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Effect of UV-B radiation on *Vigna radiata* L. Var. K-851

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ABSTRACT

Investigations on morphological, physiological and biochemical changes such as the content of proline and quantitative and qualitative changes in protein were made in the seedlings of *Vigna radiata* L. Var. K-851following regular short time (10 and 20min/day) ultraviolet B irradiation. During the initial period of ultraviolet B irradiation several morphological changes such as reduction in leaf size and increase in leaf thickness occurred. The rate of photosynthesis decreased upon ultraviolet B treatment which was found to be due to a direct action on the light reactions. The biochemical analysis of the changes in the ultraviolet B treated plants showed reduction in proteins which could be due to the low levels of photosynthates formed. Protein profile changes have been manifested as tolerant, sensitive and induced proteins.

KEYWORDS

SDS- PAGE;
Protein;
UV-stress;
Proline.

INTRODUCTION

Plants use sunlight for photosynthesis and, as a consequence, are exposed to the ultraviolet (UV) radiation that is present in sunlight. Seven percent of the electromagnetic radiation emitted from the sun is in the UV range (200-400nm). As it passes through the atmosphere, the total flux transmitted is greatly reduced, and the composition of the UV radiation is modified. Shortwave UV-C radiation (200-280nm) is completely absorbed by atmospheric gases. UV-B radiation (280-320nm) is additionally absorbed by stratospheric ozone and thus only a very small proportion is transmitted to the Earth's surface, whereas UV-A radiation (320-400nm) is hardly absorbed by ozone. In the past 50 years, the concentration of ozone has decreased by about 5%, mainly due to the release of anthropogenic pollutants such as chlorofluorocarbons[10]. Consequently, a larger proportion of the UV-B spectrum reaches the Earth's surface with serious implications for all living organisms[5,6,19]. Elevated UV-B radiation (UV-B) has pleiotropic effects on plant development, morphology, and physiology. The UV-B portion of sunlight has received much attention in recent years because irradiation from this spectral region (especially 297 to 310nm) will increase as the stratospheric ozone concentration decreases[5,6]. Currently, ozone decreases result from chlorofluorocarbon contamination of the stratosphere[11]. UV wavelengths from 320 to 390nm, which make up the UV-A region of the spectrum, are

not attenuated by ozone, so their influence will be unaffected by ozone layer reduction. Like all living organisms, plants sense and respond to UV radiation, both the wavelengths present in sunlight (UV-A and UV-B) and the wavelengths below 280nm (UV-C). AI1 types of UV radiation are known to damage various plant processes. Such damage can be classified into two categories: damage to DNA (which can cause heritable mutations) and damage to physiological processes. There has been much speculation about how increased UV radiation exposure will affect plants, but as yet, there are no definitive answers. Influences of low and high doses of UV-B radiation (280-320nm from special luminescent lamp) on cereals (barley, oat and winter wheat) were investigated by a number of physiologists[3,5-8,12,15-18]. With this background the present study was aimed to examine the physiological changes and protein profiles variations due to different exposures (0, 10 and 20 min.) of UV-B radiation.

MATERIALS AND METHODS

The seeds of *Vigna radiata* Var. K-851 were sterilized with 0.1% (w/v) $HgCl_2$ for 3 min. and rinsed thrice with distilled water. Seeds were placed on plastic tray with sterile sand and kept in the glass growth chamber for germination. On the 3rd day after planting, plants were exposed to UV-B radiation for various durations 0, 10 and 20 minutes per day. Investigations were carried out on the characteristics of plant response to irradiation such as changes in the content of proline and quantitative and qualitative changes in protein. Estimation of proline was done by Bates et al.[2] method. The proteins were estimated by the method of Lowry et al.[9]. Separation and staining of protein was carried out by Anbalagan method[1].

RESULTS AND DISCUSSION

Seeds and Seedlings of *Vigna radiata* L were grown under various UV-B exposures (0, 10 and 20 min.). UV-B exposed seeds and seedlings of *V. radiata* showed different percentage of seeds germination and seedlings growth. High percentage (100%) of seed germination was observed in all exposures. When young *V. radiata* seedlings were exposed to UV-B radiation (10 and 20 min/day) several morphological changes occurred in leaves during the first 5 days. Both leaf expansion and growth of the plant were reduced to a large extent after the commencement of the UV-B treatment. Exposure of plants to UV-B radiation for 5 days caused more than 80% inhibition of leaf expansion. Similar decreases were also noticed in fresh and dry weight of the UV-B treated leaves when expressed on unit leaf basis. The germinated seedlings were irrigated with 10x diluted Murashige and Skoog's medium (1962) with various exposures of UV-B 0, 10 and 20 min. UV-B exposed for 10 min showed high percentage tolerance compared to 20 min exposed seedlings. UV-B exposed for 20 min seedlings tolerated up to 14 days, 10 min exposed seedlings tolerated up to 18 days. Control seedlings were tolerated with out any change. The exomorphic characters such as shoot length, fresh weight and dry weight were also affected differently at different exposures (data are not included). UV-B exposed seedlings showed the leaves shedding after 10th day of germination. UV-B exposed plants showed the increased reduction in plant height progressively and consistently with increasing exposures.

