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Foliar Application of Salicylic Acid and Glycine Betaine improve Antioxidant Enzymes and Biochemical Characteristics of Potato Cultivars

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ABSTRACT

This experiment was conducted to evaluate the response of potato plants to water deficit and foliar application of salicylic acid (SA) and glycine betaine (GB). Potato cultivars were grown in pots and subjected to two irrigation regimes (30 and 60% depletion of available soil water) and three independent levels of both SA (0.0, 0.5 and 1.0 mM) and GB (0.0, 1.0 and 2.0 mM). Activities of superoxide dismutase, catalase, peroxidase, proline, photosynthesis, total chlorophyll, carotenoid along with plant dry mass and tuber yield were measured. Drought significant effects on all traits. Drought tolerance was determined as increasing in antioxidant enzymes, proline and by calculating the stress susceptibility index (SSI). As well, drought tolerant cultivars (Spirit and Born) revealed higher accumulation of proline content, carotenoid and antioxidant enzymes activities. Also, the minimum values of SSI were observed in tolerant cultivars. However, foliar applied SA and GB affected the activities of antioxidant enzymes and increased proline content, photosynthesis, total chlorophyll, carotenoid, PDM (plant dry mass) and TY (tuber yield). The range of the increases seemed more when potato cultivars were grown under water deficit conditions. All the above effects aided the PDM and TY of potato cultivars to increase by increasing SA and GB in most levels and led to the supposition that external spray of SA and GB could be improved the growth and enabled potato cultivars to withstand water deficit. Also, under 60% depletion of available soil water, the SA effects were clearer on all traits as compared with their controls and applied GB.

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INTRODUCTION

Drought, salinity, low and high temperature are the most important limiting factors among different environmental constraints which induce plant stress and diminish crop productivity in many parts of the world (Lawlor, 2002). Drought ranks first in limiting crop productivity in the majority of world agricultural fields especially in arid and semi-arid areas (Tas and Tas, 2007; Abedi and Pakniyat, 2010). Drought due to water stress impedes many physiological and biochemical aspects of plant growth.

However, growth regulators are substances that in very low concentrations influence and regulate a wide range of cellular and physiological processes. Also, they regulate the opening and closing of stomata, photosynthesis, absorption, and transfer of ions in plants (Morgan, 1990). Moreover, salicylic acid (SA), one of the growth regulators which have a positive effect on plant resistance to various environmental stresses (Farooq et al., 2009). Moreover, Khan et al., (2014) indicated that salicylic acid induced glycine betaine accumulation in Vigna radiata under salinity stress, subsequent increase of glutathione, reduction of ethylene and oxidative stress, and enhancement of photosynthesis. Likewise, glycine betaine (GB) is an organic osmolite that naturally accumulates in many plant species exposed to various stresses. Also, it is found to be abundant in chloroplasts, which play an important role in regulating and protecting the thylakoid membrane, thus maintaining the amount of photosynthesis (Genard et al., 1991). As well as GB plays an important role in osmoregulation and it can interplay with molecules and structures, preserving the activity of macromolecules, maintaining the integrity of membranes against stresses, and scavenging reactive oxygen species (Annunziata et al., 2019).

Meanwhile, many investigations have been reported that externally using of salicylic acid and glycine betaine leads to increasing and improving plant resistance to these stresses (Hayat et al., 2010; Cheng et al., 2013; Janda et al., 2014). However, results of Vicent and Plasencia (2011) and Cheng et al. (2013) showed that exogenous SA and GB regulate the activity of various antioxidant enzymes including peroxidase, superoxide dismutase, and catalase. As well, increase photosynthesis, transpiration, stomatal conductance, and carbon dioxide concentration in the sub-stomatal chamber (Monneveux et al., 2014). Nevertheless, drought stress becomes the first limiting factor in the growth and production of crops worldwide (Hojati et al., 2011). Also, increases accumulation of proline content (Shannon, 1997) and reactive oxygen species (ROS) (Boguszewska et al., 2010), which will be harmful to all components of plant cells (Hue et al., 2012). So, plants to protect their cells from the toxic effect of ROS have two antioxidant defense systems which consist of non-enzymatic antioxidant molecules (Boguszewska et al., 2010) and also antioxidant enzymes (Cervilla et al., 2007). However, potato (*Solanum tuberosum* L.) is inserted in the fourth most important food crop after wheat, maize, and rice (Vreugdenhil et al., 2007) and well known as a crop which is highly sensitive to soil drought, which could lead to significant tuber yield losses (Jefferies and MacKerron, 1989; Hijmans, 2003). However, approaches to select cultivars with improved drought tolerance while retentive the present yield potential are of great interest. In the past years, breeding for drought tolerance in potato was based on selection for high yield under stress and other phenotypic traits (Monneveux et al., 2013). Also, many researchers indicated that the activities of antioxidant enzymes i.e. (CAT, APX and SOD) were increased in tolerant genotypes (Shi et al., 2015; Askari and Ehsanzadeh, 2015). However, the activities of these antioxidant enzymes are induced as a general adaptation strategy to withstand oxidative stress (Foyer and Noctor, 2003). As well, a number of selection indicators, such as stress susceptibility index (SSI) is usually used in investigation and breeding practices for identification of cultivars which produce high dry mass and yield under both stress and non-stress environments (Fischer and Maurer, 1978). So, this investigate was conducted to study some of biochemical characters and yield of four potato cultivars in response to drought stress under greenhouse conditions. Also, study the effect of foliar application salicylic acid and glycine betaine if they could improve growth and tolerance of potato cultivars against drought stress based on the production of plant dry mass and yield of potato cultivars, the activity of antioxidant enzymes and by using the stress susceptibility index.

