

RESEARCH PAPERS

Effect of Soil Drought on Biomass Production, Physiological Attributes and Antioxidant Enzymes Activities of Potato Cultivars¹

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Abstract—The effects of water deficit on the activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX), and also on total antioxidant capacity, ion leakage, proline content, chlorophyll fluorescence, gas exchange, chlorophyll and carotenoid content and plant dry mass of potato cultivars (*Solanum tuberosum* L.) were investigated. For this purpose, the response of ten potato cultivars (Agria, Arinda, Marfona, Banba, Born, Santé, Milva, Satina, Jelly and Spirit) was studied under two irrigation regimes (70 and 20% of soil available water). Water deficit increased the activity of antioxidant enzymes, ion leakage and proline content but decreased total antioxidant capacity, F_v/F_m , gas exchanges, chlorophyll, carotenoid and plant dry mass in all potato cultivars. Based on stress tolerance index (STI), Agria and Born were the most tolerant and sensitive cultivars to water stress, respectively. Positive correlations were found between total antioxidant capacity and the activities of CAT, APX and POX under control irrigation. Proline content positively correlated with plant dry mass and negatively with photosynthesis, transpiration and carbon dioxide concentration in the sub-stomatal chamber under water deficit treatment. The highest increases in ion leakage under water deficit were observed in the most sensitive cultivars. The results of this experiment, therefore, showed that proline accumulation and ion leakage may be related to water stress tolerance of potato cultivars. Also, increased chlorophyll and carotenoid content in resistant cultivars supported potato plants to tolerate water deficit.

Keywords: *Solanum tuberosum*, antioxidant enzymes, ion leakage, proline

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INTRODUCTION

Environmental stresses are major limiting factors for plant growth in many parts of the world [1]. Damages due to water stress, salinity and temperature are more widespread as compared to the other stresses [2]. Under water deficit, the water potential of the soil falls and as a result, the plant cannot absorb water, therefore under these conditions a series of changes in the plant tissues are accrued for plant adaptation [3]. Photosynthesis as an important plant production process is affected by drought through inhabitation of RuBisCo activity and disruption of energy balance and distribution [4], and also by the changes in chlorophyll con-

tents and components [5]. The resistance of plants to drought stress is classified into three categories; the first type is escaping from drought. In this case, the period of plant growth ends quickly. The second type is drought avoidance by which some modifications occurs in plants, such as increased root growth and water absorption, decreased transpiration, twisting and reducing the leaf area, reduction in the distance between stomata and the increase of waxy compounds and the third type is resistance to drought stress through the production of some protein substances that increase the plant tolerance to stress [3]. Proline and soluble carbohydrates accumulation and ion leakage (IL) are also affected by water deficit [6]. Besides, environmental stresses with direct and indirect effects on plant water status disrupt the photosynthesis and induce oxidative stress in plants [7]. To avoid destructive effects of oxidative damage caused by the excessive production of reactive oxygen species under stress conditions plants are equipped with an antioxidant defense system designed to silence free radicals, which consists of antioxidant enzymes and non-enzymatic

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Abbreviations: APX—ascorbate peroxidase; CAT—catalase concentration in the substomatal chamber; Chl *a*—chlorophyll *a*; Chl *b*—chlorophyll *b*; Chl *a/b*—ratio chlorophyll *a/b*; C_i—carbon dioxide concentration in the substomatal chamber; DPPH—2-2-D-phenyl-1-picrylhydrazil; F_v/F_m — maximum quantum efficiency of photosystem II; Gs—stomatal conductance; IL—ion leakage; NBT—nitroblue tetrazolium; PDM—plant dry mass; POX—peroxidase; SOD—superoxide dismutase; STI—stress tolerance index; TAC—total antioxidant capacity.

components [7]. Non-enzymatic antioxidants include ascorbate, glutathione, alpha tocopherol and beta-carotene. Among the antioxidant enzymes we can refer to superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX). These antioxidants play important roles to modulate the equilibrium between the production and the elimination of free radicals [3, 8]. In areas where potato is grown under water-limited conditions, understanding of plant drought tolerance or adaptation is important. However, selection for drought tolerance is complicated by the fact that the differences in yield reduction cannot be traced back to one or a few major morphological or physiological components while they are needed to develop an efficient screening technique. Moreover, the ability to maintain a high yield under drought is determined by many characteristics and the importance of each factor varies with time and severity of the deficit [9]. In fact, the defense mechanism system often comprises the combination of antioxidant enzymes and non-enzymatic antioxidants [7]. The results of studies have shown that increasing the activity of one or more antioxidant enzymes may increase the resistance to environmental stresses [3, 8]. There are different methods for evaluating total antioxidant capacity. One of these methods is the use of free and stable radical 2,2-D-phenyl-1-picrylhydrazil (DPPH). DPPH is a lipophilic radical with a maximum absorption at 517 nm [10]. In assessing the DPPH antioxidant capacity, DPPH radicals react with antioxidants and, as a consequence, their amounts are reduced [10]. The reduction of DPPH molecules is occurred when hydroxyl groups donate one hydrogen to DPPH radical and as a result, the color of solution converts from violet-dark to light yellow. Absorption at a wavelength of 517 nm indicates the amount of DPPH remaining. Under water deficit conditions, the cell membrane degrades and therefore, water potential of plant cell decreases which end up increasing IL [10]. In addition, one of the plant's reactions to drought stress is osmotic regulation in which increasing the accumulation of some amino acids such as betaine and proline content [11]. Regarding the strategic importance of the potato crop, climate changes, declining rainfall and the shortage of water resources, especially in arid and semi-arid regions of the world, this research, therefore, was carried out on ten potato cultivars to study the relationship between the antioxidant potential and plant growth attributes with the biochemical and physiological bases of potato tolerance to water deficit.

MATERIALS AND METHODS

Plant material and growing conditions. In this pot research, ten potato cultivars (*Solanum tuberosum* L.) including Agria, Arinda, Marfona, Banba, Born, Santé, Milva, Satina, Jelly and Spirit were studied at two moisture regimes (70 and 20% of soil available

water) under greenhouse condition. An experiment based on a randomized complete block design with four replications was conducted during January, February and March of 2017. The culture medium of the pots contained a mixture of sand, garden soil and rotten animal manure with the ratio of 4 : 1 : 1, respectively. To prevent the spread of diseases (insects, nematods, fungus, etc.), disinfection of wet soil was carried out with the metam sodium ($C_2H_4NNaS_2$). Before cultivating the potato tubers, they were stored in dark condition at room temperature of 25°C for two weeks to germinate. Then in each pot, one potato tuber (about 50 g) was planted at a depth of approximately 8 cm. From the time of planting till carrying out the drought stress treatment, all pots were irrigated normally and fertilized with a complete fertilizer of 20-20-20 (a product of CIFO S.P.A., Italy). Water deficit treatment was applied at the stage of forming eight and ten leaves. The instrument for measuring and recording the moisture and soil temperature (IDRG SMS-T1) was used in order to determine the irrigation time. The plants were harvested after four weeks of applying water deficit treatment. Before harvest, the upper fully developed leaves were collected and directly incubated in liquid nitrogen (-196°C) for analysis of antioxidant enzymes, proline content and other physiological traits.

Enzyme extractions and assays. The amount of 0.1 g of leaf tissue was homogenized using a chilled mortar and pestle and added to it 1 mL of 100 mM potassium phosphate buffer (pH 7.8), containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone. The extract was centrifuged at 12000 rpm for 30 min at 4°C. The supernatant was used to assay the following antioxidant enzymes.

The activity of catalase (CAT; EC 1.11.1.6) was measured using the method described by Aebi [12]. The assay buffer contained 3 mL of 50 mM K-phosphate buffer (pH 7.0), 50 µL of enzyme extract and 4.51 µL of H_2O_2 (30%). The decrease in absorbance at 240 nm was inspected for 1 min, using a spectrophotometer U-1800 (Hitachi, Japan).

The ascorbate peroxidase (APX; EC 1.11.1.11) activity was measured based on the method defined by Nakano and Asada [13]. The assay buffer contained 3 mL of 50 mM K-phosphate buffer (pH 7.0), containing 100 µL of 5 mM ascorbate, 50 µL of enzyme extract and 4.51 µL of H_2O_2 (30%). The decrease in absorbance at 290 nm was monitored for 1 min, using a spectrophotometer U-1800 (Hitachi).

