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Research Article

IN VITRO GERMINATION OF F_1 SEEDS HARVESTED FROM TEN VARIETIES OF COWPEA GROWN UNDER *IN SITU* SUPPLEMENTARY UV-B RADIATION

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ABSTRACT
The ozone layer resides in the stratosphere surrounding the earth and absorbs the ultraviolet-B (UV-B) radiation from the solar spectrum. As a result, the amount of UV-B reaching the earth's surface is greatly reduced. Any depletion in ozone layer results in enhanced UV-B exposure which can damage terrestrial plant life and animal life including human, single cell organisms and aquatic ecosystems. The present study is to evaluate the viability of seeds from F_1 generation harvested from *in situ* control and supplementary UV-B irradiated (UV-B = 2 hours daily @ 12.2 kJ m^{-2} d^{-1}; ambient = 10 kJ m^{-2} d^{-1}) cowpea varieties *viz. viz.* CW-122, COVU-1, COFC-8, CO-1, COVU-2, KM-1, CO-6, VAMBAN, CO-3 and PUDUVAI by germinating in culture media, as an attempt to screen the seeds for germplasm conservation. Unstressed CW-122, CO-3 and VAMBAN seeds, UV-B stressed CW-122, COFC-8, KM-1, CO-6 and VAMBAN seeds did not germinate under *in vitro* culture. Only seeds harvested from *in situ* UV-B irradiated COVU-1, CO-1, COVU-2, CO-3 and PUDUVAI varieties of cowpea responded to *in vitro* germination.

Keywords: *Ultraviolet-B, Cowpea, Ten Varieties, In Vitro Seed Germination*

INTRODUCTION
Concentrations of ozone in the stratosphere fluctuate naturally in response to variations in weather conditions. In addition man-made emissions of CFCs (chlorofluorocarbons) and other chemicals used in refrigeration, aerosols and cleansing agents may cause a significant destruction of ozone in the stratosphere, thereby letting through more of the harmful ultraviolet-B (UV-B) radiation. Enhanced ultraviolet-B radiation (280-320 nm) is a dangerous atmospheric stress as it is detrimental to plant growth and development severely suppressing photosynthesis (Rajendiran and Ramanujam, 2003; Rajendiran and Ramanujam, 2004) and inhibiting nodulation and nitrogen metabolism (Rajendiran and Ramanujam, 2006; Rajendiran and Ramanujam, 2003; Sudaroli and Rajendiran, 2013a; Sudaroli and Rajendiran, 2013b; Arulmozhi and Rajendiran, 2014; Vijayalakshmi and Rajendiran, 2014) in several sensitive crops. Crop foliage receives major proportion of the ultraviolet radiation and hence always reacts immediately to prevent its entry into the internal organs (Bornman and Vogelmann, 1991; Rajendiran and Ramanujam, 2000; Kokilavani and Rajendiran, 2013; Kokilavani and Rajendiran, 2014a; Kokilavani and Rajendiran 2014b). The present work deals with the response of seeds of F_1 generation harvested from normal and UV-B stressed ten varieties of cowpea *to vitro* culture.

MATERIALS AND METHODS
Cowpea (*Vigna unguiculata* (L.) Walp.), the nitrogen fixing grain legume was chosen for the study. Viable seeds of the ten varieties of cowpea *viz.* CW-122, COVU-1, COFC-8, CO-1, COVU-2, KM-1, CO-6, VAMBAN and CO-3 were procured from Saravana Farms, Villupuram, Tamil Nadu and PUDUVAI from local farmers in Pondicherry. 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-B_{BE}) of 12.2 kJ m^{-2} d^{-1} equivalents to a simulated 20 % ozone depletion at Pondicherry (12º2'N, India). The control plants, grown under natural solar radiation, received UV-B_{BE} 10 kJ m^{-2} d^{-1}. Seeds (F_1 generation) were harvested from both unstressed and supplementary UV-B stressed parent crops grown in the *in situ* condition. The seeds were germinated in culture media to evaluate their viability.

The seeds of ten varieties of cowpea used for *in vitro* culture 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 were inoculated horizontally and 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 0.1to 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 were recorded after 15 DAI (days after inoculation) and callus proliferation after 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.

