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UV-B Radiation Impacts on Growth, Bulb Yield and Antioxidants in Onion under Salt Stress

Yasmin M. R. Abdellatif[1*] and Neima K. Al-Senosy[2]

[1]Department of Agricultural Botany, Faculty of Agriculture, Ain Shams University, Cairo 11241, Egypt.
[2]Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo 11241, Egypt.

Authors' contributions

This work was carried out in collaboration between both authors. Author YMRA designed the study, wrote the protocol, performed the statistical analysis, interpreted and prepared the manuscript. Author NKAS performed and wrote the gene expression part. Both authors read and approved the final manuscript.

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ABSTRACT

Ultraviolet radiation considered one of the environmental stresses that widely disturb most of the physio-biochemical processes in the plant. However, previous studies referred that irradiation of plant tissues with a low dose of UV-B radiation stimulates adaptive mechanisms and providing better protection to other stresses. In this approach, a pot experiment was laid out in the open field during the two growing seasons of 2014/2015 and 2015/2016. Transplants of red onion (Giza 20), six weeks old, were exposed to UV-B (280-320 nm) for 15 min, then grown plants were irrigated with three concentrations of NaCl (0, 50 and 100 mM) as a challenge. The effect of low dose of UV-B was evaluated by determination of vegetative growth characteristics, bulb yield/plant and some biochemical changes associated with UV-B induced tolerance. In general, plants irrigated with 50 and 100 mM of NaCl remarkably expressed a reduction in plant growth, yield and chlorophyll a and b. Flavonoids were significantly reduced under the highest level of salinity. Results showed that plants grown from UV-B treated transplants were less affected by salt stress which was obvious in increasing plant fresh weight, shoot height, number of leaves/plant, leaf length, leaf diameter and neck thickness. Moreover, UV treatment alleviated onion bulb yield in term of fresh bulb weight, bulb

**Corresponding author: E-mail: dr_yasminmarzouk@agr.asu.edu.eg;*

length, bulb diameter and harvest index. The enhanced tolerance was noticed by increasing organic osmolytes (free amino acids, reducing sugars and total sugars) and induced antioxidant system (total soluble phenols, carotenoids, flavonoids, peroxidase (POD), polyphenol oxidase (PPO) and ascorbate peroxidase (APX) in onion leaves and anthocyanin in bulbs. Regarding protein electrophoresis, 33% of polymorphism was produced; negative markers were detected by UV + 50 mM NaCl and UV + 100 mM NaCl. While one positive marker was detected by UV +100 mM NaCl at 87.545 kDa which indicated that the interaction between salinity stress and UV-B radiation resulted in plant salt tolerance.

Keywords: Onion (Allium cepa L.); UV-B radiation; salt stress; osmolytes; antioxidant pigments; antioxidant enzymes.

1. INTRODUCTION

Recent evidence showed that depletion of stratospheric ozone has a heightened concern about how possible ecological consequences of increasing solar ultraviolet reaching the Earth's surface [1]. Solar UV is classified into 3 regions: UV-A (320-400 nm), UV-B (280-320nm) and UV-C (200-280 nm). The most harmful effect of UV on the living system increases towards shorter wavelength [2]. In general, most studies were directed mainly toward the negative effects of high doses of UV-B irradiation which supply high energy of UV-B that can directly damage the photosynthetic apparatus, DNA lesion, leakage of membranes as well as alterations in plant morphology [3] or indirectly by affecting on photosynthetic pigments and stomatal conductance [4]. High UV-B intensities induced dimer formation on plant growth and morphogenesis, but at 22 μw cm^{-2} of UV-B that closed to a natural condition, no significant dimer formation was detected in the irradiated plants due to enhancing a protective mechanism as DNA photo repair and pigment screening [5]. It was found that higher doses of UV-B have less hazardous in DNA damage than low doses of UV-C because of the convergence between the DNA absorption peak (260 nm) and UV-C irradiated wavelength (254 nm) comparing with UV-B (280-320 nm) [6].

Soil salinization in Egypt is a further problem for agriculture. Sever salinity problem in Egypt increases because of the poor drainage system of the cultivated lands as a reason for unsuitable irrigation. River Nile is the main irrigation water resource and some other farms depend on underground water. Insufficient leaching of salt ions accumulates the dominant salt NaCl in upper soil layers [7].

Onion (*Allium cepa* L.) plant is more affected by salinity than other crops. Sta-Baba et al. [8] reported that onion is classified as a more sensitive salt plant that has 1.2 dS m^{-1} EC threshold.

Nasibi and Kalantari [9] documented that UV-B increased protein in *Brasica napus,* they attributed these increments to the synthesis of defence protein as heat shock proteins and gene expression of antioxidant enzymes or enzymes that contribute to the synthesis of UV-B absorbing molecules such as phenylalanine ammonia lyase which related with phenols synthesis especially flavonoids. The concentration of chlorophyll a, carotenoids, flavonoids and anthocyanin biosynthesis increased under solar UV-B enhancement [1]. Furthermore, they observed that low doses of UV-B stimulated firstly non-enzymatic antioxidant system after 6 h from exposure, then the enzymatic antioxidant system was detected due to a specific signal transduction induced by UV-B. Katerova et al. [10] documented that prolonged exposure to UV-B inhibits plant growth in contrast to a low dose of UV-B which enhanced the plant vigour and prolong the shelf life of yield.

