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## **CHANGES IN THE MORPHOLOGY, GROWTH AND YIELD OF VIGNA MUNGO (L.) HEPPER VAR. VAMBAN-4 UNDER ULTRAVIOLET-B STRESS**

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### **ABSTRACT**

The variations brought about by ultraviolet-B (UV-B) radiation on the morphology, growth and yield of black gram *Vigna mungo* (L.) Hepper var. VAMBAN-4 was reported. Growth of black gram after exposure to supplementary UV-B radiation (2 hours daily @  $12.2 \text{ kJ m}^{-2} \text{ d}^{-1}$ ; ambient =  $10 \text{ kJ m}^{-2} \text{ d}^{-1}$ ) was assessed on 15, 30, 45 and 60 DAS. UV-B irradiated crops had less number of leaves (17 to 33 %), reduced total leaf area (20 to 35 %) and leaf area index (2.48 to 48.65 %). Specific leaf weight under UV-B stress decreased by 16 to 66.66 %. UV-B decreased the fresh weight of leaves by 3.81 to 63.10 % and dry weight of foliage by 14.76 to 64.76 % in all stages of UV-B exposed plants. Growth of all the varieties of black gram was progressively inhibited by the UV-B radiation. Reduction in root and shoot length ranged from 11.11 to 42.6 % respectively at all stages of growth resulting in reduced plant height and S/R (2 to 3 %). Plant fresh and dry weight after UV-B stress fell below control by 25.12 to 47.89 % 60 DAS. The relative growth rate (RGR) was reduced in all varieties with age as it reached 50 % reduction on 60 DAS. UV-B exposure decreased the entire yield components per plant basis, the decreases being 60 % in the pod number, 66.59 % in pod weight, 22.22 % in pod length, 28.57 to 64.28 % in seed number and 25.66 to 62.57 % in seed mass. Harvest index was reduced under UV-B treatment which showed a reduction of 78.45 % than control. The UV-B irradiated plants recorded 4.83 % more value on shelling percentage than the normal plants.

**Keywords:** Ultraviolet-B, Black Gram, Var. VAMBAN-4, Morphology, Growth, Yield

### **INTRODUCTION**

Elevated ultraviolet-B were harmful to leaves (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b; Kokilavani and Rajendiran, 2014c), inhibiting plant growth (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and suppressing nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in a variety of crop. Although literature on UV-B and plant interaction is plenty, most of the works deal with the gross effects on growth and yield under controlled environmental conditions. Hardly 5% of the over 600 publications relate to field studies (Caldwell *et al.*, 1998). This was considered as the major defect leading to overstating the damaging influence of UV-B (Jordan 1997) as plants under natural day light conditions with high PAR (photosynthetically active radiation) are affected very little (Adamse and Britz, 1992). This study gains more importance as the plants of black gram *Vigna mungo* (L.) Hepper var. VAMBAN-4 were grown under field conditions (*in situ*) and a supplementary UV-B was given along with sunlight.

### **MATERIALS AND METHODS**

Black gram (*Vigna mungo* (L.) Hepper var. VAMBAN-4) the nitrogen fixing grain legume was chosen for the study. Viable seeds of *Vigna mungo* (L.) Hepper var. VAMBAN-4 were procured from Saravana Farms, Villupuram, Tamil Nadu, India. The seeds were selected for uniform colour, size and weight and used in the experiments. The crops were grown in pot culture in the naturally lit greenhouse (day temperature maximum  $38 \pm 2 \text{ }^\circ\text{C}$ , night temperature minimum  $18 \pm 2 \text{ }^\circ\text{C}$ , relative humidity  $60 \pm 5 \%$ , maximum irradiance (PAR)  $1400 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ , photoperiod 12 to 14 h). Supplementary UV-B radiation

