OPEN ACCESS

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN: 2091-2609

Indexing and Abstracting

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CODEN (Chemical Abstract Services, USA): IJASKD

Vol-4, Issue-1 (March, 2016)

Available online at:
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http://www.nepjol.info/index.php/IJASBT/index

SEM-Biotech Publishing

Impact factor*: **1.422**
Scientific Journal Impact factor#: **3.419**
Index Copernicus Value: **6.02**

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).
#Impact factor is issued by SJIF INNO SPACE; **Impact factor is issued by INFOBASE INDEX.

K Rajendiran et al. (2016) Int J Appl Sci Biotechnol, Vol 4(1): 44-52
DOI: 10.3126/ijasbt.v4i1.14573

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

IN VITRO SCREENING OF STEM EXPLANTS OF *IN SITU* UV-B IRRADIATED COWPEA VARIETIES FOR GERMPLASM STORAGE

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Abstract

Even though seeds form the natural progenies of all sexually reproducing plants, change in climate makes it mandatory to device *in vitro* methods for selecting germplasm for conservation and regeneration. An experiment was conducted to induct callus in stem explants (nodal region from third node 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}) three varieties of cowpea (*Vigna unguiculata* (L.) Walp.) *viz.* GOWMATHI, FOLA and NS-634 to assess their response to *in vitro* culture. Axillary bud initiation occurred only from GOWMATHI control stem explants, while callus induction occurred in GOWMATHI and FOLA control stem explants. Stem explants harvested from all the three UV-B stressed cowpea varieties did not proliferate callus. The nodal stem explants of the three varieties of cowpea are not the suitable materials for germplasm preservation for growing in UV-B elevated climate.

Key words: Callus proliferation; Cowpea; Stem explants; Three varieties; Ultraviolet-B.

Introduction

Tissue culture techniques of biotechnology are of great importance as they can be used for collection, multiplication and storage of plant germplasm thereby helping the conservation of plant biodiversity in the changing environment. In addition to conservation of wild species of plants, much attention is required to preserve the germplasm of important food crops that struggles to survive in the climate change. Ozone depletion triggered by human activities allows plenty of ultraviolet-B (UV-B) radiation (280-320 nm) into Earth's surface creating stress on plants (Caldwell *et al.,* 1998), as it affects plant foliage (Kokilavani and Rajendiran 2013; Kokilavani and Rajendiran 2014a; Kokilavani and Rajendiran 2014b; Kokilavani and Rajendiran 2014c; Kokilavani and Rajendiran 2014d; Kokilavani and Rajendiran 2014f; Kokilavani and Rajendiran 2014g; Kokilavani and Rajendiran 2014h; Kokilavani and Rajendiran 2014j; Kokilavani and Rajendiran 2014k; Kokilavani and Rajendiran 2014l; Kokilavani and Rajendiran 2014m; Kokilavani and Rajendiran 2014n; Kokilavani and Rajendiran 2015a; Kokilavani and Rajendiran 2015b) causes aberrations in the epidermis of cotyledons (Rajendiran *et al.,* 2015b; Rajendiran *et al.,* 2015c), suppresses photosynthesis (Kulandaivelu *et al.,* 1989; Sullivan *et al.,* 1994; Rajendiran 2001) causes abnormalities in morphology and retards growth (Rajendiran and Ramanujam 2003; Rajendiran and Ramanujam 2004; Kokilavani and Rajendiran 2014; Rajendiran *et al.,* 2015j; Rajendiran *et al.,* 2015l; Rajendiran *et al.,* 2015m) reduces harvest (Mark and Tevini 1997; Rajendiran and Ramanujam 2004; Kokilavani and Rajendiran 2014e; Rajendiran *et al.,* 2015m) and disturbs nodulation and nitrogen metabolism (Rajendiran and Ramanujam 2006; Sudaroli Sudha and Rajendiran 2013a; Sudaroli Sudha and Rajendiran 2013b; Kokilavani and Rajendiran 2014i; Sudaroli Sudha and Rajendiran 2014a; Sudaroli Sudha and Rajendiran 2014b; Sudaroli Sudha and Rajendiran 2014c; Arulmozhi and Rajendiran 2014a; Arulmozhi and Rajendiran 2014b; Arulmozhi and Rajendiran 2014c; Vijayalakshmi and Rajendiran 2014a; Vijayalakshmi and Rajendiran 2014b; Vijayalakshmi and Rajendiran 2014c; Rajendiran *et al.,* 2015k) in a variety of sensitive crops. This work deals with identifying the variety of cowpea whose stem explants can tolerate ultraviolet-B radiation and induct callus in culture media.

