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Differential physiological and biochemical responses of two *Vigna* species under enhanced UV-B radiation

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ABSTRACT

Differential physiological and biochemical responses of two *Vigna* spp. i.e. *Vigna mungo* (L.) and *Vigna acontifolia* (Jacq.) seedlings exposed to enhanced ultraviolet-B (ambient+supplemental, 280–320 nm) radiation were studied. UV-B radiation accelerated the generation of ROS i.e. superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) in leaves, and concomitantly damaging effects on lipid peroxidation, electrolyte leakage and growth in both *Vigna* spp. were noticed in dose dependent manner, but *V. mungo* exhibited greater UV-B damaging effects. UV-B stress induced positive response on antioxidants: superoxide dismutase (SOD) and guaiacol peroxidase (GPX) activity, and contents of proline, ascorbic acid, total phenolic contents (TPCs) and total flavonoid contents (TFCs) in leaves of both spp., however, catalase (CAT) exhibited varied activity. The study concludes that substantially higher contents of TPCs and TFCs in epidermal layer, proline and ascorbic acid, and higher CAT activity before and after enhanced UV-B exposure probably attributed greater tolerance to *V. acontifolia* species, thus exhibited lesser UV-B induced damaging effects on cellular components and growth than that of *V. mungo*. This study also suggests that *V. acontifolia* is comparatively resistant to UV-B and thus may be useful for practical cultivation.

1. Introduction

Though due to the successful implementation of Montreal Protocol on substances that deplete the ozone layer, there has been a reduction in incoming solar UV-B radiation (WMO, 2010), however, terrestrial ecosystems appear to be still sensitive due to the variations in UV-B irradiance (Ballaré, Caldwell, Flint, Robinson, & Bornman, 2011). Among the living organisms, plants are vulnerable to increased UV-B radiation as they are absolutely dependent on solar radiation for their survival. Several biologically active molecules such as

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nucleic acids, proteins and lipids absorb the high energy UV-B radiation directly and get damaged (Landry, Chapple, & Last, 1995). The findings have shown that UV-B radiation can affect growth and physiological processes in plants (Musil, Chimphango, & Dakora, 2002; Mishra, Srivastava, & Prasad, 2009).

Reduction in growth of photoautotroph may occur directly due to the effects of UV-B on various cell components or indirectly through enhanced generation of reactive oxygen species (ROS). The photosynthetic and respiratory electron transport systems, photorespiratory pathway and plasma membrane have been considered as potential sources of ROS and oxidative burst (Asada, 1999). ROS play various roles in cellular system that may be positive and related to the regulation of cell growth, intercellular signaling and synthesis of biologically important compounds (Mahalingam & Fedoroff, 2003). However, at elevated concentration, ROS can be extremely harmful to organisms (Jordan, 1996). Superoxide radical ($O_2^{\bullet -}$) is short lived and moderately reactive ROS which is readily dismutated to relatively long-lived hydrogen peroxide (H_2O_2). Hydrogen peroxide may diffuse some distance from its production site and at elevated concentration inhibits photosynthesis, and thus its scavenging is vital for the proper functioning of chloroplast (Foyer & Lelandais, 1996). Superoxide and H_2O_2 at physiological concentration do not produce negative effects, however, their toxicity arises due to formation of metal ion dependent production of hydroxyl radicals ($^{\bullet}OH$), which are capable of mutating DNA and initiating chain reaction of lipid peroxidation leading to loss of function and tissue destruction (Alscher, Erturk, & Heath, 2002).

To mitigate harmful effects of ROS, the aerobic organisms have been equipped with fine regulatory mechanisms to control their levels under limit. The antioxidant defense systems successfully scavenge ROS and protect cells from oxidative damage. The enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and peroxidases (PODs) are efficient scavengers of $O_2^{\bullet -}$ and H_2O_2. The enzyme SOD is present in all sub-cellular components susceptible to oxidative stress and determines the concentration of $O_2^{\bullet -}$ and H_2O_2, and is therefore called first line of defense against ROS (Alscher et al., 2002). The scavenging of H_2O_2 is performed by CAT and a number of PODs. CAT decomposes H_2O_2 to water and molecular oxygen without consuming reductant, thus provides cells with an efficient mechanism to remove H_2O_2 (Scandalios, 1994). PODs are monomeric haemoproteins that catalyze the oxidation of a range of substrates by H_2O_2. In addition to enzymatic antioxidants, organism also contains an important array of non-enzymatic antioxidants i.e. ascorbic acid, proline, phenols, flavonoids, glutathione etc. (Buettner & Jurkiewiez, 1996; Kováčik, Klejdus, Štork, & Malčovská, 2011).

