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Mutagenic effects of ultraviolet (UV-C) irradiation on the anatomy of three species of *Capsicum*

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Abstract

UV radiant seedlings of *Capsicum annuum, C. Chinenese* and *Capsicum frutescens* were studied anatomically to observe the UV effects on the leaf epidermis, stem and root ultrastructures. While there is a higher percentage of stomatal index in the UV-exposed plants compared to the controlled, unexposed plants, there is no correlation in the stomatal density and stomatal size between the exposed and unexposed plants to the ultraviolet irradiation. There was also no correlation between the stomatal size and the stomatal density in both treatments (exposed and unexposed) in all the plants. Significant differences were observed in the stomatal index on both leaf surfaces between the exposed and controlled plants of *C. frutescens* and *C. annuum.* Cell walls of the stem and root wereobserved to be thicker in the UV-exposed plants.

Keywords: Mutagenic; Anatomy; *Capsicum annuum; Capsicum chineneseX; Capsicum frutescens;* UV radiation

Introduction

Ultraviolet radiation (UVR) is the part of the non-ionizing region of the electromagnetic spectrum, which comprises approximately 8-9% of the total solar radiation. The spectrum of UV radiation reaching the earth's surface has been divided into lower energy (UV-A, 320-400 nm), higher energy (UV-B, 280-320 nm) and UV-C (254-280 nm) regions. UV-C (254-280 nm) is extremely harmful to living organisms. Meanwhile, UV-B (280-320 nm) is of particular interest because although this wavelength represents only approximately 1.5 % of the total spectrum, it can have a variety of damaging effects in plants. UV-A (320-400 nm) represents approximately 63 % of the incoming solar radiation and is the least hazardous part of UV radiation (Hollosy, 2002). Studies of more than 300 plant species and cultivars have been carried out, and about 50 % have been considered sensitive, 20-30 % moderately sensitive and the rest incentive to UV-B radiation (Teramura *et al.* 1990; Teramura and Sullivan, 1994).

Among these, UV-C is the radiation with the lower wavelength, or rather with the higher associated energy. It is known that UV-C can induce oxidative results and genetic mutations in plants that in turn have strong negative effects on plant morphology, flowering, pollination, transpiration and photosynthesis (Murali and Saxe, 1984).

The influence of UV radiation on growth appears to be mediated by phytohormones, either via photodestruction or enzymatic reactions.Overall, the effects of UV radiation vary, both among species and among cultivars of a given species. Ofthose plants that have been tested, a large proportion exhibited reduced plant growth, photosynthetic activity, and flowering. Stem and leaf thickness are altered in UV-treated plants. At the ultrastructural level, it has been observed that changes occur in chloroplasts and peroxisomes.

In this study, some species of Capsicumare exposed to ultraviolet radiation (UV-C). *Capsicum* (chilies and other peppers) belongs to the family Solanaceae (tribe Solaneae, subtribe Capsicinae), which also includes other economically important crops such as tomato, potato and tobacco (Dias *et al.,* 2013). They consist of annual or perennial herbs or shrubs and are native to South and Central America and the Galapagos (Walsh and Hoot, 2001). They are predominantly diploid (2n=24, infrequently 2n=26), except for a few (Moscone *et al.,* 2003). Pepper (especially, *C. annuum,* whichis the most widely cultivated species worldwide

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(Andrews, 1984) grows relatively quickly with a maturity period of 3-4 months. In Nigeria, it is grown in home gardens and convenient sites near settlements often as intercrop but it is now grown as a monocrop on a large scale by both peasant and commercial farmers.

The treatments of plants with UV irradiation previously have reflected many anatomical and morphological changes, such as the reduction of plant height and leaf length/area (Deckmyn and Impens, 1998; Pukacki, 2000), leaf bronze, glazing, chlorosis and necrotic spots (Kakani *et al.,* 2003). Thus, the effect of increased UV radiation on growth and physiology of many plants, including crop and terrestrial plant species, under both greenhouse and field conditions, has become one of the most important subjects of investigation in the last decades by the investigators. In the earlier work by Sargheini *et al*. (2008), the effect of UV-R was slightly felt on the chlorophyll-a and chlorophyll-b contents of UV-R exposed pepper (*C. longum*) but there was significant decreased on the total chlorophyll amount, especially of UV-C exposed plants of the same species. Based on this, the present work aimed to assess the effect of UV-C irradiation on the ultrastructural changes in root, stem, and leaf of *Capsicum annum*, *C. chinenese* and *C. frutescens*.