Growth of any organ is associated with an additional synthesis of protein which is building blocks of protoplasm and are again the resultant inter- mediatory metabolism. The soluble protein showed a gradual increase in their content. This may be due to the reduction in chlorophyll contents, as confirmed by our observation that after 10 days the leaves turned pale yellowish green. This is an indication of Chlorosis. The chlorophyll in photosynthetic pigments established a complex with protein when chlorophyll was decreased. The relative positions of the protein bands as revealed by SDS-PAGE of the *V. radiata* under different UV-B exposures (0, 10 and 20 min.) showed obvious changes in the banding profiles (Figure 1 & 2). In case of *V. radiata,* the number of observable polypeptides increased gradually from 0-20 min. UV- B exposures. Of which, maximum numbers of bands (17) were observed in 10 min exposed 6 days old seedlings of *V. radiata*. Followed by, 10 min exposed 8 days old seedlings of *V. radiata which s*howed sixteen bands. The majority of the bands appeared between the MW-Rf ranges 0.29 to 0.87. The protein profiles were classified in to three categories based on the expression viz.,

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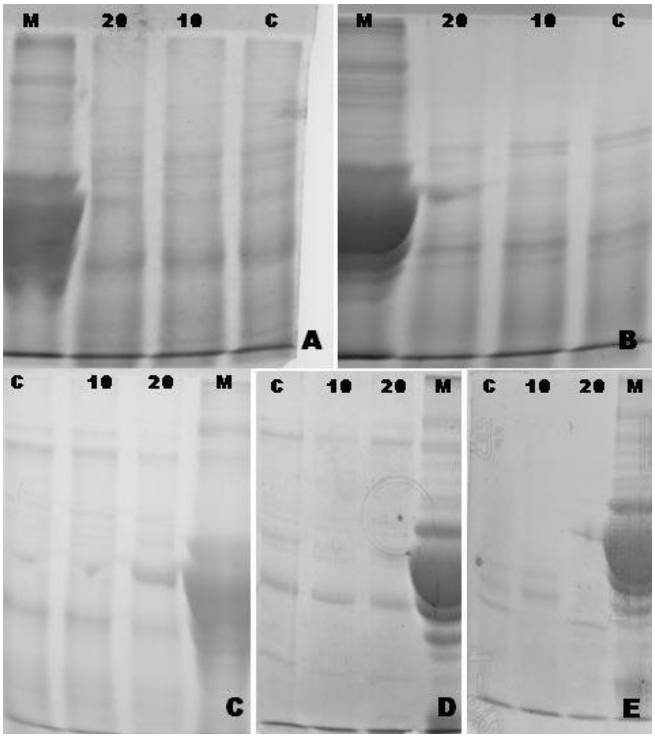

Figure 1 : Protein pattern of the *V. radiata* L. Var. K-851 under different UV-B exposures

A. 1st Harvest of *V. radiata* L.Var. K-851 under different UV-B exposures. B. 2nd Harvest of *V. radiata* L.Var. K-851 under different UV-B exposures. C. 3rd Harvest of *V. radiata* L.Var. K-851 under different UV-B exposures. D. 4th Harvest of *V. radiata* L.Var. K-851 under different UV-B exposures. E. 5th Harvest of *V. radiata* L.Var. K-851 under different UV-B exposures.

M-Marker; C-Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

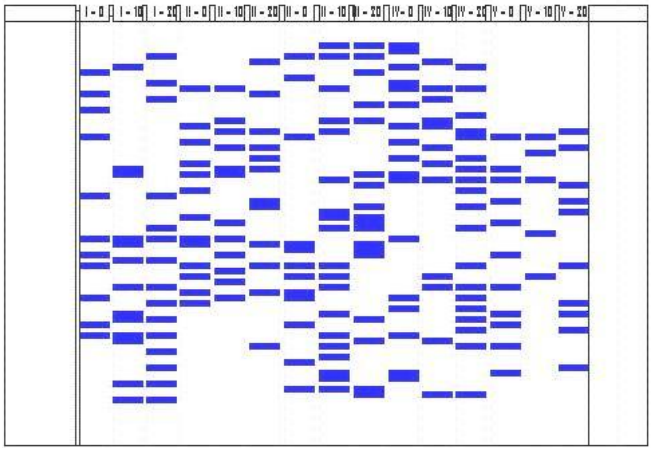

Figure 2 : Zymogram of the *V. radiata* L. Var. K-851 under different UV-B exposures

