IMPACT OF SORBITOL- INDUCED OSMOTIC STRESS ON SOME BIOCHEMICAL TRAITS OF POTATO IN VITRO

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ABSTRACT

This study had as principal objective identification of osmotic-tolerant potato genotypes by using "*in vitro*" tissue culture and sorbitol as a stimulating agent, to induce water stress, which was added to the culture nutritive medium in different concentration (0,50, 110, 220, 330 and 440 mM). The starting point was represented by plantlets culture collection, belonging to eleven potato genotypes: Barcelona, Nectar, Alison, Jelly, Malice, Nazca, Toronto, Farida, Fabulla, Colomba and Spunta. Plantlets were multiplied between two internodes to obtain microcuttings (in sterile condition), which were inoculated on medium. Sorbitol-induced osmotic stress caused a significant reduction in the ascorbic acid, while the concentration of proline, H_2O_2 and solutes leakage increased compared with the control. Increased the proline content prevented lipid peroxidation, which played a pivotal role in the maintenance of membrane integrity under osmotic stress conditions. The extent of the cytoplasmic membrane damage depends on osmotic stress severity and the genotypic variation in the maintenance of membranes stability was highly associated with the ability of producing more amounts of osmoprotectants (proline) and the non-enzymic antioxidant ascorbic acid in response to osmotic stress level. The results showed that the genotypes Jelly, Nectar, Allison, Toronto, and Colomba are classified as highly osmotic stress tolerant genotypes, while the genotypes Nazca and Farida are classified as osmotic stress susceptible ones.

Key words: proline, MDA, H_2O_2, osmotic stress, sorbitol, potato.

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تأثير الإجهاد الحلولي المصطنع بالسوربيتول في بعض الصفات البيوكيميائية في البطاطا خارج الجسم الحي

ماويا راضي صبح سليم حسين زيد فهد البيسكي أيمن الشحاذه العوده
باحثة أستاذ باحث أستاذ

المستخلص

كان الهدف الرئيس من هذه الدراسة هو تحديد طرز البطاطا المتحملة للإجهاد الحلولي باستعمال تقانة زراعة الأنسجة خارج الجسم الحي، واستعمال سكر السوربيتول كعاملٍ محرض لاستحداث الإجهاد المائي، حيث أُضيف على الوسط المغذي بعدة تراكيز متباينة (0، 50، 110، 220، 330، 440 ميللي مولر). وتمثلت نقطة البداية بزراعة نبيتات البطاطا التابعة لأحد عشرة طرازاً زراثياً من البطاطا: Barcelona، Nectar، Alison، Jelly، Malice، Nazca، Toronto، Farida، Fabulla، Colomba، وSpunta. تمّت مكاثرة النبيتات ضمن ظروفٍ عقيمة إلى سلاميتين للحصول على الأجزاء الدقيقة، التي حضنت على وسط مغذي. وضعت التجربة وفق التصميم العشوائي التام بأربعة مكررات. وسبب الإجهاد الحولي تراجعاً معنوياً في تركيز حمض الأسكوربيك (فيتامين –C)، في حين ازداد تركيز كلٍ من البرولين، والماء الأوكسجيني، ونسبة الذائبات المتسربة عبر الأغشية السيتوبلاسمية بالمقارنة مع الشاهد. ومنع ازدياد محتوى الأوراق من البرولين تخريب المواد الدهنية الداخلة في تركيب الأغشية السيتوبلاسمية، الأمر الذي أدى إلى المحافظة على سلامتها تحت ظروف الإجهاد الحلولي. وتوقفت سلامة الأغشسة السيتوبلاسمية على شدّة الإجهاد الحلولي والتباين الوراثي بين طرز البطاطا، الذي ارتبط بمقدرتها على تصنيعه كميةٍ أكبر من الواقيات الحلولية (البرولين)، ومضادات الأكسدة غير الأنزيمية (حمض الأسكوربيك). بيّنت النتائج أنّ الطرز الوراثية Jelly، Nectar، Allison، Toronto، Colomba تُصنف كطرزٍ عالية التحمل للإجهاد الحلولي، في حين تَصنف الطرازان الوراثيان Nazca، Farida كطرزٍ حسّاسة.