Materials and methods

Collection of study materials

The seeds of three species of *Capsicum* namely *C. annuum*, *C. chinenese* and *C. frutescens* were obtained from Institute of Agricultural Research and Training (IAR & T), Ibadan, Oyo State, Nigeria. The UV irradiation lamp was gotten from the Biology Laboratory,the soil and water used were obtained from the Botanical Garden and Oyun River, all at the University of Ilorin, Ilorin, Nigeria.

Planting and exposure of study materials to UV-C irradiation

A total of 12 pots was arranged and labeled for the seed planting. The base of the pots was perforated to allow the drainage of water and aeration.The pots were filled with topsoil,which was mixed thoroughly to ensure homogeneity and used as the growth medium. The seeds were planted in the pots and watered at two days interval. Each variety has different pots for controlled and UV-exposed seedlings. Each treatment has three replicates. TheUV-exposed seedlings were exposed to UV-C (254 nm) radiation for 5 days, by means of a low pressure mercury vapour discharge hand held lamp (Phillips germicidal sterile) with a tubular glass envelope emitting short wave UV radiation with a peak of 254 nm (UV-C) with 3.6 kJ/m2 for 45 minutes placed at approximately 14 cm from the seedlings in the UV-C chamber. The pots were laid out in a completely randomized block design (CRBD).

Plants were grown at 24/20ºC (day/night) 16h of light and 8h of dark, and were alternately watered.

Anatomical study: Light microscopy

Isolation of Leaf epidermal layer

Fresh matured leaves of the plants (exposed and non-exposed) to the UV light were harvested, washed in clean water and dried. The leaves were then painted with nail varnish on the upper (adaxial) and lower (abaxial) surfaces and allowed to dry. A short, clean cellophane tape was firmly pressed over the dried surface. The tape was carefully peeled from the leaf. The obtained cuticular peel was stained in 10% safranin for 10 minutes and mounted in glycerol on a clean slide and covered with clean cover slip for microscopic study.

Stomatal frequency and identification

Using the fields of view at X40 objective as quadrats, the numbers of subsidiary cells per stoma was noted to determine the frequency of the different stomatal complex types and was expressed as the percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). Terminologies for naming stomatal complex types followed Dilcher (1974).

Stomatal density and size

The stomatal density was determined as the number of stomata per square millimeter (Stace, 1965). The mean stomatal size was determined following the method of Franco (1939). Samples of 32 stomata were used.

SS = L x B x K, L = length, B = breadth, K = Franco's constant = 0.79

Anatomy of stem and root

The stems of plants (controlled and treated plants) were harvested with the aid of a sharp knife. Thereafter, transverse and longitudinal sections were obtained by cutting along the radial and longitudinal planes of a cylindrical portion of the stem with the aid of a razor blade. The sections were stained in safranin for 3 minutes. The sections were then placed in 70%, 80%, 90% and absolute alcohol (100%) for 5 minutes each. The sections were mounted and observed under microscope and photographed using Kodax Easy Share C913, 9.2 mega pixels.

