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UV-B Radiation, Soil Salinity, Drought Stress and Their Concurrent Effects on Some Physiological Parameters in Mize Plant

Susan Javadmanesh, Fatemeh Rahmani and Latifeh Pourakbar

Biology Department and Biotechnology Research Centre of Urmia University, Urmia, Iran

Abstract: Continuous climate change can lead to several stress factors. Among these, UV-B radiation, drought and soil salinity are the most concerning issues that affect plant productivity and survival. Maize is a main food and the third most important world-wide crop plant. In the present study, 21-day-old maize plants (*Zea mays* L. cv. SC. 704) were exposed to 8 days UV-B radiation, drought and salinity stress (100 mM NaCl), alone or combined and some physiological parameters such as pigment content, RWC, lipid peroxidation, total protein content, soluble sugar content, enzyme activity of ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and nitrate reductase (NRA) were investigated. In this study RWC decreased but carotenoid content, enzyme activity of APX and GPX as well as MDA content increased markedly during all stresses. Our results showed a significant decrease in NRA for both shoots and roots of salinity and salinity-UV-treated plants. Oppositely, chlorophyll content increased under salinity and simultaneous stress, but decreased slightly in UV-B-treated and sharply in drought-treated plants. Furthermore, plants under unfavorable conditions reduced their protein content in roots but this parameter did not alter in shoot tissue. Our results demonstrated that among these factors, plants exhibit higher adaptive potential under UV-B radiation and drought has the most detrimental effect on maize plant. Furthermore, we found that UV-B radiation discounts the deleterious effects of soil salinity and drought in this plant.

Abbreviations: APX-Ascorbate peroxidase · Chl-Chlorophyll · Cx+c-Total Carotenoid · GPX-Guaiacol Peroxidase · MDA-Malondialdehyde · NRA-Nitrate Redutase Activity · Pro-Total Protein Content · RWC-Relative Water Content · Sug-Sugar Content

Key words: Antioxidant enzyme activit · Lipid peroxidation · Nitrate reductase activity · Pigment content · RWC · Soluble sugar content · Total protein content

INTRODUCTION

The term 'abiotic stress' includes numerous stresses caused by complex environmental condition, such as strong light, UV radiation, high and low temperatures, freezing, drought, salinity, nutrient deficiency, anaerobic stresses, heavy metals and hypoxia. Climate change has been identified as a serious risk factor for the future, is continuing to increase [1]. Therefore, understanding abiotic stress responses is one of the most important topics in plant research [2]. Most of the abiotic stresses (e.g. salinity and drought) are related to anthropogenic activities, which are clearly causing major changes in atmospheric chemistry and climate [3]. There is much concern about increasing levels of earth's stratospheric ozone layer destruction because this protective layer is the primary attenuator of solar UV-B radiation. UV-B amount and intensity depends on atmospheric and geographic factors [4, 5]. However, some of plant responses to UV-B radiation can be modified in combination with other abiotic stresses. Plants acclimation to a combination of different stress conditions depend on their appropriate response to each of these different stresses, individually [6]. However, the effects of these combined stresses on plants are still largely unknown. An increasing number of studies have been designed to test the interactions of environmental factors on plants, such as the interaction between UV-B and osmotic stress [7, 8], interactions between salinity and Fe deficiency [9-11], interaction between UV-B radiation and

Corresponding Author: Susan Javadmanesh, Biology Department and Biotechnology Research Centre of Urmia University, Urmia, Iran. Tel: +989112703550- +989356987328.

N nutrient [12] and interaction between UV-B radiation and Fe deficiency [13, 14].