الكلمات المفتاحية: البرولين، المالوندي ألدهيد، الماء الأوكسيجيني، السوربيتول، البطاطا.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is often considered as a drought sensitive crop and its sustainable production is threatened due to frequent drought episodes. Potato is of great economic value and ranks as the fourth most important food crop in the world (11). Potatoes are grown in over 125 countries and more than a billion people worldwide consume them on a daily basis (24). Potato represents an excellent source of nutrients including carbohydrates, proteins, vitamin C, several forms of vitamin B, and minerals (6). Drought stress considerably decreases potato yield, making water availability a limiting factor in the production of this crop (27), so one of the great challenges for the next decade is to mitigate any effect of climate change on crop production with a main focus being to maintain crop production levels with reduced availability of water. In the Mediterranean regions during the heat- free periods of the year, potato yields will go down dramatically as the suitable periods become shorter and higher evaporative demands will minimize water use efficiently causing a remarkable decline in tuber potato yield in the temperate environments (14). Globally, for potato, drought will decrease potential potato yield by 18–32% in the projected period of 2040–2069 (16). Plants respond to limiting water availability through a complex series of adaptive changes often accompanied by deleterious pleiotropic effects (17). Water stress reduces plant growth through a reduction in photosynthesis, mainly caused by a stomatal limitation (28). This decrease in the photosynthetic rate under water deficit conditions leads to an increase in the production of reactive oxygen species (ROS) (9). ROS can alter the normal functioning of plants due to the damage caused to lipids, proteins, nucleic acids, photosynthetic pigments and enzymes (18). Biochemical responses of potato to drought are complex with levels of antioxidants showing increases, decreases, or no effect, depending on the genotype and kind of antioxidant (36). Plants adapt to drought conditions either by decreasing water loss or by maintaining water uptake. Osmotic adjustment (OA) results in an increase in solutes in plant cells leading to a lowered osmotic potential, which in turn can improve cell hydration, help maintain cell turgor in leaf tissue, maintain metabolic processes and thus enhance plant growth and yield under drought conditions (33). Studies in potato have demonstrated that drought stress leads to accumulation of osmotically active solutes including proline. The principal damage caused by ROS during water stress is lipid peroxidation, which decreases the stability of cellular membranes and increases their permeability, thereby modifying cellular metabolism (38). In order to overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidants (8). Proline serves as an ROS scavenger and is used as a non-enzymatic antioxidant to counteract the damaging effect of different ROS members helps in water stress survival through osmoprotection (34). Ascorbic acid (AsA) is a naturally occurring compound with antioxidant activity that plays a pivotal role in plant cell adaptation (1). The generation of H_2O_2 is increased due to a wide variety of stresses, and some authors have suggested that H_2O_2 is a key factor mediating the phenomena of acclimation and cross-tolerance (26). It plays a dual role in plants: at low concentrations, it acts as an acclamatory signal, triggering tolerance to various stresses (10). Therefore, biochemical and physiological behavior of the plants, even at early developmental stage under osmotic stress could provide information on their capacity to tolerate drought stress. Resistance to water stress by conventional methods takes time, is costly and labor-intensive. *In vitro* tissue culture allowed a deeper understanding of the physiology and biochemistry of plants grown under unfavorable environmental (5). The most widely used method for the selection of genotypes tolerant to abiotic stress is the *in vitro* selection pressure technique (29). *In vitro* simulation drought was made to identify varieties with optimum tolerance at drought (3). In Syria, potato is cultivated in highly mountainous areas with few or no available water, suggesting that this crop is often subjected to drought stress conditions, so it is of great importance to evaluate the response of the available genetic material for drought stress to identify the most drought adaptive

genotypes and drought-associated traits. The objective of this study is to evaluate the response of eleven potato genotypes to sorbitol-induced osmotic stress *In vitro* based on some biochemical traits.

MATERIALS AND METHODS

Plant material and site of experimentation: The experiment was conducted at the laboratories of Plant Biotechnology, Faculty of Agriculture, Damascus University, during the growing season 2017 – 2018, in order to evaluate the response of eleven potato genotypes (Barcelona, Nectar, Alison, Jelly, Malice, Nazca, Toronto, Farida, Fabulla, Colomba and Spunta) to osmotic stress *In Vitro*, using sorbitol as a stimulating agent, based on some biochemical traits.

Treatments: In this study, to induce *in vitro* water stress, sorbitol was used with the basic medium MS, considered control. Osmotic stress was imposed through the addition of different concentrations of sorbitol ($C_6H_{14}O_6$) to the growth media (0, 50, 110, 220, 330 and 440 mM), which equivalent to osmotic potentials (- 0.82, -1.09, - 1.44, - 1.79 and - 2.14 Mpa respectively). Sorbitol was used to exert a water deficiency in the nutrient medium necessary for growth and development of the plantlet with the purpose of cause changes of growth, similar to those produced by the drying of the soil.

