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Intensified UV-B Radiation Effect on Germination and biochemical Components of Maize (*Zea mays* L)

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In the present investigation, Maize seeds of hybrid NK 6240 and 900M Gold were exposed to UV-B (280-320 nm) for periods of 40 and 60 minutes and compared with the control without exposer to UV-B. The biochemical changes associated with UV-B induced resistance were investigated by determination of proline concentration, total soluble sugars, total soluble protein, malondialdehyde content and free amino acids from leaves and roots. Also comparison for germination percentage between control and treated seeds was carried along with biochemical traits. Analysis of variance explains both the hybrids were significantly different in germination percentage, total soluble sugars, proline and free amino acids. Whereas both treatments showed high significant variation for all the studied traits, but genotype × treatment interaction was non-significant for all the traits. 40 minutes UV-B treated seeds showed reduced total soluble sugars and increased malondialdehyde, proline and total soluble protein content. In 60 minutes UV-B treatment, decrease in free amino acids, proline, percentage of germination, and total soluble protein and increase in total soluble sugars was observed.

Keywords: UV radiation, Maize, Total soluble sugars, Total soluble protein, Proline.

Maize (Zea mays L.) is the third most important cereal crop in the world after wheat and rice. The superior position of maize is due to its wide and variety utilization. During the centuries maize plant was known for multifarious uses. Maize is used like a food, fodder for livestock, for producing alcohol and non alcohol drinks, built materials like a fuel and like medicinal and ornamental plant[1-3].

UV-B effects plant development, growth and productivity[4]. Still, a low number of investigations considered the possible subject of UV on induced resistance in plant against biotic and abiotic stress[5-6].

The responses of plants to UV irradiation include physiological, biochemical, and morphological changes. In general, UV radiation deleteriously affects plant growth, reducing leaf size, determining the field available for solar energy capture. These determinations have been accomplished primarily through studies in the greenhouse and exposure to artificial sources of UV radiation, extrapolation to changes on crop production as a result of increase in terrestrial solar UV radiation is difficult. Tolerant plants have evolved mechanisms to overcome these harmful changes. The outcomes of most subject fields have shown that resistance to abiotic stresses is usually correlated with a more efficient antioxidant system[7]. Also, proline accumulation is seen as a biomarker for plant tolerance to drought and salt stresses due to its osmoprotective function[8]. Thus, the present investigation is aimed to examine the force of different doses of UV-B on the antioxidant systems in Maize seeds and biochemical changes and the level of UV stress tolerance.

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MATERIALS AND METHODS

The experiment was conducted at the Department of Biochemistry of the Osmania University. Maize seeds were irradiated using a UV hood source of 125W (15PSIG) for 40 and 60 minutes.

Seed Material

The popular maize hybrids NK 6240 and 900M Gold were used for study as these hybrids are widely accepted by farmers due to high yield and stability.

UV-B Treatment

UV irradiation is an important stress factor for plants resulting in damage to genetic system and cell membranes and various metabolic processes. UV-B has greater damaging effects on plants because the cell macromolecules like DNA and protein have a strong absorption at 280-320 nm. The seeds were exposed to UV-B radiation (280-320 nm) from an artificial source situated at 20 cm distance over the seeds. Durations of exposure time were 40 and 60 min. After treatment, petridishes containing the germinated seeds were enwind with aluminum foil until sowing to minimize any possible photo reactivation processes[9-10].

Biochemical analysis

The leaves of 10th day seedlings were collected from each treatment for the analysis of protein[11], Malondialdehyde (MDA), Proline, Free amino acids (FAA), and Total soluble sugars (TSS). Protein estimation was carried out by

Table 1. Analysis of variance for genotypes, treatment and Genotype × Treatment interactions

	Germination	MDA	TSS	Proline	FAA	Protein
Genotype	34.72*	0.25^{NS}	6384.5**	1.3*	0.00092**	0.057^{NS}
Treatment	751.38**	24.85**	115211.05**	26.92**	0.025**	92.65**
G x T	10.05^{NS}	1.32^{NS}	393.16^{NS}	0.573^{NS}	0.000078^{NS}	0.53^{NS}
C.V	2.74	15.42	4.32	5.73	11.77	5.54
L.S.D	2.65	1.41	13.63	0.49	0.0098	0.8316

MDA= Malondialdehyde, TSS= Total Soluble Sugars, FAA= Free Amino Acids
*Significant at 5% level, ** Significant at 1% level, ^{NS} non-significant.

