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Secondary metabolites and phenylpropanoid pathway enzymes as influenced under supplemental ultraviolet-B radiation in *Withania somnifera* Dunal, an indigenous medicinal plant

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ABSTRACT

The present study aims to investigate the effects of supplemental ultraviolet B (3.6 kJ m^{-2} day^{-1} above ambient) radiation on secondary metabolites and phenylpropanoid pathway enzymes of *Withania somnifera* under field conditions at 40, 70, and 100 days after transplantation. Secondary metabolites' (alkaloids, anthocyanins, carotenoids, flavonoids, lignin, phytosterols, saponins, and tannins) concentrations were analysed at the end of the treatments. Activities of phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), 4-coumarate-CoA ligase (4CL), chalcone–flavanone isomerase (CHI), and dihydroflavonol reductase (DFR) were also determined. In treated plants, secondary metabolite-concentrations generally increased (higher concentrations being recorded in roots compared to leaves). Anomalies were recorded for lycopene in roots and phytosterols in leaves (all sampling ages); β-carotene declined in leaves at third sampling age. s-UV-B-treated plants depicted decrease in withanolide A content with concomitant increase in withaferin A (two major alkaloids analysed by HPLC) compared to their respective controls. Phenylpropanoid pathway enzyme-activities increased in leaves and roots under s-UV-B treatment, the latter showing greater increase. The study concludes that s-UV-B is a potent factor in increasing the concentrations of secondary metabolites and their biosynthetic pathway enzymes in *W. somnifera*.

1. Introduction

Ultraviolet (UV) radiation, comprising three components (UV-A, UV-B, and UV-C), is a natural component of sunlight of which UV-B (315–280 nm) has been a potent factor in influencing all aspects of life on Earth since the depletion of ozone layer primarily due to anthropogenic emissions. Though ozone layer depletion and consequent UV-B levels are directly related, the changing climatic conditions and land-use patterns may influence supplementary levels of UV-B reaching the earth much more than ozone depletion itself [1,2]. Laube et al. [3] have detected and quantified three CFCs and one HCFC (emission sources yet unknown) which are being destroyed very slowly in the atmosphere and may be instrumental in increased levels of UV-B reaching the earth. Photosynthetic organisms need sunlight for their survival and hence they require protective structures and mechanisms to shield and/or adapt themselves against this stress factor. One of the mechanisms includes the biosynthesis of more secondary metabolites involved in the normal development and survival of plants. Secondary metabolites such as alkaloids, phenolics, flavonoids, anthocyanins and terpenoids, produced in medicinal plant tissues serve different functions acting as growth regulators, antioxidants, enzyme inhibitors, chemical signals and toxins, and UV-B screens [4,5].

UV-B radiation is an important factor enhancing the production of secondary metabolites in plants [6], and their increased concentrations make them more suited from human health perspective. UVR8 (UV RESISTANCE LOCUS8), has been identified as a UV-B-specific photoreceptor implicated in UV-B perception, and induction and regulation of genes pertaining to plant photomorphogenic responses and phenylpropanoid pathway enzymes. Transcription factor HY5 and MYB also act as regulators of flavonoid biosynthesis pathway genes [7–9]. An overview of the procedure by which UVR8 functions in the plant cell on UV-B perception is shown in Fig. 1.

Withania somnifera is an indigenous medicinal plant and used as a source of natural medicine since ancient times because of the secondary metabolites (notably withanolides) present in it. Withanolides have a wide range of therapeutic applications including antimicrobial, anti-tumour, anti-inflammatory, anti-oxidant,

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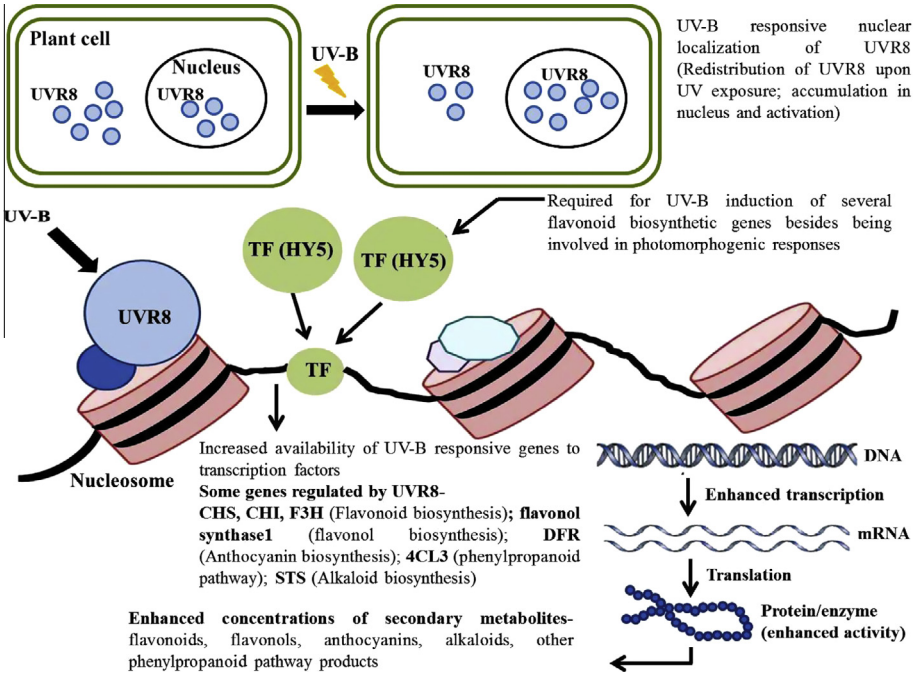

