Similarities in the biochemical changes between solar UV exclusion and GA application in *Amaranthus caudatus*

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ABSTRACT

Seedlings of *Amaranthus caudatus* grown under UV exclusion filters (-UV -B <320nm; -UV -B/A <400nm) in the ambient solar radiation showed an enhancement in growth. This was accompanied by reduction in the synthesis of betacyanin but enhancement in the level of amino acids and proteins. The growth promotion and the accompanying biochemical changes were similar to the external application of GA₃ to the seedlings under ambient solar radiation. The results are discussed in the light of similarities between UV exclusion and GA₃ application in terms of diversion of amino acid precursors towards enhanced primary metabolism. [*Physiol. Mol. Biol. Plants* 2009; 15(4) : 367-370] E-mail : knguruprasad@hotmail.com

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Abbreviations : UV: Ultra violet radiation, GA₃: Gibberellic acid

INTRODUCTION

Studies have demonstrated the deleterious effects of UV-B (280-315 nm) radiation on photosynthesis, plant growth and development, yield and biomass production (Caldwell, 1971; Teramura 1983; Tevini and Teramura, 1989). These studies used elevated levels of UV-B either as a supplement to natural solar radiation or by artificial UV-B light sources (Caldwell, 1971). The small portion of UV-B present in the natural solar radiation has profound photo biological effects on the plants. In tropical regions, the plants are exposed to high ambient levels of solar UV-B radiation compared to the temperate zones. The biochemical changes for adaptation to ambient UV-B can be studied by excluding the UV-B component from the natural solar radiation. Such exclusion studies done earlier on soybean have shown an enhancement in growth and biomass of the plants (Varalakshmi *et al.*, 2003; Guruprasad *et al.*, 2007). These growth changes were also accompanied by enhancement in the total soluble proteins.

Growth and biomass of plants can also be enhanced by the application of hormones like GA₃. Amongst the GA₃ responsive plants, *Amaranthus* is the most sensitive (Stobart *et al.*, 1970; Kinsman *et al.*, 1975; Laloraya *et al.*, 1976). The enhancement of growth in *Amaranthus* is also accompanied by an enhancement in the total soluble proteins (Sweet and Guruprasad, 1993), but reduction in the level of betacyanin pigment (Stobart *et al.*, 1970; Laloraya *et al.*, 1976). Using a radioactive precursor of betacyanin, tyrosine, an amino acid, Sweet and Guruprasad (1993) demonstrated that GA₃ diverted this amino acid to enhance protein synthesis, thereby causing reduction in the synthesis of betacyanin.

Since betacyanin replaces anthocyanin in higher plants like *Amaranthus* and is functionally similar to anthocyanin in protecting the plants by attenuating UV components of solar radiation, the present investigation was aimed to study the impact of UV exclusion on betacyanin synthesis in *Amaranthus*. The biochemical changes in the level of betacyanin and total soluble amino acids and proteins were investigated in the UV-excluded plants and compared to the plants treated with GA₃ under ambient levels of UV.

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MATERIAL AND METHODS

Seeds of *Amaranthus caudatus* purchased from Suttons India Ltd, Kolkata were sown in 12 inch pots exposed to sunlight. The pots were covered with wooden frames (3ft. X 1 ½ ft. X 1 ½ ft.) wrapped with polyester cut off filters for the exclusion of UV-A and UV-B from the solar radiation (see Varalakshmi *et al.*, 2003). 10 ml of different concentrations of GA₃ (0.1 to 10 µg/ml) were sprayed once in a day on the seedlings grown in the open field. Growth and Biochemical estimations were done on 4th and 8th day after seedling emergence. The average values of 20 seedlings were taken for fresh and dry weight. Since hypocotyls are most sensitive in their response to GA₃, hypocotyl length was measured on day 4 and day 8.

Betacyanin was extracted by the method of Malviya and Laloraya (1966). According to the method of Schneider (1957), proteins were extracted from hypocotyl and cotyledons together omitting roots and estimated by the method of Lowry *et al.* (1951). Similarly, for the extraction of total amino acids, the method of Noorudeen and Kulandaivelu (1982) was followed. Amino acids were estimated following the method of Troll and Cannan (1953).

RESULTS

Hypocotyl growth

Amaranthus seedlings grown under filters that excluded UV-B and UV-B/A showed an enhancement in the length of the hypocotyl measured on 4th and 8th day after the emergence of the seedlings. It was to the extent of ca 38 % (4th day) and 33 % (8th day) (-UV B) and 63 % (4th day) and 68 % (8th day) (-UV-B/A) (Fig. 1).

Fig. 1. Effect of UV exclusion and GA₃ application on Hypocotyl length in *Amaranthus caudatus*

Seedlings grown under ambient UV- A/B and treated with different concentrations of GA₃ showed much higher enhancement with increased concentrations, ca 27 % (GA₃ 0.1µg/ml) to 64 % (GA₃ 10µg/ml) on the 4th day and from ca 50 % (GA₃ 0.1µg/ml) to 75 % (GA₃ 10µg/ml) on the 8th day (Fig. 1). Thus UV exclusion produced an enhancement in the hypocotyl length comparable to 0.1 µg/ml to 1 µg/ml concentration of GA₃ (Fig. 1).

