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Research Paper

CROSS-LINKING OF PROTEINS – A KEY FACTOR IN DETERMINING THE UV-B SENSITIVITY OF PLANTS

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Abstract

The level of ambient solar UV-B radiation is very high in countries lying near equator regions. In south India, the influx of solar UV-B is maximum twice a year. UV-B is known to affect photosynthesis by acting on multiple target sites. Irrespective of the sites, plants try to stabilize the vulnerable photosystem II (PS II) by cross-linking the proteins to other proteins. The study mainly focuses the effect of enhanced solar UV-B radiation during the periods of high solar fluxes viz., summer (March to May) and monsoon (September to December) season. Two tropical legumes viz., cluster bean (*Cyamopsis tetragonoloba* L.) and black gram (*Phaseolus mungo* L.) were grown under enhanced UV-B radiation at two different climatic seasons behaved differently. In cluster bean, during monsoon period, a rapid induction of cross-linking had occurred whereas in summer season not much difference was observed. In black gram, the PSII complex existed in the cross linked condition throughout the early stages of growth.
Key words: UV-B radiation: photosynthesis, photosystem-II, D1 protein.

INTRODUCTION

Over the last 50 years, stratospheric ozone has decreased by about 5%, mainly due to the release of ozone-destroying anthropogenic pollutants such as chlorofluorocarbons (1) resulting in higher levels of UV-B (280–320 nm) radiation at the Earth's surface. Increases in solar UV-B have raised concerns regarding the damaging impact of UV-B radiation on crop plants (2, 3). Current global terrestrial UV-B radiation range between 2 and 12 kJm^{-2} d^{-1} on a given day with near equator and mid-latitudes receiving higher doses which includes an increase of 6–14% since 1980s (UNEP, 2002). The man made pollutants have the potential to cause a depletion of ozone layer which is responsible for the increase in fluctuation of UV-B radiation on the earth's surface has been proposed by Molina and Rowland 1974(4). UV-B flux is not uniform throughout the earth's surface but vary gradually from high to low latitude regions. Several investigations of the effects of UV-B on plants have been carried out in response to concern over decreasing global stratospheric ozone and a concomitant increase in incoming UV-B radiation (5-7). It has been shown that UV-B radiation has developmental and morphological effects on

many plants including *Arabidopsis* (8). Accumulation of proline and ABA in wheat exposed to UV-B was likely due to the synthesis of phenolic secondary metabolites (9, 10).

The enhanced solar radiation show significant inhibition in growth and loss in yield. Such changes are mainly caused by the overall loss in the photosynthetic activity. Since photosynthetic antennae are highly sensitive to UV-B, chloroplast integrity of the thylakoid membrane seems to be much more sensitive than other photosynthetic apparatus. Several workers have shown that the PSII of the thylakoid membrane is the primary target of UV-B radiation (11-16). Most of the studies focus on PS II rather than PS I as the major site of UV-B action. In PS II the D1 protein was assumed to be the main target of UV-B (17, 18). The enhanced solar UV-B radiation strongly induces photodamage to PSII complex especially D1 protein and water oxidation complex as a result the PSII electron transport was inhibited (19). The damaged D1 protein, do not usually accumulate in the thylakoid membrane. The repair cycle operated to replace damaged subunits within PSII. The repair of PSII involved many steps including degradation and removal of the D1protein synthesis *de novo* of the precursor of D1 protein assembly of the PSII complex. The degradation of D1 protein is not the only major event that occurs after photo damage of the D1 protein. Many investigation have demonstrated significant cross-linking of the D1 protein withstand damaged D1 protein cross linked with neighboring polypeptides namely, D2, CP43 and *psbA* protein under photoinhibitory conditions *in vitro*.(20,21). D1 protein prevented and may act as dissipative conducts o protect function other functional PSII centres (22, 23).

In this work is a survey of the plant response to UV-B radiation revealed that the action of UV-B varies from plant to plant and season to season. Generally the effect of UV-B was higher during monsoon season than in summer season. The aim of the study is to investigate takes place in the PSII organization of black gram and cluster bean.

MATERIAL AND METHODS

Plant materials and growth conditions

Certified seeds of black gram (*Vigna mungo* L.) and cluster bean (*Cyamopsis tetragonoloba* L.) were obtained from Agricultural College, Tamil Nadu Agriculture University. The seeds were sown in the experimental plots (6m x 4 m) in the University Botanical garden. The experiments were conducted during January-April 2012 and July-October 2012 in randomized complete block design for field black gram and cluster been. Seedlings were grown in 3 plots (6 m x 4m). Each 2 main plots was divided in to 10 sub plots. The plants were irrigated throughout with ground water. One week after germination, the seedlings were thinned to promote uniform growth. One set of the plants was grown under the ambient solar radiation. The day/night temperature were 32±2°C /28±2°C (summer season) and 28 ± 2°C / 26 ± 2°C (monsoon season). The natural photoperiod was 10-13 hrs.