Methodology: The genotypes were first cultivated in pots under greenhouse conditions. Explants from each genotype were grown *in vitro* in test tubes containing 13.5 ml of solid Murashige and Skoog (25) medium, supplemented with 30 g l^{-1} sucrose and 7 g l^{-1} plant agar. The pH of the medium was adjusted to 5.8 before autoclaving for 20 min at 120°C. Then, 1.5-2.0 cm long stem cuttings with one or two axillary buds were prepared by excluding the basal and apical portions of the plantlets, to be evaluated for osmotic stress tolerance. The experiment was laid out as a randomized complete design in a factorial arrangement and replicated four times in two rounds. The experimental materials were cultured for 30 days at a constant temperature of 22 ± 2°C, 16/8 hours light/dark photoperiod with a photosynthetically active photon flux density of approximately 35 µmol m^{-2} s^{-1}.

Investigated traits

Membrane permeability: Solutes leakage (SL) was measured as described by Leopold *et al.*, (20). Plant material (0.5 g) was washed with deionized water, placed in tubes with 10 ml of deionized water and left on a shaker for 3 h at 25°C. Subsequently, the initial absorbance of the solution (A1) was determined at an optical density of 273 nm. Samples were then boiled in a boiling bath at 100 °C for 20 min and the final absorbance of the solution (A2) was determined at the same optical density. The percentage of the solutes leakage was measured according to the following formula: SL (%) = (A1/A2) × 100

Proline content: Free proline content was measured by the method of Bates *et al.* (4). Plant tissue (100 mg) was homogenized in 3% (w/v) sulphosalycylic acid and centrifuged at 700 ´ *g* for 3 min. After addition of ninhydrin reagent, mixtures were heated at 100 °C for 1 h and cooled in an ice-bath. The chromophore obtained was extracted from liquid phase with toluene and the absorbance of organic layer was read at 520 nm. Proline concentration was determined from calibration curve using L-Proline as standard and expressed as µg (proline) g^{-1} fresh weight.

MDA and H_2O_2 content: Lipid peroxidation was determined by estimating the amount of malondialdehyde (MDA) content using the thiobarbituric acid method described by Heath and Packer (15). The crude extracts were mixed with 0.25% (w/v) thiobarbituric acid solution containing 10% (w/v) trichloroacetic acid, heated at 95 °C for 30 min and the reaction was stopped in an ice bath. The cooled mixtures were centrifuged at 10000 ´ *g* for 10 min and the MDA content calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for non-specific turbidity) by using extinction coefficient of 155 $mM^{-1}cm^{-1}$. H_2O_2 was extracted from 100 mg fresh tissue in ice-cold acetone and estimated according to the method of Mukherjee and Choudhuri (23). After addition of titanyl-sulphate and conc.NH_4OH solution, the formed peroxide-titanium precipitate was dissolved in 2M H_2SO_4 and absorbance of the mixtures read at 415 nm. The H_2O_2 content was calculated from a

standard curve and expressed as nmol (H_2O_2) g^{-1} fresh weight.

Ascorbate content: Ascorbate was isolated by extraction with 6% trichloroacetic acid from 100 mg plant tissue, following the method of Mukherjee and Choudhuri (23). Extracts were mixed with 2% dinitrophenyl hydrazyne followed by the addition of 1 drop of 10% thiourea solution and boiled for 15 min in a water bath. After adding 80% (w/v) H_2SO_4 (in an ice-bath), the absorbance of the mixtures containing hydrazone complex was read at 530 nm. Ascorbate concentration was determined using calibration curve and expressed as mmol (ascorbate) g^{-1} fresh weight.

RESULTS AND DISCUSSION

Membrane permeability (solutes leakage %): Results showed significant differences (P≤0.01) in the solutes leakage through the cytoplasmic membranes among genotypes and the osmotic stress levels and their interaction. Solutes leakage increased significantly and proportionately with the increment of sorbitol in the growth media, where it was significantly the highest at the highest concentration of sorbitol (82.39%), while it was the lowest in the control (without sorbitol) (39.01%). The percentage of the solutes leakage was significantly higher in the genotype Colomba (93.91%), while it was significantly lower in the genotype Fabulla (28.10%), followed by Toronto, Alison and Barcelona (39.73, 40.51 and 47.41% respectively) (Table, 1). This increase in the solutes leakage could be due to the peroxidation of lipids caused by an increase in ROS, as has been reported for plants such as tomato (*Lycopersicon esculentum* Mill.) (32), and potato (9).