Fresh and Dry weight

UV exclusion enhanced fresh and dry weight of seedlings. Exclusion of UV B enhanced fresh weight by 30 (4th day) and 43 % (8th day) and -UV B/A by 51 % (4th day) and 28 % (8th day). The dry weight enhanced by -UV B was ca 23 (4th day) and 29 % (8th day) and by -UV B/A ca 49 (4th day) and 101 % (8th day). Fresh weight (47-102 % 4th day; 73-103 % 8th day) and dry weight (31-76 % 4th day; 51-146 % 8th day) also increased by various concentrations of GA (Table 1).

Betacyanin

UV exclusion resulted in the inhibition of the synthesis of betacyanin in the seedlings. The inhibition was higher by the exclusion of -UV B/A (60 % at day 4 and 32 % at day 8) compared to -UV B (Fig 2). Reduction of betacyanin in the cotyledons was up to 32 and 47 % (-UV B at 4th and 8th day) and 47 and 57 % (-UV A/B)

Fig. 2. Effect of UV exclusion and GA₃ application on Betacyanin content (A) Hypocotyl and (B) Cotyledons

Table 1. Fresh and Dry Weight in mg/seedling after day 4 and day 8

Treatment	Fresh Weight		Dry Weight	
	Day 4	Day 8	Day 4	Day 8
Control	7.50 (100 %)	16.85 (100 %)	0.92 (100 %)	2.48 (100 %)
- UV -B	9.35 (124 %)	24.15 (143 %)	1.14 (123 %)	3.20 (129 %)
- UV -B/A	11.75 (156 %)	30.00 (178 %)	1.38 (150 %)	4.99 (201 %)
0.1µg/ml GA	11.05 (147 %)	29.20 (173 %)	1.21 (131 %)	3.90 (157 %)
1.0µg/ml GA	13.35 (178 %)	31.15 (184 %)	1.44 (156 %)	5.06 (204 %)
10 µg/ml GA	15.15 (202 %)	34.35 (203 %)	1.63 (177 %)	6.11 (246 %)

on 4th and 8th day. Application of GA₃ further reduced the betacyanin content from *ca* 50 (0.1 µg/ml GA₃) to 85 - 90 % at higher concentrations (1 µg/ml GA₃ and 10 µg/ml GA₃) in both the hypocotyl and cotyledons (Fig. 2). Thus the enhancement of growth of hypocotyl was accompanied by inhibition in the level of betacyanin in both the UV excluded or GA₃ treated seedlings.

Soluble proteins and amino acids

Exclusion of UV B and UV B/A enhanced the level of total soluble proteins as well as free amino acids (Figs. 3, 4). Compared to UV B, enhancement was higher with UV B/A. Exclusion of UV B enhanced the total proteins to 48 (4th day) and 34 % (8th day) while exclusion of UV B/A enhanced the level to 78 (4th day) and 66 % (8th day) (Fig. 3). GA enhanced the protein level from 70 to 146 % (4th day) and 62 to 125 % (8th day).

There was also an increase in the total free amino acids (Fig. 4). With different concentrations of GA₃ the enhancement in total free amino acid varied (Fig. 4).

Fig. 3. Effect of UV exclusion and GA₃ application on total protein content in the seedlings.

DISCUSSION

The impact of exclusion of solar UV on the seedlings of *Amaranthus caudatus* was similar to the application of GA₃ to the seedlings grown in ambient solar UV. The similarity is in the enhancement of hypocotyl growth, fresh and dry weight, level of free amino acids and soluble proteins and reduction in the synthesis of betacyanin.

Betacyanins are functionally similar to anthocyanins with a heavy absorption in the UV part of the solar spectrum (data not given) and have a protective role against ambient solar radiation. Exclusion of UV reduced the synthesis of betacyanin as the protection was not required. Since tyrosine (Monohydroxy phenylalanine) and dopa (di hydroxy phenylalanine), are the direct precursors of betacyanin synthesis (Garay and Tower, 1966; Giudici *et al.*, 1975) and these aromatic amino acids are synthesized from phenylalanine by hydroxylation of the aromatic rings, non utilization

Fig. 4. Effect of UV exclusion and GA₃ application on total free amino acids content in the seedlings.

of the precursors of betacyanin in the absence of UV makes them available for enhanced protein synthesis. In the absence of solar UV there is a signal transduction that causes a change in metabolism and diverts the amino acid precursors to enhance the free amino acid pool and protein synthesis.

A similar diversion of amino acid precursors of betacyanin to protein synthesis by application of GA₃ has earlier been demonstrated in *Amaranthus* (Laloraya *et al* 1976; Sweet and Guruprasad, 1993). The diversion of metabolites by GA has been further proved by the use of radioactive tyrosine (Sweet and Guruprasad, 1993). In the present investigation GA was externally applied to seedlings grown in ambient solar radiation. GA enhanced growth and protein synthesis and reduction in the synthesis of betacyanin even in the presence of UV components in the solar radiation. The present investigation indicates that UV exclusion may also induce the diversion of precursors, similar to GA. Ambient UV thus seems to be limiting the impact of endogenous GA on primary metabolism. Conversely, exclusion of solar UV potentiates endogenous GA resulting in enhanced primary metabolism (enhanced protein synthesis) at the cost of secondary metabolism leading to betacyanin synthesis. Presence of UV in the natural solar radiation limits primary metabolism by necessitating the synthesis of betacyanin (a product of secondary metabolism) required for protection from UV.

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