Effect of enhanced solar UVB (280-320nm) radiation on secondary pigment synthesis in some plants

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Abstract— *The investigation was carried out to find out whether enhanced UVB radiation increases the synthesis of secondary metabolites in some plants grown under tropical region. Like tropical plant temperate plant also grown successfully in the tropical region. Tropical plants are thought to have an inherent resistance to abiotic stress. The abiotic stress that enhances the ability of the plant by increasing the content of the secondary bioactive components. While the plants exposed to enhanced UVB radiation in the field condition, the synthesis of secondary pigments like flavonoids and anthocyanin level varied among species. In radish and beet- root the synthesis of flavonoid and anthocyanin the content was increased, whereas, in the onion and carrot, the level of synthesis of secondary pigment was less. But compared to ambient plants the UVB the irradiated the plant has more secondary pigment. So it proves that the UVB radiation increases the synthesis of secondary pigment content by activating the defence mechanism and to protect plants against UVB damage.*

Keywords— Anthocyanin, UV-B radiation, Carrot, Radish, Beetroot, Onion, Seconary pigment.

I. INTRODUCTION

Due to the depletion of the stratospheric ozone layer increased the ultraviolet radiation in the biosphere, especially in the range of UV--B(280-320nm) is increasing in the earth's surface. Such increases were very high in the low latitude region when compared to the high latitude region. The increases in solar UV-B radiation have a great impact on the agricultural crops in the tropical region. The effect of UV-B on plants includes inhibited growth, morphology ,Chl biosynthesis, photosynthetic activity, electron transport, damage the DNA, protein, lipid membrane, decrease biomass of plants and increases in the level of secondary pigment synthesis in plants.(1,2).Tropical plants reside in an environment which possesses a naturally high amount environmental stress. When the plant exposed to enhanced UVB radiation continuously means the plants to synthesis secondary or protective pigment by defence mechanism. Naturally tropical plant synthesis, high amount photoprotective pigment compares to temperate plants.When they exposed to the addition of UV-B radiation means it increases the synthesis of secondary metabolites. The continuous exposure plants to UV-B radiation increase photobleaching of chlorophyll pigment, it affects photosynthetic activity and other physiological processes. Such damages are reduced in some level by these protective pigments like anthocyanin and flavonoids.(3)

Flavonoids are the class of secondary phenolics with significant antioxidant and chelating properties widespread in various plants. Accumulation of the UV-B absorbing pigments one of the ways by which plants alleviate the harmful effect of UV-B light (4). Lie et al 1995 observed that the largest UV-B absorbing compound in barley plants. It has been shown that the photo-induced accumulation of these flavanoids precedes by n some enzyme phenylpropanoid biosynthetic pathway such as phenylalanine ammonialyase and chalcone synthase of flavanoids biosynthetic pathway(6,7) . However,the flavonoid concentration may be higher (8) in tropical species growing at high elevated regions.

Anthocyanin is another important secondary metabolites it accumulated in leaf tissue.

Many studies have shown that very small amount of UV-B radiance differences have a large effect on the accumulation of anthocyanin and flavonoids. The Anthocyanin also, act as UV-B attenuators in protecting the cellular components against radiation damage. High UV-B radiation is known to increase anthocyanin production is several crop species (9,10). Drumm-Herrel and Mohr (1981) have demonstrated that anthocyanin synthesis in sorghum mesocotyls the involved interaction between UV-A, UV-B and phytochrome photoreceptors . UV-B induced anthocyanin production has also been reported in mustard hypocotyls, corn, wheat, and rye coleoptiles (12Wellmann, 1982). Although this pigment production represents a specific UV-B effect, anthocyanin biosynthesis may not be particularly adapted since it has little absorption in the UV-B waveband. The effect of UVB on plant physiological has been widely studied. But limited studies only focus on the synthesis of secondary metabolites in plants grown for agriculture purpose. In the present work, an attempt has been made to study the effects of enhanced solar UV-B radiation on the temperate vegetative crops, which are successfully grown in the tropical regions and are also economically important. After screening a wide range of plants with underground storage plants for their UV-B induced responses, there is a need to analyze synthesis of secondary metabolites.In Daucus carota L. (Carrot), Raphanus sativus L. (radish) Allium cepa L (onion) and Beta vulgaris L (beet -root) a comparative investigation has been carried out to understand the impact of ambient solar radiation and UV-B enhanced solar radiation (20% above the ambient UV-B level).

