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Effect of abiotic stress factors on growth, physiology and total withanolide production in *Withania somnifera* (L.) Dunal

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ABSTRACT

An investigation was carried out to study the effects of abiotic stress factors on growth, physiology and total withanolide production in Withania somnifera (L.) Dunal. The abiotic stresses were provided in the form of three levels of light stress (25%,50% and 75% shade) and three levels of water stress (25%,50% and 75% FC) along with control under optimum conditions. Withanolide production was significantly affected by various stress factors. Maimum values for plant height (57.75cm), length of tap root (28.00cm) total dry matter production (28.08g/plant) and specific leaf area(93.40 cm^2/g)were recorded in plants grown under 75% shade. The secondary metabolite withanolide was found to be significantly affect by abiotic stress factors and was also recorded maximum (64.75mg/g) in plants grown under 75% shade.

INTRODUCTION

The medicinal actions of plants are unique to particular species or groups, owing to the pressure of specific secondary metabolites. The secondary metabolites have a pivotal role to play in the ecophysiology of plants and the ecological functions. Many of such phytomolecules have also found to process important health benefits for humans. Plant secondary metabolites also have protective actions in relation to plant's defence against abiotic stress [2]. The efficiency of these phytoceutical molecules is less menifosed a particular environment.

Withania somnifera (L.) Dunal known as Aswagandha is a medicinal plant belonging to the family Solanaceae. Alkaloids and withanaloids (steroidal lactones) are the major groups of the secondary metabolites of medicinal interest isolated and characterised from this plant [6]. They have been reported to possess wide range of biological activities including antimicrobial, antitumour, anti-inflammatory, antistress adaptogenic and rejuvenating actions.

In plants the biosynthesis of secondary metabolites, although controlled genetically, is affected to a large extent by various endogeneous and exogeneous factors. Understanding how the environmental factors affects phytomedicinal chemical production will be of great importance towards optimising field growth conditions for maximum recovery of phytomedicinal chemicals. It also helps to know the extent to which the level of phytochemical production is determined by genetic potential versus environmental modulation. Such information will help us to understand how the plant respond to environmental stress affecting their survival and how humans can alter plants

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metabolism to favour the synthesis of a particular metabolite of a medicinal economical value. In this scenario the present study is undertaken to study the influence of selected abiotic stress factors like low light intensity and low water availability on growth and secondary metabolite production in *Withania somnifera.*

MATERIALS AND METHODS

Withania somnifera seeds (variety:Jawahar Aswagandh-20) were collected from National Centre for Medicinal and Aromatic Plants Anand,Gujarat, India.The seeds were sown in a tray filled with potting mixture consisting of soil, cowdung, and sand in the ratio1:1:1. One month old seedlings were transplanted into pots of 10 kg volumes which were filled with same potting mixture The design were used as completely randomised block design. The seedlings were grown under optimum conditions following the agronomic practises for medicinal and aromatic plants. Five month old plants were exposed to different treatments for a period of 30 days. The treatment level included 3 levels each of light and water stress .The light stress levels were 75%, 50%, 25% shade and control under open conditions. The water stress level was 75%, 50%, 25% of field capacity level and control at field capacity .Growth parameters such as plant height, leaf area, length of tap root and dry matter production, physiological parameters such as specific leaf area, stomatal conductance and total withanolide content were recorded from the treatments at the end of stress period. The data obtained were analyzed statistically.

The total withanolide were extracted in absolute alcohol and determined calorimetrically as suggested by Mishra Mishra [5].The detailed method of withanolide extraction is as follows. Sun dried root were powdered by using liquid nitrogen. One hundred mg of dried root powder was extracted with 5ml of ethyl alcohol and kept overnight at room temperature. Withanoldes were extracted with occasional shaking. The materials were then filtered through whatman no.1 filter paper. The combined filterate was adjusted to 50ml and an aliquot of one ml solution was used for development of colour by adding 2ml of glacial acetic acid (AR grade) and 2ml of colour reagent [8ml of stock solution solution ($2.5gFecl_3.6H_2O$) dissolved in 100ml of orthophosphoric acid].After keeping the test tube for 5 minutes in an ice bath, in an ice bath, the O D was recorded in the spectrophotometer at 540nm.The concentration of withanolide calculated using standard graph for cholesterol.

