**Volume 54(1):37-43, 2010**
**Acta Biologica Szegediensis**
http://www.sci.u-szeged.hu/ABS

**ARTICLE**

# Contrastive response of *Phlomis tuberosa* to salinity and UV radiation stresses

Ghader Habibi[1], Roghieh Hajiboland[1,2]*, Gholamreza Dehghan[1]

[1]Plant Science Department, University of Tabriz, Tabriz, Iran, [2]Excellent Center of Biodiversity, University of Tabriz, Tabriz, Iran

**ABSTRACT** Growth, photosynthetic characteristics and antioxidant defense system were investigated under salinity stress and UV radiation in *Phlomis tuberosa* (Lamiaceae) grown under environmentally controlled conditions for two weeks. Salinity at 40 mM results in significant reduction of shoot growth up to 20%, while UV radiation at 10 kJ $m^{-2}$ $d^{-1}$ did not affect plants dry matter production. Salinity did not influnce PSII photochemistry, while UVA+B radiation caused a significant reduction of maximum quantum yield of PSII. The net photosynthesis rate was inhibited by both salinity and UV stress following reduced stomatal conductance. Leaf osmotic and water potential were decreased by salinity but not UV radiation. Activity of antioxidant enzymes increased under both salinity and UV radiation stress, however, membrane damage was occurred only under UV stress. Our data implied that, high salinity sensitivity in this species was mainly attributable to the salt-induced disturbance in water relations and reduced assimilation rate rather than to other factors such as damage to PSII, oxidative stress or membrane damage. However, PSII photoinhibition, membrane damage and significant reduction of net assimilation rate were not able to affect negatively plants performance under UV stress implying involvement of other factors in high UV stress tolerance in *Phlomis tuberosa*.

**Acta Biol Szeged 54(1):37-43 (2010)**

**KEY WORDS**

Antioxidant defense system
leaf photochemistry
gas exchange
water relations
salinity
UV stress

Plants, in their natural habitats, are often subjected to various stress conditions such as high light intensity, UV radiation, temperature extremes, drought and salinity. Native vegetations consist of most adapted plant species to their local environmental conditions. A wide spectrum of biochemical and metabolic adaptations are found in plants under these stress conditions.

Environmental stress conditions enhance generation of reactive oxygen species (ROS) and cause imbalance between production of ROS and quenching activity of the antioxidants, resulting in oxidative damage (Mano 2002). ROS causes lipid peroxidation, membrane destruction, protein denaturation and DNA damage (Creissen and Mullineaux 2002). Activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidases (POD) as the most important components in scavenging and prevention of ROS damage are increased under stress conditions (Dat et al. 2000). Considering multiple roles that ROS play in plant metabolism, it is plausible that protective pathways in the plant are indeed shared, than separate pathways. Thus, tolerance mechanisms to several kinds of stress such as drought, salinity and UV radiation are interconnected and partially overlapping. Physiological studies found correlations between levels of antioxidants and the level of stress tolerance among plant species (Perl-Treves and Perl 2002).

The general response of plants to increasing salt concentration includes osmotic stress, specific ion toxicity and nutrient deficits that affect a range of physiological processes involved in cell metabolism (Ashraf and Harris 2004). An increase in salinity causes reduction of water and osmotic potential, leaf area and stomatal density (Parida and Das 2005). Similar with other abiotic stresses, salinity is known to negatively influence $CO_2$ assimilation via affecting both stomatal and non-stomatal components of photosynthesis (Kao et al. 2003). Moreover, a correlation exists between activity of antioxidant enzymes and salt tolerance of plants (Parida and Das 2005).

Elevated UV radiation at high altitudes in mountain areas causes distinct effects on plants growing at high elevations (Filella and Penuelas 1999). Deleterious effects of UV radiation on the growth, productivity and photosynthesis of higher plants have been extensively studied (Germ et al. 2005; Xu et al. 2008; Surabhi et al. 2009). Evidences suggest that ROS are involved in the damages caused by UV radiation. Like the antioxidant pools, the activity of several key enzymes involved in ROS metabolism such as CAT and APX is altered by UV radiation (Yannarelli et al. 2006). UV radiation impairs all major processes of photosynthesis including photochemical reactions and stomatal conductance (Allen et al. 1998).

Accepted May 20, 2010
*Corresponding author. E-mail: ehsan@tabrizu.ac.ir

However, UVB inhibits maximum net photosynthesis rate in a variety of plants without direct correlation with chlorophyll fluorescence or PSII activity, suggesting that photodamage to PSII is not the primary reason for reduced rates of net assimilation rate (Allen et al. 1998; Fedina et al. 2003).

The Mediterranean climate regions of the world occupy less than 5% of the Earth's surface, but harbor about 20% of the world total known vascular plant species (Cowling et al. 1996). The genus *Phlomis* L. comprises over 100 species including herbs, shrubs and sub-shrubs of the family Lamiaceae (Albaladejo et al. 2005) and is distributed mainly in the Mediterranean region of Anatolia (Turkey) and Iran (Azizian and Moore 1982). *P. tuberosa* is a summer perennial herb growing in an altitudinal gradient of increasing UV radiation of mountains in NW of Iran. Considering conditions of natural habitat, this species is expected to be adapted well with high UV radiation, however, is likely sensitive to salinity similar with other species from Lamiaceae.

