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Research Article

IN VITRO PROPAGATION OF EXPLANTS FROM ULTRAVIOLET-B STRESSED *SPINACIA OLERACEA* L.

***Rajendiran K., Periyalakshmi D., Vijayalakshmi R. and Kokilavani V.**
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry - 605 008, India
**Author for Correspondence*

ABSTRACT
The *in vitro* regeneration was carried out in spinach (*Spinacia oleracea* L.) after *in situ* UV-B irradiation for the first time. Seeds, nodal stem explants and leaf explants (third from top of canopy) harvested on 30 days after seed germination from *in situ* control and supplementary ultraviolet-B irradiated (UV-B = 2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) spinach to analyze their viability for germplasm storage. UV-B suppressed the growth parameters in spinach at 15 DAS as well as on 30 DAS of growth. UV-B stress induced different types of abnormalities in the leaves of spinach. Unstressed spinach seeds responded *in vitro* germination. UV-B exposed dry spinach seeds failed to germinate under *in vitro* culture. UV-B exposed wet spinach seeds germinated under *in vitro* culture. UV-B irradiation suppressed height of seedlings at wet conditions compared with control. Plant biomass of seedlings from UV-B stressed wet seeds was 11.37 % below control. Callus induction did not occur in both control and UV-B irradiated stem explants. Stem explants from control and UV-B stressed *in situ* plants did not proliferate axillary buds. Callus induction did not occurred both in control and UV-B stressed spinach leaf explants. To conclude, only the seeds of spinach are suitable for germplasm conservation and regeneration for cultivating in enhanced UV-B environment.

Keywords: *Ultraviolet-B, Spinach, In Situ Growth, Seeds, Leaf Explants, Stem Explants, In Vitro Regeneration*

INTRODUCTION
Thickness of green house gases and ozone depleting substances (ODS) released by human activities around the earth continue to deplete ozone layer by direct as well as indirect methods. The heat which would normally escape the troposphere and enter the stratosphere was retained, making the stratosphere cooler. Very low temperatures in this layer enhance ozone reduction, increasing the UV-B radiation thereby affecting the ecosystems. Ultraviolet-B (UV-B) radiation (280-320 nm) is a dangerous atmospheric stress (Caldwell *et al.*, 1983; Jordan, 1997; Caldwell *et al.*, 1998) as it affects foliar epidermis (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000a; Rajendiran and Ramanujam, 2000b; Rajendiran, 2001; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b), suppresses photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and inhibits nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b, Sudaroli and Rajendiran, 2014; Arulmozhi and Rajendiran, 2014a; Arulmozhi and Rajendiran, 2014b; Arulmozhi and Rajendiran, 2014c; Vijayalakshmi and Rajendiran, 2014a; Vijayalakshmi and Rajendiran, 2014b; Vijayalakshmi and Rajendiran, 2014c) in sensitive plants. Hence screening methods have to be developed to select the best varieties of crops that are suitable for surviving in elevated UV-B environment and to conserve their germplasm. On this line, the present work is an attempt for the first time to find out the spinach that can tolerate supplementary UV-B irradiation and to identify the germplasm of the crop for conservation and regeneration through tissue culture method.

MATERIALS AND METHODS
Spinach (*Spinacia oleracea* L.) **(**Paalakerrai in Tamil) was chosen for the study. Viable seeds of spinach were procured from Madagadipet Seeds Depot, Pondicherry. 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 ± 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} equivalent to a 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}.

The responses of spinach in control and supplementary UV-B irradiation under *in situ* condition were assessed in terms of growth on 15 and 30 DAS.

Supplementary UV-B radiation was provided by one UV-B lamp (Philips TL 20W/12 Sunlamps, The Netherlands) which was suspended horizontally over the seeds. UV-B dose was maintained by adjusting the distance (30 cm) between seeds and the lamp. The lamp was wrapped with cellulose diacetate filters (0.076 mm) to filter UV-C radiation (< 290 nm). The filters were changed periodically to maintain uniform optical properties. UV-B exposure to seeds was given only once for two hours duration with one hour recovery time in between. Seeds received a biologically effective UV-B dose ($UV\text{-}B_{BE}$) of 12.2 kJ m^{-2} d^{-1}. The control seeds were exposed to sunlight for same duration receiving $UV\text{-}B_{BE}$ 10 kJ $m^{-2}d^{-1}$ with one hour recovery time in between (Caldwell, 1971).