Kubiś and Rybus-Zając [11] recorded that one of the stresses reduced the effects caused by simultaneous application of the other. The combined effect of UV-B radiation and drought stress improved the osmotic tolerance and stimulate the antioxidant enzymes activity. Low ambient levels of UV-B improved the survival of plant under heat stress and increased plant growth [12]. Also, preconditioning of jack pine seedlings with near ambient levels of UV-B reduced membrane injury and showed freezing tolerance [13]. They stated that the reason for this noticed reasonable to induction of scavenging enzymatic and non-enzymatic antioxidant defence system, particularly ascorbate-glutathione cycle.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein (SDS-PAGE), as a biochemical marker, was the inexpensive and simplest technique that offer sufficient information and serve as a starting point for DNA-based studies. Protein electrophoresis method has been used to study the possible role of UV-B irradiation to stimulate salinity tolerance**.** These studies proved that exposure of plant leaves to UV-B radiation produced a UV-dependent accumulation of 65 KDa polypeptide as a result of forming a non-disulphide subunit of ribulose bisphosphate carboxylase [14].

In this connection, the purpose of the present investigation was

1- Study the effect of low dose of UV-B and salinity stress, individually, on plant growth, onions yield and the osmoregulatory and antioxidant system.
2- Evaluate the ability of low UV-B dose pretreatment under the levels of salinity stress to induce salinity stress tolerance in onion plants and enhancing plant growth and productivity.

2. MATERIALS AND METHODS

2.1 Plant Culture

Transplants of red onion (*Allium cepa* L.), Giza 20 cultivar, six weeks age, at the 3-4 leaf stage and 12-14 cm height were purchased from Arid Land Agriculture Research Institute, Faculty of Agriculture, Ain Shams University. A pot experiment was laid out in the open field of Experimental Farm of Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Shoubra El Kheima, Qalyubia Governorate during the two growing seasons of 2014/ 2015 and 2015/2016. Half number of the seedlings was subjected to UV-B radiation and the second half was used as control treatment (without UV-B supplement). Plastic pots with dimensions 30 x 40 cm with 5 bottoms drainage holes were filled with 20 Kg clay loamy soil (Table 1) and divided into two groups. Each group contained 3 subgroups. On December 21th of 2014 and 2015 the pots were irrigated with well tap water, then the UV-B treated transplants and non-UV-B treated ones were sown in the previous two main groups of pots, ten transplants per each pot.

Supplement UV- B radiation was provided by two white lamps of artificial ultraviolet-B 20 W/ 01 RS (Philips, Holland) with wavelength 320 – 280 nm which was situated at 20 cm over the transplants in a dark closed bench for 15 minutes exposure. Transplants were stored in complete darkness place directly after treatment for 5 days to minimize any photo-reactivation process [15]. On January 5, 2015, and 2016 plants were thinned to five similar plants per pot, in the same time, plants were irrigated with artificially salinized water with three different levels of NaCl (50 and 100 mM) and the third level was well tap water as a non-saline control treatment, each level was applied in a subgroup with three replicates for each treatment, each replicate contained 3 pots.

The experimental design was a complete randomized block design. Salted irrigation was maintained three weeks interval for the first two months and then the period has been approximated to one-week interval until 21 days to harvest when irrigation was stopped completely. All other agricultural practices were applied as recommended by the Egyptian Ministry of Agriculture and Land Reclamation. Harvesting of onion bulbs was performed when 70 % of plants show neck fall.

2.2 Data Collection

On February 22, 2015, and 2016 three plants were taken randomly from each replicate. Samples of fresh leaves were weighted and immediately placed in -20°C for 8 hours and moves to -80°C freezer until determining some biochemical constituents of plants as an indicator of enhancing the plant tolerance.

Table 1. Mechanical and chemical analyses of the experimental soil

%			Soil texture	EC dS m^{-1}	pH	Soluble anions (meq $^{-1}$)			Soluble cations (meq $^{-1}$)			
Clay	Silt	Sand				HCO_3^-	SO_4^{--}	Cl^-	K^+	Na^+	Ca^{++}	Mg^{++}
50.00	28.40	21.60	Loamy	0.951	7.15	3.45	3.83	2.24	0.83	2.54	4.50	2.93

Soil and Water Research Centre, ARC, Giza, Egypt

Total soluble free amino acids, total soluble phenols, total and reducing sugars and flavonoids were extracted by the method of Ackerson [16] using ethanol 80% at 70°C.

In the ethanol extract, the soluble free amino acids concentration was spectrophotometrically analyzed by using reagent of ninhydrin and acetate buffer (pH 5.5) at 570 nm according to Swamy [17] and calculated as mg g^{-1} f.wt. using glycine as a standard.

Total soluble phenols in the metabolic extracts were colourimetrically determined by using a modification method of the Folin – Ciocalteu according to the procedure of Ozyigit et al. [18]. The concentrations were calculated as mg gallic acid g^{-1} leaf fresh weight.

Reducing sugars concentration was determined using 3,5-dinitrosalsylic acid as the strategy portrayed by Miller [19] using glucose as a standard. Total sugars concentration was measured in the previous extract by adding 5 ml HCl (2N) to 15 ml extract and heated in a water bath at 60ºC for 30 min. Then the solution was cooled, neutralized and reached the total volume to 50 ml with distilled water. Total sugars were analyzed spectrophotometrically by using 3,5- dinitrosalsylic acid solution according to Miller [19]. Non-reducing sugars concentration was calculated by subtracting reducing sugars from total sugars.

Flavonoids concentration was measured by aluminium chloride colourimetric assay by the method of Marinova et al. [20]. Quercetin was used as a standard.

Chlorophylls a and b (Chl a & Chl b) and carotenoids concentrations were extracted and estimated as the procedure portrayed by (Costache et al. [21]. The samples were grounded and pestled in dimethyl formamide. Then extractions were incubated in tubes for overnight in the dark. The tubes were centrifuged at 1000 rpm for 5 min. Chl a & Chl b and carotenoids were determined at 662, 645 and 440 nm, respectively. Formula and extinction coefficients were calculated using the following equations and expressed as mg g^{-1} fresh weight:

Chlorophyll a = 9.784 A662 – 0.99 A645
Chlorophyll b = 21.426 A645 – 4.65 A662
Total chlorophylls = 20.2 A662 + 8.02 A645
Carotenoids = 4.695 A440 – 0.268 total chlorophylls.

