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Stress Induced Enhancement of Secondary Metabolite Production in *Withania somnifera* (L).

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ABSTRACT: *In this study, an attempt was made to exploit the role of light and moisture stress on the secondary metabolite – Withanolide- production in* Withania somnifera *(L). Dunal. Pot culture experiments were conducted by imposing different levels of shade (25%, 50% and 75% shade) and water stress (75%, 50% and 25% field capacity) along with control under optimum conditions. Exposure of plants to these abiotic stress factors for a period of 30 days was found to enhance the production of withanolide, with a maximum accumulation under 75% shade condition. Analyses of plant growth and translocation pattern also showed favorable changes under low light conditions. An understanding of the role of abiotic stress factors like low light and moisture stress, as associated with secondary metabolite production is of great importance in phytomedical enrichment programmes.*

Keywords: *Withania somnifera, Withanolide, Light stress, Water stress.*

INTRODUCTION

Secondary metabolites are organic compounds produced by plants which have no generally recognized, direct roles in the primary metabolism. They are unique in that particular secondary metabolites are found in only one species or related group of species. In the plant system, they act as defense compounds and have ecological functions by protecting plants against herbivores and microbial pathogens. They also serve as attractants for pollinators and seed dispersing animals. The role of secondary metabolites in the signal transduction pathways has also been established. By virtue of their biological activities, many secondary metabolites are employed commercially as insecticides, fungicides and pharmaceuticals, while others find uses as fragrances, flavoring agents, medicinal drugs and industrial materials. Because of these commercial applications, induction of increased levels of secondary metabolites into specific plant species is gaining tremendous importance.

For enhancing secondary metabolites production, many approaches are used in different systems including plant cell culture, organ culture and use of elicitors in cell suspension cultures. Use of biotechnology and molecular approaches also has wide applications in enhancing the secondary metabolites production. The nature and quantity of secondary metabolite in each plant species will depend upon its genetic makeup. Apart from this, the environmental factors also play an important role in the secondary metabolite accumulation in a system at a particular period of time. Plants can allocate assimilated carbon to any process like synthesis of secondary metabolites, if such diversions are more useful for survival (Lorio, 1986). Considering the functions of secondary metabolites in plant system under stress exposure, an investigation was made into the role of shade and water stress in inducing withanolide production in *Withania somnifera*.

Withania somnifera (L.) Dunal is one of the best known and sought after herbs which has been studied extensively for its biologically active constituents. This is a well known medicinal plant in Indian system of medicine and is of increasing demand in the global market. It is also known as 'Indian ginseng' or 'Winter cherry' belongs to the family Solanaceae. It is called 'Aswagandha' in Sanskrit, Aswagand or Punir in Hindi, Amukkira in Tamil and Amukkuram in Malayalam. Withanolides (C_{28}-steroidal lactones

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derived from triterpenoids) are the major groups of the secondary metabolites of medicinal interest; isolated and characterized from the plant (Patra *et al*., 2004). Besides these, several medicinally important compounds are also isolated from the plants. It has been reported to possess wide range of biological activities including antimicrobial, anti-tumor, anti-inflammatory, antistress, and rejuvenating properties. Since root of the Withania plant is the part most commonly used in herbal medicines, the impact of stress factors on translocation pattern as reflected on root/shoot ratio was also analysed.

In this paper we report the efficiency of low light and moisture stress on improving the withanolide status as well as root/ shoot ratio in *Withania somnifera.*

MATERIALS AND METHODS

Planting Material- *Withania somnifera* seeds (variety: Jawahar aswagandh-20) were collected from the National Centre for Medicinal and Aromatic Plants, Anand, Gujarat, India. The seeds were sown in a tray filled with potting mixture consisting of soil, cow dung and sand in the ratio 1:1:1. One month old seedlings were transplanted in to pots of 10 kg capacity which were filled with the same potting mixture.

Lay Out of the Experiment- The experiment was laid out in CRD with six treatments and four replications per treatment.

