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In Vitro Screening of Different Potato Genotypes for Heat Stress Tolerance

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ABSTRACT

In vitro screening of 30 potato genotypes for heat stress was conducted. *In vitro* potato plants were evaluated on the basis of their growth, microtuberization and biochemical analysis under different temperature treatments (12ºC, 24ºC and 30ºC). Both *in vitro* high (30ºC) or less than normal (12ºC) temperature degrees significantly affected plantlet length, number of nodes/plantlet and root number. Plantlet height at high temperature (heat stress) was significantly reduced (ave. 2.9 cm) while, less than normal temperature did not decrease plant height and was comparable to the control treatment (24ºC) (ave. 5.7 vs. 5.5 cm). In addition, microplants grown under 30ºC had significantly less both number of nodes and root number per plantlet in contrast to control condition, supporting the idea of the unfavorable effect of heat stress on potato growth. As an average over all tested genotypes, the number of microtuber/jar was significantly affected by temperature treatment. The lowest recorded number (ave. 1.44 microtubers) was observed when *in vitro* potato plants were grown under high temperature as a heat stress treatment. Potato genotypes were found to be significantly different in their microtuberization potential. Our results indicated profound significant interaction between *in vitro* temperature degrees and genotypes concerning microtuber formation and development. All genotypes produced microtubers at 24ºC and at 12ºC, while some genotypes were not able to form microtubers due to the heat stress exposure. On the other hand, the cvs. Fridor, Lady Rosetta, Agria and Picasso had higher microtuber number/ jar at 30ºC compared to the control or low temperature and may be considered as tolerant to high temperature stress. Under high temperature stress, all photosynthetic pigments were significantly lower than the control. At this high temperature, chl. a, b, total chl. and carotenoids represented 82.4%, 69.3%, 76.3% and 70.5% of the control, respectively. These results indicated that heat stress adversely affecting photosynthetic pigment contents compared with growth under normal or less than normal temperature degrees. Heat stresses significantly decreased total amino acids and proline contents; however it activates SOD and CAT enzymes. In current investigation, results showed heat stress significantly reduced tuberization in the majority of genotypes. However, few genotypes showed superior growth and microtuberization under heat stress condition and may be used as stock genetic material in breeding programs for producing elite potato genotypes adapted to heat stress.

Keywords: *Solanum tuberosum* L., cultivars, microtuberization, abiotic stress, total amino acids, superoxide dismutase, catalase.

INTRODUCTION

Abiotic stresses such as extreme temperature, drought and high salinity often result in significant yield losses of economically important crops. Plants constantly exposed to variable conditions have adapted at the molecular, cellular, physiological and biochemical level, enabling them to survive and cope with adverse environmental stresses. Genotypic selection for adaptation to such environments has already played an important role in agriculture. Recent scientific advances make exploration of these mechanisms more feasible and could result in large gains in productivity.

Examining the field performance of genotypes under a particular stress is the usual method for evaluation, but the results are often inconclusive. Field evaluation requires considerable space, time, labor, equipment and planting material resources (Arvin and Donnelly, 2008). In Egypt, several new potato cultivars have been introduced to farmers during the past decades, of these; more than 30 cultivars are micropropagated in the Plant Tissue Culture Lab., of the Horticulture Department, Suez Canal University which represent a valuable germplasm stock material to be evaluated against several abiotic stresses (El-Magawry, 2015).

High temperatures, which prevail during most of the year, are a major limitation of potato production in many developing countries (Dodds, 1990). However, potato cultivars or clones which are able to maintain relatively high yield at high temperatures have been identified in field trials (Malik *et al*., 1992), but less efforts were made to screen large number of potato genotypes under *in vitro* condition in particular with the growing concerns related to the global warming and temperature extreme fluctuation.

Screening to heat stress has been performed in 46 potato genotype in young *in vitro*-derived plants under 33/25ºC day/night temperature regime compared to control which grow under 20/10ºC day/night (Midmore and Prange, 1991). The results showed that in all tested genotypes, plants grow under heat stress had significantly lower values comparing with control plants for relative growth rate and net assimilation rate (Midmore and Prange, 1991). A number of heat tolerant potato clones have been obtained by Gamma-irradiation which was able to withstand growth and microtuber formation at 28ºC, however, microtuber under this temperature had distorted shape, and 60% of plants had abnormal leaf morphology while the control (heat sensitive) plants showed high percentage of damage leaves (Das *et al*., 2000).

Temperature is the single most important uncontrollable factor affecting growth and yield of potato (Levy and Veilleux, 2007). Studies of naturally

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occurring variation in heat stress responses aimed to uncover the nature of the cellular mechanism responsible for enhanced tolerance to high temperatures. Genotypic differences in the heat stress response and thermo-tolerance in four potato cultivars have been detected (Ahn *et al*., 2004). High temperature stress reduces total dry-matter accumulation in potatoes and diverts photosynthate from tubers to shoots, especially stems (Borah and Milthorpe, 1962; Ewing, 1981). Potato cultivars differ in heat tolerance with respect to yield (Ben-Khedher and Ewing, 1985; Levy, 1978).

Heat stress lower potato tuber yields through reduction in the net amount of photosynthate available for total plant growth or through reduced partitioning to the tubers. There are significant genetic differences in response to high temperatures in potato (Ewing, 1981). Under high ambient temperatures, yield losses in various genotypes ranged from 0-96% compared with yield under favorable conditions, indicating the extreme susceptibility of some genotypes to heat stress (Levy, 1986). When potato cultivars were evaluated for their heat tolerance, differences have been noted among the various genotypes. Tolerance to heat, as assessed by the capacity to form tubers under high temperatures, was genetically controlled and it appears that tolerance to heat is associated with earliness (Levy *et al*., 1991). Certain genotypes have the ability to initiate tubers at high temperature (Ewing, 1981) and yield loss is limited (Levy, 1986).

Crop plants experience high temperature stresses, which result in the formation of various reactive oxygen species (ROS) that responsible for the oxidative stress (Cadenas, 1989). The detoxification of ROS is of prime importance in any defence mechanism. Plants protect cell and sub-cellular systems from these ROS using antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) (Larson, 1988). Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzymes activity (Rui *et al*., 1990; Gupta *et al*., 1993a; Zhau *et al*., 1995).

Superoxide Dismutase is one of the vital enzymes in living organisms and plays a key role in cellular defence mechanisms against ROS (Alscher *et al*., 2002). Transgenic tobacco expressing Cu/Zn-SOD have been shown to tolerate chilling and heat stress (Gupta *et al*., 1993b). Transgenic *Arabidopsis* with Mn-SOD showed tolerance to heat (Im *et al*., 2009). Enhanced tolerance of transgenic potato plants expressing both SOD and ascorbate peroxidase against oxidative stress and high temperature has been observed (Tang *et al*., 2006).

Plant catalases play a role in stress tolerance against oxidative stress. Catalases are involved in eliminating hydrogen peroxide generated by different environmental stresses (Kim *et al*., 2008; Ahmad *et al*., 2010). Lopez-Delgado *et al.* (1998) found that high temperature stress (35ºC) reduced CAT in both adaptive and control plantlets of potato. Hertwig *et al*. (1992) have demonstrated that CAT was hampered at 40°C. Anderson (2002) showed that high temperature is responsible for the decrease in CAT activity in pepper plants. In comparison, the desert plant *Retama raetam* exposed to heat shock temperature showed only a minor inactivation of CAT activity (Streb *et al*., 1997). Scandalios *et al*. (2000) have also observed a reduced CAT activity in maize on exposure to elevated temperatures of 35-40°C. Also, in tomato, more proline was found in tissues of heat tolerant lines after exposure to super-optimum level of temperature (30/22ºC day/night) (Nautiyal *et al*., 2005).

Decrease in chlorophyll (chl.) content due to high temperature has been observed (Feirabend, 1977; Liv and Su, 1985). Bhullar and Jenner (1983) reported accelerated chl. degradation under high temperature stress. Chlorophyll biosynthesis in heat (42°C)-stressed cucumber seedlings was reduced by 60% (Tewari and Tripathy, 1998). In heat-bleached leaves of rye and oat, chl. synthetase activity was partially reduced by 40% (Hess *et al*., 1992). A shift in the ratio of chl. (a) and chl. (b) was observed in heat-bleached model organism *Euglena gracilis* (Thomas and Ortiz, 1995). In tomato, heat led to adaptation response of the photosynthesis pigment apparatus for the thermo-tolerant genotype, but not for sensitive genotype, and thus an increase in chl. a/b ratio and a decrease in chlorophyll/carotenoid ratio were shown in thermo-tolerant stressed plants (Camejo *et al*., 2005).