Proline content (µg g^{-1} fresh wt.): Results showed significant differences (P≤0.01) in the proline content among genotypes and the osmotic stress levels and their interaction. The proline content in the leaves increased significantly and roportionately with the increment of sorbitol in the growth media. The free amino acid proline content in the leaves was significantly higher in the highest

Table 1. Solutes leakage (%) under different osmotic stress levels of some potato genotypes

Genotypes	Treatments (mM sorbitol)						Mean
	Control	55	110	220	330	440	
Barcelona	24.310	90.740	25.883	35.205	39.415	68.885	47.406^{H}
Fabula	4.568	12.220	10.925	19.465	45.840	75.613	28.105^{K}
Nazca	68.195	59.155	94.410	94.405	92.580	88.403	82.858^{B}
Farida	4.067	17.640	68.275	62.665	76.365	77.728	51.123^{G}
Colomba	94.368	88.392	94.288	97.580	94.510	94.330	93.911^{A}
Malice	76.642	71.650	78.575	66.382	61.833	87.180	73.710^{D}
Jelly	87.658	88.303	26.845	94.345	94.270	95.197	81.103^{C}
Allison	31.818	54.813	11.445	22.787	56.955	65.262	40.513^{I}
Nectar	9.787	15.508	19.697	75.330	95.785	95.153	51.877^{F}
Toronto	10.800	8.555	4.270	92.350	28.675	93.758	39.735^{J}
Spunta	16.868	67.367	93.195	97.783	95.587	64.832	72.605^{E}
Mean	39.007^{F}	52.213^{D}	47.983^{E}	68.936^{C}	71.074^{B}	82.395^{A}	-
Increase ratio (%)	-	25.29	18.70	43.41	45.11	52.66	-

Different letters in front of means in the rows and columns indicates significant differences at 0.01.

Statistical variable	Treatments	Genotypes	Interaction
LSD (0.01)	0.1911	0.2772	0.6789
CV (%)		0.61	

Table 2. Proline content (µg g^{-1} fresh wt.) under different osmotic stress levels of some potato genotypes

Genotypes	Treatments (mM sorbitol)						Mean
	Control	55	110	220	330	440	
Barcelona	5.931	16.260	23.748	21.105	20.889	17.169	17.52^{I}
Fabula	30.345	32.012	32.290	32.615	33.192	33.778	32.37^{A}
Nazca	7.756	10.388	23.242	24.086	32.572	34.681	22.12^{E}
Farida	4.232	10.742	14.456	19.197	29.738	32.083	18.41^{H}
Colomba	5.706	11.177	17.743	26.735	33.393	35.019	21.63^{F}
Malice	7.687	13.876	21.097	30.650	31.607	32.431	22.89^{D}
Jelly	15.313	19.599	27.223	30.633	31.428	32.500	26.12^{C}
Allison	12.194	23.716	28.041	29.771	30.327	31.334	25.90^{C}
Nectar	14.081	23.201	28.584	29.914	31.533	33.336	26.77^{B}
Toronto	8.614	10.688	16.620	25.355	30.977	32.659	20.82^{G}
Spunta	12.934	19.338	20.608	31.942	35.143	35.560	25.92^{C}
Mean	11.34^{F}	17.36^{E}	23.06^{D}	27.45^{C}	30.98^{B}	31.87^{A}	-
Increase ratio (%)	-	34.67	50.82	58.68	63.39	64.41	-

Different letters in front of means in the rows and columns indicates significant differences at 0.01.

Statistical variable	Treatments	Genotypes	Interaction
LSD (0.01)	0.2455	0.2891	0.7082
CV (%)		1.62	

sorbitol concentration in the nutritive media (440 mM) (31.87µg g^{-1} fresh wt.) and decreased proportionally and significantly with decreasing the osmotic stress intensity, where it was the lowest in the control (without sorbitol) (11.34µg g^{-1} fresh wt.). Proline content was significantly higher in the genotype Fabulla (32.37µg g^{-1} fresh wt.), followed with the genotype Nectar (26.77µg g^{-1} fresh wt.), while it was significantly lower in the genotype Barcelona (17.52µg g^{-1} fresh wt.) (table, 2). In water-deficit conditions, proline retains osmotic potential (22) and redox balance of cells (37), scavenges free radicals and ROS as an antioxidant, protects macromolecules from denaturation as a chemical chaperone (31). Under stressful conditions, proline is considered as a nitrogen and carbon provider after rehydration (2), source of energy (35). Proline can protect plants from stress through different mechanisms,

MDA content (nmol g^{-1} fresh wt.): Results revealed significant differences ($P \leq 0.01$) in the MDA content in the leaves among genotypes and the osmotic stress levels and their interaction. MDA content was including osmotic adjustment, detoxification of ROS, protection of membrane integrity, and stabilization of proteins/enzymes (19). In general, Proline accumulation improves stress tolerances without disrupting cellular structure. When the cellular water content decreases, proline and other compatible solutes can act as water substitutes to stabilize cellular structure through hydrophilic interactions and hydrogen bonding.