II. MATERIALS AND METHODS

2.1 Plant materials

Certified seeds of *Daucus carota* L (Early nanties), *Raphanus sativus* L.(Pusa chetki), *Allium cepa* L. (Nasik N-53) and *Beta vulgaris* L. (Hy-Pronto) obtained from the Agriculture Department, Madurai were sown in experimental plots in the Madurai Kamaraj University Botanical Garden. One set of plants was grown under ambient solar radiation and other under 20% UV-B enhanced solar radiation.

2.2 Plant growth and UV-B treatment

The seeds were soaked overnight, in running water. Separate soil beds were prepared for control (ambient) and UV-B treatment and seeds were sown in these experimental plots. The plants were watered regularly and care was taken to avoid microbial or pest infection during the experimental period. Plants with the first foliage leaf stage were used for UV-B treatment. UV-B treatment was given to these plants for 4 hours daily from 10 am to 2 pm. Treatment was continued under ambient solar radiation and 20% UV-B enhanced solar radiation supplemented by a Philips TL40W/12 sunlamp (Gloelampenfabrieken, Holland). The first formed leaves were collected at different time periods and all the physiological and biochemical analyses were carried out.

2.3 Measurement of radiation

A Li-Cor Li-188B quantum/radiometer (Li-Cor., Inc., USA) with suitable photodetector was used to measure all the visible and photosynthetically active radiation. Radiation below 400 nm was determined by an IL 700 radiometer with a SEE 400 photodiode detector (International Light Inc., USA)

A. Flavonoids

Fresh leaf samples equivalent to 100 mg were cut into small pieces and incubated overnight in 5 ml of 80% acidified methanol (80:20:1 of methanol:water:HCl) at 4^oC in the dark. After centrifugation to remove debris, the absorbance at 315 nm was taken and the flavonoid content was expressed as µg/g leaf fresh weight (13Mirecki and Teramura, 1984).

B. Anthocyanins

Anthocyanins were extracted from the leaves by grinding the leaves in 80% acidified methanol (80:20:1 of methanol:water:HCl). After centrifugation, the clear extract was used to estimate the concentration of anthocyanin by measuring the absorbance at 530 and 657nm, according to Mancinelli *et al*. (1975).

$$\mu g/g \text{ fresh weight} : (A_{530}) – (0.3 \times A_{657})$$

III. RESULT

3.1 Changes in non-photosynthetic pigments

3.1.1 Flavonoids

Application of abiotic stress, such as enhanced UV-B treatment can induce distinct changes in the plants secondary metabolism. The flavonoid content in the leaves of the crops was also estimated in all the three crops. The flavonoid content was more in all the four crops that were exposed to enhanced UV-B .The changes in the level of flavonoid content in crops grown under ambient and enhanced UV-B radiation is shown in Fig. 1.The flavonoids are UV-B protecting pigments and they are known to accumulate plants grown under enhanced UV-B radiation. The increase in flavonoid content was more in all the four vegetative crops throughout the plant growth. Among the four plants the UVB treated radish and beet root has synthesis high amount of flavonoids when compared to the remaining two plants. Such synthesis was 10%high in in beet-root. In carrot and onion the changes in flavonoid content were only marginal. In onion and carrot in particular stage the synthesis of the flavanoid content was decreased, but when compared to ambient the UVB- treated plants show high flavanoid content such changes most probably due to the impact of the initial senescence.