The present work was aimed to study the effect of salinity and UV radiation stresses on growth, photosynthesis and antioxidant defense capacity of *P. tuberosa*. Involvement of stomatal and non-stomatal factors in the response of plants photosynthesis to salinity and UV radiation was studied. In addition, functional significance of antioxidant defense system in plants adaptation to UV radiation in comparison with that to salinity was investigated.

## Materials and Methods

### Plant materials and treatments

Seeds of *Phlomis tuberosa* were collected from Mishoudagh, near the city of Marand, 65 km south of Tabriz (45°38' E, 38°22' N) at an elevation of 1800 m, East-Azarbaijan Province (NW of Iran). Seeds were surface sterilized and germinated on filter paper moistened with distilled water and $CaSO_4$ at 0.05 mM. Ten-day-old seedlings were transferred to Hoagland nutrient solution (Johnson et al. 1957) and were pre-cultured for 45 days prior to the start of treatments. Thereafter, plants with uniform size were selected and subjected to either salinity or UV radiation treatments. Salinity treatment consisted of control, 20 mM and 40 mM NaCl. For UV radiation treatments, in addition to the photosynthetic active radiation (PAR, 400-700 nm) supplied by cool white fluorescent lamps throughout the day time, UVAB fluorescent lamps (30 W, Hagen, Japan) was used without filter for UVA+B, with transparent Plexiglass filter cutting wavelength under 320 nm for UVA and with yellow colored Plexiglass filter for cutting wavelength under 400 nm for control plants with 6 h irradiance periods centered midway through the photoperiod. The spectral outputs of the three lighting conditions were measured with a calibrated spectrophotometer (Shimadzu, UV-2450) and biologically effective UV doses employed were 10 kJ $m^{-2}$ $d^{-1}$ calculated based on Caldwell's generalized plant damage action spectrum normalized to 300 nm (Caldwell 1971).

Plants were grown in a growth chamber under environmentally controlled conditions at about 150 µmol $m^{-2}$ $s^{-1}$ light intensity, 18/6 h light/dark photoperiod, 25/17ºC day/night temperature and relative humidity of 60/70%.

### Harvest

Two weeks after treatment, plants were harvested. Leaf samples were washed with distilled water, blotted dry on filter paper and dried for 48 h at 70°C for determination of dry weight.

### Determination of chlorophyll fluorescence and gas exchange parameters

Chlorophyll fluorescence parameters were recorded using a portable fluorometer (OSF1, ADC Bioscientific Ltd., UK) for both dark adapted and light adapted leaves. Measurements were carried out on the second youngest, fully expanded and attached leaf. An average of 4 records from different parts of each individual leaf was considered for each replicates. Leaves were acclimated to dark for 30 min using leaf clips before measurements were taken. Initial ($F_0$), maximum ($F_m$), variable ($F_v=F_m-F_0$) fluorescence as well as maximum quantum yield of PSII ($F_v/F_m$) and $F_v/F_0$ ratios were recorded. Light adapted leaves (400 µmol $m^{-2}s^{-1}$) were used for measurement of steady-state ($F_s$) and maximum ($F'_m$) fluorescence. Calculations were made for $F'_0$ ($F'_0=F_0/[(F_v/F_m)+(F_0/F'_m)]$), photochemical quenching, $qP$ $[(F'_m- F_s)/(F'_m-F'_0)]$, non-photochemical quenching, $qN$ $(1-[(F'_m-F'_0)/(F_m-F_0)])$ and effective quantum yield of PSII, $\Phi_{PSII}$ $[(F'_m-F_s)/F'_m]$ (Krall and Edwards 1992).

Leaf gas exchange parameters were determined in parallel with Chl fluorescence measurements in the same leaf with a calibrated portable gas exchange system (LCA-4, ADC Bioscientific Ltd., UK) between 10:00 A.M. and 13:00 P.M at harvest. The measurements were conducted with PPFD at the leaf surface of 350 µmol $m^{-2}s^{-1}$ measured by a quantum sensor attached to the leaf chamber of the gas exchange unit. The net photosynthesis rate by unit of leaf area ($A$, µmol $CO_2$ $m^{-2}s^{-1}$), transpiration rate ($E$, mmol $H_2O$ $m^{-2}s^{-1}$) and stomatal conductance to water vapor ($g_s$, mol $m^{-2}s^{-1}$) were measured by the infrared gas analyzer of the portable photosynthesis system. Water use efficiency was calculated using the values of $A$ and $E$ ($WUE=A/E$, µmol $mmol^{-1}$).