Water deficit stress caused by drought, soil salinity and low-temperature, affect negatively plant growth and development. It cause to a series of physiological and molecular responses that will enable plants to overcome this adverse situation. Water stress, has been shown to either increase or mask the UV-B radiation effects. Salinity affects 19.5% of irrigated land and 2.1% of dry land agriculture existing on the globe [15]. Moreover, drought stress affects 40 to 60% of the world's agriculture lands [16]. Hence, increasing of drought and soil salinity have become a critical topic. Plants response to water deficit is complicated and involves changes in their morphology, physiology and metabolism such as growth, photosynthesis, enzyme synthesis, induction of proteins and compatible osmolytes and lipid metabolism. Maize (*Zea mays* L.) is considered as a moderately salt-sensitive plant [17]. It is a main food and economical crop and of the most important crops throughout the world. Therefore, it is urgent to increase maize yields even under the unfavorable conditions [18]. The concurrency of different stresses is rarely addressed by molecular biologist and its physiological aspects are insufficient understood [6]. In order to investigate the effects of UV-B irradiation, salinity and drought, applied alone or combined, on some physiological parameters and to find whether the combination of UV-B and these factors have any physiological effect on each other, this experiment has been carried out on *zea mays* L. (cv. single cross 704).

MATERIALS AND METHODS

Growth Condition: Maize *(Zea mays* L. cv. SC. 704) was used as plant entries in this study. Its seeds were obtained from Urmia Agriculture Research Centre. They were graded and the big and uniform shaped ones used and their surface sterilized with 2% sodium hypochlorite for 15 min. After sterilization, seeds were washed with distilled water three times. Plants were grown in plastic pots kept in a greenhouse with controlled environmental conditions (32/27°C day/night temperature cycle; light intensity of 400 µmol m^{-2} s^{-1} with 16/8 h light/dark period and 70-80% relative humidity) for 29 days. The pots were furnished with a hole at the bottom and three seeds were planted. 21-day-old maize seedlings were divided into 4 groups and undergone following treatments for 8 days:

Control (C), plants were watered regularly and grown normally until the end of experiment period; Drought (D), plants were prevented from irrigation throughout the experiment; Salinity (S), plants were watered with 25 mM NaCl on the first day of treatment. To avoid osmotic shock, NaCl was added to the growth medium in 25 mM increments every 24 h, until the final concentrations of 100 mM was reached.; UV-B radiation (UV), UV-B was artificially provided by UV-B fluorescent tubes (Philips 30W LF-215M. France) positioned 45 cm above leaf level. During UV-B treatment, no white light was applied. Maize plants were irradiated at midday for 10 min on the first day of treatment and then its duration increased every day by 10 min to reach the final dose obtained in 40 min; UV-B radiation and Drought (UV-D), plants were treated with both UV-B radiation and drought, simultaneously; UV-B radiation and Salinity (UV-S), plants were treated with both UV-B radiation and salinity, simultaneously. After 8 days applying treatments, the shoots and roots were harvested and the following experiments were conducted:

Relative Water Content (RWC): To estimate the relative water content, Smart and Bingham [19] method was used. Leaves were excised, weighed fresh (FW) and placed in distilled water in the dark for 24 hours to re-hydrate. The following morning, leaf turgid weight (TW) was measured. Leaves were dried at 80°C for 48 hours and dry weight (DW) was determined. The RWC was calculated following this formula:

$$RWC = [(TW - FW)/ (TW- DW)] 100$$

Estimation of Chl a,b and Cx+c: Chlorophyll content was determined according to Dere *et al*. [20]. Leaf fresh materials (1 g) were ground properly in 50 ml of 100% acetone then centrifuged for 10 min at 2500 g. The absorbance was read spectrophotometrically at 662, 645 and 470 nm. Pigment content was estimated following this formula:

$$Chla = 11.75\ A662 - 2.350\ A645$$
$$Chlb = 18.61\ A645 - 3.960\ A662$$
$$Cx+c = 1000\ A470 - 2.270\ Chla - 81.4\ Chlb/227$$

Estimation of Enzyme Activity: To estimate the enzyme activity, kang and Saltveit [21] method was used. Leaf fresh materials (0.5 g) were ground properly in 3 ml of 0.05 mM tris-HCl buffer (PH=7, $MgCl_2$ 3 mM, EDTA 1 mM) and centrifuged for 20 min at 5000 g. The supernatant was used as the source of enzymes assays.

Estimation of APX Enzyme Activity: The activity of APX was assayed according to Chen and Asada [22]. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.5 mM H_2O_2 and 0.1 ml enzyme extract. The reaction was started by the addition of H_2O_2. The activity of enzyme was assayed by measuring the decrease in absorbance at 290 nm for 1 min of ascorbic as ascorbic acid oxidized.

