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. Chinense 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 were observed to be thicker in the UV-exposed plants.

Keywords: Mutagenic; Anatomy; Capsicum annuum; Capsicum chinense; 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 insensitive 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. Of those 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, which is the most widely cultivated species worldwide.

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Capsicum annum, C. chinense

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. chinense and C. frutescens.

Materials and methods

Collection of study materials

The seeds of three species of Capsicum namely C. annum, C. chinense 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. The UV-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 24/20°C (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.

\[ SS = L \times B \times K, \quad L = \text{length}, \quad B = \text{breadth}, \quad K = \text{Franco's constant} = 0.79 \]

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf surface</th>
<th>Stomatal complex types</th>
<th>Frequency (%)</th>
<th>Stomatal density (mm²)</th>
<th>Stomatal size (μm)</th>
<th>Stomatal index (%)</th>
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</thead>
<tbody>
<tr>
<td>Capsicum frutescens</td>
<td>Adaxial</td>
<td>Anisocytic</td>
<td>77.49</td>
<td>2.20±0.20de</td>
<td>26.96±2.95</td>
<td>2.16±0.47ef</td>
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<td></td>
<td></td>
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<td>22.51</td>
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<td></td>
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<td>3.40±0.51d</td>
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<td>9.13±2.61d</td>
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<td>6.70±1.67def</td>
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<td>Anisocytic</td>
<td>80.87</td>
<td>2.60±0.24d</td>
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<td>11.02±1.62c</td>
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<td>3.20±0.37d</td>
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<td>Capsicum chinense</td>
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<td>Anisocytic</td>
<td>86.67</td>
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<td>48.66±5.58</td>
<td>1.91±0.45f</td>
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</table>

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 tetracytic stomatal complex type (Tt), anisocytic stomatal 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 ultraviolet 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 1). The effect was noted in the frequency of stomatal complex types, stomatal density, index and size as shown in Table 1. 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 shown significant difference at p≤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. frutescens with significant difference at p≤0.05 between the adaxial and abaxial surfaces in all the species. 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. frutescens. Also, there are significant differences at p≤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

![Image](image-url)
the control as observed in transverse and longitudinal sections. All structures such as xylem, phloem, parenchymatous cells, collateral 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.

**Acknowledgement**

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