2.3 Antioxidant Enzymes Extraction

One-gram of frozen tissue leaves were ground using cold mortar and pestle in cold potassium phosphate buffer (100 mM, pH=7.0), 0.1 mM EDTA and polyvinyl pyrrolidone (PVP) 1 % (W/V). Homogenate was centrifuged at 15000 ×g / 15 min under cooling (4°C). The supernatant was separated as enzyme crude extract and used to determine the activities of guaiacol peroxidase (POD), polyphenol oxidase (PPO). For extraction of ascorbate peroxidase (ASP) leaf tissues were separately homogenized with ascorbic acid (20mM) added to the previous ingredients.

2.4 Antioxidant Enzymes Assay

Guaicol peroxidase, POD (E.C 1.11.1.7) activity was estimated according to the method of Hammer Schmidt et al. [22]. The assay mixture included 50 mM potassium phosphate buffer, 1% (v/v) guaicol and 0.3% H_2O_2. The volume of crude enzyme was added to start the reaction. The absorbance changes were recorded every 30 sec for 3 min at 470 nm by spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). One unit of enzyme (IU) was expressed as ΔOD = 0.01.

Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was quantified according to Oktay et al. [23]. The reaction mixture included 200 µl crude enzyme, 2.2 ml phosphate buffer (0.1 M, pH 6.5) and 600 µl catechol. The absorbance at 420nm was measured at 0 times and after 1 min. One unit of PPO activity was expressed as the amount of enzyme which causes an increase in absorbance of 0.001 / min.

Ascorbate peroxidase (APX) (E.C 1.11.1.11) activity was determined by the method of Nakano and Asada [24]. The total volume of the reaction mixture was 3 ml consisted of crude enzyme extract, 50 mM potassium phosphate buffer with pH 7.0, 0.1 mM EDTA, 0.5 mM ascorbic acid and then added 0.1 mM H_2O_2 which initiates the reaction. One unit of enzyme activity of APX was defined as the amount of enzyme that required for the oxidation of 1 µmol of ascorbate/min. Absorbance was recorded for 3 min with 3 sec intervals at 290 nm using spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech). The activity of ascorbate oxidation was calculated from the extinction coefficient (ε = 2.8 mM^{-1} cm^{-1}). All enzymes activities were expressed as unit mg^{-1} protein.

Also, protein concentration was estimated in the crude extract by the method of Bradford [25] using bovine serum albumin as a standard.

On May 15, 2015 and 2016, another three randomized plants from each treatment were harvested at the physiological maturity stage (140 days after transplanting) to record the plant f.wt. (g), plant height (cm) from the soil surface to the top of the longest mature leaf, number of leaves/plant, the length and diameter (cm) of the longest mature leaf, the neck thickness (cm) at the narrowest point. Also, bulb f.wt. (g), bulb length and diameter (cm) per plant were recorded. Harvest index (HI) was calculated by dividing bulb dry weight (g) / total plant dry weight (g).

Three replicates of fresh bulbs were weighted from each treatment to determine total anthocyanin concentration in red bulbs according to the method of Connor et al. [26]. The values were expressed as mg cyanidin-3-glucoside (c3g) equivalents per 1 g f.wt. by using a molar extinction coefficient of 27.900.

2.5 Protein Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for soluble proteins, extracted from green leaves of treated plants, was used to detect the effect of salinity and low dose of UV-B treatments on gene expression.

Extraction of Total Protein: bulked leaf sample (0.5 g) from each treatment was ground with liquid nitrogen mixed with extraction buffer pH 7.5 (50 mM Tris-HCl, 5% glycerol and 14 mM ß-mercaptoethanol) in a mortar with pestle, left overnight, then vortexed for 15 sec and centrifuged at 12000 ×g at 4°C for 10 min. The supernatants were transferred to new eppendorf tubes and kept at -20°C until use, protein electrophoresis analysis was performed according to Laemmli [27]. The gel was stained with Coomassie Brilliant Blue R-250 (0.05% Coomassie Brilliant Blue R-250, 50% methanol and 10 % acetic acid) for 12 h and re-stained with staining solution (30 % methanol and 10 % acetic acid) for 24 h. The gel was scanned using the Gel Doc 2000 Bio-Rad system and analyzed with the Gel Analyzer 3 software.

2.6 Statistical Analysis

Data of all treatments were arranged and presented as mean from three replicates. Data were statistically analyzed for significance in Statistix (8^{th} edition, Analytical Software, USA, Steel et al. [28]) using analysis of variance (ANOVA). Significance between means was compared by Duncan's multiple range test at $p<0.05$ probability according to the method of Gomez and Gomez [29].

3. RESULTS

Data presented in Table (2) showed that all vegetative growth parameters, *i.e.* plant f.wt., shoot tissue height, the number of leaves/plant, leaf length, leaf diameter and neck thickness were decreased gradually with increasing NaCl concentration in irrigation water.

These results agreed with Sta-Baba et al. [8] who found that onion plants exhibited an initial reduction in growth under salt stress gradually with increasing salt stress from 3.70 to 9.71 dSm^{1} with 50% reduction in number of leaves at 3.70 dSm^{-1}. The author reported that this reduction of plant growth was due to inducing an excess absorption of Na^{+} in antagonism of K^{+} causing double in NaCl accumulation in the root zone. Several factors like ion toxicity, ionic disequilibrium, osmotic stress, nutritional disorder, alteration of primary and secondary metabolic processes, oxidative stress, disorganization and reduction in cell division and cell expansion negatively affect plant growth under salt stress [30].