Estimation of GPX Enzyme Activity: GPX activity was determined according to Maehly and Chene [23] by the oxidation of guaiacol in presence of H_2O_2. The increase in absorbance due to formation of tetra guaiacol was recorded at 470 nm. The reaction solution was recorded 3 ml containing 10 mM KH_2PO_4/ K_2HPO_4, PH 7.0, 10 mM H_2O_2, 20 mM guaiacol and 0.5 ml enzyme extracted.

Estimation of Malondialdehyde (MDA) Content: MDA in the leaves was analyzed following Heath and Packer [24]. This method is based on the reaction with thiobarbituric acid. Fresh leaves (1.0 g) were ground properly in 2.5 ml of 0.1% tri-chloroacetic acid solution and centrifuged for 20 min at 15000 g. Equal volume of the supernatant with 20% TCA solution comprising 0.5% thiobarbituric acid was reacted, then it was heated for 30 min at 95°C in a water bath and then immediately cooled on ice for 5 min. After centrifugation for 5 min at 10000 g, the absorbance of the supernatant was read at 532 and 600 nm.

Estimation of Nitrate Redutase Activity (NRA): NRA was determined using the method described by Xiong *et al*. [25]. One g of fresh tissue was ground properly with 9 ml KNO_3 100 mM and vacuum infiltrated for three times. The incubation was conducted for 30 min at 25°C in the dark. To estimate the amount of nitrite formed, 1 ml of 1% sulfanilamide in 1 m mol L^{-1} HCl and 0.02% naphthyl ethylene diamine dihydrochloride was added to 2 ml of incubated solution. After 15 min the solution was centrifuged for 5 min at 1200 g. Absorbance of the last solution was recorded at 540 nm.

Estimation of Total Protein Content: To measure total protein content Folin-lowry [26] method was used. 0.02 g dried tissue was added to 4 ml Tris-HCl buffer and shacked for 30 min. Afterward, the suspension was centrifuged for 30 min at 5000 g. One ml supernatant was added to 4 ml reagent C and left for 5 min at room temperature and then added 1.5 ml reagent D with instant mixing. After 30 min at dark place and room temperature, the absorbance was measured at 660 nm.

Reagent C: [Reagent A (2% Na2CO3 in 0.4 N NaOH and 2% Potassium Sodium Tartrate) + Reagent B (0.5% CuSO4.5H2O) in 50:1].

Reagent D: (Folinciocolteu's reagent, diluted with distilled water in 1:9).

Estimation of Soluble Sugar Content: Soluble sugars were measured using phenol-sulfuric method [27]. Fresh materials of roots and leaves (0.5 g) were ground in 5 ml distilled water and then filtered. One ml phenol 5% + 3ml sulphuric acid 98% were added to 2 ml of filtered solution. Absorbance of this resulting solution was recorded at 485 nm.

Statistical Analysis: The experiment was conducted by completely randomized design with three replications for sampling and statistical analysis was performed using SPSS 19 program. The data represent means calculated from three replicates. The analysis of variance procedure (ANOVA) was used to compare the effect of these stresses to control and statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Many studies have shown the detrimental effects of UV-B radiation, salinity and drought on plants development, morphology and physiological processes. In general, to cope with the different types of stresses, plants respond differently by stimulating protection or activating repair mechanisms [28]. These factors cause changes in photosynthetic rate [29-31]. Decrease of photosynthesis is often related to reduction of pigment content caused by inhibition of their synthesis or increased destruction as well as damage to chloroplasts [31, 32]. In the present study, The concentrations of chlorophyll a, b and a+b in plant tissue under salt stress were higher than under the other stresses (Figure and Table 1) which can be attributed to an important mechanism lead to higher photosynthetic capacity and carbohydrate formation under salinity [33]. The increasing of chlorophyll content under salinity stress was observed on previous study as well [34, 35]. Nevertheless, chlorophyll a, b and a+b concentration decreased in drought and UV-treated plants though it wasn't significant for UV-B chlorophyll a content. In agreement with our results, chlorophylls of *Quercus rubra* and *Zea mays* L. were not affected by enhanced UV-B radiation [36] and it reduced markedly in chickpea