### Leaf water and osmotic potential

Leaf osmotic potential ($\psi_s$) and water potential ($\psi_w$) was determined in the second youngest leaf harvested at 1 h after light on in the growth chamber. Leaves were homogenized in pre-chilled mortar and pestle and centrifuged at 4000 g for 20 min at 4°C. The osmotic pressure of samples was measured

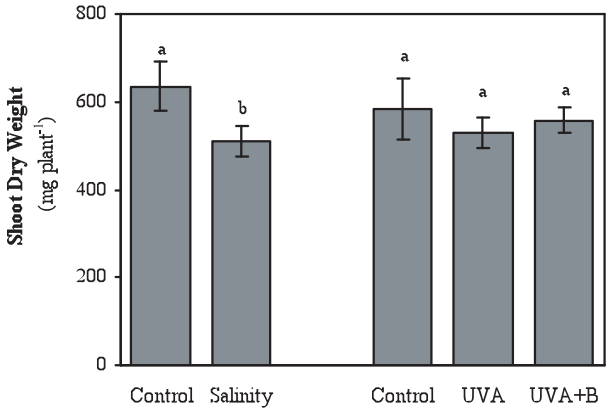


**Figure 1.** Shoot dry weight (mg plant-1) of Phlomis tuberosa grown for two weeks in nutrient solution with 40 mM NaCl salinity or under UV radiation at 10 kJ m-2 d-1. Values are the mean ± SD (n=4). Bars indicated by the same letters are not significantly different (P<0.05).

by an osmometer (Micro-Osmometer, Heman Roebling MESSTECHNIK, Germany), and the miliosmol data were recalculated to MPa. Water potential was measured using a pressure chamber (DTK-7000, Japan).

### Assay of antioxidant enzymes and related metabolites

Determination of the activity of antioxidant enzymes and concentration of related metabolites were undertaken according to optimized protocols described elsewhere (Hajiboland and Hasani 2007). Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by determining ascorbic acid oxidation, one unit of APX oxidizes ascorbic acid at the rate of 1 μmol $min^{-1}$ at 25˚C. Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of $H_2O_2$ at 240 nm, unit activity was taken as the amount of enzyme, which decomposes 1 μmol of $H_2O_2$ $min^{-1}$. Peroxidase (POD, EC 1.11.1.7) activity was assayed using the guaiacol test, the enzyme unit was calculated as enzyme protein required for the formation of 1 μmol tetraguaiacol $min^{-1}$. Total superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using monoformazan formation test. One unit of SOD was defined as the amount of enzyme required to induce a 50% inhibition of NBT reduction as measured at 560 nm, compared with control samples without enzyme aliquot. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid (Sigma) at 532 nm. MDA levels were calculated from a 1,1,3,3-tetraethoxypropane (Sigma) standard curve. The concentration of $H_2O_2$ was determined using potassium titanium-oxalate at 508 nm. Proline was extracted with 3% sulfosalicylic acid, after centrifugation the supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h, and then absorbance at 520 nm was determined. Proline (Sigma) was used for production of standard curve. Soluble proteins were determined using a commercial Bradford reagent (Sigma) and BSA (Merck) as standard (Hajiboland and Hasani 2007).

Experiments were under taken in complete randomized block design with 4 replications. Statistical analyses were carried out using sigma stat (3.02) with Tukey test (P<0.05).

**Table 1.** Leaf photochemical parameters including photochemical efficiency of PSII ($F_v/F_m$), ratio of variable to initial fluorescence ($F_v/F_0$), photochemical quenching ($q_p$), non-photochemical quenching ($q_N$) and quantum yield of PSII ($\Phi_{PSII}$) and gas exchange parameters including net photosynthetic rate (*A*, μmol $m^{-2}$ $s^{-1}$), transpiration rate (*E,* mmol $m^{-2}$ $s^{-1}$), stomatal conductance to water vapor ($g_s$, mol $m^{-2}$ $s^{-1}$) and water use efficiency (*WUE*, μmol $mmol^{-1}$) in *Phlomis tuberosa* grown for two weeks in nutrient solution with 0, 20 and 40 mM NaCl salinity. Values are the mean ± SD (n=4). Data of each row indicated by the same letters are not significantly different (P<0.05).

| | Treatment | | |
|---|---|---|---|
| Photochemistry | Control | 20 mM NaCl | 40 mM NaCl |
| $F_v/F_m$ | 0.84 ± 0.01 [a] | 0.82 ± 0.02 [a] | 0.80 ± 0.04 [a] |
| $F_v/F_0$ | 5.17 ± 0.07 [a] | 4.61 ± 0.67 [a] | 4.17 ± 1.00 [a] |
| *qP* | 0.898 ± 0.004 [a] | 0.936 ± 0.027 [a] | 0.962 ± 0.057 [a] |
| *qN* | 0.080 ± 0.019 [a] | 0.146 ± 0.077 [a] | 0.160 ± 0.087 [a] |
| $\Phi_{PSII}$ | 0.746 ± 0.004 [a] | 0.748 ± 0.010 [a] | 0.748 ± 0.015 [a] |
| Gas exchange | | | |
| *A* | 4.74 ± 0.72 [a] | 4.60 ± 0.26 [a] | 2.97 ± 0.51 [b] |
| *E* | 0.90 ± 0.44 [a] | 0.73 ± 0.20 [a] | 0.54 ± 0.03 [a] |
| $g_s$ | 1.33 ± 0.898 [a] | 0.293 ± 0.126 [b] | 0.160 ± 0.010 [b] |
| *WUE* | 6.16 ± 2.81 [a] | 6.67 ± 2.26 [a] | 5.48 ± 0.76 [a] |

## Results

Shoot dry weight was inhibited by salinity at 40 mM NaCl up to 20% in *Phlomis tuberosa* plant. In contrast, UV radiation did not affect dry matter production of plants significantly (Fig. 1).