Table 3. MDA cont (nmol g^{-1} fresh wt.) under different osmotic stress levels of some potato genotypes.

Genotypes	Treatments (mM sorbitol)						Mean
	Control	55	110	220	330	440	
Barcelona	5.931	16.260	23.748	21.105	20.889	17.169	17.52^{I}
Fabula	30.345	32.012	32.290	32.615	33.192	33.778	32.37^{A}
Nazca	7.756	10.388	23.242	24.086	32.572	34.681	22.12^{E}
Farida	4.232	10.742	14.456	19.197	29.738	32.083	18.41^{H}
Colomba	5.706	11.177	17.743	26.735	33.393	35.019	21.63^{F}
Malice	7.687	13.876	21.097	30.650	31.607	32.431	22.89^{D}
Jelly	15.313	19.599	27.223	30.633	31.428	32.500	26.12^{C}
Allison	12.194	23.716	28.041	29.771	30.327	31.334	25.90^{C}
Nectar	14.081	23.201	28.584	29.914	31.533	33.336	26.77^{B}
Toronto	8.614	10.688	16.620	25.355	30.977	32.659	20.82^{G}
Spunta	12.934	19.338	20.608	31.942	35.143	35.560	25.92^{C}
Mean	11.34^{F}	17.36^{E}	23.06^{D}	27.45^{C}	30.98^{B}	31.87^{A}	-
Increase ratio (%)	-	34.67	50.82	58.68	63.39	64.41	-

Different letters in front of means in the rows and columns indicates significant differences at 0.01.

Statistical variable	Treatments	Genotypes	Interaction
LSD (0.01)	0.2455	0.2891	0.7082
CV (%)		1.62	

significantly higher at the two osmotic stress levels 330 and 440 mM sorbitol without significant differences between them (44.27 and 43.87 nmol g^{-1} fresh wt. respectively), while it was significantly lower in the control treatment (without sorbitol) (24.88 nmol g^{-1} fresh wt.). MDA content was significantly higher in the genotype Malice (74.87nmol g^{-1} fresh wt.), followed by Nazca (48.83nmol g^{-1} fresh wt.), while it was significantly lower in the genotype Barcelona (17.71nmol g^{-1} fresh wt.0 followed by the genotypes Colomba, Toronto and Spunta without significant Differences among them (20.31, 20.40 and 20.76 nmol g fresh wt. respectively) (table, 3). It can be noticed that the genotypes which could maintain the cytoplasmic stability accumulated the least quantity of MDA, such as Barcelona (17.71 nmol g^{-1} fresh wt.), followed with the genotypes Colomba, Toronto and Spunta (20.31, 20.40and 20.76 nmol g^{-1} fresh wt. respectively), indicating the relevance of estimating MDA to assess the membranes integrity and the capacity of the genotypes to recover growth as aconsequence.

H_2O_2 content (µ mol g^{-1} fresh wt.): Results revealed significant differences ($P \leq 0.01$) in the H_2O_2 content in the leaves among genotypes and the osmotic stress levels and their interaction. H_2O_2 content was significantly higher at the osmotic stress level 440 mM sorbitol (60.667 µmol g^{-1} fresh wt.), while it was significantly lower in the control treatment (without sorbitol) and the lowest osmotic stress level (55 mM sorbitol) without significant differences between them (26.19 and 26.36 µmol g^{-1} fresh wt. respectively). H_2O_2 content was significantly higher in the genotype Malice (72.92µmol g^{-1} fresh wt.), while it was significantly lower in the genotype Toronto (15.35µmol g^{-1} fresh wt.). (table, 4). H_2O_2 content increased with increasing the sorbitol level in the growth medium, particularly at the osmotic stress level 220 mM and the higher concentrations. Generally, H_2O_2 is produced in response to many abiotic stresses, suggested that H_2O_2 is a key factor mediating the phenomena of acclimation and cross-tolerance (26). Recent investigations have revealed that H_2O_2 is a central component of the signal transduction cascade involved in plant adaptation to a changing environment (26). Results showed that the genotype Malice produced the highest concentration of H_2O_2, so it was more susceptible to osmotic stress conditions, due to formation of more amount of ROS, where the MDA level in such genotype was significantly higher (74.87nmol g^{-1} fresh wt.), indicating a higher level of lipid peroxidation. On the other hand, the concentration of H_2O_2 was significantly lower in the two genotypes Toronto and Colomba (15.35and 16.92umol g^{-1} fresh wt. respectively), but unfortunately