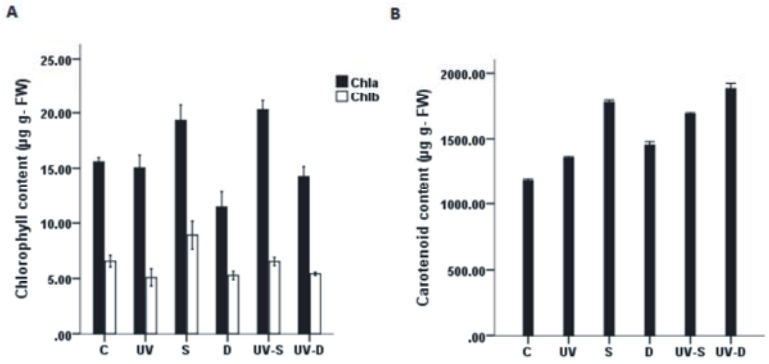

Fig. 1: Chlorophyll (A) and carotenoid (B) content of Maize leaves and roots under UV-B radiation, drought, NaCl (100 mM) and combination of two treatments. (C) Control; (UV) UV-B treatment; (D) Drought treatment; (S) Salinity treatment; (UV-D) UV-B and Drought concurrent treatment; (UV-S) UV-B and Salinity concurrent treatment. Data are means of three replicates ±SE at P <0.05.

Table 1: The effects of UV-B radiation, salinity and concurrent stress on Chla, Chlb, Chla+b, C c+x and RWC% in shoots of Zea mays L. cv. SC. 704.

Treatments	Chla	Chlb	Chla+b	Car	RWC%	RWC reduction%
C	15.57	6.56	22.13	1184.36	99.33	_
UV	15.03	5.08	20.11	1354.76	95	4.33%
S	19.29	8.91	28.2	1685.48	88	11.33%
D	11.47	5.27	16.74	1452.53	64	34.66%
UV_S	20.28	6.54	26.82	1685.48	93	6.33%
UV-D	14.22	5.42	19.64	1882.4	77	22.33%

Data represent the mean value of three replicates. Within a column, all the mean values are statistically different based on Duncan's range test at P < 0.05.

cultivars under drought stress [37]. The chlorophyll components, thylakoids and grana are sensitive to the incoming solar radiation. So, reduction in chlorophyll content under UV-B can be attributed to a breakdown of the structural integrity of chloroplasts [38]. Damage to chloroplasts caused by active oxygen species is the main reason of chlorophyll decrease under drought stress [37].

Carotenoids are a big group of isopernoid molecules that synthesize by all photosynthetic and many of non-photosynthetic organs [39]. In our experiment, maize plants responded to all stresses by increasing carotenoid concentration (Figure and Table 1). This point to the photo-protection role of carotenoids in photosynthetic systems by dissipating excess excitation energy through the xanthophylls cycle [40]. In the present study, the lowest level of carotenoid content was observed in plants exposed to UV-B radiation, representing the greater resistance of plants to this stress. Reduction of this parameter under drought stress compared to salt stress is indicating the considerable oxidative stress by accumulation of ROS under this condition.

Relative water content (RWC) is the most appropriate measure of plant water status. It has a close relation with several physiological parameters like turgor, growth, stomatal conductivity, transpiration, respiration and photosynthesis [41]. Reduction of RWC is an important adaptation strategy against environmental stress [42]. In this study, RWC showed a decrease under all three stresses, but it was more significant under drought treatment (Figure 2). We concluded that rapid induction

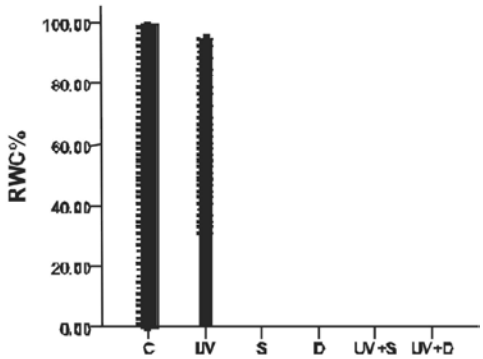

Fig. 2: RWC percentage of Maize leaves and roots under UV-B radiation, drought, NaCl (100 mM) and combination of two treatments. (C) Control; (UV) UV-B treatment; (D) Drought treatment; (S) Salinity treatment; (UV-D) UV-B and Drought concurrent treatment; (UV-S) UV-B and Salinity concurrent treatment. Data are means of three replicates ±SE at P <0.05.