UV-B radiation treatment

Seven day old seedlings of second set were exposed to UV-B radiation source (Wm^{-2}) for 4 h daily between 10 am and 2 pm. UV-B radiation was provided by Philips Holland TL20S/12 (max 315 nm) sunlamps. The lamps were suspended above and perpendicular to the row of sub plots. The control plots were placed away from the irradiation. The quantity of radiation was controlled by maintaining the height of lamps at a distance, 2 feet above the tops of the plants throughout the experimental period.

Isolation of chloroplasts

Type II chloroplasts were isolated from fresh leaves as described by Reeves and Hall 1973(24) using an assay medium consisted of 20 mM Tris-HCl, pH 7.8, 0.4 M sucrose, 5 mM $MgCl_2$ and 10 mM NaCl at 4°C.

PSII activity

PSII mediated O_2 evolution in the presence of BQ was measured using a Hansatech UK oxygen electrode as described by Noorudeen and Kulandaivelu 1982(25). The reaction mixture was the same as followed by Lingakumar and Kulandaivelu 1996(26).

Isolation of PSII particles

PSII membrane fragments were prepared according to the modified procedure of Kuwabara and Murata 1979(27).

Analysis of protein of PSII particles by SDS-PAGE

PS2 polypeptides were analyzed by SDS-PAGE on a slab gel of 15% acrylamide. Samples were solubilized at 20°C for 5 min in 2% SDS containing 60 mM DTT and 8% sucrose at a SDS-Chl ratio of 20:1.

RESULTS

Changes in the photosynthetic electron transport

To determining the possible effects of UV-B on photosynthesis activity was analyzed by using an O_2 electrode PSII mediated electron transport was assayed individually. The PSII mediated electron transport, measured as O_2 evolution, was found to be of the same magnitude in both ambient and enhanced UV-B condition irrespective of the crop and season (Fig. 1).

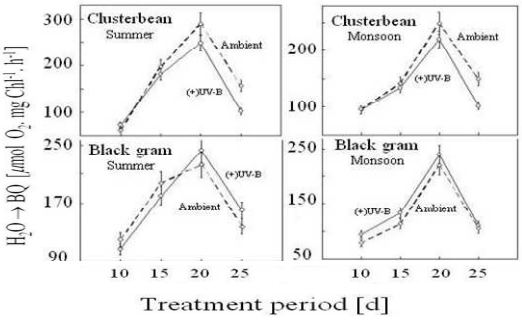


Fig.1 Changes in the PSII mediated ($H_2O \rightarrow BQ$) electron transport activities in chloroplast isolated from cluster bean and black gram plants grown under ambient and enhanced UV-B radiation in both summer and monsoon seasons. The values represent an average of 5 independent measurements and are significant at ±5% level.

The rate of O_2 evolution was found to increase with the age of the seedlings till 20 days of treatment and declined thereafter. This trend was noticed in all the two crops grown under ambient condition. Enhanced UV-B was found to increase the PSII activity in black gram only at the mid period of treatment whereas cluster bean exhibited a decline in activity right from the 10th day of treatment and such a decrease was found to be large at the later stages of treatment. Similar to the summer period, black gram plants under enhanced UV-B showed higher rate of activity than those under ambient light where as in cluster bean enhanced UV-B had caused inhibition of PSII activity .such changes was high in monsoon season than in summer season .

Effect of enhanced UV-B on the PSII polypeptides

To find out the specific changes in the polypeptides associated with the PSII core complex, thylakoid membranes were solubilized and the polypeptides composition was analysed in cluster bean and black gram. The polypeptides composition of ambient and enhanced UV-B treated seedlings was different in monsoon period at different treatment periods. Accumulation of 32 kDa polypeptide was noticed in black gram both summer and monsoon seasons. The presence of a 183 kDa polypeptide was observed in both the seasons at all stages of growth (Fig.2&3).

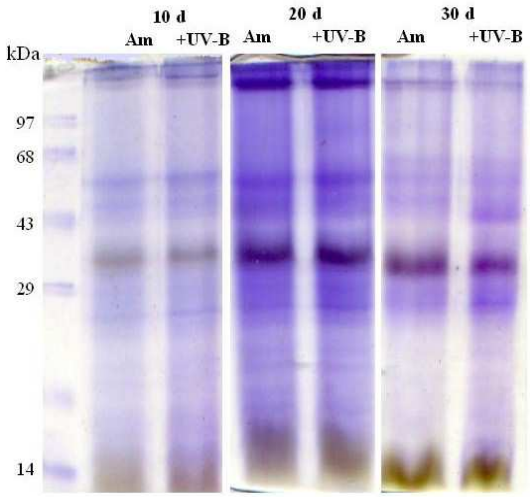


Fig. 2 SDS-PAGE analysis of PSII polypeptides isolated from black gram plants grown under ambient and enhanced UV-B radiation in monsoon season. Protein sample equivalent to 25µg was loaded in each well. Other details are as under Materials and Methods. Am= ambient control; +UV-B= enhanced UV-B.