Pre-treated transplants with UV-B radiation significantly improved the previously mentioned growth characters under both saline and non-saline conditions as compared to control plants at both seasons. Under salt stress, the effect of UV-B was more pronounced with 50 mM NaCl salinity than 100 mM NaCl (Table 2).

In the same manner, Kacharava et al. [31] results on sugar beet, cabbage and kidney bean showed an increase in plant fresh weight, dry weight, plant height and leaf area as a result of UV pre-sowing treatment for seeds. In this context, it was proposed that the application of the optimal doses of UV-B should cause a repairable damage in DNA and this slight shock should activate the repair mechanisms for radiation-induced DNA damage as photoreactivation, excision repair and post-replication repair and stimulate another vital processes inside the cells like over compensation of normal metabolic processes and induced the basic physiological functions

Table 2. Effect of UV-B irradiation on growth parameters of red onion under salinity stress and their combinations during 2014/2015 and 2015/2016 growing seasons

Treatments	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean
	1st season				2nd season			
Plant f.wt. (g)								
Control	119.33^{b}	76.26^{d}	47.55^{f}	81.05^{B}	121.80^{a}	78.53^{c}	50.07^{d}	83.47^{B}
UV radiation	123.91^{a}	102.40^{c}	52.04^{e}	92.78^{A}	126.13^{a}	105.43^{b}	66.77^{c}	99.44^{A}
Mean	121.62^{A}	89.33^{B}	49.80^{C}		123.97^{A}	91.98^{B}	58.42^{C}	
Shoot height (cm)								
Control	57.80^{c}	47.99^{d}	33.05^{e}	46.28^{B}	54.33^{b}	42.40^{c}	28.57^{d}	41.77^{B}
UV radiation	67.87^{a}	61.38^{b}	59.55^{bc}	62.93^{A}	65.70^{a}	62.40^{a}	61.87^{a}	63.32^{A}
Mean	62.83^{A}	54.69^{B}	46.30^{C}		60.02^{A}	52.40^{B}	45.22^{C}	
Number of leaves / plant								
Control	10.67^{b}	8.33^{d}	6.67^{e}	8.56^{B}	11.33^{a}	8.67^{b}	7.67^{b}	9.22^{B}
UV radiation	12.67^{a}	10.33^{bc}	9.67^{c}	10.89^{A}	12.67^{a}	11.33^{a}	8.33^{b}	10.78^{A}
Mean	11.67^{A}	9.33^{B}	8.17^{C}		12.00^{A}	10.00^{B}	8.00^{C}	
Leaf length (cm)								
Control	44.57^{bc}	42.40^{bc}	31.47^{d}	39.48^{B}	46.23^{bc}	41.53^{cd}	34.57^{e}	40.78^{B}
UV radiation	52.53^{a}	46.23^{ab}	38.73^{c}	45.83^{A}	53.13^{a}	47.50^{ab}	38.13^{de}	46.26^{A}
Mean	48.55^{A}	44.32^{A}	35.10^{B}		49.68^{A}	44.52^{B}	36.35^{C}	
Leaf diameter (cm)								
Control	1.53^{a}	1.27^{b}	0.97^{c}	1.25^{B}	1.43^{ab}	1.14^{bc}	0.89^{c}	1.16^{B}
UV radiation	1.70^{a}	1.57^{a}	1.27^{b}	1.51^{A}	1.78^{a}	1.32^{ac}	1.32^{ac}	1.47^{A}
Mean	1.62^{A}	1.42^{B}	1.12^{C}		1.61^{A}	1.23^{B}	1.10^{B}	
Neck thickness (cm)								
Control	1.67^{a}	1.33^{b}	1.13^{bc}	1.38^{A}	1.67^{ab}	1.60^{ab}	1.24^{c}	1.50^{A}
UV radiation	1.80^{a}	1.30^{bc}	1.07^{c}	1.39^{A}	1.80^{a}	1.43^{bc}	1.11^{c}	1.44^{A}
Mean	1.73^{A}	1.32^{B}	1.10^{C}		1.73^{A}	1.52^{A}	1.17^{B}	

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan's multiple range test; capital letters for mean of UV-B treatment or salinity, whereas lowercase letters for interaction

that had previously been repressed, all these changes directing the homeostasis of the plant to a positive change that lead to growth augmentation [32].

Irrigation with a high concentration of NaCl decreased red onion yield (Table 3). Bulb fresh weight significantly decreased by (39.74 and 47.65)%, bulb length decreased by (17.11 and 30.03)% and bulb diameter decreased by (18.89 and 36.86)% at 50 and 100 mM NaCl, respectively, as compared to control (well tap water). A similar trend was recorded in the second season.

On the other hand, pre-treatment of transplants with UV-B radiation for 15 min significantly increased all bulb studied characters in comparing with un-irradiated plants. The highest bulb fresh weight (87.63 and 90.9) g, bulb length (5.43 and 5.51) cm and bulb diameter (6.37 and 6.69) cm were observed in unsalted treated plants exposed to UV-B, whereas the lowest values were recorded in plants irrigated with 100 mM NaCl without supplemental of UV-B radiation in both seasons (Table 3).

Katerova et al. [10] proposed that a low dose of UV-B do not impair both growth and productivity in different plants and reduce the severity against other stresses. Based on this concept, the ability of the optimal UV-B radiation to induce plant tolerance makes plants that underlie one stress appears a cross-protection to others. Whereas high doses of UV decreased the physiological performance causing the death of plant [12].