Materials and Methods

In situ UV-B radiation

Cowpea (*Vigna unguiculata* (L.) Walp.) belonging to the family Fabaceae which is a nitrogen fixing grain legume was chosen for the study. Viable seeds of the three varieties of cowpea *viz.* GOWMATHI, FOLA and NS-634 (Namdhari Seeds) were procured from Saravana Farms, Villupuram, Tamil Nadu and from local farmers in Pondicherry, 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 ± 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 5 DAS (day after seed germination). 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}. Nodal shoot segments (stem explants) from third node from top of canopy were harvested from 30 DAS crops that received supplementary UV-B irradiation and sunlight in the *in situ* condition.

In vitro culture with stem explants

Nodal shoot segments (stem explants) after appropriate aseptic treatment were used for *in vitro* culture. Stem 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 stem 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 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.

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

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

The anatomical features were viewed through Nikon Labomed microscope under incident and translucent light and photographed using Sony digital camera fitted with Olympus adaptor. The culture tubes with stem explants and callus were photographed in daylight using a Sony digital camera fitted with appropriate close-up accessories.

Dendrogram

At least three replicates were maintained for all treatments and control. The experiments were repeated to confirm the trends. The result of single linkage clustering (Maskay 1998) was displayed graphically in the form of a diagram called dendrogram (Everstt 1985). The similarity indices between the three varieties of cowpea under study were calculated using the formula given by Bhat and Kudesia (2011).

$$\text{Similarity index} = \frac{\text{Total number of similar characters}}{\text{Total number of characters studied}} \times 100$$

Based on the similarity indices between the three varieties of cowpea, dendrogram was draw to derive the interrelationship between them and presented in Table 2 and Plate 5.

Results and Discussion

Standardisation of culture medium for stem explant

For the standardisation of culture media, stem explants from GOWMATHI variety of cowpea NIRMAL-7 grown under control condition were used. The explants were inoculated on MS medium for culture initiation containing different concentration and combination of cytokinins (6-benzyl amino purine - BAP = 2.0 mgL^{-1} and Kinetin = 0.1, 0.25 and 0.5 mgL^{-1}) and auxins (IAA - Indole acetic acid = 1.0 mgL^{-1}). The combination of cytokinins (6-benzyl amino purine - BAP = 2.0 mgL^{-1} and Kinetin = 0.25 mgL^{-1}) and auxins (IAA - Indole acetic acid = 1.0 mgL^{-1}) was found to be best suited for initiating callus and axillary buds in stem explants (Plate 1) and used for stem explants of all varieties of cowpea (Plate 2).

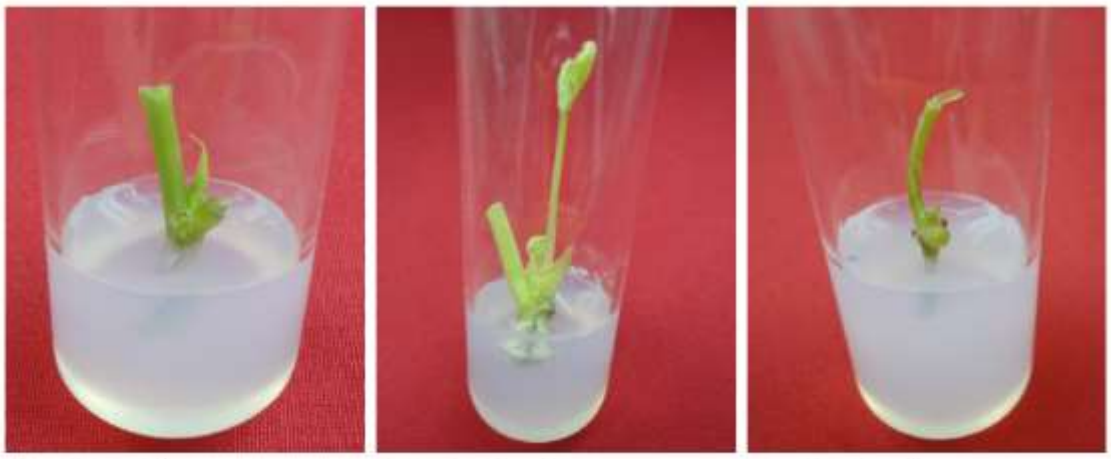

Plate 1: Standardisation of Kinetin (K) concentration in culture media for *in vitro* regeneration from stem explants using *Vigna unguiculata* (L.) Walp. var. GOWMATHI control samples. (7 DAI - Days after inoculation)

Table 1: Characteristics of callus / axillary bud proliferation in stem explants of three varieties of 30 DAI Vigna unguiculata (L.) Walp. from control and supplementary UV-B exposed conditions – In vitro.

