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UVB Tolerance Mechanisms in Medicinally Important Plant Simarouba Glauca: Epicuticular Wax and Lipid Peroxidation

KEYWORDS: UVB radiation, epicuticular wax, lipid peroxidation

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ABSTRACT *Simarouba glauca is medicinally important oil yielding evergreen tree which is environmentally sturdy. 10h/day UVB (280-320nm) irradiation treatments of 4, 8,12and16days were applied to one year old seedlings. While control plants were kept in normal sunlight. It was noticed that, the epicuticular wax content was elevated by 40% over the control and which further remains stable in 8, 12, and 16 days of UV-B exposed plants. The slight increase in MDA level in response to UV-B irradiations was noticed and this increase was about 5.20% over the control. The plants exposed to UV-B irradiation showed the stable lipid peroxidation indicating 5.20% increase in the Malone di aldehyde (MDA) which is directly proportional to lipid peroxidation. It indicates that the membrane fluidity is stable showing slight leakiness exhibiting a slight damage to the functional proteins, which is further reflected in the slight (2 to 5%) increase in electrolyte leakage. The elevation in epicuticular wax might be contributing for the development of thick waxy cuticle and it helps to reflect the UV-B radiations and protects the internal tissues from UV-B damage.This property of S. glauca leaves might be helpful to develop thick green belts in the regions of which uvb radiations.Thus this species is suitable for the development green belts to reduce UVBradiation effects on human beings*

Introduction

The cuticle is mainly composed of epicuticular wax or bloom containing straight chain of aliphatic hydrocarbons with different types of substituted functional groups .Epicuticular wax contain a crystalline projections from the plant surface which helps in water repellency (Holloway.1969). This forms a self cleaning property known as lotus effect (Barthlott and Neinhuis 1997) and also reflects UV radiations. Jenks and Ashworth (1999) noticed that wax reeduces solar energy load on plant by enhancing reflectance as well as it resist reduced water potential over control. Intra cuticular waxes are enclosed in the cutin and epicuticular waxes which are present on the surface (Jetter *et al.,* 2000).

Lipid Peroxidation is one of most serious sequel of oxidative stress. When ROS levels of plants pass over the capacity to scavenge, it increases the lipid peroxidation in biological membranes, which leads to affect the physiological processes of the cell. Thus, lipid peroxidation is metabolic process which indicates the. Lipid peroxidation divides into three stages. (1) Initiation (2) Propogation oxidative damage (3) Termination (Schiach 1992, Shewfelt and Purvis 1995). Generation of reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide, singlet oxygen or the hydroxyl radical takes place in the initiation phase, which are byproducts produced after electron transport in mitochondria. Lipid peroxidation and degradation leads under a stress condition due to increased level of ROS (Foyer *et al.,* 1994). Fronkel (1985) reported that, propogation phase related with involvement of new molecules of PUFA in the mechanism by peroxyl radicals. One of the last products of oxidative modification of lipid is Malondialdehyde (MDA) which mainly causes the cell membrane damage, changes in internal properties of the membrane like fluidity, ion transport and loss of enzyme activity as well as protein cross – linking. These alternations results in cell death (Sharma *et al.,* 2012). A component of membrane phospholipids are polyunsaturated fatty acid (PUFA) susceptible to ROS activity. These polyunsaturated fatty acids can reacts with ROS species and generates conjugated dienes (CD) or trienes (CT), lipid peroxyl radical as well as lipid hydro peroxides. The lipid hydroperoxide easily generates and breakdowns into many reactive species such as lipid alkoxyl radicals, aldehydes (Malondialdehyde) alkanes, lipid peroxides and alcohols (Davies, 2001 and Fam and Morrow, 2003), thus, in a chain reaction the output of a single initiation event yields multiple peroxide molecules. Finally resulting into the lowering the membrane fluidity, leakiness get high and various functional proteins like receptors and enzymes in the membrane get damaged. As well known the one of the decomposition product of PUFA is Malondialdehyde. Hence, the degree of MDA production is directly proportional to the lipid peroxidation forming a basis of TBARS (Thiobarbaturic acid Reactive substance) Assay, which has been most extensively used for calculating lipid peroxidation (Devasagayam *et al.,* 2003). Various studies have been performed to evaluate the sensitive and tolerant nature of several other plants species to UVB radiations and to understand protective mechanisms. The aim of present study is to evaluate the UVB tolerance mechanisms in medicinally important plant *S. glauca*.

Materials and Methods

One year old seedlings of *S.glauca* were purchased from Nursory of Department of social forestry Kagal. Seedlings with plastic bags were kept in polyhouse under minimum and maximum air temperature 21 to 31^{0}c respectively and relative humidity of air up to 55%. The seedlings were exposed to UVB radiations artificially supplied by UVB tubes (Philips TL20 W/16NV, Holland). For current study experiments were carried out at irradiation level 10 h/day for 4,8,12 and 16 days as per the method described by Lydon et al. (1986). The tubes were suspended perpendicular to the seedlings. Tubes were wrapped with 13 mm cellulose diacetate (CA) film to remove out UVC radiation shorter than 290 nm. CA paper was changed per week to avoid photo degradation. Treatments of UVB radiations were given from 8:00 am to 6:00pm. Epicuticular wax was determined according to the method described by Ebercon (1977). The epicuticular wax was determined from standard graph and expressed as mg/100g fresh tissue. The method described by Carkmak and Hort (1991) was applied for determination of Lipid Peroxidation. The Lipid Peroxidation was expressed as moles MDA h^{-1} g^{-1} fresh tissue.