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was provided in UV garden by three UV-B lamps (*Philips TL20W/12 Sunlamps*, The Netherlands), which were suspended horizontally and wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 280 nm). UV-B exposure was given for 2 h daily from 10:00 to 11:00 and 15:00 to 16:00 starting from the 5th day after sowing. Plants received a biologically effective UV-B dose (UV-B<sub>BE</sub>) of 12.2 kJ m<sup>-2</sup> d<sup>-1</sup> equivalent to a simulated 20 % ozone depletion at Pondicherry (12°2'N, India). The control plants, grown under natural solar radiation, received UV-B<sub>BE</sub> 10 kJ m<sup>-2</sup> d<sup>-1</sup>. Ten plants from each treatment were carefully uprooted on 15, 30, 45 and 60 DAS and their axial growth (roots and shoot length and plant height) and fresh biomass were measured. They were then dried in an oven at 80° C for 48 h and weighed again for dry mass measurements. Alongside, morphological and developmental abnormalities if any, caused by UV-B radiation were also recorded. Assessment of growth of test plant on 15, 30, 45 and 60 DAS were recorded and calculated using standard methods. Ten plants were selected at random from each of the treatments. The leaf area (the leaflets from all the nodes) was determined at various stages using Area meter (Analytical Development Corporation, UK, model AM100). The total leaf area per plant was obtained by summing up the area of the leaves from all the nodes of the plant. Leaf area index (LAI) (Williams 1946), specific leaf weight (SLW) (Pearce *et al.*, 1968), relative growth rate (RGR) (Williams 1946) and shoot / root ratio (Racey *et al.*, 1983) were calculated using the following formulae.

$$\text{LAI} = \frac{\text{Leaf area of the plants (cm}^2\text{)}}{\text{Ground area occupied (cm}^2\text{)}}$$

$$\text{SLW} = \frac{\text{Leaf dry weight (g)}}{\text{Leaf area (m}^2\text{)}}$$

$$\text{RGR} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{t_2 - t_1}$$

where, W<sub>1</sub> and W<sub>2</sub> are dry masses of whole plants at t<sub>1</sub> and t<sub>2</sub> (time in days) respectively.

$$\text{S/R ratio} = \frac{\text{Shoot weight (g)}}{\text{Root weight (g)}}$$

Mature fruits were harvested periodically from each plant and the length and weight of the pod, number of seeds per pod and number of seeds per plant and weight of seeds per plant were recorded. Harvest index (Mohan *et al.*, 1992) and shelling percentage (Francis *et al.*, 1978) were calculated using the following formulae.

$$\text{Harvest index} = \frac{\text{Yield of the plant (g)}}{\text{Biomass of the plant (g)}} \times 100$$

$$\text{Shelling percentage} = \frac{\text{Seed wt. plant}^{-1}}{\text{Fruit wt. plant}^{-1}} \times 100$$

At least ten replicates were maintained for all treatments and control. The experiments were repeated to confirm the trends.

### RESULTS AND DISCUSSION

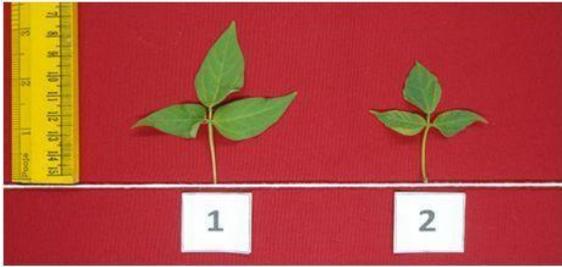
The responses of black gram were assessed in terms of growth on 15, 30, 45 and 60 DAS. There were fewer leaves only (17 to 33 %) under UV-B stress on 45 and 60 DAS, but plants under normal ambience had more number of leaves (Table 1). Supplementary UV-B irradiation reduced the total leaf area (20 to 35 %) throughout the growth period (Table 1; Plate 1, Figure 1 to 4). The LAI was reduced by UV-B exposure to a larger extent (2.48 to 48.65 %) below control (Table 1). The SLW in UV-B irradiated decreased with age (16 to 66.66 %). UV-B stress decreased the fresh weight of leaves by 3.81 to 63.10 %, and