Varieties	Treatment	Time taken for initiation (d)	Fresh weight (g)	Dry weight (g)	Parenchyma cell Frequency (µm)	Parenchyma cell size (µm)	
						Length	Breadth
GOWMATHI	Control	25	1.456	0.532	618.45± 1.27	126.45± 0.18	77.34± 0.45
	UV-B	-	-	-	-	-	-
FOLA	Control	23	0.765	0.154	285.47± 0.63	165.57± 1.68	128.36± 1.19
	UV-B	-	-	-	-	-	-
NS-634	Control	-	-	-	-	-	-
	UV-B	-	-	-	-	-	-

Table 2: The similarity indices in callus / axillary bud proliferation from stem explants of three varieties of *Vigna unguiculata* (L.) Walp. after supplementary UV-B exposure – *In vitro.*

Varieties	GOWMATHI	FOLA	NS-634
GOWMATH	100%	100%	12.5%
FOLA	100%	100%	12.5%
NS-634	12.5%	12.5%	100%

In vitro callus induction

Axillary bud initiation occurred from the stem explants of GOWMATHI excised from cowpea crops grown under *in situ* control condition only (Plate 3). All the explants from *in situ* grown FOLA and NS-634 both under control and UV-B failed to initiate axillary buds. Proliferation of callus occurred in stem explants of only in two out of three varieties of cowpea taken for study (Table 1; Plate 3). Callus induction was observed in GOWMATHI and FOLA both in control stem explants only. Callus formation did not occur in stem explants harvested from *in situ* supplementary UV-B irradiated crops. Stem explants from both control and UV-B exposed NS-634 variety of cowpea did not proliferate callus (Table 1; Plate 3 to 4). The induction of callus was delayed by two days in explants harvested from GOWMATHI compared with those of FOLA (Table 1). A reduction in the fresh biomass of callus by 47.46 % in FOLA occurred compared with the fresh weight of callus from GOWMATHI, both under control conditions. The trend observed with fresh weight continued in dry weight also, as GOWMATHI variety accumulated 71 % more dry weight than the callus from FOLA stem explants (Table 1).