Chemical composition of MS medium (Murashige and Skoog, 1962)

Constituents	Quantity (mg L^{-1})
Macronutrients	
NH_4NO_3	1650
KNO_3	1900
$CaCL_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Na.EDTA	37.23
$FeSO_4.7H_2O$	27.95
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4,5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Meso-Inositol	100
Glycine	2.0
Thiamine. HCl	0.1
Nicotinic acid	0.5
Pyridoxine. HCl	0.5
Sucrose (%w/v)	3 %
pH	5.8

Seeds, nodal shoot segments (stem explants) and leaf discs (leaf explants) after appropriate aseptic treatment were used for *in vitro* culture. The explants were thoroughly washed with water containing 0.1% Bavistin (a systemic fungicide BASF, India Ltd., Bombay) for 4-5 minutes. They were surface sterilized with 0.1% $HgCl_2$ for 4-5 minutes and washed 6 to 8 times with autoclaved water under Laminar Air Flow Cabinet (Technico Systems, Chennai) and inoculated aseptically onto culture medium. The final

wash was given with aqueous sterilized solution of (0.1%) ascorbic acid. The surface sterilized explants were dipped in 90% ethanol for a short period (40 seconds).

The seeds stem and leaf explants were inoculated vertically on MS medium for culture initiation. Different concentration and combination of cytokinins (6-benzyl amino purine – BAP and Kinetin ranging from 0.1 to 5.0 mgl^{-1}) and auxins (IAA - Indole acetic acid ranging from 0.1 to 1.0 mgl^{-1}) were incorporated in the medium for inducing bud breaking. These cultures were incubated at 28 ± 2˚ C in the dark for 2-3 days. Subsequently these were kept under diffused light (22 µ mol m^{-2} s^{-1} SFP- spectral flux photon) for 8 to 10 days. The light was provided by fluorescent tubes and incandescent bulbs. Temperature was maintained by window air conditioners. Positive air pressure was maintained in the culture rooms, in order to regulate temperature and to maintain aseptic conditions.

The cultures were regularly monitored and the growth parameters and callus proliferation were recorded after 15 DAI (days after inoculation) and 30 DAI. The experiments were carried out with three replicates per treatment. The plant tissue culture media generally comprise of inorganic salts, organic compounds, vitamins, gelling agents like agar-agar. All the components were dissolved in distilled water except growth regulators. Auxins were dissolved in 0.5N NaOH or ethanol and cytokinins were dissolved in dilute 0.1N HCl or NaOH. For the present study MS basal medium (Murashige and Skoog, 1962) was used as nutrient medium. MS basal medium was used either as such or with certain modification in their composition. Sucrose and sugar cubes were added as a source of carbohydrate. The pH of the media was adjusted to 5.8 ± 2 with 0.5N NaOH or 0.1N HCl before autoclaving. The medium was poured in the culture vessels. Finally the medium was steam sterilized by autoclaving at 15 psi pressure at 121˚C for 15 minutes.

Preparation of MS Medium

Approximately 90 % of the required volume of the deionized-distilled water was measured in a container of double the size of the required volume. Dehydrated medium was added into the water and stirred to dissolve the medium completely. The solution was gently heated to bring the powder into solution. Desired heat stable supplements were added to the medium solution. Deionized-distilled water was added to the medium solution to obtain the final required volume. The pH was adjusted to required level with NaOH or HCl. The medium was finally dispensed into culture vessels. The medium was sterilized by autoclaving at 15 psi (pounds per square inch) at 121˚C for appropriate period of time.

Photography

Plants grown under *in situ* condition and *in vitro* cultures tubes were photographed in daylight using a Sony digital camera fitted with appropriate close-up accessories.

RESULT AND DISCUSSION

In situ Studies

In spinach there were fewer leaves only showing a reduction by 6.67 to 11.11 % under UV-B stress on 15 DAS and 30 DAS respectively, but plants under normal ambience had greater number of leaves (Table 1 to 2; Plate 1). Supplementary UV-B irradiation reduced the total leaf area throughout the growth period, the maximum being 75.01 % on 30 DAS. The LAI was reduced by UV-B exposure to a larger extent (56.01 %) on 15 DAS, the maximum being 62.29 % below control on 30 DAS. The SLW in UV-B irradiated increased with age. An average increase of 91.51 and 75.42 % were observed on 15 and 30 DAS, respectively. UV-B stress decreased the fresh weight of leaves by 4.95 % on 15 DAS, with the maximum reduction being 12.92 % on 30 DAS. The dry weight of foliage decreased by 36.97 to 57.44 % on 15 and 30 DAS of UV-B exposed plants (Table 1 to 2; Plate 1). Reductions in leaf area and mass were observed in the field-grown sweetgum plants exposed to elevated UV-B radiation (Sullivan *et al.*, 1994) and *in situ* pot-grown ten varieties of cowpea (Kokilavani and Rajendiran, 2014a; 2014b, 2014c, 2014d, 2014e, 2014f, 2014g, 2014h, 2014i, 2014j, 2014k, 2014l, 2014m, 2014n, 2014o). 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. According to Britz and Adamse (1994) inhibitions are part of general UV-B effects.