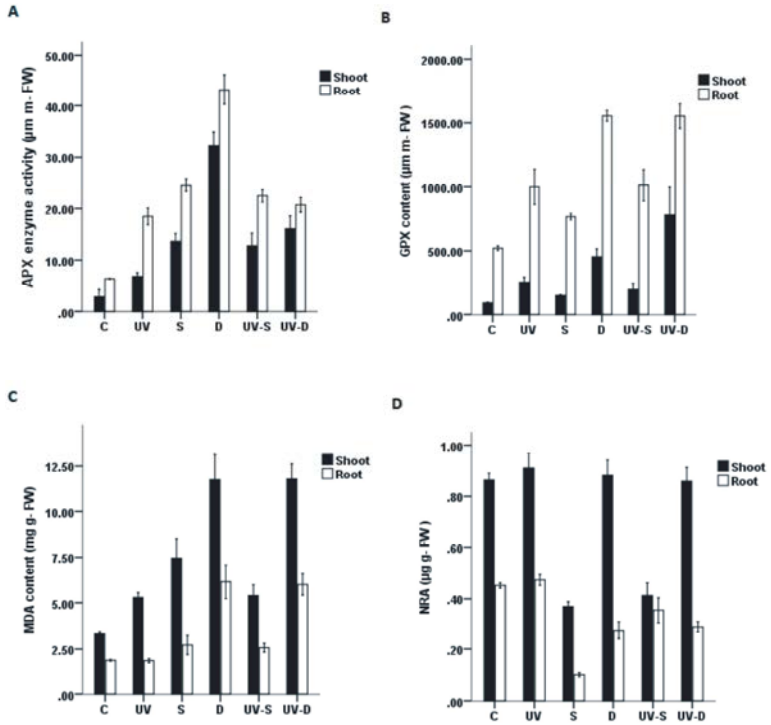

Fig. 3: APX content (A) GPX content (B) MDA content (C) and NRA (D) of Maize leaves and roots under UV-B radiation, drought, NaCl (100 mM) and combination of two treatments. (C) Control; (UV) UV-B treatment; (D) Drought treatment; (S) Salinity treatment; (UV-D) UV-B and Drought concurrent treatment; (UV-S) UV-B and Salinity concurrent treatment. Data are means of three replicates ±SE at P <0.05.

of osmolytes and stress proteins are responsible for high level of RWC in plants treated with UV-B. It is suggested that UV-B regulate the effect of drought by rapid induction of dehydrine proteins and compatible osmolytes in Arabidopsis [43]. Our data is similar to Zhao *et al*. [44] (red kidney bean) under UV-B radiation and Meloni *et al*., [45] (*Prosopis alba*) under salinity stress. Our result demonstrated.

That UV-B reduce the sever effect of drought and salinity by keeping more plants relative water content (Table 1).

Environmental stresses limiting photosynthesis can increase oxygen-induced cellular damage due to increased ROS generation [46, 47]. The degree of damage by ROS depends on the balance between the product of ROS and its removal by these antioxidant-scavenging systems [48, 49]. It has been found that the levels of ROS are elevated after exposure to UV-B radiation [50]. Furthermore, a correlation between the antioxidant enzyme activity and salinity tolerance was demonstrated by comparison of tolerant cultivars with sensitive cultivars in several plants. This effect was also reported for plants under drought stress [51]. In the present study, the APX and GPX activity was increased in the roots and leaves under these stresses, but plant response pattern was different. The most induction of enzyme activity was observed in drought treated plants, that showing the deleterious effect of this factor on maize plants. This parameter fallen in combination with UV-B radiation that was similar to Kubis and Rybus-Zajac [51] in cucumber leaves under drought and Heidari and Mesri [52] in three wheat cultivars under salinity stress. According to Kim *et al*. [53], the activities of barley antioxidant enzymes were increased in the root and shoot under NaCl stress. However, the increase was more significant and consistent in the root system. Our results indicated that the APX activity in the roots for UV-B was about 3 times and for salinity and combination of two stresses was about 4 and 3.5 times and for drought and combination of two stresses stress was about 7 and 3 times higher, respectively in comparison to control (Figure 3). In addition, induction of roots GPX activity under drought stress alone or combine was 3, for UV-B and concurrent stress was two and for salinity was about 1.5 times higher in maize plants compared to control (Figure 3 and Table 2). The results suggest that different

