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Research Article

IMPACT OF SUPPLEMENTARY UV-B RADIATION ON THE MORPHOLOGY, GROWTH AND YIELD OF *VIGNA MUNGO* (L.) HEPPER VAR. ADT-3

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ABSTRACT
The effect of ultraviolet-B (UV-B) radiation on the morphology and growth of black gram *Vigna mungo* (L.) Hepper var. ADT-3 was evaluated. Screening of black gram after exposure to supplementary UV-B radiation (2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) was carried out in terms of growth on 15, 30, 45 and 60 DAS. Supplementary UV-B irradiation reduced the number of leaves by 20 to 60 %. Total leaf area (83.23 %) and leaf area index (48.65 %). Specific leaf weight in UV-B irradiated increased by 18.08, 3.81 and 10.67 % on 15, 30 and 45 respectively, while it showed a reduction on 60 DAS. UV-B stress decreased the fresh weight of leaves by 7.93 to 80.74 % and dry weight of foliage by 33.33 to 76.08 % in all stages of UV-B exposed plants. Growth of all the varieties of black gram was progressively inhibited by the UV-B radiation. Suppression of root and shoot length ranged from 8.50 to 36.70 % respectively at all stages of growth resulting in reduced plant height and S/R (4.52 to 30.99 %). However, S/R ratio showed an increase of 38.91 % over control on 315 DAS. Plant dry weight after UV-B stress fell below control by 76.97 % 60 DAS. The relative growth rate (RGR) was reduced in all varieties with age as it reached 42.46 % reduction on 60 DAS. UV-B exposure decreased the entire yield components per plant basis, the decreases being 50 % in the pod number, 66.28 % in pod weight, 22.22 % in pod length, 61.29 % in seed number and 21.26 to 61.87 % in seed mass. Harvest index was the least under UV-B treatment which showed a reduction by 14.66 % compared with control. A similar pattern was obtained for data on shelling percentage also.

Keywords: *Ultraviolet-B, Black Gram, var. ADT-3, Morphology, Growth, Yield*

INTRODUCTION
If a stress like ultraviolet-B (UV-B) radiation is imposed on the plant during the active growth phase, the impact would be reflected on the gross morphology mediated through the metabolic labyrinth, as the plant struggles to negotiate the adverse effects and perform normally (Rajendiran, 2001). Elevated ultraviolet-B severely damages leaves (Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b; Kokilavani and Rajendiran, 2014c), inhibits plant growth (Rajendiran and Ramanujam, 2003, Rajendiran and Ramanujam, 2004) and suppresses 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 sensitive plants. Although literature on UV-B and plant interaction is voluminous, 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 has been blamed 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). The present work assumes further significance as the plants of black gram *Vigna mungo* (L.) Hepper var. ADT-3 has been grown under field conditions (*in situ*) allowing the interaction of PAR and other environmental factors.

MATERIALS AND METHODS
Black gram (*Vigna mungo* (L.) Hepper var. ADT-3) the nitrogen fixing grain legume was chosen for the study. Viable seeds of *Vigna mungo* (L.) Hepper var. ADT-3 was procured from Saravana Farms,

Villupuram, Tamil Nadu, India. The seeds were selected for uniform color, size and weight and used in the experiments. The crops were grown in pot culture in the naturally lit greenhouse (day temperature maximum 38 ± 2 ºC, night temperature minimum 18 ± 2 ºC, relative humidity 60 ± 5 %, maximum irradiance (PAR) 1400 µmol m^{-2} s^{-1}, photoperiod 12 to 14 h). Supplementary UV-B radiation 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 ($UV\text{-}B_{BE}$) of 12.2 kJ m^{-2} d^{-1} equivalents to simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received $UV\text{-}B_{BE}$ 10 kJ m^{-2} d^{-1}. 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 80º 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_1 and W_2 are dry masses of whole plants at t_1 and t_2 (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

