UV Irradiation Effects on Seed Germination and Growth, Protein Content, Peroxidase and Protease Activity in Portulaca grandiflora and Portulaca oleracea

Babak Peykarestan, Mohammadreza Seify, Mohsen Shoukat Fadaei and Mohammad Hatim

Department of Agriculture Payam Noor University, Iran, P.O. Box: 3813616486, Milajerd, Iran
Young Researchers Club, Arak Branch, Islamic Azad University, Arak, Iran

Abstract: Ultraviolet radiation is energetically capable of disrupting proteins. Ultraviolet radiations are divided into three bands included UV-A (320-390 nm), UV-B (280-320 nm) and UV-C (254-280nm). Several studies have indicated that enhanced UV-B radiation can deleteriously affect physiological processes and overall growth in some plants species. Portulaca grandiflora and Portulaca oleracea seeds irradiated with 220 to 400 nm UV rays were grown in incubator for 8 days at 25°C. Germination, growth (seedling fresh weight, root shoot length and their ratio), lipid peroxidation, protease and peroxidase activity were measured in leaves. Results showed that percent germination of the seeds and the rates of growth of sprouts were inversely related to the irradiation doses. In Portulaca oleracea, peroxidase and protease activities (two folds) and malondialdehyde (MDA) contents were higher as compared to Portulaca grandiflora while vice versa for protein contents, revealing inherent differences between two types. Results of protein contents, peroxidase and protease activities suggested that irradiation dose should not under 300 nm UV in Portulaca oleracea and also 300 nm UV in Portulaca grandiflora. In Portulaca oleracea 320 to 400 nm UV irradiation dose non-significantly affected the protein contents, peroxidase activity, MDA contents and protease activity. In Portulaca grandiflora 300 nm UV irradiation dose increased the peroxidase activity and MDA contents, while it affects in protein content and protease activity.

Key words: Portulaca grandiflora • P. oleracea • UV radiation • Protease • Peroxidase

INTRODUCTION

The increase in solar ultraviolet-B (UV-B) radiation (280-320 nm) reaching the Earth’s surface as a consequence of depletion of the ozone layer raises concerns since it may have deleterious effects on both animals and plants. Enhanced UV-B radiation can alter plant growth and development as well as reproduction [1]; this has serious implications for plant yields and economics. Physiological and biochemical processes in plants are significantly affected by UV irradiation stress. The irradiation of seeds with high doses of UV rays disturbs the synthesis of protein [2], hormone balance [3], leaf gas-exchange [4], water exchange and enzyme activity [5]. The morphological, structural and the functional changes depend on the strength and the duration of the UV-irradiation stress. In the case of moderate stress, the adaptability capacity of the plants is preserved and the observed changes are reversible. Antioxidants and peroxidase are involved in the compensatory mechanisms for the inhibition of free radicals formed upon UV irradiation of seeds [6]. Correlation between growth and antioxidant enzyme activity of seedlings after UV and neutron irradiation of Portulaca seeds has been reported. Depending on the UV radiation dose between 300 and 380 nm the height of Portulaca seedlings was found shorter and parallel with this peroxidase activities were higher than in the unirradiated controls [7]. Similarly an increased level of the glutathione peroxidase activity after low doses of UV irradiation has also been reported in corn (Zea mays L.).

Protein breakdown and recycling, which depend on the levels of proteolytic enzymes, are an essential part of the plant response to environmental stress [8]. In response to environmental abiotic and biotic factors cellular proteins should be rebuilt. Degradation of damaged, misfolded and potentially harmful proteins provides free amino acids required for the synthesis of new proteins [8]. After UV and neutron irradiation of Portulaca seeds, an inverse correlation between growth and degrading enzyme activity has also been reported [7].
A dose-dependent decrease in the triacylglycerol content and a concomitant increase in free fatty acids were observed after UV-irradiation of nutmeg (Myristica fragrans Houtt.). Similarly a prolonged irradiation of seeds with UV light (for 1-6 h) led to an increase in the level of lipid peroxidation in wheat sprouts [6]. This suggested a breakdown of acylglycerols during radiation processing, resulting in the release of free fatty acids.

A number of radiobiological parameters are commonly used in early assessment of effectiveness of irradiation to induce mutations. Seed germination, seedling survival and pollen and ovule sterility have been used extensively. Fluorescence and light absorption spectra of chlorophyll attributed to different doses treatments of corn grains have been used to find out the superior irradiation doses in stimulating corn plants [10]. Previously on the basis of increase in free fatty acids it have also been suggested that radiation processing of nutmeg should be limited to a dose of 5 kGy. However protein contents, peroxidase, protease and lipid peroxidation, which have an important role in oxidative stress and are indicators of cellular damage should have been taken, as an important criterion, in the purslane mutation breeding studies.