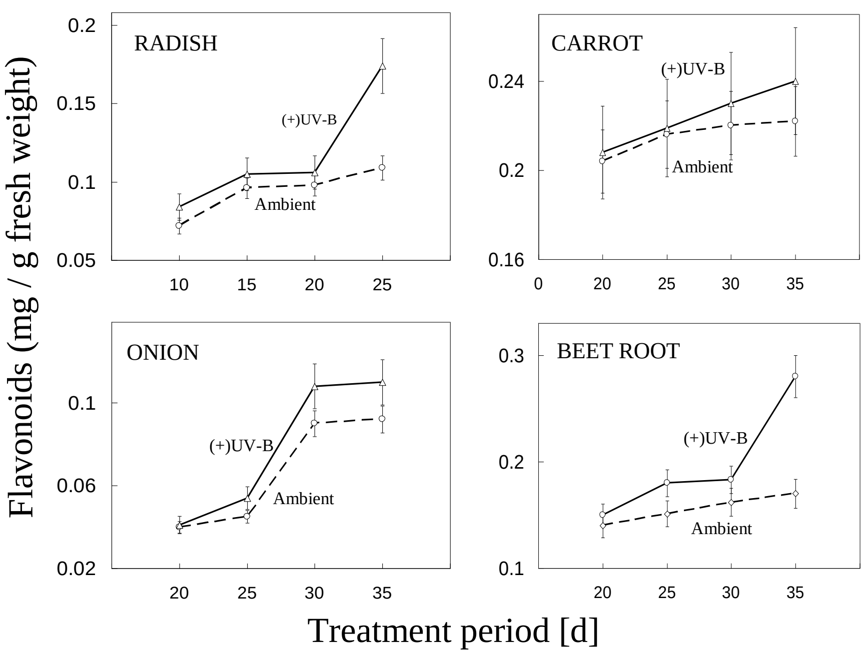

FIG. 1: CHANGES IN THE FLAVONOID CONTENT OF RADISH, CARROT, ONION AND BEET ROOT PLANTS GROWN UNDER AMBIENT AND ENHANCED UV-B RADIATION. THE VALUES REPRESENT AN AVERAGE OF 3 INDEPENDENT MEASUREMENTS. MEAN ± SE, n=3.

3.1.2 Anthocyanin

The anthocyanin content in the leaves was estimated in all the crops at different stages of plant growth. Similar to flavonoids, the accumulation of anthocyanin was more throughout the plant growth in all the four crops grown under enhanced UV-B radiation. The extent of such increase varied largely from plant to plant (Fig. 2). In, radish more than 40% increase over the level found in ambient light grown plants was observed in plants grown under the UV-B enhanced radiation**.** In carrot crops grown under enhanced UV-B radiation shows gradual decrease in anthocyanin content during a late senescence stage, which leads to decline in photosynthetic activity in plants.

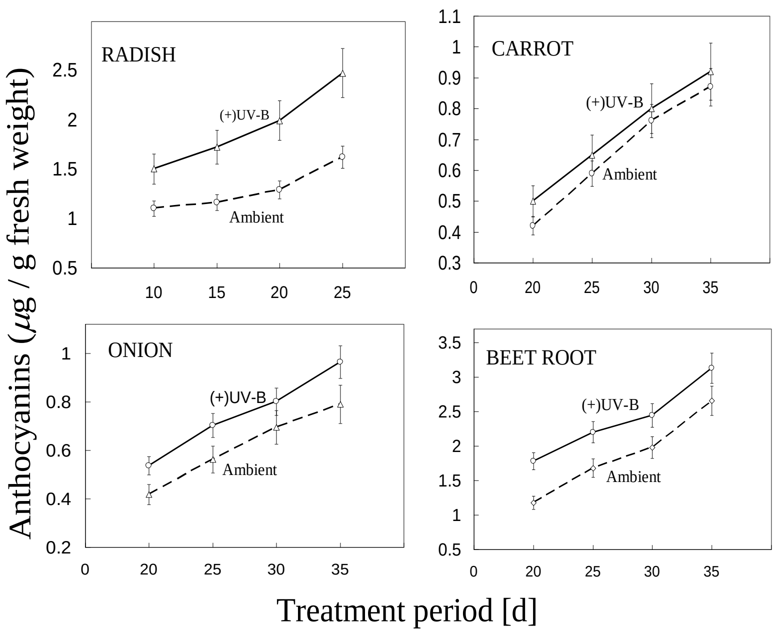

FIG. 2 CHANGES IN THE ANTHOCYANIN CONTENT OF RADISH, CARROT, ONION AND BEET-ROOT PLANTS GROWN UNDER AMBIENT AND ENHANCED UV-B RADIATION. THE VALUES REPRESENT AN AVERAGE OF 3 INDEPENDENT MEASUREMENTS. MEAN ± SE, n=3.