I-0-Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

I -10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

I- 20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

II-0 - Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

II-10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

II-20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

III-0-Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

III-10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

III-20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

IV-0-Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

IV-10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

IV-20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

V-0-Seedlings of *V. radiata* L. Var. K-851 exposed to ordinary fluorescent lamp

V-10-10 minutes exposed seedlings of *V. radiata* L. Var. K-851

V-20-20 minutes exposed seedlings of *V. radiata* L. Var. K-851

UV- B tolerant protein; UV- B induced proteins and UV-B sensitive proteins. UV- B induced proteins (MW-Rf. 0.15, 0.25, 0.29, 0.37, 0.41 , 0.42, 0.52, 0.56, 0.57, 0.60, 0.61, 0.66, 0.68, 0.70, 0.80, 0.82, 0.85, 0.88, 0.89, 0.92, 0.95, 0.97 and 0.98) were observed only in the UV-B exposed seedlings of *V. radiata*, they failed to express in the control seedlings. UV-B tolerant proteins (MW-Rf. 0.11, 0.14, 0.16, 0.17, 0.19, 0.21, 0.26, 0.35, 0.39, 0.44, 0.45, 0.47, 0.49, 0.51, 0.53, 0.54, 0.55, 0.58, 0.59, 0.62, 0.63, 0.64, 0.65, 0.67, 0.69, 0.72, 0.73, 0.74, 0.75, 0.84, 0.87, 0.93, 0.94 and 0.96) were observed in control seedlings and UV-B exposed seedlings. UV-B sensitive proteins (MW-Rf. 0.12, 0.18, 0.19, 0.26, 0.27, 0.40, 0.47, 0.49, 0.59, 0.62, 0.65, 0.72, 0.73, 0.75, 0.81, 0.84, 0.87, 0.90, 0.93 and 0.94) were observed only in control seedlings. They were denatured and failed to express in the UV-B exposed seedlings. Several prominent protein bands were present in the UV-B exposed plants. It is evident from the result present in this report that the metabolism of *V. radiata* seedlings respond to the changes in the environmental conditions. The changes in the normal over a period of 24 h reflects continuous metabolism occurring in the leaves/plants.

Proline generally alleviated in the inhibitory effect of UV-B stress on the studied parameters. This alleviation was generally associated with K^+/Na^+ ratio of shoots and roots. It is clear from our observations that proline levels are very high at every period of UV-B stress. Our results depicting proline accumulations are in agree-

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TABLE 1: Protein pattern of the *V. radiata* L. Var. K-851 under different UV-B exposures

Mw- Rf	I Harvest			II Harvest			III Harvest			IV Harvest			V Harvest		
	0	10	20	0	10	20	0	10	20	0	10	20	0	10	20
0.11	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
0.12	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
0.14	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-
0.15	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
0.16	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-
0.17	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
0.18	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
0.19	-	-	+	-	-	-		-	-	+	-	-	-	-	-
0.21	-	-	-	+	+	-	-	+	-	+	+	+	-	-	-
0.24	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
0.25	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-
0.26	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
0.27	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.28	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
0.29	-	-	-	-	+	-	-	+	+	-	+	-	-	-	-
0.35	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-
0.37	-	-	-	-	+	+	-	+	-	-	-	+	-		+
0.39	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-
0.40	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
0.41	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+
0.42	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
0.44	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-
0.45	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
0.47	-	+	-	-	+	+	-	-	-	-	-	+	+	-	-
0.49	-	+	-	+	+	-	-	-	+	+	-	-	-	-	-
0.51	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-
0.52	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
0.53	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
0.54	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
0.55	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+
0.56	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-
0.57	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
0.58	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-
0.59	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-
0.60	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-
0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
0.62	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-
0.63	-	+	-	+	-	+	+	-	+	-	-	-	-	-	-
0.64	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
0.65	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-
0.66	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
0.67	+	-	-	+	-	+	+	+	-	-	-	+	-	-	+