Figure 1: On 15 DAS

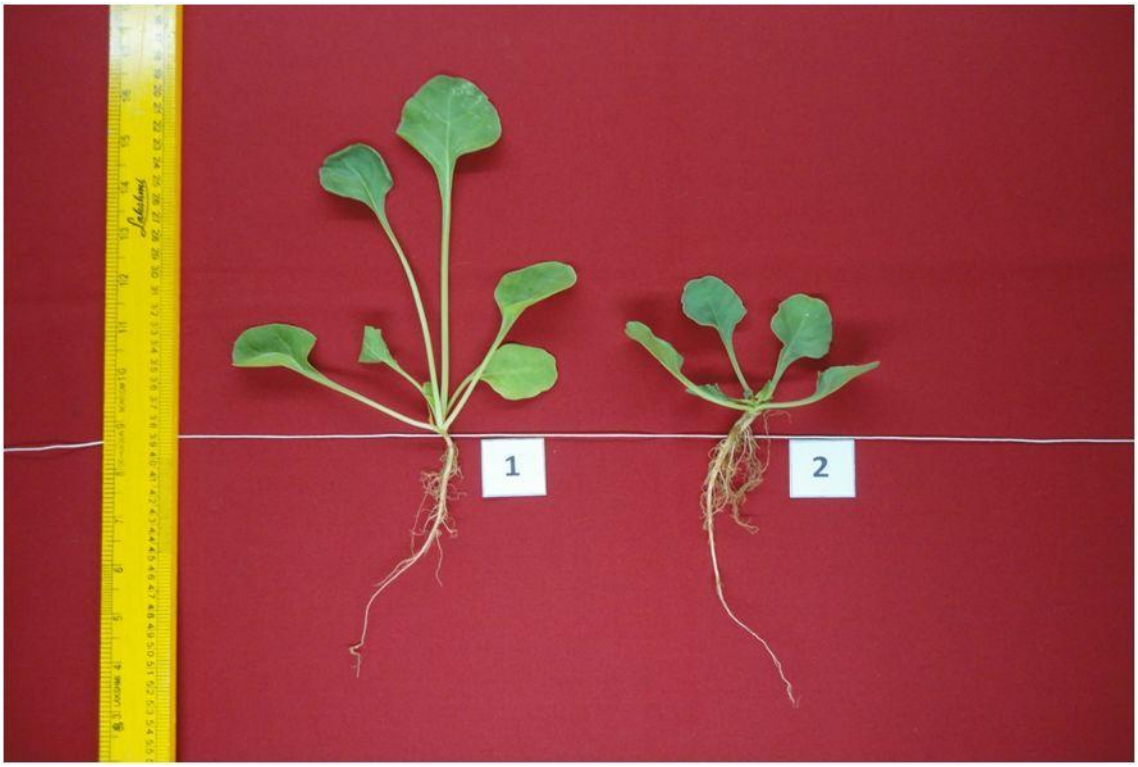

Figure 2: On 30 DAS

Plate 1: The control and supplementary UV-B stressed plants of *Spinacia oleracea* L. (1: Control, 2: UV-B)