Chemical Composition of MS Medium (Murashige and Skoog 1962)

Constituents	Quantity (mgl^{-})
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 culture tubes 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 term dendrogram is used in numerical taxonomy for any graphical drawing giving a tree-like description of a taxonomic system. The similarity indices between the ten 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 ten varieties of cowpea, dendrograms were draw to derive the interrelationship between them and presented in tables and plates.

RESULT AND DISCUSSION

The seeds were selected for uniform colour, size and weight and used in the experiments (Plate 1). Out of the ten varieties of cowpea suffering under *in situ* supplementary UV-B radiation, plant growth was at its best in KM-1 and CO-1 varieties of cowpea on 30 DAS (days after seed germination) followed by CO-3, COVU-1 and VAMBAN (Plate 2). Even though the number of seeds harvested per plant basis from *in situ* UV-B stressed crops was less than that of controls irrespective of the varieties, the F_1 seeds of all the ten varieties were tested on culture media for their chance of survival against the respective controls. This report is the first of its kind to test the viability of seeds belonging to F_1 generation harvested from *in situ* control and supplementary UV-B irradiated cowpea varieties on in *vitro* media. Unstressed CW-122, CO-3 and VAMBAN failed to respond *in vitro* germination. The cowpea varieties CW-122, COFC-8, KM-1, CO-6 and VAMBAN of *in situ* UV-B exposed F_1 cowpea seeds also failed to germinate under *in vitro* culture (Plate 3). Only five varieties of F_1 cowpea seeds harvested from *in situ* supplementary UV-B irradiated parents *viz.*, COVU-1, CO-1, COVU-2, CO-3 and PUDUVAI germinated under *in vitro* culture (Plate 3).

The varieties CO-1, COVU-1, COVU-2 and PUDUVAI as one group yielded 75 % similarity as both the control as well as UV-B stressed F_1 seeds of these four varieties germinated well in culture media (Table 1; Plate 4).

Figure 1: CW-122

Figure 2: COVU-1

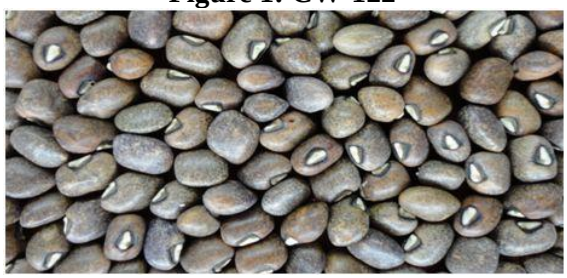

Figure 3: COFC-8

Figure 4: CO-1

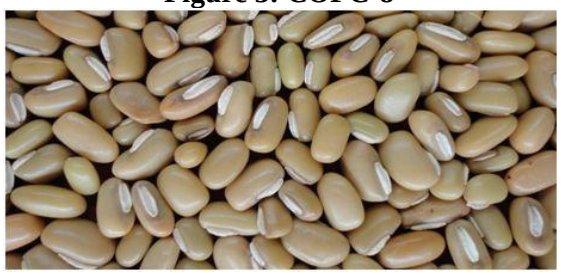

Figure 5: COVU-2

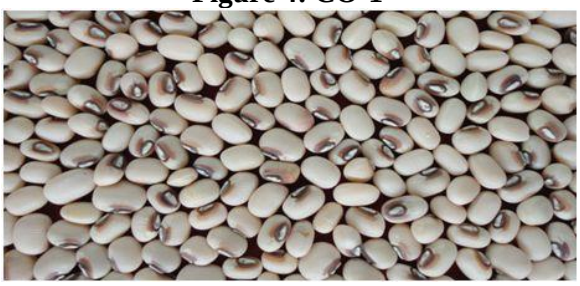

Figure 6: KM-1

Figure 7: CO-6

Figure 8: VAMBAN

Figure 9: CO-3

Figure 10: PUDUVAI

Plate 1: Seeds of the ten varieties of *Vigna unguiculata* (L) Walp.