Stress Treatments

Light stress- 3 levels of light stress - 25% (T1), 50% (T2) and 75% shade (T3)- were given for a period of 30 days, to 5 month old plants keeping all other factors under optimum conditions. Shade levels were provided using high density polyethylene net purchased from Kerala Agro Industries Corporation which was spread over pandals. Control plants were maintained in open sun light.

Water stress- For imposing water stress, prior to the treatment all the pots were irrigated at evening and left overnight to bring them to field capacity. The exposed part of the soil on the pot was completely covered with the broken pieces of pot. The drainage holes were closed with cement. The weight of the individual pot with soil, pot pieces and plant was recorded with the help of an electronic digital top loading weighing balance. The water holding capacity of the soil was calculated gravimetrically. All the pots in each shade level were grouped into three sets with one set at field capacity (FC) and others at 25% FC (T4), 50% FC (T5) and 75% FC (T6). The weights of the pots to be kept throughout the stress period to maintain the soil moisture levels at 75%, 50%, 25% FC were calculated based on water holding capacity using the equations below.

Total Pot weight at FC (wFC) = A+B+Q100

Total Pot weight at 75%FC ($wFC_{75\%}$) =A+B+Q75

Total Pot weight at 50%FC ($wFC_{50\%}$) =A+B+Q50

Total Pot weight at 25% FC ($wFC_{25\%}$) =A+B+Q25

(A = soil dry weight with pot, B =broken pot pieces (spread on each pot surface to prevent evaporation), Q100, Q75, Q50 and Q25 = Quantity of water present at FC, 75%, 50% and 25% of FC respectively).

The plants were kept at respective soil moisture level by adding same quantity of water which was lost by transpiration during the previous day calculated by weighing the pots daily and by finding out of the difference with the respective reference weights. Stress treatments were given for a period of 30 days and on the thirty first day samples were collected for various analyses.

Study of root characters

Length of Tap Root- The lengths of tap roots were measured using a twine of 1m length. The root length was marked in the twine. The marked length was measured with a scale.

Number of thick roots- Number of thick roots is taken by counting the roots which measured highest in diameter (1-1.5 cm).

Number of medium roots- Number of medium roots is taken by counting the root which measured higher in diameter (0.5-1.0 cm).

Number of thin roots- Number of thin roots is counted by counting the roots which measured lower than medium roots in diameter (<0.5 cm).

Root-shoot ratio- The ratio of root dry weight to shoot dry weight was expressed as root-shoot ratio. The root and shoot portions of uprooted sample plants were cut and separated. The root and shoot portions were dried separately in oven at 85^{o}C for three days, the dry weights of root and shoot were recorded and the ratios were worked out.

Total Dry matter production- The sum of dry root and dry shoot weights were expressed as the total dry matter production.

Estimation of Withanolide Content- Dried root samples were powdered with the addition of liquid nitrogen and the root powder was taken for withanolide estimation. Withanolide estimation was carried out as per the procedure described by Mishra, (1994). The concentration of withanolide was expressed as mg/g. Data are expressed as mean ± standard deviation (SD) of all replicates.

RESULTS AND DISCUSSION

The present investigation was undertaken to study the effect of different levels of light and water stress on production of withanolide. Withania plants were exposed to three levels each of shade intensity and moisture regimes. Significant increase in withanolide content was observed under all the treatments over control. The mean withanolide content was 25.25 mg/g under open and field capacity level. Among the treatments, 75% shade induced maximum accumulation of withanolides (Fig. 1).

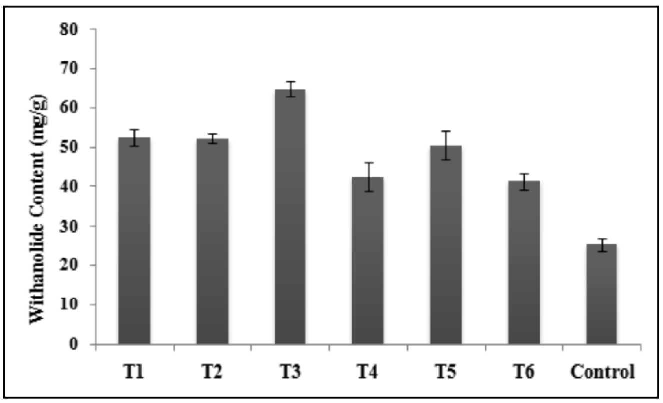

Figure 1: Withanolide content in different abiotic stress treatments (T1- 25% shade, T2-50% shade, T3- 75% shade, T4- 25% FC, T5- 50% FC and T6- 75% FC). Data are expressed as mean ± standard deviation (SD) of all replicates. Values are statistically significant at P<0.05