In the present investigation, the deleterious effect of salinity stress on yield/plant in term of harvest index (HI) and yield characteristics was maxillary ameliorated at the moderate concentration of NaCl with UV-B exposure. The highest value of HI under non-saline stress condition was recorded with UV-B radiation treatment in the first season. Also, UV-B radiation improved the HI under salt stress. However, there was insignificant difference between HI in plants treated with the combination of UV-B and 50 mM

NaCl and unstressed plants. In the second season, the insignificant difference was shown in the treatments of non-saline irrigated water with or without UV irradiation and 50 mM NaCl supplemented with UV. Pre-treatment with UV exceeded the HI of plants treated with the high concentration of NaCl to become there was an insignificant difference between this treatment and the individual treatment of the low concentration of NaCl at both seasons (Table3). This data was compatible with that obtained by Brown et al. [33] who recommended that the application of dry seeds of cabbage with a low dose of UV produced the highest weight head and largest head diameter as compared with control.

Exposure of plants to salinity stress influenced the total soluble free amino acids, soluble phenols, reducing sugars and total sugars concentrations in leaves of onion plants during the two seasons (Table 4). On the other hand, gradually insignificant decrease was shown in non- reducing sugars with increasing salt stress. Application of 50 mM NaCl increased soluble free amino acids and reducing sugars concentrations more than 100 mM NaCl. However, the high concentrations of total soluble phenols and total sugars were recorded with the high dose of NaCl. It should be noted that total soluble phenols, reducing sugars and total sugars concentrations gradually increased with increasing salt stress in the second season. It was stated by more than evidence that salinity stress causes oxidative damage to cell components due to over production of reactive oxygen species (ROS). Total soluble phenolic compounds, soluble amino acids, total sugars and soluble proteins exhibited an increasing value with salt stress [34,35]. Elevation in amino acids and phenols protect plant cells under stress from oxidative damage and encourage stabilization of cell membrane [36].

Moreover, individual treatment of transplants with UV-B radiation significantly elevated the soluble free amino acids, total soluble phenols, reducing sugars and total sugars concentrations in leaves as compared with control at both seasons, while insignificant increase was noticed in non-reducing sugars. The increase in amino acid concentration was more remarkable in leaves of plants grown under 50 mM NaCl with previous UV-B radiation in comparing with other treatments (Table 4). However, during the two seasons, the interaction between the UV-B application and the highest level of NaCl exhibited the maximum concentration of phenols and total sugars (Table 4). Previous treatment of UV-B irradiation under both 50 and 100 mM NaCl insignificantly decreased non-reducing sugars at both seasons.

Table 3. Effect of UV-B irradiation on bulb yield and yield attributing parameters on red onion under salinity stress and their combinations during 2014/2015 and 2015/2016 growing seasons

Treatments	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean
	1st season				2nd season			
	bulb f.wt. (g)							
Control	81.77^{b}	38.50^{e}	32.07^{f}	50.78^{B}	77.03^{b}	34.49^{d}	32.16^{d}	47.89^{B}
UV radiation	87.63^{a}	63.58^{c}	56.61^{d}	69.27^{A}	90.90^{a}	63.73^{c}	55.95^{c}	70.19^{A}
Mean	84.70^{A}	51.04^{B}	44.34^{C}		83.96^{A}	49.10^{B}	44.06^{B}	
	bulb length (cm)							
Control	4.50^{ab}	3.80^{bc}	2.93^{c}	3.74^{A}	4.62^{b}	3.85^{c}	2.97^{d}	3.81^{B}
UV radiation	5.43^{a}	4.43^{ab}	3.23^{bc}	4.36^{A}	5.51^{a}	4.50^{b}	3.47^{cd}	4.49^{A}
Mean	4.97^{A}	4.12^{A}	3.08^{B}		5.06^{A}	4.18^{B}	3.22^{C}	
	bulb diameter (cm)							
Control	6.07^{a}	4.63^{b}	3.73^{c}	4.81^{B}	5.93^{b}	4.83^{c}	3.73^{d}	4.83^{B}
UV radiation	6.37^{a}	6.10^{a}	4.63^{b}	5.70^{A}	6.69^{a}	6.03^{b}	4.37^{c}	5.70^{A}
Mean	6.62^{A}	5.37^{B}	4.18^{C}		6.31^{A}	5.43^{B}	4.05^{C}	
	Harvest index							
Control	0.63^{b}	0.58^{c}	0.43^{d}	0.55^{B}	0.63^{a}	0.58^{b}	0.43^{c}	0.55^{B}
UV radiation	0.64^{a}	0.62^{b}	0.57^{c}	0.61^{A}	0.63^{a}	0.62^{a}	0.56^{b}	0.60^{A}
Mean	0.63^{A}	0.60^{B}	0.50^{C}		0.63^{A}	0.60^{B}	0.49^{C}	

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan's multiple range test; capital letters for mean of UV-B treatment or salinity, whereas lowercase letters for interaction

Table 4. Effect of UV-B irradiation on some biochemical constituents of red onion under salinity stress and their combinations during 2014/2015 and 2015/2016 growing seasons