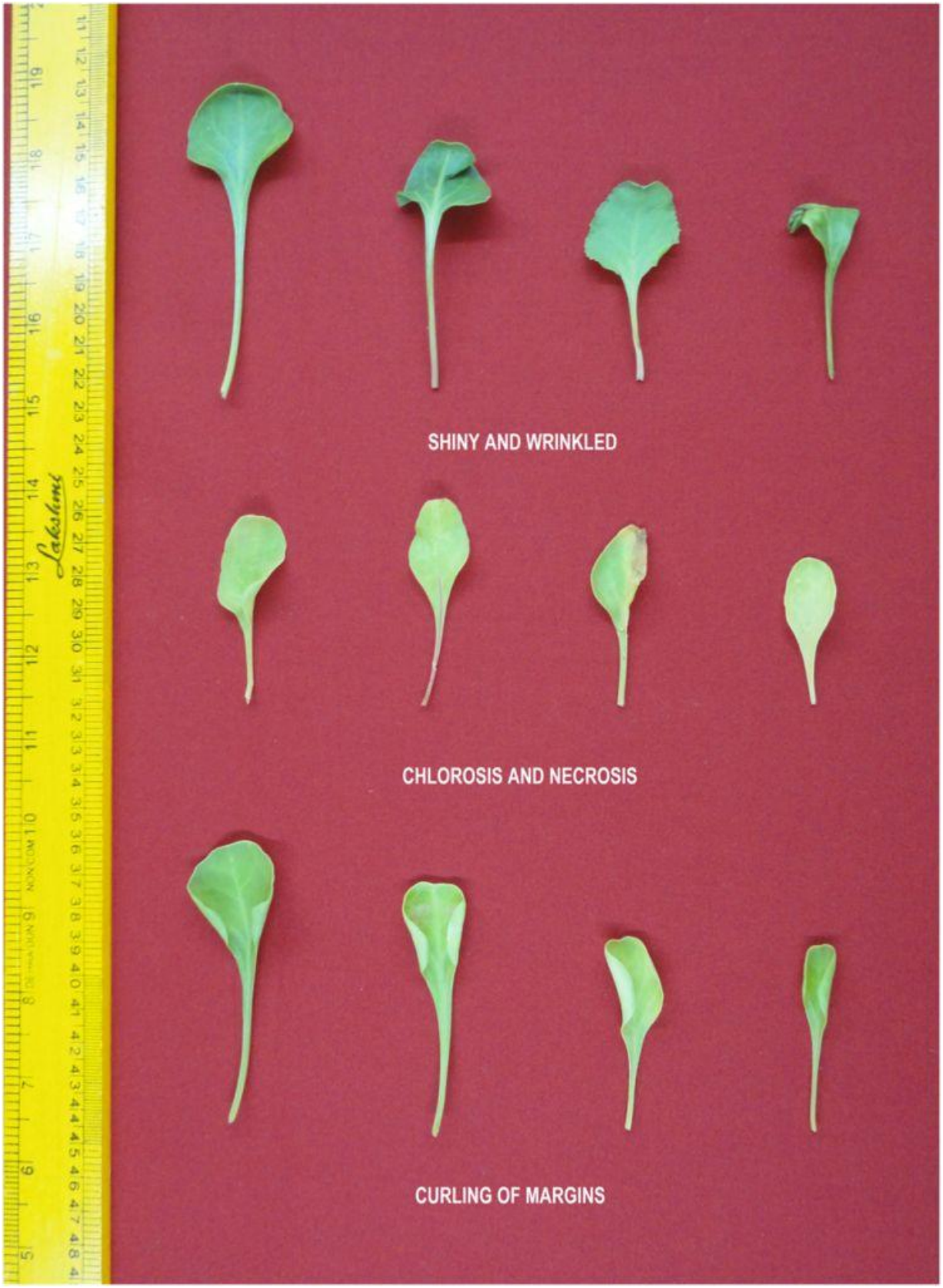

Plate 2: Types of foliar injury caused by elevated UV-B radiation in *Spinacia oleracea* L. on 30 DAS

1 DAI Figure 1: Control

Figure 2: UV-B on dry seed

Figure 3: UV-B on soaked seed

7 DAI Figure 4: Control

Figure 5: UV-B on dry seed

Figure 6: UV-B on soaked seed

Plate 3: *In vitro* seed germination and growth of *Spinacia oleracea* L. in control and UV-B irradiated dry and soaked seeds (DAI - Days after inoculation)

7 DAI: Stem explants Figure 1: Control

Figure 2: UV- B

7 DAI: Leaf explants Figure 3: Control

Figure 4: UV- B

Plate 4: *In vitro* callus proliferation from stem and leaf explants of control and UV-B irradiated *Spinacia oleracea* L. plants. (DAI - Days after inoculation)

Table 1: Changes in foliage of 15 DAS *Spinacia oleracea* L. in control and UV-B irradiated plants– *In situ*

Treatment	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)
Control	5.00	122.02	0.200	0.273	1.161	0.495
UV-B	4.66	60.51	0.379	0.023	0.103	0.312

Table 2: Changes in foliage of 30 DAS *Spinacia oleracea* L. in control and UV-B irradiated plants– *In situ*

Treatment	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)
Control	6.00	323.30	0.331	0.409	3.230	1.857
UV-B	5.33	89.78	0.125	0.100	2.812	0.790

Table 3: Changes in growth parameters of 15 DAS *Spinacia oleracea* L. in control and UV-B irradiated plants– *In situ*

Treatment	Plant height (cm)	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	8.16	5.93	1.93	2.91	0.395	1.154	2.711	0.212	0.506	1.214	0.02
UV-B	4.3	4.00	0.63	0.78	0.224	0.282	1.629	0.171	0.149	0.632	0.01