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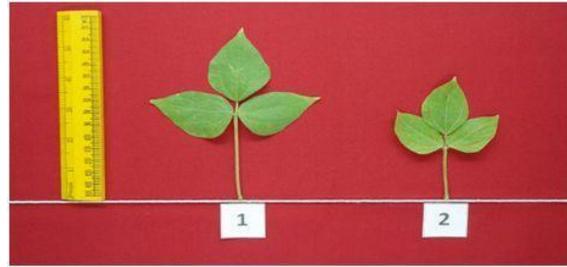
with the maximum reduction being on 45 DAS. The dry weight of foliage also decreased by 14.76 to 64.76 % in all stages of UV-B exposed plants (Table 1). Reductions in leaf area and mass were observed in the field-grown sweetgum plants (Sullivan *et al.*, 1994), black gram (Rajendiran and Ramanujam, 2000) and green gram (Rajendiran and Ramanujam, 2003) and ten varieties of cowpea (Kokilavani and Rajendiran, 2014d) exposed to elevated UV-B radiation. According to Britz and Adamse (1994) changes in the leaf area and dry mass indicated that cell elongations as well as cell contents were affected. On prolonged exposure to UV-B, the leaves of black gram exhibited various kinds of abnormalities. The leaves became generally pale which at times occurred in patches. The yellowing intensified into complete chlorosis (Plate 1, Figure 5). On continuous exposure to UV-B the leaves developed necrosis in the tips and margins (Plate 1, Figure 6). UV-B exposure reduced root length significantly on all stages of growth till 60 DAS. UV-B stressed plants showed maximum reduction of root growth 42.60 % on 45 DAS which recovered after 60 DAS (Table 2). Shoot length of UV-B stressed plants decreased by 11 to 42.6 % till 60 DAS (Table 2). The S/R ratio was decreased by UV-B stress by 2 to 3 % (Table 2). Fresh weight of roots increased with age in all treatments. But the biomass accumulation in root and shoot was inhibited by UV-B treatment by 25.12 to 47.89 % on all stages of growth. The trends observed in root and shoot biomass pattern were reflected at the whole plant level too (Table 2, Plate 1, Figure 3, 4, 7, 8). The relative growth rate (RGR) was reduced in all varieties with age as it reached 50 % reduction on 60 DAS (Table 2). Inhibition of growth indicated by reductions in root and shoot length and biomass content due to UV-B stress were apparent at all stages. Such inhibitions are characteristic of UV-B stressed legumes as in *Vigna unguiculata* (Kulandaivelu *et al.*, 1989), *Phaseolus vulgaris* (Mark and Tevini, 1997), *Vigna mungo* (Rajendiran and Ramanujam, 2000), *Vigna radiata* (Rajendiran and Ramanujam, 2003) and ten varieties of cowpea (Kokilavani and Rajendiran, 2014d). The stunting of UV-B stressed plants is attributed to destruction of endogenous IAA whose photo-oxidative products may be inhibitory (Kulandaivelu *et al.*, 1989, Tevini and Teramura, 1989) as indicated by a decrease in IAA content concomitant with a corresponding increase in IAA oxidase activity in rice leaves (Huang *et al.*, 1997). Total biomass represents a long-term integration of all biochemical, physiological and growth parameters. It is a good indicator of UV-B radiation effects on growth as low biomass appears to be an even response. Analysed for the whole plant or plant parts, fresh or dry, shoot or root biomass accumulations or shoot / root ratios are often substantially reduced by UV-B (Nedunchezian and Kulandaivelu, 1997; Noguez *et al.*, 1998). Brandle *et al.*, (1977) found that the dry weight of pea was significantly reduced after nine days of UV-B irradiance. Similar results were obtained in *Phaseolus vulgaris* (Dumpeert and Knacher, 1985), *Helianthus annuus* (Tevini *et al.*, 1991), *Oenothera stricta*, *Plantago lanceolata* and *Chamaesyce celastroides* (Ziska *et al.*, 1992), *Liquidamber styraciflua* (Sullivan *et al.*, 1994) and *Vigna radiata* (Rajendiran and Ramanujam, 2003) and in ten varieties of cowpea (Kokilavani and Rajendiran, 2014d). Leaf biomass increased by UV-B irradiance while contribution of root and stem decreased (Jain *et al.*, 1999). RGR was reduced with age as it reached 25 to 50 % reduction on 60 DAS (Table 2). Similar inhibitions of RGR by UV-B were observed by Jain *et al.*, (1999) in mungbean and Rajendiran, Ramanujam (2003) in green gram and in ten varieties of cowpea (Kokilavani and Rajendiran 2014d). Supplemental UV-B exposure consistently decreased the entire yield components per plant basis, the decreases being 60 % in the pod number, 66.59 % in pod weight, 22.22 % in pod length, 28.57 to 64.28 % in seed number and 25.66 to 62.57 % in seed mass (Table 3, Plate 1, Figure 9, 10). Analysed on the basis of number of seeds per pod, only the UV-B treated plants had more fruits containing fewer number of seeds (Table 3). Harvest index was the least under UV-B treatment which showed a reduction by 78.45 % compared with control. The UV-B stressed crops recorded 4.83 % more value on shelling percentage than the controls (Table 3).

