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Impact of uv-b radiations on morphology of Ocimum and Mentha (family-Lamiaceae) by ascorbic acid

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Abstract
Ultraviolet-B radiation is an important component of the environment, acting as an ecophysiological factor with the potential to alter plant growth and photosynthesis. Plant response to UV-B is effectively regulated by photo protective mechanisms that dissipate excess radiation. At the morphological & physiological level these photo repair mechanisms and antioxidant responses mop up reactive oxygen species, UV-B screening compounds and adaptation in leaf morphology, stop UV-B from reaching the chloroplasts. These mechanisms contribute to a coordinated response to UVB, some of which can be enhanced by the UV-A portion of the photosynthetically active radiation.

Keywords: Ultraviolet-B, environment, ecophysiological factor.

1. Introduction
UV-B affects various aspects of photosynthesis (Jansen *et al*., 2010) [1]. Photosystem (PS II) rather than PS I, is considered to be the most vulnerable target of UV-B. Reductions inCO_2 assimilation rate may be further mediated through reduction in light-harvesting complexes, disruption of thylakoid membrane integrity, and/or degradation and inactivation of Rubisco (Takeuchi *et al*., 2002) [2]. Several studies have also shown that reduction in CO_2 assimilation is caused by UV-induced changes in stomatal conductance (Jansen and van den Noort, 2000) [3].

In general, plant response to UV-B depends on the biologically effective dose applied (Kotilainen *et al*., 2011) [4] and interactions with other environmental stimuli (Caldwell *et al*., 2007) [5]. In particular, the spectral balance between PAR (400–700 nm), UV-A (320–400 nm) and UV-B has been shown to be important in determining plant sensitivity in field studies (Krizek, 2004) [6]. In several studies, elevated UV-B radiation has produced negligible or even stimulating effects on plant growth and/or photosynthesis when PAR was also high (Searles *et al*., 2001; Nithia *et al*., 2005) [7, 8]. There is general agreement that PAR can alleviate some of the negative effects of enhanced UV-B radiation. Blue light stimulates the production of photolyases which are involved in the repair of UV-induced cyclobutane pyrimidine dimers (CPD) of DNA (Mazza *et al*., 1999; Ballaré *et al*., 2011) [9, 10]. Some of those UV-screening compounds accumulated in plant leaves due to UV radiation also respond to PAR (e.g., flavonoids, ferulic acids, hydroxycinnamic acids etc.; reviewed in (Burchard *et al*., 2000) [11], whereas under low PAR intensities, UV-A can be particularly effective in mitigating UV-B damage (Jenkins, 2009) [12]. However, when PAR is high, effective UV-B damage mitigation seems to proceed irrespective of UV-A dose. (Götz *et al*., 2010) [13] found that high PAR intensities confer basic UV protection through quercetin accumulation, which is enhanced when UV radiation is also received by the plant. Some phenolic compounds are constitutively synthesized in the leaf, others respond to UV-A, while a third strata respond only when UV-B is also present. On the other hand, (Pfündel *et al*., 1992) [14] found that high PAR intensities together with enhanced UVB lead to inhibition of violaxanthin de-epoxidation that represents an important part of plant photoprotection.

UV-B radiation induces a range of morphogenic responses including epidermal and leaf thickening and curling, inhibition of hypocotyl, stem, and leaf elongation, axillary branching, and shifts in the root-shoot ratio reviewed in (Jansen, 2002) [15]. Photomorphogenic responses are mediated by UV-B-specific signalling compounds (e.g., UV RESISTANCE LOCUS 8) and pathways stimulating the expression of genes involved in UV-protection and hence promoting plant survival under UV-B (Jenkins *et al*, 2009) [16]. Only a little is known about the relative importance of constitutive (i.e., genetic) and inducible UV protection in plants (Rizzini *et al*., 2011) [17]. However, it has been reported that variation in constitutive UV protection is small as compared to the amplitude of environmentally induced changes in UV protection (Jansen *et al.*, 2010) [18]. In addition to inherent UV protection, (Fedina *et al*., 2009) [19] reported that the effects of UV-B radiation on barley plants are related to the developmental stages of their photosynthetic apparatus.

1.1. Ultraviolet Radiations

The Sun is the source of light and energy. The rays of light reach us in the form of electro-magnetic radiations. They are known as gamma rays, x rays, ultraviolet rays, visible rays, infra-red rays, microwaves, radiowaves etc. Of all the above rays, the rays of light that have affected our plants in a dangerous way are ultraviolet radiations. They have a wavelength of 200-400 nm. UV Radiations can be of three categories:

i. UV-A (315-400 nm.)
ii. UV-B (290-315 nm.)
iii. UV-C (100-290 nm.)