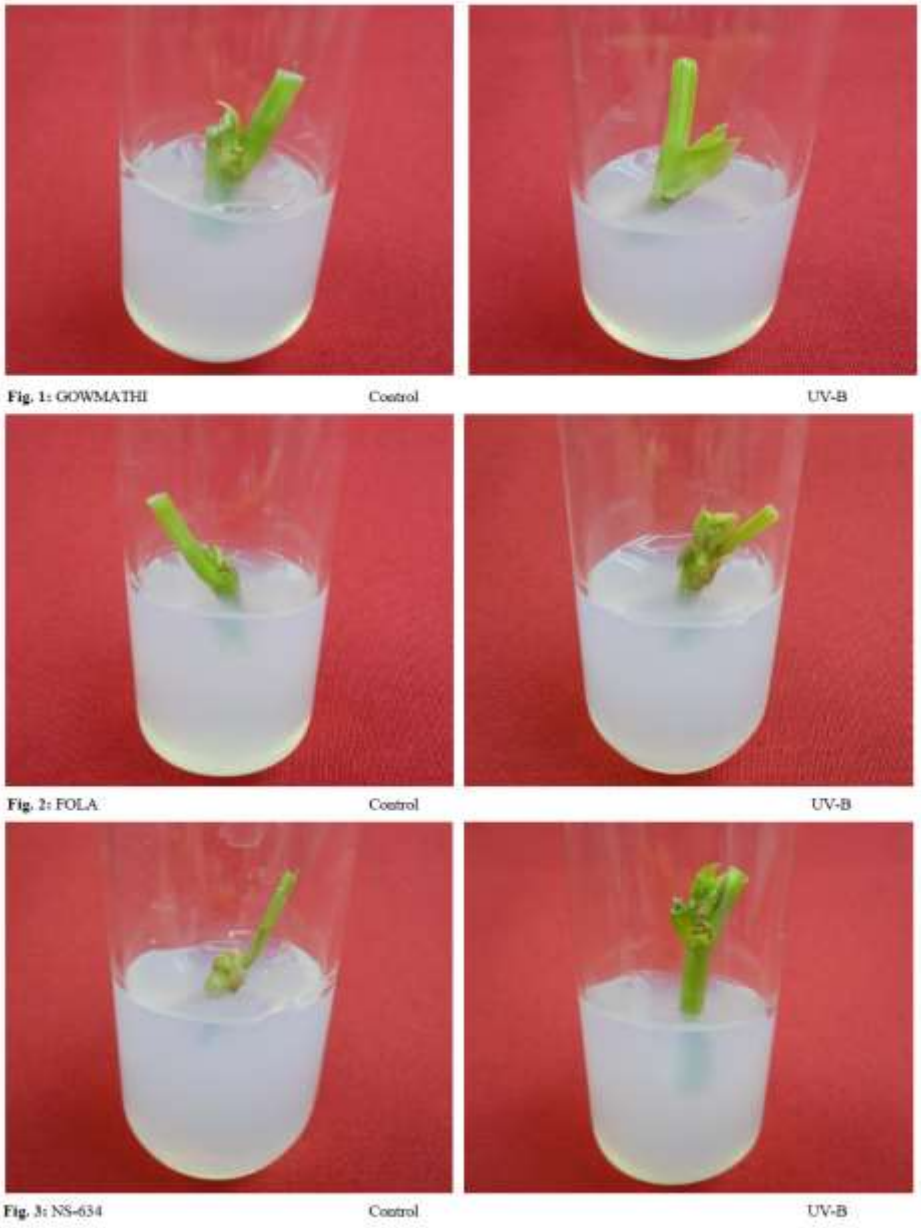

Plate 2: Innoculum for *in vitro* regeneration of three varieties of *Vigna unguiculata* (L.) Walp. from stem explants of control (C) and Ultraviolet-B (UV-B) irradiated plants.

The parenchyma cells appeared isodiametric with thin cell walls and were distributed evenly all through the callus in control samples (Plate 4). The parenchyma cells that have proliferated in the GOWMATHI callus were comparatively smaller (31 %) and more in number by 53.84 % than FOLA, both from stem explants harvested from controls (Table 1; Plate 4). Similar results were reported in *Amaranthus dubius* Mart. Ex. Thell. (Rajendiran *et al.*, 2015a), *Macrotyloma uniflorum* (Lam.) Verdc. (Rajendiran *et al.*, 2015d), *Momordica charantia* L. (Rajendiran *et al.*, 2015e), *Spinacia oleracea* L. (Rajendiran *et al.*, 2015f), *Trigonella foenum-graecum* (L.) Ser. (Rajendiran *et al.*, 2015g), *Benincasa hispida* (Thunb.) Cogn. (Rajendiran *et al.*, 2015h) and in *Portulaca oleracea* L. (Rajendiran *et al.*, 2015i) stem explants harvested from plants after UV-B irradiation. However, varied results in callus induction have been reported by Rajendiran *et al.*, (2014a) in leaf explants, in axillary bud and callus initiation by Rajendiran *et al.*, (2014b) in stem explants and *in vitro* germination of seeds by Rajendiran *et al.*, (2014c) using ten varieties of cowpea irradiated with UV-B rays.

