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Uv irradiation effects on seed germination and growth, protein content, peroxidase and protease activity in redbean

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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.Bean Sayad and Bean Derakhshan 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 Derakhshan, peroxidase and protease activities (two folds) and MDA contents were higher as compared to Bean Sayad while vice versa for protein contents, revealing inherent differences between two types. Data for protein contents, peroxidase and protease activities therefore suggested that irradiation dose should not under 300nm UV in Bean Derakhshan and also 300nm UV in Bean Sayad. In Bean Derakhshan 320 to 400 nm UV irradiation dose non-significantly affected the protein contents and peroxidase activity and uppered MDA contents and protease activity. In Bean Sayad 300 nm UV irradiation dose increased the peroxidase activity,uppered the MDA contents and affect the protein content and protease activity. It was concluded that protein contents, protease, peroxidase and lipid peroxidation may be used in early assessment of effectiveness and superiority of radiation dose to induce mutations.

Keywords: Sayad, Derakhshan, UV radiation, protease, proxidase

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 (Caldwell, Teramura and Tevini, 1989; Tevini and Teramura, 1989); 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 (Xiuzher, 1994), hormone balance (Rabie *et al.,* 1996),leaf gas-exchange (Stoeva & Bineva, 2001), water exchange and enzyme activity (Stoeva *et al.,* 2001). The morphological, structural and the functional changes depends 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 arereversible.Antioxidants and peroxidase are involved in the compensatory mechanisms for the inhibition of free radicals formed upon UV irradiation of seeds (Rogozhin *et al.,* 2000). Correlation between growth and antioxidant enzyme activity of seedlings after UV andneutron irradiation of Bean seeds has been reported. Depending on the UV radiation dose between 300 and 380 nm the height of Bean seedlings was found shorter and parallel

with this peroxidase activities were higher than in the unirradiated controls (Bagi *et al.,* 1988).Similarly an increased level of the glutathione peroxidase activity (Marchenko *et al.,* 1996) 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 (Hieng *et al.,*2004). 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 (Schaller, 2004; Grudkowska and Zagdanska, 2004). After UV and neutron irradiation of Bean seeds, an inverse correlation between growth and degrading enzyme activity has also been reported (Bagi *et al.,* 1988).

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.) (Niyas *et al.,* 2003). 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 (Rogozhin *etal.,* 2000). This suggested a breakdown of acylglycerols during radiation processing,resulting in the release of free fatty acids (Niyas *et al.,* 2003).

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 (Al-Salhi *et al.,*2004). 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 (Niyas *et al.,*

2003). 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: (1) to observe the effects of different doses of UV rays on seed germination, seedling growth, protease, peroxidase and lipid peroxidation in purslane. (2) Investigating the feasibility of UV rays irradiation of seeds using lipid peroxidation, peroxidase and protease as an index of mutation frequency and to determine the possible role of these biochemical parameters in determination of appropriate radiation dose for inducing mutation in Bean Sayad and Bean Derakhshan. (3) Based on radiobiological effects on lipid peroxidation, protease and peroxidase activity an attempt was made to study differential radiosensitivity of Bean varieties.

Material and Methods

Seeds of Sayad and Derakhshan 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 25oC. Number of germinated seeds was recorded after 1, 2, 3, 4, 5 and 6 days. Different parameters like final percent 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 50mM Potassium phosphate buffer. The homogenate was centrifuged at 14000rpm 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 Drapurslaneu *et al.,* (1974). 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 & Huang (2000). The POD reaction solution (3ml) contained 50mM potassium phosphate buffer (pH 7.8), 20mM guaiacol, 40mM H2O2, and 100µl enzyme extract. Changes in absorbance of the reaction solution at 470nm were determined every 20 sec. 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.,* (1981) and Zhang &Kirkham (1994). A 2ml 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 95oC for 30min., cooled to room temperature and then centrifuged at 14,000rmp for 10 min. The absorbance of supernatant at 532nm was determined and nonspecific absorbance at 600nm was subtracted from it. The MDA content was

calculated by using extinction coefficient of 155mM-1 cm –1 (Heath & Packer, 1968).

Protein contents: Total soluble protein contents were measured using Bradford's method (Bradford, 1976).

Statistical analysis: All experiments were repurslaneted 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. All the statistical calculations were performed using computer software Microsoft Excel 2000.