Under saline conditions, chlorophyll fluorescence parameters were not influenced significantly (Table 1). However, slight reduction of maximal efficiency of PSII in dark-adapted leaves ($F_v/F_m$) and the proportion of active Chl associated with the reaction centers (RCs) of PSII ($F_v/F_0$) as well as increase in photochemical quenching (*qP*), non-photochemical quenching (*qN*) and quantum yield of PSII ($\Phi_{PSII}$) were detectable in leaves of salt-stressed plants. Net assimilation rate (*A*) was not influenced by 20 mM salinity stress, but reduced up to 37% by 40 mM salt concentration (Table 1). Although a slight reduction was detected in the transpiration rate (*E*), it was not affected significantly by the used NaCl concentrations, while the stomatal conductance to water vapor ($g_s$) was reduced strongly by both salt stresses (about 88%).

**Table 2.** Leaf photochemical parameters including photochemical efficiency of PSII ($F_v/F_m$), ratio of variable to initial fluorescence ($F_v/F_0$), photochemical quenching ($q_P$), non-photochemical quenching ($q_N$) and quantum yield of PSII ($\Phi_{PSII}$) and gas exchange parameters including net photosynthetic rate (*A*, µmol $m^{-2}$ $s^{-1}$), transpiration rate (*E,* mmol $m^{-2}$ $s^{-1}$), stomatal conductance to water vapor ($g_s$, mol $m^{-2}$ $s^{-1}$) and water use efficiency (*WUE*, µmol $mmol^{-1}$) in *Phlomis tuberosa* grown for two weeks under UV radiation at 10 kJ $m^{-2}$ $d^{-1}$. Values are the mean ± SD (n=4). Data of each row indicated by the same letters are not significantly different (P<0.05).

| | Treatment | | |
|---|---|---|---|
| Photochemistry | Control | UVA | UVA+B |
| $F_v/F_m$ | 0.84 ± 0.01 [a] | 0.81 ± 0.04 [a] | 0.75 ± 0.06 [b] |
| $F_v/F_0$ | 4.82 ± 0.66 [a] | 4.55 ± 1.04 [a] | 3.27 ± 0.59 [a] |
| *qP* | 0.968 ± 0.011 [a] | 0.950 ± 0.071 [a] | 0.994 ± 0.023 [a] |
| *qN* | 0.073 ± 0.070 [a] | 0.097 ± 0.059 [a] | 0.201 ± 0.112 [a] |
| $\Phi_{PSII}$ | 0.786 ± 0.002 [a] | 0.759 ± 0.025 [a] | 0.766 ± 0.001 [a] |
| Gas exchange | | | |
| *A* | 4.84 ± 0.52 [a] | 4.36 ± 0.33 [ab] | 3.65 ± 0.70 [b] |
| *E* | 1.21 ± 0.25 [a] | 0.80 ± 0.23 [b] | 0.61 ± 0.11 [b] |
| $g_s$ | 1.22 ± 0.742 [a] | 0.327 ± 0.144 [b] | 0.217 ± 0.081 [b] |
| *WUE* | 4.18 ± 1.42 [a] | 5.76 ± 1.51 [a] | 5.97 ± 0.67 [a] |

**Table 3.** Water potential ($\psi_w$) and osmotic potential ($\psi_s$) (MPa) in the leaves of *Phlomis tuberosa* grown for two weeks in nutrient solution with 0, 20 and 40 mM NaCl salinity or under UV radiation at 10 kJ $m^{-2}$ $d^{-1}$. Values are the mean ± SD (n=4). Data of each column within each treatment indicated by the same letters are not significantly different (P<0.05).

| Treatment | $\psi_s$ | $\psi_w$ |
|---|---|---|
| Control | –0.640 ± 0.086 [a] | –0.737 ± 0.097 [a] |
| 20 mM | n.d. | n.d. |
| 40 mM | –0.933 ± 0.043 [b] | –1.34 ± 0.108 [b] |
| Control | –0.711 ± 0.054 [a] | –0.777 ± 0.205 [a] |
| UVA | –0.668 ± 0.052 [a] | –0.812 ± 0.185 [a] |
| UVA+B | –0.708 ± 0.031 [a] | –0.837 ± 0.254 [a] |

Water use efficiency (*WUE)* showed only a slight reduction by salinity.