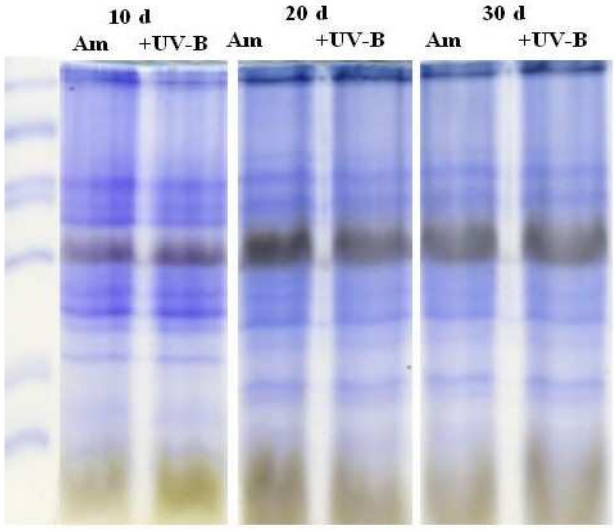


Fig.3 SDS-PAGE analysis of PSII polypeptides isolated from black gram plants grown under ambient and enhanced UV-B radiation in summer season.

In cluster bean, the composition and level of polypeptides was different from that of black gram (Figs.4& 5) After 10 days of treatment, the level of a transitory polypeptide of 72 kDa size which was low under ambient condition showed large increase under enhanced UV-B radiation. The appearance of the 72 kDa polypeptide was evident in both the seasons. After 20 days of treatment, the 183 kDa cross linked polypeptide appeared with concomitant disappearance of 73, 23 and 21 kDa polypeptide.

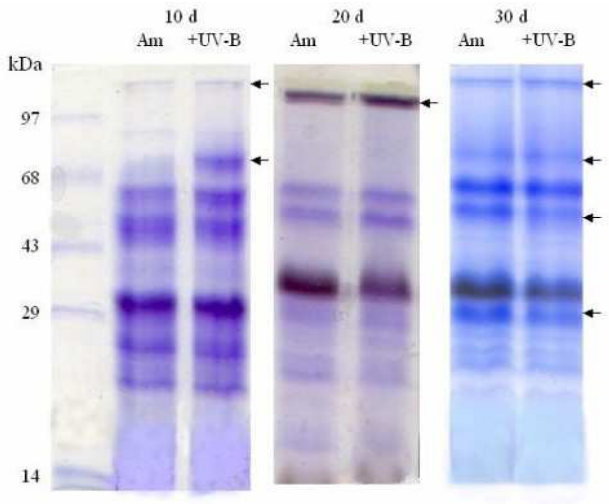


Fig.4 SDS-PAGE analysis of PSII polypeptides isolated from cluster bean plants grown under ambient and enhanced UV-B radiation in monsoon season. Protein sample equivalent to 25µg was loaded in each well. Other details are as under Materials and Methods. Am= ambient control; +UV-B= enhanced UV-B.