Table 2: The effects of UV-B radiation, salinity and concurrent stress on enzyme activity of APX, GPX and NRA in shoots and roots of Zea mays L. cv. SC.704.

	APX		GPX		MDA	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
C	2.89[a]	6.38[a]	92.68[a]	521.56[a]	3.33[a]	1.88[a]
UV	6.8[a]	18.47[b]	250.94[ab]	999.57[c]	5.30[ab]	1.86[a]
S	13.61[b]	22.57[c]	151.21[a]	766.63[b]	7.45[b]	2.72[a]
D	32.23[c]	43.23[d]	453.54[b]	1555.78[d]	12.06[c]	6.15[b]
UV_S	12.76[b]	22.14[c]	197.05[a]	1012.21[c]	5.39[ab]	2.57[a]
UV-D	16.09[b]	20.71[bc]	780.35[c]	1552.98[d]	12.44[c]	6.00[b]

Data represent the mean value of three replicates. Within a column, mean values followed by different letters are statistically different based on Duncan's range test at $P < 0.05$.

Table 3: The effects of UV-B radiation, salinity and concurrent stress on MDA, Pro and Sug content in shoots and roots of Zea mays L. cv. SC.704.

	NRA		Pro		Sug	
Treatments	Shoot	Root	Shoot	Root	Shoot	Root
C	0.86[b]	0.45[d]	114.10[a]	86.43[c]	13.09[a]	15.17[ab]
UV	0.91[b]	0.47[d]	118.91[a]	80.77[c]	15.72[a]	13.33[a]
S	0.36[a]	0.10[a]	108.13[a]	53.23[a]	23.66[bc]	34.33[d]
D	0.88[b]	0.27[b]	110.17[a]	50.88[a]	30.43[c]	19.67[bc]
UV_S	0.41[a]	0.35[c]	113.27[a]	68.74[b]	15.38[a]	16.03[ab]
UV-D	0.85[b]	0.29[bc]	112.85[a]	60.28[ab]	23.34[b]	24.49[c]

Data represent the mean value of three replicates. Within a column, mean values followed by different letters are statistically different based on Duncan's range test at $P < 0.05$.

regulatory mechanisms may exist in the regulation of antioxidant enzyme activity under different situations and tissues.

Leaf Malondialdehyde (MDA) content, the product of lipid peroxidation, is a prominent indicator of membrane impairment and free radical production [54, 55]. The lipid peroxidation in all stresses was estimated and data is presented in Figure 3 and Table 3. We observed that MDA content was increased in all treatments except in roots exposed to UV-B that there was no significant difference compared to control plants (Figure 3). However, the content of MDA in shoots was higher than roots. Some studies indicate that this parameter is dropped [56, 57] or induced [58-60] under these situations. MDA is a product of peroxidation of unsaturated fatty acids in phospholipids and its level is used as an indicator of free radical damage to cell membranes under stress conditions. We concluded that plants under drought condition had higher level of MDA content due to high amount of ROS production. It is obvious that combined with UV-B radiation, have deduced the production of ROS and thereby resulted in reduction of MDA level in maize plant (Table 2).