the percentage of solutes leakage was significantly higher in the genotype Colomba (93.91%), while it was significantly lower in the genotype Toronto (39.73%), indicating that the denaturation of the cytoplasmic membranes in the genotype Colomba was not due to the ROS rather than the indirect effect of osmotic stress on the membrane proteins.=

Ascorbate content (µg g^{-1} fresh wt.): The ascorbic acid (AsA) content was significantly higher at the osmotic stress level 110 mM sorbitol (485.30µg g^{-1} fresh wt.), followed by the osmotic level 55 mM sorbitol (472.50µg g^{-1} fresh wt.), while it was significantly lower at the sorbitol concentration 440 mM (286.40 µg g^{-1} fresh wt.), followed by the two sorbitol concentrations 220 and 330 mM (345.50and 347.20µg g^{-1} fresh wt. respectively). Increasing the osmotic stress intensity in the growth medium above 220 mM was found to inhibit the biosynthesis of ascorbic acid, which in turns explains the detrimental effects of higher levels of osmotic stress on all the investigated traits. Generally, ascorbic acid plays a pivotal role in improving the capacity of the plant cells to endure abiotic stresses (7). The concentration of ascorbic acid was significantly higher in the genotype Toronto (614.8µg g^{-1} fresh wt.), while it was significantly lower in the genotype Colomba (63.45µg g^{-1} fresh wt.) (table, 5). AsA is known to play a role in response to oxidative stress, although the regulatory molecular mechanism of AsA synthesis has not been yet well understood. Plants have four major H_2O_2-scavenging pathways. Two of these pathways, the water-water cycle and the ascorbate-glutathione cycle (21), are related to AsA, which is known to have roles in plant stress responses (39). In addition, AsA also has a role in ROS detoxification, as an antioxidant and H_2O_2-scavenger in plant cell to avoid accumulation of ROS under stress conditions (12). In our study, the increased AsA content in some genotypes with increased osmotic stress levels suggests its importance in improving tolerance to osmotic stresses, which in turn promotes the scavenging of excess H_2O_2. According to Puthur (30), the ascorbic acid could react directly with hydroxyl radicals, superoxide, and singlet oxygen and thus provides protection by scavenging free radicals. The genotypic variation in the H_2O_2 concentration is partially attributed to to the variability in the AsA content, where the genotypes which synthesized significantly higher amount of AsA, such as Toronto, Nictar and Barcelona (614.80, 544.40and 460.70µg g^{-1} fresh wt. respectively) produced significantly lower H_2O_2 (15.35, 20.17and 35.72µmol g^{-1} fresh wt. respectively), indicating the importance of AsA in detoxifying the ROS, especially in potato (12).

Table 4. H_2O_2 content (µmol g^{-1} fresh wt.) under different osmotic stress levels of some potato genotypes

Genotypes	Treatments (mM sorbitol)						Mean
	Control	55	110	220	330	440	
Barcelona	30.085	33.250	10.500	15.168	50.333	75.000	35.72^{E}
Fabula	30.333	30.750	16.832	40.583	67.000	82.500	51.33^{B}
Nazca	8.250	23.668	56.583	37.250	19.668	41.500	31.15^{F}
Farida	28.168	28.500	23.500	67.750	62.000	68.167	46.35^{C}
Colomba	27.835	12.000	18.168	21.832	47.500	64.168	16.92^{J}
Malice	47.250	45.583	36.665	61.165	118.650	128.200	72.92^{A}
Jelly	18.082	15.918	50.417	42.083	110.332	34.500	21.89^{H}
Allison	28.500	34.833	38.417	35.168	28.332	33.168	26.40^{G}
Nectar	14.132	18.250	29.168	47.833	49.168	42.500	20.17^{I}
Toronto	26.500	16.250	15.168	20.332	47.500	42.332	15.35^{K}
Spunta	29.000	31.500	22.168	66.500	49.500	60.667	43.22^{D}
Mean	26.19^{E}	26.36^{E}	28.87^{D}	41.42^{C}	59.08^{B}	61.15^{A}	-
Increase ratio (%)	-	0.64	9.28	36.76	55.67	57.17	-

Different letters in front of means in the rows and columns indicates significant differences at 0.01.