RESULTS AND DISCUSSION

The data pertaining to the effect abiotic stress factors on growth parameters were presented in the table-1 and physiological parameters and total withanolide content were presented in table-2.

Growth parameters

Plants under 75% shade showed highest plant height. It was increased with shade level. Water stress at different levels reduced the plant height. This finding was in agreement with the result reported by Ancy[1] and George et al.,[4]. Plants grown under 50%shade recorded maximum leaf area. The lowest leaf area was registered when plants grown under different water stress level. This result was in accordance with the study of Pratima [7].The length of tap root increased in all treatments compared to the control. Among the treatments plants grown under 75%shade showed highest length of tap root. The result was the same for the

Table-1 Effect of abiotic stress factors on growth parameters and wittanolide content in *Withania somnifera*

Treatment	Relative water content (%)	Specific leaf area (cm2/g)	Stomatal conduc-tance (cm/s)	Witha-nolide content mg/g
25% FC	77.50	36.40	0.030	42.50
50%FC	81.50	47.37	0.038	50.50
75%FC	85.50	55.95	0.046	41.50
25%Shade	93.60	69.65	0.235	52.50
50%Shade	94.60	85.75	0.125	52.25
75%Shade	95.50	93.40	0.082	64.75
Control	95.00	42.34	0.081	25.25
Grand mean CD(0.05)	88.668 1.994	61.261 4.999	0.101 0.095	47.035 2.182

Table-2 Effect of abiotic stress factors on physiological parameters in *Withania somnifera*

Treatment	Plant height (cm)	Leaf area cm^2	Length of tap root (cm)	Total Dry Matter Production (g/plant)
25% FC	38.75	2.05	15.00	20.03
50%FC	43.25	1.40	12.67	23.00
75%FC	49	3.73	11.14	22.07
25%Shade	53	10.25	15.00	26.14
50%Shade	54	10.26	20.32	22.44
75%Shade	57.75	6.63	28.00	28.68
Control	47.75	7.75	6.70	22.87
Grand mean	47.428	5.985	14.895	23.690
CD(0.05)	10.193	2.656	5.609	6.587

total dry matter production and it was in agreement with the findings of Ancy [1].

The specific leaf area was high in 75% shade and was very low in different field capacity levels. The highest stomatal conductance recorded under 25% shade condition. There was a reduction in the stomatal conductance corresponding to the increase in water stress from 75%to 25% field capacity reported by Else et al [3]. Since whole part of *Withania somnifera* is equally important for medicinal purposes all theseresults were important for the medicinal compounds enrichment and accumulation programme.

Withanolide accumulation

The effect of light and water stress on total withanolide content of *Withania somnifera* was significant. Shade stress increased the withanolide content. The maximum was recorded in the treatment 75% shade followed by 25% shade and it showed a decreasing trend when field capacity decreased from 75% to 25%. However the total withanolide content was higher in field capacity levels compared to control plants. The metabolite withanolide was reported to have antioxidant activity and thus plants under shade and water stress accumulated more withanolide to protect them from low light stress and oxidative stress Salma [8]. It has also been reported that, even though the withanolide synthesis is governed by genes, fluctuations in the concentrations and amount of withanolides produced by plant is highly influenced by environmental conditions [9]. It effects the general growth of the plants as well as formation of alkaloids. As there is direction relationship between CO_2 fixation and growth, with light, there can be remarkable differences in the production of secondary metabolites in the plants under varying conditions of light.

CONCLUSION

The present investigation has shown that exposure to abiotic stress factors invariably leads to better growth and physiological characters and accumulation of total withaolide. It has shown that apart from gene controlling the synthesis of withanolide and environmental factors alters biosynthesis and degradation of the secondary metabolite. Therefore understanding the role of abiotic stress factors is of great importance towards the enrichment of phytomedicinal programme.

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