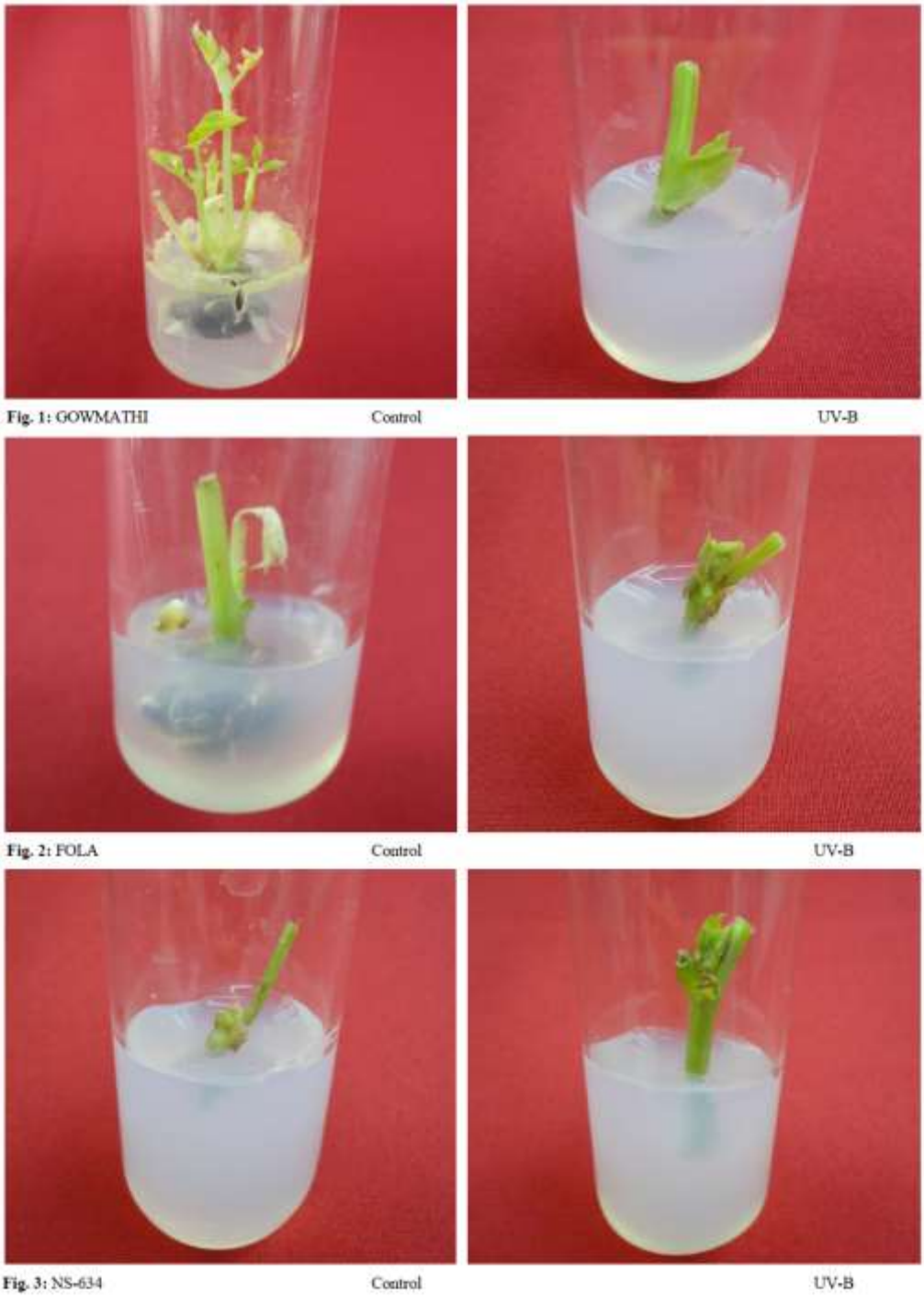

Plate 3: Comparison of *in vitro* callus / axillary bud proliferation from stem explants of three varieties of *Vigna unguiculata* (L.) Walp. on 30 DAI (Days after inoculation).