In plants grown under UV radiation treatments, the maximal efficiency of PSII in dark adapted leaves ($F_v/F_m$) was not affected by UVA, while UVA+B treatment caused a significant reduction up to 11% in the $F_v/F_m$ ratio (Table 2). UVA+B affected $F_v/F_0$ ratio only slightly. In light adapted leaves, *qP*, *qN* and $\Phi_{PSII}$ remained unchanged under UV treatments. Net assimilation rate (*A*) was reduced by UVA treatment slightly. In contrast, effect of UVA+B treatment on *A* was significant and reached up to 24% compared with control plants without UV treatment. Simultaneous with reduction of *A*, transpiration rate (*E*) and stomatal conductance ($g_s$) were reduced strongly by both UVA and UVA+B treatments. Effect of UVA+B was more pronounced than UVA. *WUE* was not affected by UV treatment significantly (Table 2).

**Table 4.** Leaf content of MDA (nmol $g^{-1}$ FW) and $H_2O_2$ (nmol $g^{-1}$ FW) in *Phlomis tuberosa* grown for two weeks in nutrient solution with 0, 20 and 40 mM NaCl salinity or under UV radiation at 10 kJ $m^{-2}$ $d^{-1}$. Values are the mean ± SD (n=4). Data of each column within each treatment indicated by the same letters are not significantly different (P<0.05).

| Treatment | MDA | $H_2O_2$ |
|---|---|---|
| Control | 12.67 ± 1.37 [a] | 494 ± 49 [b] |
| 20 mM | 16.37 ± 1.48 [a] | 501 ± 39 [b] |
| 40 mM | 14.20 ± 3.73 [a] | 680 ± 66 [a] |
| Control | 12.84 ± 0.51 [b] | 467 ± 16 [a] |
| UVA | 12.98 ± 1.42 [b] | 545 ± 27 [a] |
| UVA+B | 17.91 ± 3.39 [a] | 507 ± 94 [a] |

Leaf water potential ($\psi_w$) was lowered by about 45% with 40 mM salinity, similarly, leaf osmotic potential ($\psi_s$) decreased up to 31% (Table 3). In contrast, UV treatments did not affect significantly $\psi_w$ and $\psi_s$. However, a slight reduction of $\psi_w$ and $\psi_s$ was detected in leaves of *Phlomis tuberosa* particularly under UVA+B treatment compared with control (Table 3).

Specific activity of all studied enzymes was increased by both applied salinity levels (Fig. 2). Activity of APX was influenced by salinity (up to 70%), more than other enzymes. SOD, CAT and POD activities increased by 40 mM salinity with similar extent (by about 40%). Concentration of MDA remained unchanged under salinity, while $H_2O_2$ concentration increased under 40 mM NaCl salinity significantly (Table 4).

Under UV treatments, only activity of CAT and APX was influenced significantly. Activity of APX was increased by UV stress up to 53% and CAT by 30% (Fig. 2). UVA+B treatment, in contrast to salinity, caused a significant increase of MDA content of leaves, while did not influence $H_2O_2$ concentration (Table 4).

## Discussion

### Contrastive response of *Phlomis tuberosa* to salinity and UV radiation

*Phlomis tuberosa* showed a high sensitivity to salinity. Studies on various Lamiaceae species often demonstrated a high to moderate salt sensitivity in these species (Tabatabaie and Nazari 2007). However, these works have mainly focused on the effects of salinity on the quality and quantity of secondary metabolites (Karray-Bouraoui et al. 2009; Ben Taarit et al. 2009). Data regarding the effect of salinity on photosynthesis and water relations are rare and little attention has been paid on the mechanisms of high salt susceptibility in these species.

In contrast, UV radiation stress did not affect dry matter production of plants, a response characteristic for plants growing at high elevations (Filella and Penuelas 1999).