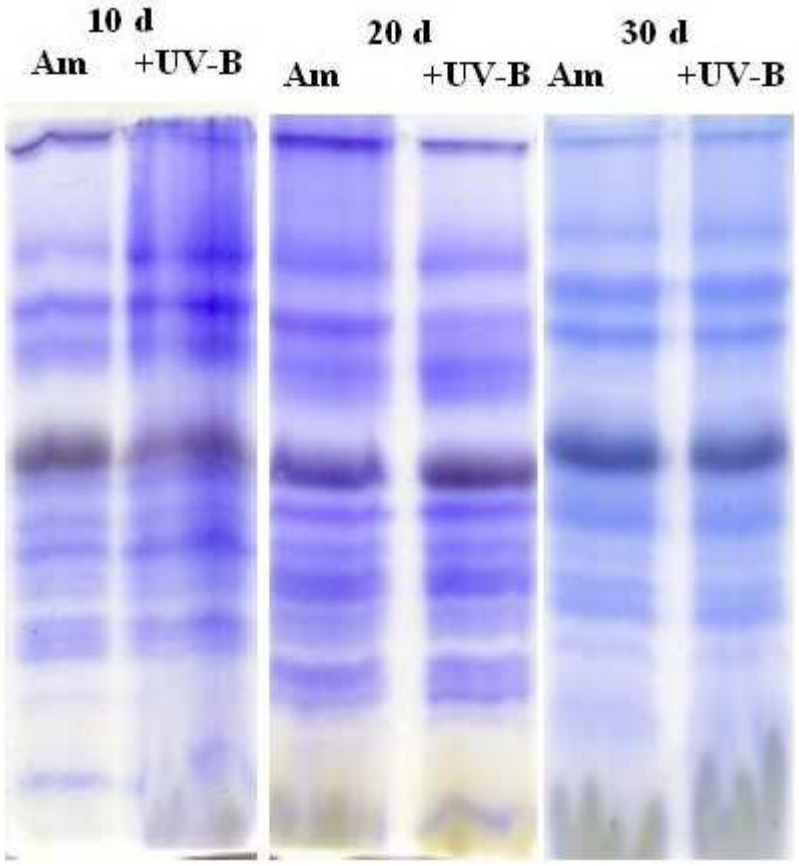


Fig.5 SDS –PAGE analysis of PSII polypeptides isolated from cluster bean plants grown under ambient and enhanced UV-B radiation in summer season.

The level of 183 kDa polypeptide was slightly more under UV-B enhanced radiation than under ambient light. During the later stages of treatment, a loss in the level of 183 kDa polypeptide with concomitant increase in the level of 62, 55, 33 and 28 kDa polypeptide had occurred in

both the seasons in UV-B enhanced as well as ambient light grown plants. The polypeptide pattern of PSII fraction of cluster bean plants grown during monsoon period showed large variation at different period of treatment. These changes were comparatively less in summer plants.

To understand the possible polypeptides involved in the formation of the cross linked 183 kDa product (Fig.6), the polypeptide was lysed by elution buffer, (0.1% SDS, 0.5M Tris-HCl, pH 7.9, 0.1M EDTA,5mM DTT, 0.15M NaCl) lyophilized and separated on a 15% linear SDS-PAGE and detected by silver staining.

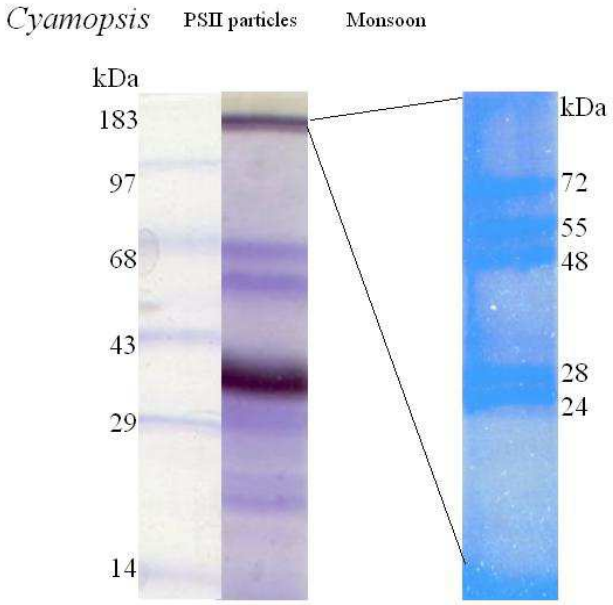
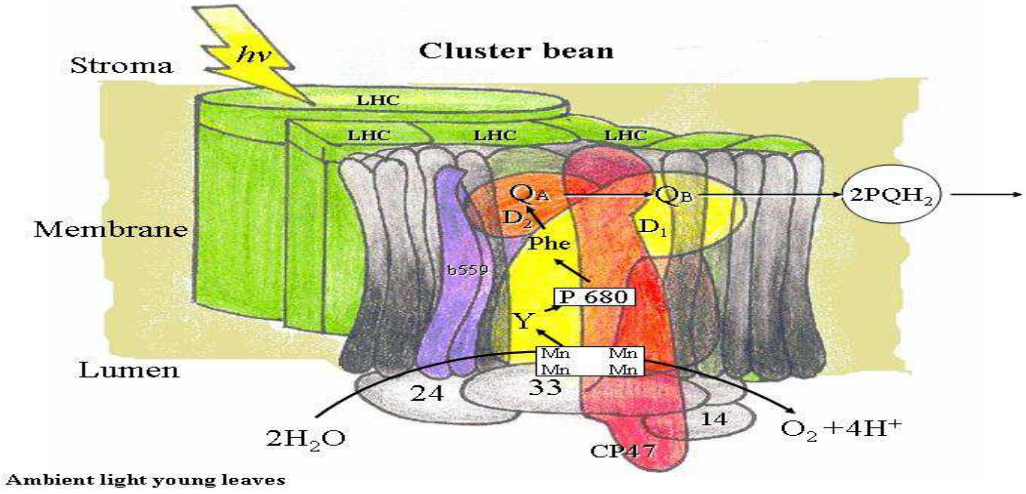


Fig. 6 SDS-PAGE analysis of 183 kDa PSII polypeptides of cluster bean plants grown under ambient radiation for 20d in monsoon season. The 183 kDa cross-linked protein was extracted in elution buffer and separated using a 15% linear gel.