Statistical variable	Treatments	Genotypes	Interaction
LSD (0.01)	0.8935	1.080	2.645
CV (%)		4.14	

Conclusions

In vitro screening of potato genotypes for osmotic stress tolerance evaluated in the current study demonstrated that increased osmotic stress levels due to sorbitol treatment in the growth medium resulted in an increased of the solutes leakage, proline content, ascorbic acid, MDA and H_2O_2 with observed genotypic variation among the tested potato genotypes. However, some of the genotypes were observed to have tolerance to the osmotic stress, indicating the possibility to select genotypes that could adapt to drought in field conditions. Generally, that the *in vitro* method could be particularly helpful to screen a large number of plant genotypes within a short period of time. However, the effectiveness of *in vitro* screening should be further tested under field conditions on promising potato genotypes for better root yield and quality production capacity under different moisture stress regimes. Therefore, evaluating the potato genotypes that showed *in vitro* osmotic stress tolerance further under moisture-stressed conditions in the field may validate the result and could be a step in the direction to develop potato varieties that can be cultivated in drought-prone areas in Syria in particular and temperate environments in general. *In vitro* screening of a large number of genotypes for osmotic stress can be carried out by adding sorbitol to the Murashige and Skoog (MS) medium to reduce the osmotic potential (25). Such assays can identify genotypes based on osmotic stress tolerance, are less costly, less time consuming than field trials, and easier to reproduce (13).

REFERENCES

1. Alhasnawi, A.N., C.M.Z. Che Radziah, A.A. Kadhimi, A. Isahak, A. Mohamad, W.M.W. Yusoff 2016. Enhancement of antioxidant enzymes activities in rice callus by ascorbic acid under salinity stress. Biologia Plantarum, 13: 1-5.

2. An, Y., M. Zhang, G. Liu, R. Han, and Z. Liang. 2013. Proline Accumulation in Leaves of Periploca sepium via Both Biosynthesis Up-Regulation and Transport during Recovery from Severe Drought. PLOS ONE, 8:e69942.

3. Andreea, T., C. Mihaela, C. Nicoleta, and B. Carmen. 2016. Influence of Different *In Vitro* Simulators For Hydric Stress For Growth And Development of Potato. Scientific Bulletin. Series F. Biotechnologies, Vol. XX, 2016.

4. Bates L.S., R.P. Waldren and I.D. Tears. 1975. Rapid determination of free proline in water stress studies. Plant Soil, *39*: 205–207.

5. Benderradji L., F. Brini, K. Kellou, N. Ykhelf, A. Djekoun, K. Masmoudi and H. Bouzerour. 2012. Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum Aestivum* L.) genotypes under saline and heat stress conditions. ISRN Agronomy, Article ID 367851. Bobr. Plant Cell Tissue and Organ Culture; 102 387–395.

6. Birch, P.R.J., G. Bryan, B. Fenton, E.M. Gilroy, I. Hein and J.T. Jones. 2012.Crops that feed the world 8: potato: are the trends of increased global production sustainable? Food Secur. 4,477–508.

7. Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, and E. Francia, 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Res. 105, 1–14.

8. Cruz de Carvalho, M.H. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal. Behav. 3, 156-165.

9. Farhad, M.S., A.M. Babak, Z.M. Reza, R.S.M. Hassana, and T. Afshin. 2011. Response of proline, soluble sugars, photosynthetic pigments, and antioxidant enzymes in potato (*Solanum tuberosum* L.) to different irrigation regimes in greenhouse condition. Aust. J. Crop. Sci. 5(1), 55-60.

10. Fukao, T., J. Bailey-Serres. 2008. Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proceedings of the National Academy of Science of the United States of America, 105: 16814-16819.

11. Gastelo, M., U. Kleinwechter, and M. Bonierbale. 2014. *Global Potato Research for a Changing World. Working Paper*. Available on line at:http://cipotato.org/ wp-content/uploads/2014/06/006151.