IV. DISCUSSION

4.1 UV-B induced changes in Flavonoids content:

The flavonoid content in both UV-B tolerant and sensitive species grown under enhanced UV-B radiation showed an increase in leaves and the extent of such increase varied largely from plant to plant. Such increase is one of the adaptive responses of plant species exposed to enhanced UV-B radiation (15). In addition, the composition of flavonoids also varies according to altitude and latitude (16,17). Beggs and Wellmann (1994) reported that many herbaceous plants do not have efficient UV protection, but respond to high UV flexes by stimulating flavonoid synthesis. At the same time Kakani *et al*. (2003) reported that the UV-B absorbing compounds might not offer a total protection against UV-B radiation. In radish the accumulation of flavonoid has increased and this probably has enhanced the growth under enhanced UV-B radiation. A few earlier reports indicate that high concentrations of flavonoids were produced in response to UV-B radiation by seedlings of tolerant species like barley and radish and very little flavonoid production occurs in a UV-B sensitive species like bean (20). Furthermore, radish seedlings which are resistant to UV-B radiation had a high level of leaf flavonoids,which possibly protect the seedlings by absorbing UV in the leaf epidermis (21). In carrot and onion, in the early stages of plant growth, the flavonoid content increased. But in the later stages the plants showed significant decrease in the flavonoid level. A similar change was reported in barley where a strong decline with increasing leafage, in the activity of phenylalanine ammonialyase, a key enzyme of flavonoid metabolism (22). The young leaves showed a significant increase of flavonoids in barley (23). Carrot, onion and beet-root grown under enhanced UV-B radiation show increased levels of flavonoids, but these plants showed high sensitivity towards UV-B enhancement. The accumulation of UV-B absorbing flavonoids, in the leaf epidermis can be either in the cuticle, cell wall or in the vacuole (24,25Caldwell *et al*., 1983, Krauss *et al*., 1999). The extractable UV-B absorbing compounds may not provide protection if they are located in the mesophyll (26,27).

4.2 UV-B indued changes in Anthocyanin content

The accumulation of anthocyanin in the leaf epidermis increased in UV-B irradiation plants. Similar to flavonoids, anthocyanins also have an UV-B protective role in plants, it acts as UV-B attenuators in protecting the cellular components against UV-B radiation damage. Tevini *et al*. (1991) suggested that flavonoids and anthocyanin absorb UV radiation and keeps UV radiation from reaching the photosynthetic tissues. The epidermis blocks transmittance of 95 to 99% of incoming UV radiation (292). In all the four vegetative crops the anthocyanin concentration increased under UV-B irradiation. An increase in the anthocyanin was higher in radish when compared to other three crop carrot, onion and beet- root. High UV-B radiation on magnesium deficient cowpea seedlings had caused a marked accumulation of anthocyanin (30). The action spectrum for anthocyanin inhibition peaks at 263 nm and this inhibition does not involve phytochrome (31). Phytochrome, a blue light photoreceptor, and a UV-B photoreceptor are shown to be involved in flavonoid induction (32).Anthocyanin pigment production represents a specific UV-B effect. Anthocyanin biosynthesis may not be particularly adapted since it has little absorption in the UV-B wave bands. Increased anthocyanin levels were shown to reduce the formation of pyrimidine dimmers upon exposure of cultured cells to UV-B irradiation (33). Leaves of plants exposed to UV-B irradiation were denser and thicker and had higher concentration of UV-B absorbing and protecting pigments (34,35).

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