Figure 1: CW-122

Figure 2: COVU-1

Figure 3: COFC-8

Figure 4: CO-1

Figure 5: COVU-2

Figure 6: KM-1

Figure 7: CO-6

Figure 8: VAMBAN

Figure 9: CO-3

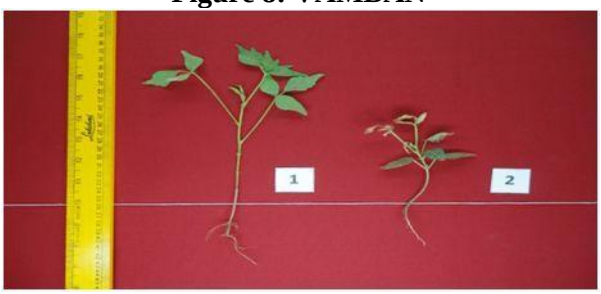

Figure 10: PUDUVAI

Plate 2: The control and supplementary UV-B stressed plants of ten varieties of *Vigna unguiculata* (L) Walp. on 30 DAS. (1: Control, 2: UV-B)


Figure 1: CW-122 C


UV-B


Figure 2: COVU-1 C


UV-B


Figure 3: COFC-8 C


UV-B


Figure 4: CO-1 C


UV-B


Figure 5: COVU-2 C


UV-B


Figure 6: KM-1 C


UV-B


Figure 7: CO-6 C


UV-B


Figure 8: VAMBAN C


UV-B


Figure 9: CO-3 C UV-B


Figure 10: PUDUVAI C UV-B

Plate 3: *In vitro* germination (F_1 generation) of *Vigna unguiculata* (L.) Walp. seeds harvested from *in situ* control (C) and Ultraviolet-B (UV-B) irradiated plants

