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Research Paper

Effect of enhanced solar UV-B radiation (280-320nm) on chloroplast polypeptide profile of some leguminous plants at different growing seasons

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Abstract

UV-B radiation (280-320nm) is harmful to living organism and has detrimental effects on plants growth, development photosynthetic activity and chloroplast protein expression. In this work we examined response of some legume plants like Black gram and Cluster bean(*V. mungo and C. tetragonoloba* L) to enhanced UV-B radiation at two different growing seasons (summer and monsoon).we analyzed the impact of UV-B radiation chloroplast polypeptide profile in two seasons. The Cluster bean plants sensitive to enhanced UV-B radiation, which appeared in the decreases chloroplast polypeptide expression reduced gradually from low to high molecular protein from the early stage to later stage. Such reduction was very high in monsoon season than summer season. Where as in Black gram UVB radiation promote the polypeptide expression gradually from early to later stage in both the season.

Keywords: Blackgram, Clusterbean, Chloroplast protein, Light harvesting chlorophyll, protein, Rubisco, Ultravioler B radiation.

Introduction

The depletion of the stratospheric ozone layer is enhancing fluxuation of the solar UVB radiation at the earth's surface. The range of UV-B (280-320nm) corresponds to a minor percentage of the solar energy, but it potentially harmful because these shortwave lengths are capable of causing deleterious effects on human health and ecosystem. The enhanced UV-B exposure is potentially detrimental to all living things, but it is particularly harmful to plants due to obligatory requirement for sunlight to survive and this inability to move.

The amount of UV-B incident at the plant depends upon spatial, temporal factors such as time of day, seasonality, latitude, altitude, cloud cover, and aerosol and canopy coverage. Many studies have shown that plants exhibit a tremendous variety to UV-B radiation[1,2] response that occur include changes anatomy and morphology, reduction and growth in the net carbon assimilation capacity (photosynthesis) and Changes in biomass allocation and growth [3].

However the knowledge of the effects of UV-B at the biochemical and molecular level are limited. It is well established that the major site of damage by UV-B in the choloroplast and integrity of the thylakoid membrane and structure. The effect of UVB on chloroplast protein seems to be even more sensitive than the activities of the photosynthetic apparatus bound within [4,5]. Negative effects on several photosynthetic components are known, including through suppression of chlorophyll synthesis

[6], the inactivation oxygen evolution, LHCII, PSII reaction centres functionality and the thylakoid electron flux. Due to the key role of LHCII in light absorption and energy transfer to the reaction center, as well as on thylakoid stacking, any damage to these structures can result in multiple effects on the photosynthetic functioning. Furthermore, it must be considered that UV-B radiation inhibition of LHCII [7,8,9,5] is also eventually linked to a decrease in the transcription of the cab gene responsible for the synthesis of the chlorophyll a/b-binding proteins of LHCII, which may lead to the functional disconnection of LHCII from PSII [10], its leading to impairment of photosynthetic function[11].

In few studies have focused the molecular mechanism underlying UV-B sensitivity of photosynthesis [12,13]. The deleterious effect of UV-B on the efficiency of this process can be attributed to specific reductions in expression of important photosynthetic genes [14], a reduction in Rubisco activity [15], changes in ion permeability of thylakoid membranes[16] and in the level of chlorophyll and carotenoids [17,18]. Changes in gene expression reported in response to supplemental UV-B include reduction in expression and synthesis of key photosynthetic genes including Rubisco (rbcS and rbcL). D1 polypeptide of photosystem II (psbA), chlorophyll a/b binding protein (Lhcb or cab) a decline ATP ase complex[18]. This is manifested as low rates of PSII electron transport [19] and low variable fluorescence yield [20]. The inhibition of photosynthesis is closely associated with PSII and PSI and coincident with ultra structure damage of chloroplast protein.

In the present investigation we selected Black gram and Cluster bean (*Vinga mungo and Cyamopsis tetragonoloba L*) plants. These plants are typical tropical species and are grown normally in low latitudes. For the experiments these plants were grown under field conditions and are exposed to either ambient UV-B or enhanced UV-B radiation in two seasons, one during the months of March-May and the second during September-January. The first season is (summer) coupled with dry weather and high temperature (35-40^{o}C) and the second (monsoon) with rainy and slightly low temperature (28-32^{o}C). The ambient UV-B levels in both seasons vary only marginally. These two plants grown under ambient and 20% UV-B enhanced solar radiation and the changes in photosynthetic electron transport and chloroplast polypeptide profile were followed.