Current evidence suggests that, when plants are exposed to potentially harmful environmental conditions such as elevated temperatures, carotenoids (zeaxanthin) partition between the light-harvesting complexes and the lipid phase of membranes to increase its thermostability (Havaux, 1998). The exposure of potato plants to 35°C for 20 min significantly increased the stability of photosystem II to heat stress (Havaux, 1993). This rapid acclimation was attributed to the accumulation of zeaxanthin in the leaves, which stabilizes the lipid phase of membranes (Havaux, 1998). When potato transgenic plants were treated with high temperature (42°C for 20 h), the photosynthetic activity decreased by only 6%, whereas that of non- transgenic plants decreased by 29% (Tang *et al*., 2006).

The objectives of the present study were directed towards screening large number of newly introduced potato genotypes and breeding lines, as well as some potato genotypes already cultivated in Egypt for heat stress *in vitro*. Growth and microtuber formation under heat stress will be examined. Morphological, physiological and biochemical variations among stressed and unstressed genotypes will also be analyzed which finally will allow to group potato genotypes into tolerant, moderate, and sensitive to heat stress.

MATERIALS AND METHODS

The current investigation was conducted at the Plant Tissue Culture laboratory of the Horticulture Department, Suez Canal University, Ismailia, Egypt

during the period of 2013 and 2014. This experiment was conducted to study the effect of heat stress on morphological (shoot and root growth characters) and microtuber-forming capacity of different potato cultivars under *in vitro* conditions.

Plant materials and culture conditions

The experiment included 30 potato genotypes of three maturity groups, early, moderate, and late maturity genotypes. Six early genotypes (Safran, Margod, Universa, Alaska, Spunta and Elodi), eight mid-early genotypes (Triumph, Lady Rosetta, Nicola, Fridor, Naga, May Flower, Oceania and Diamant), one late maturity genotype (Agria) and seven potato lines (97-980, 94F, 97F-267, 94F-81.1, 96F-25-25, 95K-94 and 99-981), German varieties (Jelly, Presto and Marabel) and new locally-grown old cultivars (Picasso, Proventa, Arinda, Bolista and Santa).

The new locally-grown cultivars were kindly provided by The Vegetable Research Department, Horticulture Research Institute, Giza, Egypt. The newly introduced genotypes were kindly provided by the seed potato production support project, Ministry of Agriculture, Central Administration for Seed Testing and Certification funded by the French Food Aid Counterpart Fund.

Potato tubers from the different genotypes were cultivated in pots containing wet vermiculite under glasshouse conditions until sprouting. Sprout of 5 cm long were collected for sterilization with 10% commercial bleach (2.5% hypochlorite) for 10 min, and then washed with sterile distilled water three times in a laminar air flow hood. Meristem tip explants (0.3 mm) were excised from sprout shoot tips under binuclear microscope. Cultures were incubated at 25±2°C with 16/8 h day/night at 40 µmol m^{-2} s^{-1} photon flux density (cool white fluorescent light). For micropropagation, MS (Murashige and Skoog, 1962) basal salts and vitamin (Duchefa Biochemi, Netherlands) was used, supplemented with 3% sucrose and 0.7% agar. The medium pH was adjusted to 5.8 before the addition of agar.

In vitro grown plants were propagated by sub-culture with 4 weeks interval for three sub-cultures before starting the experiment. Ten single node explants (about 1 cm) were sub-cultured into 350 ml ca. jars containing 30 ml MS medium. The proliferated cultures of 4 weeks old and approximately 10 cm long, avoiding the top and bottom node segments were used as the starting materials for subsequent trials. Media were sterilized by autoclaving at 121ºC and 1.05 kg/cm^2 for 20 min., and then dispensed into the tissue culture jars.

Plantlet growth under heat stress

The study utilized 30 cultivars and breeding lines under heat stress. Two nodes (1cm long) were cultured per test tube containing 20 ml MS medium with 30 g l^{-1} sucrose and no hormones. Test tubes were kept either in incubator at 12ºC and 30ºC (heat stress) for all genotypes. The third treatment was growth under 24±1 ºC as a control. The pH of the media was adjusted to 5.7 ± 0.1 before being solidified with 7 g l^{-1} agar. After 6 weeks, 2 plantlets were taken from each genotype × temperature treatment combination and data were taken on plantlet length, number of nodes, root number, fresh and dry weight/plantlet.

Microtuberization under low and high temperature stress

Fifteen ml sterilized liquid MS media amended with high sucrose level (80 g l^{-1}) were added to each test tube containing the growing plantlets after removal of the test tube cap in a laminar air-flow hood. Cultures were incubated in the dark for 2 months under (12ºC), (30ºC) and control (24±1ºC). Microtubers produced from each treatment were harvested after 8 weeks and data were taken on microtuber diameter, number and weight (yield) of microtuber/jar and the average single microtuber weight were calculated by dividing weight/ number.

Biochemical analysis of potato plants grown *in vitro* under heat stress

The plantlet samples (weight 0.1-0.4 g) were weighed and stored at -20ºC, and then processed as described in Ni *et al*. (2001). Briefly, the enzymes from the frozen plant sample were extracted using cold potassium phosphate buffer (0.1 M, pH 7.0) containing 1% (w/v) polyvinylpyrrolidone and 1% (v/v) Triton X-100. Two ml of the extracting buffer was used for each sample. An aliquot (1.5 ml) of the extract was centrifuged at 10 000 g for 10 min at 4°C. The supernatant was immediately frozen for future enzyme activity assays.

Free total amino acids determination

Total amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). The reaction mixture consists of 1 ml sample and 1.9 ml ninhydrin solution in 0.5 M citrate buffer (pH 5.5); 0.2 ml of 0.5 M citrate buffer (pH 5.5) and 1.2 ml glycerol. The mixture was heated in a boiling water bath 10 min and cooled in a tap water bath. The developed color was read at 570 nm. The amino acids were expressed as µg alanine per g fresh weight. Proline was estimated using the method described by Sadasivam and Manickam (1991). Superoxide dismutase level was determined by using biodiagnostic kit No.MD2529 which is based on the spectrophotometric (U/V Spectrophotometer spectronic 1201, Milton Roy, U.S.A) method of Nishikimi et al. (1972). Catalase activity was measured using biodiagnostic kit NO.CA 2517 which is based on the spectrophotometric method described by Aebi (1984). The amount of photosynthetic pigments (chlorophyll a, b, total and carotenoids) was determined according to the method of Lichtenthaler (1987) .

Statistical analysis

The experiment was conducted twice with five replicates each, using a Randomized Complete Block Design in factorial fashion. Data were combined and subjected to ANOVA using CoStat-software program (CoHort Software, Monterey, CA, USA) and the means were separated by Least Significant Differences (LSD) at probability level= *0.05*.

RESULTS

The present work included experiment on the effects of *in vitro* heat stress on the growth and microtuber induction and development in large number of potato genotypes. Plant growth performance was examined based on plantlet length, number of nodes/plantlets, and rooting characteristics. In the same solid medium, liquid microtuber induction medium was added. High temperature (30ºC) was tested against normal laboratory temperature (24ºC) and less than normal temperature (12ºC) in factorial approach in RCBD to show the main and interaction effects of the heat by genotypes. Screening the large number of potato genotypes depended on the relative growth performance to the control treatment. Morphological, physiological and biochemical characteristics, including photosynthetic pigment contents and proline, free amino acids and antioxidant enzymes, were analyzed. Thirty potato genotypes were examined for their *in vitro* plantlet growth and tuberization under different temperature regimes (30ºC, 24ºC and 12ºC).

Differential effects of temperature on plantlet growth of potato genotypes:

Both high (30ºC) or less than normal (12ºC) temperature degrees significantly affected plantlet length, number of nodes/plantlet and root number (Tables 1-3, Fig. 1). As averaged over all genotypes, the difference in plant height was found significant among the tested temperature degrees (Table 1). Plant height at high temperature (30ºC) (heat stress) was significantly reduced (ave. 2.9 cm) while, less than normal temperature (12ºC) did not decrease plant height and was comparable to the control treatment (24ºC) (ave. 5.7 vs. 5.5 cm). In addition, plants grown under 30 ºC had significantly less number of nodes per plantlet (ave. 4.8 cm) in contrast to plants grown under less than normal temperature which produced number of nodes per plantlet not significantly different than those under the control (7.7 vs. 8.0 cm) (Table 2). Similarly, regarding root number per plantlet, plants grown under 30 ºC had significantly less number (ave. 0.45 root) compared with plants grown under control (24ºC) (ave. 2.3 root) or under less than normal temperature (12ºC) (ave. 3.3 roots) (Table 3).

Difference among potato genotypes were detected in several growth and rooting characters (Tables 1-3, Fig. 1). With respect to plantlet height (Table 1), the cv. Arinda recorded the highest length of plantlet (7.6 cm), followed by Picasso and Nicola while the least heights were recorded in genotypes 99.981 (1.617 cm), 97F.267 (2.0 cm) and Santa (ave. 2.7 cm). Concerning the number of nodes per plantlet, the cv. Fridor recorded the highest number (ave. 11.2) followed by Naga (9.6), Universa (9.4), Proventa (8.6) and Arinda (8.5). The least numbers of nodes were recorded on genotypes 99.985, 97.980 and 97F.267, showing between 3.9 -4.6 nodes per plantlet (Table 2).