The activity of peroxidase (POX; EC 1.11.1.7) was based on the process described by Zaharieva et al. [14]. The assay buffer contained 3 mL of 50 mM K-phosphate buffer (pH 7.0), containing 3.35 µL guaiacol, 50 µL of enzyme extract and 4.51 µL of H_2O_2 (30%). The increase in absorbance at 470 nm was monitored every 30 s for 2 min, using a spectrophotometer U-1800 (Hitachi).

Activity of catalase, ascorbate peroxidase and peroxidase was determined according to the following relationship:

$$\text{Enzyme activity } (\mu\text{mol}/(\text{min g protein})) \\ = \Delta abc \times 1000 \times (1/(A \times \epsilon)) \times ((B + P)/P), \quad (1)$$

where Δabc —the absorption difference of each enzyme; A —the amount of enzyme extract in the reaction solution (μL); ϵ —extinction coefficient of the enzyme (39.4, 2.8 and 26.6 mM/cm for CAT, APX and POX, respectively); B is weight of buffer + weight of plant material; P —fresh weight of plant leaves (g).

The activity of superoxide dismutase (SOD; EC 1.15.1.1) was measured using the modified method described by Giannopolitis and Ries [15] and the activity was determined in 1 mL of general phosphate buffer, 33 μL of nitroblue tetrazolium (NBT) and 33 μL of riboflavin. The absorbance at 570 nm was measured using a spectrophotometer U-1800 (Hitachi). Protein content was evaluated for all antioxidant enzymes by using bovine serum albumin as the standard method described by Bradford [16].

Total antioxidant capacity (TAC). The amount of 0.1 g leaf tissue was homogenized using a chilled mortar and pestle in extraction buffer containing 1 mL of alcohol (96%). The extract was centrifuged at 4000 rpm for 5 min at 4°C. The supernatant was used to assay the total antioxidant capacity which was measured using the modified method reported by Bettaieb et al. [17]. The assay buffer contained 2 mL of 2,2-diphenyl-1-picrylhydrazyl solution and 50 μL of extract. The mixture and reaction took place in the dark for half an hour. The absorption wavelength of the 517 nm in spectrometer was set. Then, the inhibition was calculated based on the following equation:

$$\text{Inhibition } (\%) = (A_0 - A_1)/A_0 \times 100, \quad (2)$$

where A_0 is the control absorption (non-extract added) and A_1 is the sample absorption (extract added).

Ion leakage. The measurement of IL was performed according to the method defined by Lutts et al. [18]. From young leaves, 3 discs with a diameter of 1 cm in each replicate were prepared and washed three times with distilled water. The samples were then placed in test tubes and 10 mL of distilled water was added in each tube and placed on a shaker for 24 h at room temperature. Then, the electrical conductivity (L_t) was measured by the electrical conductivity meter (CC-501). Containers containing specimens were placed in an autoclave for 1 h at 120°C, and again the electrical conductivity of the samples was measured (L_0). Finally, the percent of IL was calculated from the following equation:

$$\text{Ion leakage } (\%) = L_t/L_0 \times 100. \quad (3)$$

Proline content. The method described by Bates et al. [19] was used to measure proline content. The absorbance of the samples was measured using a spectrophotometer U-1800 (Hitachi) at 520 nm. Proline concentration was determined according to the standard chart and expressed using the following formula in terms of $\mu\text{mol}/\text{g}$ of fresh weight of plant leaves:

$$\text{Proline} = [(\Delta abc \times 4 \text{ mL toluene})/115.5] \\ \times [\text{sample weight}/5], \quad (4)$$

where Δabc is the absorption of samples.

Chlorophyll, carotenoid content, fluorescence and gas exchange. Chlorophyll and carotenoid content were measured by the method of Lichtenthaler [20] using the acetone extracts of leaves (0.1 g of leaves per 10 mL of 100% acetone cooled to 2–4°C). The contents of chlorophylls a , b , total chlorophyll and carotenoids were determined spectrophotometrically at 661.6, 644.8 and 470 nm after extraction.

Parameter of chlorophyll fluorescence, maximum quantum efficiency of photosystem II (F_v/F_m) was measured applying a fluorometer (Walz, Germany).

Gas exchanges (photosynthesis (CO_2 absorbance), stomatal conductance, transpiration, carbon dioxide concentration in the sub-stomatal chamber) were measured by using a portable apparatus model (L.C.I. Software version 1:10 UK), on the youngest adult leaves in plants. All measurements were made between 10 and 13 h.

Plant dry mass. All plant parts (shoots and roots) of each cultivar and in each replicate were dried in an oven at 80°C for 48 h to specify a fill dry weight.

Stress tolerance index (STI). This index used to identify cultivars that produce high plant dry mass under both drought and non-drought conditions and expressed using the following formula [21]:

$$\text{STI} = (Y_{pi} \times Y_{si})/(\bar{Y}_p)^2, \quad (5)$$

where Y_{pi} and Y_{si} are yields of cultivar under control and stress conditions, respectively. \bar{Y}_p is total yield mean of cultivars under control condition.

Data analysis. Data were tested and subjected to analysis of variance (ANOVA) by using SAS and MSTATC programs to determine the difference in both treatments and cultivars and based on a randomized complete block design. Comparison of means was performed by using LSD test ($P < 0.05$) and the correlation coefficients between the traits were done by using PROC CORR of SAS. In the other hand, principle component analysis (PCA) was performed based on the correlation matrix to reduce the multiple dimensions of data space and the biplot was drawn using Stat Graphics software.

Table 1. Analysis of variance for the activity of antioxidant enzymes, total antioxidant capacity, ion leakage, proline content, chlorophyll fluorescence, chlorophyll and carotenoids content, and plant dry mass under 70 and 20% of soil available water

Source of variation	Mean square																	df	
	SOD	CAT	APX	POX	TAC	IL	Pro	F_v/F_m	CO ₂ absorbance	Gs	E	Ci	Chl <i>a</i>	Chl <i>b</i>	Chl (<i>a</i> + <i>b</i>)	Chl <i>a/b</i>	Carot		PDM
Drought	3057.1**	2515.1**	3.98**	244.8**	528.1**	3761**	368.7**	0.753**	3780.7**	0.119**	43.06**	460258**	0.184**	0.138**	0.637**	6.53**	0.049**	2267.5**	1
Cultivar	40.48**	80.85**	0.092**	8.23**	62.1**	254.2**	11.86**	0.004 ^{ns}	12.13**	0.001**	0.27 ^{ns}	9619**	0.023**	0.013**	0.064**	0.547**	0.004**	800.9**	9
Cultivar × drought	43.29**	42.32**	0.053**	4.65**	23.7 ^{ns}	48.65 ^{ns}	10.45**	0.001 ^{ns}	13.38**	0.0006**	0.26 ^{ns}	5894**	0.005**	0.002 ^{ns}	0.006 ^{ns}	0.398**	0.0003 ^{ns}	300.9**	9
Error	2.67	3.75	0.0048	0.522	8.78	35.36	0.46	0.0031	3.80	0.0001	0.154	1432.2	0.0015	0.0012	0.005	0.121	0.0002	3.61	54

SOD—superoxide dismutase activity; CA—catalase activity; APX—ascorbate peroxidase activity; POX—peroxidase activity; TAC—total antioxidant capacity; IL—ion leakage; Pro—proline; F_v/F_m —maximum quantum efficiency of photosystem II; CO₂ absorbance; Gs—stomatal conductance; E—transpiration; Ci—carbon dioxide concentration in the sub-stomatal chamber; Chl *a*—chlorophyll *a* content; Chl *b*—chlorophyll *b* content; Chl (*a* + *b*)—total chlorophyll *a* and *b* content; Chl *a/b*—ratio chlorophyll *a/b*; Carot—carotenoids content; PDM—plant dry mass; *df*—degree of freedom. ** Significant at $P < 0.01$, ^{ns}—non-significant.