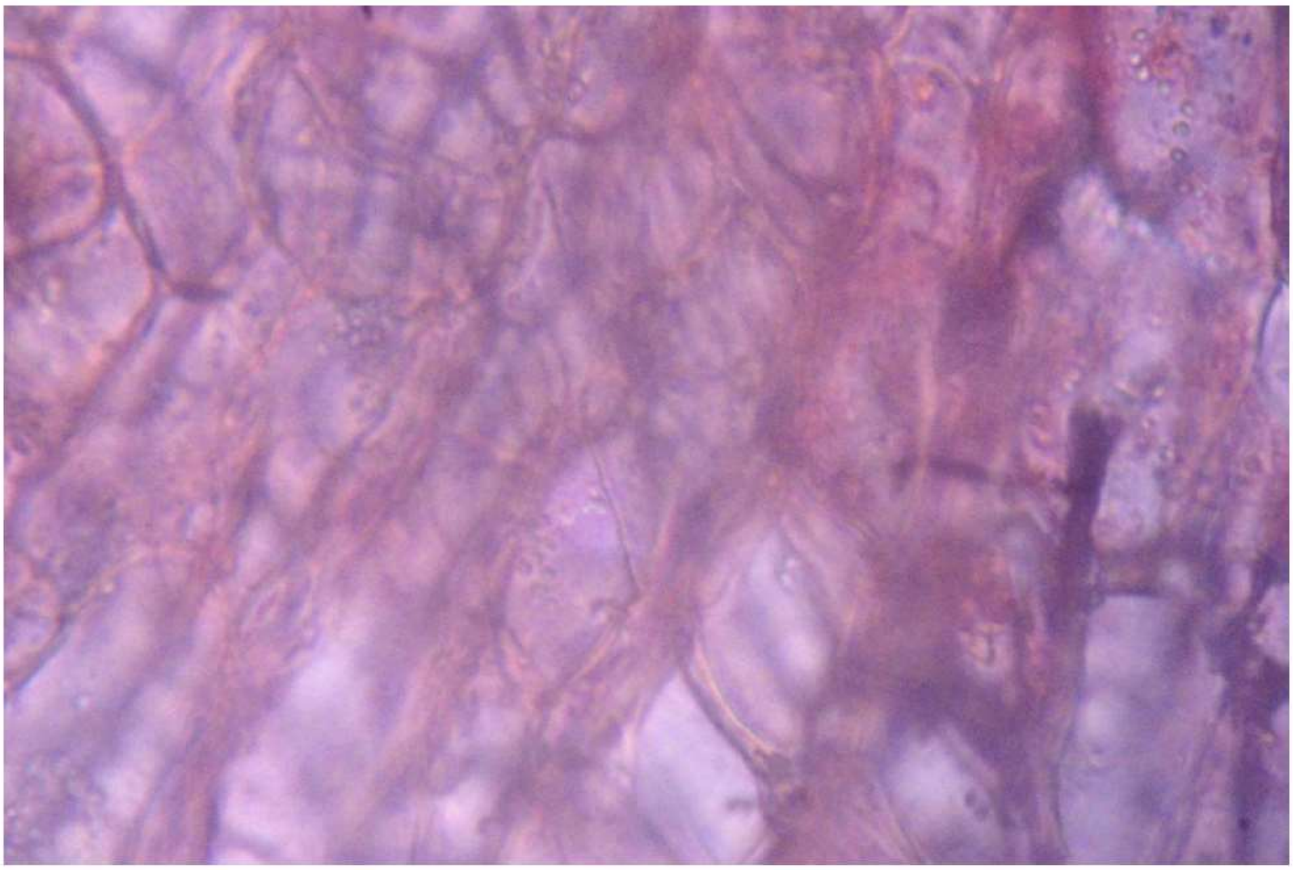

Fig. 1: GOWMATHI Control

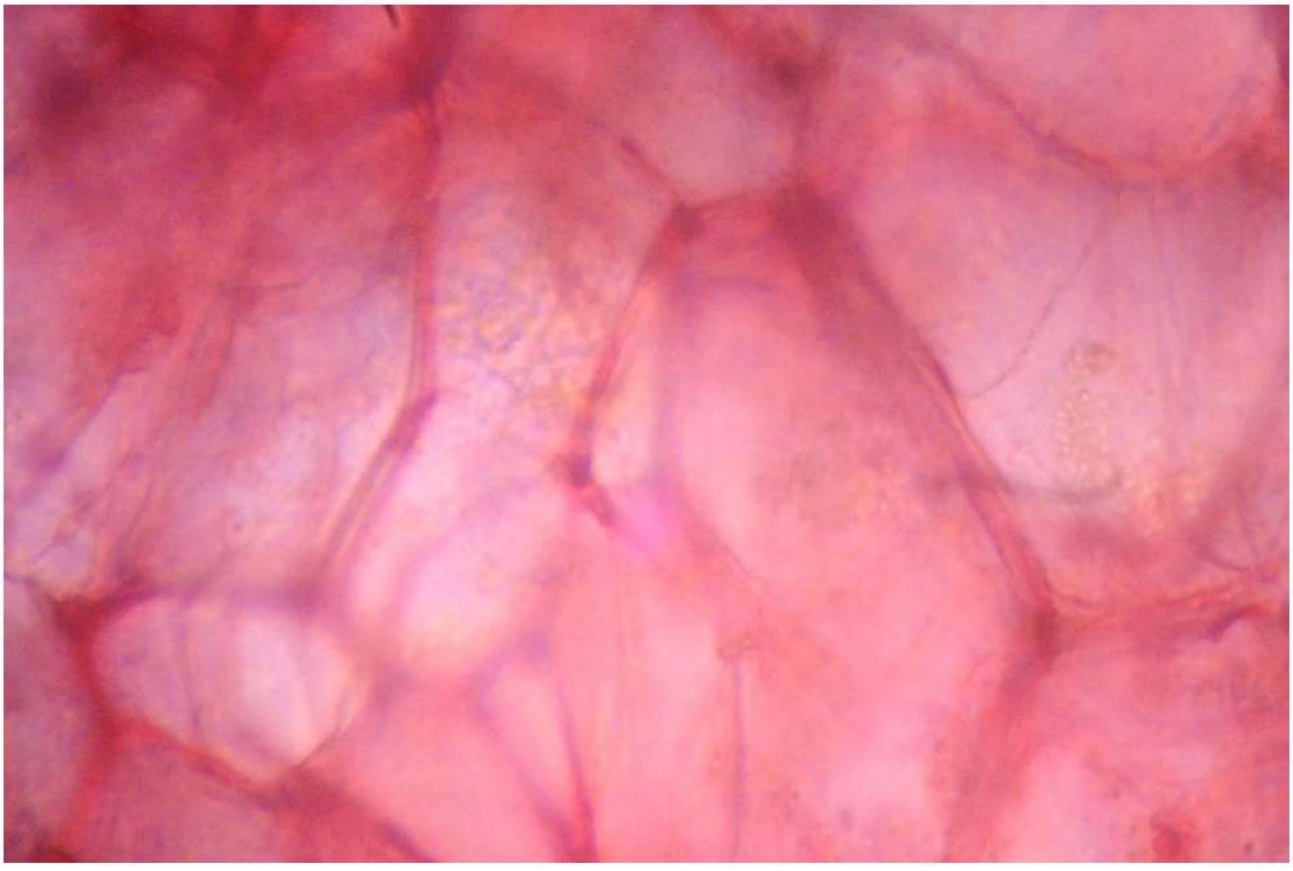

Fig. 2: FOLA Control

Plate 4: Cross section of callus formed in two out of three varieties of *Vigna unguiculata* (L.) Walp. from stem explants of control and UV-B irradiated plants. (All figs. 400x)

Dendrogram

The similarity index between two varieties *viz.*, FOLA and GOWMATHI was 100 % and they formed one group as the stem explants harvested from UV-B stressed crops of these two varieties failed to produce callus (Table 2). NS-634 remained alone showing only 12.5 % with other varieties as both the control and UV-B stressed stem explants failed to induct callus and axillary bud (Table 2; Plate 5).

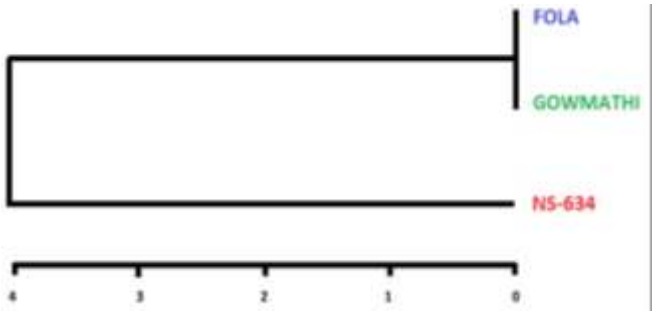

Plate 5. Dendrogram showing the interrelationship between the three varieties of *Vigna unguiculata* (L.) Walp. in callus / axillary bud proliferation from stem explants of control and supplementary UV-B irradiated plants - *In vitro.*

Conclusion

In view of callus induction occurring only in GOWMATHI and FOLA control stem explants and not in UV-B stressed stem explants, it is concluded that other explants from the three cowpea varieties need to be screened for *in vitro* regeneration before arriving at a decision on germplasm storage.

Acknowledgement

The authors thank Prof. Dr. Thamizharasi Tamizhmani, Director, KMCPGS, Pondicherry, India, for providing research facilities and Dr. M. P. Ramanujam for his support and encouragement.

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)**: 343-346.

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.

Bhat TM and Kudesia R (2011) Evaluation of Genetic Diversity in Five Different Species of Family Solanaceae using Cytological Characters and Protein Profiling. *Genetic Engineering and Biotechnology Journal*. 1- 8.

Caldwell MM, Bjorn LO, Bornman JF, Flint SD, Kulandaivelu G, Teramura AH, Tevini M (1998) Effects of increased solar ultraviolet radiation on terrestrial ecosystems. *Photochem. Photobiol.* **46:** 40 - 52. DOI: 10.1016/S1011-1344(98)00184-5