Table 2. Mean values for two hybrids with different treatments

	NK 6240			900M Gold		
	control	40MinUV-B	60 Min UV-B	control	40 Min UV-B	60 Min UV-B
Germination	100	100	80	100	93.33	78.33
MDA	7.65	11.72	7.17	7.68	10.43	7.71
TSS	154.6	273.3	416.3	174	317	466.3
Proline	7.54	11.33	6.81	7.16	10.11	6.3
FAA	0.143	0.028	0.045	0.163	0.035	0.061
Protein	12.1	18.6	12.4	12.1	19	11.6

MDA= Malondialdehyde, TSS= Total Soluble Sugars, FAA= Free Amino Acids

Table 3. Effect of UV-B treatment on biochemical traits compared using Duncan's multiple range test

Treatment	Germination	MDA	TSS	Proline	FAA	Protein
Control	100^{a}	7.66^{b}	164.33^{c}	7.34^{b}	0.152^{a}	12.06^{b}
UV-B (40 min)	96.66^{b}	11.07^{a}	295.16^{b}	10.71^{a}	0.031^{c}	18.82^{a}
UV-B (60 Min)	79.167^{c}	7.44^{b}	441.33^{a}	6.8^{b}	0.053^{b}	11.97^{b}

Values a, b, c represent significant differences compared to controls at $P < 0.05$ according to Duncan's multiple range test

Lowry method[11] and MDA was analyzed by Lipid Peroxidation method[12], Proline and FAA were analyzed by Ninhydrin method[13]. Total Soluble Sugars was analyzed by the Phenol Sulphuric Acid method[14].

Statistical analysis

The data are shown as mean ± SE from five replicates. Data was subjected to two-way ANOVA to examine the issue of seed treatment with different dosages of UV-B irradiation on the grown plants and the levels of significance are represented by *P d" 0.05, **P d" 0.01, and non significant (NS) and combined means of both the hybrids were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

The amount of UV-B radiation reaching to earth surface is increasing. Plants which use sunlight for photosynthesis cannot avoid the exposer to enhanced level of UV-B radiation and they are at risk. Hence understand the effect of UV-B radiation on various biochemical parameters in Maize would be useful to researchers to breed maize crop according to current climate change conditions. Two popular Maize hybrids were studied to understand the impact of UV-B radiation on Maize seed germination and biochemical parameters. Two factor analysis of variance showed significant difference for UV-B treatment in all the studied traits, whereas two hybrids were significantly different in Germination, TSS, Proline content and FAA. The Genotype × Treatment interactions were non-significant for all traits (Table 1).

Germination

Both treatments showed reduce germination, although both treatments were significantly different compared to control 40 minute UV-B treatment showed less reduction in germination compared to 60 min UV-B treatment (Table 3 and Figure 1A).

Malondialdehyde (MDA)

For 40 min UV-B treatment both the hybrids showed enhanced level of MDA compared to control (Figure 1B).There was no significant difference observed between control and 60 minute UV-B treated seeds (Table 3).This notice was previously found in leaves of Arabidopsis challenged with the fungus Botrytis where MDA levels were correlated with the survival of tissues. The results suggest that trienoic fatty acids (the major precursors of MDA) contribute to ROS control via non enzymatic oxidation[15].