RESULTS

Plant Dry Mass

The interaction effects of cultivars and irrigation levels were significant ($P < 0.01$) on plant dry mass (PDM) (Table 1). Under normal conditions of irrigation (70% of soil available water), the most and least values of PDM were attained in Agria (25.9 g/plant) and Born (9.37 g/plant). Nevertheless, under drought treatment (20% of soil available water), the maximum and minimum values of PDM were obtained in Marfona (7.95 g/plant) and Born (2.18 g/plant). PDM was significantly reduced in all cultivars at 20 compared to 70% of soil available water. Maximum and minimum reductions were observed (Fig. 1) in Born (76.7%) and Santé (35.2%). Stress tolerance index (STI) was calculated to assess drought tolerance of potato cultivars. The values of STI for cultivars were 0.760 (Agria), 0.617 (Satina), 0.598 (Marfona), 0.351 (Spirit), 0.303 (Sante), 0.277 (Banba), 0.224 (Jelly), 0.207 (Arinda), 0.201 (Milva) and 0.081 (Born).

The Activity of Antioxidant Enzymes

The interaction effects of cultivars and irrigation levels were significant ($P < 0.01$) on the activities of SOD, CAT, APX and POX (Table 1).

The most and least activities of SOD under control irrigation were observed in cultivars Agria (6.57 U/(min mg protein)) and Born (3.77 U/(min mg protein)) and under drought treatment were achieved in Born (26.8 U/(min mg protein)) and Satina (10.7 U/(min mg protein)). The activity of SOD was increased in all cultivars. Maximum and minimum increases were detected in cultivars (Fig. 2) Born (610%) and Satina (129%).

The most and least activities of CAT under control level of irrigation were observed in cultivars Marfona (12.1 $\mu\text{mol}/(\text{min mg protein})$) and Jelly (5.02 $\mu\text{mol}/(\text{min mg protein})$) and under drought treatment were achieved in Born (27.3 $\mu\text{mol}/(\text{min mg protein})$) and Milva (11.8 $\mu\text{mol}/(\text{min mg protein})$). CAT activity was increased in all cultivars. Maximum and minimum increases (Fig. 3) were noticed in cultivars Born (410%) and Satina (85.0%).

The most and least activities of APX under control level of irrigation were observed in cultivars Marfona (0.347 $\mu\text{mol}/(\text{min mg protein})$) and Agria (0.120 $\mu\text{mol}/(\text{min mg protein})$) and under drought treatment were observed in Born (0.829 $\mu\text{mol}/(\text{min mg protein})$) and Agria (0.369 $\mu\text{mol}/(\text{min mg protein})$). APX activity was increased in all cultivars. The highest and the lowest increases were observed in cultivars Born (548%) and Satina (105%) (Fig. 4).

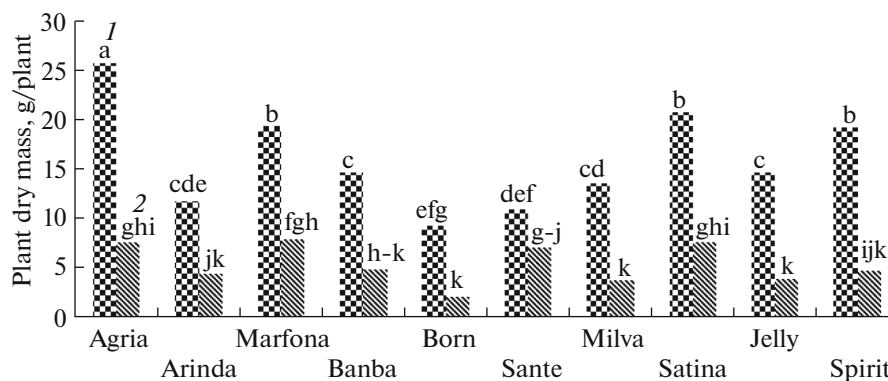


Fig. 1. Plant dry mass of 10 potato cultivars under two irrigation regimes 70% (1) and 20% (2) of soil available water.

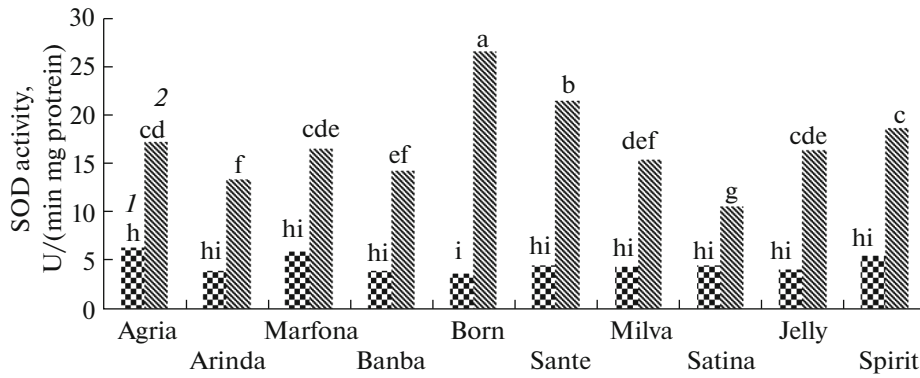


Fig. 2. SOD activity of 10 potato cultivars under two irrigation regimes 70% (I) and 20% (2) of soil available water.

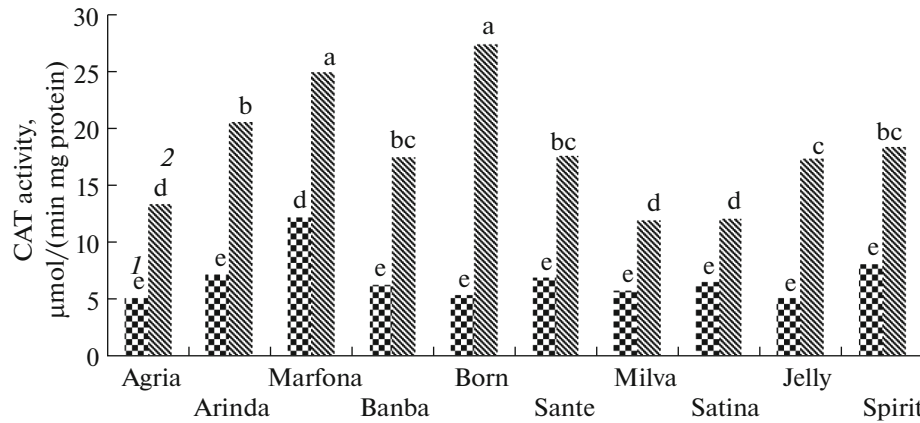


Fig. 3. CAT activity of 10 potato cultivars under two irrigation regimes 70% (I) and 20% (2) of soil available water.

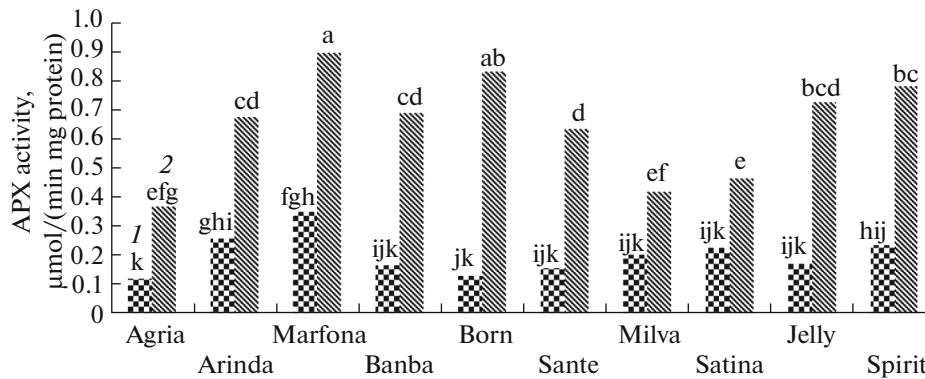


Fig. 4. APX activity of 10 potato cultivars under two irrigation regimes 70% (I) and 20% (2) of soil available water.

The maximum and minimum POX activities under control irrigation were observed in cultivars Marfona (2.933 $\mu\text{mol}/(\text{min mg protein})$) and Banba (1.277 $\mu\text{mol}/(\text{min mg protein})$) and under drought treatment were obtained in Marfona (7.787 $\mu\text{mol}/(\text{min mg protein})$) and Milva (2.617 $\mu\text{mol}/(\text{min mg protein})$). POX activity was increased in all cultivars. The maximum and minimum significant increases (Fig. 5) were observed in cultivars Born (393%) and Milva (86.5%).