Genotypic difference in root number per plantlet was also detected (Table 3). The average number of roots in cv. Picasso was (ave. 3.97 roots/ plantlet), followed by cv. Naga (3.2 roots) and cv. Proventa (3.1 roots). The least rooting formation was found in cvs. Marabel, Presto and lines 97F.267, 99-98, as well as in cvs. Oceana and Lady Rosetta (Table 3).

The interactions between different temperature degree and genotypes were significant in all three recorded growth characters. The temperature degree and genotypes interaction significantly affected plantlet length (Table 1). The highest plantlet height was recorded on cv. Nicola at 24ºC (ave. 10.2 cm), followed by Proventa (9.0 cm) and 94F (9.05 cm). However, shoot length was higher in cv. Margod at 30 ºC than the control or at 12ºC. The other cvs. (Alaska, Triumph, Elodi, 95K, Agria, 97-980) had higher plantlet length at reduced temperature treatment (12ºC) than the control (24ºC) or high temperature (30ºC). Numbers of nodes were also affected by the interaction between different temperature degrees and genotypes (Table 2). The highest no. of nodes/plantlet was observed in cv. Fridor (15.1 nodes), followed by Nicola (12.7), Bolista (12.9) at 24ºC. However, in cvs. Margod, Safran, Elodi, Naga and Marabel, number of nodes were higher at 12ºC than the control. Under heat stress (30ºC), nodes number were higher (ave. 8.2 nodes/plantlet) than at 24ºC or 12ºC (ave. 5.9 nodes). Rooting was also affected by the interaction between different temperature degree and genotypes (Table 3). The highest root number/ plantlet was found in cv. Naga (6 roots/plantlet) at 12ºC, followed by Picasso (ave. 5.2 roots) at 24ºC, and Margod at 12ºC (ave. 5.4 roots), while no roots were formed in the other 16 genotypes at 30ºC (Table 3).

Differential effects of temperature on microtuberization of potato genotypes:

The three tested temperature degrees significantly affected the *in vitro* microtuberization of potato (Tables 4-6, Fig. 2). As an average over all tested genotypes, the number of microtuber/jar was significantly affected by temperature treatment (Table 4). The lowest recorded number (ave. 1.44 microtubers) was observed when *in vitro* potato plants were grown under high temperature (30ºC) as a heat stress treatment. Unexpectedly, at less than normal temperature (12ºC), the number of microtubers was significantly higher (ave. 2.88 microtuber/jar) than under control condition (24ºC) (ave. 2.2 microtuber). In accordance with the previous results, average microtuber weight was higher at 12ºC

(ave. 204 mg/tuber) when compared to the microtuber weight obtained under 24ºC or 30ºC (ave. 71.0 mg) (Table 5). Heat stress also affected microtuber diameter as microtuber diameter was higher at 12ºC (ave. 5.0 mm) than the control (ave. 3.0 mm) or at high temperature (30ºC) (Tale 6).

Table (1): Effect of *in vitro* heat stress on shoot (plantlet) length in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	Plantlet length (cm)					
Safran	4.400	4.000	1.200	27.273	30.000	3.200
94F-81.1	5.600	7.900	4.900	87.500	62.025	6.133
Margod	5.000	4.950	6.150	123.00	124.242	5.367
Universa	6.100	6.050	4.150	68.033	68.595	5.433
Alaska	6.550	4.050	3.050	46.565	75.309	4.550
Spunta	5.700	5.250	2.900	50.877	55.238	4.617
Elodi	5.650	4.100	3.500	61.947	85.366	4.417
96F-25-25	5.250	5.550	5.450	103.810	98.198	5.417
Triumph	7.150	5.500	2.700	37.762	49.091	5.117
Lady Rosetta	6.300	4.090	2.250	35.714	55.012	4.213
95K-94	8.450	5.000	3.200	37.870	64.000	5.550
Oceania	3.350	4.600	3.450	102.985	75.000	3.800
Nicola	8.250	10.200	1.100	13.333	10.784	6.517
Fridor	7.100	8.000	3.850	54.225	48.125	6.317
Naga	6.800	7.800	2.050	30.147	26.282	5.550
May Flower	4.050	5.300	1.200	29.630	22.642	3.517
Diamant	3.400	4.850	0.500	14.706	10.309	2.917
Agria	5.200	4.200	0.750	14.4236	17.857	3.383
99-981	2.900	1.450	0.500	17.2416	34.483	1.617
97-980	5.650	4.700	1.600	28.319	34.043	3.983
94F	5.950	9.050	2.450	41.177	27.072	5.817
Jelly	6.050	6.050	1.500	24.794	24.793	4.533
Presto	3.050	5.500	2.200	72.131	40.000	3.583
Marabel	4.000	3.050	2.450	61.250	80.328	3.167
97F-267	2.100	2.900	1.150	54.762	39.655	2.050
Picasso	8.300	8.200	4.950	59.6386	60.366	7.150
Proventa	7.650	9.000	2.300	30.0656	25.556	6.317
Arinda	7.900	8.600	6.500	82.279	75.581	7.667
Bolista	3.850	7.050	6.350	164.935	90.071	5.750
Santa	3.850	2.500	1.500	38.961	60.000	2.617
Mean Temp.	**5.518**	**5.648**	**2.860**			
Cultivars LSD 0.05				**1.000**		
Temperature LSD 0.05				**0.330**		
Interaction LSD 0.05				**1.727**		

Under the conditions of this study, potato genotypes were found to be significantly different in their microtuberization potential (Tables 4-6, Fig. 2). As an average over the tested temperature degrees, cv. Nicola (ave. 5.56 microtuber/jar), followed by the potato line 95K-94 (ave. 5.0 microtuber), and cv. Picasso (ave. 4.06 microtubers) showed the highest significant average microtuber number (Table 4).

Moderate numbers of microtubers were detected in cvs. Universa, 96f 25-25, Fridor, and Arinda (between 3.2-3.5 microtuber/jar). Finally, the cvs. Santa and line 94F recorded the least number of microtubers/ jar (< 1.0) (Table 4). Average microtuber weight was at the highest point in cvs. Universa and Picasso, followed by Fridor and Arinda (ave. 287-342 mg per tuber). The least microtuber weight was recorded in cvs. Lady Rosetta, May Flower, 99-981, 94F and Proventa (21-51 mg) (Table 5).Genotypic differences in microtuber size were also observed (Table 6). As tested over all temperature treatments, the average microtuber diameter was the highest in cv. Picasso (ave. 7.22 mm), followed by line 96F. 25.25 (6.11 mm), Universa (5.58 mm) and Fridor (5.38 mm), while genotypes 97.980, 94F, Jelly and Santa recorded the least microtuber diameter (1.16-2.83 mm). Our results indicated profound significant interaction between *in vitro* temperature degrees and genotypes concerning microtuber formation and development (Tables 4-6). With regard to microtuber number/jar, it was found that all genotypes produced microtubers at 24ºC and at 12ºC, while some genotypes were not able to form microtubers due to the Oceana, May Flower, 99-981, 97-980, Jelly, 97-F-267 and Santa (Table 4). Several genotypes produced more microtuber at 1ºC (18 genotypes) than at 24ºC, while other (8

genotypes) had higher microtuber number at 24ºC than at 12ºC, including Spunta, Lady Rosetta, Oceana, Diamant, Jelly, Presto, Proventa and Bolista. On the other hand, cvs. Fridor, Lady Rosetta, Agria and Picasso had higher microtuber number/ jar at 30 ºC compared to the control (24ºC) or low temperature (12ºC) (Table 4)