Table I. Stomatal features for the controlled and exposed *Capsicum* species

Species	Leaf surface	Stomatal complex types	Frequency (%)	Stomatal density (mm^{-2})	Stomatal size (µm)	Stomatal index (%)
Capsicum frutescens controlled	Adaxial	Anisocytic Tetracytic	77.49 22.51	2.20±0.20^{de}	26.96±2.95^{d}	2.16±0.47^{ef}
	Abaxial	Anisocytic Tetracytic	73.57 26.43	5.20±0.20^{c}	54.82±5.83^{abc}	8.46±1.47^{cd}
Capsicum frutescens exposed to UV light	Adaxial	Anisocytic Tetracytic	77.50 22.50	3.40±0.51^{d}	52.35±8.74^{abc}	9.13±2.61^{cd}
	Abaxial	Anisocytic Tetracytic	72.67 27.33	5.00±0.32^{c}	50.56±8.54^{bc}	6.70±1.67^{cdef}
Capsicum annuum controlled	Adaxial	Anisocytic Tetracytic	80.87 19.13	2.60±0.24^{de}	67.62±4.43^{a}	11.02±1.62^{c}
	Abaxial	Anisocytic Tetracytic	79.55 20.45	12.00±0.71^{a}	26.54±6.06^{d}	48.50±2.53^{a}
Capsicum annuum exposed to UV light	Adaxial	Anisocytic Tetracytic	84.05 15.95	3.20±0.37^{d}	61.77±2.74^{ab}	7.14±1.89^{cde}
	Abaxial	Anisocytic Tetracytic	82.71 17.29	7.40±0.24^{b}	46.13±2.55^{bc}	20.94±0.84^{b}
Capsicum chinense controlled	Adaxial	Anisocytic Tetracytic	83.82 16.18	2.40±0.24^{de}	45.34±2.63^{bc}	4.81±0.76^{def}
	Abaxial	Anisocytic Tetracytic	81.02 18.98	6.00±0.45^{c}	23.38±4.67^{d}	6.51±0.84^{cdef}
Capsicum chinense exposed to UV light	Adaxial	Anisocytic Tetracytic	86.67 13.33	1.60±0.24^{e}	48.66±5.58^{bc}	1.91±0.45^{f}
	Abaxial	Anisocytic Tetracytic	86.96 13.04	5.60±0.51^{c}	44.08±3.84^{c}	9.35±1.82^{cd}

Means with the same letters along columns are not significantly different at P≤0.05

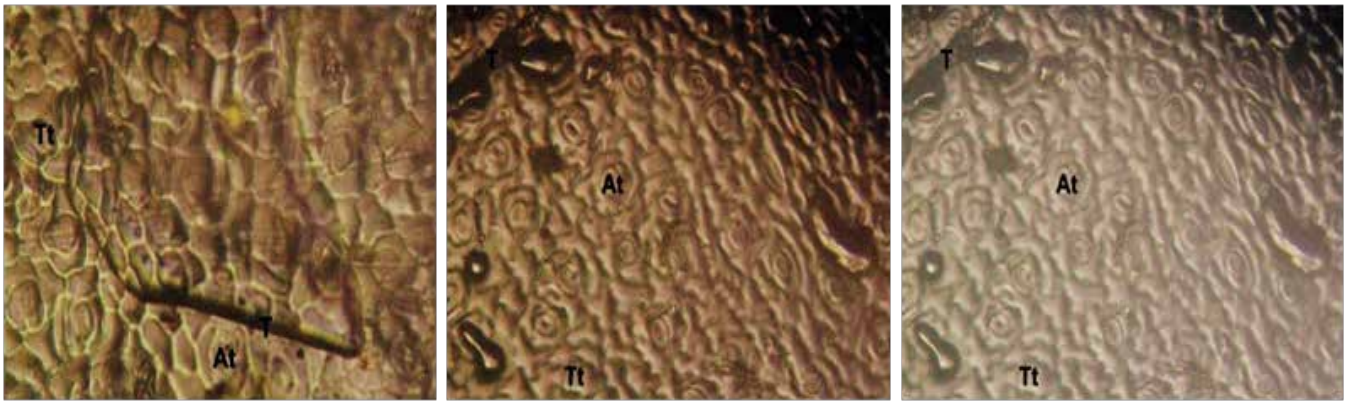

Fig. 1. Leaf epidermis (a) abaxial-not exposed of *Capsicum* frutescens; (b) adaxial-exposed; (c) abaxial - exposed) of *Capsicum* annuum UV light showing tetracyticstomatal complex type (Tt), anisocyticstomatal complex type (At) and trichome (T) x400

Data analysis

The data generated were subjected to analysis of variance (ANOVA). Duncan multiple range test (DMRT) was used to separate the means.