UV-C radiations are absorbed by the super atmospheric ozone layer. UV-B radiations are absorbed by the ozone layer. The penetration of UV-B radiations through the atmosphere due to ozone depletion is having devastating effects. The results of European studies have shown several implications of UV-B radiations on vegetation. Some of the findings are:

- UV radiations inhibit the growth processes of almost all the green plants.
- The reduction in total dry weight, with reduction in growth of plants in the pea and cabbage family
- Reduction in plant size, leaf area, fresh and dry weight, lipid content and photosynthetic activity.
- Decrease in crop yield, seed production etc.
- There are good evidences of potential yield loss from increased UV-B intensity in Soybean and Pea.
- Several scientists investigated UV-B effects on flowering. UV-B radiations absorbing pigments and diffusing conductance of water vapours through stomata also have been reported by (Tevini, 1993) [20].

1.2. Ascorbic Acid

Ascorbic acid is also known as vitamin C, Cevitamic acid and Antiscorbutic acid. Ascorbic acid is most abundant antioxidant in plants. In recent years, due to its antioxidant properties, it is one being increasingly investigated for their possible role in protecting plants against air polluting gases.

1.2.1. Occurrence & Properties

1. Vitamin C occurs abundantly in fruits such as citrus fruits, lemons, limes and grapes.
2. The leafy green vegetables such as spinach, cabbage, tomatoes etc. contain plenty of vitamin C.
3. It is a colourless, odourless, crystalline substance, slightly sour in taste and optically active.
4. Melting point - 190-192 °C.
5. It is soluble in water & insoluble in ether, alcohol and sterols.
6. Molar mass- 176.1256 g/mol.
7. Density-1.7 g/ml.
8. Alkalies destroy its activity but it is stable in weak acid solutions.
9. It spares vitamin A, E, & some B complex from oxidation.

1.2.2. Mechanism of Ascorbic Acid as Antioxidant

1. Antioxidant is a chemical that reduces the rate of a particular oxidation reaction. Oxidation reactions are chemical reactions, which involve transfer of electrons from a substance to an oxidizing agent.
2. Ascorbate acts as antioxidant by donating electrons and hydrogen ions and reacting with Reactive Oxygen Species of free radical. Free radical oxidizes ascorbate first to monohydroascorbate and then dehydroascorbate. The free radical reduces to water, while the oxidized form of ascorbate is stable and does not cause cellular damage.

2. Review of Literature

Reviews of UV-B effects in terrestrial plants have been published during the last decade by (Kasat, 1979) [21] etc. Many biological effects and physiological changes in terrestrial plants exposed to UV-B radiations have been reported, but much is yet to be learned. Most of the work has been conducted using crop plants primarily from temperate regions, whereas tropical and nonagricultural plants have been more or less neglected. Studies of more than 300 plant species and cultivars have been carried out and about 50% of these plants have been considered as UV-sensitive, where sensitivity is defined as any negative morphological, physiological, or biochemical changes induced by UV-B.

In many sensitive plant species reduced leaf area and stem growth have been found, for example in Wheat, Rice, Maize, Rye, Soybean, Sunflower and Cucumber. Growth parameters like plant height and leaf area were significantly reduced under high UV-B radiation. For leaf area and hypocotyl length of Cucumber and Sunflower seedlings, grown in growth chamber under artificial UV-B radiation, an influence response relationship was demonstrated.

Several scientists investigated UV-B effects on flowering, for example, it has been demonstrated that exclusion of UV-B radiation by plastic films or glass stimulated flowering in *Melilotus*, *Trifolium dasyphyllum* and *Tagetes*. The inhibition of photoperiodic flower induction was found to be dependent on UV- B influence rate and influence on the long day-plant *Hyoscyamus niger*, where a 20% decrease of flowering resulted from irradiation with, 100 mwm-2 UV-B, a 50% reduction from 300 mwm-2 UV-B compared to plants merely irradiated with white light.

Tree species, especially conifers in the seedling stage are also susceptible to enhanced UV-B radiation. Growth physiological function and biochemical composition of sunflower and corn seedlings were intensively studied over

several summer seasons using the ozone filter technique. For both Sunflower and Corn seedlings, growth parameters like plant height and leaf area were significantly reduced under high UV-B radiation. These observations confirm results obtained in previous studies on other plant species with artificial UV-B application in green house and in the fields (Teramura, 1986) [22] or in growth chambers. Multiple sites of UV-B inhibition have been demonstrated throughout the years with the most sensitive site around photosystem II. Photosystem I seems much more resistant to UV-B radiation. In contrast, ribulose 1, 5-biphosphate carboxylase (Rubisco), the key enzyme of the Kelvin cycle, has recently been shown in pea leaves to be very UV- sensitive.