Mw- Rf	I Harvest			II Harvest			III Harvest			IV Harvest			V Harvest		
	0	10	20	0	10	20	0	10	20	0	10	20	0	10	20
0.68	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
0.69	-	-	-	+	-	-	+	+	-	-	+	-	-	+	-
0.70	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
0.72	-	+	+	-	-	-	-	+	-	-	+	+	+	-	-
0.73	-	-	-	+		+	+	-	-	-	-	-	-	-	-
0.74	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-
0.75	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+
0.76	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
0.79	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+
0.80	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-
0.81	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-
0.82	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
0.84	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-
0.85	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
0.87	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-
0.73	-	-	-	+		+	+	-	-	-	-	-	-	-	-
0.74	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-
0.75	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+
0.76	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
0.88	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
0.89	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
0.90	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
0.92	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
0.93	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-
0.94	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
0.95	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
0.96	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
0.97	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
0.98	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

ment with other reports[4,16]. The greater accumulation in *V. radiata*, presumably render them drought tolerant. A possible reason for these, increased levels of proline during UV-B stress could be an alternation in the activities of the enzymes involved in the biosynthesis and degradation of proline. Proline is thought to play a multifunctional role in the defense mechanism. It acts as a mediator of osmotic adjustments, a stabilizer of subcellular structure, a scavenger of free radicals, an energy salt and stress related signal[14]. A strong correlation between the accumulation of proline and tolerance of drought stress has been demonstrated by over expression of the Δ' pyrroline-5-carboxylate synthesis

gene p5cs or by anti-sense suppression of the proline dehydrogenease (pro DH) gene in various plants. In conclusion, elevated UV-B stress significantly enhanced the accumulation of protein and proline in *V. radiata* var. K-851. The UV-B exposed seedlings of *V. radiata* var. K-851 exhibited substantial changes in protein and proline levels. The data on various parameters of plant performance obtained in this study revealed a significant effect on UV-B sensitive varieties. The protein banding pattern was used to identify the UV-B tolerant and induced proteins.

It is intended to further solve the structure of the few selective proteins using techniques like nuclear magnetic resonance (NMR) study, X-ray diffraction (XRD) analysis of crystals or bio-informatics.

REFERENCES

[1] K.Anbalagan; An introduction to electrophoresis, Electrophoresis Institute Yercaud, amil Nadu, India, **(1999)**.

[2] L.S.Bates, R.P.Waldren, I.D.Teare; Plant and Soil, **39**, 205-207 **(1973)**.

[3] B.O.Blum, S.A.Gorobetes, A.A.Blum; Effect of UV-B radiation on some cereals BMC Plant Biol., **5(1)**, **(2005)**.

[4] J.D.A Britto; J.Ecotoxiccol.Environ.Monit., **5(2)**, 113-17 **(1995)**.

[5] M.M.Caldwell, A.H.Teramura, M.Tevin; Trends Ecol.Evol., **4**, 363-367 **(1989)**.

[6] M.M.Caldwell, C.L.Ballaré, S.D.Flint, L.O.Björn, A.H.Teramura, G.Kulandaivelu, M.Tevini; Photochem Photobiol Sci., **2**, 29-38 (**2003**).

[7] I.Fedina, M.Velitchkova, K.Georgieva, D.Nedeva, H.Cakirlar; Acta Biologica Hungarica, **60(2)**, 195-210 **(2009)**.

[8] K.Lingakumar, G.Kulandaivelu; L.Photosynthetica, **29(3)**, 341-351 **(1993)**.

[9] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randal; J.Biol.Chem., **193**, 265-275 **(1951)**.

[10] S.Madronich, F.R.deGruijl; Photochemistry and Photobiology, **59**, 541-546 **(1994)**.

[11] M.McFarland, J.Kaye; Photochem.Photobiol., **55**, 911-929 **(1992)**.

[12] N.S.Murali, A.H.Teramura; Journal of Plant Nutrition, **10(5)**, 501-515 **(1987)**.

[13] T.Murashige, F.Skoog; Physiologia Plantarum, **15**, 473-497 **(1962)**.

[14] T.Nanjo, M.Fujita, M.Seki, T.Kato, S.Tabata, K.Shinozaki; Plant and Cell Physiology, **44**, 541-548 (**2003**).

[15] N.Nedunchezhian, K.Annamalainathan, G.Kulandaivelu; Photsynthetica, **31(1)**, 21-29 **(1995)**.

[16] A.M.Noorudeen, G.Kulandaivelu; Plant Physiology and Biochemistry, **9(1)**, 33-40 **(1982)**.

[17] A.Strid; Plant Cell Physiol., **34(6)**, 949-953 **(1993)**.

[18] A.H.Teramura, L.H.Ziska, A.E.Sztein; Physiologia Plantarum, **83**, 373-380 **(1991)**.

[19] F.S.Xiong, T.A.Day; Plant Physiol., **125**, 738-751 **(2001)**.