Table (2): Effect of *in vitro* heat stress on number of nodes per plantlet in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	No. of nodes/plantlets					
Safran	8.800	7.700	4.300	48.864	55.844	6.933
94F-81.1	5.900	5.900	8.200	138.9834	138.983	6.667
Margod	10.100	6.300	6.500	64.3564	103.175	7.633
Universa	10.800	10.800	6.600	61.1114	61.111	9.400
Alaska	7.700	5.000	2.600	33.7664	52.000	5.100
Spunta	7.000	6.900	5.400	77.143	78.261	6.433
Elodi	10.900	6.900	5.700	52.294	82.609	7.833
96F-25-25	7.400	5.700	5.500	74.324	96.491	6.200
Triumph	9.600	7.100	4.800	50.000	67.606	7.167
Lady Rosetta	8.900	5.400	4.100	46.067	75.926	6.133
95K-94	8.900	6.000	3.700	41.573	61.667	6.200
Oceania	6.400	6.800	5.000	78.125	73.529	6.067
Nicola	11.900	12.700	2.200	18.487	17.323	8.933
Fridor	11.600	15.100	7.000	60.345	46.358	11.233
Naga	12.000	11.400	5.500	45.833	48.246	9.633
May Flower	6.200	6.000	4.200	67.742	70.000	5.467
Diamant	8.800	4.700	3.500	39.773	74.468	5.667
Agria	7.800	7.300	2.900	37.180	39.726	6.000
99-981	5.000	3.900	3.000	60.000	76.923	3.967
97-980	4.000	6.500	3.200	80.000	49.231	4.567
94F	7.200	7.700	3.500	48.611	45.455	6.133
Jelly	7.700	8.000	3.400	44.156	42.500	6.367
Presto	4.200	7.800	4.800	114.286	61.538	5.600
Marabel	7.900	5.600	4.300	54.430	76.786	5.933
97F-267	5.100	4.600	4.400	86.275	95.652	4.700
Picasso	9.500	11.100	7.400	77.895	66.667	9.333
Proventa	8.500	9.700	7.500	88.235	77.320	8.567
Arinda	9.100	9.500	7.150	78.571	75.263	8.583
Bolista	5.400	12.900	3.900	72.222	30.233	7.400
Santa	6.200	6.800	3.900	62.903	57.353	5.633
Mean Temp.	**8.037**	**7.727**	**4.785**			
Cultivars LSD 0.05				**1.564**		
Temperature LSD 0.05				**0.512**		
Interaction LSD 0.05				**2.702**		

Average microtuber weight was also significantly affected by interaction between *in vitro* temperature degrees and genotypes (Table 5). Under less than favorable temperature (12ºC), the cv. Universa recorded the highest microtuber weight (ave. 869 mg/ tuber), followed by Triumph (ave. 611 mg) and Arinda (527 mg), while, at heat stress (30ºC), the cv. Fridor had more microtuber weight (ave. 549 mg) than at low (12ºC) temperature, or the control. In contrast, some genotypes had higher microtuber fresh weight at 24ºC than at 12ºC, which includes Diamant, Agria, 99-981, Presto, Jelly, 97F.267, Proventa and Bolista (Table 5).

Significant interaction between *in vitro* temperature degrees and genotypes was detected regarding microtuber diameter (Table 6). The potato line 94F recorded the highest microtuber diameter (ave. 7.0 mm) when experienced growth under heat stress (30ºC), which was significantly higher than at 24ºC (4.8 mm) or 12ºC (6.5 mm) for this genotypes. On the other hand, the highest microtuber size was found in cv. Triumph (ave. 8.5 mm), followed by Picasso (8.0 mm) at 12ºC, while at 24ºC, the highest diameter was recorded on line 99-981 (8.16 mm), followed by cv. Jelly (ave. 7.83 mm).

Classification of potato genotypes into heat sensitive vs. tolerant groups

According to the results, the 30 potato genotypes were ranked and grouped for heat tolerance based on performance at high temperature stress as % of control (Table 7). Based on plantlet height at high temperature stress as % of control, cvs. Margod, 96F-25-25 and Bolista ranked the highest three genotypes, while, cvs. Agria, Nicola and Diamant ranked the lowest three genotypes (Table 7). When potato genotypes were ranked based on microtuber number as % of control,

Table (3): Effect of *in vitro* heat stress on root number per plantlet in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	Root no./plantlets					
Safran	3.600	2.600	0.400	11.111	15.385	2.200
94F-81.1	2.700	3.100	1.400	51.852	45.161	2.400
Margod	5.400	1.800	1.700	31.482	94.444	2.967
Universa	3.600	3.100	1.100	30.556	35.484	2.600
Alaska	3.800	0.400	0.200	5.263	50.000	1.467
Spunta	2.900	1.700	0.000	0	0.000	1.533
Elodi	4.300	3.300	1.100	25.58	33.333	2.900
96F-25-25	3.800	1.000	0.000	0	0.000	1.600
Triumph	4.500	2.100	0.000	0	0.000	2.200
Lady Rosetta	1.900	0.600	0.000	0	0.000	0.833
95K-94	5.500	2.000	0.000	0	0.000	2.500
Oceania	1.200	0.400	1.000	83.333	250.000	0.867
Nicola	3.500	4.200	1.000	28.571	23.810	2.900
Fridor	3.600	2.800	0.600	16.667	21.429	2.333
Naga	6.000	3.400	0.200	3.333	5.882	3.200
May Flower	4.600	2.400	0.000	0	0.000	2.333
Diamant	3.500	0.300	0.000	0	0.000	1.267
Agria	2.700	0.600	0.000	0	0.000	1.100
99-981	1.500	1.000	0.000	0	0.000	0.500
97-980	2.400	0.700	0.300	12.500	42.857	1.133
94F	2.400	3.900	0.000	0	0.000	2.100
Jelly	2.800	3.500	0.000	0	0.000	2.100
Presto	1.000	1.400	0.000	0	0.000	0.467
Marabel	1.500	1.000	0.000	0	0.000	0.500
97F-267	1.100	1.000	0.000	0	0.000	0.367
Picasso	4.300	5.200	2.400	55.814	46.154	3.967
Proventa	4.100	3.700	1.500	36.585	40.541	3.100
Arinda	3.700	3.500	0.700	18.919	20.000	2.633
Bolista	1.900	4.900	0.000	0	0.000	2.267
Santa	4.200	2.000	0.000	0	0.000	2.067
Mean Temp.	**3.267**	**2.253**	**0.453**			
Cultivars LSD 0.05				**0.734**		
Temperature LSD 0.05				**0.240**		
Interaction LSD 0.05				**1.269**		

cvs. Agria, Universa and Fridor ranked the highest three genotypes, while, cvs. Jelly, Santa and May Flower ranked the lowest three genotypes (Table 7).

Differential effects of temperature on pigment contents of potato genotypes

Analysis of variance results indicated that all tested photosynthetic pigments were significantly affected by the *in vitro* growth temperature, as shown in Table (8). Under high temperature stress (30ºC) all pigments were significantly lower than the control. At this high temperature, chl. a, b, total chl. and carotenoids represented 82.4%, 69.3%, 76.3% and 70.5% of the control (24ºC), respectively. With regard to low temperature (12ºC), the chl. a, b and total chl., as well as carotenoids were significantly decreased as compared to the control (24ºC). Pigment contents at 12ºC represented 89.1%, 78.8%, 85.4% and 87.8% of the control, for chl. a, b, total chl. and carotenoids, respectively.

As an average over the tested temperatures, results show significant differences among potato genotypes on pigment contents (Table 8). The cv. Bolista, followed by Safran recorded the highest chl. a, b and total chl. The least chl. content was recorded in cv. Nicola. Carotenoids were the highest in cv. Triumph, followed by Universa and Nicola while cv. Agria showed the least value.

The interaction between *in vitro* temperature degrees and genotypes were found significant for all examined

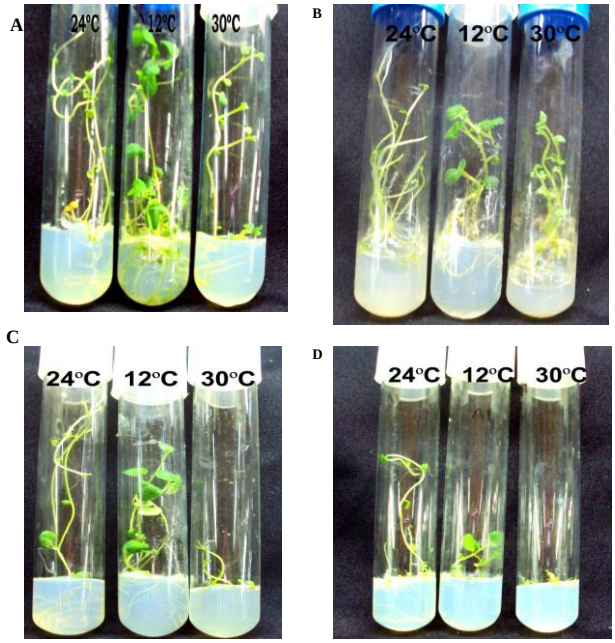

Figure (1): *In vitro* shoot growth of potato under different temperature regimes (24°C, 12°C, and 30°C). A: tolerant to heat stress cv. (Margod), B: tolerant to heat stress cv. (Oceania), C: sensitive to heat stress cv. (Nicola), D: sensitive to heat stress cv. (Bolista).

pigments (Table 8). Under high temperature treatment, chl. a, b and total chl. were significantly less at 30ºC than at 24ºC in all genotypes (Table 8). Total chl. content, as % of control were 85.7%, 88.1%, 82.0%, 83.7%, 69.6%, 61.9% and 75.0% in cvs. Safran, Universa, Triumph, Nicola, Diamant, Agria and Bolista, respectively. Carotenoids under 30ºC were also less than the control in several genotypes (Table 8).