The present study was designed with following objectives:

- To observe the effects of different doses of UV rays on seed germination, seedling, protease, peroxidase and lipid peroxidation in purslane.
- To investigate the feasibility of UV rays irradiation of seeds using lipid peroxidation, peroxidase and protease as an index of mutation frequency.
- To determine the possible role of these biochemical parameters in determination of appropriate radiation dose for inducing mutation in Portulaca grandiflora and Portulaca oleracea.
- To study differential radiosensitivity of Portulaca varieties.

**MATERIAL AND METHODS**

Seeds of Portulaca grandiflora and Portulaca oleracea genotypes were treated with 5 doses of UV rays ranging from 220 to 400 nm with an interval of 320 nm source. After irradiation, thirty seeds were sown per port filled with autoclaved sand along with untreated controls in three replicates with completely randomized design. The pots were placed in an incubator at 25°C. Number of germinated seeds was recorded after 1, 2, 3, 4, 5 and 6 days. Different parameters like final percent of germination (FPG), mean germination time (MGT) and time to 50% germination (T50) were calculated from resulting data. One week after sowing, root shoot lengths (cm), root/shoot ratio and seedling fresh eight were recorded. For different biochemical estimations leaves were grounded with a mortar and pestle under chilled condition in 50 mM Potassium phosphate buffer. The homogenate was centrifuged at 14000 rpm for 10 min at 0°C. The supernatant was separated and used for assay of enzyme activities and the level of lipid peroxidation.

**Proteases Activity:** Protease activity was determined by the casein digestion assay described by Drapeau [11]. Briefly by this method one unit is that amount of enzyme, which releases acid soluble fragments equivalent to 0.001 A280 per minute at 37°C and pH 7.8.

**Peroxidase Activity:** Peroxidase (POD) activity was determined as described by Liu and Huang [12]. The POD reaction solution (3 ml) contained 50mM potassium phosphate buffer (pH 7.8), 20 mM guaiacol, 40 mM H2O2 and 100 µl enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20 min. One unit of peroxidase activity was defined as an absorbance change of 0.01 units per min.

**MDA Contents:** The lipid peroxidation level was determined in terms of malondialdehyde (MDA) content by the method of Dhindsa et al. [13]. A 2 ml aliquot of enzyme solution was added to a tube containing 1ml 20% (v/v) trichloroacetic acid and 0.5% (v/v) thiobarbituric acid. The mixture was heated in a water bath at 95°C for 30 min. cooled to room temperature and then centrifuged at 14,000 rpm for 10 min. The absorbance of supernatant at 532 nm was determined and nonspecific absorbance at 600 nm was subtracted from it. The MDA content was calculated by using extinction coefficient of 155 /mM/cm [14].

**Protein Contents:** Total soluble protein contents were measured using Bradford’s method [15].

**Statistical Analysis:** All experiments were repurpslaneted three times (90 and 30 seedlings per replication for germination and biochemical studies respectively). The descriptive statistics were applied to analyze and organize the resulting data. The F-test was applied to find differences in variance among samples. The significance of differences between means (irradiated and non-irradiated) for different parameters was measured using Student’s t-Test (two tailed) at 0.01 and where applicable at 0.05 significance level.
RESULTS

Germination and Growth: Seed germination test after UV irradiation of seeds (220 to 400nm UV) revealed that mean germination time was increased with decreasing irradiation dose for both Portulaca grandiflora and Portulaca oleracea. The delay in germination was more pronounced in case of Portulaca oleracea as compared to Portulaca grandiflora purslane (Fig.1). Final germination percentage was non-significantly affected in Portulaca grandiflora with all irradiation doses. However in Portulaca oleracea, final germination percentage was decreased significantly in lower irradiation doses ranging from 220 to 300 nmUV. Maximum decrease in germination percentage was observed before 240 nm UV dose.

Shoot length was decreased in both Portulaca grandiflora and Portulaca oleracea after all doses of UV irradiation of seed as compared to non-irradiated. Generally shoot length of seedling was decreased gradually with decreasing dose. Maximum decrease in shoot length was observed in both portulaca types between 220 to 260 irradiation doses of UV. Root length also decreased after all doses of irradiation as compared to non-irradiated control in both Portulaca grandiflora and Portulaca oleracea. Maximum decrease in root length was observed in 220 and 240 nmUV dose in Portulaca grandiflora and in 240 uv dose in Portulaca oleracea. Root/shoot ratio was increased at 280nm UV dose in Portulaca grandiflora and at 300 nm UV dose in Portulaca oleracea.