Proline Content

The interaction effects of cultivars and irrigation levels were significant ($P < 0.01$) on proline content (Table 1). The highest and the lowest contents of proline under control level of irrigation were obtained in cultivars Jelly and Spirit (4.05 and 4.04 $\mu\text{mol}/\text{g fr wt}$, respectively) and Agria (0.51 $\mu\text{mol}/\text{g fr wt}$) and under water stress treatment were achieved in cultivars Mar-

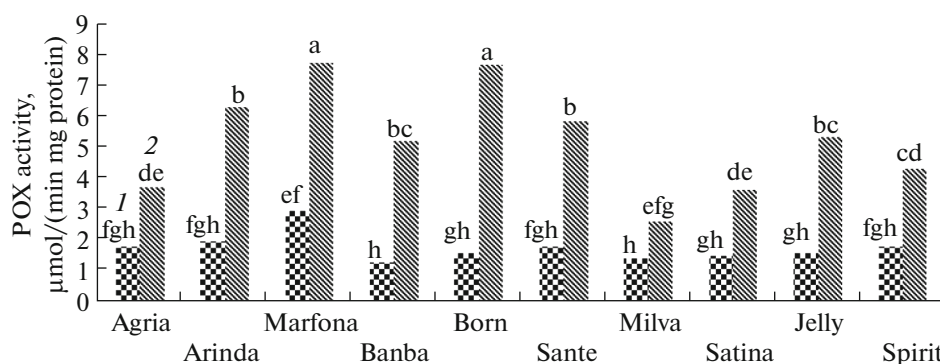


Fig. 5. POX activity of 10 potato cultivars under two irrigation regimes 70% (I) and 20% (2) of soil available water.

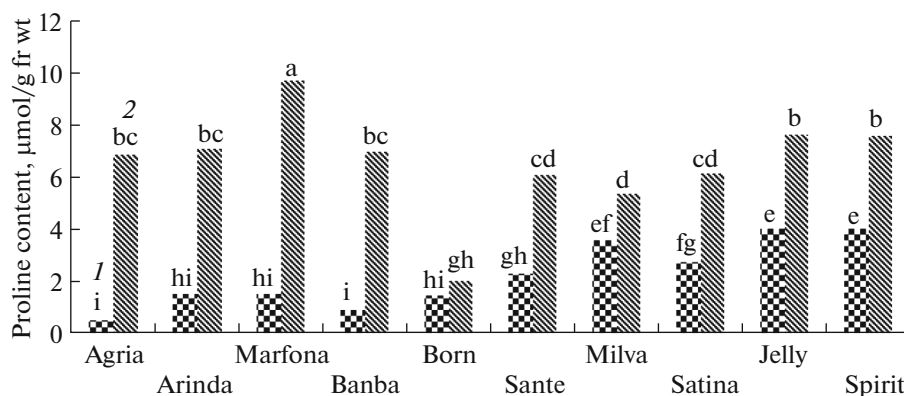


Fig. 6. Content of proline of 10 potato cultivars under two irrigation regimes 70% (I) and 20% (2) of soil available water.

fona (9.74 $\mu\text{mol/g fr wt}$) and Born (2.04 $\mu\text{mol/g fr wt}$). The content of proline was increased in all cultivars and the highest and the lowest increases (Fig. 6) were observed in cultivars Agria (1247%) and Born (37.8%).

Total Antioxidant Capacity and Ion Leakage

The effects of cultivars and irrigation levels were significant ($P < 0.01$) on total antioxidant capacity and IL (Table 1). Water deficit was associated with decreased total antioxidant capacity and increased percent of IL by about 23.3 and 76.7%, respectively (Table 2). However, the interactions between cultivars and irrigation levels were not significant for these two attributes (Table 1). The highest and the lowest total antioxidant capacities (Table 3) were obtained in cultivars Banba (25.03 $\mu\text{g/mL}$) and Milva (15.66 $\mu\text{g/mL}$), the most and least values of IL were observed in cultivars Arinda (34.92%) and Satina (19.51%) respectively.

Chlorophyll, Carotenoids Content and Fluorescence

The interaction effects of cultivars and irrigation levels were significant ($P < 0.01$) on chlorophyll *a* and the ratio of chlorophyll *a/b* (Table 1). The highest and

the lowest contents (Table 4) of chlorophyll *a* under control level of irrigation were obtained in cultivars Jelly, Satina (0.500 mg/g fr wt) and Arinda (0.373 mg/g fr wt). The corresponding values for the ratio of chlorophyll *a/b* were achieved in Milva (2.84) and Agria (1.58). However, under water stress treatment, these values were obtained for Chl *a* in cultivars Satina (0.466 mg/g fr wt) and Santé (0.263 mg/g fr wt), and for Chl *a/b* in cultivars Satina (3.14) and Marfona (2.20). The effects of cultivars and irrigation levels were significant ($P < 0.01$) on F_v/F_m , Chl *b*, Chl (*a + b*) and carotenoids (Table 1). Water deficit was associated with decreased F_v/F_m , Chl *b*, Chl (*a + b*) and carotenoids by about 25.35, 37.90, 26.68 and 32.68%, respectively (Table 2). However, except F_v/F_m , the differences among cultivars were significant in terms of other measured attributes (Table 1).

Gas Exchange

The effects of cultivars and irrigation levels were significant ($P < 0.01$) on photosynthesis (CO_2 absorbance), stomatal conductance (Gs) and carbon dioxide concentration in the sub-stomatal chamber (Ci) (Table 1). The maximum and the minimum rate (Table 4) of photosynthesis under control level of irrigation were obtained in cultivars Arinda (19.53 μmol

Table 2. Means of total antioxidant capacity, ion leakage, chlorophyll fluorescence, chlorophyll and carotenoids content in 10 potato cultivars under two levels of irrigation 70 and 20% of soil available water

Treatment	TAC, $\mu\text{g/mL}$	IL, %	F_v/F_m	Transpiration, $\text{mmol H}_2\text{O}/(\text{m}^2 \text{ s})$	Chl <i>b</i> , mg/g fr wt	Chl (<i>a + b</i>), mg/g fr wt	Carot, mg/g fr wt
Control	22.06 ^a	17.89 ^b	0.777 ^a	2.39 ^a	0.219 ^a	0.671 ^a	0.153 ^a
Drought	16.92 ^b	31.61 ^a	0.58 ^b	0.92 ^b	0.136 ^b	0.492 ^b	0.103 ^b
Variances, %	23.30	76.69	25.35	61.51	37.90	26.68	32.68
LSD, %	2.25	3.17	0.041	0.43	0.01	0.041	0.011

TAC—total antioxidant capacity; IL—ion leakage; F_v/F_m —maximum quantum efficiency of photosystem II; Chl *b*—chlorophyll *b* content; Chl (*a + b*)—total chlorophyll *a* and *b* content; Carot—carotenoids content. Mean followed by the same letter in each column are not significant different according LSD-test (probability level of 5%).

Table 3. Means of total antioxidant capacity, ion leakage, chlorophyll and carotenoids content in 10 potato cultivars

Cultivar	TAC, $\mu\text{g/mL}$	IL, %	Chl <i>b</i> , mg/g fr wt	Chl (<i>a + b</i>), mg/g fr wt	Carot, mg/g fr wt
Agria	18.19 ^{cd}	24.16 ^{bc}	0.26 ^a	0.723 ^a	0.177 ^a
Arinda	17.95 ^{cd}	34.92 ^a	0.151 ^{de}	0.477 ^f	0.108 ^{ef}
Marfona	21.78 ^b	33.57 ^a	0.187 ^{bc}	0.581 ^{cd}	0.117 ^{def}
Banba	25.03 ^a	27.56 ^b	0.191 ^{bc}	0.631 ^{bc}	0.133 ^{bc}
Born	20.49 ^{bc}	22.63 ^{bc}	0.137 ^{de}	0.476 ^f	0.102 ^f
Santé	15.91 ^d	19.83 ^c	0.145 ^{de}	0.507 ^{ef}	0.125 ^{cde}
Milva	15.66 ^d	19.94 ^c	0.132 ^e	0.512 ^{def}	0.108 ^{ef}
Satina	20.34 ^{bc}	19.51 ^c	0.203 ^b	0.685 ^{ab}	0.135 ^{bc}
Jelly	20.26 ^{bc}	24.87 ^{bc}	0.208 ^b	0.659 ^{ab}	0.150 ^b
Spirit	19.32 ^{bc}	20.57 ^c	0.168 ^{cd}	0.566 ^{cde}	0.128 ^{cd}
LSD, %	2.97	5.96	0.034	0.073	0.016

TAC—total antioxidant capacity; IL—ion leakage; Chl *b*—chlorophyll *b* content; Chl (*a + b*)—total chlorophyll *a* and *b* content; Carot—carotenoids content. Mean followed by the same letter in each column are not significant different according LSD-test (probability level of 5%).