The cv. Bolista had the highest chl. a, b and carotenoids at 24ºC, followed by Safran. Under low growth temperature (12ºC), three cvs. had less chl. a than control, viz, Safran, Universa and Bolista (70.8%, 94.3% and 77.0% of the control), respectively. However, cv. Nicola and Diamant at 12ºC had more chl a. than control, while in cv. Triumph, chl. a was not significantly changed compared to the control treatment (Table 8). Regarding chl. b, it was also lower than the control in cvs. Safran, Universa, Diamant, Agria and Bolista, but higher than the control in cv. Nicola. Carotenoids under low temperature (12ºC) were not significantly different than control in cvs. Safran and Triumph, while it was less than the control temperature in cvs. Universa, Diamant, Agria and Bolista (73.6%,

91.3% and 45.7% of the control), respectively. Only cv. Nicola recoded higher carotenoids than the control (Table 8).

Differential effects of temperature on contents of amino acids and proline and activities of SOD and CAT of potato genotypes

Heat stresses significantly decreased total amino acids and proline contents; however it activates SOD and CAT enzymes (Table 9, Fig. 3). Growth under heat stress (30ºC) resulted in potato tissues with 94.1% of free amino acids content relative to growth below 24ºC. Proline, SOD, and CAT under 30ºC were 71.5%, 98.4% and 88.7% of the control, which demonstrate that proline and CAT decreased more than SOD and total amino acids under high temperature stress (30ºC). Under below normal temperature (12ºC) the % amino acid contents (as relative to control) were 76.3%, while it was 64.1%, 92.6% and 79.9% for proline, SOD and CAT, which reveal that, SOD declined less than proline, total amino acids and CAT at less than favorable temperature. Results indicated that cv. Bolista, followed by Diamant and Agria showed higher free amino acids than Safran, Universa, Triumph and Nicola. Bolista also recorded higher proline, followed by Agria, Nicola, Diamant, while the least proline was detected in cvs. Safran, Universa (Table 9, Fig. 3). In contrast, the cv. Safran, followed by Universa, Triumph and Nicola had higher SOD than Bolista, Agria and Diamant. CAT activity was the highest in cv. Bolista, followed by Agria, Diamant and Safran, while it was the least in cvs. Triumph and Universa

Significant interaction between *in vitro* temperature degrees and genotypes has been detected by ANOVA for the content and activities of proline, free amino acids and antioxidant enzymes (Table 9). Under high temperature stress (30ºC), shoot tissue of cv. Safran showed higher CAT activity, but lower SOD, free amino acids and proline than the control. At 30ºC, free amino acids were lower than at 24ºC in cvs. Safran, Triumph, Diamant, Agria and Bolista. Proline contents were higher than control in cvs. Universa, Triumph and Diamant. CAT activity was higher at 30ºC compared to 24 ºC in cv. Safran, Universa and Bolista. Additionally, under 12ºC, total free amino acids, proline, SOD and CAT were less than the control (24ºC) in cv. Safran, while in cv. Universa, total free amino acids and SOD were higher than the control temperature. In cv. Triumph, SOD was higher at low temperature, while CAT was equal to the control. Proline and free amino acids were less than at 24ºC

Significant interaction between *in vitro* temperature degrees and genotypes has been detected by ANOVA for the content and activities of proline, free amino acids and antioxidant enzymes (Table 9). Under high temperature stress (30ºC), shoot tissue of cv. Safran showed higher CAT activity, but lower SOD, free amino acids and proline than the control. At 30ºC, free amino acids were lower than at 24ºC in cvs. Safran, Triumph, Diamant, Agria and Bolista. Proline contents were higher than control in cvs. Universa, Triumph and Diamant. CAT activity was higher at 30ºC compared to 24 ºC in cv. Safran, Universa and Bolista. Additionally, under 12ºC, total free amino acids, proline, SOD and

CAT were less than the control (24ºC) in cv. Safran, while in cv. Universa, total free amino acids and SOD were higher than the control temperature. In cv. Triumph, SOD was higher at low temperature, while CAT was equal to the control. Proline and free amino acids were less than at 24ºC.

Table (4): Effect of *in vitro* heat stress on average microtuber number /jar in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	Average microtuber no./jar					
Safran	2.667	1.667	1.000	37.495	60.000	1.778
94F-81.1	2.667	1.667	1.000	37.495	60.000	1.778
Margod	2.333	2.000	1.000	42.863	50.000	1.778
Universa	6.000	1.333	2.333	38.883	175.000	3.222
Alaska	3.000	2.333	1.000	33.333	42.857	2.111
Spunta	2.000	3.000	2.000	100.000	66.667	2.333
Elodi	1.667	1.000	1.000	59.988	100.000	1.222
96F-25-25	6.000	3.000	1.000	16.667	33.333	3.333
Triumph	4.333	1.000	1.000	23.079	100.000	2.111
Lady Rosetta	1.333	2.000	2.667	200.075	133.333	2.000
95K-94	8.333	4.000	2.667	32.005	66.667	5.000
Oceania	3.667	5.000	0.000	0	0.000	2.889
Nicola	9.000	4.667	3.000	33.333	64.286	5.556
Fridor	2.667	3.000	5.000	187.477	166.667	3.556
Naga	4.667	1.000	1.000	21.427	100.000	2.222
May Flower	1.000	1.000	0.000	0	0.000	0.667
Diamant	2.333	3.500	1.000	42.863	28.571	2.278
Agria	1.000	1.667	4.000	400.000	240.000	1.889
99-981	1.000	2.000	0.000	0	0.000	1.000
97-980	2.000	1.333	0.000	0	0.000	1.111
94F	1.333	1.000	1.000	75.0188	100.000	0.778
Jelly	1.000	4.667	0.000	0	0.000	1.556
Presto	1.000	2.667	1.000	100.000	37.500	1.556
Marabel	2.333	1.000	1.000	42.863	100.000	1.444
97F-267	3.000	1.000	0.000	0	0.000	1.333
Picasso	4.000	3.167	5.000	125.000	157.895	4.056
Proventa	1.000	2.000	1.000	100.000	50.000	1.000
Arinda	5.333	2.000	2.667	50.009	133.333	3.333
Bolista	1.333	3.333	1.000	75.018	30.000	1.889
Santa	1.333	1.000	0.000	0	0.000	0.778
Mean Temp.	**2.978**	**2.267**	**1.444**			
Cultivars LSD 0.05				**0.969**		
Temperature LSD 0.05				**0.373**		
Interaction LSD 0.05				**2.045**		

DISCUSSION

Abiotic stress is considered to be the most serious growth-limiting factor for potato crop (Boyer, 1982; Vinocur and Altman, 2005). Heat stress due to increased temperature affects plant growth and development and may lead to a severe reduction in yield. In addition, it may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for the start of the season and crop maturity to reach earlier (Porter, 2005). The adverse effects of heat stress can be mitigated by developing crop plants with improved thermotolerance using various genetic approaches. Tremendous variation within and between species is existed, providing opportunities to improve crop heat-stress tolerance through genetic means. The use of genetic stocks with different degrees of heat tolerance is promising approach to understand the genetic basis of thermotolerance. Most commercially important cultivars of potato have been developed in temperate climates however, due to the current expansion of potato cultivation to subtropical, semiarid and arid regions, the need to explore and identify heat-tolerant cultivars is mandatory (Veilleux *et al*., 1997).

An important prerequisite of successful breeding program is a reliable method of screening to identify genotypes tolerant to high temperatures. Various screening procedures for the selection of heat-tolerant types have been employed. Field screening trials inherently carry some major confounding effects, such as unpredictable weather, variability in soil type, moisture and mineral distribution, disease incidence, etc., and can usually accommodate only a limited number of clones (Tai *et al*., 1994). *In vitro* tuber formation, or microtuberization, under elevated