Separation of this revealed the involvement of 72, 55, 48, 28 and 24 KDa polypeptides. The presence of 183 kDa cross linked polypeptide was also confirmed by eluting and running a SDS-PAGE with out lysis. This had produced only a single band of 183 KDa size. The schematic diagram showing the cross-linking of PSII complex at various stages of growth of cluster bean plants is presented in Fig. 7.



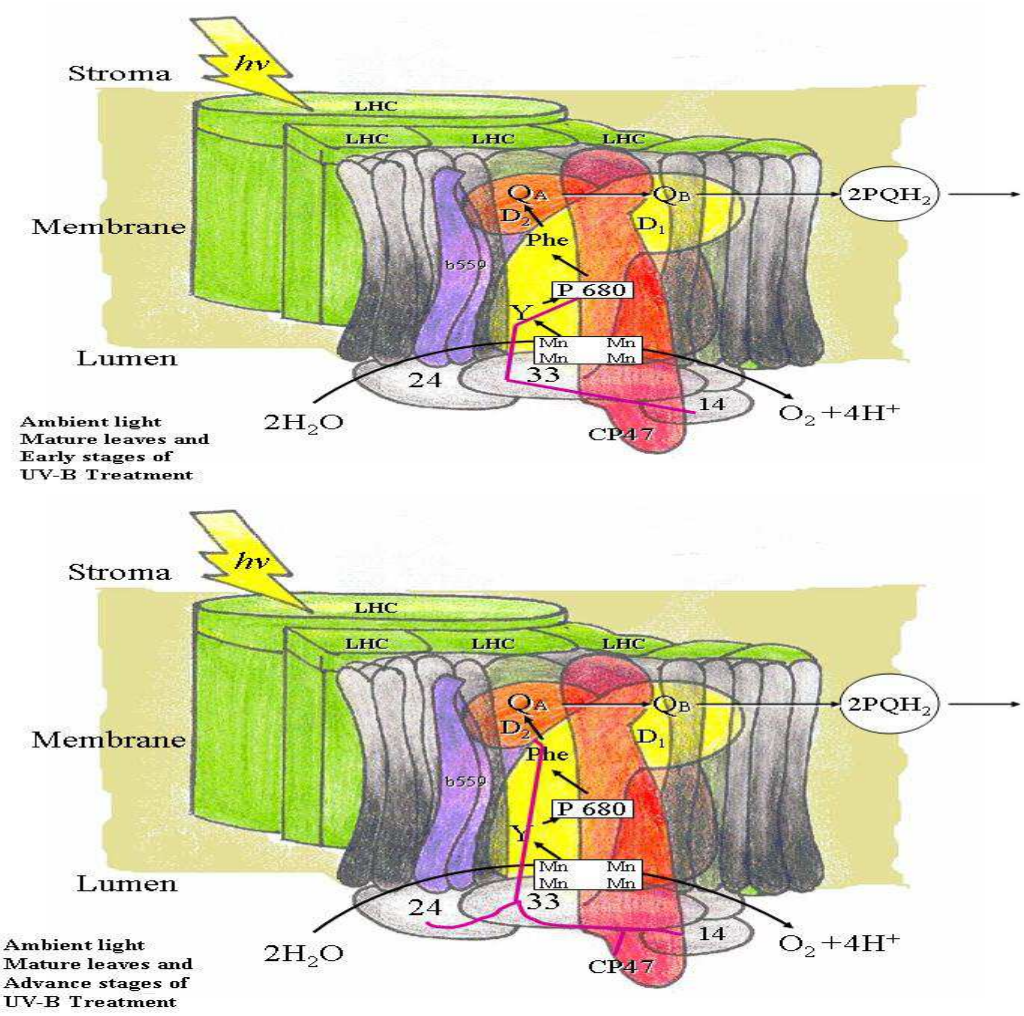


Fig.7 Schematic representation of cross-linking in PSII complex at various stages of growth (early, mid stage and later stage) in cluster bean Cross-linking in PSII complex offers stabilization to enhanced UV-B radiation.

DISCUSSION

Photosynthesis is one of the most sensitive physiological processes. The PSII activity in both cluster bean and black gram increased steadily up to plant maturation and there after declined rapidly. UV-B treatment decreased the PSII activity at all stages of growth in cluster bean while in black gram no definite change was seen. Recently Shi *et al*. (28) reported in S*aussurea superba*, a strong UV-B radiation had a potential negative impact in photo physiological process of alpine plant. Further these changes were significantly more during monsoon periods. When compared to that in summer season most the reported that PSII as the most vulnerable site for the action of UV-B. Several studies have shown a similar decrease in PS2 activity (29, 30). It was suggested that changes may be due to the damage caused to the oxidizing side of PSII to the reaction center itself (31,32,33).