Materials and Methods

Plant growth and UV-B radiation treatment: Certified seeds of *V. mungo* and *C. tetragonoloba* L obtained from the Farm Aids, Madurai, India was sown in experimental plots in the University Botanical garden. One set of plants was grown under the ambient solar radiation and another set under 20% UV-B radiation, artificially provided for 4 h using PhilipsTL40W/12 sunlamps (Philips, Holland) centered at solar noon. The plants were grown under field conditions. Experiments were carried out in two seasons, one during the month of March-May (summer) and second during September-January (monsoon). The experimental plots were maintained for three years (2001-2004) continuously without any disturbance.

Protein preparation for SDS-PAGE

Membrane were precipitated with 10%TCA and left on ice for 30min before centrifugation to collect the pellet. Traces of TCA left behind in the pellet were removed by three washings in ice-cold acetone. The final pellet was air dried and solublised in small volume of 10%-SDS to which equal in a small volume of sample buffer was added. The samples were boiled for 2min and centrifuged 3000g for 5 min to remove unsolublised materials.

Estimation of protein: Total protein content estimated by Lowry et al.(1951) [21], respectively.

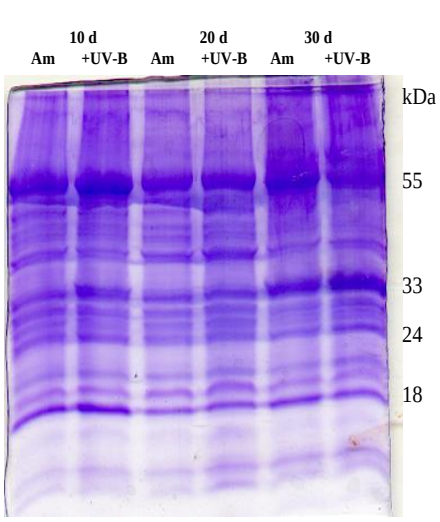
SDS-PAGE

SDS.PAGE was done by the gel system described by Laemmli (1970) [22] using Polyacrylamide gradient of 8-16% of chloroplast protein.

Results and Discussion

The effect of enhanced solar UV-B radiation on the chloroplast polypeptides were studied in *V. mungo and C. tetragonoloba* L that had been grown under field condition in the summer and monsoon seasons. The polypeptide composition of cluster bean chloroplasts isolated from the plant grown for various periods under ambient and enhanced UV-B radiation (Figure 1 and 3).

In the monsoon period, plants grown under the ambient light condition showed an increase in polypeptides in the range of 18-24 and 43-55k Da at different period of growth. UV-B enhanced radiation caused an overall decrease in the level of 55, 47, 33, 27 and 20 kDa polypeptides. Such a decrease was evident up to 20 days of treatment with enhanced UV-B. At the later stages of treatment (30 days) the summer period failed to provoke any reduction in the level of above mentioned polypeptides under the enhanced UV-B radiation.



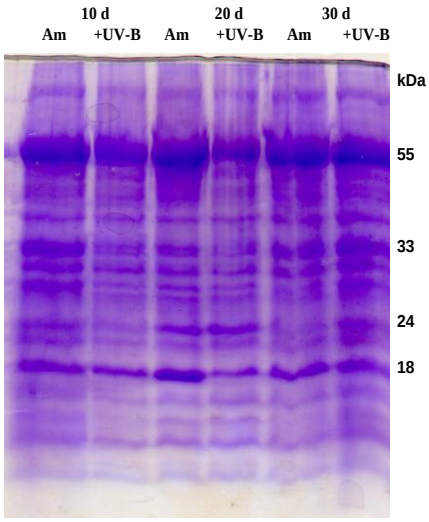


Figure 1 and 2: SDS –PAGE profile chloroplast polypeptides of Black gram and Cluster bean plants grown under ambient and enhanced UV-B radiation in monsoon season. Protein sample equivalent to 300µg was loaded in each well

The recent studies also reported that UVB radiation decrease [23] protein synthesis in some annual desert. Allen et al (1998) [24] a suggested that effects on photosynthesis was primarily associated with loss of Calvin cycle enzyme and adaxial stomata closure.

UV-B radiation denatures protein and also damages nucleic acids leading to reductions in the amount of stromal and thylakoid proteins. The damages of nucleic acid and thereby the protein synthesis affecting Rubisco, which constitutes about 50% of the total soluble proteins, by enhanced UV-B radiation have been reported [8,9]. In many higher plants and macroalgae the Rubisco subunit degradation upon exposure to UV-B have been reported [25,26]. Suppression of gene expression of key proteins involved in photosynthesis (like *rbc*L encoding for LSC of Rubisco and *psb*A for D1) has also been shown [27,13]. High UV-B irradiance in combination with low PAR produced significant reduction in the concentration of carboxylating enzyme [28]. High UV-B levels were reported to decrease the amount of carboxylating enzyme and the level of both subunits had decreased [9,29,30] reported the UVB radiation decrease the initial carboxylation velocity of Rubisco which was further correlation with large reduction in the expression and abundance of both large and small subunits of Rubisco. UV-B induced inactivation of Rubisco could be due to the modification of the peptide chain or degradation of the protein and diminished transcription of gene [27,11,31].

