal (7.0g/l) agar in the tissue culture medium was compared to high level (10.0g/l). As average over the 7 examined genotypes, results indicated significant decline in chl. a, chl. b and total (a+b) chl and carotenoids under high concentration of agar. As % of control, chl. a, chl. b, total chl and carotenoids contents were 79.6%, 73.4%, 77%, and 86% of control, respectively.

d.2. Main effect of genotypes:

Potato genotypes tested over the two levels of agar indicated significant differences in their pigment contents (Table 5). Chlorophyll a, b and total chl. as well as carotenoids contents were higher in cv. Bolista compared to the rest of genotypes, while cv. Nicola recorded the least accumulation of photosynthetic pigments.

d. 3. Effect of agar x genotype interaction

Agar x genotype interactions were found significant for the contents of photosynthetic pigments (Table 5). The accumulation of chl. a at high agar level was significantly less than the control for all potato genotypes, except in cv. Nicola which had no change in chl. a under agar-induced water stress. The trend of reduction in chl. a was different among the different cvs. At high agar level (10.0g/l), chl. a contents as % of control were 74.2, 82.3, 63.0, 88.0, 86.4 and 72.4% for the cvs. Safran, Universa, Triomph, Diamant, Agria and Bolista, respectively. The contents of chl. b were always lower than the control in all examined potato genotypes, except cv. Nicola. Thus, total chl. had the same trend and represented 77% of control.

Carotenoids were also, and significantly lower under high agar level than the control in all cvs. However, the degrees of reductions were different among the different cvs. As percent of control, carotenoids were 84, 74.8, 62.6, 95.7, 81.0, and 80.8% of control in cvs. Safran, Universa, Triomph, Daimant,Agria, and Bolista respectively. Results indicated increase in carotenoids contents under water stress in cv. Nicola as opposite to the rest of genotypes.

e. Effect of agar and genotypes on some biochemical contents:

e. 1. Main effect of agar:

In this analysis, free amino acids and proline contents, as well as the activities of antioxidant enzymes (SOD and CAT) in the plantlet tissues were studied in relation to the water stress induced by high agar level in the medium. Results revealed that high agar (10g/l) significantly increased the average free amino acids and CAT activity as an average over the tested genotypes, however, SOD was significantly reduced and proline content was unaffected (Table 6).

Table (5). Effect of *in vitro* agar- induced water stress on chlorophyll and carotenoid contents in 7 potato cultivars.

CV	Agar	Chl. a		Chl. b		Chl. Total	Carotenoids	
Safran	7g\l	35.15*	b	21.12	e	56.26	13.37	f
	10 g\l	26.07	h	15.05	k	41.12	11.24	i
	Mean CV	30.61	c	18.08	d	48.69	12.31	e
Universa	7g\l	29.84	e	23.36	d	53.19	16.51	c
	10 g\l	24.56	j	11.32	m	35.88	12.35	g
	Mean CV	27.20	d	17.34	e	44.53	14.43	b
Triomph	7g\l	29.84	e	23.36	d	53.19	16.51	c
	10 g\l	18.80	m	16.69	g	35.48	10.34	k
	Mean CV	24.32	e	20.03	b	44.35	13.43	c
Nicola	7g\l	21.34	l	15.95	i	37.27	9.39	l
	10 g\l	21.56	k	16.49	h	38.05	14.65	d
	Mean CV	21.45	f	16.22	g	37.67	12.02	f
Diamant	7g\l	28.91	i	19.23	f	48.12	11.45	h
	10 g\l	25.46	f	14.89	l	40.35	10.96	j
	Mean CV	27.18	d	17.06	f	44.24	11.20	g
Agria	7g\l	33.27	c	23.47	c	56.72	14.12	e
	10 g\l	28.75	g	15.59	j	44.34	11.44	h
	Mean CV	31.01	b	19.53	c	50.54	12.78	d
Bolista	7g\l	41.58	a	35.99	a	77.55	20.66	a
	10 g\l	30.09	d	29.22	b	59.31	16.70	b
	Mean CV	35.84	a	32.60	a	68.44	18.68	a
Mean agar at 7.0 g\l		31.42	a	23.21	a	54.63	14.57	a
Mean agar at 10.0 g\l		25.04	b	17.04	b	42.08	12.53	b

* Means with the same letters are not significantly different at $p \leq 5\%$.

e. 2. Main effect of genotypes:

Averaged over the two agar levels, differences among potato genotypes were detected in their contents of free amino acids, proline, SOD and CAT (Table 6). With respect to total free amino acids, the cvs. Bolista and Diamant (drought sensitive) had significantly higher total free amino acids, followed by cv. Agria, while the cvs. Nicola, followed by Triomph, Universa and Safran had the least free amino acids. Proline contents were also the highest in cvs. Bolista, followed by Agria and Nicola, while the least proline accumulation was recorded in cvs. Safran and Universa, followed by Triomph and Diamant. However, SOD activity was higher in cv. Safran followed by cv. Universa (drought tolerant), and the least activities were found in the tissues of cvs. Bolista and Diamant. The activity of CAT enzyme followed the same trend of free amino acids and proline, with the cv. Bolista having the highest CAT, followed by cvs. Agria and Diamant. The least CAT activity was detected in cv. Nicola followed by cv. Safran (Table 6).

e. 3.Effect of agar x genotype:

Results indicated that potato genotypes included in this analysis had different responses to the water stress induced by high agar level in the medium. Regarding the contents of free amino acids, the cvs. Safran, Universa, Triomph and Diamant had increased free amino acids contents under 10.0g/l agar as compared to the control, while cvs. Nicola and Agria had significantly less free amino acids at the highest level of agar (Table 6).

With respect to proline contents, the cvs.Safran, Triomph, Diamant and Agria accumulated higher proline at high agar level, while the other cvs. (Universa and Bolista) had lower proline content under water stress imposed by high agar concentration in the medium. In cv. Nicola, the proline content at high level of agar was not significantly different than at the control. The activity of SOD was increased at high agar level in cv. Diamant, while all other cvs had less SOD at higher agar level, compared to control. The activity of CAT showed almost similar pattern to proline. The cvsSafran, Universa, Triomph, Diamant and Bolista, respectively, showed higher CAT activities under high agar-induced water stress, while the reverse was true in cv. Agria. The cv. Nicola had the activity of CAT at high agar (10.0g/l) similar to the control (7.0g/l).

Table (6). Free amino acids, proline and antioxidant profile analysis of seven potato cultivars under high level of agar *in vitro*

CV	Agar g\l	Free amino acids mg/g FW		Proline mg\g FW		Superoxide dismutase (u/gm fresh weight)		Catalase (u/gm fresh weight)	
Safran	7g\l	909.00*	gh	30.870	fgh	6947.00	a	1.370	g
	10 g\l	2629.67	e	42.330	efg	6493.00	b	4.360	f
	Mean CV	1769.34	d	36.600	d	6720.00	a	2.860	e
Universa	7g\l	772.67	h	39.200	efg	6633.00	b	1.180	g
	10 g\l	1062.99	g	34.370	i	6434.33	b	7.030	ef
	Mean CV	917.83	f	36.790	d	6533.67	b	4.110	d
Triomph	7g\l	624.00	i	26.630	ghi	6657.33	b	1.140	g
	10 g\l	3302.33	d	52.500	ef	6093.67	c	7.150	d
	Mean CV	1963.17	c	39.570	cd	6375.50	c	4.140	d
Nicola	7g\l	2596.00	e	66.670	e	5983.00	c	1.510	g
	10 g\l	137.33	j	67.500	e	4737.00	e	1.190	g
	Mean CV	1366.67	e	67.080	c	5360.00	c	1.350	f
Diament	7g\l	3507.67	c	34.970	fg	5617.00	d	2.010	g
	10 g\l	4226.33	a	42.670	efg	5972.33	c	8.390	d
	Mean CV	3867.00	ab	38.820	cd	5794.67	d	5.200	c
Agria	7g\l	3870.33	bc	122.670	d	6610.00	b	13.520	c
	10 g\l	1482.67	f	139.330	c	6126.67	c	7.970	d
	Mean CV	2676.50	b	131.000	b	6368.33	c	10.705	b
Bolista	7g\l	3932.33	bc	197.330	a	4645.33	e	17.290	b
	10 g\l	4008.33	b	170.670	b	6427.67	b	19.010	a
	Mean CV	3970.33	a	184.000	a	5536.50	e	18.150	a
Mean agar at 7.0 g\l		2316.00	b	74.050	a	6156.10	a	5.430	b
Mean agar at 10.0 g\l		2406.80	a	78.480	a	6040.67	b	7.870	a

* Means with the same letters are not significantly different at $p \leq 5\%.0$.

DISCUSSION

In vitro screening of different potato genotypes for drought stress induced by high level of agar was performed using single node and microtuberization bioassays. Physiological and biochemical changes associated with agar stresses factor were examined in selected genotypes, representing different tolerant groups. Result of this study revealed that potato plantlet grown under high concentration (10g/l) of agar in the medium also showed reduced growth compared to the control (7.0g/l agar) and the highest percentage of decline was noticed in root number. This large decline in rooting may cause restricted water and nutrients uptake from the medium, leading to the observed decrease in plantlet growth. These results are in harmony with those of Daneshmand *et al*. (2010), Hassanpanah (2009) in potato, and Azami *et al*. (2010) in tomato tissue culture under PEG- induced water stress. Using high agar level as water stress agent, Shafqatulla *et al*. (2007), Gopal *et al*. (2008) and Nistor *et al*. (2014) also reported reduction in plantlet height, number of nodes and rooting.