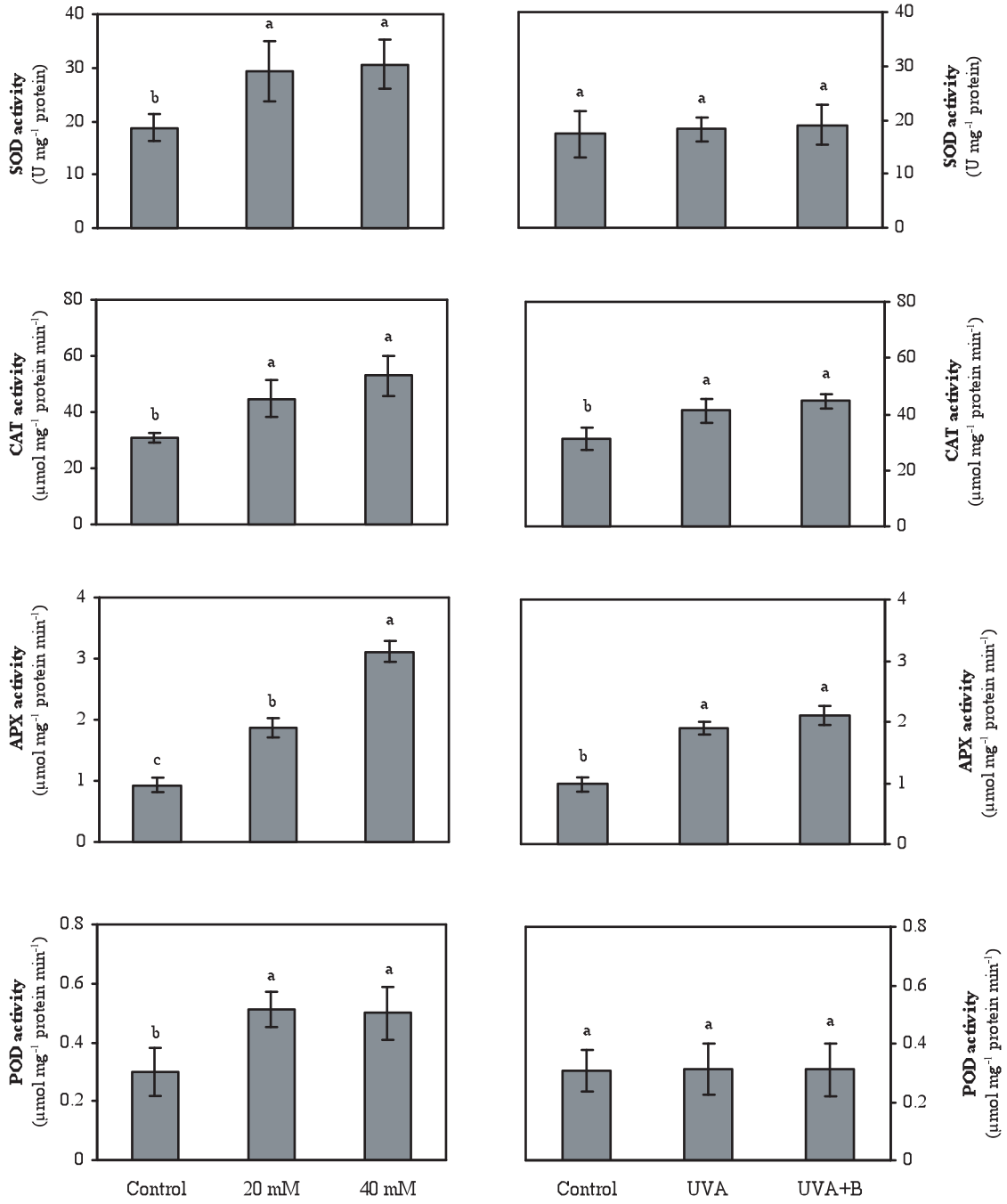


**Figure 2.** Specific activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) in the leaves of *Phlomis tuberosa* grown for two weeks in nutrient solution with 0, 20 and 40 mM NaCl salinity or under UV radiation at 10 kJ $m^{-2}$ $d^{-1}$. Values are the mean ± SD (n=4). Bars indicated by the same letters are not significantly different (P<0.05).

## Leaf photochemistry and gas exchange under salinity and UV radiation stress

Though a high sensitivity to salt as judged by changes in plants dry matter production, PSII photochemistry was not affected under these conditions. Significant change in leaf photochemical parameters such as $F_v/F_m$ due to salinity was reported for some salt sensitive (Wang et al. 2007) but not salt tolerant (Megdiche et al. 2008) species. Many authors suggested application of chlorophyll a fluorescence analysis as a reliable method to assess the changes in the function of

PSII under stress conditions (Maxwell and Johnson 2000). Fluorescence parameters have been used to screen for salinity tolerance in some plant species (Jiang et al. 2006). Lack of change in photochemical parameters in *Phlomis tuberosa* under growth-inhibiting salinity levels in this work demonstrated that PSII was highly resistant to salinity stress in this species and damage to PSII was not involved in plants response to salinity. In contrast to leaf photochemistry, gas exchange parameters were influenced strongly by salinity. Net assimilation rate (*A*) is an important photosynthetic parameter that represents the maximal photon utilization capacity of plants and thus, reflects the net primary productivity (Surabhi et al. 2009). Reduction of net assimilation rate (*A*) under salinity was correlated well with elevated stomatal resistance in our work as was reported by other authors (Jiang et al. 2006; Wang et al. 2007). Salinity-induced partial closure of stomata may improve the efficiency of plants for an economical use of water for growth. Accordingly, an improvement of water use efficiency (*WUE*) has been reported in plants subjected to salinity (Parida and Das 2005). In this work, however, concomitant with only a slight reduction of transpiration rate, *WUE* remained unchanged under salinity. In addition, significant reduction of leaf osmotic potential and water potential revealed a serious salt-induced disturbance in water relations (Romeroaranda et al. 2001) that was correlated with reduction of leaf growth under salinity.

UV radiation stress did not cause any change in leaf photochemistry with the exception of reduction of $F_v/F_m$, an indicator of photoinhibitory damage (Maxwell and Johnson 2000) caused by light or other environmental stresses under UVA+B stress. This change revealed higher sensitivity of PSII to UVA+B radiation in *Phlomis tuberosa*. UVB radiation can impair all major processes of photosynthesis including photochemical reactions in thylakoid membranes as well as stomatal conductance (Surabhi et al. 2009). A remarkable drop in *A* especially in the UVA+B treatment in this work was associated with significant reduction of stomatal conductance.