Results and discussion

The leaves of the 3 *Capsicum* species are amphistomatic i.e. having stomata on both adaxial and abaxial surfaces. The effect of ultraviolent irradiation (UV) on the leaf anatomy was not noticed in the stomatal complex types, i.e. no variation on the stomatal types present.The types of stomatal complex, i.e. anisocytic and tetracytic present on both the abaxial and adaxial leaf surfaces are same in all the studied varieties of the 3 species (Figs. 1a – c; Table I). The effect was noted in the frequency of stomatal complex types, stomatal density, index and size as shown in Table I. The anisocytic stomatal complex type is more frequent on both leaf surfaces than the tetracytic type in all species. The frequency of the two stomatal types is closely related in a species between the exposed and controlled plants. But in all the three species studied, the stomatal density is higher in the adaxial surface than the abaxial surface, and the density of stomata showna significant difference at $p \leq 0.05$ between the leaf surfaces. There is no significant difference between the exposed and controlled stomatal density in all the species. There are bigger stomata on the adaxial surface than on the abaxial surface in the three species except in the control *C. frutenscens* with significant difference at $p \leq 0.05$ between the adaxial and abaxial surfaces in all the spieces. The percentage stomatal occurrence (i.e. stomatal index) was higher on the abaxial surface than on the adaxial surface in all the treatments in all the species except in the UV-treated *C. frutenscens.* Also, there are significant differences at $p \leq 0.05$ in the percentage stomatal occurrence between the two leaf surfaces.

There is no structural deformity in the roots of *C. annuum, C. chinense* and *C. frutescens* (Figs 2a – h and k and l), and stems of *C. annuum* (Figs. 2i and j) in both the treatments and