Treatments	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean
	1st season				2nd season			
	Soluble free amino acids (mg g^{-1} f.wt.)							
Control	1.38^{c}	1.71b^{c}	1.54^{bc}	1.54^{B}	1.34^{c}	1.74^{bc}	1.69^{bc}	1.59^{B}
UV radiation	1.70^{bc}	2.61^{a}	2.13^{ab}	2.15^{A}	1.65^{bc}	2.51^{a}	2.15^{ab}	2.10^{A}
Mean	1.54^{B}	2.16^{A}	1.83^{AB}		1.50B	2.12A	1.92A	
	Total soluble phenols (mg 100 g^{-1} f.wt.)							
Control	239.11^{b}	324.24^{a}	350.71^{a}	304.69^{B}	241.78^{b}	344.57^{a}	351.04^{a}	312.47^{B}
UV radiation	346.09^{a}	367.80^{a}	377.32^{a}	363.73^{A}	344.09^{a}	383.51^{a}	391.65^{a}	373.08^{A}
Mean	292.60^{B}	346.02^{AB}	364.01^{A}		292.93^{B}	364.04^{A}	371.35^{A}	
	Reducing sugars (mg g^{-1} f.wt.)							
Control	16.09^{b}	18.33^{b}	17.00^{b}	17.14^{B}	15.96^{d}	18.81^{cd}	18.54^{d}	17.77^{B}
UV radiation	20.87^{ab}	25.61^{a}	25.31^{a}	23.93^{A}	22.78^{bc}	26.57^{ab}	26.92^{a}	25.42^{A}
Mean	18.48^{A}	21.97^{A}	21.16^{A}		19.37^{B}	22.69^{A}	22.73^{A}	
	Non- reducing sugars (mg g^{-1} f.wt.)							
Control	6.15^{a}	8.14^{a}	6.66^{a}	6.99^{A}	6.95^{a}	7.32^{a}	7.13^{a}	7.13^{A}
UV radiation	9.68^{a}	7.03^{a}	8.40^{a}	8.37^{A}	9.44^{a}	6.41^{a}	6.47^{a}	7.44^{A}
Mean	7.91^{A}	7.59^{A}	7.53^{A}		8.19^{A}	6.86^{A}	6.80^{A}	
	Total sugars (mg g^{-1} f.wt.)							
Control	22.24^{c}	26.47^{bc}	23.67^{c}	24.13^{B}	22.91^{b}	26.14^{b}	25.67^{b}	24.91^{B}
UV radiation	30.55^{ab}	32.65^{a}	33.72^{a}	32.30^{A}	32.21^{a}	32.98^{a}	33.38^{a}	33.86^{A}
Mean	26.39^{A}	29.56^{A}	28.70^{A}		27.56^{A}	30.23^{A}	30.36^{A}	

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan's multiple range test; capital letters for mean of UV-B treatment or salinity, whereas lowercase letters for interaction

In this regard, Tiwari et al. [37] declared that UV seed treatment successfully promoted phenols which acted as ROS scavengers directly as a non-enzymatic antioxidant or indirectly as substrates for antioxidant enzymes. These responses of phenols accumulation were attributed to the activation of the key enzyme of phenyl propanoid pathway. The enriched cellular levels of free amino acids, total sugars and soluble proteins in leaf tissues play a role in osmoregulation for adaptive mechanisms of plants to salt-induced toxicity. Shetty et al. [38] found that UV-B treatment enhanced the activity of glucose-6-phosphate dihydrogen which considers the key precursor for pentose phosphate pathway and erythrose-4-phosphate for shikimate pathway that converts simple carbohydrates to phenyl alanine (aromatic amino acid) that attribute for phenyl propanoid pathway.

Chlorophyll is the central player in absorbing light energy for photosynthesis. Data in (Table 5) referred that salt stress led to significantly decrease in both Chl a and Chl b. The maximum reduction was detected in plants treated with 100 mM NaCl. The aforementioned results agree with Kao et al. [39] who reported that salinity reduced the efficiency of photosynthesis. Individual treatment with 50 mM NaCl imposed significant decrease in Chl a & Chl b concentrations [40]. The degradation of leaf chlorophylls was attributed to increase chlorophyllase enzyme activity [41] or due to the interference between salt ions and *Novo* protein synthesis that salt-induced weakling of protein- pigment - lipid complex [42].

Whereas, plants responded to UV-B radiation by an insignificant reduction in the concentrations of Chl a & Chl b in comparing with un-irradiated plants as shown in Table (5). Also, UV-B treatment elevated the concentrations of Chl a & Chl b under the two different levels of salt stress.

In this concept, it was expected that many plants reduced photosynthetic function and induced chlorosis as a symptom of UV-B radiation [43]. On contrary, other plants retain higher photosynthetic integrity due to maintaining chlorophyll levels during UV-B exposure [44].

Data in Table 5 also showed that carotenoids insignificantly increased gradually with increasing salt stress.

Table 5. Effect of UV-B irradiation on some pigments concentration of red onion under salinity stress and their combinations during 2014/2015 and 2015/2016 growing seasons

Treatments	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean
	1st season				2nd season			
Chlorophyll a (mg g^{-1} f.wt. leaves)								
Control	1.32^{a}	0.91^{ab}	0.67^{b}	0.97^{A}	1.33^{a}	0.95^{bc}	0.68^{c}	0.99^{A}
UV radiation	1.19^{a}	1.14^{a}	1.10^{a}	1.14^{A}	1.30^{a}	1.07^{ab}	1.09^{ab}	0.15^{A}
Mean	1.25^{A}	1.03^{AB}	0.88^{B}		1.32^{A}	1.01^{B}	0.89^{B}	
Chlorophyll b (mg g^{-1} f.wt. leaves)								
Control	0.90^{a}	0.61^{bc}	0.46c	0.66^{A}	0.97^{a}	0.60^{b}	0.52^{b}	0.70^{A}
UV radiation	0.84^{ab}	0.86^{ab}	0.74ac	0.81^{A}	0.91^{a}	0.93^{a}	0.71^{ab}	0.85^{A}
Mean	0.87^{A}	0.73^{AB}	0.60^{B}		0.94^{A}	0.77^{AB}	0.62^{B}	
Carotenoids (mg g^{-1} f.wt. leaves)								
Control	0.39^{a}	0.48^{a}	0.43^{a}	0.41^{A}	0.38^{a}	0.40^{a}	0.47^{a}	0.42^{A}
UV radiation	0.38^{a}	0.43^{a}	0.44^{a}	0.42^{A}	0.36^{a}	0.50^{a}	0.50^{a}	0.45^{A}
Mean	0.38^{A}	0.41^{A}	0.43^{A}		0.37^{B}	0.45^{AB}	0.48^{A}	
Flavonoids (mg g^{-1} f.wt. leaves)								
Control	2.24^{a}	2.46^{a}	0.09^{b}	1.60^{B}	2.10^{ab}	2.28^{a}	1.24^{b}	1.87^{B}
UV radiation	2.47^{a}	2.91^{a}	2.50^{a}	2.62^{A}	2.62^{a}	2.97^{a}	2.81^{a}	2.80^{A}
Mean	2.36^{A}	2.69^{A}	1.29^{B}		2.36^{A}	2.63^{A}	2.02^{A}	
Anthocyanin (mg 100 g^{-1} f.wt. bulb)								
Control	4.37^{b}	6.67^{ab}	6.99^{a}	6.01^{B}	5.55^{b}	6.65^{b}	6.70^{b}	6.30^{B}
UV radiation	6.98^{ab}	9.97^{a}	9.99^{a}	8.98^{A}	7.76^{ab}	7.87^{ab}	9.85^{a}	8.49^{A}
Mean	5.67^{A}	8.32^{A}	8.49^{A}		6.65^{A}	7.26^{A}	8.27^{A}	