Table (5): Effect of *in vitro* heat stress on average microtuber weight in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	Average microtuber weight / jar(mg)					
Safran	0.437	0.027	0.110	25.172	415.075	0.191
94F-81.1	0.437	0.027	0.110	25.172	415.075	0.191
Margod	0.054	0.006	0.010	18.519	160.104	0.023
Universa	0.869	0.020	0.117	13.464	576.733	0.335
Alaska	0.134	0.062	0.032	23.881	52.592	0.076
Spunta	0.201	0.103	0.084	41.791	82.250	0.129
Elodi	0.066	0.067	0.065	98.485	96.466	0.066
96F-25-25	0.227	0.114	0.015	6.608	13.322	0.119
Triumph	0.611	0.008	0.031	5.074	398.305	0.217
Lady Rosetta	0.030	0.066	0.057	190.000	87.583	0.051
95K-94	0.273	0.008	0.029	10.623	352.191	0.104
Oceania	0.160	0.036	0.000	0	0.000	0.066
Nicola	0.250	0.074	0.129	51.600	173.041	0.151
Fridor	0.194	0.089	0.549	282.99	613.908	0.278
Naga	0.244	0.007	0.058	23.771	882.653	0.103
May Flower	0.037	0.025	0.000	0	0.000	0.021
Diamant	0.017	0.068	0.006	35.294	8.120	0.030
Agria	0.009	0.031	0.149	1655.556	478.648	0.060
99-981	0.028	0.035	0.000	0	0.000	0.021
97-980	0.180	0.009	0.000	0	0.000	0.063
94F	0.087	0.009	0.052	59.770	574.444	0.046
Jelly	0.009	0.219	0.000	0	0.000	0.073
Presto	0.005	0.088	0.007	140.000	7.625	0.033
Marabel	0.235	0.002	0.026	11.064	1120.143	0.088
97F-267	0.092	0.179	0.000	0	0.000	0.090
Picasso	0.591	0.284	0.149	25.212	52.332	0.342
Proventa	0.009	0.037	0.034	377.778	94.080	0.024
Arinda	0.527	0.051	0.294	55.788	580.921	0.291
Bolista	0.039	0.245	0.004	10.256	1.813	0.096
Santa	0.082	0.148	0.000	0	0.000	0.077
Mean Temp.	**0.205**	**0.071**	**0.071**			
Cultivars LSD 0.05				**0.080**		
Temperature LSD 0.05				**0.027**		
Interaction LSD 0.05				**0.146**		

Table (6): Effect of *in vitro* heat stress on average microtuber diameter in 30 potato cultivars.

CV.	Temperature					Mean cv.
	12°C	24°C	30°C	% of at 30°C/ at 12°C	% of at 30°C/ at 24°C	
	Average microtuber diameter/jar (cm)					
Safran	6.000	4.333	4.333	72.217	100.000	4.889
94F-81.1	6.000	4.333	4.333	72.217	100.000	4.889
Margod	3.500	2.110	2.333	66.657	110.585	2.648
Universa	5.887	6.000	5.167	87.770	86.111	5.684
Alaska	7.667	3.833	2.833	36.951	73.913	4.778
Spunta	5.500	4.553	2.000	36.364	43.924	4.018
Elodi	6.333	5.000	3.667	57.903	73.333	5.000
96F-25-25	6.500	4.833	7.000	107.69	144.828	6.111
Triumph	8.500	3.000	4.833	56.859	161.111	5.444
Lady Rosetta	2.500	4.667	2.833	113.320	60.714	3.333
95K-94	6.953	6.167	1.667	23.975	27.027	4.929
Oceania	7.167	5.833	0.000	0	0.000	4.333
Nicola	6.207	2.667	3.167	51.023	118.750	4.013
Fridor	6.500	4.320	5.333	82.046	123.457	5.384
Naga	5.833	2.333	5.167	88.582	221.429	4.444
May Flower	5.000	6.167	0.000	0	0.000	3.722
Diamant	5.500	4.667	2.000	36.364	42.857	4.056
Agria	1.000	4.833	4.167	416.700	86.207	3.000
99-981	5.500	8.167	0.000	0	0.000	4.556
97-980	2.000	1.500	0.000	0	0.000	1.167
94F	4.333	1.000	3.500	80.775	350.000	2.611
Jelly	1.000	7.833	0.000	0	0.000	2.611
Presto	2.000	5.833	3.500	175.000	60.000	3.778
Marabel	4.000	3.000	3.000	75.000	100.000	3.333
97F-267	6.000	5.333	0.000	0	0.000	3.778
Picasso	8.000	6.167	7.500	93.750	121.622	7.222
Proventa	1.000	4.000	4.833	483.300	120.833	2.944
Arinda	5.667	3.000	5.667	100.000	188.889	4.778
Bolista	3.167	4.833	2.500	78.939	51.724	3.500
Santa	4.833	3.667	0.000	0	0.000	2.833
Mean Temp.	**5.002**	**4.466**	**4.433**			
Cultivars LSD 0.05				**0.828**		
Temperature LSD 0.05				**0.309**		
Interaction LSD 0.05				**1.694**		

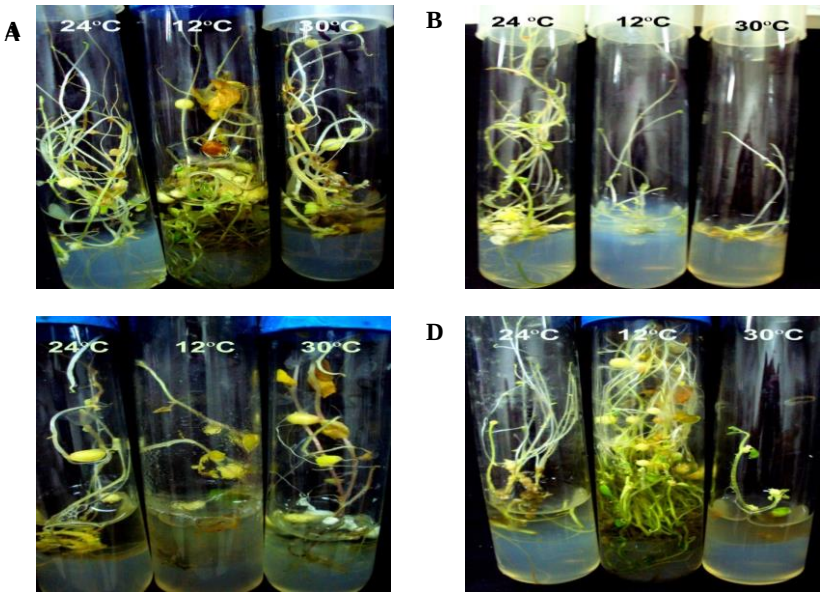

Figure (2): *In vitro* microtuber of potato under different temperature regimes (24°C, 12 °C, and 30°C). A: tolerant to heat stress cv. (Universa), B: sensitive to heat stress cv. (Agria), C: tolerant to heat stress cv. (Safran), D: sensitive to heat stress cv. (Triumph)

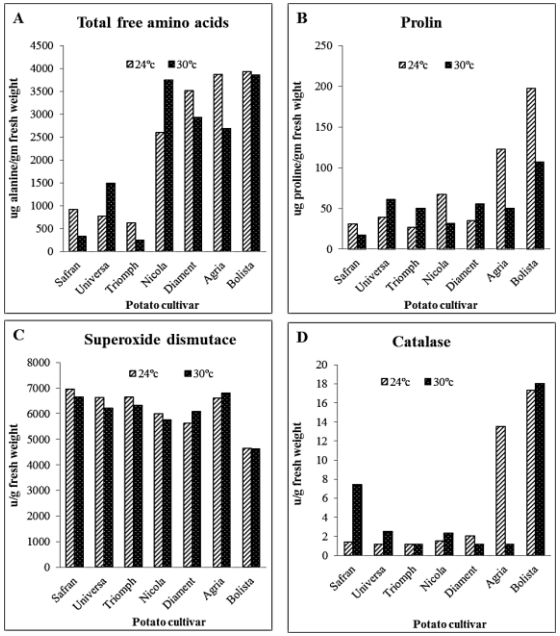

Figure (3): Change in total free amino acids (A), proline (B), SOD (C) and CAT (D) activities under *in vitro* high temperature (30 °C) stress in different potato genotypes.

Table (7): Grouping potato genotypes for response to heat stress (30ºC).

Grouping	**High Tolerant**	**Tolerant**	**Moderately Tolerant**	**Sensitive**
Character	**> 100% of control**	**70-100% of control**	**50-100% of control**	**<50% of control**
Plantlet Height	Margod	Alaska, Elodi, 96F, Oceana, Marabel, Arinda, Bolista.	94F, Universa, Spunta, Lady Rosetta, 95K, Santa.	Safran, Triumph, Nicola, Fridor, Naga, May Fl, Diamant, Agria, 99-980, 97-980, 94F,
No. of Nodes	Margod, 94F 81.1	Elodi, 96F, Lady Rosetta, Oceana, May Margod	Safran, Universa, 95K, Alaska	Nicola, Fridor, Naga, Agria, 97.980, 94F-
No. of Roots	Oceana			Safran, 94F, Universa, Elodi, Nicola, Fridor,
No. of	Universa, Lady Rosetta, Fridor, Agria,	Elodi, Triumph, 94F, Naga, Marabel.	Safran, Margod, Spunta,	Alaska, 96F, Diamant, Presto, Bolista. (cv.
Ave.	Safran, 94F-81.1, Margod, Universa,	Spunta, Elodi, Lady Rosetta, Proventa.	Alaska, Picasso.	96F.25-25, Diamant, 94F, Presto, Bolista. **
Microtuber	Margod, 94F-81.1, Triumph, Nicola,	Safran, 94F-81.1, Universa, Alaska,	Lady Rosetta, Presto,	Spunta, 95K, Diamant. **

* Other genotypes had zero rooting

** Jelly and May Flower did not form microtubers.

Table (8): Effect of *in vitro* heat stress on chlorophyll and carotenoid contents in 7 potato cultivars.