The loss of 47 kDa polypeptides might be due to the disruption of the PSII complex [32,19]. The 33, 23 and 17 kDa polypeptide associates are required for optimal functioning of the oxygen evolving machinery. These three polypeptides are present in equimolar amounts 33,34,35,36. Seidler (1994) [37] have suggested that the catalytic cluster of water oxidation and especially the 33 kDa polypeptide containing the Mn cluster is highly sensitive to UV-B radiation. Rengeret al. (1989) [38] reported that UV-B radiation induced destruction of 33, 23 and 17 kDa polypeptides in *Vigna* chloroplasts. Decrease in the level of these polypeptides or release of any one of these polypeptides from the thylakoid could result in low efficiency of the O_2 evolving apparatus.

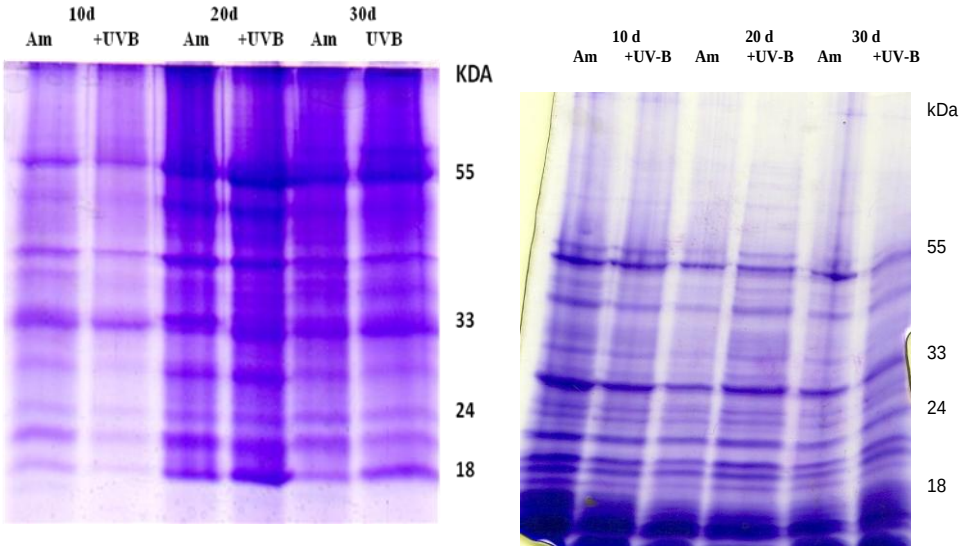


Figure 3 and 4: SDS –PAGE profile chloroplast polypeptides of Black gram and Cluster bean plants grown under ambient and enhanced UV-B radiation in summer season. Protein sample equivalent to 300µg was loaded in each well

When compared to cluster bean, black gram was found to be resistant to enhanced UV-B (Figure 2 and 4). Plants grown in both monsoon and summer periods, did not show much change in the composition and quantity of the chloroplast polypeptides, although changes in the level of some polypeptides had occurred at different treatment periods[39]. Sharma et al. (1998)[16] suggested that in some plants are more tolerant to UV than others because they produce a variety of secondary metabolites including flavonoids and anthocyanins. These compounds often accumulate in the upper epidermis cells of leaves and effectively absorb UV radiation, thus preventing it from penetrating the leaf mesophyll cells. Plants also have developed a complex antioxidant system this prevent the plant from UVB damage. Such kind of plant is Blackgramt, naturally, is more tolerant to UVB radiation because leaves very thick and to produce more secondary pigment which are given resistance against UVB radiation. So in Black gram highly stable nature of the oxygen evolving complex was observed and less decrease in the level of extrinsic proteins was observed under UV-B treatments.

Conclusion

From the above results it is confirmed that the Cluster bean as UV-B sensitive whereas Black gram is UV-B resistant plant. The polypeptides of UV-B resistant plant black gram and UV-B sensitive cluster bean plant chloroplasts were studied in order to find out if any specific polypeptides are responsible for this differential behaviour. In cluster bean UV-B radiation significantly reduced many high and low molecular proteins in the initial and mid stages of plant growth treatment. Such reduction was large in monsoon season. In contrast to this, in black gram there was no change in the level of these polypeptides. This study confirms the effect of UVB radiation depending upon the highly variable.

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