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan's multiple range test; capital letters for mean of UV-B treatment or salinity, whereas lowercase letters for interaction

Plants responded to a high level of NaCl by significant decreasing in flavonoids concentration in leaves in the first season. This decreasing became insignificant in the second season. UV radiation significantly enhanced the concentration of flavonoids at both seasons. Except the leaves samples collected from the individual treatment of 100 mM NaCl, there was an increase in flavonoids concentration in compared to control plants at both seasons. Furthermore, anthocyanin concentration (in onion bulbs) was more elevated by UV-B pre-treatment especially at the high level of salt stress.

As known, salt stress faced the plant to alter the cellular redox balance to cope the oxidized state. Leaf carotenoid concentration in plants exposed to low UV-B was 8% higher than control plants [45]. Their results suggested that carotenoids have antioxidant properties which play a main role in the antioxidant defense system which depletes carotenoids to scavenge excess ROS resulting from both salt stress and UV exposure. Thus, Nithia and Shanthi [46] showed an increase in the synthesis of secondary pigments concentration by enhancing the defense mechanisms of the plant against UV-B radiation. UV-B induced accumulation of flavonoids due to activating of phenylalanine ammonia lyase enzyme, a key enzyme of flavonoid biosynthesis in phenylpropanoid biosynthesis pathway [47]. Seedling of *Capsicum annum* L. responded to a low dose of UV-B irradiation by elevating of flavonoids and UV- absorbing compounds in leaves [1]. High levels of flavonoids and anthocyanin possibly protect the plant by absorbing UV in the leaf epiderm and prevent it from reaching to the photosynthetic tissues [48]. Increasing phenols, especially flavonoids, were noticed to be located in the epidermis to empower plant cell to cope oxidative stress and filter out UV radiation then absorb only UV rays to attenuate penetration to following tissues [49]. Furthermore, treatment with UV enhanced the concentration of plastid pigments and anthocyanin biosynthesis in leaves of bean [31]. They added that these responses elevate ROS scavenging capacity which induced better protection against Na^{+} and Cl^{-}.

It should be noticed in (Table 6) that the activities of antioxidant enzymes elevated as a result of salt stress but with different patterns comparing with control. Activities of POD, PPO and APX increased under both concentrations of salt stress in compared to control. The increasing in

POD activity reached 2.5 fold times more than control in plants irrigated with 50 mM NaCl. It was detected that POD and PPO activities at 50 mM NaCl were pronounced insignificant increase more than 100 mM NaCl at both seasons. UV-B application stimulated both POD and PPO more than un-irradiated treatments under the two concentrations of salinity (Table 6). The highest activities of POD and PPO were recorded in irradiated plants which irrigated with 50 mM NaCl.

Data in Table (6) also indicated that there was no APX activity detected in unsalted stressed plants whether under irradiated or un-irradiated conditions. Salinity stress gradually induced the activity of APX to (29.39 and 39.11) and (102.27 and 107.49) units mg^{-1} protein with 50 mM NaCl and 100 mM NaCl, respectively, during the two seasons. An insignificant increase in APX activity was noticed when the transplants were exposed to UV-B radiation. However, the maximum APX activity was recorded with 100 mM NaCl, which reached the level of significance.

POD, PPO and APX were reported to be correlated with enhancing salinity stress tolerance in wheat [50]. Also, Kumari et al. [51] found that enzymatic antioxidant system (POD, PPO and APX) enhanced due to supplementary to UV-B. Zacchini and de Agazio [52] mentioned that generated H_2O_2 after calli exposure to ultraviolet activated the antioxidant enzyme by spreading through cell layers that were not exposed to ultraviolet. The relationship between POD, PPO and APX is integrated. In stressed plant tissues, POD catalyzes H_2O_2 detoxification in the presence of aromatic electron donor as guaicol and pyrogalol and plays a vital role in lignin biosynthesis [53]. Gerdemann et al. [54] found that PPO was expressed as a catalase-like activity which plays a role in the directing removal of H_2O_2. The importance of APX resulting from its role in ascorbate- glutathione cycle (Mittler, [55]) due to catalyzing scavenging of H_2O_2 by utilizing ascorbic acid as an electron donor [56].