In a series of experiments, the responses of black gram were assessed in terms of growth on 15, 30, 45 and 60 DAS. There were fewer leaves only (20 to 60 %) 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 throughout the growth period, the maximum being 83.23 % on 45 DAS (Table 1; Plate 1, Figure 1 to 4). The LAI was reduced by UV-B exposure to a larger extent (48.65 %) below control on 60 DAS (Table 1). The SLW in UV-B irradiated increased with age, showing a dip on 60 DAS (22.22 %). However, an increase of 18.08, 3.81 and 10.66 % were observed on 15, 30 and 45 DAS respectively. UV-B stress decreased the fresh weight of leaves by 7.93 to 80.74 %, with the maximum reduction being on 45 DAS. The dry weight of foliage decreased by 33.33 to 76.08 % 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 and became discretely chlorotic (Plate 1, Figure 5). Browning developed in patches indicating necrosis of the underlying tissues during later stages. Necrotic lesions appeared in older leaves which have received UV-B over a long time (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 36.70 % on 15 DAS which recovered after 45 DAS (Table 2). Shoot length of UV-B stressed plants decreased by 8.50 % within 15 DAS and continued so till 60 DAS with 18.80 % reduction (Table 2). The S/R ratio was decreased by UV-B stress by 4.52 to 30.99 %. However, S/R ratio showed an increase of 38.91 % over control on 15 DAS (Table 2). Fresh weight of roots increased with age in all treatments. But the biomass accumulation in root was inhibited by UV-B treatment by 1 to 45.34 % on all stages of growth. The trends observed in root and shoot biomass pattern were reflected at the whole plant level too with inhibitions at UV-B, little improvement in later stages with maximum reduction of 76.97 % on 45 DAS (Table 2, Plate 1, Figure 7). 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. Analyzed 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 (Nedunchezhian and Kulandaivelu, 1997; Nogues *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* (Dumpert and Knacher, 1985), *Helianthus annuus* (Tevini *et al*., 1991), O*enothera 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 22.82 to 42.46 % 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 50 % in the pod number, 66.28 % in pod weight, 22.22 % in pod length, 61.29 % in seed number and 21.26 to 61.87 % in seed mass (Table 3, Plate 1, Figure 9, 10). Analyzed on the basis of number of seeds per pod, only the UV-B treated plants had more fruits containing less number of seeds (Table 3).

Harvest index was the least under UV-B treatment which showed a reduction by 14.66 % compared with control. A similar pattern was obtained for data on shelling percentage also (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). Since the success of pollination is linked to the availability of pollinators, any phonological 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 % 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.

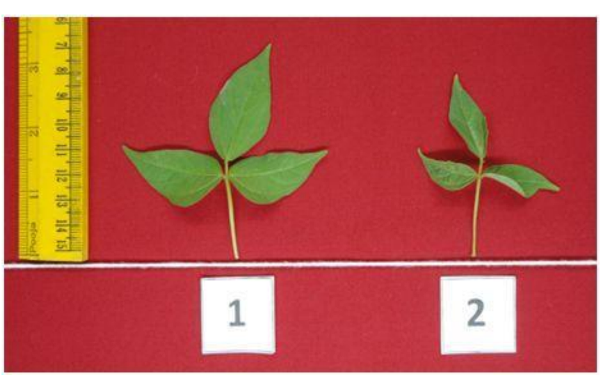

Figure 1: 15 DAS leaves

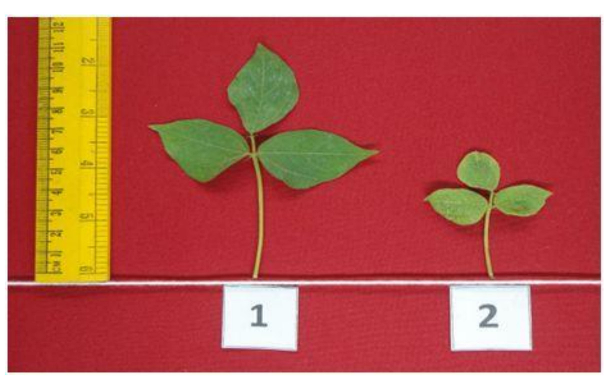

Figure 2: 30 DAS leaves

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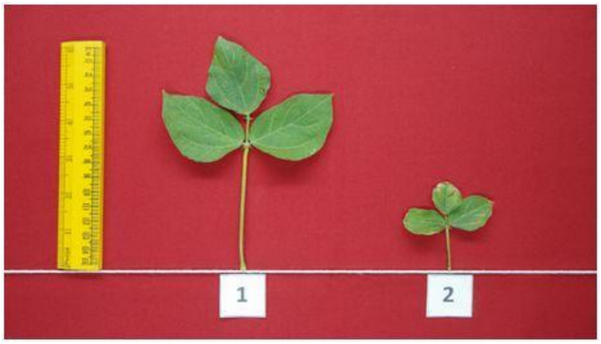