$\text{CO}_2/\text{m}^2 \text{ s}$) and Banba (11.72 $\mu\text{mol CO}_2/\text{m}^2 \text{ s}$), Gs in Jelly (0.125 mol $\text{CO}_2/(\text{m}^2 \text{ s})$) and Santé (0.072 mol $\text{CO}_2/(\text{m}^2 \text{ s})$) and Ci in Satina (164.5 $\mu\text{mol/mol}$), Agria (162.8 $\mu\text{mol/mol}$) and Jelly (44.5 $\mu\text{mol/mol}$). However, under water stress treatment, these values were obtained for photosynthesis in cultivars Born (2.57 $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$) and Spirit (0.93 $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$), Gs in Born (0.027 $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$), Jelly (0.025 $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$) and 0.02 $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$) in other cultivars and Ci in Born (371.3 $\mu\text{mol/mol}$) and Marfona (187.0 $\mu\text{mol/mol}$). The effects of cultivars and irrigation levels (Table 1) were significant ($P < 0.01$) on transpiration. Water deficit was associated with decreased transpiration by about 61.51% (Table 2).

Relationship between the Traits

Correlation coefficients between different traits are presented in Table 5. Under control conditions (70% of soil available water), SOD activity, Chl *b*, Chl (*a + b*) and carotenoids content were highly and positively correlated with PDM. Also, APX activity was posi-

tively correlated with SOD and CAT activities. Too, POX activity was correlated with CAT and APX activities. Proline content was negatively correlated with IL. Also, transpiration was positively correlated with photosynthesis and proline content. But it was negatively correlated with IL. At the same time under drought condition (20% of soil available water), there were high positive correlations among antioxidant enzymes, chlorophyll, carotenoid contents and also between proline content and PDM. Also, a positive correlation was obtained between transpiration and photosynthesis (CO_2 absorbance), STI and PDM. But the negative correlation was achieved proline, photosynthesis, transpiration and Ci. As well, we observed a positive correlation between carotenoid contents and the contents of Chl *a*, Chl *b* and Chl (*a + b*) under both 70 and 20% of soil available water.

Biplot Analysis

Principle component analysis (PCA) explained that the first and second components revealed more than 55 and 61% of the variation in 70 and 20% of soil available water, respectively (Table 6). Under 70% of

Table 4. Interaction between cultivar and drought on chlorophyll *a* content and ratio Chl *a/b*, photosynthesis (CO₂ absorbance), stomatal conductance, carbon dioxide concentration in the sub-stomatal chamber and under two levels of irrigation 70 and 20% of soil available water

Cultivar	Chl <i>a</i> , mg/g fr wt		Chl <i>a/b</i>		CO ₂ absorbance, μmol CO ₂ /(m ² s)		Gs, mol CO ₂ /(m ² s)		Ci, μmol/mol	
	control	drought	control	drought	control	drought	control	drought	control	drought
Agria	0.478 ^{abc}	0.445 ^{a-f} (-6.9)	1.58 ^g	2.33 ^{b-f} (+47.19)	15.41 ^{bc}	1.15 ^f (-92.5)	0.075 ^{cd}	0.02 ^e (-73.3)	162.8 ^{ef}	270.5 ^{bc} (+66.2)
Arinda	0.373 ^{ghi}	0.280 ^{jk} (-24.93)	2.09 ^{efg}	2.96 ^a (+41.63)	19.53 ^a	1.53 ^f (-92.2)	0.108 ^b	0.02 ^e (-81.5)	83.8 ^{hi}	253.0 ^{bc} (+202)
Marfona	0.418 ^{c-g}	0.374 ^{ghi} (-10.53)	2.33 ^{b-f}	2.20 ^{c-f} (-5.58)	12.35 ^{de}	1.33 ^f (-89.2)	0.087 ^c	0.015 ^e (-82.8)	99.5 ^{gh}	187.0 ^{de} (+87.9)
Banba	0.497 ^{ab}	0.384 ^{f-i} (-22.74)	2.16 ^{def}	2.76 ^{abc} (+27.78)	11.72 ^e	1.47 ^f (-87.5)	0.077 ^{cd}	0.017 ^e (-77.9)	95.3 ^{ghi}	224.5 ^{cd} (+136)
Born	0.397 ^{e-h}	0.282 ^{jk} (-28.97)	2.30 ^{b-f}	2.99 ^a (+30.00)	15.82 ^b	2.57 ^f (-83.7)	0.115 ^{ab}	0.027 ^e (-76.5)	115.3 ^{fgh}	371.3 ^a (+222)
Santé	0.463 ^{a-d}	0.263 ^k (-43.2)	2.72 ^{a-d}	2.60 ^{a-e} (-4.41)	12.74 ^{cde}	2.07 ^f (-83.7)	0.072 ^d	0.015 ^e (-79.2)	98.0 ^{ghi}	252.0 ^{bc} (+157)
Milva	0.436 ^{b-g}	0.325 ^{ijk} (-25.46)	2.84 ^{ab}	3.06 ^a (+7.75)	16.11 ^b	1.50 ^f (-90.7)	0.083 ^{cd}	0.02 ^e (-75.9)	147.5 ^{efg}	265.0 ^{bc} (+79.7)
Satina	0.500 ^a	0.466 ^{a-d} (-6.8)	1.95 ^{fg}	3.14 ^a (+61.03)	17.41 ^{ab}	1.53 ^f (-91.2)	0.117 ^{ab}	0.02 ^e (-82.9)	164.5 ^{ef}	263.0 ^{bc} (+59.9)
Jelly	0.500 ^a	0.403 ^{d-g} (-19.2)	2.07 ^{efg}	2.80 ^{ab} (+35.27)	15.07 ^{bcd}	1.87 ^f (-87.6)	0.125 ^a	0.025 ^e (-80.0)	44.5 ⁱ	229.5 ^{cd} (+416)
Spirit	0.457 ^{a-e}	0.340 ^{hij} (-25.60)	2.08 ^{efg}	3.02 ^a (+45.19)	17.30 ^{ab}	0.93 ^f (-94.6)	0.112 ^b	0.02 ^e (-82.1)	89.0 ^{hi}	301.3 ^b (+238)

Chl *a*—chlorophyll *a* content; Chl *a/b*—ratio chlorophyll *a/b*; Gs—stomatal conductance, Ci—carbon dioxide concentration in the sub-stomatal chamber. Means followed by the same letter in each column are not significant different according LSD test (probability level of 5%). Values in the parentheses are percent decrease (-) or increase (+) as compared with the control (70% of soil available water).

soil available water, principle component 1 (PC1) had higher and negative correlation with SOD, chlorophyll, carotenoid contents and PDM. But it had a positive correlation with transpiration. This PC could be named as photosynthetic capacity. Principle component 2 (PC2) had higher and positive correlation with CAT, APX, POX, TAC and IL. But it had higher and negative correlation with proline, photosynthesis, Gs and transpiration. This PC could be named as antioxidative potential. Therefore, higher values of these characters indicated higher amount of PDM varieties. As a result, PC1 could be called «potential biomass of plant dry weight». To categorize the varieties based on PCA, the biplot of PC1 and PC2 was constructed (Fig. 7a). As a result, cultivars Agria, Satina and Marfona were found to have the highest SOD activity, chlorophyll pigments and plant dry mass, but Born, Santé, Arinda and Milva were found to have the lowest plant dry mass (low PC2). In other side, variety Marfona had high potential in terms of CAT, APX and POX enzyme (high PC1) under 70% of soil available water conditions. However, under 20% of soil available water, PC1 had positive correlations with SOD, F_v/F_m ,

photosynthesis (CO₂ absorbance), transpiration and Ci and negative correlations with chlorophyll, carotenoid contents, PDM and STI. Therefore, selection based on higher and negative PC1 values may lead to sensitive cultivars. On the other hand, PC2 was positively correlated with CAT, APX, POX activities, IL and proline content. So, cultivars with high PC2 are not suitable for drought stress conditions. According to the biplot analysis of PC1 and PC2 (Fig. 7b), cultivars Born, Arinda, Santé and Spirit had high PC2 but varieties Agria, Satina and Marfona had high PC1 and were hence identified as preferable tolerant cultivars at the vegetative growth stage under 20% of soil available water conditions.