12. Gill, S.S., and N. Tuteja 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48:909–30.

13. Gopal J. and K. Iwama. 2007. *In vitro* screening of potato against water stress

mediated through sorbitol and polyethylene glycol. Plant Cell Rep., 26, 693-700.
14. Haverkort, A.J., and A. Verhagen. 2008. Climate change and its repercussions for the potato supply chain. *Potato Res.* 51, 223–237.
15. Heath, R.L. and L.Packer. 1968. Photoperoxidation in isolated chloroplasts. I-kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys 125:* 189–198.
16. Hijmans, R.J. 2003. The effect of climate change on global potato production. *Am. J. Potato Res.* 80, 271–279.
17. Jones, H.G. 2014. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. Cambridge: Cambridge University Press.
18. Kar, R.K. 2011. Plant responses to water stress: role of reactive oxygen species. Plant Signal. Behav. 6, 1741-1745.
19. Kishor, P.B., S. Sangam, R.N. Amrutha, P.S. Laxmi, K.R. Naidu, K.R.S.S. Rao, S. Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu, 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science, 88: 424-438.
20. Leopold, A. C., M. E. Musgrave and K. M. Williams. 1981. Solute Leakage resulting from leaf desiccation. Plant Physiology 68:1222-1225.
21. Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci.; 7:405–10.
22. Molinari, H. C., C. J. Marur, J. B. Filho, A. K. Kobayashi, M. Pileggi, R. L. Júnior, and L. E. Vieira. 2004. Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (Citrus sinensis Osb. x Poncirus trifoliata L. Raf.) overproducing proline. Plant Sci., 167:1375-1381.
23. Mukherjees, P. and M.A. Choudhuri. 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol Plant 58*:* 166–170.
24. Mullins, E., D. Milbourne, C. Petti, B.M. Doyle-Prestwich, and C. Meade. 2006. Potato in the age of biotechnology. *Trends Plant Sci.* 11,254–260.
25. Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473- 497.
26. Neill, S., R. Desikan, J. Hancock. 2002. Hydrogen peroxide signaling. Current Opinion in Plant Biology, 5: 388-395.
27. Obidiegwu, J.E., G.J. Bryan, H.G. Jones, and A. Prashar. 2015. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. Front. Plant Sci. 6(542), 1-23
28. Parent, B., C. Hachez, E. Redondo, T. Simonneau, F. Chaumont, and F. Tardieu. 2014. Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. Plant Physiol. 149, 2000-2012.
29. Pérez-Clemente R. M. and A. Gómez-Cadenas. 2012**.** *In vitro* Tissue Culture, a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions. In: Annarita Leva and Laura M. R. Rinaldi(Eds.) Agricultural and Biological Sciences ."Recent Advances in Plant *in vitro* Culture", Chapter 5, 91-108.
30. Puthur, J.T., 2016. Antioxidants and cellular antioxidation mechanism in plants. South Indian Journal of Biological Sciences, 2(1): 9-13.
31. Rajendrakumar, C.S., B.V. Reddy and A.R. Reddy. 1994. Proline–protein interactions: Protection of structural and functional integrity of M4 lactate dehydrogenase. Biochem. Biophys. Res. Commun., 201:957-963.
32. Sanchez-Rodriguez, E., M.M. Rubio-Wilhelmi, L.M. Cervilla, B. Blasco, J.J. Rios, M.E. Rosales, L. Romero, and J.M. Ruiz. 2010. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci. 178, 30-40
33. Sanders, G., and S. Arndt. 2012. Osmotic adjustment under drought conditions. In *Plant Responses to Drought Stress*, ed. R. Aroca (Berlin; Heidelberg: Springer), 199–229.
34. Vanková, R., J. Dobrá, and H. Štorchová. 2012. Recovery from drought stress in tobacco: an active process associated with the reversal of senescence in some plant parts and

the sacrifice of others. Plant Signal. Behav. 7, 19–21.

35. Verbruggen, N. and C. Hermans, 2008. Proline accumulation in plants: a review. Amino Acids, 35:753-759.

36. Wegener, C.B., and G. Jansen. 2013. Antioxidants in different potato genotypes: effect of drought and wounding stress. Agriculture 3, 131–146

37. Yamada, M., H. Morishita, K. Urano, N. Shinozaki, K. Yamaguchi-Shinozaki, K. Shinozaki, and Y. Yoshiba, 2005. Effects offree proline accumulation in petunias under drought stress. J. Exp. Bot., 56:1975-1981.

38. Yordanov, I., V. Velikova, and T. Tsonev. 2003. Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol. Special Issue, 187-206

39. Zhang, Z., J. Wang, R. Zhang, and R. Huang. 2012. The ethylene response factor AtERF98 enhances tolerance to salt through the transcriptional activation of ascorbic acid synthesis in Arabidopsis. Plant J.; 71:273–87.