In vitro tuberization of potato was significantly affected by agar-induced water stress. Using agar at 10g/l as water stress factor, microtuber number and the average tuber weight were decreased, reaching 49% and 7.1% of the control, respectively. Recent report (Pino *et al*., 2013) showed that cultivated potato plants are generally sensitive to water stress, had reduced yield and tuber quality.

Under water stress induced by agar, a marked reduction in chlorophyll and carotenoides was observed, in agreement with the results of Daneshmand *et al*. (2010). The reduction in total chlorophyll was higher in cvs. Universa, Triomph and Bolista than in cvs. Agria, Diamant and Safran, while no decline in chlorophyll content occurred in cv. Nicola (moderately tolerant), indicating that chlorophyll contents were not associated with water stress tolerance in the examined potato genotype.

Biochemical analysis of potato plantlet under water stress indicated that amino acids contents were generally higher under water stress in tolerant - classified genotypes (cvs. Safran and Universa) in addition to cv. Triomph, but decreased sharply in moderate tolerant (cv. Nicola) and was almost the same, compared to the control, in sensitive (cv. Bolista). Proline showed general increase under water stress in tolerant (cv. Safran), moderate (cvs. Triomph, Nicola and Agria) and sensitive (cv. Diamant) but decreased under water stress in cvs. Universa and Bolista. SOD decreased in all cvs. under water stress, except cv. Diamant and Bolista (drought sensitive). However, CAT activities under water stress were higher than that of the control. CAT increased shapely in tolerant and moderate cvs., but decreased in the water stress sensitive ones.

In accordance with these results, Pino *et al*. (2013) and Heuer and Nadler (1998) reported increase proline contents under water stress in potato plantlet *in vitro*, similar to the finding of Azami *et al*. (2010) in tomato, but it is not necessarily be associated with the tolerance mechanism. Free amino acids and proline contents were also found to be higher in drought stress tolerant tomato line (Srivastava e*t al*., 1995).

The increase in antioxidant enzyme activities under water stress, including CAT, SOD, GPOX and peroxidase in water stress tolerant genotypes was previously reported by Daneshmand *et al*. (2010); Soni *et al*. (2011) and Yu-Jie *et al.* (2013). However, the decrease in SOD activity in our experiment was in contrast with the previous report. Perhaps CAT has more protective role against water stress than SOD in potato plantlet under the *in vitro* condition of our study.

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مسح معملي لعديد من التراكيب الوراثية المختلفة من البطاطس للإجهاد المائي باستخدام مستويات مرتفعة من الآجار في بيئة زراعة الأنسجة

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فيهذه الدراسة تم حصر وتقييم ٢٧ صنف وسلالة من البطاطس للتركيزات العالية من الآجار المضافة لبيئة زراعة الأنسجة كمصدر للإجهادالمائي. حيث قيمت استجابة الأجزاء النباتية المنزرعة (عقد بها برعم منفرد) لتركيزات الآجار المختلفة (٧-١٠ جم /لتر) وذلك على مستوى النمو النباتي والمقدرة على تكوين الدرينات. حيث أوضحت النتائج أنة بزيادة تركيز الآجار في بيئة الزراعة أنخفض طول النبات و عدد الجذور وكذلك عددالدرينات بدرجات مختلفة حسب الأصناف والسلالات المختبرة. أوضحت النتائج أن سفران وينفرسا كانت الأصناف الأكثر مقاومة للإجهادالمائيفي حين أن أصناف نيكولا و تريامف و أجريا قيمت من الأصناف متوسطة التحمل للجفاف الناتج عن الإجهادالمائي (آجار مرتفع) وكانت الأصناف الأكثر حساسية للجفاف هي ديامنت و بوليستا.

أظهرت النتائج أن صبغات التمثيل الضوئيانخفضت تحت ظروف التركيز المرتفع من الآجار في حين أن أعلى انخفاض وجد في صنف ينفرسا مقارنا بصنفى ديامنت و بولستا. كذلك أوضحت النتائج أن التركيزات الأعلى من البرولين و الأحماض الامينية وكذلك نشاط إنزيم الكتاليز وجدت في النباتات المعرضة للإجهادالمائي (١٠ جم/لتر آجار) مقارنا بالمعاملة الكنترول في حين أن نشاط أنزيم السوبر أوكسيد دسميوتز أنخفض في النباتات المعرضة للإجهادالمائي مقارنا بالكنترول. وبناءا على ما سبق فأن الأحماض الأمينية ونشاط أنزيم الكتاليز ترافقت ايجابيا مع ميكانيكية مقاومة أصناف البطاطس تحت الدراسة للإجهاد المائي.