MATERIALS and METHODS

Plant Material and Growing Conditions

A pot greenhouse experiment based on a randomized complete block design with three replications was conducted from September to December 2017 in the research greenhouse of college of agriculture, Isfahan University of Technology located in Isfahan (51º 28' E, 42º 33' N and 1626.2 m above the mean sea level) to study the effects of foliar application of salicylic acid (three levels including 0, 0.5, and 1 mM) and glycine betaine (three levels including 0, 1, and 2 mM) for three times (after two weeks of starting drought treatment and every five days) separately under two irrigation regimes (30 and 60% depletion of available soil water) on four potato cultivars (Spirit, Born, Arinda and Banba) class super elite (SE). Tubers (50-60 g) were kept at room temperature of 25°C for two weeks to germinate and planted at a depth of approximately 8 cm in pots with dimensions (35×25 cm) in the culture medium which was prepared of sand, garden soil and rotten animal manure with the ratio of 4: 1: 1, respectively. In addition to, some samples of the medium culture were prepared for analyzing (Table 1). Also, it sterilized with the metam sodium ($C_2H_4NNaS_2$) to prevent the spread of diseases. All pots were irrigated normally and fertilized with a complete floral fertilizer of 20-20-20 (a product of CIFO S.P.A., Bologna, Italy). Thus, water deficit treatment was applied at the stage of forming eight and ten leaves. Furthermore, the instrument for measuring and recording the moisture and soil temperature (IDRG SMS-T1) was used to determine the irrigation time. At the end of six weeks of applying water deficit treatment plants were harvested. Before that, the upper fully developed leaves were collected and directly incubated in liquid nitrogen (-196^{o}C) for analysis of antioxidant enzymes, proline content and other physiological traits. The available soil water and the volume of irrigation water were calculated based on Askari and Ehsanzadeh (2015).

Table 1. Physical and chemical properties of the experimental soil.

pH	Electrical conductivity (dSm^{-1})	Organic carbon %	Available K (mg kg^{-1})	Available P (mg kg^{-1})	Total N (mg kg^{-1})	Type of soil	Organic content %
7.75	1.1	0.76	381.5	55.1	342	sandy	1.33

Measurement of Growth Parameters

Enzyme Extractions and Assays

To determine the activity of superoxide dismutase, catalase and peroxidase enzymes the amount of 0.1 g of the upper fully developed leaves tissue were collected and homogenized using a chilled mortar and pestle and the mixture was prepared 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^{o}C. The supernatant was used to assay the following antioxidant enzymes.

Superoxide Dismutase (SOD; EC 1.15.1.1): SOD activity was assayed by measuring its ability to inhibit the photo reduction of nitro blue tetrazolium (NBT) using the modified method of Giannopolitis and Ries (1977) and determined in 1 ml of phosphate buffer, 33 µl of nitro blue tetrazolium and 33 µl of riboflavin, respectively. The absorbance at 570 nm was measured using a spectrophotometer U-1800 (Hitachi, Japan).

Catalase (CAT; EC 1.11.1.6): The activity of CAT was measured using the method of Aebi (1984). The assay buffer contained 3 ml of 50 mM K-phosphate buffer (pH 7.8), 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).

Peroxidase (POX; EC 1.11.1.7): The activity of this enzyme was based on the process described by Rao et al. (1996). The assay buffer contained 3 ml of 50 mM K-phosphate buffer (pH 7.8), 50 µl of enzyme extract, 4.51 µl of H_2O_2 (30%), and 3.35 µl Guiacol. In this method increasing absorbance at 470 nm was monitored every 30 s for 2 min, using a spectrophotometer U-1800 (Hitachi, Japan).

All extraction stages were carried out at 4 ºC, and protein content was evaluated for all antioxidant enzymes by using bovine serum albumin as the standard method described by Bradford (1976).

Leaf Proline Content, Total Chlorophyll and Carotenoid Contents

The method described by Bates et al. (1973) was used to measure free proline content in the leaves of potato plants.

Chlorophyll and carotenoid contents were measured by the method of Lichtenthaler (1987) using the acetone extracts of leaves (0.1 g of leaves per 10 mL of 100% acetone cooled to 2–4°C).

Photosynthesis

Photosynthesis rate was 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 hours.

Plant Dry Mass and Yield Measurement

Aerial parts of potato plant cultivars in each replicate were dried separately in an oven at 80ºC for 72 h to specify a fixed dry weight. Also, the yield was weighed on the basis of gram per plant.

Stress Susceptibility Index (SSI)

SSI index was used to select the susceptible cultivars. Lower SSI corresponds to higher drought tolerance and this index was calculated according to the relationship of Fischer and Maurer (1978).

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.

RESULTS

Analysis of variance for antioxidant enzymes, photosynthesis (Photo), total chlorophyll (Chl *tot*), carotenoid (Car), Leaf proline content (Pro), plant dry mass (PDM) and tuber yield (TY) of potato cultivars showed significant ($p < 0.01$) between all interactions (drought × cultivar, drought × treatment, cultivar × treatment and drought × cultivar × treatment) except PDM at the interaction drought × treatment (Table 2).

Plant Dry Mass and Tuber Yield

Results of PDM and TY of potato cultivars showed significant effect between interactions (drought × cultivar × treatment) and these attributes were affected by SA and GB levels (Figure 1a, b). Our results indicated that PDM of cultivars Spirit and Banba under control and water deficit was decreased with increase in GB level (2.0 mM).