DISCUSSION

Among different environment stresses such as UV radiation, low or high temperature, mechanical injuries, herbicides, flooding and salinity, drought has the highest rank in limiting crop production in many agricultural areas of the world [1]. Efforts for searching of resistant cultivars to water deficit are thus necessary.

Table 5. Correlation coefficients between different traits under 70% (below diagonal) and 20% of soil available water (above diagonal)

Characters	SOD	CAT	APX	POX	TAC	IL	Pro	F_v/F_m	CO ₂ absorb.	Gs	E	Ci	Chl <i>a</i>	Chl <i>b</i>	Chl (<i>a</i> + <i>b</i>)	Chl <i>a/b</i>	Carot	PDM	STI
SOD	1	0.58**	0.44**	0.48**	-0.14 ^{ns}	-0.23 ^{ns}	-0.51**	0.32 ^{ns}	0.60 ^{ns}	0.33 ^{ns}	0.34 ^{ns}	0.62*	-0.57 ^{ns}	-0.48 ^{ns}	-0.56 ^{ns}	-0.12 ^{ns}	-0.36 ^{ns}	-0.33*	-0.40 ^{ns}
CAT	0.35*	1	0.88**	0.87**	0.06 ^{ns}	0.26 ^{ns}	-0.03 ^{ns}	0.35 ^{ns}	0.43 ^{ns}	0.17 ^{ns}	0.43 ^{ns}	0.20 ^{ns}	-0.50 ^{ns}	-0.24 ^{ns}	-0.44 ^{ns}	-0.21 ^{ns}	-0.62 ^{ns}	-0.29 ^{ns}	-0.35 ^{ns}
APX	0.41**	0.71**	1	0.79**	0.22 ^{ns}	0.27 ^{ns}	0.16 ^{ns}	0.28 ^{ns}	0.24 ^{ns}	0.05 ^{ns}	0.21 ^{ns}	-0.01 ^{ns}	-0.39 ^{ns}	-0.21 ^{ns}	-0.35 ^{ns}	-0.13 ^{ns}	-0.60 ^{ns}	-0.23 ^{ns}	-0.37 ^{ns}
POX	0.21 ^{ns}	0.81**	0.60**	1	0.13 ^{ns}	0.31*	0.01 ^{ns}	0.31 ^{ns}	0.49 ^{ns}	0.03 ^{ns}	0.44 ^{ns}	0.003 ^{ns}	-0.45 ^{ns}	-0.10 ^{ns}	-0.35 ^{ns}	-0.34 ^{ns}	-0.49 ^{ns}	-0.12 ^{ns}	-0.26 ^{ns}
TAC	0.04 ^{ns}	0.31*	0.35*	0.37*	1	0.30 ^{ns}	0.18 ^{ns}	-0.20 ^{ns}	-0.09 ^{ns}	-0.07 ^{ns}	0.25 ^{ns}	-0.30 ^{ns}	0.37 ^{ns}	0.36 ^{ns}	0.38 ^{ns}	-0.03 ^{ns}	0.19 ^{ns}	0.01 ^{ns}	-0.01 ^{ns}
IL	0.06 ^{ns}	0.41**	0.23 ^{ns}	0.38**	0.12 ^{ns}	1	0.41**	-0.10 ^{ns}	-0.16 ^{ns}	-0.10 ^{ns}	0.001 ^{ns}	-0.45 ^{ns}	-0.08 ^{ns}	0.32 ^{ns}	0.04 ^{ns}	-0.30 ^{ns}	-0.15 ^{ns}	0.02 ^{ns}	-0.003 ^{ns}
Pro	-0.08 ^{ns}	-0.05 ^{ns}	0.05 ^{ns}	-0.21 ^{ns}	-0.33*	-0.49**	1	-0.47 ^{ns}	-0.71*	-0.60 ^{ns}	-0.69*	-0.84**	0.36 ^{ns}	0.62*	0.45 ^{ns}	-0.52 ^{ns}	0.31 ^{ns}	0.48**	0.50 ^{ns}
F_v/F_m	-0.23 ^{ns}	-0.09 ^{ns}	-0.14 ^{ns}	0.08 ^{ns}	0.33 ^{ns}	0.42 ^{ns}	-0.49 ^{ns}	1	0.44 ^{ns}	0.50 ^{ns}	0.28 ^{ns}	0.64*	-0.30 ^{ns}	-0.50 ^{ns}	-0.37 ^{ns}	0.53 ^{ns}	-0.41 ^{ns}	-0.29 ^{ns}	-0.30 ^{ns}
CO ₂ absorb.	-0.12 ^{ns}	-0.25 ^{ns}	0.08 ^{ns}	-0.17 ^{ns}	-0.28 ^{ns}	-0.32 ^{ns}	0.30 ^{ns}	0.37 ^{ns}	1	0.46 ^{ns}	0.81**	0.42 ^{ns}	-0.46 ^{ns}	-0.47 ^{ns}	-0.48 ^{ns}	0.19 ^{ns}	-0.38 ^{ns}	-0.44 ^{ns}	-0.59 ^{ns}
Gs	-0.35 ^{ns}	-0.12 ^{ns}	0.12 ^{ns}	-0.11 ^{ns}	0.31 ^{ns}	-0.31 ^{ns}	0.50 ^{ns}	0.38 ^{ns}	0.60 ^{ns}	1	0.50 ^{ns}	0.66*	0.01 ^{ns}	-0.22 ^{ns}	-0.06 ^{ns}	0.49 ^{ns}	-0.10 ^{ns}	-0.71**	-0.42 ^{ns}
E	-0.47 ^{ns}	-0.41 ^{ns}	-0.14 ^{ns}	-0.45 ^{ns}	-0.52 ^{ns}	-0.69*	0.68*	-0.01 ^{ns}	0.70*	0.53 ^{ns}	1	0.37 ^{ns}	-0.16 ^{ns}	-0.25 ^{ns}	-0.18 ^{ns}	0.23 ^{ns}	-0.34 ^{ns}	-0.45 ^{ns}	-0.44 ^{ns}
Ci	0.32 ^{ns}	-0.17 ^{ns}	-0.16 ^{ns}	-0.16 ^{ns}	-0.15 ^{ns}	-0.33 ^{ns}	-0.28 ^{ns}	-0.19 ^{ns}	0.15 ^{ns}	-0.32 ^{ns}	-0.04 ^{ns}	1	-0.32 ^{ns}	-0.59 ^{ns}	-0.42 ^{ns}	0.52 ^{ns}	-0.32 ^{ns}	-0.55 ^{ns}	-0.36 ^{ns}
Chl <i>a</i>	0.15 ^{ns}	-0.32 ^{ns}	-0.35 ^{ns}	-0.44 ^{ns}	-0.02 ^{ns}	-0.35 ^{ns}	0.22 ^{ns}	-0.48 ^{ns}	-0.36 ^{ns}	-0.08 ^{ns}	-0.20 ^{ns}	0.09 ^{ns}	1	0.80**	0.98**	-0.17 ^{ns}	0.73**	0.48 ^{ns}	0.73**
Chl <i>b</i>	0.54 ^{ns}	-0.24 ^{ns}	-0.30 ^{ns}	-0.07 ^{ns}	0.16 ^{ns}	-0.01 ^{ns}	-0.21 ^{ns}	0.07 ^{ns}	-0.04 ^{ns}	0.003 ^{ns}	-0.42 ^{ns}	0.23 ^{ns}	0.65*	1	0.90**	-0.65*	0.78**	0.61*	0.77**
Chl <i>a+b</i>	0.40 ^{ns}	-0.31 ^{ns}	-0.35 ^{ns}	-0.27 ^{ns}	0.09 ^{ns}	-0.18 ^{ns}	-0.01 ^{ns}	-0.19 ^{ns}	-0.20 ^{ns}	-0.04 ^{ns}	-0.35 ^{ns}	0.19 ^{ns}	0.89**	0.92**	1	-0.33 ^{ns}	0.78**	0.54 ^{ns}	0.77**
Chl <i>a/b</i>	-0.37 ^{ns}	0.15 ^{ns}	0.13 ^{ns}	-0.02 ^{ns}	-0.33 ^{ns}	-0.19 ^{ns}	0.32 ^{ns}	-0.51 ^{ns}	-0.28 ^{ns}	-0.28 ^{ns}	0.31 ^{ns}	-0.11 ^{ns}	-0.28 ^{ns}	-0.85**	-0.65*	1	-0.40 ^{ns}	-0.59 ^{ns}	-0.54 ^{ns}
Carot	0.57 ^{ns}	-0.24 ^{ns}	-0.35 ^{ns}	-0.08 ^{ns}	0.11 ^{ns}	-0.08 ^{ns}	-0.05 ^{ns}	-0.05 ^{ns}	-0.15 ^{ns}	-0.03 ^{ns}	-0.39 ^{ns}	0.07 ^{ns}	0.72**	0.96**	0.93**	-0.73**	1	0.50 ^{ns}	0.65*
PDM	0.84**	0.17 ^{ns}	0.16 ^{ns}	0.17 ^{ns}	0.11 ^{ns}	-0.01 ^{ns}	-0.10 ^{ns}	-0.18 ^{ns}	0.02 ^{ns}	-0.12 ^{ns}	-0.45 ^{ns}	0.46 ^{ns}	0.46 ^{ns}	0.81**	0.72**	-0.67*	0.75**	1	0.88**
STI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