In recent time, gradual change in environment such as enhanced UV-B radiation on the Earth's surface has become an unavoidable fact and thus, causing real threat to the existing organisms. The chlorofluorocarbons, which can deplete the ozone layer and can remain in the upper atmosphere for 40–150 years, hence, the global UV-B radiation will not recover to the levels of the pre-industrialization era by the 2050, even if all the nations implement the Montreal Protocol. Furthermore, it is known that organisms may show differential responses to the stress factors which can be explained on the basis of their morphological, genetical, biochemical and physiological features. In the present study, an attempt has been made to understand the differential responses of growth, oxidative stress and antioxidants system in two species of *Vigna* i.e. *Vigna mungo* and *Vigna acontifolia* at their early stage of growth against enhanced UV-B radiation. The study is significant because (i) the crop is important in maintaining the nitrogen economy of agricultural fields and nutrient value in vegetarian diet due to high protein content, and also (ii) the early stage of seedlings that play a decisive role in the crop yield being used as vegetables and pulses appears to be most vulnerable stage of growth to enhanced UV-B radiation.

2. Materials and methods

2.1. Plant materials and growth conditions

The seeds of *V. mungo* (L.) cv. IPU- 94 -1 and *V. acontifolia* (Jacq.) cv. RM 570 were obtained from Indian Institute of Pulses Research (IIPR), Kanpur and Regional Research Centre, Jaipur, India, respectively. Healthy seeds were surface sterilized in sodium hypochlorite solution for 15 min and washed thoroughly with sterilized double distilled water. Thereafter, seeds were soaked for 2 h in distilled water, wrapped in moistened cotton cloth and left overnight in dark for germination. The germinated seeds were sown in plastic trays containing acid washed sterilized sand and incubated in dark at 28 ± 2 °C for a day. The seedlings were grown in a growth chamber at 28 ± 2 °C under 13:11h light and dark periods (550 μmol photons m^{-2} s^{-1}, PAR) with relative humidity of 60–80%. The seedlings were watered daily with double distilled water. After 3 days of growth, equal sized seedlings were gently transferred in 0.2 strength Rorison nutrient medium (pH 7.5). The nutrient medium was aerated intermittently with sterile air to avoid the anaerobic condition around roots.

2.2. UV-B treatment

After acclimatization in nutrient medium for two days, the seedlings were given two successive exposures of UV-B on 6th and 7th day. UV-B radiation was provided by fluorescent UV-B tube (TL − 40 W/12, Philips, Holland) with its main output at 312 nm together with white light (550 μmol photons m^{-2} s^{-1}, PAR). The UV-B tube was covered with 0.127 mm cellulose diacetate filters (Johnston Industrial plastics, Toronto, Canada to remove radiation below 280 nm) for enhanced UV-B (eUV-B, ambient + supplemental UV-B) radiation. Cellulose diacetate filter was changed regularly to avoid aging effects on the spectral transmission of UV-B. The UV-B irradiance at the top of the plant under the tube was measured with Power Meter (Spectra Physics, Model 407, A-2, USA). At study place, the ambient UV-B radiation was 8.6 kJ m^{-2} d^{-1} on the summer solstice weighted against generalized plant response action spectrum. The plants beneath cellulose diacetate film received different levels of biologically effective UV-B radiation ($UV\text{-}B_{BE}$) i.e. ambient+1.2 kJ m^{-2}, ambient+2.4 kJ m^{-2}, ambient+3.6 kJ m^{-2}, ambient+4.8 kJ m^{-2} and