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 Sayad and Derakhshan.The delay in germination was more pronounced in case of Bean Derakhshan as compared to Sayad purslane (Fig.1).Final germination percentage was non-significantly affected in Sayad with all irradiation doses. However in Derakhshan, final germination percentage was decreased significantly in lower irradiation doses ranging from 220 to 300 nm UV.Maximum decrease in germination percentage was observed before 240 nm UV dose.

Shoot length was decreased in both Sayad and Derakhshan 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 bean types Between 220 to 260 irradiation dose of UV.Root length also decreased after all doses of irradiation as compared to non-irradiated control in both Sayad and Derakhshan. Maximum decrease in root length was observed in 220 and 240 nmUV dose in Sayad and in 240 uv dose in Derakhshan. Root/shoot ratio was increased at 280nm UV dose in Sayad and at 300nm UV dose in Derakhshan.

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

Seedling dry weight was increased in Derakhshan 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 Derakhshan and Sayad after different doses of UV irradiation of seeds (Fig. 8). In Derakhshan genotype, leaf protein contents were slightly decreased after different levels of UV irradiation of seeds as compared with non-irradiated control. However in Sayad genotype, protein contents were lower before 220 to 240 nm uv dose as compared with non-irradiated control. However lower radiation doses (220 to 300 nm UV) caused an decrease in leaf protein contents in Sayad as compared with non-irradiated control.

Maximum decrease in protein contents as compared to control was observed before 240 nm uv dose in Sayad as well as Derakhshan genotypes, however difference was highly significant in former one (Fig. 8).

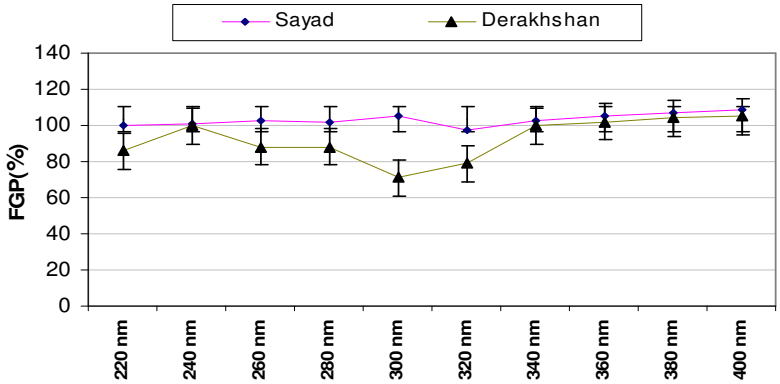

Figure 1: UV radiation dose (nm)

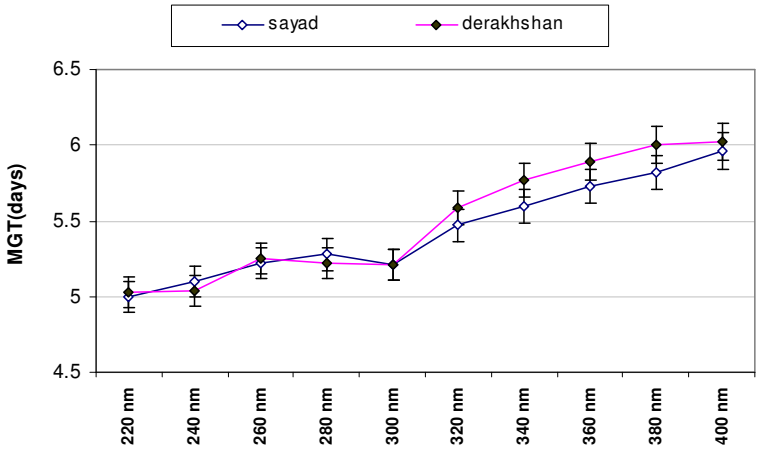

Figure 2: UV radiation dose (nm)

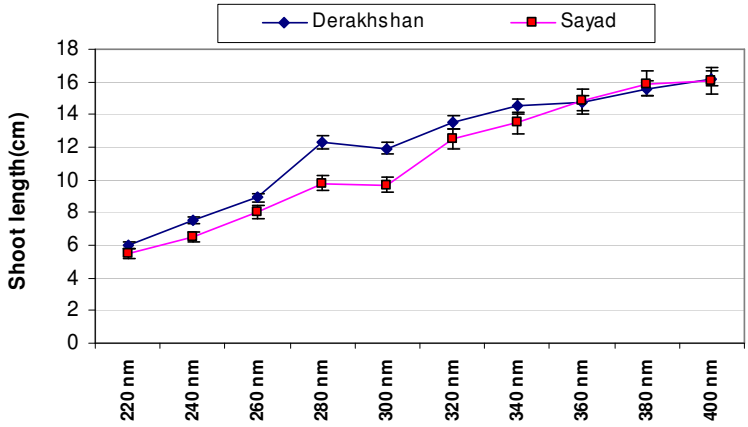

Figure 3: UV radiation dose (nm)

Peroxidase activity: Leaf peroxidase activity was also affected after UV irradiation of seeds (Fig. 9). In Sayad change in leaf peroxidase activity was dose dependent.