3.1 SDS-PAGE Leaf Proteins

Analysis of gels based on electrophoretic pattern indicated alterations in the protein profile of UV-B treated and salinity stress as compared to control. Certain new protein bands appeared and a few were lost after exposure. Leaf protein analysis was carried out on two controls and four treatments of *onion* (Tables 7 and 8) and illustrated in Fig. 1. The results indicated that the total numbers of six protein bands were observed. Moreover, three bands were monomorphic and two bands were polymorphic revealing 33.3% of polymorphism. A negative marker was detected by the interaction of UV + 50 mM NaCl and UV + 100 mM NaCl at 114.132 kDa. Also, a negative marker was detected by UV + 100 mM NaCl at 11.656 kDa, while one positive marker was detected by UV + 100 mM NaCl at 87.545 kDa. These results revealed that proteins were expressed in specific regions of onion plants adapted to UV and salt stress

Table 6. Effect of UV-B radiation treatment on some pigments concentration of red onion under salinity stress during 2015/2016 and 2016/2017 growing seasons

Treatments	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	Mean
	1st season				2nd season			
	POD specific activity (unit mg^{-1} protein)							
Control	741.92^{c}	1872.42^{bc}	1779.42^{bc}	1464.60^{B}	841.80^{c}	1507.69^{c}	1815.85^{bc}	1388.5^{B}
UV radiation	1697.54^{bc}	3846.18^{a}	2885.04^{ab}	2809.60^{A}	1676.76^{c}	3965.76^{a}	2784.43^{b}	2809.00^{A}
Mean	1219.70^{B}	2859.00^{A}	2332.20^{AB}		1259.30^{B}	2736.70^{A}	2300.10^{A}	
	PPO specific activity (unit mg^{-1} protein)							
Control	64.48^{b}	84.89^{ab}	77.46^{ab}	75.61^{A}	62.06^{b}	89.25^{ab}	78.18^{ab}	76.24^{B}
UV radiation	78.64^{ab}	106.57^{a}	102.21^{a}	95.80^{A}	85.96^{ab}	111.67^{a}	112.10^{a}	103.24^{A}
Mean	71.56^{A}	95.72^{A}	89.83^{A}		74.01^{A}	100.46^{A}	95.14^{A}	
	APX specific activity (unit mg^{-1} protein)							
Control	0.00^{b}	7.27^{b}	117.10^{a}	41.46^{A}	0.00^{c}	13.54^{bc}	119.29^{a}	44.27^{A}
UV radiation	0.00^{b}	51.50^{ab}	87.45^{ab}	46.32^{A}	0.00^{c}	$64.68a^{bc}$	95.69^{ab}	53.46^{A}
Mean	0.00^{B}	29.39^{AB}	102.27^{A}		0.00^{B}	39.11^{B}	107.49^{A}	

Means followed by different letters are significantly different at P ≤ 0.05 level; Duncan's multiple range test; capital letters for mean of UV-B treatment or salinity, whereas lowercase letters for interaction

Table 7. SDS-PAGE patterns of total soluble proteins among the six treatments of salinity and UV during 2015/2016 growing season

MW	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Band types
149.374	1.000	1.000	1.000	1.000	1.000	1.000	Monomorphic
114.132	1.000	1.000	1.000	0.000	1.000	0.000	Polymorphic
87.545	0.000	0.000	0.000	0.000	0.000	1.000	Unique
58.583	1.000	1.000	1.000	1.000	1.000	1.000	Monomorphic
25.527	1.000	1.000	1.000	1.000	1.000	1.000	Monomorphic
11.656	1.000	1.000	1.000	1.000	1.000	0.000	Polymorphic

(MW) Molecular Weight; control = (1), control UV = (2), salinity 50 = (3), UV + salinity 50 = (4), salinity 100 = (5), and UV + salinity 100 = (6)

together. The data of protein analysis obtained in this study suggested that UV-B enhanced expression of certain protein that confers tolerance to onion against salt stress.

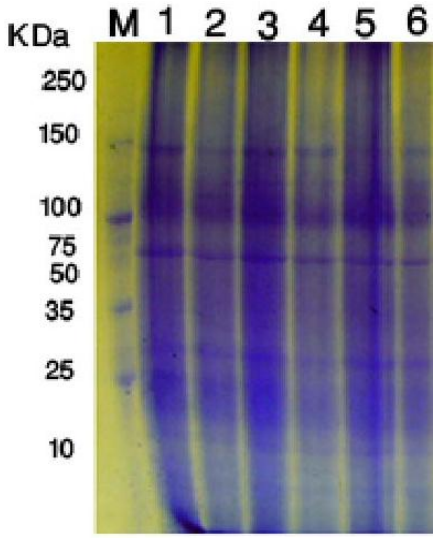

Fig. 1. SDS-PAGE profiles of soluble protein among the treatments

(M) Maker; control = (1), control UV = (2), salinity 50 = (3), UV + salinity 50 = (4), salinity 100 = (5), and UV + salinity 100 = (6)

From the above results, the authors can deduce that the individual treatment of UV-B radiation or the different concentration of NaCl didn't appear any electrophoretic pattern of onion plant samples, while the interaction between them produced some changes which induced plant tolerance. Babele et al. [57] declared that a few new proteins appeared and certain proteins were lost in bacterium *E.coli* expressed to UV-B radiation. They recommended that alterations in quantity and quality of proteins are expected as it has been an essential step of adaptive mechanism developed by any organism under UV-B stress. On the other hand, Beltagi et al. [58] reported that NaCl treatments reduced the total number of protein bands in *Phaseolus vulgaris* while low dose of gamma irradiation induced stability in the total numbers of protein bands. A synergistic interaction between salinity stress and a non-lethal dose of irradiation resulted in salt tolerance in *Phaseolus vulgaris*.

Table 8. Number, types and polymorphism percentage of leaf soluble protein bands

Gel polymorphism	
Monomorphic bands	3
Polymorphic (without Unique)	2
Unique bands	1
Total number of bands	6
Polymorphism (%)	33.3%

Taken all together, it was assumed that salt stress negatively affected growth, productivity and chlorophylls concentration in onion plants. Low doses of UV-B enhanced plant tolerance due to more elevation of osmoregulators (soluble amino acids, total sugars), antioxidants (total soluble phenols, carotenoids, flavonoids and anthocyanin) and antioxidant enzymes (POD, PPO, APX) of salt-stressed plants. Therefore, pre-treatment of transplants with the optimum dose of UV-B seems to be an effective way to induce plant tolerance against salinity stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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