Analysis of the PSII proteins in plants grown under ambient light showed significant changes at different periods of the growth in two different seasons. Accumulation of 32 kDa polypeptide was noticed in black gram during monsoon season than in summer. In cluster bean, the composition and level of polypeptides was different from that of blackgram. In clusterbean, after 10 days of growth, a transitory polypeptide of 72kDa was synthesized under enhanced UV-B treatment. This was not observed under ambient light condition. The appearance of the 72 kDa was evident in both the seasons. The formation of this new polypeptide could be due to cross-linking of a protein. Protein cross-linking of PSII core polypeptide occurred with few low molecular weight polypeptides. The damaged D1 polypeptide is not only degraded, but also

cross-linked to neighboring polypeptides. The damaged D1 cross-linking process was studied extensively *in vitro* which was found to be physiologically significant (33,21). Cross-linking of the D1 polypeptide with CP43 was first indicated by Mori and Yamamoto 1992(34), the amount of CP43 decreased in coomassie band following SDS/urea-PAGE after strong illumination of PSII membrane (34). Western blot analysis using antibodies against both the D1 polypeptide and CP43 showed that the D1 polypeptide from cross-linked product with CP43 in the range of 70-100 kDa with an aid of low molecular weight polypeptide (35).

In black gram 183 kDa polypeptide was observed with the season at all stages of growth whereas in clysterbean after 20th day treatment, the 183 kDa a cross-linked polypeptide appeared with disappearance of 43, 33, 23 and 21 kDa polypeptide .The level of 183 kDa polypeptide was slightly more under UV-B enhanced radiation than under ambient light. The 183 kDa appears to be a cross-linked product formed due to interaction with a few low molecular weight polypeptides. The expression of such cross-linked product was very prominent only in the mid stage of growth subjected to high irradiation. This could be considered as an adaptive mechanism to maintain the stability of the PSII core and water oxidizing complex both under ambient and also under UV-B enhanced radiation.

The cross-linking of the D1 and D2 protein, Cyt B559 and CP43 occurs in parallel with degradation of the D1 polypeptide during illumination of intact chloroplast thylakoids and PS2 enriched membranes (21). Cross-linking of the D1 and D2 polypeptides, with the α-subunit of Cyt b559 and with CP43 indicates that these polypeptides are located in close proximity to each other. A D1 cross linked of 160 kDa product was reported by the high light illumination of *Dunaliella salina* (36).

The high molecular weight cross linked product (183 kDa) was eluted and it was separated in SDS-PAGE .Separation of this product revealed the involvement of 72, 55, 48, 28 and 24 kDa polypeptides. Yamamoto *et al*.1998 (37) observed photodamaged D1 polypeptide cross links with D2, the α-subunit of Cyt b559 and CP43. Previous report of high molecular weight 160 kDa polypeptide also originated from the aggregation or cross-linking of damaged PS2 reaction center which probably occurs as unsolublised D1/D2/Cyt b559 dimers (38). Prasil *et al*. (39) suggested that the cross-linking formed by disassembly of PSII and in the absence of sufficient capacity in *de novo* biosynthesis, neighboring PS2 RC polypeptides may aggregate either by hydrophobic interaction or by cross-linking of constituent polypeptides. In *Cyamopsis* during later stages of treatment, a loss in the level of 183 kDa polypeptide with a concomitant increase in the level of 62, 55, 33 and 28kDa polypeptide occurred in both the season in UV-B enhanced as well as ambient light grown plants. The D1 polypeptide completely recovered from damage and restored the PS2 activity through repair mechanism. Previous studies reported that the damaged D1 protein degraded rapidly (40) and is replaced by a newly synthesized protein (41,441). Yamamoto (21) has reported that synthesis of new polypeptides after protective response to overcome photo inhibition. These observations indicate that UV-B enhanced radiation try to stabilize the sensitive PSII complex by cross-linking the proteins. This process occur even under ambient solar radiation where the UV-B content is fairly high and UV-B enhanced radiation only accelerated the process plants like black gram show resistance against UV-B radiation as the proteins are already present in the cross-linked condition.

REFERENCES

1. Pyle, J.A., 1997, Global ozone depletion: observation and theory. In: Lumbsden, P.J. (Ed.), Plants and UV-B: Responses to Environmental Change. Cambridge University Press, Cambridge, pp. 3–12.
2. Caldwell, M.M., Bjorn, L.O., Bornman, J.F., Flint, S.D., Kulandaivelu, G., Teramura, A.H. and Tevini, M., 1998, Effect of increased solar UV-B on terrestrial ecosystems, J. Photochem. Photobiol. Biol. 46: 40–52.
3. Kakani, V.G., Reddy, K.R., Zhao, D. and Sailaja, K., 2003, Field crop responses to ultraviolet-B radiation: a review, Agricultural and Forest Meteorology. 120:191–218.
4. Molina, M. J. and Rowland, F.S., 1974, Stratospheric sink for chlorofluoromethanes: chlorine atom-catalyzed destruction of ozone, Nature. 249: 810 - 812.