Leaf peroxidase activity in Sayad was higher after 300, 340and 380 nm dose while lower after all other doses as compared with non-irradiated control.

Leaf peroxidase activity was generally decreased in Derakhshan after seed irradiation as compared with non-irradiated control. In Derakhshan, initially leaf peroxidase activity was increased after 260 nm dose followed by a gradual increase in activity up to 400 nm dose.The proxidase growth dramatically in activity after between 280 and 300 nm dose (Fig. 9).

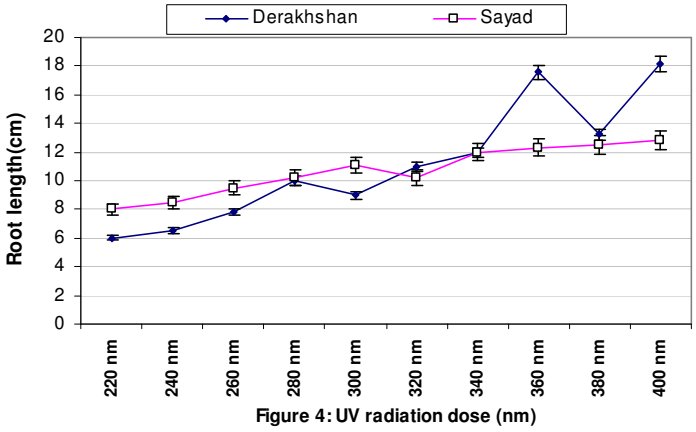

Figure 4: UV radiation dose (nm)

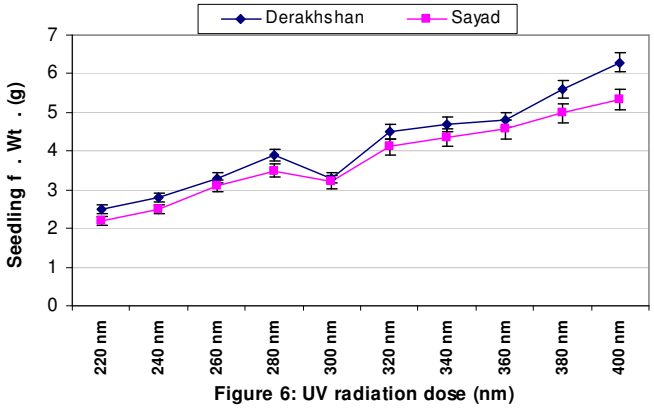

Figure 6: UV radiation dose (nm)

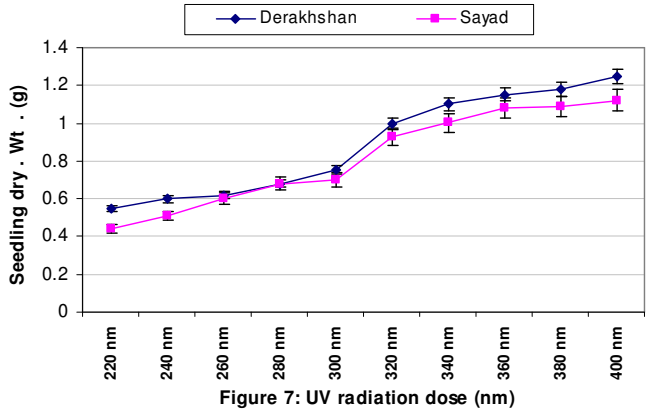

Figure 7: UV radiation dose (nm)

Figures: Germination (fig 1 , 2) and seedling growth (3, 4, 5, 6,7) in.Derakhshan and .Sayad genotypes after UV irradiation of seeds.