2.1. Impact of Ascorbic Acid on Plants

According to (Foyer and Halliwell, 1976) [23] a major function of ascorbate in plant cells was identified at the end of 1970s when the participation of ascorbate in the scavenging of hydrogen-peroxide was demonstrated.

A possible relationship between ascorbic acid content and tolerance to O_3 was also observed in different Spinach. (Lee, 1991) [24] suggested that in the leaves of soybean ascorbic acid, because of its free radical scavenging property may protect the leaf cells from injury by ozone or other oxyradical products.

According to (Foyer *et al*., 2005) [25], in its role as an antioxidant, ascorbate is a component of the regulatory system that serves to coordinate supply and demand in photosynthesis. The ascorbate system is involved not only as an antioxidant protecting against H_20_2 and other oxygen radicals, but it is also intimately associated with the process of photosynthetic control and energy dissipation. In green plant tissues ascorbic acid is highly concentrated in the chloroplast. It has been estimated that 35-40 % of the cells total ascorbic acid may be present in its chloroplast (Cuadra *et al*., 1997) [26] in *Gnaphalium luteo-album* reported that low UV irradiance treatment showed increase in stem elongation and in absorbance of vacuolar phenolics. High irradiance treatments inhibited plant growth. Negative effects of enhanced UV-B radiation have been demonstrated in plants but impacts under realistic field conditions remain uncertain. Adverse impacts to major crops such as rice (*Oryza sativa* L.) that were grown in areas with currently high ambient levels of UV-B could have consequences for the food security. (Stapleton *et al*., 1997) [27] observed that short term UV radiation damages specific cellular component in *Zea mays*.

(*Dai et al*., 1997) [28] conducted an intensive and extensive series of field experiments, using irrigated rice cultivars under tropical conditions to address the response of rice (*Oryza sativa* L.) to UV-B radiation. This multi season study indicated that supplemental UV-B radiation had no significant effects on rice (*Oryza sativa* L.) grain yield and growth parameters. The absence of UV-B effects was consistent across seasonal environment (4 dry and 3 wet seasons), cultivars and types of exposure system. Thus yields were likely to be affected by increases in UV-B under realistic field conditions.

(Karousou *et al*., 1998) [29] studied that due to the decreasing trends in stratospheric ozone concentration, UV-B radiation has increased at the surface of the earth, which has led to much research on the effects of enhanced UV-B radiation on some changes in *Mentha spicata* plants. (Yue *et al*., 1998) [30] reported a higher reduction in the biomass of leaves than that of stem or spikes in *Triticum aestivum* L. due to enhanced ultraviolet-B (UV-B) radiation. (Jansen *et al*., 1998) [31] observed that UV-B radiation above ambient levels may inhibit plant growth, development, reproduction and depress photosynthesis. (Ambasht and Agrawal, 1998) [32] observed that over 275 percent increase in the anthocyanin 5 content in maize (*Zea mays* L.). Anthocyanins have very weak absorption in the UV-B regions and regarded as UV screens only at very high concentration.

(Furness *et al.*, 1999) [33] observed that there is limited information on the impact of present day solar ultraviolet-B (UV-B) radiation on biomass, grain yield of field crops and on the mechanisms that confer tolerance to UV-B radiation under field conditions. Leaf numbers, leaf area, leaf area ratio, root shoot ratio, specific leaf weight and plant dry weight was decreased under solar UV-B radiation while specific leaf area, number of nodes, auxiliary branching and leaf curling was increased in 3 species of ronglond weeds under solar UV-B radiation.

(Golaszewska *et al*., 2003) [34] conducted an experiment in green house under different doses of supplemental UV-B radiation in two species *Avena fatua* and *Setaria viridis*. They observed a decrease of plant height, fresh mass of leaves, shoots and roots as well as leaf area and leaf curling in both of the species. The significant differences between *Avena fatua* and *Setaria viridis* in the studied traits were mainly due to the tillering ability of the species. The content of chlorophyll varied considerably. Supplemental UV-B radiation did not reduce leaf weight ratio, shoot dry matter, shoot to root ratio and leaf area ratio.

(Hong *et al*., 2008) [35] observed that enhanced supplemental UV-B radiation did not affect seed germination of common alpine grass species and the order affecting seed germination. (Chang *et al*., 2009)[36] observed that supplemental ultraviolet-B (UV-B) radiation significantly reduced plant height but increased the number of shoots and plant dry matter in *Ocimum basilicum* L. (Ravindran *et al*, 2005) [37] investigated that peroxidase activity was also an important component of antioxidant defense system for scavenging H_2O_2. The peroxidase activity in *Phyllanthus amarus* was increased with increasing treatment period of supplemental UV-B radiation. (Hongmei *et al*., 2010) [38] systematically studied the response of 16 day old rice (*Oryza sativa* L.) seedlings to UV (0.67 Wm-2) biologically effective UV-B and 0.28Wm-2 UV-A exposure for 6, 12 and 24 hr. Supplemental ultraviolet exposure resulted in the appearance of light brown patches on leaves, a decrease in the net photosynthetic rate, lipid per oxidation, accumulation of UV-absorbing compounds (including flavonoids and other phenolic pigments) and differential expression of 22 proteins. Both physiological and molecular responses became stronger with increasing supplemental UV exposure time, indicating the effects of UV accumulation on plants. (Takahashi *et al*., 2011) [39] reported the photoreactivation of cyclobutane pyrimidine dimers (CPDs) in chloroplast and mitochondrial DNA in rice (*Oryza sativa* L.). The results indicated that rice may have evolved a CPD photolyase that functions in chloroplasts, mitochondria and nuclei, and that contains DNA to protect cells from the harmful effects of supplemental UV-B radiation.