Table 4: Changes in growth parameters of 30 DAS *Spinacia oleracea* L. in control and UV-B irradiated plants – *In situ*

Treatment	Plant height (cm)	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	14.43	10.7	3.73	3.41	1.89	6.476	11.605	1.602	1.797	5.257	0.04
UV-B	12.46	11.4	1.06	1.56	2.277	3.564	8.655	1.652	1.611	4.054	0.04

Table 5: Changes in growth parameters of 15 DAI *Spinacia oleracea* L. in control and UV-B irradiated soaked seeds– *In vitro*

Treatment	Plant height (cm)	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	10.0	1.8	7.0	1.16	1.657	1.925	3.582	0.688	0.738	1.427	0.03
UV-B	7.5	1.5	6.0	1.13	1.489	1.685	3.175	0.475	0.579	1.055	0.03

On prolonged exposure to UV-B the leaves of spinach exhibited different types of abnormalities (Plate 2). The leaves became generally pale which became waxy, shiny and wrinkled. The yellowing intensified and became discretely chlorotic. 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. The leaves exhibited curling of margins and became dry and brittle. More abnormalities were reported in black gram (Kokilavani and Rajendiran, 2013), cucumber (Kokilavani and Rajendiran, 2014b) and several varieties of cowpea grown under *in situ* UV-B exposure (Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran, 2014b; Kokilavani and Rajendiran, 2014c; Kokilavani and Rajendiran, 2014e; Kokilavani and Rajendiran, 2014f; Kokilavani and Rajendiran, 2014g; Kokilavani and Rajendiran, 2014j; Kokilavani and Rajendiran, 2014k; Kokilavani and Rajendiran, 2014l; Kokilavani and Rajendiran,

2014m; Kokilavani and Rajendiran, 2014n; Kokilavani and Rajendiran, 2014o; Kokilavani and Rajendiran, 2015a; Kokilavani and Rajendiran, 2015b).

Root length was reduced after UV-B exposure by 1.68 % on 15 DAS, where as root growth was increased by 6.54 % till 30 DAS (Table 3 to 4; Plate 1). Shoot length of UV-B stressed plants decreased by 67.24 % within 15 DAS and continued so till 30 DAS with 71.43 % reduction. The S / R ratio was decreased by UV-B stress by 73.05 % on 15 DAS and by 54.18 % on 30 DAS compared with control plants. Fresh weight of roots increased with age in all treatments. The biomass accumulation in root of spinach was inhibited by UV-B treatment by 12.16 % on 15 DAS, however, an increase of 19.99 % was noticed on 30 DAS. A general decrease of 75.51 % in shoot fresh weight of UV-B treated plants was observed. The same trend was maintained till 30 DAS (44.96 %) of growth. The trends observed in root and shoot biomass pattern were reflected at the whole plant level too with inhibitions at UV-B by 36.06 % on 15 DAS and a reduction of 25.43 % on 30 DAS. A gradual reduction in the root biomass content (19.63 %) was recorded on 15 DAS after UV-B treatment, which showed an enhancement by 3.12 % over control on 30 DAS. UV-B exposure suppressed dry weight of shoot by 70.57 % on 15 DAS, reaching a minimum of 10.05 % on 30 DAS over control. Plant dry weight increased with age but after UV-B stress, it fell below control by 47.91 % on 15 DAS and 22.88 % on 30 DAS.

Inhibitions of growth indicated by reductions in root and shoot length and biomass content on UV-B exposure are characteristic of UV-B stressed plants as in *Vigna unguiculata* (Kulandaivelu *et al.,* 1989), *Phaseolus vulgaris* (Mark and Tevini, 1997), *Vigna mungo* (Rajendiran and Ramanujam, 2000a) and *Vigna radiata* (Rajendiran and Ramanujam, 2003) and ten varieties of cowpea (Kokilavani and Rajendiran, 2014o). 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).

The relative growth rate (RGR) was lowered by 44.98 % in 15 DAS UV-B irradiated plants which showed an enhancement in RGR by 10.34 % over control on 30 DAS (Table 3 to 4). Similar inhibitions of RGR by UV-B were observed by Jain *et al.,* (1999) in mungbean and in ten varieties of cowpea (Kokilavani and Rajendiran, 2014o).