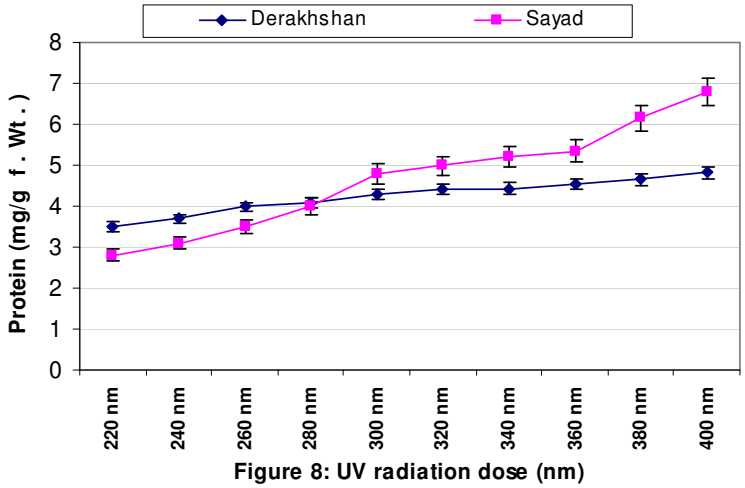

Fig. 8. Comparison of total soluble protein contents in.Derakhshan and.Sayad.

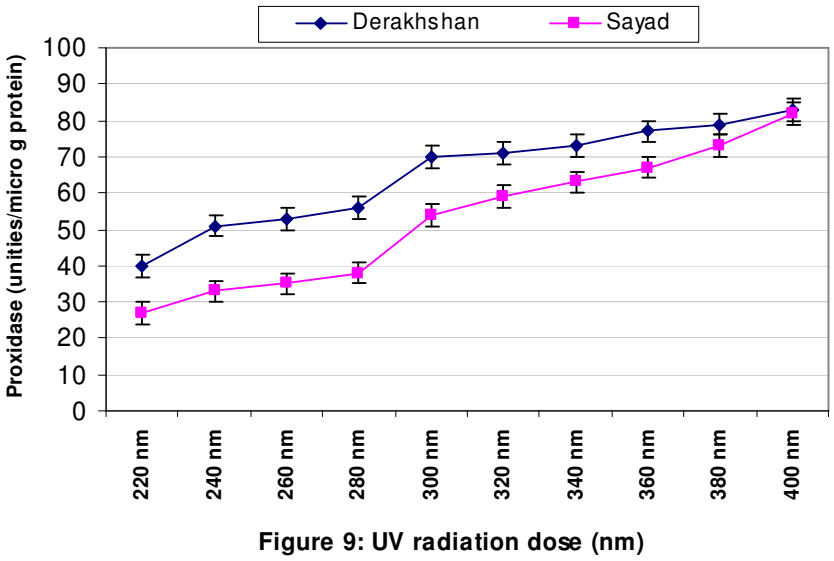

Fig. 9. Comparison of peroxidase activity in.Derakhshan and.Sayad.

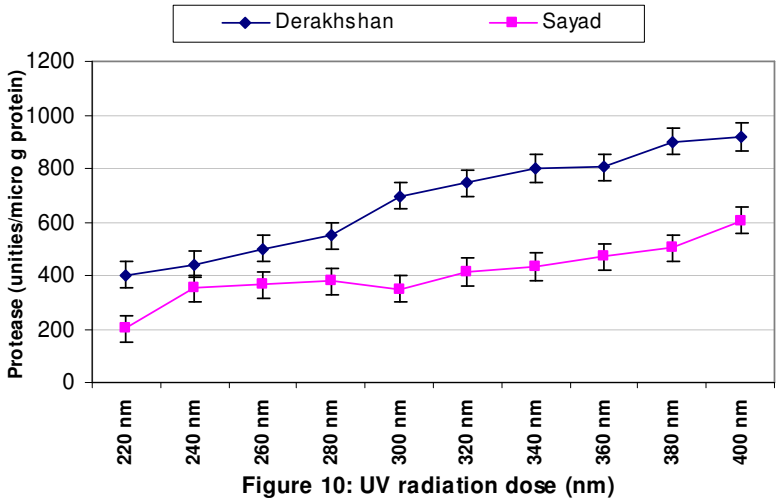

Fig. 10. Comparison of protease activity in Derakhshan and Sayad.

Protease activity: Leaf protease activity was also affected by UV irradiations of seeds in both Sayad and Derakhshan (Fig. 10). In Sayad change in leaf peroxidase activity was dose dependent. Leaf protease activity in Sayad was higher after 240, 260 and 280nm 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 Derakhshan after seed irradiation except after 300 nm dose where activity was same as compared with nonirradiated 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 Sayad and Derakhshan genotypes (Fig. 11). In Sayad 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 Derakhshan leaf MDA contents were higher as compared with Sayad in non-irradiated control as well as after all irradiation doses. Among irradiated seeds, maximum MDA increasing contents were measured after 300nm dose in Derakhshan and 320 nm dose in Sayad.