Total soluble Sugars (TSS)

The TSS results were observed in UV-B seed treatment grown plants at the doses of 40 and 60 min. The highest increment (~ 3.5-fold increase) was observed at the dosage of 60 min followed by a dose of 40 min which led to a ~ 1.5- fold increase over the control (Figure1C), Duncans multiple

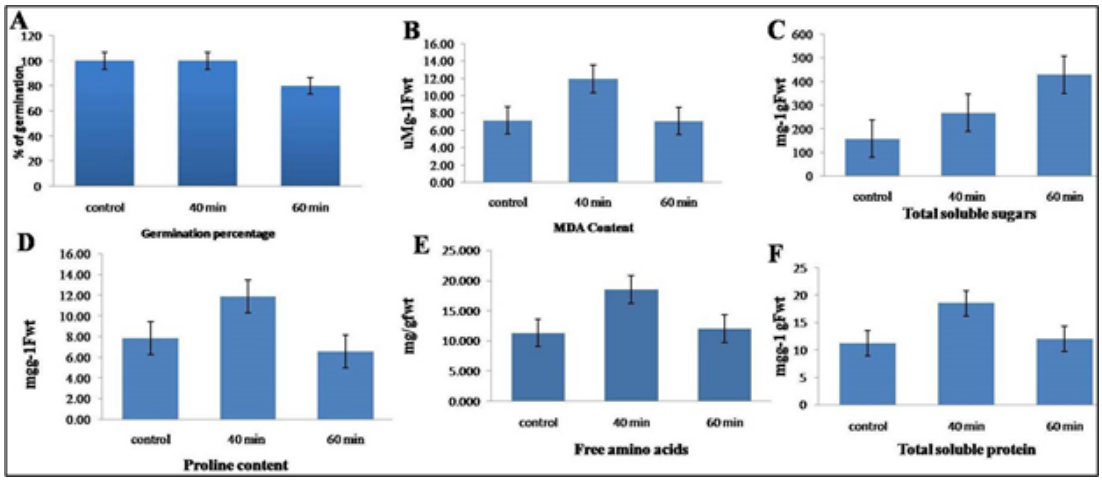

Fig.1. Studied biochemical traits under 40 minutes and 60 minutes of UV-B treatment in maize seeds A) Germination pattern of UV-B treated seeds B) Malondialdehyde content in UV-B treated seeds C) Total soluble sugars levels in UV-B treated seeds D) Proline concentration in UV-B treated seeds E) FAA content in UV-B treated seeds and F) Total soluble proteins in UV-B treated seeds

range test showed both the treatments significantly differ from control. In both hybrids UV-B treated plants had a universal propensity to lower sink capacity[16].

Proline Concentration

Proline concentration increased in both hybrids in 40 min treatment, whereas in 60 min treatment there was no significant change observed compared to control (Figure 1D). Proline has many roles in water stress resistance, being an osmoregulator proline also protects protein from damage and enhances enzyme activities[17-19]. High levels of proline synthesis maintain the NADP+/ NADPH ratio in the cell ascities under normal conditions[20]. In response to UV-B stress, FAA content was declined in 40 and 60 min compared to the control (Figure1E).In previous studies a dose dependent decrease in the triacylglycerol content and a concomitant increase in FAA content were observed after UV-irradiation in nutmeg[21].

Total Soluble Proteins

Plants produced from UV-B treated seeds for 40 min showed a considerable elevation in total soluble protein levels as compared to control plants, whereas 60 min UV-B treated seeds were on par with control (Figure 1F). Physiological and biochemical processes in plants are significantly affected by UV irradiation. The irradiation of seeds with high dosages of UV rays disturbs the synthesis of protein[22], UV-B radiation promoted the aggregation of a novel 66-KD protein in pea, love apple, tobacco and brassica napus[23].

CONCLUSIONS

In most of the studied biochemical traits 40 minutes of UV-B treatment showed more variation than 60 minutes of UV-B treatment granting to the obtained results, it could be reasoned that the exposure of Maize seeds to UV-B enhanced accumulation of proline in the leaves and root which lessened the effect of osmotic stress. In the present study, we demonstrated that the optimal dose for maize seeds was at 40 min at which achieved the enhanced level of stress tolerance.

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