Table 2. Analysis of variance for the activity of antioxidant enzymes, proline content, photosynthesis, chlorophyll and carotenoids content, plant dry mass and tuber yield of potato cultivars under two levels, 30% (control) and 60% (drought) depletion of available soil water, and three levels of salicylic acid and glycine betaine treatments

Source of variation	*df*	**Mean square**								
		SOD	**CAT**	**POX**	**Pro**	**Photo**	**Chl *tot***	**Car**	**PDM**	**TY**
Drought	1	577.19**	415.41**	6.16**	284.96**	4082.05**	3.28**	0.597**	762.55**	1078255.2**
Cult	3	22.36**	17.29**	3.82**	10.71**	8.18**	0.013**	0.001**	15.65**	36228.54**
Treat	4	16.53**	59.45**	2.34**	4.86**	184.84**	0.093**	0.005**	7.15**	1995.52**
Drought*Cult	3	29.52**	59.65**	2.97**	6.09**	25.89**	0.02**	0.001**	28.54**	33329.1**
Drought*Treat	4	23.24**	24.78**	0.298**	6.09**	13.44**	0.012**	0.0005**	0.807^{ns}	1831.77**
Cult*Treat	12	1.43**	13.20**	0.297**	1.18**	4.36**	0.01**	0.001**	3.475**	2082.19**
Drought*Cult*Treat	12	1.46**	5.05**	0.177**	0.821**	5.57**	0.005**	0.001**	1.280**	1993.16**
Error	80	0.081	1.34	0.029	0.049	1.20	0.0005	0.0001	0.455	238.54
C.V	-	6.89	8.58	8.20	9.69	5.85	4.37	6.00	8.33	8.20

cultivar (Cult), treatment (Treat), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), proline (Pro), photosynthesis (Photo), total chlorophyll (Chl tot), carotenoid (Car), plant dry mass (PDM) and tuber yield (TY), freedom degree (df). ** Significant at $P < 0.01$, * Significant at $P < 0.05$, ^{ns}: non-significant, respectively.

The maximum TY under control conditions was observed in Arinda at (1.0, 2.0 mM GB) and Banba and Arinda at (0.5 mM SA), respectively. Also, the maximum TY under drought conditions was observed in Born at (0.5 and 1.0 mM SA). The stress susceptibility index (SSI) was calculated to evaluate the level of drought tolerance of potato cultivars; the results showed variation with cultivars, ranging from 0.83 (Spirit) to 0.86 (Born), 1.08 (Arinda) and 1.11 (Banba).

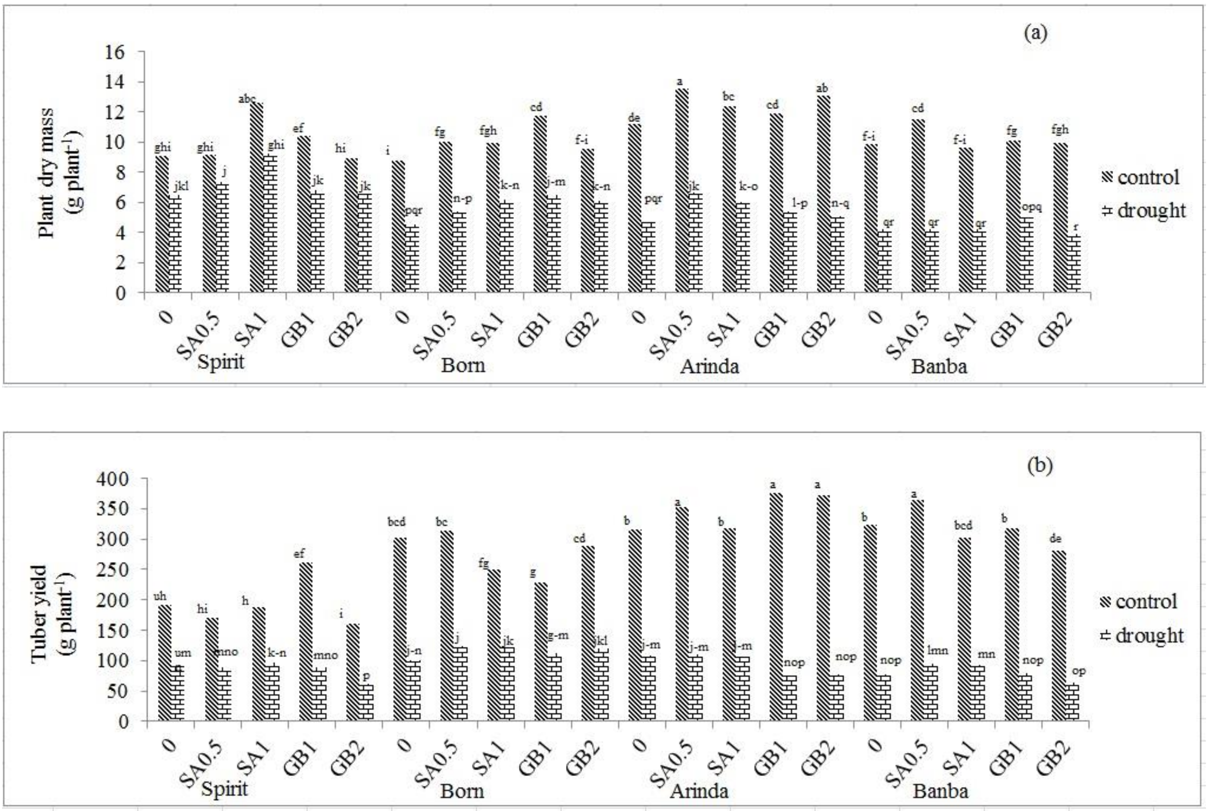

Figure 1. Effect of foliar application salicylic acid and glycine betaine on plant dry mass (a) and tuber yield (b) of four potato cultivars under two irrigation regimes, 30% (control) and 60% (drought) depletion of available soil water.

Photosynthesis, Chlorophyll and Carotenoid Contents

The interaction effects of drought × cultivar × treatment were significant ($p < 0.01$) on the rate of Photo, Chl *tot* and Car content and affected by SA and GB levels (Table 3). Photo, Chl *tot* and Car of all cultivars under control and water deficit were increased with the increasing SA and GB levels. The maximum Photo under control conditions was observed in Spirit, Arinda and Banba at (2.0 mM GB), Chl *tot* and Car in Arinda at (2.0 mM GB), respectively. Also, under drought conditions the maximum Photo was observed in Born at (1.0 mM SA), Spirit and Born at (2.0 mM GB), Chl *tot* and Car in Spirit and Born at (1.0 mM SA), Born in at (2.0 mM GB), also, Born and Arinda at (1.0 and 2.0 mM GB), respectively.