5. Bornman, J.F, and Teramura, A.H., 1993, Effect of ultraviolet-B radiation on terrestrial plants. In: Young, A.R., Bjorn, L.O.,Moan, J., Nulttsch, W. (Eds.), Environmental UV Photobiology, Plenum Press, New York, pp. 427–472.
6. Caldwell, M.M. and Flint, A.H, 1995, Lighting considerations in controlled environments for non-photosynthetic plant responses to blue and ultraviolet radiation, In: T. W. Tibbitts (ed.) Proceedings, International lighting in controlled environments Workshop, pp.113-124.
7. Bjorn, L.O., Callaghan, T.V., Johansen, I., Lee, J.A, Manetas ,Y., Paul, N.D., Sonessen, M, Wellburn, A.R., Coop, D., Heide-Jorgensen, H.S, Gehrke, ,C., Gwynn-Jonnes, D., Johanson, U, Kyparissis, A, Levizou, E,, Nikolopoulos, D, Petropoulou, Y. and Stephanou, M., 1997, The effects of UV-B radiation on European heathland species. Plant Ecol. 128:252–264.
8. Kim, V.C., Tennessen, D.J. and Last, R.L., 1998, UV-B induced photo morphogenesis in *Arabidopsis thaliana*, Plant J 15(5): 667–674.
9. Yang, J.H., Chen, T. and Wang, X., 2000, Effects of enhanced UV-B radiation on endogenous ABA and free proline in wheat leaves. Acta Ecol Sin 20: 39–42.
10. Shetty, P., Atallah, M.T. and Shetty, K., 2002, Effects of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-DOPA synthesis in dark germinated *Vicia faba*. Process Biochem. 37: 1285–1295
11. Brandle, J.R.,Campbell, W.F., Sisson, W.B. and Caldwell, M.M.,1977, Net photosynthesis, electron transport capacity and ultra structure of *Pisum sativum L*.exposed to ultraviolet-B radiation Plant Physiol. 60:165-169.
12. Noorudeen, A.M, and Kulandaivelu, G., 1982, On the possible site of inhibition of photo synthetic electron transport by ultraviolet-B (UV-B) radiation, Physiol. Plant. 55:161–166.
13. Renger, G., Volker, M., Eckert, M.J., Trome, R., Hohmveit, S. and Graber, P., 1989, On the mechanism of photosystem II deterioration by UV-B radiation, Photochem. Photobiol.49:97–105.
14. Kulandaivelu, G., Nedunchezhian, N. and Annamalainathan, K., 1991, Ultraviolet-B radiation induced changes in photochemical activities and polypeptide components of C_3 and C_4 chloroplasts. Photosynthetica ,25:333 – 339.
15. Correia, C.M., Areal, E.L.V., Torres, P.M.S. and Torres, P.J.M.G.,1999, Intra specific variation in sensitivity to ultraviolet-B radiation in maize grown under field condition, Field Crop Res. 62: 97–105.
16 Savitch, Pocock, L.V. Krol, T., Wilson, M., Greenberg, B.M. and Huner, N.P.A., 2001, Effects of growth under UV-A radiation on CO_2 assimilation carbon portioning PS II photochemistry and resistance to UV-B radiation in *Brassica napus* cv.Topas Ouality control of Photosystem II, Aust. J. Plant Physiol. 28:203–212.
17 Greenberg, B.M., Gaba, V., Canaani, O., Malkin, S. Mattoo, A.K. and Edelman, M., 1989, Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions, Proc Natl. Acad. Sci.USA. 86*:*6617–6620.
18. Van Rensen, J. J. S. Vredenberg, W.J., and Rodrigues, G. C., 2007, Time sequence of the damage to the acceptor and donor sides of photosystem II by UV-B radiation as evaluated by chlorophyll *a* fluorescence, Photosynth Res .94:291–297.
19. Chu, Z. Yi, W. and ZongYu, C., 2009, Effects of strong solar UV-B radiation on photosynthesis and photosynthetic pigment contents of *Saussurea superba* on Qinghai-Tibet Plateau, Journal of Yunnan Agricultural University.24:895-903.
20. Ishikawa, H., Hasenstein, K.H. and Evans, M.L., 1991, Computer-based video digitizer analysis of surface extension in maize roots, Planta .183: 381–390.
21. Yamamoto, Y., 2001, Quality control of photosystem II, Plant Cell Physiol.42:121-128.
22 Henmi, T. Yamasaki, H. Sakuma, S. Tomokawa, Y. Tamura, N. Renshen, J.and Yamamoto, Y., 2003, Dynamic interaction between the D1 protein CP 43 and OEC33 at the lumenal side of photo system II in spinach chloroplast evidence from Light induced cross-linking of the protein in the donor side photo inhibition. Plant Cell Physiol. 44:451-456.