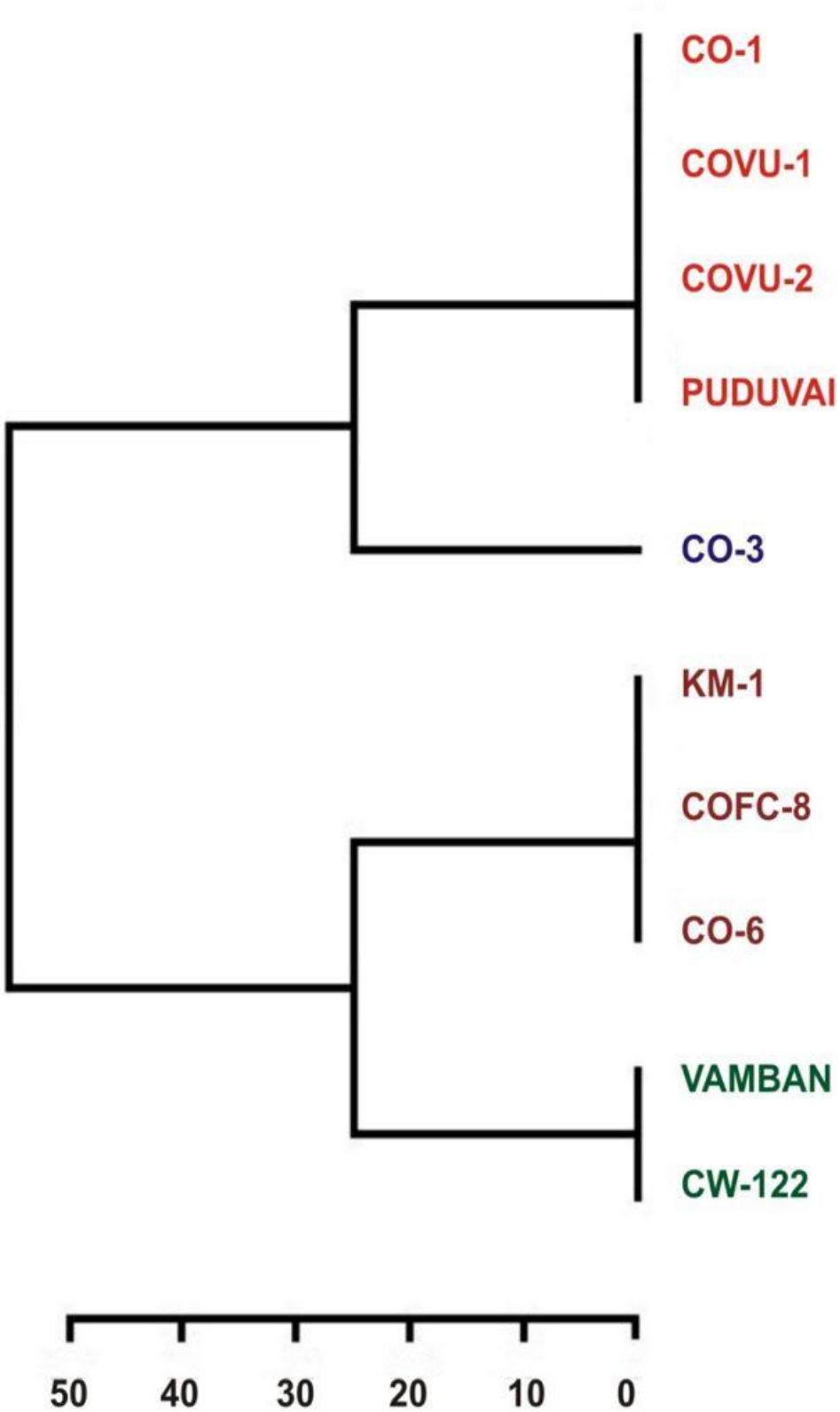

Plate 4: Dendrogram showing the interrelationship between the ten varieties of *Vigna unguiculata* (L.) Walp. in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* control and UV-B irradiated parents

CO-3 remained with this group as a separate cluster showing 62.5 % similarity as the seeds of this variety harvested from UV-B exposed F_1 seeds alone germinate under *in vitro* culture. Control seeds of KM-1, COFC-8 and CO-6 varieties germinated under *in vitro* condition, while the UV-B irradiated F_1 seeds of these varieties failed. Due to this feature, they formed another group with only 37.5 % similarity value between them. VAMBAN along with CW-122 remained separated from other varieties of cowpea as the F_1 seeds from both control and UV-B stressed parents did not germinate in culture media (Table 1; Plate 4).

Table 1: The similarity indices in the *in vitro* germination of seeds of F_1 generation harvested from *in situ* grown ten varieties of *Vigna unguiculata* (L.) Walp. after supplementary UV-B exposure

Varieties	CW-122	COVU-1	COFC-8	CO-1	COVU-2	KM-1	CO-6	VAMBAN	CO-3	PUDUVAI
CW-122	-	-	-	-	-	-	-	-	-	-
COVU-1	-	100%	50%	75%	75%	50%	50%	-	62.5 %	75%
COFC-8	-	50%	100%	62.5%	62.5%	37.5%	37.5%	-	50%	62.5%
CO-1	-	75%	62.5%	100%	75%	50%	50%	-	62.5 %	75%
COVU-2	-	75%	62.5%	75%	100%	50%	50%	-	62.5 %	75%
KM-1	-	50%	37.5%	50%	50%	100%	37.5%	-	50%	62.5%
CO-6	-	50%	37.5%	50%	50%	37.5%	100%	-	62.5 %	75%
VAMBAN	-	-	-	-	-	-	-	-	-	-
CO-3	-	62.5%	50%	62.5%	62.5%	50%	62.5%	-	100 %	62.5%
PUDUVAI	-	75%	62.5%	75%	75%	62.5%	75%	-	62.5 %	100%

The present study suggests that out of the ten varieties of cowpea taken for screening, the progenies of COVU-1, CO-1, COVU-2 and PUDUVAI possessed more survival value as their F_1 seeds harvested from both normal and supplementary UV-B irradiated *in situ* grown parents showed greater viability in culture media.

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REFERENCES

Arulmozhi D and Rajendiran K 2014. 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.

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.

Bornman JF and Vogelmann TC (1991). Effect of UV-B radiation on leaf optical properties measured with fibre optics. *Journal of Experimental Botany* **42** 647 - 554.

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). 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 (2014b). Alterations in leaf architecture of *Ocimum sanctum* L. under elevated ultraviolet-B stress. *Global Journal of Bio-Science and Biotechnology* **3**(4) 374-378.
Maskay N (1998). Single linkage clustering. In: *Encyclopedia of Biostatistics*, edited by Armintage P and Cotton T (Wiley, New York) **5** 4121- 4122.
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 and Ramanujam MP (2000). Efficacy of triadimefon treatment in ameliorating the UV-B stress in green gram. In: *National Symposium on Environmental Crisis and Security in the New Millennium*, edited by Khan M (National Environmental Science Academy, New Delhi) 41-42.
Rajendiran K and Ramanujam MP (2003). Alleviation of ultraviolet-B radiation-induced growth inhibition of green gram by triadimefon. *Biologia Plantarum* **46** 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** 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.
Vijayalakshmi R and Rajendiran K (2014). 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.