Antioxidant Enzymes

The interaction effects of drought × cultivar × treatment were significant ($p < 0.01$) on the activities of SOD, CAT and POX and affected by SA and GB levels (Table 2). with the exception of SOD activity in Born at (0.5 and 1.0 mM SA) and Arinda at (0.5, 1.0 mM SA and 1.0 mM GB) under control conditions, SOD, CAT and POX activities of all cultivars under control and water deficit were increased with the increased of SA and GB levels. The maximum SOD activity under control conditions was observed in Arinda and Banba at (2.0 mM GB), CAT and POX activities in Arinda at (1.0 mM SA), respectively. Also, under drought conditions the maximum activities of SOD at (0.5

and 1.0 mM SA) and CAT at (0.5 mM SA) were observed in Spirit cultivar. While the highest POX activity was detected in Spirit and Born cultivars at level (1.0 mM SA). In the contrary, the minimum values of antioxidant enzymes activity were obtained in Banba cultivar at (0.0 mM SA and GB) treatment (Figure 2 a, b and c).

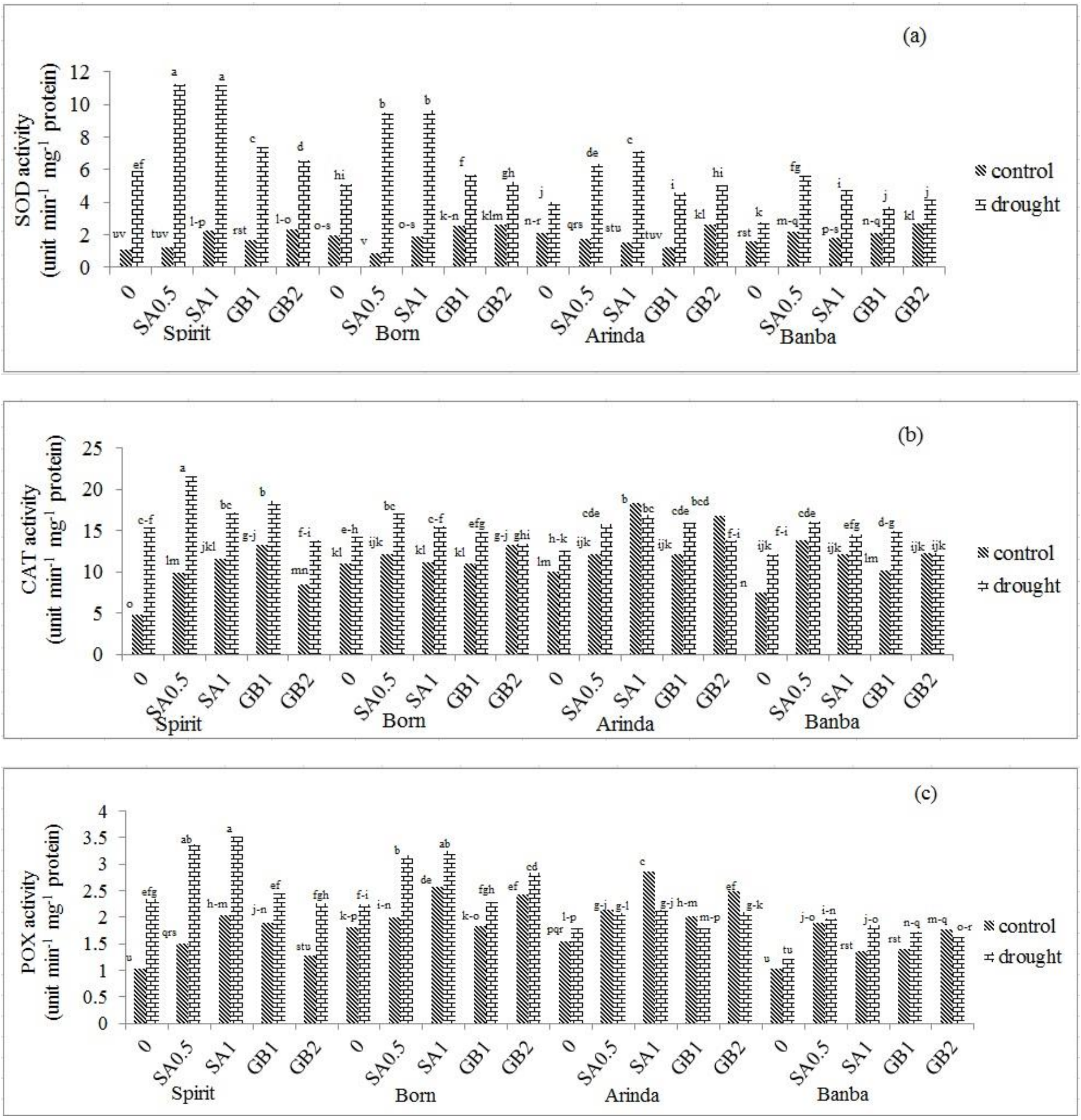

Figure 2. Effect of foliar application salicylic acid and glycine betaine on SOD (a), CAT (b) and POX (c) activity of four potato cultivars under two irrigation regimes, 30% (control) and 60% (drought) depletion of available soil water.