Everstt B (1985) Clustering analysis, John Wiley and Sons, New York.

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) Alterations in leaf architecture of *Ocimum sanctum* L. under elevated ultraviolet-B stress. *Global Journal of Bio-Science and Biotechnology.* **3 (4):** 374-378.

Kokilavani V and Rajendiran K (2014d) 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 (2014e) 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 (2014g) 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 (2014h) 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 (2014i) 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 (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.

Kokilavani V and Rajendiran K 2014f. 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.

Kulandaivelu G, Maragatham S, Nedunchezhian N (1989) On the possible control of ultraviolet - B induced response in growth and photosynthetic activities in higher plants. *Physiol. Plant.* **76:** 398 - 404. DOI: 10.1111/j.1399-3054.1989.tb06210.x

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. DOI: 10.1023/A:1009798528605

Maskay N (1998) Single linkage clustering. In: Armintage P and Cotton T (Eds) *Encyclopedia of Biostatistics*, ***Chapter 5,*** Wiley, New York, 4121- 4122.

Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant.* **15:** 473 - 497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

Rajendiran K (2001) Amelioration of Ultraviolet-B radiation impacts in green gram by Triadimefon. PhD. Thesis, Pondicherry University.

Rajendiran K and Ramanujam MP (2003) Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum.* **46:** 621-624. DOI: 10.1023/A:1024840301092

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:** 145-148. DOI: 10.1023/B:BIOP.0000024293.31266.a2

Rajendiran K, Ariswary D, Arulmozhi D and Kokilavani V (2015a) Screening of explants of ultraviolet-B exposed *Amaranthus dubius* Mart. Ex. Thell. for *in vitro* propagation. *International Journal of Food, Agriculture and Veterinary Sciences.* **5 (2):** 33-45.