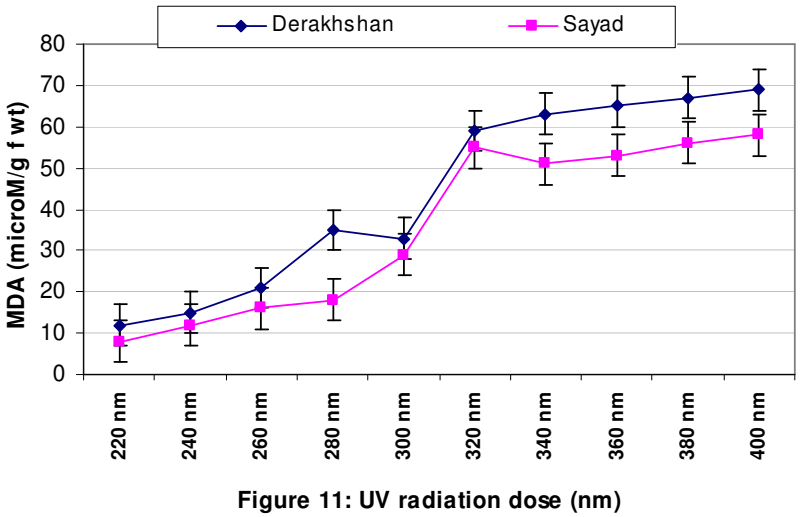

Fig. 11. Comparison of MDA contents in P.Derakhshan and P.Sayad.

Discussion

Knowing that water radiolysis, the predominant effect of ionizing radiation in organisms, induces reactive oxygen species (ROS) formation (De-Vita *et al.,* 1993), 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 (Zaka *et al.,* 2002). Antioxidants and peroxidase are involved in the compensatory mechanisms of inhibition of free radicals formed upon irradiation of seeds (Rogozhin *et al.,* 2000).

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 (Bagi *et al.,* 1988). Similarly in our case, leaf peroxidase activity in Sayad was higher after 300, 320, 340 and 380 nm UV irradiation doses and also shoot length was incresed in both Sayad and Derakhshan 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 (Rogozhin *et al*., 2000). A dose-dependent decrease in the triacylglycerol content and a concomitant increase in free fatty acids was observed (Niyas *et al.,* 2003). Similarly in present study a dose dependent increase in lipid peroxidation was also observed in Sayad. Based on lipid peroxidation data it is suggested that irradiation of Sayad should be limited to a dose of 300nm while 320nm for Derakhshan. 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 300nm UV (Niyas *et al.,* 2003).

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 (Grudkowska & Zagdanska, 2004). The rapidly growing amount of information indicates that proteases participate in turnover of proteins during response to abiotic stresses (Grudkowska & Zagdanska, 2004). Protein breakdown and recycling, which depend on the levels of proteolytic enzymes, are an essential part of the plant response to environmental stress (Hieng *et al.,* 2004). Similarly in present study higher proteolytic activity in Sayad after 220nm to 300 nm dose 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 Sayad and Derakhshan. Sayad has almost more protein content as compared to Derakhshan inheritably. On the other hand peroxidase and protease activities (two folds) and MDA contents were inheritably higher in Derakhshan as compared to Sayad.

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

It was evident from all biochemical parameters included in the present study that Sayad and Derakhshan respond deferentially to UV irradiation. Firstly, there was a substantial loss in protein contents before 300 nm UV irradiation dose Sayad , which itwas seen in 280 nm UV Derakhshan. Secondly peroxidase activity was enhanced by 260 and 300 UV irradiation dose in Sayad while activity was suppressed after 220nm irradiation doses in Derakhshan. Thirdly minimum MDA contents were observed after 220 nm dose in Derakhshan while after 240 nm dose in Sayad. Further MDA contents increased steadily from 300 to 400 nm dose in Sayad

while there was a decrease in MDA contents with increasing dose from 260 to

400 nm in Derakhshan. Inherent differences in Sayad and Derakhshan 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. (Al-Salhi *et al.,* 2004).

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 Derakhshan (non-effected protein contents and peroxidase activity and lower MDA contents and protease activity) and 400 nm for Sayad (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 Sayad while 300 for the Derakhshan. Peroxidase and protease activities were higher (two folds) in Derakhshan as compared with Sayad 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.

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