CV.	Temp.	Chl. a (mg/g FW)	Chl. b (mg/g FW)	Chl. Total (mg/g FW)	Carotenoids (mg/g FW)
	12ºC	24.94	17.95	42.88	15.31
Safran	24ºC	35.15	21.12	56.26	13.37
	30ºC	27.58	20.80	48.36	5.29
	Mean CV.	**29.22**	**19.96**	49.17	11.33
	12ºC	28.15	17.76	45.89	12.16
Universa	24ºC	29.84	23.36	53.19	16.51
	30ºC	26.25	20.69	46.94	11.41
	Mean CV.	**28.08**	**20.60**	**48.68**	**13.36**
	12ºC	29.15	23.37	52.52	16.43
Triumph	24ºC	29.84	23.36	53.19	16.51
	30ºC	27.74	15.88	43.63	16.46
	Mean CV.	**28.91**	**20.87**	**48.68**	**16.47**
	12ºC	28.47	19.45	47.92	17.90
Nicola	24ºC	21.34	15.95	37.27	9.39
	30ºC	20.83	10.41	31.24	12.78
	Mean CV.	**23.55**	**15.27**	**38.81**	**13.36**
	12ºC	29.61	12.53	42.14	10.46
Diamant	24ºC	28.91	19.23	48.12	11.45
	30ºC	24.41	9.11	33.50	11.50
	Mean CV.	**27.64**	**13.62**	**41.25**	**11.14**
	12ºC	23.82	18.55	42.38	8.06
Agria	24ºC	33.27	23.47	56.72	14.12
	30ºC	22.47	12.69	35.16	5.61
	Mean CV.	**26.52**	**18.24**	**44.75**	**9.26**
	12ºC	32.04	19.57	51.61	9.45
Bolista	24ºC	41.58	35.99	67.55	20.66
	30ºC	32.26	18.46	50.72	4.03
	Mean CV.	**35.29**	**24.67**	**56.63**	**11.38**
Mean of 12ºC		**28.02**	**18.29**	**46.31**	**12.82**
Mean of 24ºC		**31.43**	**23.21**	**54.16**	**14.57**
Mean of 30ºC		**25.93**	**15.43**	**41.36**	**10.29**
Cultivars LSD 0.05		**1.16**	**0.65**	**1.81**	**1.53**
Temperature LSD 0.05		**0.95**	**0.77**	**1.72**	**0.73**
Interaction LSD 0.05		**2.51**	**2.03**	**4.54**	**1.92**

Table (9): Free amino acids, proline and antioxidant profile analysis of seven potato cultivars under *in vitro* heat stress.

CV.	Temp.	AA* (µg alanine/g FW)	Proline (µg/g FW)	SOD# (µg/g FW)	Catalase (µg/g FW)
Safran	12ºc	280.00	21.10	6786.67	1.14
	24ºc	909.00	30.87	6947.00	1.37
	30ºc	330.33	17.00	6650.00	7.44
	Mean CV	**506.44**	**22.99**	**6794.56**	**3.32**
Universa	12ºc	1551.67	28.90	6665.00	1.14
	24ºc	772.67	39.20	6633.00	1.18
	30ºc	1481.67	60.67	6216.00	2.52
	Mean CV	**1268.67**	**42.92**	**6504.67**	**1.61**
Triumph	12ºc	389.67	24.80	6772.33	1.14
	24ºc	624.00	26.63	6657.33	1.14
	30ºc	240.00	49.57	6329.67	1.13
	Mean CV	**417.89**	**33.67**	**6586.44**	**1.14**
Nicola	12ºc	2387.00	55.33	5954.67	8.28
	24ºc	2596.00	66.67	5983.00	1.51
	30ºc	3741.67	31.37	5748.67	2.31
	Mean CV	**2908.22**	**51.12**	**5895.44**	**4.03**
Diamant	12ºc	3754.67	50.00	5109.33	8.25
	24ºc	3507.67	34.97	5617.00	2.01
	30ºc	2929.00	55.33	6073.67	1.15
	Mean CV	**3397.11**	**46.77**	**5600.00**	**3.80**
Agria	12ºc	3054.33	74.67	6236.67	2.60
	24ºc	3870.33	122.67	6610.00	13.52
	30ºc	2688.00	49.67	6793.67	1.18
	Mean CV	**3204.22**	**82.33**	**6546.78**	**5.77**
Bolista	12ºc	3972.00	77.67	6027.00	7.82
	24ºc	3932.33	197.33	4645.33	17.29
	30ºc	3854.33	107.00	4615.33	18.01
	Mean CV	**3919.56**	**127.33**	**5095.89**	**14.37**
Mean of 12ºc		**2198.48**	**47.50**	**6221.67**	**4.34**
Mean of 24ºc		**2316.00**	**74.05**	**6156.10**	**5.43**
Mean of 30ºc		**2180.71**	**52.94**	**6061.00**	**4.82**
Cultivars LSD 0.05		**106.69**	**3.49**	**83.3**	**0.36**
Temperature LSD 0.05		**81.45**	**4.15**	**77.34**	**0.27**
Interaction LSD 0.05		**215.49**	**10.99**	**204.62**	**0.71**

*= Total free Amino Acids; # SOD= Superoxide Dismutase

temperatures has been suggested as a screening tool for heat tolerance (Nowak and Colborne, 1989; Gopal and Minocha, 1998). Using microtuberization at 28°C subsequent to a radiation treatment of two cultivars of potato, Das *et al*. (2000) identified heat-tolerant mutants after the microtubers were planted in the field under naturally occurring heat stress.

In vitro tuberization as a potential screening method for heat stress tolerance in potato was assessed in different genotypes and heat stress significantly reduced tuberization. Certain genotypes failed to tuberize under heat stress (Nowak, and Colborne, 1989). Similarly, in our experiment, seven cvs. including Santa and May Flower failed to form microtubers at high temperature treatment and thus may consider as heat sensitive.

The results of our study revealed that high temperature stress markedly affected *in vitro* potato growth and tuberization. As an average over the 30 tested genotypes, plantlet length decreased about 50% and root length also severely decreased (80%) at high temperature (30ºC) compared to growth at 24ºC. The highest root number/plantlet was found at 12ºC, followed by at 24ºC, while no roots were formed in several genotypes at 30ºC supporting the idea of the unfavorable effect of heat stress on potato growth. Under heat stress (30ºC), nodes number were higher than at 24ºC or 12ºC, supporting the assumption of different response of potato genotypes to the different temperature degrees.

In vitro tuberization as well showed 39% decline in microtuber number/jar. These observed reductions could be explained on the bases of decrease in stomatal resistance and net photosynthesis with an increase in transpiration under high temperature stress in potato (Wolf *et al*., 1990). Similar results of decreased growth and tuberization of potato plantlets under high temperature (30-32ºC) were also found by Nowak and Colborne (1989); Midmore and Prande (1991); Lafta and Lorenzen (1995); Das *et al.* (2000) and Khan *et al*. (2015).

Our results also indicated differences among potato genotypes in their response to heat stress. However, some genotypes were found tolerant based on their improved growth under 30ºC (cv. Bolista), but were not tolerant based on their microtuber number produced under prolonged exposure to high temperature. On the other hand, cv. Agria was found tolerant based on the increase of microtuber number under heat stress, but was ranked as sensitive based on its relative plantlet growth. The cvs. Universa and Triumph may be ranked tolerant or moderately tolerant to heat stress based on both growth and tuberization ranking criteria. Such difference in genotype response to heat stress is not well understood, although other research efforts reported similar finding (Nowak and Colborne 1989; Arvin and Donnelly 2008). Comparable to our results, Savić *et al*. (2012) ranked cv. Agria as heat sensitive and cv. Marabel as moderately heat sensitive.

Based on the results, we attempt to rank and group potato genotypes for heat tolerance. Based on plantlet height at high temperature stress as % of control, the examined potato genotypes could be ranked as follow: Margod > 96F-25-25 > Bolista > Elodi > Marabel > Arinda > Alaska > Oceania > Universa > 95K-94 > 94F-81.1 > Picasso > Santa > Spunta > Lady Rosetta > Triumph > Fridor > Presto > 97F-267 > 99-981 > 97-980 > Safran > 94F > Naga > Proventa > Jelly > May Flower > Agria > Nicola > Diamant. Based on microtuber number as % of control, potato genotypes could be ranked as follow: Agria > Universa > Fridor > Picasso > Lady Rosetta > Arinda > Elodi= Triumph= Naga > 94F > Marabel > Spunta= Presto > 95K-94 > Nicola > Safran > 94F-81.1 > Margod > Proventa >Alaska > 96F-25-25 > Bolista >Diamant > Oceania=99-981= 97-980 > Jelly = 97F-267 > Santa > May Flower.