Rajendiran K, Gowsalya L, Vidya S and Thiruvarasan K (2015b) Influence of elevated ultraviolet-B radiation on the cotyledonary epidermis of F_1 seedlings of *Lagenaria siceraria* (Molina) Standl. var. Warad. *International Journal of Food, Agriculture and Veterinary Sciences.* **5 (2):** 98-103.

Rajendiran K, Gowsalya L, Vidya S and Thiruvarasan K (2015c) Modifications in the cotyledonary epidermis of F_1 seedlings of *Lablab purpureus* (L.) Sweet var. Ankur under UV-B stress. *International Journal of Geology, Earth and Environmental Sciences.* **5 (2):** 188-193.

Rajendiran K, Iswarya R, Arulmozhi D and Kokilavani V (2015d) Evaluation of ultraviolet-B stressed explants of *Macrotyloma uniflorum* (Lam.) Verdc. For *in vitro* propagation. *International Journal of Innovative Research and Review.* **3 (2):** 19-31.

Rajendiran K, Julie Soniya F, Vijayalakshmi R and Kokilavani V (2015e) *In vitro* propagation of ultraviolet-B stressed *Momordica charantia* L. *International Journal of Geology, Earth and Environmental Sciences.* **5 (2):** 92-104.

Rajendiran K, Kokilavani V and Murugananthan P (2014a) *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, Kokilavani V and Murugananthan P (2014b) *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 (2014c) *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, Periyalakshmi D, Vijayalakshmi R and Kokilavani V (2015f) *In vitro* propagation of explants from

ultraviolet-B stressed *Spinacia oleracea* L. *International Journal of Innovative Research and Review.* **3 (2):** 32-44.

Rajendiran K, Priyadarsini V, Sudaroli Sudha J and Kokilavani V (2015g) *In vitro* propagation of *Trigonella foenum-graecum* (L.) Ser. after supplementary UV-B irradiation. *International Journal of Food, Agriculture and Veterinary Sciences.* **5 (2):** 46-57.

Rajendiran K, 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. DOI: 10.1007/s10535-006-0112-3

Rajendiran K, Riswan A, Sudaroli Sudha J and Kokilavani V (2015h) *In vitro* propagation of *Benincasa hispida* (Thunb.) Cogn. explants after enhanced UV-B exposure. *International Journal of Innovative Research and Review.* **3 (2):** 45-56.

Rajendiran K, Shanmathy M, Sudaroli Sudha J and Kokilavani V (2015i) Assessment of ultraviolet-B tolerance in *Portulaca oleracea* L. for *in vitro* propagation. *International Journal of Biotechnology.* **4 (2):** 32-42.

Rajendiran K, Thiruvarasan K and Vijayalakshmi R (2015j) Influence of elevated ultraviolet-B radiation on the morphology and growth of three varieties of black gram. *International Journal of Geology, Earth and Environmental Sciences.* **5 (3)**: 62-76.

Rajendiran K, Thiruvarasan K and Vijayalakshmi R (2015k) Assessment of nodulation in three varieties of black gram under elevated UV-B radiation. *International Journal of Geology, Earth and Environmental Sciences.* **5 (3)**: 77-81.

Rajendiran K, Vidya and Arulmozhi D (2015l) Impact of supplementary UV-B radiation on the morphology and growth of *in situ* grown three green gram varieties. *International Journal of Geology, Earth and Environmental Sciences.* **5 (3)**: 82-97.

Rajendiran K, Vidya S, Gowsalya L and Thiruvarasan K (2015m) Impact of supplementary UV-B radiation on the morphology, growth and yield of *Vigna mungo* (L.) Hepper var. ADT-3. *International Journal of Food, Agriculture and Veterinary Sciences.* **5 (2):** 104-112.

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 (2014a) 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.

Sudaroli Sudha J and Rajendiran K (2014b) Ultraviolet-B induced reduction in nodulation and nitrogen metabolism in *Vigna mungo* (L.) Hepper var. T-9. *Global Journal of Bioscience and Biotechnology.* **3 (4):** 370-373.

Sudaroli Sudha J, Rajendiran K (2014c) Ultraviolet-B induced reduction in nodulation and nitrogen metabolism in *Vigna unguiculata* (L.) Walp. cv. CO-1. *Global Journal of Bioscience and Biotechnology.* **4 (3):** 10-14.

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. DOI: 10.2307/2445763

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.