Seedling fresh weight was increased in Portulaca grandiflora as well as Portulaca oleracea as compared with non-irradiated control after almost all irradiation doses. Minimum seedling fresh weight was observed in 400 irradiation doses of UV in Portulaca oleracea and also in Portulaca grandiflora (Fig. 6).

Seedling dry weight was increased in Portulaca oleracea after all irradiation doses as compared to non-irradiated control. However seedling dry weight was significantly affected by seed irradiation and it was slightly increased after some irradiation doses as compared with non-irradiated control (Fig. 7).

Total Soluble Protein Contents: Leaf protein contents were estimated in Portulaca oleracea and Portulaca grandiflora after different doses of UV irradiation of seeds (Fig. 8). In Portulaca oleracea genotype, leaf protein contents were slightly decreased after different levels of UV irradiation of seeds as compared with non-irradiated control. However in Portulaca grandiflora genotype, protein contents were lower before 220 to 240 nm uv dose as compared with non-irradiated control.
Maximum decrease in protein contents as compared to control was observed before 240 nm UV dose in Portulaca grandiflora as well as Portulaca oleracea genotypes, however difference was highly significant in former one (Fig. 8).

Peroxidase activity: Leaf peroxidase activity was also affected after UV irradiation of seeds (Fig. 9). In Portulaca grandiflora, change in leaf peroxidase activity was dose dependent. Leaf peroxidase activity in Portulaca grandiflora was higher after 300, 340 and 380 nm dose while lower after all other doses as compared with non-irradiated control. Leaf peroxidase activity was generally decreased in Portulaca oleracea after seed irradiation as compared with non-irradiated control. In Portulaca oleracea, initially leaf peroxidase activity was increased after 260 nm dose followed by a gradual increase in activity up to 400 nm doses. The proxidase growth dramatically in activity after between 280 and 300 nm doses (Fig. 9).

Protease Activity: Leaf protease activity was also affected by UV irradiations of seeds in both Portulaca grandiflora and Portulaca oleracea (Fig. 10). In Portulaca grandiflora change in leaf peroxidase activity was dose dependent. Leaf protease activity in Portulaca grandiflora was higher after 240, 260 and 280 nm dose while lower after in 300 nm and higher again after 320 nm doses as compared with non-irradiated control. The difference was significant as compared to non-irradiated control after 400 nm (higher) and 220 nm (lower) dose only.
Leaf protease activity was generally decreased in *Portulaca oleracea* after seed irradiation except after 300 nm dose where activity was same as compared with non-irradiated control. Leaf protease activity was minimum at 220 nm dose where it was many fold lower as compared to non-irradiated control (Fig. 10).

**Lipid Peroxidation (MDA Contents):** Leaf MDA contents were decreased significantly by all doses of UV irradiation of seed in both *Portulaca grandiflora* and *Portulaca oleracea* genotypes (Fig. 11). In *Portulaca grandiflora* MDA contents were many folds lower between 220 and 280 nm dose. A gradual increase in MDA contents was observed with increasing radiation dose. In *Portulaca oleracea* leaf MDA contents were higher as compared with *Portulaca grandiflora* in non-irradiated control as well as after all irradiation doses. Among irradiated seeds, maximum MDA increasing contents were measured after 300 nm dose in *Portulaca oleracea* and 320 nm dose in *Portulaca grandiflora*.

**DISCUSSION**

Knowing that water radiolysis, the predominant effect of ionizing radiation in organisms, induces reactive oxygen species (ROS) formation, one can assume that plant, bacterial and animal enzymes that are involved in cell protection against oxidative stress will display similar responses under ionizing radiation stress as under other stress factors [16]. Antioxidants and peroxidase are involved in the compensatory mechanisms of inhibition of free radicals formed upon irradiation of seeds [6].

Inverse correlation between growth and peroxidase enzyme activity of seedlings after UV and neutron irradiation of purslane seeds has been reported. Depending on the UV dose between 320 and 360 nm UV the height of purslane seedlings was found shorter and parallel with this the peroxidase activities were higher than in the un-irradiated controls [7]. Similarly in our case, leaf peroxidase activity in *Portulaca grandiflora* was higher after 300, 320, 340 and 380 nm UV irradiation doses and also shoot length was increased in both *Portulaca grandiflora* and *Portulaca oleracea* after same all doses of UV irradiation of seed.