UV-B exposure delayed the flowering and reduced the yield in crop plants in general (Caldwell and Flint, 1994; Rajendiran and Ramanujam, 2004; Kokilavani and Rajendiran, 2014e). Both the timing of flowering and the number of flowers produced in maize cultivars, *Petunia hybrida*, *Brassica rapa* and several mono- and dicotyledonous ephemerals have been altered by UV-B (Staxen and Bornman, 1994; Musil, 1995; Klaper *et al.*, 1996; Mark *et al.*, 1996; Rajendiran and Ramanujam, 2004).

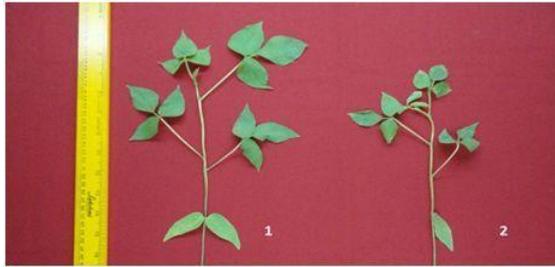
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**Figure 1: 15 DAS leaves**



**Figure 2: 30 DAS leaves**



**Figure 3: 30 DAS plants**



**Figure 4: 45 DAS plants**



**Figure 5: Chlorosis**



**Figure 6: Necrosis**



**Figure 7: 60 DAS plant tops**



**Figure 8: Root system and nodules**



**Figure 9: Pods**



**Figure 10: Seeds**

**Plate 1: First fully expanded trifoliate leaves, foliar injuries, plant tops and yield of *Vigna mungo* (L.) Hepper var. VAMBAN-4 (1: Control, 2: UV-B)**

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**Table 1: Changes in foliage of *Vigna mungo* (L.) Hepper var. VAMBAN-4 under control and supplementary UV-B exposed conditions**

Treat ment	Days after seed sowing	Number of leaves	Total area (cm <sup>2</sup> )	leaf area	Leaf index	Specific leaf weight (g <sup>-2</sup> )	Fresh weight of foliage (g)	Dry weight of foliage (g)
Control	15	2	83.34		0.447	0.009	1.698	0.081
	30	4	346.18		0.745	0.009	0.346	0.238
	45	6	677.55		1.025	0.007	1.982	0.479
	60	6	1172.37		1.593	0.006	0.927	0.718
UV-B	15	2	37.06		0.436	0.003	1.639	0.064
	30	3	225.02		0.555	0.003	0.176	0.118
	45	3	242		0.726	0.006	0.731	0.170
	60	4	937.88		0.818	0.005	0.763	0.612