Fig. 1. Perception and functioning of UVR8 in plants.

anti-stress properties; for instance withanolide A possesses immune-modulatory potential while withaferin A is mainly valued for its anti-cancerous properties [10,11]. Though carbohydrate- and elicitor-induced concentrations of withanolide A and withaferin A have been studied in hairy root cultures and in *in vitro* propagated plants [12,13], the secondary metabolite profile of this plant, as well as the concentration and distribution of these alkaloids in different developmental stages of leaves and roots of this plant under field conditions are not reported yet. The objectives of the present research were to study the (i) effects of supplementary dose of UV-B radiation (sUV-B) on some secondary metabolites produced by the plant including alkaloids (along with specific determination of withanolide A and withaferin A via HPLC), anthocyanins, carotenoids (with separate quantitation of lycopene and beta-carotene), flavonoids, lignin, sterols (or phytosterols), and tannins (ii) to determine their role in conferring protection to the plant against this oxidative stress and (iii) to study the activity of some of the enzymes including PAL (phenylalanine ammonia lyase), CAD (cinnamyl alcohol dehydrogenase), 4CL (4-coumarate CoA ligase), CHI (chalcone-flavanone isomerase), and DFR (dihydroflavanol reductase) of the phenylpropanoid pathway, leading to the synthesis of flavonoids, anthocyanins, lignin, and tannins.

2. Materials and methods

2.1. Experimental site

The experiments were conducted in the Botanical Garden, Department of Botany, Banaras Hindu University, Varanasi (25°18′N, 82°03′E, and 76 m above mean sea level), India. The meteorological conditions during the experimental period are outlined in Table 1. The average minimum temperature recorded during the season was 24.4 °C, maximum temperature being 37.8 °C. Relative humidity varied from 41.9% to 61.3%.

2.2. Experimental design

One month old *W. somnifera* plants obtained from nursery were planted in triplicate plots (1 m × 1 m) for control as well as sUV-B treatments. Twelve plants (in 3 rows with 4 plants in each row) were planted in each plot. Under planting conditions, distance between the ridges was 30 cm, between ridges and plot border 15 cm, and between plants 20 cm. Irrigation was done at regular intervals as per the requirement.

2.3. sUV-B treatment

Plants were exposed to sUV-B radiation using UV-B lamps (Q Panel UV-B 313 40 W fluorescent lamps, Q panel Inc., Cleveland, OH, USA) directly after their establishment in the experimental plots. The lamps were wrapped with a 0.13 mm cellulose diacetate filter (Cadillac Plastic Co., Baltimore, MD, USA) (transmission down to 280 nm). Thus, the treated plants received a supplementary UV-B dose of +3.6 kJ m^{-2} d^{-1} above ambient (9.6 kJ m^{-2} d^{-1}) (biologically effective UV-B (UV-B_{BE}) simulating 10% ozone depletion over Varanasi) while the control plants received only ambient UV-B doses. Due to the photo-degradation of filters by UV-B, they were replaced each week. Also, the distance between the plant canopy and UV-B lamps was kept constant throughout the experiment. UV-B was provided to the plants during the solar noon period (11:00 to 14:00 h). UV-B irradiance and biologically effective UV-B (UV-B_{BE}) were measured using Ultraviolet Intensity Meter (UVP Inc., San Gabriel, CA, USA) and Spectropower-meter (Scientech, Boulder, USA) respectively.

2.4. Plant sampling and analysis

The plants were sampled at 40, 70, and 100 DAT (days after transplantation). They were dug out in the form of monoliths with roots intact, washed thoroughly with tap water, and plant parts separated. Fresh leaves and roots were used for all analyses using seven replicates for each treatment.

2.4.1. Analysis of secondary metabolites

2.4.1.1. Alkaloids. The alkaloid content was determined as per the method of Harborne [14]. 5 g of sample was weighed in 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to it. The beakers were covered and allowed to stand for 48 h. The