SOD—superoxide dismutase activity, CAT—catalase activity, APX—ascorbate peroxidase activity, POX—peroxidase activity, TAC—Total antioxidant capacity, IL—ion leakage, Pro—proline content, F_v/F_m —maximum quantum efficiency of photosystem II, Gs—stomatal conductance, E—transpiration, Ci—chlorophyll *a* and *b* content, Chl (*a* + *b*)—total chlorophyll *a* and *b* content, Chl *a/b*—ratio chlorophyll *a/b*, Carot—carotenoids content, PDM—plant dry mass, STI—stress tolerance index; ns—non-significant, * significant at 5% level of probability and ** significant at 1% level of probability.

Table 6. Principle component loadings for the traits measured in 10 potato cultivars under two levels of soil available water in the greenhouse conditions

Characters	70% of soil available water		20% of soil available water	
	PC1	PC2	PC1	PC2
SOD, U/(min mg protein)	-0.282	0.170	0.248	0.061
CAT, $\mu\text{mol}/(\text{min mg protein})$	0.026	0.415	0.211	0.384
APX, $\mu\text{mol}/(\text{min mg protein})$	0.085	0.327	0.162	0.401
POX, $\mu\text{mol}/(\text{min mg protein})$	-0.018	0.416	0.167	0.426
TAC, $\mu\text{g}/\text{mL}$	-0.101	0.253	-0.076	0.135
IL, %	-0.031	0.381	-0.036	0.381
Pro., $\mu\text{mol}/\text{g fr wt}$	0.123	-0.235	-0.259	0.270
F_v/F_m	0.034	0.086	0.218	-0.070
CO_2 absorb., $\mu\text{mol CO}_2/(\text{m}^2 \text{ s})$	0.111	-0.171	0.264	0.004
Gs, $\text{mol CO}_2/(\text{m}^2 \text{ s})$	0.092	-0.138	0.187	-0.192
E, $\text{mmol H}_2\text{O}/(\text{m}^2 \text{ s})$	0.261	-0.313	0.209	0.013
C_i , $\mu\text{mol}/\text{mol}$	-0.127	-0.081	0.247	-0.253
Chl <i>a</i> , $\text{mg}/\text{g fr wt}$	-0.285	-0.220	-0.272	-0.120
Chl <i>b</i> , $\text{mg}/\text{g fr wt}$	-0.403	-0.082	-0.298	0.119
Chl (<i>a + b</i>), $\text{mg}/\text{g fr wt}$	-0.384	-0.161	-0.291	-0.047
Chl <i>a/b</i>	0.313	0.011	0.171	-0.319
Carot, $\text{mg}/\text{g fr wt}$	-0.392	-0.103	-0.272	-0.148
PDM, g	-0.369	0.050	-0.269	0.087
STI	—	—	-0.292	0.007
Eigenvalue	5.59	4.44	8.05	3.68
Percent of variation	31.05	24.68	42.39	19.39
Cumulative percentage	31.05	55.72	42.39	61.78

SOD—superoxide dismutase activity, CAT—catalase activity, APX—ascorbate peroxidase activity, POX—peroxidase activity, TAC—Total antioxidant capacity, IL—ion leakage, Pro—proline content, F_v/F_m —maximum quantum efficiency of photosystem II, Gs—stomatal conductance, E—transpiration, C_i —carbon dioxide concentration in the sub-stomatal chamber, Chl *a*—chlorophyll *a* content, Chl *b*—chlorophyll *b* content, Chl (*a + b*)—total chlorophyll *a* and *b* content, Chl *a/b*—ratio chlorophyll *a/b*, Carot—carotenoids content, PDM—plant dry mass, STI—stress tolerance index.

Potato is among the plants that are susceptible to water scarcity [22]. The current research was therefore attended to evaluate the response of some potato cultivars to water shortage under greenhouse condition in terms of changes in plant biomass, antioxidant enzymes, chlorophyll pigments, gas exchange, proline content and IL.

In the present study, under control irrigation (based on 70% of soil available water), among ten tested potato cultivars, the highest biomass production was achieved in Agria (25.9 g/plant) followed by Satina (20.8 g/plant), Marfona (19.43 g/plant) and Spirit (19.4 g/plant), respectively (Fig. 1). These cultivars, therefore, growth better under conditions where water is not limited. Plant dry mass of all cultivars were significantly decreased at 20% of soil available water as compared to the control irrigation. Studies [23, 24] indicate that water deficit affects plant canopy architecture by reducing leaf size and leaf expansion rate

and increasing the rate of senescence. Also, stomatal closure occurs as a result of water shortage which in turn limits plant CO_2 intake and leads to reduced biomass production. There was significant variation among tested potato cultivars in response to water deficit, as the reduction in biomass production under water stress treatment ranged from 35 (Santé) to 76% (Born). Based on the stress tolerance index, the most drought tolerant cultivar was Agria, followed by Satina, Marfona, Spirit, Santé, Banba, Jelly, Arinda, Milva and Born. Consequently, Agria was the most tolerant and Born was the most sensitive cultivar in terms of reduction in biomass production under water stress imposed during plant vegetative growth stage. Other researchers [25] have also reported the existence of significant variation among potato cultivars in response to water stress. Similar to the findings [5], we detected a decrease in the contents of chlorophyll and carotenoid but an increase in Chl *a/b* under water