Results and Discussion

The effect of UV-B radiation on epicuticular wax content in *S.glauca (*fig.1) indicates elevation by 40% over the control and this increase was stable in 8, 12, and 16 days of UV-B exposed plants. Epicuticular wax layer is an important character which response to various types of environmental stresses (Bondada *et al.,* 1996; Rao and Reddy, 1980; Baker, 1982). According to Tevini and Steinmuller, (1987) and Barnes *et al.,* (1996) the epicuticular wax layer acts as interface between the environment and internal structures of the leaf.

Increased waxy layer concentration might provide a protective function against UV-B and helps to which reflects up to 10% -30% UV-B radiations in *Eucalyptus.* (Caldwell, *et al.,* 1983; Holmes, 1997). As Clark and Lister (1975) confirmed that epicuticular wax increased reflectance markedly due to UV-B radiation. Under UV-B radiation waxy layer concentration increased up to 23% in barley plants and similarly up to 28% in bean plants(Steinmuller and Tevini, 1985).They observed that barley leaves had five times higher amount of wax content than bean. Soybean N-15 cultivar canopy with a higher wax concentration reflects more UV-B radiation as compared to cultivar BM-15 that have a low wax content (Grant, 1999).Exposure of cotton to UV-B radiation resulting, 200% increase of epicuticular wax concentration was noticed by Kakani *et al.,* (2003) . In the present observation the epicuticular wax was increased by 10-40%over the control in response to UV-B stress, Hence the slight elevation in epicuticular wax might be responsible for the development of thick waxy cuticle which acts as a interphase between environment and internal structure of leaf as indicated by Barnas *et al.,(*1986).As well as it helps to reflects the UV-B radiations and protects the internal tissues from UV-B damage.

The effect of UV-B radiations on the lipid peroxidation in S *glauca* (fig. 2) indicate slight increase in MDA level in response to UV-B irradiations and this increase was about 40% higher over the control. Costa *et al.,* (2002) stated that in *Conocarpus lancifolius* plants under radiation stress MDA accumulation was most advantageous after 10-20 DAT and further declined. In the present study *S. glauca* grown under UV B stress condition showed 10-20% elevation in MDA level due to 4 to 16 days of treatments. Thus, the plants exposed to UV-B irradiation showed the stable lipid peroxidation indicating 20% increase in the Malone di aldehyde (MDA) which is directly proportional to lipid peroxidation. It indicate that the membrane fluidity is stable showing slight leakiness exhibiting a slight damage to the functional membrane proteins, which is further reflected in the slight (2 to 5%)increase in electrolyte leakage (Patil 2015) under UV stress, this might be responsible for the development of stress tolerance to UV-B radiations.

Conclusion-

It has been concluded that the elevated levels of UVB radiation results in an increase in the epicuticular wax, with stable lipid peroxidation helps to develop thick waxy cuticle and also due to scavenging of ROS by synthesizing antioxidative compounds (Patil 2015), in leaves of *S. glauca* and is considered as evolved mechanism for UVB tolerance. This might be helpful for development of green belts of S*. glauca for* protection against the UV-B radiations, also helps to reduce green house effects in future.

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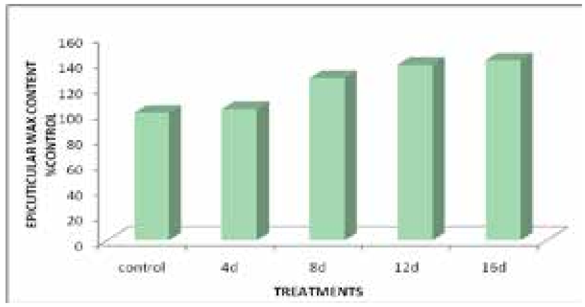
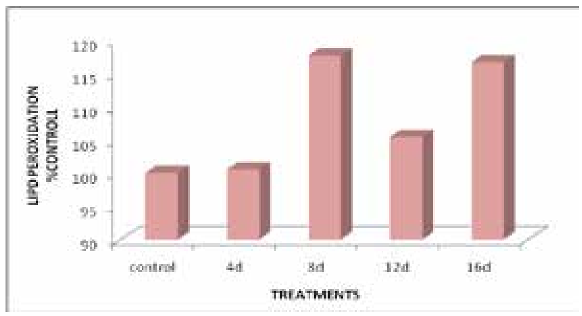


Control value-27.57 mg 100^{-1}g fresh wt.
Figure.1. Effect of UV-B radiation on Epicuticular wax of leaves of *S. glauca*.



Control value -9.23 µmole MDA g^{-1} fresh wt.
Figure 2. Effect of UV-B radiation on lipid peroxidation of leaves of *S. glauca*.

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