In vitro Studies

The seeds of unstressed spinach responded *in vitro* germination. The UV-B stressed dry seeds did not respond. However, UV-B irradiated wet seeds of spinach responded differently from the dry seeds as they germinated profusely under *in vitro* condition. Only UV-B stressed wet seeds responded to *in vitro* germination along with control. UV-B exposure reduced root length significantly by 16.67 % on 15 DAI. Unstressed plants showed profuse root growth on 15 DAI (days after inoculation). UV-B stressed wet seeds of spinach responded to *in vitro* germination but performed poorly showing a reduction in root length by 16.67 % on 15 DAI. UV-B exposure in wet seeds reduced shoot length by 25.71 % on 15 DAI compared to unstressed seeds, which showed profuse shoot growth on 15 DAI (Table 5; Plate 3). Overall, the height of the seedling was suppressed by UV-B irradiation under wet conditions in spinach compared with control. UV-B stressed wet seeds of spinach showed enhanced S / R ratio by 2.61 % over control on 15 DAI. Biomass accumulation in root was inhibited by UV-B irradiation by 10.11 % in wet seed treatments on 15 DAI. A general decrease of 12.46 % in shoot fresh weight after UV-B exposure to wet seeds was observed on 15 DAI. The trends observed in root / shoot biomass pattern were reflected at the whole seedling level too with inhibitions by 11.37 % in UV-B irradiated wet seeds. Reduction in the root biomass content by 30.92 % on 15 DAI was caused by UV-B treatment in wet seed treatments. UV-B exposure suppressed dry weight of shoot by 21.57 % on 15 DAI over control in wet seed treatment. Plant dry weight increased with age in control and in all treatments. But after UV-B stress, it fell below control by 26.08 % on 15 DAI after wet seed exposures. The relative growth rate (RGR) of seedlings was lowered by 9.21 % in UV-B irradiated wet seeds. This is in accordance with the report of Rajendiran *et al.,* (2014a, 2014b) after experimenting with the *in vitro* regeneration of UV-B stressed seeds in ten varieties of cowpea.

Proliferation of axillary buds did not occur in stem explants of control and stem explants from UV-B stressed spinach taken for study. Callus induction did not occur both in control stem explants as well as in stem explants harvested from *in situ* supplementary UV-B irradiated plants (Plate 4, Figure 1 to 2). Similar experiments were carried out by Rajendiran *et al.,* (2014c) with *in vitro* regeneration of stem explants harvested from *in situ* grown UV-B stressed ten varieties of cowpea.

Callus induction was not observed in spinach both in control leaf explants as well as in leaf explants harvested from *in situ* supplementary UV-B irradiated crops (Plate 4, Figure 3 to 4). However Rajendiran *et al.,* (2014d) reported callus proliferation with leaf explants harvested from *in situ* grown UV-B stressed ten varieties of cowpea.

From all the parameters assessed *viz.,* growth under *in situ* condition and the responses of seeds and explants under *in vitro* culture, the present study recommended that only the seeds of spinach are best suited for germplasm conservation and regeneration for cultivating in elevated UV-B environment.

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REFERENCES

Arulmozhi D and Rajendiran K (2014a). Effect of supplementary ultraviolet-B radiation on nodulation and nitrogen metabolism in *Lablab purpureus* L. var. Goldy. *International Journal of Advanced Biological Research* **4**(3) 43-346.

Arulmozhi D and Rajendiran K (2014b). Supplementary ultraviolet-B induced reduction in nodulation and nitrogen metabolism in hyacinth bean. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 73-77.

Arulmozhi D and Rajendiran K (2014c). Effect of elevated ultraviolet-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. COFC-8. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(2) 184-188.

Bornman JF and Vogelmann TC (1991). Effect of UV-B radiation on leaf optical properties measured with fibre optics. *Journal of Experimental Botany* **42** 547-554.

Britz SJ and Adamse P (1994). UV-B induced increase in specific leaf weight of cucumber as a consequence of increased starch content. *Photochemistry and Photobiology* **60** 116-119.

Caldwell MM (1971). Solar UV irradiance and the growth and development on higher plants. In: *Photophysiology*, edited by Giese AC **(**Academic Press), New York **6** 131-177.

Caldwell MM, Bjorn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH and Tevini (1998). Effects of increased solar ultraviolet radiation on terrestrial ecosystems. *Photochemistry and Photobiology* **46** 40-52.

Caldwell MM, Gold WG, Harris G and Ashurst CW (1983). A modulated lamp systam for solar UV-B (280-320 nm) Supplementation studies in the field. *Photochemistry and Photobiology* **37** 479 - 485.

Huang S, Dai Q, Peng S, Chavez AO, Miranda MLL, Visperas RM and Vergara BS (1997). Influence of supplementary ultraviolet-B on indole acetic acid and calmodulin in the leaves of rice (*Oryza sativa* L.). *Plant Growth Regulation* **21** 59-64.