Table 3 Interaction between drought, cultivar, treatment of salicylic acid and glycine betaine on photosynthesis, total chlorophyll and carotenoid under two levels of irrigation, 30% (control) and 60% (drought) depletion of available soil water in the pots

Cult		**Treat**	**Photo (µmol CO2 m^{-2} s^{-1})**		**Chl *tot* (mg g^{-1} leaf)**		**Car (mg g^{-1} leaf)**	
			30%	60%	30%	60%	30%	60%
Spirit	**SA (mM)**	0.0	19.60^{hi}	11.21^{op}	0.534^{i}	0.224^{r}	0.163^{j}	0.034^{q}
		0.5	21.26^{gh}	15.41^{jk}	0.661^{ef}	0.386^{lm}	0.200^{fgh}	0.065^{nop}
		1.0	25.94^{def}	14.51^{jkl}	0.749^{bc}	0.456^{j}	0.211^{c-f}	0.085^{k}
	GB (mM)	1.0	22.61^{g}	15.53^{jk}	0.696^{de}	0.346^{nop}	0.203^{fg}	0.077^{k-n}
		2.0	29.51^{a}	15.68^{j}	0.678^{e}	0.385^{lm}	0.193^{ghi}	0.077^{k-n}
Born	**SA (mM)**	0.0	19.65^{hi}	8.44^{qr}	0.589^{h}	0.229^{r}	0.199^{fgh}	0.038^{q}
		0.5	25.96^{def}	12.90^{l-o}	0.635^{fg}	0.434^{jk}	0.204^{fg}	0.070^{mno}
		1.0	27.01^{b-f}	15.67^{j}	0.670^{ef}	0.460^{j}	0.223^{c}	0.081^{klm}
	GB (mM)	1.0	26.17^{c-f}	13.82^{k-n}	0.748^{bc}	0.403^{kl}	0.211^{c-f}	0.083^{klm}
		2.0	27.33^{b-e}	15.95^{j}	0.727^{cd}	0.457^{j}	0.207^{ef}	0.086^{k}
Arinda	**SA (mM)**	0.0	18.43^{i}	9.71^{pq}	0.735^{c}	0.215^{r}	0.220^{cde}	0.037^{q}
		0.5	22.91^{g}	12.26^{no}	0.597^{h}	0.298^{q}	0.183^{i}	0.055^{p}
		1.0	25.58^{ef}	14.21^{j-m}	0.715^{cd}	0.396^{l}	0.207^{ef}	0.076^{k-n}
	GB (mM)	1.0	25.48^{f}	12.93^{l-o}	0.734^{c}	0.316^{opq}	0.221^{cd}	0.071^{l-o}
		2.0	28.09^{ab}	13.28^{lmn}	0.827^{a}	0.402^{kl}	0.286^{a}	0.084^{kl}
Banba	**SA (mM)**	0.0	18.17^{i}	7.37^{r}	0.575^{h}	0.213^{r}	0.188^{hi}	0.035^{q}
		0.5	27.61^{bcd}	12.28^{no}	0.676^{e}	0.328^{opq}	0.206^{fg}	0.060^{op}
		1.0	26.72^{b-f}	12.39^{no}	0.777^{b}	0.374^{lmn}	0.208^{def}	0.075^{k-n}
	GB (mM)	1.0	26.00^{def}	12.68^{mno}	0.667^{ef}	0.310^{pq}	0.241^{b}	0.076^{k-n}
		2.0	27.82^{abc}	12.33^{no}	0.602^{gh}	0.351^{mno}	0.188^{hi}	0.074^{k-n}
LSD%			1.78		0.036		0.013	

Cultivar (Cult), treatment (Treat), photosynthesis (Photo), total chlorophyll (Chl tot), carotenoid (Car), salicylic acid (SA) and glycine betaine (GB). Mean followed by the same letter in each column are not significant different according LSD test (probability level of 5 %).

Leaf Proline Content

The interaction effects of drought × cultivar × treatment was significant ($p < 0.01$) on the proline content of potato cultivars and affected by SA and GB levels (Table 2). With the exception of proline content under control conditions in Arinda at (1.0 mM SA) and Banba at (1.0, 2.0 mM GB) the proline content in all cultivars were decreased as compared with their controls. Also, under drought conditions the maximum proline content was observed in Spirit at (1.0 mM GB) and Born at (1.0 mM SA) treatment, respectively. In the contrary, the minimum values of proline content were obtained in Arinda and Banba cultivars at (0.0 mM SA and GB) treatment (Figure 3).

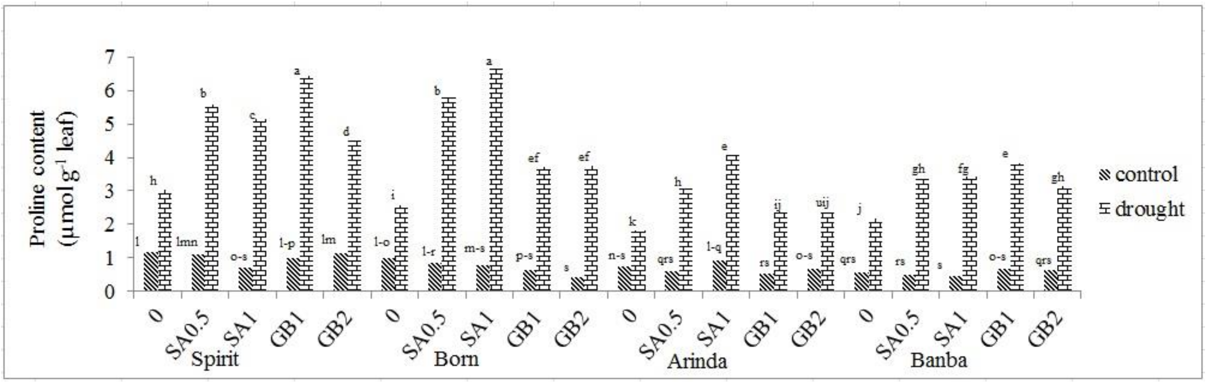

Figure 3 Effect of foliar application salicylic acid and glycine betaine on proline content of four potato cultivars under two irrigation regimes, 30% (control) and 60% (drought) depletion of available soil water.

Relationship Between the Traits

In order to determine relationships among potentially useful characteristics to be used in collecting potato cultivars, for arid and semi-arid regions of the word which be branded with declining rainfall and the shortage of water resources, the correlation coefficients between SSI and the measured traits were calculated and are presented in Table 4. Except Chl *tot* and Car under drought conditions (60% depletion of available soil water), SSI index was negatively correlated with all traits especially PDM and TY. Higher values of SSI correspond to higher sensitively cultivars to water deficit. Also, our result indicates that there are a positive correlation between PDM and antioxidant enzymes, proline, photosynthesis, Chl *tot* and TY (Table 4).