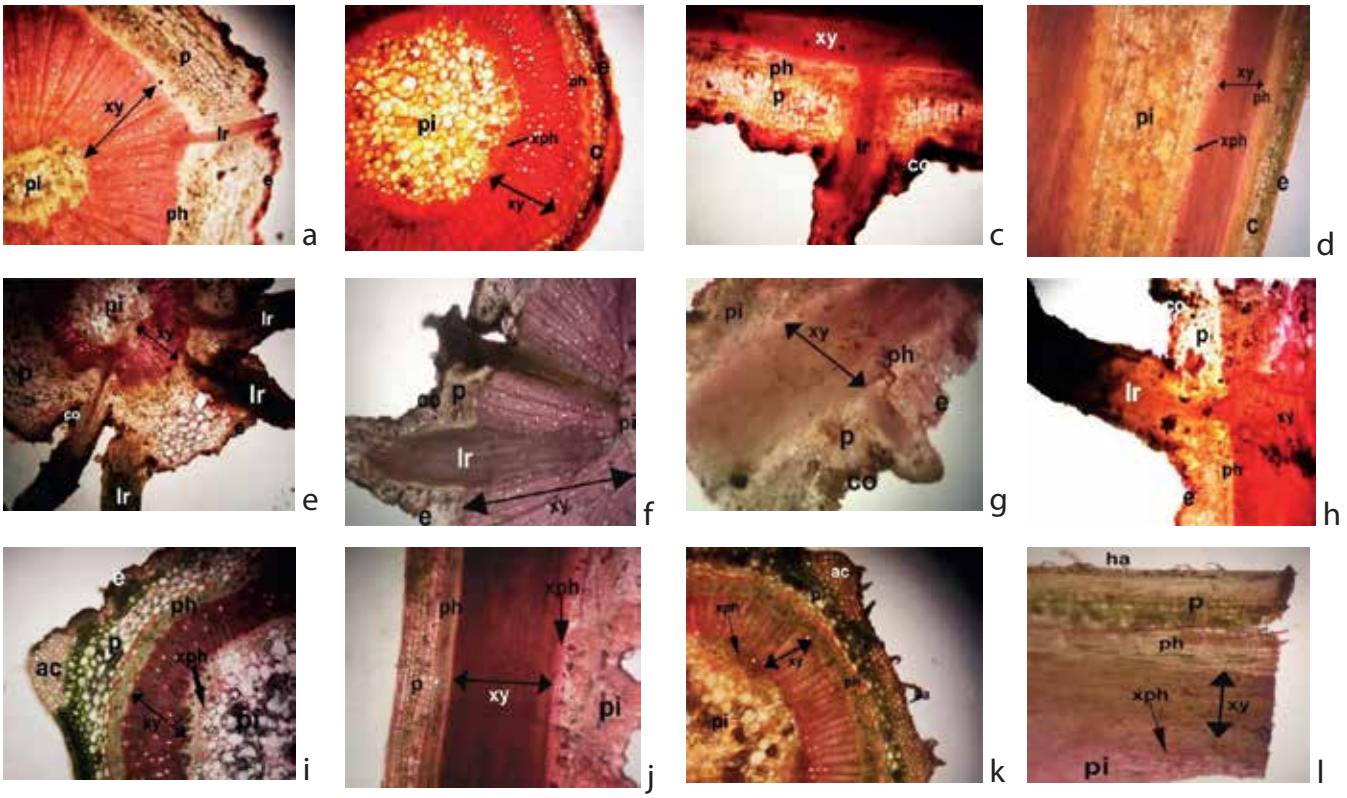

Fig. 2. Transverse (a-exposed; b-not exposed) and longitudinal (c-exposed; d-not exposed) sectionsof the root of *Capsicum* frutescens; transverse (e-exposed; f-not exposed) and longitudinal (g-exposed; h-not-exposed) sections of the root of *Capsicum* annum; and transverse (i-exposed) and longitudinal (j-not exposed) sections of the stem of *Capsicum* annum exposed transverse (k-not exposed) and longitudinal (l-not exposed) sections of the root of *Capsicum* chinense to UV light. Pi-pith, xy-xylem, ph-phloem, p-parenchyma, co-cork cells, e-epidermis, lr-lateral root, xph-intraxylary phloem, e-epidermis, ac-angular collenchyma, ha–hair x400

the control as observed in transverse and longitudinal sections. All structures such as xylem, phloem, parenchymatous cells, collenchymatous cells, bark and pith are firmly intact.Meanwhile, the cell walls of the treated plants were thicker than those of non-treated plants.

The plants are the primary producer and as such must expose to sunlight to ensure photosynthesis, and are therefore exposed to ultraviolet radiation. It is obvious that increased exposure to UV-B radiation has specific effects on terrestrial ecosystems, aquatic ecosystems and biogeochemical cycles, and thus on human health and plants. There is a particularly concerned about the potential impact of increased UV radiation on plants, simply because they form the basis of our food supply. Significant changes in the health or growth of these plants negatively may reduce the amount of available food.

While there is a higher percentage of stomatal index in the UV-exposed plants compared to the controlled, unexposed plants, there is no such correlation in the stomatal density and stomatal size between the exposed and unexposed plants to the ultraviolet irradiation. There was also no correlation between the stomatal size and the stomatal density in both treatments (exposed and unexposed) in all the plants. This is contrary to earlier observations where small stomata gives the higher stomatal density and vice versa (Metcalfe and Chalk, 1988; Oyeleke *et al*., 2004; AbdulRahaman and Oladele, 2003). In a similar work on *Capsicum longum*, Sargheini *et al*. (2011) reported an increase in the length of stomatal as a result of exposure to UV irradiation when compared to the control plants. A similar pattern was observed in the adaxial surface of *C.frutescens* and on abaxial surface of *C. annuum* and *C. chinense*. Worthy of notice is the significance differences observed in the stomatal index on both leaf surfaces between the exposed and controlled plants of *C.frutescens* and *C. annuum*. There was no such effect on C. *chinense,* the stomatal distribution in term of percentage occurrence was even in both treatments and on both surfaces. One of the effects of UV radiation on plants is increased in number and size of stomata of UV-C exposed plants. The incident was reported in *C. longum* (Sargheini *et al*., 2011). In this present work, the UV-C radiation did not produce the same effects; there are situations where stomatal number and size were higher and larger in the controlled plants than in the UV-exposed plants.

Though no deformity in the organization and arrangement of structures of the stem and root internally, there may be some slight variations in the thickness of the cells in these two organs as observed by Sargheini *et al*. (2011). In their work, there was increase in the stem thickness in UV-C exposed plants than in UV-A exposed plants of *C. longum*. The reason for such an increase was captured in the report of Ros and Tevini (1995) where they stated that UV radiation cause increase in the production of a hormone called ethylene which decreases stem elongation but increases stem thickness in plants. This was contrary to the occurrence in *Fagopyrum tataricum* where UV exposure caused a decrease in the stem thickness (Yao *et al*., 2006).

Conclusion

In conclusion, UV-C irradiation has effects on the foliar anatomy as shown in the frequency of the stomatal complex types, stomatal density, index and size of *C. annuum, C. chinense* and *C. frutescens* studied in this work. There was no effect on the stomatal complex types. Also, there was no deformity on the structures of the roots and stems in both the treated and non-treated plants; though the cell walls of the stems and roots of the treated plants were observed to be thicker than those of non-treated plants.

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References

AbdulRahaman AA and Oladele FA (2003), Stomatal complex types, stomatal size, density and index in some vegetable species in Nigeria, *Nig. J. Bot.* **16**: 144 -150.