3. Materials

To investigate the effects of UV-B radiations in combination with ascorbic acid, the following medicinal plants of Lamiaceae-family were selected for the experiment.

(a) *Ocimum basilicum*: The *Ocimum* is an erect, softy, hairy, aromatic herb or under shrub, found throughout India. The plant grows to a height of 50-80 cm. Stem is erect, herebaceous, woody, branched, hairy, sub quadrangular, extremely purplish brown to black. Leaf is 2.5-5 cm long, 1.6-3.2 cm wide, ovate, acute, entire or serrate. Petiole is thin- 1.5-3.0 cm long, aromatic. Flower is purplish, bract- 3 mm long & broad, pedicel longer than calyx. Fruit is in the form of nutlets, each nutlet is one seeded. The leaves are expectorant, stomachic, diaphoretic and aromatic; their decoction is given in malaria, gastric diseases of children and liver disorders. As a prophylactic agent against malaria, fresh leaves are taken with black pepper in the morning. The leaf juice is given in chronic fever, hemorrhage dysentery and dyspepsia. It is also used to check vomiting and as an anthelmintic.

(b) *Mentha piperata*: It is an aromatic perennial herb, growing to 100-120 cm tall, with wide spreading underground rhizomes and erect branched stems. The leaves are arranged in opposite pairs, simple oblong to lanceolate, often downy and with serrate margins. The flowers are produced in clusters (Verticals) on an erect spike, white to purple, the biliped corolla with subequal lobes. Mint is used as a medicinal herb, to whiten teeth, as a diuretic, in perfumes and cosmetics etc.

4. Methodology

UV-B lamp / UV-B filter will be used to expose the plant to UV-B radiations, ascorbic acid will be used as foliar spray. The various methods which will be used are as follows.

A) Epidermal Studies

Epidermal peels will be obtained by mechanical method or by putting leaf pieces in a mixture of 40 ml H_2O_2, 40 ml acetic acid, and 20 ml, water for 24 hrs. at 40°-50°C. Epidermal peels will be stained in Delafield hematoxylin and mount in 50% Glycerin, and borders of cover slip shall be sealed by DPX.

(B) Leaf Architecture

Leaf architecture studies shall be made by clearing leaves in 5% KOH at 60°C followed by lactic acid or 1:1 mixture of saturate aqueous solutions of H_2O_2 and chloral hydrate. Then the clear leaves will be washed with water and then dehydrated in alcohol xylol series. These leaves will be stained with 1% basic fuschin dissolved in 50 % xylol.

(C) Fresh and Dry Weight of Leaves

The leaves shall be plucked and fresh and dry weight of control and treated leaves shall be taken.

(D) Estimation of Chlorophyll

We shall cut 1 gram fresh leaves in to small pieces and homogenize in acetone with a mortar and pestle. Then we shall decant and filter the supernatant in a Buckner funnel through what-man no. 42 filter paper. We shall then add a sufficient quantity of 80% acetone and repeat the extraction. Then we shall transfer the content from the mortar to Buckner funnel & wash the brief with acetone until it is colourless. We shall then put the filtrates and make up the volume 100 ml in a volumetric flask. Then we shall transfer 5 ml of extract into a 50 ml. volumetric flask and dilute it by making up the volume with 80% acetone. We shall measure the absorbance at 645 and 663 nm by spectrophotometer, for the determination of chlorophyll a, chlorophyll b and total chlorophyll. For routine measurement of total chlorophyll content, we shall only measure the absorbance of the extract at 652 nm using a light path of 1 cm.

(E) Seed Viability

For seed viability, seeds will be harvested from control and experimental plants and will be surface sterilized with 0.1% mercuric chloride solution for 2 to 3 minutes. Then they will be imbibed in distilled water for 24 hours. Embryos will be taken out by peeling off the seed coat. These embryos will be kept submerged in 0.1% 2, 3, 5, tetrazolium chloride for 24 hours in dark due to which a reddish colour will be developed in the viable seeds.

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