23. Mohanty, P. Suleyman I. Allakhverdiev, I. and Murata, N., 2007, Application of low temperatures during photoinhibition allows characterization of individual steps in photodamage and the repair of photosystem II, Photsynth.Res 94:217-224.
24. Reeves, S.G. and Hall, D.O., 1973, The stoichiometry (ATP: 2e-ratio)of non-cyclic photophosphorylation in isolated spinach chloroplasts, Biochim. Biophys. Acta 314: 66–78.
25. Noorudeen, A.M. and Kulandaivelu, G., 1982, On the possible site of inhibition of photosynthetic electron transport by ultraviolet-B (UV-B) radiation Physiol. Plant. 55:161-166.
26. Lingakumar, K. and Kulandaivelu, G., 1996, Reversal of ultraviolet-B (280-320 nm) radiation induced changes in *Vigna unguiculata* L chloroplasts by supplemental white light by 32:463-469.
27. Kuwabara, T. and Murata, N., 1979, Purification and characterization of 33 kilodalton protein of spinach chloroplasts. Biochim. Biophys. Acta 581:228-236.
28. Shi, S, Xia Shang, Y. Zhu, P.J. and Zhang, D.G, 2011, Effects of strong solar UV-B radiation on photosynthesis and photosynthetic pigment contents of *Saussurea superba* on Qinghai-Tibet Plateau, J. Appl. Ecol.22:53-60.
29. Tevini, M., Fieser, G. and Grausemann, P., 1988, In: Applications of Chlorophyll Fluorescence, (Lichtenthaler, H.K., ed.) Kluwer, Dorecht, pp. 229-238.
30. Van Hasslet, P.R., Chow, W.S. and Andersson, J.M., 1996, Short-term treatment of pea leaves with supplementary UV-B at different oxygen concentrations: impacts on chloroplast and plasma membrane bound processes. Plant Sci. 120:1-9.
31. Bornman, J.F., Bjorn, L.O. and Akerlund, H.E., 1984, Action spectrum for inhibition by ultraviolet-B radiation of photosystem II activity in spinach thylakoids Photochem. Photobiophys. 8:304-313.
32. Demmig, B. and Bjorkman, O., 1987, Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants, Planta171 – 184.
33. Ishikawa ,Y., Nakatani, E., Henmi, T., Ferjani, A., Harada,Y., Tamura, N and Mori H, Yamashita Y, Akasaka T, Yamamoto, Y., 1995, Further characterization of the loss of antenna chlorophyll-binding protein CP43 from Photosystem II during donor-side photoinhibition, Biochim Biophys Acta 1228: 37–42.
34. Mori, T.and Yamamoto, Y., 1992, Deletion of antenna chlorophyll-*a*-binding proteins CP43 and CP47 by Tris-treatment of PSII membranes in weak light: evidence for a photo-degradative effect on the PSII components other than the reaction center-binding proteins, Biochim Biophys Acta 1100: 293–298.
35. Mori, H. and Yamashita, Y. Akasaka, T. and Yamamoto, T.,1995, Further characterization of the loss of antenna chlorophyll-binding protein CP43 from photosystem II during donor-side photoinhibition, Biochim. Biophys. Acta 1228: 37-42.
36. Baroli, I. and Melis, A., 1996, Photoinhibition and repair in *Dunaliella salina* acclimated to different growth irradiances, Planta 198*:* 640–646*.*
37. Yamamoto, Y.,Ishikawa, Y.,Nakatani, E.,Yamada, M.,Zhang, H. and Wydrzynski, T., 1998, Role of an Extrinsic 33 Kilodalton Protein of Photosystem II in the Turnover of the Reaction Center-Binding Protein D1 during Photoinhibition, Biochemistry 37: 1565-1574.
38. Kim, J.H., Nemson,A. and Melis, A., 1993, Photosystem II reaction center damage and repair in *Duanliella salina* (green alga). Plant Physiol.103:181-189.
39. Prasil, O., Adin, N. and Ohad, I., 1992, In: The Photosystem: Structure, Function and Molecular Biology. (Barber, J. ed) Elsevier, Amsterdam, London, New York, Tokyo. pp 295 – 348.
40. Ohad, I., Kyle, D.J. and Arntzen, C.J., 1984, Membrane protein damage and repair; Removal and replacement to inactivated 32 kilo Dalton polypeptide in chloroplast membrane.J.Cell Biol. l99:481-485.
41. Mattoo, A.K., Hoffman-Falk, H., Marder, J.B and Edelman, M., 1984, Regulation of protein metabolism coupling of photosynthetic electron transport to *in vivo* degradation of

rapidly metabolized 32-Kilodalton protein of the chloroplast membrane. Proc. Natl. Acad. Sci. USA 81: 1380-1384.