Jain VK, Dhingra GK and Ambrish K (1999). Changes in productivity and biomass partitioning in field grown mungbean with response to supplementary UV-B radiation. In: *Plant Physiology for Sustainable Agriculture*, edited by Srivastava GC, Singh K and Pal M (Pointer Publishers) Jaipur, India 301-308.

Jordan BR (1997). Effects of UV-B radiation on plants - An overview. In. UV radiation and its Effects: An update. Report of a workship sponsoredby the National Science Strategy Committee for Climate Change. *The Royal Society of New Zealand* **49** 27-28.

Kokilavani V and Rajendiran K (2013). Ultraviolet-B induced changes in the leaf epidermal and anatomical characteristics of *Vigna mungo* L.var. KM-2. *International Journal of Science and Nature* **5**(1) 126-130.

Kokilavani V and Rajendiran K (2014a). Changes in leaf architecture of *Vigna unguiculata* (L.)Walp. cv. BCP-25 after exposure to elevated ultraviolet-B radiation. *International Journal of Science and Nature* **5**(3) 542-546.

Kokilavani V and Rajendiran K (2014b). Ultraviolet-B induced changes in the leaf architecture of *Cucumis sativus* L. var. CO 1. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 208-215.

Kokilavani V and Rajendiran K (2014c). Ultraviolet-B induced changes in the leaf epidermal and anatomical characteristics of *Vigna mungo* L. var. KM-2. *International Journal of Advanced Biological Research* **5**(1) 126-130.

Kokilavani V and Rajendiran K (2014d). Effect of supplementary UV-B radiation on the yield of ten varieties of cowpea. *International Journal of Geology, Earth and Environmental Sciences* **4**(3) 65-73.

Kokilavani V and Rajendiran K (2014e). Influence of elevated Ultraviolet-B radiation on foliar organisation in *Vigna unguiculata* (L.) Walp. c.v. CW-122. *International Journal of Innovative Research and Review* **2**(4) 53-60.

Kokilavani V and Rajendiran K (2014f). Evaluation of the impact of Ultraviolet-B radiation on the foliar epidermal and anatomical characteristics of *Vigna unguiculata* (L.) Walp. c.v. COVU-1. *International Journal of Innovative Research and Review* **2**(4) 61-68.

Kokilavani V and Rajendiran K (2014g). Variation in leaf architecture of *Vigna unguiculata* (L.) Walp. c.v. COFC-8 induced by supplementary UV-B exposure. *International Journal of Innovative Research and Review* **2**(4) 69-76.

Kokilavani V and Rajendiran K (2014h). Ultraviolet-B induced reduction in nodulation in ten varieties of cowpea. *International Journal of Innovative Research and Review* **2**(4) 77-82.

Kokilavani V and Rajendiran K (2014i). *In vitro* regeneration of apical shoot explants from *in situ* grown UV-B stressed *Ocimum sanctum* L. *International Journal of Biotechnology* **3**(4) 67-71.

Kokilavani V and Rajendiran K (2014j). Efficacy of *Vigna unguiculata* (L.) Walp. cv. Vamban leaves to withstand supplementary ultraviolet-B irradiation. *International Journal of Geology, Earth and Environmental Sciences* **4**(3) 203-210.

Kokilavani V and Rajendiran K (2014k). Anatomical and epidermal alterations in the leaves of *Vigna unguiculata* (L.) Walp. cv. CO-6 due to UV-B exposure. *International Journal of Geology, Earth and Environmental Sciences* **4**(3) 211-218.

Kokilavani V and Rajendiran K (2014l). A survey on the adaptive mechanism in leaf architecture of *Vigna unguiculata* (L.) Walp. cv. KM-1 under ultraviolet-B radiation. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 50-57.

Kokilavani V and Rajendiran K (2014m). Modifications in leaf architecture of *Vigna unguiculata* (L.) Walp. cv. COVU-2 to defend from ultraviolet-B radiation. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 65-72.

Kokilavani V and Rajendiran K (2014n). Analysis of the UV-B induced changes in morphology, anatomy and epidermis of *Vigna unguiculata* (L.) Walp. cv. CO-1 leaves. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 87-94.

Kokilavani V and Rajendiran K (2014o). Influence of elevated ultraviolet-B radiation on the morphology and growth of ten varieties of cowpea. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 171-189.

Kokilavani V and Rajendiran K (2015a). Variations in foliar morphology and anatomy of *Vigna unguiculata* (L.) Walp. c.v. CO-3 after supplementary ultraviolet-B exposure. *International Journal of Advanced Biological Research* **5**(1) 23-28.