### Responses of antioxidant defense system to salinity and UV radiation

A large number of studies on various species indicated that salt stress alters the amount of the enzymes involved in scavenging ROS (Parida and Das 2005). SOD is reported to play an important role in cellular defense against oxidative stress, as its activity directly modulates the amount of $H_2O_2$ (Sudhakar et al. 2001). In the present work, a pronounced increase was observed in the activity of all antioxidant enzymes studied even at salinity as low as 20 mM NaCl. As expected, $H_2O_2$ was accumulated under salinity likely following an imbalance between production and scavenging of $H_2O_2$. In contrast, leaf MDA content remained unchanged under salinity presumably due to the increased activity of antioxidant enzymes which was sufficient to avoid a substantial elevation in lipid peroxidation. Accordingly, salinity did not cause membrane damage and lipid peroxidation in leaves of *Phlomis tuberosa* consistent with the unaffected leaf photochemistry in the leaves of salinized plants.

Under UV radiation, in contrast, activity of CAT and APX but not SOD or POD was enhanced. Under similar doses of UVB radiation applied in our work, activity of SOD was reported to be increased, decreased or remained unaffected depending on plant species (Xu et al. 2008). Increased APX activity in our work due to UV radiation is consistent with the results of several other studies and may suggest an important role for this enzyme in the response and adaptation of plants to UV stress (Xu et al. 2008 and refs therein). Accordingly, leaf concentration of $H_2O_2$ did not change by UV stress likely because of an efficient scavenging by APX and CAT under these conditions. Some studies report an increase (Kalbina and Strid 2006) while others report no change (Xu et al. 2008) of tissue content of $H_2O_2$ due to UVB stress. In contrast to $H_2O_2$, a significant increase of leaf MDA content was observed under UVA+B stress. It implied that an oxidative damage has been occurred under UVA+B stress likely by ROS other than $H_2O_2$ e.g. superoxide anions ($O_2^{\cdot-}$), the dominant ROS in UV-irradiated leaves (Hideg et al. 2002). Although the $O_2^{\cdot-}$ was not determined in this work, it is likely that elevated production without an accompanying increase in the ability to scavenge the formed $O_2^{\cdot-}$ results in accumulation of this radical in UVA+B affected leaves.

## Conclusion

*Phlomis tuberosa* was shown to be highly sensitive to salinity but relatively resistant to UV radiation stress as judged by dry weight data of plants. Information from leaf photochemistry and gas exchange as well as antioxidant defense system of plants revealed that, the cause of high salinity sensitivity of *Phlomis tuberosa* is not damage to PSII or membrane destruction. Indeed, salinity induced stomatal limitation and strong reduction of net assimilation rate, without efficient limitation of water loss from salt affected leaves. Accordingly, disturbance in water relations rather than the effect of other factors such as oxidative stress, inhibited leaf photochemistry or membrane damage was involved in determination of responses of *Phlomis tuberosa* to salinity.

In contrast, UV stress, which caused PSII damage, increased MDA content and significant reduction of net assimilation rate, did not significantly influence plants growth. This implies that these factors were not able to affect negatively plants performance under UV stress at least during the short period of exposure in this work.

There are few published works on the effect of UV radiation in Lamiaceae species (Chang et al. 2009). Plants are known to react to UV radiation by radical scavenging and pigmentation (Jacobs et al. 2007). Screening out UV-B radia-

tion by accumulation of flavonoids in the leaf epidermis was suggested as mechanisms for resistance to UV-B radiation (Jacobs et al. 2007). Members of Lamiaceae are well known to produce flavonoids (Tomás-Barberán and Wollenweber 1990) and accumulate these compounds in the leaf surface (Jamzad et al. 2003). Our results suggested that since antioxidant defense system is obviously inefficient in protection of plants against UV radiation, biochemical defense plays presumably a determinant role in high resistance to UV stress in *Phlomis tuberosa*.

## References


Albaladejo RG, Aguilar JF, Aparicio A, Feliner GN (2005) Contrasting nuclear-plastidial phylogenetic patterns in the recently diverged Iberian *Phlomis crinita* and *P. lychnitis* lineages (Lamiaceae). Taxon 54:987-998.

Allen DJ, Nogues S, Baker NR (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? J Exp Bot 49:1775-1788.

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:3-16.

Azizan D, Moore DM (1982) Morphological and palynological studies in *Phlomis* L. and *Eremostachys* Bunge (Labiatae). Bot J Linn Soc 85:249-281.

Ben Taarit M, Msaada K, Hosni K, Hammami M, Kchouk ME, Marzouk M (2009) Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions. Ind Crop Prod 30:333-337.

Caldwell MM (1971) Solar UV irradiation and the growth and development of higher plants. In Giese AC, ed., Photophysiology. Academic Press, New York, NY, USA, 131-137.

Chang X, Alderson PG, Wright CJ (2009) Enhanced UV-B radiation alters basil (*Ocimum basilicum* L.) growth and stimulates the synthesis of volatile oils. J Hort Forest 1:27-31.

Cowling RM, Rundel PW, Lamont BB, Arroyo MK, Arianoutsou M (1996) Plant diversity in Mediterranean-climate regions. Trees 11:362-366.

Creissen GP, Mullineaux PM (2002) The molecular biology of the ascorbate-glutathione cycle in higher plants. In Inzé D, Montganu MV, ed., Oxidative Stress in Plants. Taylor & Francis, UK, 247–270.