Figure 3: 45 DAS leaves

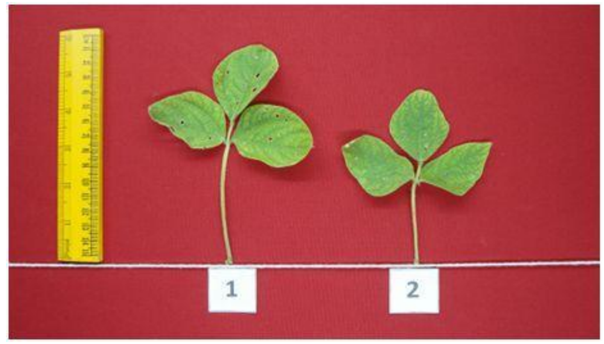

Figure 4: 60 DAS leaves

Figure 5: Chlorosis under UV-B exposure

Figure 6: Necrosis under UV-B exposure

Figure 7: 45 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 control (1) and ultraviolet-B (2) irradiated *Vigna mungo* (L.) Hepper var. ADT-3

Table 1: Changes in foliage of *Vigna mungo* (L.) Hepper var. ADT-3 under control and supplementary UV-B exposed conditions

Treatment	Days after seed sowing	Number of leaves	Total leaf area (cm^2)	Leaf area index	Specific leaf weight (g^{-2})	Fresh weight of foliage (g)	Dry weight of foliage (g)
	15	2	73.60	0.348	0.001	1.729	0.083
Control	30	3	249.97	0.509	0.006	1.921	0.155
	45	5	644.78	0.735	0.007	1.743	0.501
	60	5	521.20	0.747	0.001	1.464	0.944
	15	2	29.77	0.411	0.001	1.591	0.255
UV-B	30	3	63.83	0.428	0.008	1.424	0.053
	45	4	108.10	0.814	0.001	0.271	0.142
	60	2	157.87	0.383	0.001	0.282	0.225

Table 2: Changes in growth parameters of *Vigna mungo* (L.) Hepper var. ADT-3 under control and supplementary UV-B exposed conditions

Treatment	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
	15	7.9	17.5	2.21	1.311	2.115	4.826	0.029	0.136	0.165	-
Control	30	9.5	21	2.21	1.781	2.614	4.395	0.055	0.289	0.344	0.12
	45	10.5	36	3.42	4.381	4.258	6.639	0.200	1.043	1.243	0.12
	60	12.5	44.5	3.56	7.359	6.343	13.702	0.908	2.376	3.284	0.13
	15	5.2	16	3.07	2.703	1.824	4.127	0.013	0.091	0.104	-
UV-B	30	8.5	18	2.11	1.762	1.911	4.248	0.022	0.122	0.144	0.11
	45	9.5	22.5	2.36	3.234	2.191	5.068	0.078	0.329	0.407	0.11
	60	10	36.1	3.00	4.022	4.022	5.711	0.321	0.625	1.138	0.13

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

Treatment	Days after seed sowing	Pod number $plant^{-1}$	Single pod wt. (g)	Pod wt. $plant^{-1}$ (g)	Length of the pod (cm)	Seed number pod^{-1}	Seed number $plant^{-1}$	Seed mass pod^{-1} (g)	Seed mass $plant^{-1}$ (g)	Shelling percentage $plant^{-1}$	Harvest index
Control	60	6	0.460	2.192	4.5	7	31	0.221	1.023	25.95	66.76
UV-B	60	3	0.297	0.739	3.5	5	12	0.175	0.390	55.94	54.66

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).
A technique to ameliorate the UV-B induced suppression in crops needs to be designed in near future as the food security will be in danger if any further increase in ozone depletion occurs in the Earth's atmosphere.

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