Biplot Analysis

Principle component analysis (PCA) exposed that the first and second components explained more than 59.05 and 78.61% of the variation in 30% (control) and 60% (drought) depletion of available soil water, respectively (Table 5). However, under 30% depletion of available soil water, PC1 had higher correlation with SOD, CAT, POX, Photo, Chl *tot*, Car and PDM. As higher values of these characteristic may show higher photosynthetic capacity and antioxidant activity. This PC could be named as “anti-oxidative potential and photosynthetic capacity”. Also, PC2 had higher correlation with TY.

Table 4. Correlation coefficients of different traits under 60% depletion of available soil water in the greenhouse conditions

Traits	**SOD**	**CAT**	**POX**	**Pro**	**Photo**	**Chl*tot***	**Car**	**PDM**	**TY**	**SSI**
SOD	1	-	-	-	-	-	-	-	-	-
CAT	0.78^{**}	1	-	-	-	-	-	-	-	-
POX	0.92^{**}	0.64^{**}	1	-	-	-	-	-	-	-
Pro	0.81^{**}	0.66^{**}	0.76^{**}	1	-	-	-	-	-	-
Photo	0.60^{**}	0.51^{*}	0.62^{**}	0.71^{**}	1	-	-	-	-	-
Chl*tot*	0.61^{**}	0.33^{ns}	0.66^{**}	0.65^{**}	0.83^{**}	1	-	-	-	-
Car	0.34^{ns}	0.22^{ns}	0.38^{ns}	0.51^{*}	0.83^{**}	0.89^{**}	1	-	-	-
PDM	0.76^{**}	0.61^{**}	0.74^{**}	0.55^{**}	0.60^{**}	0.44^{*}	0.33^{ns}	1	-	-
TY	0.36^{ns}	0.19^{ns}	0.49^{*}	0.29^{ns}	0.11^{ns}	0.28^{ns}	0.02^{ns}	0.20^{**}	1	-
SSI	-0.74^{**}	-0.47^{*}	-0.81^{**}	-0.59^{**}	-0.43^{*}	-0.39^{ns}	-0.12^{ns}	-0.73^{**}	-0.50^{*}	1

superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), proline (Pro), photosynthesis (Photo), total chlorophyll (Chl tot), carotenoid (Car), plant dry mass (PDM), tuber yield (TY) and stress susceptibility index (SSI). ^{ns} non-significant, * significant at $p < 0.05$ and ** significant at $p < 0.01$, respectively.

Table 5. Principle component loadings for the traits measured on 4 potato cultivars under two levels 30% (control) and 60% (drought) depletion of available soil water

Characters	**30% depletion of available soil water**		**60% depletion of available soil water**	
	PC1	PC2	PC1	PC2
SOD (unit min^{-1} mg^{-1} protein)	0.245	-0.265	0.339	-0.021
CAT (unit min^{-1} mg^{-1} protein)	0.417	0.021	0.255	0.031
POX (unit min^{-1} mg^{-1} protein)	0.360	0.035	0.349	-0.045
Pro (μmol g^{-1} leaf)	-0.292	-0.099	0.306	0.134
Photo (μmol CO$_2$ m^{-2} s^{-1})	0.296	-0.356	0.268	0.360
Chl *tot* (mg g^{-1} leaf)	0.374	-0.306	0.258	0.338
Car (mg g^{-1} leaf)	0.367	0.130	0.170	0.500
PDM (g plant^{-1})	0.336	0.119	0.300	0.004
TY (g plant^{-1})	0.275	0.536	0.178	-0.226
SSI	-	-	-0.322	0.222
Eigenvalue	4.25	1.65	7.38	2.83
Percent of variation	42.52	16.53	56.81	21.80
Cumulative percentage	42.52	59.05	56.81	78.61

superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), proline (Pro), photosynthesis (Photo), total chlorophyll (Chl tot), carotenoid (Car), plant dry mass (PDM), tuber yield (TY) and stress susceptibility index (SSI).

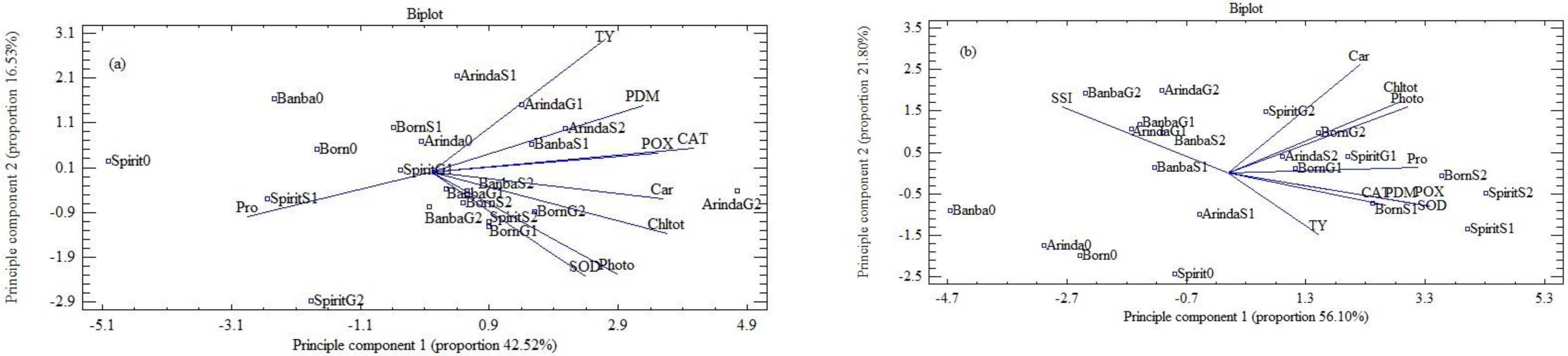

Figure 4 The biplot display of the traits measured on 4 potato cultivars under 30% (control) (a) and 60 % (drought) (b) depletion of available soil water. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), proline (Pro), photosynthesis (Photo), total chlorophyll (Chl tot), carotenoid (Car), plant dry mass (PDM) and tuber yield (TY) and stress susceptibility index (SSI). Cultivars with 0 (control), Salicylic acid at 0.5 and 1.0 mM (S1, S2, respectively), glycine betaine at 1.0 and 2.0 mM (G1 and G2, respectively)