It has been reported that prolonged irradiation of wheat (*Triticum aestivum* L.) seeds with UV light (for 1-6 h) led to an increase in the level of lipid peroxidation in sprouts [6]. Similarly in present study a dose dependent increase in lipid peroxidation was also observed in *Portulaca grandiflora*. Based on lipid peroxidation data it is suggested that irradiation of *Portulaca grandiflora* should be limited to a dose of 300 nm while 320 nm for *Portulaca oleracea*. Previously on the bases of increase in free fatty acids it has also been suggested that radiation processing of nutmeg should be limited to a dose of 300 nmUV.

Recent results have shown the complexity of cellular regulation in plants by proteolysis. They are involved in protein maturation, degradation and protein rebuilt in response to different external stimuli and to remove abnormal, misfolded proteins. The rapidly growing amount of information indicates that proteases participate in turnover of proteins during response to abiotic stresses. Protein breakdown and recycling, which depend on the levels of proteolytic enzymes, are an essential part of the plant response to environmental stress [8]. Similarly in present study higher proteolytic activity in *Portulaca grandiflora* after 220 nm to 300 nm doses indicates protein degradation by proteases, for removal of abnormal, misfolded proteins and for rebuilt processes in response to UV irradiation. Degradation of damaged, misfolded and potentially harmful proteins provides free amino acids required for the synthesis of new proteins. Inherent differences in all biochemical parameters studied were observed in *Portulaca grandiflora* and *Portulaca oleracea*. *Portulaca grandiflora* has almost more protein...
content as compared to *Portulaca oleracea* inherently. On the other hand peroxidase and protease activities (two folds) and MDA contents were inheritably higher in *Portulaca oleracea* as compared to *Portulaca grandiflora*.

In growth parameters root shoot lengths and seedling fresh and dry weights were higher in *Portulaca oleracea* as compared with *Portulaca grandiflora*. So it can be concluded that *Portulaca oleracea* has higher antioxidant value (peroxidase activity) while *Portulaca grandiflora* has higher protein contents.

It was evident from all biochemical parameters included in the present study that *Portulaca grandiflora* and *Portulaca oleracea* respond deferentially to UV irradiation. Firstly, there was a substantial loss in protein contents before 300 nm UV irradiation dose *Portulaca grandiflora*, which it was seen in 280 nm UV *Portulaca oleracea*. Secondly peroxidase activity was enhanced by 260 and 300 UV irradiation dose in *Portulaca grandiflora* while activity was suppressed after 220nm irradiation doses in *Portulaca oleracea*. Thirdly minimum MDA contents were observed after 220 nm doses in *Portulaca oleracea* while after 240 nm doses in *Portulaca grandiflora*. Further MDA contents increased steadily from 300 to 400 nm dose in *Portulaca grandiflora* while there was a decrease in MDA contents with increasing dose from 260 to 400 nm in *Portulaca oleracea*. Inherent differences in *Portulaca grandiflora* and *Portulaca oleracea* may be the basis for their differential response to UV irradiation.A number of radiobiological parameters are commonly used in early assessment of effectiveness of irradiation to induce mutations. Seed germination, seedling survival and pollen and ovule sterility have been used extensively. Fluorescence and light absorption spectra of chlorophyll attributed to different doses treatments of corn grains has confirmed the superiority of UV irradiation dose in stimulating corn plants. [10].

Similarly in present study biochemical parameters like protein and MDA contents and protease and peroxidase activities has pointed towards the superiority of 500 UV irradiation dose for *Portulaca oleracea* (non-effected protein contents and peroxidase activity and lower MDA contents and protease activity) and 400 nm for *Portulaca grandiflora* (higher peroxidase activity, non-effected protein and protease activity and lower MDA contents).

**CONCLUSION**

Collective data for protein contents, peroxidase and protein activities therefore suggested that seed irradiation should be limited to a dose of 280 nm UV for *Portulaca grandiflora* while 300 for the *Portulaca oleracea*. Peroxidase and protease activities were higher (two folds) in *Portulaca oleracea* as compared with *Portulaca grandiflora* while **vice versa** for protein contents. It was concluded that peroxidase was involved in the compensatory mechanisms of inhibition of free radicals formed upon UV irradiation of seeds. Biochemical parameters like protein contents, protease, peroxidase and lipid peroxidation may be helpful in early assessment of effectiveness and superiority of irradiation dose.

**REFERENCES**