Exposing plants to any type of stress factor will induce the production of reactive oxygen species which are known to have a damaging effect on membranes, organelles and macromolecules. The role of secondary metabolites as antioxidants and as a protective mechanism against oxidative compounds is a proven fact (Munn and Penuels, 2004). This class of compounds plays a major role in the adaptation of plants to non-optimal growing environments and in overcoming stress constraints (Edreva *et al.*, 2008).

Shade induced enhancement of secondary metabolite production has been reported in few medicinal plants and tree species. In *Centella asiatica* 50% shading of plants resulted in higher yields of herbage and asiaticoside (Shalini *et al.*, 2000). A survey done at tropical rain forest in Gabon revealed that the quantity of biologically active molecules of plants were more in shade compared to open grown plants (Downum *et al.*, 2001). In shade grown *Uncarina rhynchophyll*, the plant growth rate and alkaloid contents in the hooks increased with the degree of shade (Kawazoe *et al.*, 1989).

Withania plants are well adapted to open field conditions. In the present study, unlike the natural habitat, the growing conditions provided for the experimental plants was of very low flux density and of very poor water status which are sufficient for an oxidative burst in the system. As protective antioxidant machinery, there was an activation of withanolide biosynthesis. Salma and Sharma (2005) also reported a protective role of withanolide upon exposure to stress factor. Apart from its role as an anti oxidant, accumulation of withanolides under low light conditions also proves its role in defense against herbivores and pathogens.

This study also give evidence for an altered translocation pattern under stress conditions reflecting upon better root yield in terms of root length, number of roots as well as improved root/shoot ratio. A similar alteration in root-shoot ratio has also been reported in the case of cowpea upon exposure to stress condition (Ashok *et al.*, 2001). Since root of the Withania plant is the part most commonly used in herbal industries, this proves to be highly advantageous (Fig. 2 and Table 1).

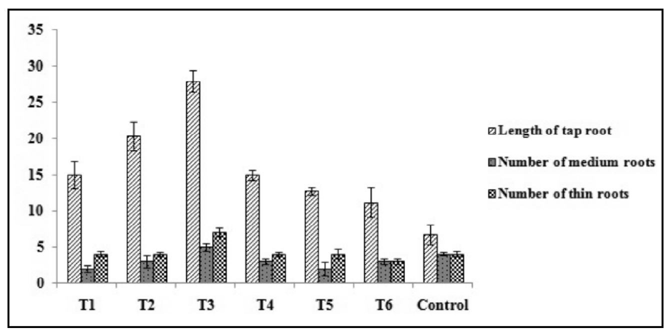

Figure 2: Root characters in different stress treatments. (T1- 25% shade, T2-50% shade, T3- 75% shade, T4- 25% FC, T5- 50% FC and T6- 75% FC). Data are expressed as mean ± standard deviation (SD) of all replicates. Values are statistically significant at P<0.05

Table 1
Root-shoot ratio and total dry matter production

	Root-shoot ratio	*Total Dry matter production*
T1	0.2035	26.14
T2	0.099	22.44
T3	0.2353	28.68
T4	0.0708	20.03
T5	0.1053	23.0
T6	0.1261	22.7
Control	0.1228	22.87
CD(0.05):	0.0681	6.587

The biosynthesis of secondary metabolites, although controlled genetically, is affected to a large extent by various exogenous and endogenous factors. This extends possibilities of altering the biochemical profiles by manipulating the growing environment. In the case of Withania somnifera, exposure of plants to light stress proves to be an efficient and economic technology for phytomedicinal enrichment

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