Therefore, higher value of this trait indicated higher yield productivity of the varieties. As a result, PC2 could be named "potential of yield production". To classify the cultivars based on PCA, the biplot of PC1 and PC2 was constructed (Figure 4a). As a result, cultivars Born and Arinda at (0.5 and 1.0 mM SA), Banba and Spirit at (0.5 and 1.0 mM SA), Arinda at (1.0 and 2.0 mM GB) and Banba at (1.0 mM GB) were found to have high anti-oxidative potential, photosynthetic capacity, PDM and TY under 30% depletion of available soil water, respectively. On the other hand, under 60% depletion of available soil water, PC1 had higher and positive correlation with SOD, CAT, POX, proline, PDM and TY. But it had negatively correlated with SSI index. Therefore, selection based on high PC1 values can lead to tolerant cultivars with high anti-oxidative potential and photosynthetic capacity. In the contrary, PC2 was positively correlated with photo, Chl *tot* and Car. Therefore, cultivars with high PC2 are suitable for drought stress conditions. According to the biplot analysis of PC1 and PC2 (Figure 4b), cultivars Born and Spirit at (0.5, 1.0 mM SA and 1.0 and 2.0 mM GB), Arinda at (1.0 mM SA), respectively had high PC1 and PC2 and were hence identified as preferable cultivars for 60% depletion of available soil water.

DISCUSSION

All over the world, 85 percent of fresh water is used in agriculture (Levy et al., 2013) and potato production in arid and semi-arid regions is highly dependent on irrigation water supplies to produce high yields of marketable potatoes (Stark et al., 2013). So, potato yield losses in the world due to climate change are expected to range between 18 and 32% during the first three decades of this century (Monneveux et al., 2014). In the present study, under control irrigation regime (30% depletion of available soil water), we observed variation in production PDM and TY of potato cultivars and foliar applied of SA and GB in some concentrations increased them, i.e. the higher PDM was obtained in Arinda (13.46 and 13.01 g plant^{-1}) at (0.5 and 2.0 mM SA and GB), respectively followed by Spirit (12.58 g plant^{-1}) at 1.0 mM of SA treatment (Figure 1a). Also, the higher TY was obtained in Arinda (375 and 371.7 g plant^{-1} at 1.0 and 2.0 mM of GB, respectively and 351.7 g plant^{-1} at 0.5 mM of SA), also Banba (363.3 g plant^{-1} at 0.5 mM SA). These cultivars could be suitable for cultivation in regions where water is not limited for crop production (Figure 1 b). Similar to the findings Nouri et al. (2016), our results indicated that PDM and TY were significantly reduced at 60% depletion of soil available water as compared to the control irrigation. With the exception of PDM of Banba at 2.0 mM GB, exogenous of SA and GB separately increased it at all levels and in all cultivars (Figure 1a). But the results for TY under this irrigation regime were different. However, the higher TY was obtained in Born cultivar, especially after exogenous of SA and GB at all levels. In fact, water deficit reduced plant growth (Kumar et al., 2007), dry matter (Stiller, 2008) and chlorophyll pigments (Sarani *et al.,* 2014) and water deficit results in stomatal closure, decreases photosynthetic efficiency, transpiration rates (Yordanov et al., 2000) and CO_2 assimilation is greatly reduced in susceptible cultivars of potato (Schafleitner, 2009). In the other hand, salicylic acid and glycine betaine are described to be biosynthesized in plants in response to environment stresses and to improve the growth of plants and their resistance to these stresses if applied externally (Allard et al., 1998; Janda et al., 2014). Furthermore, the findings of Vicent and Plasencia (2011) and Annunziata et al. (2019), showed that SA and GB appear to be 'effective therapeutic agent' for plants and it plays a crucial role in the regulation of physiological and biochemical processes.

Still, water deficit leads to increase accumulation of ROS in plants (Finkel and Holbrook, 2000; Hue et al., 2012). So, plants have many mechanisms of adaption to water deficit such as activation of antioxidant enzymes to clean these destructive free radicals (Boguszewska et al., 2010; Cervilla et al., 2007). However, plants have two antioxidant enzymes defense system to protect their cells from the toxic effect of ROS includes superoxide dismutase (SOD), catalase (CAT) and peroxidases (POXs) (Cervilla et al., 2007). Similar to the findings Shi et al. (2015), in the current study, potato cultivars reacted differently to water deficit in terms of antioxidant enzyme activities. This displays that potato cultivars have various adaptive mechanisms to control their redox status. Also, Shi et al. (2015) reported that the activities of SOD, POX and CAT were increased under water stress in two resistant and one susceptible varieties of potato. In our study, the activities of SOD, CAT and POX enzymes were increased in most cases in response to water deficit. Also, with some exceptions, exogenous different concentrations of SA and GB were significantly increased the activities in all cultivars under both control and water deficit conditions (Figure 2 a, b and c). The extents increased of SOD activity in tolerant cultivars Spirit (11.2 unit min^{-1} mg^{-1} protein) followed by Born (9.5 and 9.7 unit min^{-1} mg^{-1} protein) at (0.5 and 1.0 mM SA), respectively. CAT activity was obtained in Spirit (21.6 unit min^{-1} mg^{-1} protein at 0.5 mM SA and 18.6 unit min^{-1} mg^{-1} protein at 1.0 mM GB, respectively) followed by Born (17.1 unit min^{-1} mg^{-1} protein) at 0.5 mM of SA level and the highest values of POX activity was obtained in Spirit (3.5 and 3.4 unit min^{-1} mg^{-1} protein) and Born (3.2 unit min^{-1} mg^{-1} protein) at both levels 0.5 and 1.0 mM of SA (Figure 2 a, b and c). However, the minimum values of antioxidant enzymes activity were obtained in susceptible cultivars of potato (Banba and Arinda). Similar to our results Cheng et al. (2013), Askari and Ehsanzadeh (2015), reported increased in SOD, CAT and POX in terms of water deficit and exogenous of SA and GB. However, many researchers showed that GB specifically increases its own content and improves the activity of antioxidant enzymes and metabolites, i.e. SOD, CAT, APX, reducing H_2O_2 and malondialdehyde (MDA) in *O. sativa* (Yao et al., 2016). Moreover, Annunziata et al. (2019) indicated that exogenous GB applications have been confirmed to encourage the expression of genes involved in oxidative stress responses, with a restriction of reactive oxygen species (ROS) accumulation and lipid peroxidation in cultured tobacco cells under drought stress, and even steadying photosynthetic structures under stress. Also, antioxidant enzymes were negatively correlated with SSI index and positively with PDM. This is indicating to the main role of these enzymes in improving resistance potato cultivars against water deficit. More studies indicated that SOD enzyme is the first plant defense barrier against oxidative stresses and it converts the free radical O_2^- into H_2O_2