**Table 2: Changes in growth parameters of *Vigna mungo* (L.) Hepper var. VAMBAN-4 under control and supplementary UV-B exposed conditions**

Treat ment	Days after seed sowing	Root length (cm)	Shoot length (cm)	Shoot / root ratio	Root fresh wt. (g)	Shoot fresh wt. (g)	Plant fresh wt. (g)	Root dry wt. (g)	Shoot dry wt. (g)	Plant dry wt. (g)	Relative growth rate
Control	15	7.5	16.3	2.17	1.401	2.005	3.687	0.028	0.129	0.157	-
	30	9	28.3	3.13	0.091	1.077	1.168	0.056	0.445	0.501	0.03
	45	11.5	39.5	3.43	0.855	4.031	4.886	0.186	0.948	1.134	0.04
	60	42	45	1.07	3.778	4.093	7.151	0.746	2.206	0.852	0.02
UV-B	15	5.8	12.2	2.16	1.754	1.933	3.406	0.011	0.106	0.117	-
	30	8	23.5	2.93	0.042	0.593	0.635	0.035	0.226	0.261	0.03
	45	6.6	27.5	4.17	0.229	2.143	2.372	0.114	0.492	0.606	0.03
	60	35	36.8	1.05	0.557	3.373	4.652	0.159	1.750	1.909	0.01

**Table 3: Changes in yield components of *Vigna mungo* (L.) Hepper var. VAMBAN-4 under control and supplementary UV-B exposed conditions**

Treat ment	Days after seed sowing	Pod number plant <sup>-1</sup>	Singl e pod wt. (g)	Pod wt. plant <sup>-1</sup> (g)	Lengt h of pod (cm)	Seed number pod <sup>-1</sup>	Seed number plant <sup>-1</sup>	Seed mass pod <sup>-1</sup> (g)	Seed mass plant <sup>-1</sup> (g)	Shellin g percent age plant <sup>-1</sup>	Harv est index
Control	60	5	0.458	1.350	4.50	7	28	0.265	0.929	68.81	63.07
UV-B	60	2	0.153	1.359	3.50	5	10	0.197	0.259	72.14	13.45

Since the success of pollination is linked to the availability of pollinators, any phenological dislocation would affect the reproductive success greatly (Caldwell, 1968; Ziska *et al.*, 1992; Staxen and Bornman, 1994; Musil, 1995; Klaper *et al.*, 1996; Mark *et al.*, 1996; Rajendiran and Ramanujam, 2004). In fact, the reproductive organs like pollen and ovules have effective protective features. The UV-B absorbing compounds are abundant in the floral parts (sepals and petals) and more specifically on the walls of pollens and ovaries (Day and Demchik, 1996). Though very little work has been done on the phenological changes, even minor alterations could adversely affect the agricultural systems. Simulating a 40 % ozone reduction, Esser (1980) found the yield of potato reduced by 41 %, spinach by 66 %, cabbage by 49 %

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and bean by 75 %, for a 20 % ozone depletion the yield of green gram was decreased by 25 to 45 % (Rajendiran and Ramanujam, 2004) and 20 to 56 % reduction in cowpea (Kokilavani and Rajendiran, 2014e). In 1991, Giller observed the yield of cotton and soybean reduced by 23 and 25 % respectively under field conditions also. Supplementary UV-B altered the DNA and protein, which in turn altered the vital metabolisms including photosynthesis reflecting them in the form of reduced yield and nutrition content in the grains (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004). From the results obtained in this study, it is evident that an increase in UV-B flux into the Earth's surface would have a direct impact on crops thereby causing threat to food availability.

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