The cv. that had microtuber number > 100% of the control when produced at 30°C was considered tolerant to high temperature stress, while cvs. that were not able to form microtubers at high temperature treatment were considered heat sensitive. The cv. Universa was found among heat tolerant group based on both plantlet height and microtuber number, while Diamant was sensitive based on both parameters. Safran and Nicola may be considered moderately tolerant to high temperature stress. The cv. Bolista was ranked tolerant based on plantlet height, but among the sensitive group based on microtuberization under high temperature.

Photosynthesis pigment contents were markedly reduced under heat stress in potato as compared to the control. The reduction in *in vitro* growth and tuberization may be the result of reduced chlorophyll contents and subsequent reduction in net assimilation and photosynthesis. The results of Midmore and Prang (1991) and Wolf *et al*. (1990) support such assumption. Heat sensitivity of potato photosynthesis was attributed with accelerated senescence, chlorophyll loss and reduced stomatal conductance (Reynolds *et al*., 1990).

The significant differences among potato genotypes on pigment contents and the different response of genotypes to heat stress regarding photosynthetic pigments observed in this study were also documented in tomato as an increase in chlorophyll a/b ratio and a decrease in chlorophyll/carotenoid ratio were reported in thermotolerant stressed genotypes (Camejo *et al*., 2005). Similarly, cvs. Triumph, Nicola and Diamant showed the same trend (data not shown).

Results of photosynthetic pigments analysis indicated that heat stress adversely affecting photosynthetic pigment contents compared with growth under normal or less than normal temperature degrees and confirming the negative effect of heat stress on *in vitro* potato growth.

Biochemical analysis indicated different genotypic response due to heat stress in potato. Few genotypes (such as Agria and Bolista) showed slight decline in free

amino acids and proline contents, as well as SOD and CAT activities under high temperature stress. While other genotypes such as cvs. Universa and Nicola had more free amino acids content under heat stress. The cvs. Universa, Triumph and Diamant also accumulated more proline under heat stress. In cvs. Diamant and Agria, CAT increased, while SOD decreased under heat stress. These results indicate no clear association between the changes in amino acids, proline, SOD and CAT activates and the tolerance to heat stress in potato plantlets. The cv. Universa and Triumph (heat tolerant) had more proline under 30ºC than 24ºC, in agreement with the results of Nautiyal *et al*. (2005) who detected more proline in heat tolerant tomato line and De Ronde *et al.* (2000) who reported an increase in proline concentrations in five cotton genotypes in response to heat stress and different proline profiles were observed for the different genotypes. Also, Lopez-Delgado (1998) found a decrease in CAT activity in heat adaptive and control potato plantlets.

In conclusion, *In vitro* growth and microtuberization were adversely affected by high temperature stress at 30°C during the entire growth (6 weeks) and tuberization (10 weeks) period. Significant differences among the 30 tested potato genotypes were detected for their growth and microtuber induction when tested over the examined temperature regimes (12°C, 24°C and 30°C). Significant interaction between genotype and temperature was detected in plantlet growth parameters and tuberization. Number of microtuber was significantly reduced at 30°C compared to the control (24°C) in all cvs., except Universa, Lady Rosetta, Fridor, Agria, Picasso and Arinda, while several cvs. did not produce microtubers under 30°C. Results of screening potato genotypes under heat stress indicated that the cvs. Margod, Safran and Universa were considered among heat tolerant cvs., and the cvs. Oceana, Triumph, Diamant and Picasso could mark as moderately tolerant, while the cvs. Agria and Nicola were ranked among the sensitive genotype to heat stress. Analysis of photosynthetic pigment contents indicated that heat stress resulted in reduction in chl. a, chl. b, total chl. and carotenoids. Heat stress also induced reduction in total amino acid, proline contents and activities of the antioxidant enzymes SOD and CAT.

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الملخص العربي

مسح معملي لتراكيب وراثية مختلفة من البطاطس لتحمل الإجهاد الحرارى

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تم اجراء مسح معملي لعدد ثلاثين تركيبا وراثيا من البطاطس لتحمل الإجهاد الحراري. تم تقييم نباتات البطاطس النامية معمليا على أساس النمو، وانتاج الدرنات داخل المعمل وتحليلات بيوكيماوية تحت معاملات مختلفة لدرجات الحرارة (٣٠، ٢٤، و١٢ درجة مئوية). كل من درجتي الحرارة المرتفعة (٣٠ درجة مئوية) أو الأقل من الطبيعية (١٢ درجة مئوية) أثرت بشكل كبير على طول Plantlets، عدد العقد /Plantlet وعدد الجذور. ارتفاع Plantlet تحت ظروف درجة الحرارة المرتفعة (٣٠ درجة مئوية) (الإجهاد الحراري) قد انخفض بشكل معنوي (في المتوسط ٢.٩ سم)، في حين ان درجة الحرارة العادية (١٢ درجة مئوية) لم تخفض من ارتفاع Plantlet وكانت مقاربة لمعاملة الكنترول (٢٤ درجة مئوية) (بمتوسط ٥.٧ سم مقابل ٥.٥ سم). بالإضافة إلى ذلك، النباتات التي نمت تحت ٣٠ درجة مئوية كونت عدد أقل من العقد والجذور/plantlet على النقيض من معاملة الكنترول وهو ما يدعم فكرة التأثير الضار للإجهاد الحراري على النمو في البطاطس. تم الكشف عن فروق بين التراكيب الوراثية للبطاطس بالنسبة لصفات النمو والتجذير. كانت التفاعلات بين درجات الحرارة المختلفة والتراكيب الوراثية معنوية في جميع صفات النمو المدروسة. كمتوسط عام لكل التراكيب الوراثية المختبرة، فان عدد الدرنات الصغيرة /وعاء زراعة قد تأثر بشكل معنوي بمعاملات درجات الحرارة. وقد سجل أدنى عدد (١.٤٤ درنة صغيرة) عندما تم تنمية نباتات البطاطس تحت درجة حرارة المرتفعة (٣٠ درجة مئوية) كمعاملة للإجهاد الحراري. وجد ان التراكيب الوراثية للبطاطس تختلف إلى حد معنوي في إمكانية تكوين الدرنات الصغيرة الخاصة بهم. وأشارت النتائج الى وجود تفاعل كبير بين درجات الحرارة والتراكيب الوراثية عند تكوين الدرنات الصغيرة وتطورها. جميع التراكيب الوراثية أنتجت درنات صغيرة في ٢٤ درجة مئوية وفي ١٢ درجة مئوية ، في حين أن بعض التراكيب الوراثية لم تكن قادرة على تكوين الدرنات الصغيرة بسبب التعرض للإجهاد الحراري عند ٣٠ درجة مئوية مثل اصناف Oceana, May Flower, 99-981, 97-980, Jelly, 97F-267, Santa. ومن ناحية أخرى، فان اصناف Fridor, Lady Rosetta, Agria, Picassoاعطت عدد اكبر من الدرنات الصغيرة عند ٣٠ درجة مئوية مقارنة بمجموعة المقارنة (٢٤ درجة مئوية) أو تحت درجة الحرارة المنخفضة (١٢ درجة مئوية) ويمكن اعتبارها اصناف متحملة للإجهاد بسبب ارتفاع درجة الحرارة. تحت ظروف الاجهاد بسبب ارتفاع درجة الحرارة (٣٠ درجة مئوية) كانت كل صبغات البناء الضوئي أقل بكثير من مجموعة المقارنة. تحت درجة الحرارة المرتفعة فان كلوروفيل (ا) و(ب) والكلوروفيل الكلي و الكاروتينات قد مثلت ٨٢.٤٪، ٦٩.٣٪، ٧٦.٣٪ و ٧٠.٥٪ من مستواها عند (٢٤ درجة مئوية) على التوالي. وتشير هذه النتائج إلى أن الإجهاد الحراري يؤثر سلبا على المحتوي من صبغات البناء الضوئي مقارنة مع النمو الظروف الطبيعة أو الأقل من الطبيعية. الاجهاد الحرارى خفض بشكل ملحوظ المحتوى من الأحماض الأمينية الكلية ومن البرولين ولكنة نشط انزيمات SOD وCAT. في الدراسة الحالية فان تكوين الدرنات معمليا تحت درجة الحرارة غير المواتية للنمو كوسيلة محتملة للمسح لتحمل الإجهاد الحراري في عدد من التراكيب الوراثية المختلفة للبطاطس قد تم وأظهرت النتائج ان الإجهاد الحراري خفض معنويا من تكوين الدرنات في معظم التراكيب الوراثية. ومع ذلك، أظهرت بعض التراكيب الوراثية نموا وقدرة فائقة على تكوين الدرنات معمليا تحت ظروف الإجهاد الحراري ويمكن استخدامها كمادة وراثية في برامج التربية لإنتاج تراكيب وراثية متميزة من البطاطس متكيفة مع ظروف الإجهاد الحراري.