(Halliwell, 1999). Under environmental stresses, accumulation proline is considered one of the main roles of adapting plants to stress by removing reactive oxygen species, increasing antioxidants activity and osmotic regulation inside plants (Shannon, 1997; Ashraf and Foolad, 2007). In the current study, water deficit increased leaf proline content in all tested cultivars but to different extents. Also, this increase was significant in all tested cultivars and in all different concentrations of exogenous SA and GB as compared with control (Figure 3). Moreover, under drought conditions the maximum proline content was observed in Born (6.6 µmol g^{-1} leaf) and Spirit (6.4 µmol g^{-1} leaf) at 1.0 mM level of SA and GB, respectively (Figure 1c). As well as a positive relationship was detected in this investigation between proline content and PDM under water deficit that suggests higher proline content could have a relative increase in PDM under stress conditions (Table 4). Depending on the SSI index, the higher proline content accumulation was obtained in tolerant cultivars and the lowest in sensitive ones. Meanwhile, findings of Askari and Ehsanzadeh (2015) in fennel genotypes; Bandurska and Stroinski (2005) in barley revealed that exogenous SA increased proline content in terms to water stress. As well, results of Yao et al. (2016) indicated that existing GB increased proline content and y-amino butyric acid in *O. sativa*. In fact, under water deficit and other abiotic stresses, proline plays a main role in many processes of plants, i.e. it represents as a mediator of osmotic adjustment, as a preservative of macromolecule, as a well-matched solute to protect enzymes and as a store for carbon and nitrogen. In the other hand, Juan et al. (2005) revealed that chlorophyll pigments are one of the key factors in determining the rate of photosynthesis and dry matter accumulation. Similar to the findings Yordanov et al. (2000) and Sarani et al. (2014), our outcomes showed that photosynthesis, total chlorophyll and carotenoid were affected with water deficit. Also, exogenous of SA and GB increased photosynthesis and chlorophyll pigment under both control and water deficit conditions as compared to the control (Table 3). Meanwhile, under 60% depletion of available soil water, all levels of exogenous of SA and GB increased photosynthesis, Chl *tot* and Car. The higher values of photosynthesis were detected in Born (15.95 µmol CO_2 m^{-2} s^{-1} at 2.0 mM GB and 15.67 µmol CO_2 m^{-2} s^{-1} at 0.5mM of SA followed by Spirit (15.68 and 15.5 µmol CO_2 m^{-2} s^{-1} at 2.0 and 1.0 mM of GB and 15.4 µmol CO_2 m^{-2} s^{-1} at 0.5 mM of SA, respectively. Likewise, the highest values of Chl *tot* and Car were obtained in Spirit at 1.0 mM of SA level and Born at all levels of SA and GB as compared with control, respectively (Table 3). However, results Khan et al. (2003) on maize, Askari and Ehsanzadeh (2015) on fennel and Cheng et al. (2013) on potato plants, indicated that exogenous of SA and GB increased chlorophyll pigments and photosynthesis in terms to drought stress as compared with control.

Many researchers indicated that salicylic acid is an imperative regulator of photosynthesis for the reason that it affects leaf and chloroplast structure (Uzunova and Popova, 2000), stomatal closure (Melotto et al., 2006), chlorophyll and carotenoid contents (Chandra and Bhatt, 1998; Fariduddin et al., 2003), and the activity of enzymes such as RuBisCO (ribulose-1,5bisphosphate carboxylase/oxygenase) and carbonic anhydrase (Pancheva and Popova, 1998). As well, glycine betaine protects photosynthesis by modifying the lipid composition of the thylakoid membranes in *Triticum aestivum* (Zhao et al., 2007).

Also, under water stress a positive correlation was detected between photosynthesis, total chlorophyll and PDM that suggests higher these traits could have a comparative increase in PDM (Table 4). Moreover, under 60% depletion of soil moisture, Biplot analysis proved that the higher values of measured traits were obtained in Born and Spirit cultivars (Figure 4b).

CONCLUSION

Drought stress in the current study increased the activity of antioxidant enzymes and leaf proline content but decreased photosynthesis, total chlorophyll, carotenoid, biomass and tuber yield of potato cultivars. Also, our study revealed that exogenous of SA and GB had played a main role and promoting effect on PDM, TY and some biochemical characteristics of potato cultivars under both drought and control conditions. Else, SOD, CAT, POX enzymes, and proline content showed to be related to stress tolerance of potato cultivars as the extents of increases in the activities of these traits were greater in tolerant cultivars (Born and Spirit) and lower in sensitive cultivars (Banba and Arinda). The outcomes suggest that the external spray with SA and GB not only positively affected photosynthesis and chlorophyll pigments, but also significantly changed the activities of antioxidant enzymes that all these traits supported potato cultivars to withstand drought stress.

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Conflict of Interest

The authors have declared that there are no competing interests.

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