Kokilavani V and Rajendiran K (2015b). Study of leaf architecture of *Vigna unguiculata* (L.) Walp. cv. Puduvai under elevated ultraviolet-B radiation. *International Journal of Advanced Biological Research* **5**(1) 34-39.

Kulandaivelu G, Maragatham S and Nedunchezhian N (1989). On the possible control of ultraviolet - B induced response in growth and photosynthetic activities in higher plants. *Physiologia Plantarum* **76** 398 - 404.

Mark SM AND Tevini M (1997). Effects of solar UV-B radiation on growth, flowering and yield of central and southern European bush bean cultivars (*Phaseolus vulgaris* L.). *Plant Ecology* **128** 114 - 125.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15** 473 - 497.

Rajendiran K (2001). Amelioration of ultraviolet-B radiation impacts in green gram by triadimefon. Ph.d. Thesis. Pondicherry University.

Rajendiran K, Kokilavani V and Murugananthan P (2014a). *In vitro* seed germination and growth of ten varieties of cowpea after ultraviolet-B irradiation. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 200-217.

Rajendiran K, Kokilavani V and Murugananthan P (2014b). *In vitro* germination of F_1 seeds harvested from ten varieties of cowpea grown under *in situ* supplementary UV-B radiation. *International Journal of Innovative Research and Review* **2**(4) 90-98.

Rajendiran K, Kokilavani V and Murugananthan P (2014c). *In vitro* callus proliferation from stem explants of ten varieties of cowpea after *in situ* ultraviolet-B exposure. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(3) 190-199.

Rajendiran K, Kokilavani V and Murugananthan P (2014d). *In vitro* callus proliferation from leaf explants of ten varieties of cowpea after *in situ* ultraviolet-B irradiation. *International Journal of Geology, Earth and Environmental Sciences* **4**(3) 268-277.

Rajendiran K and Ramanujam MP (2000a). Efficacy of Triadimefon treatment in ameliorating the UV-B stress in green gram. *National Symposium on Environmental Crisis and Security in the New Millennium, National Environmental Science academy, New Delhi, Abstract* 41 - 42.

Rajendiran K and Ramanujam MP (2000b). Growth and biochemical responses of black gram (*Vigna mungo* L. Hepper cv. T-9) to supplementary UV-B radiation. *State level seminar on Environmental Pollution and Bioremediation, PSGR Krishnammal College Coimbatore Abstract* 10.

Rajendiran K and Ramanujam MP (2003). Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* **46**(4) 621 - 624.

Rajendiran K and Ramanujam MP (2004). Improvement of biomass partitioning, flowering and yield by triadimefon in UV-B stressed *Vigna radiata* (L.) Wilczek. *Biologia Plantarum* **48**(1) 145 - 148.

Rajendiran K and Ramanujam MP (2006). Interactive effects of UV-B irradiation and triadimefon on nodulation and nitrogen metabolism in *Vigna radiata* plants. *Biologia Plantarum* **50**(4) 709 - 712.

Sudaroli Sudha J and Rajendiran K (2013a). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Sesbania grandiflora* (L.) Pers. *International Journal of Science and Nature* **4**(4) 664 - 667.

Sudaroli Sudha J and Rajendiran K (2013b). Effect of elevated UV-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. c.v. BCP-25. *International Journal of Food, Agriculture and Veterinary Sciences* **3**(3) 77 - 81.

Sudaroli Sudha J and Rajendiran K (2014). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. COVU-1. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 224-230.

Sullivan JH, Teramura AH and Dillenburg LR (1994). Growth and photosynthetic responses of field-grown sweetgum (*Liquidalmbar styraciflua*) seedlings to UV-B radiation. *American Journal of Botany* **81** 826 - 832.

Tevini M and Teramura AH (1989). UV-B effects on terrestrial plants. *Photochemistry and Photobiology* **50** 479 - 487.

Vijayalakshmi R and Rajendiran K (2014a). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Cyamopsis tetragonoloba* (L.) Taub. var. PNB. *International Journal of Geology, Earth and Environmental Sciences* **4**(2) 78 - 82.

Vijayalakshmi R and Rajendiran K (2014b). Impact of ultraviolet-B radiation on nodulation and nitrogen metabolism in *Phaseolus vulgaris* L. cv. Prevail. *International Journal of Advanced Biological Research* **4**(3) 339 - 342.
Vijayalakshmi R and Rajendiran K (2014c). Effect of elevated ultraviolet-B irradiation on the nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. CW-122. *International Journal of Food, Agriculture and Veterinary Sciences* **4**(2) 189-193.