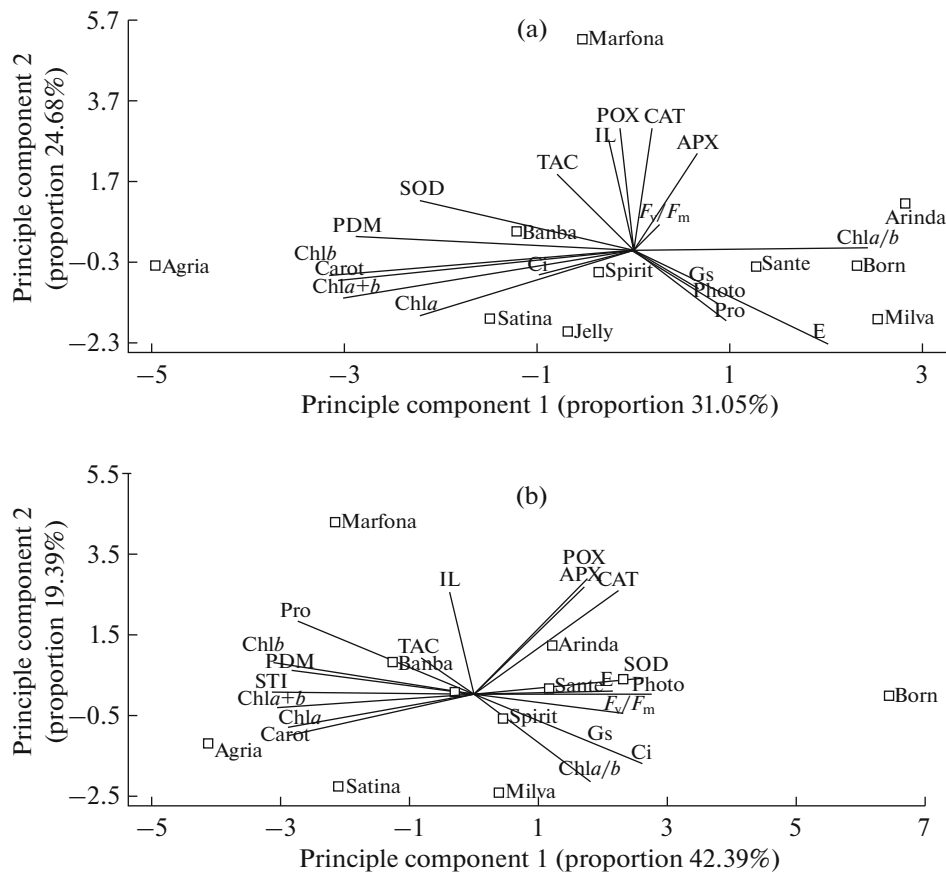


Fig. 7. The biplot displays the traits measured on 10 potato cultivars under 70% (a) and 20% (b) of soil available water. SOD—superoxide dismutase activity, CAT—catalase activity, APX—ascorbate peroxidase activity, POX—peroxidase activity, TAC—total antioxidant capacity, IL—ion leakage, Pro—proline content, F_v/F_m —maximum quantum efficiency of photosystem II, Photo—photosynthesis (CO_2 absorbance), Gs—stomatal conductance, E—transpiration, Ci—carbon dioxide concentration in the sub-stomatal chamber, Chl a—chlorophyll a content, Chl b—chlorophyll b content, Chl (a + b)—total chlorophyll a and b content, Chl a/b—ratio chlorophyll a/b, Carot—carotenoids content, PDM—plant dry mass, STI—stress tolerance index.

deficit (Tables 2, 4). The highest values of Chl b, Chl (a + b) and carotenoids content among cultivars belonged to Agria, Satina, Jelly and the lowest values were observed in Milva and Born, respectively (Table 3). Also, similar to the results of [5, 26], we detected a decrease in the photosynthesis, transpiration and Gs but increases in Ci under water deficit (Tables 2, 4). The maximum values of photosynthesis, Gs and Ci were obtained in Born and the minimum values of these attributes were achieved for photosynthesis in Spirit, Ci in Marfona and with the exception of Gs in Born and Jelly, the lowest values were observed in other cultivars (Table 4). Chlorophyll pigments and gas exchange (photosynthesis (CO_2 absorbance), transpiration, Gs and Ci) as an important plant production process are affected by water deficit [4, 5]. In fact, carotenoids are pigments which play a major role in the protection of plants against photo-oxidative processes. They are involved in protecting of the photosynthetic apparatus by quenching single oxygen and other harmful free radicals which are synthesized during photosynthesis [27]. Also, many

researchers [26] indicated that under stress conditions photo-damage for photosynthetic system occurred and the higher Ci was directly related to CO_2 absorbance and Gs.

The distribution of potato cultivars in the biplot constructed based on PCA, revealed that tolerant cultivars had high chlorophyll, carotenoid, proline contents, PDM and STI (high PC1) but had lower activities of antioxidant enzymes and rates of gas exchange (PC2) (Table 6). In the other hand, susceptible cultivars of potato plants had the lowest chlorophyll, carotenoid, proline contents, PDM and high negative STI value but higher activity of antioxidant enzymes and gas exchange. Accordingly, the resistant cultivars may include Agria, Satina and Marfona. In the contrary, susceptible cultivars are Born, Milva, Arinda and Santé (Fig. 7b).

Water deficit imposed on potato plants in this study was complemented by significantly increase in the activities of antioxidant enzymes and proline content (Figs. 2–6). In fact, water deficit increases the pro-

duction of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH^-). Meanwhile, increasing the amount of ROS destroys lipids, cell membranes, macromolecules, DNA and carbohydrates, and thus prevents photosynthetic activities. The mechanism of adaption of plants to water deficit involves the activation of antioxidant enzymes to clean these destructive free radicals under stress [28]. Similar to our findings in the studies of [3, 8] on potato and [15] on corn, the activities of SOD, CAT, APX, POX were increased under drought conditions (Figs. 2–5). Similar to the outcomes of [8], we detected a decrease in the antioxidant scavenging capacity of free radical DPPH under water deficit (Table 2). The highest and the lowest value in total antioxidant capacity between cultivars belonged to Banba (25.03 $\mu\text{g/mL}$) and Milva (15.66 $\mu\text{g/mL}$) (Table 3). This reduction occurs during drought stress because of the damage of cell membrane that leads to a decrease in the scavenging ability in plants [8]. However, the interaction between cultivar and drought stress was not significant in this regard (Table 1). In the current study, positive correlations were found between total antioxidant capacity and the activities of CAT, APX and POX under control irrigation. This may indicate that these enzymes have significant contribution in determination of total antioxidant capacity of potato plants under non-stress condition. However, there were no significant correlations between total antioxidant capacity and the activities of measured antioxidant enzymes under stress condition (Table 4). This may imply that under water-limited irrigation other enzymatic, i.e. monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase [29] and non-enzymatic antioxidants such as ascorbate, glutathione and alpha tocopherol [8] are involved in the determination of total antioxidant capacity of potato plants. Like other enzymes, SOD activity was increased in all cultivars under water deficit treatment (Fig. 2). However, the extent of increases was smaller in Agria, Satina, Marfona and Spirit that were among the most tolerant cultivars. This means that the increased activity of SOD could not lead to increased tolerance of potato cultivars. Yet, negative correlations were found between the activity of SOD and plant dry mass under water-limited irrigation (Table 5). This may suggest that increased activity of SOD under stress condition was ended up some energy costs for the plants. However, there was positive correlation between the activity of SOD and plant dry mass under control irrigation, indicating the positive role of this enzyme in plant biomass production under none-stress condition. Similar to the findings of Bansal and Nagarajan [11] in potato plants, we observed increasing of proline content in all tested cultivars under water deficit (Fig. 6). Many researchers [11] have reported the genes induced for biosynthesis of proline in potato plants under water deficit conditions. Proline accumulation in plants is one of the

common acclimating mechanisms to drought stress. In fact, plants regulate their osmotic potential and adjust to low soil water availability by accumulating osmoregulators such as proline. Moreover, plant stress tolerance is improved by protecting and stabilizing membranes and enzymes during stress by these organic compatible compounds [23]. In the current study, we observed a positive correlation between proline content and plant dry mass under water deficit treatment (Table 5). This indicates that in this study proline had significant role in manipulation of plant biomass production under water stress. However, some studies [30] reported that proline accumulation in potato under water stress was primarily occurred in sensitive cultivars.

Although no significant correlations between IL and plant dry mass were found within the two environments, e.g. normal and water-limited conditions (Table 5), the highest and the lowest value in IL between cultivars belonged to Arinda (34.92%) and Satina (19.51%) that according to stress tolerance index were among the most sensitive and tolerant cultivars, respectively (Table 3). This may indicate that cell membrane damage due to increased ROS and enhanced IL under water stress was more prominent in sensitive cultivars. According to Lu et al. [6] it was also reported increased IL in potato cultivars under drought condition. This may, therefore, verify the use of this trait as a precise index for cultivar selection under drought stress.

In conclusion, in this study we have been able to gather evidence that water deficit increased IL, proline content and the activity of antioxidant enzymes SOD, CAT, APX and POX while decreased total antioxidant capacity, F_v/F_m , gas exchange, chlorophyll, carotenoid contents and plant biomass in potato cultivars. However, the increased activities of antioxidant enzymes did not induce tolerance in potato cultivars under water stress. Accordingly, it seems that under circumstances of this experiment other enzymatic and non-enzymatic antioxidants are involved in the tolerance mechanism of the tested cultivars. Yet, results indicated that these antioxidant enzymes had some contributions in plant growth under non-stress condition. Significant correlations between proline content and low IL with plant biomass revealed the important role of these two attributes in biomass production of potato plants under water stress. Also, increases carotenoids content in resistant cultivars supported potato plants to tolerate water deficit.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies

involving animals or human participants performed by any of the authors.

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