Salinity causes reduction in ion uptake, particularly $NO3^-$, at plasma membrane and tonoplast level, perhaps because of competitive mechanisms and/or membrane alteration [61, 62]. Decrease in $NO3^-$ flux from root to leaf and the stomatal closure at the beginning of salinity treatment are apparently responsible for the decrease in the NR enzyme activity [63] (Figure 3). Decrease of NRA under salt stress has been shown by most of pervious study [45, 63, 64]. Our results demonstrated that both $NO3^-$ (data not shown) and NRA reduced significantly in maize plant (Figure 3). Previous studies have provided different results, as some have shown reducing [65, 66] and other have indicated increasing [67, 68] of this parameters. Our results showed that NRA remains unchanged under UV-B radiation due to a modulator mechanism responsible for plant resistance against the adverse conditions.

Plants facing adverse situation such as high salt concentration, lower their osmotic potential by accumulating osmolytes. Accumulation of these compatible solutes (osmoprotectant) such as proline, glycinebetaine and soluble sugars, allow turgor maintenance and/or stabilization of proteins and membranes against destabilization effects of abiotic stresses, which cause cellular water depletion and do not interfere with normal biochemical reaction [52, 69]. Soluble sugar accumulation in plant cell under salt or drought stress is a widespread response. It may functions as a typical osmoprotectant, stabilizing

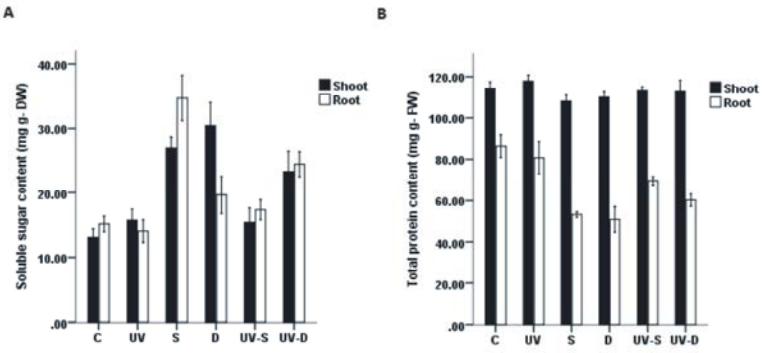

Fig. 4: Soluble sugar (A) and protein content (B) of Maize leaves and roots under UV-B radiation, drought, NaCl (100 mM) and combination of two treatments. (C) Control; (UV) UV-B treatment; (D) Drought treatment; (S) Salinity treatment; (UV-D) UV-B and Drought concurrent treatment; (UV-S) UV-B and Salinity concurrent treatment. Data are means of three replicates ±SE at P <0.05.

cellular membranes and maintaining turgor pressure. Our experiment showed that drought and 100 mM NaCl cause significant increase on soluble sugars level. Increased accumulation of sugar has been reported in many plant species exposed to these stresses, too [70-73]. Several studies have been carried out on the effects of UV-B radiation on plant carbohydrates but they have been contradictory, some indicating increase in response to UV-B (74) or decrease [75-77] and others indicating no alter [78]. This may be due to diversity of plant tissue or experimental conditions. In the present work, drought and salinity lead to induction of soluble sugar content that it was more prominent under drought condition. By contrast, UV-B radiation did not change the level of this parameter in maize plant. Moreover, plants exposed to concurrent treatment indicated a significant reduction compared to single stresses. This exhibits a very high tolerance of maize plant to ultraviolet-B radiation and the mechanisms that mitigate the detrimental effects of drought and salinity on plant under these situations.

In our experiment, there was no significant effect on total protein content in leaves of maize plants but it reduced in the roots under applied stresses (Figure 4). In agreement with our experiment, total soluble protein content was not affected in *Lupinus angustifolius* plant exposed to both drought and salt stress, but the decrease in protein content was shown in root, young and old leaves of *Helianthus annuus* and *Coleus blumei* plants [79-81]. These different results on salt stress showed that the responses to salt stress depends on plant species even in varieties of same plant species, plant developmental stage, duration and severity of the salt application [82].

CONCLUSION

Maize has a prominent role in the world economy and food industry. It is now second in importance after rice in terms of cultivated area and production. Therefore, it can clearly explain the importance of maize conserve during environmental conditions. Our results indicated that plants exposed to UV-B radiation have an efficient mechanism to tolerate this condition. In addition, our data demonstrated that UV-B radiation reduce the deleterious effects of soil salinity and drought in maize plant.

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