Dat J, Vandenabeele S, Vranova´ E, van Montagu M, Inze´ D, van Breusegem F (2000) Dual action of active oxygen species during plant stress responses. Cell Mol Life Sci 57:779-795

Fedina IS, Grigorova IT, Georgieva K (*2003*) Response of barley seedlings to UV-B radiation as affected by NaCl. J Plant Physiol 160:205-208.

Filella I, Penuelas J (1999) Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. Plant Ecol 145:157-165.

Germ M, Kreft I, Osvald J (2005) Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). Plant Physiol Biochem 43:445-448.

Hajiboland R, Hasani BD (2007) Responses of antioxidant defense capacity and photosynthesis of bean (*Phaseolus vulgaris* L.) plants to copper and manganese toxicity under different light intensities. Acta Biol Szeged 51:93-106

Hideg É, Barta C, Kálai T, Vass I, Hideg K, Asada K (2002) Detection of singlet oxygen and superoxide with fluorescent sensors in leaves under stress by photoinhibition or UV radiation. Plant Cell Physiol 43:1154-1164

Jacobs JF, Koper GJM, Ursemb WNJ (2007) UV protective coatings: A botanical approach. Prog Org Coat 58:166–171

Jamzad Z, Grayer RJ, Kite GC, Simmonds MSJ, Ingrouille M, Jalili A (2003) Leaf surface flavonoids in Iranian species of *Nepeta* (Lamiaceae) and some related genera. Biochem Syst Ecol 31:587-600.

Jiang Q, Roche D, Monaco TA, Durham S (2006) Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. Field Crop Res 96:269-278.

Johnson CM, Stout PR, Broyer TC, Carlton AB (1957) Comparative chlorine requirements of different plant species. Plant Soil 8:337-353.

Kalbina I, Strid A (2006) Supplementary ultraviolet-B irradiation reveals differences in stress responses between *Arabidopsis thaliana* ecotypes. Plant Cell Environ 29:754-763.

Kao WY, Tsai TT, Shih CN (2003) Photosynthetic gas exchange and chlorophyll a fluorescence of three wild soybean species in response to NaCl treatments. Photosynthetica 41:415-419.

Karray-Bouraoui M, Rabhi M, Neffati M, Baldan B, Ranieri A, Marzouk B, Lachaâl M, Smaoui A (2009) Salt effect on yield and composition of shoot essential oil and trichome morphology and density on leaves of *Mentha pulegium*. Ind Crop Prod 30:338–343

Krall JP, Edwards GE (1992) Relationship between photosystem II activity and $CO_2$ fixation in leaves. Physiol Plant 86:180-187.

Mano J (2002) Early events in environmental sresses in plants induction mechanisms of oxidative stress. In Inzé D, Montganu MV, ed., Oxidative Stress in Plants. Taylor & Francis, UK, 217–246.

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence–a practical guide. J Exp Bot 51:659-668.

Megdiche W, Hessini K, Gharbi F, Jaleel CA, Ksouri R, Abdelly C (2008) Photosynthesis and photosystem 2 efficeincy of two salt-adapted halophytic seashore *Cakile maritime* ecotypes. Photosynthetica 46:410-419.

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324-349.

Perl-Treves R, Perl A (2002) Oxidative stress: an introduction. In Inzé D, Montganu MV, ed., Oxidative Stress in Plants. Taylor & Francis, UK, 1–32.

Romeroaranda R, Soria T, Cuartero J (2001) Tomato plant-water relationships under saline growth conditions. Plant Sci 160:265-272.

Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzymes efficacy in two high yielding genotypes of mulberry (Morus alba L.) under NaCl salinity. Plant Sci 161:613-619.

Surabhi GK, Reddy KR, Singh SK (2009) Photosynthesis, fluorescence, shoot biomass and seed weight response of three cowpea (*Vigna unguiculata* L. Walp.) cultivars with contrasting sensitivity to UV-B radiation. Environ Exp Bot 66:160-171.

Tabatabaie SJ, Nazari J (2007) Influence of nutrient concentrations and NaCl salinity on the growth, photosynthesis, and essential oil content of peppermint and lemon verbena. Turk J Agr Forest 31:245-253.

Tomás-Barberán FA, Wollenweber E (1990) Flavonoid aglycones from the leaf surfaces of some Labiatae species. Plant Syst Evol 173:109-118.

Wang R, Chen S, Deng L, Fritz E, Hüttermann A, Polle A (2007) Leaf photosynthesis, fluorescence response to salinity and the relevance to chloroplast salt compartmentation and anti-oxidative stress in two poplars. Trees 21:581-591

Xu C, Natarajan S, Sullivan JH (2008) Impact of solar ultraviolet-B radiation on the antioxidant defense system in soybean lines differing in flavonoid contents. Environ Exp Bot 63:39-48.

Yannarelli GG, Gallego SM, Tomaro ML (2006) Effect of UV-B radiation on the activity and isoforms of enzymes with peroxidase activity in sunflower cotyledons. Environ Exp Bot 56:174-181.