Andrews J (1984), Peppers: *The domesticated Capsicums*. Austin: University of Texas Press, pp 125.

Deckmyn G and Impens I (1998), Effects of solar UV-B irradiation on vegetative and generative growth of *Bromus catharicus*, *Environ Exp Bot*. **40**: 179-185. DOI:org/10.1016/S0098-8472(98)00035-5

Dias GB, Gomes VM, Moraes TMS, Zottich UP, Rabelo GR, Carvalho AO, Moulin M, Goncalves LSA, Rodrigues R and Da Cunha M (2013), Characterization of *Capsicum* species using anatomical and molecular data, *Genet. Mol. Res.* **4(2)**: 1-14 DOI: org/10.4238/2013.February.28.29

Dilcher DL (1974), Approaches to the identification of angiosperm leaf remains, *Bot. Rev.* **40(1)**: 1-57.

Franco C (1939), Relation between chromosome number and stomata in *Coffea*, Bot. gaz. **100**: 817-818.

Hollosy F (2002), Effects of ultraviolet radiation on plant cells, *Micron*. **33**(2): 179- 197.

Kakani VG, Reddy KR, Zhao D and Sailaja K (2003), Field crop responses to ultraviolet-B radiation: a review. *Agric. For. Meteorol.* **120**: 191 - 218.

Metcalfe CR and Chalk L (1988), Anatomy of Dicotyledons, 2nd Ed., Oxford University Press, Oxford, pp 97 - 177.

Moscone EA, Baranyi M, Ebert I, Greilhuber J, Ehrendorfer F and Hunziker AT (2003), Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by Flow Cytometry and Feulgen Densitometry, *Ann. Bot.* **92**: 21-29. DOI: org/10.1093/aob/mcg105

Murali NS and Saxe H (1984), Effects of ultraviolet-C radiation on net photosynthesis, transpiration and dark respiration of *Spathiphyllum wallisii*, *Physiol. Plant.* **60**(2): 192-196.

Obiremi EO and Oladele FA (2001), Water-conserving stomatal systems in selected Citrus species, *S. Afr. J. Bot.* **67**(2): 258-260.

Oyeleke MO, AbdulRahaman AA and Oladele FA (2004), Stomatal anatomy and transpiration rate in some afforestation tree species, *NISEB Journal* **4(2)**: 83 - 90.

Pukacki PM (2000), Effects of sulphur, fluoride and heavy metal pollution on the chlorophyll fluorescence of Scots pine (*Pinus sylvestris* L.) needles, *Dendrobiology* **45**: 83-96.

Ros J and Tevini M (1995), Interaction of UV radiation and IAA during growth of seedlings and hypocotyl segments of sunflower, *J. Plant Physiol.* **146**: 295-302.

Sargheini SH, Carapetian J and Khara J (2011), The effects of UV radiation on some structural and ultrastructural parameters in pepper (*Capsicum longum* A.DC), *Turkish J. Biol.* **35**: 69 - 77. DOI: 10.3906/biy-0903-11

Sargheini SH, Carapetian J and Khara J(2008), Effects of UV-Radiation on Photosynthetic Pigments and UV Absorbing Compounds in *Capsicum longum* (L.), *International Journal of Botany* **4**: 486-490.

Stace CA (1965), Cuticular studies as an aid to plant taxonomy, *Bull. Br. Mus. Nat. Hist. Bot*. **4**: 3 - 78.

Teramura AH and Sullivan JH (1994), Effects of UV-B radiation on photosynthesis and growth of terrestrial plants, *Photosynt Res*. **39**: 463-473.

Teramura AH, Sullivan JH and Lydon J (1990), Effects of UV-B radiation on soybean and seed quality: 6 year field study, *Physiol. Plant*. **30**: 5-11.

Walsh BM and Hoot SB (2001), Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast atpb-rbcl spacer region and nuclear waxy introns, *Int. J. Plant Sci.* **162(6)**: 1409-1418.

Yao Y, Xuana Z and Li Y*et* (2006), Effects of ultraviolet-B radiation on crop growth, development, yield and leaf pigment concentration of tartary buckwheat (*Fagopyrum tataricum*) under field conditions, *Eur J Agron.* **25**: 215 - 222. DOI: rg/10.1016/ j.eja.2006.05.004