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A Study on ABA Induced Changes in Growth and Photosynthetic Pigments of *Withania somnifera*

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Abstract: ***Ashwagandha (Withania somnifera L.) is an important medicinal plant; the root is the official part. ABA is the stress hormone, application of ABA cause stress symptoms. Secondary metabolites are increased under stressed condition. Thus, ABA may indirectly improve the medicinal value of the plants. An attempt has been made here to study the growth and photosynthetic pigments of Withania somnifera plants treated with ABA. The plants were raised in the pots. ABA 10-5 M solution was exogenously applied as a foliar spray at vegetative stage. DW was also sprayed as a control of spray treatment. After one month of spray treatment various growth parameters of control and treated plants were noted. The photosynthetic pigments were estimated from the leaf of control and treated plants. ABA spray reduced the root elongation but it was remarkably thick. Root-shoot weight was enhanced by ABA. DW spray increased the elongation of root and shoot but decreased the leaf number. Photosynthetic pigments especially chlorophyll were more in ABA treated plants. Root growth improvement may be correlated with higher amount of photosynthetic pigments. The present data suggest that better root growth in ABA treated plants may increase the medicinal value of plant.***

Keywords: ABA, Vegetative growth, Photosynthetic pigments, Withania somnifera.

I. INTRODUCTION

Abscisic acid (ABA) is a growth inhibitor (Goodwin, 1978). The regulatory role of ABA in plants is best documented for responses to stress, especially water stress. ABA level is increased under water stress, rewatering restores normal level (Leopold and Kriedemann, 1988). The rise in ABA level brings about a rapid closure of stomata, thus rate of transpiration is reduced and the plant is able to conserve its internal water. The function of ABA also includes lateral bud dormancy, leaf abscission, senescence, xylem transport, the inhibition of seed germination, growth and IAA transport etc. (Matthysse and Scott 1984). It is not clear how ABA influences the water status of the guard cells. The effect of an external spray of ABA on guard cells is not long lived but usually is lost in several days. ABA has a role in the normal regulation of the opening and closing of stomata. ABA is a kind of universal stress hormone, its production is controlled or triggered by several mechanisms. In all the cases, it seems to reduce growth and metabolism and thus conserve resources, which will then be available during recovery if and when the stress is removed. Metabolism and physiology of ABA in plants is discussed (Zeevaart and Creelman, 1988). It is known that ABA is increased under stress, thus ABA application as a foliar spray is used to induce stress in plants

II. MATERIALS AND METHODS

Seeds were sown in the pots and transferred at 3 to 4 leaf stage in another pots and raised using normal practice. At 7 to 8 leaf plants were sprayed with 10-5 M ABA. The spray was given thrice within a week. DW was also sprayed as a control for foliar spray. Hand sprayer was used for exogenous application and spraying was done till the leaf surface became wet on both sides. The plants without any spray were considered as control. The effects of ABA induced changes on *Withania somnifera* were studied as follows

(A) Study on growth
(B) Study on photosynthetic pigments

(A) Study on growth

Ten plants of control and treated at random were selected. These plants were carefully uprooted (minimizing the damage to the root) and brought to the laboratory, thoroughly washed with water and gently pressed against blotting sheets to remove moisture from their surface. Root length and stem height of each plant were measured and expressed as cm/plant, leaf number was also noted and expressed as no/plant. Plant parts viz root, stem and leaf were separated and fresh weight (g/plant) of each part was recorded. These were then placed in a paper bag and transferred to oven at 800c for a period of one week for complete drying. Dry weight (g/plant) was recorded.

Following parameters were studied:

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1. Root length (cm/plant)
2. Shoot length (cm/plant)
3. Leaf number (no/plant)
4. Fresh weight of root, stem and leaf (g/plant)
5. Dry weight of root, stem and leaf (g/plant)

(B) Study on photosynthetic pigments

The fourth leaf in replicate from control, DW and ABA sprayed plants at 7 to 8 leaf stage (110 DAS) was selected and photosynthetic pigments, viz. chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoides were estimated using the method of Arnon (1949).Leaf was analyzed for photosynthetic pigments. Weighed fresh leaf material was crushed in 80 % acetone (80 ml acetone + 20 ml DW) with a pinch of sand. The homogenate was filtered using Whatman filter paper No. 1 and the filtrate was made upto a specific volume. The absorbance of the chlorophyll suspension was read on Systronics106 spectrophotometer at 480, 510, 645 and 663nm.

III. RESULTS AND DISCUSSION

(A) Study on Vegetative Growth:

Table-1represents the effect of foliar spray of ABA at vegetative stage on root length, stem height plant height, and leaf number of *Withania somnifera*. The root length and stem height of control, DW and ABA sprayed plants were increasing with time. Spray treatment lowered elongation of root, it was more in 205 days old plants DW sprayed plants in comparison to that in control, stem elongation and plant height were lowered by ABA spray while DW spray promoted it. DW Spray lowered the leaf number but in 205 days old plants it was more than control. ABA spray decreased number of leaf. The above data suggests that foliar spray of ABA at 6 to 7 leaf stage inhibited the elongation as well as number of leaf. It is known that ABA inhibits the growth of the plants (Zeevaart and Creelman, 1988; Moore 1989). ABA is well known for its growth inhibition properties.

Table: 1 Effect of Foliar Spray of ABA at Vegetative Stage of *Withania somnifera*.

Parameter	Treatment	Growth period (days) 75	130	145	160	175	190	205
Root length cm/plant	Control	3.7	4.21	5.13	11.1	12.63	13.2	13.8
	DW sprey	3.7	4.21	5.36	6.83	11.6	12.0	16.3
	ABA sprey	3.7	4.21	5.13	10.73	11.8	12.75	14.05
Stem height cm/plant	Control	4.51	6.45	7.23	10.13	13.6	20.1	21.8
	DW sprey	4.51	6.45	10.6	11.96	15.56	22.9	24.95
	ABA sprey	4.51	6.45	9.9	14.7	19.93	27.85	28.8
Leaf number no/plant	Control	4	6	8	12	15	19	21
	DW sprey	4	6	10	10	13	15	24
	ABA sprey	4	6	9	11	15	17	19

Table -2 represents the effect of foliar spray of ABA at vegetative stage on fresh weight and dry weight of early sown *Withania somnifera*. Fresh weight of root, stem, leaf and whole plant was increasing with time. The ABA spray treatment lowered fresh weight of root, stem, leaf thus whole plant fresh biomass was also lower than fresh biomass of control. DW spray enhanced root and stem fresh weight in old plants but lowered leaf biomass. ABA induced the symptom of stress in plants. As leaf is the site of application, it may be affected first by spray treatment. DW spray also significantly lowered leaf fresh weight and it may be due to oxygen deficiency thus growth may be inhibited under such condition.

Dry weight of root, stem, leaf and whole plant was increasing with time. Generally ABA Spray lowered dry weight. DW spray also lowered dry weight. The above data suggests that ABA may cause inhibitory effect on dry biomass of ashwagandha. I'ma Neerakal et. al., (1994) reported that application of ABA before two months of harvest of Gerenium thumbergii reduced the dry biomass of aerial part, when ABA is sprayed on leaves, guard cell loose turgor, stomata close, transpiration is chacked and ultimately growth will be inhibited (Moore 1989). ABA inhibited the photosynthesis in pea seedling and it was due to adverse effect on chloroplast structure (Krendeleva and Makeev, 1987). ABA accelerates the abscision process (Singh and Murty, 1984). ABA is an inhibitor of expansion.

Table: 2 Effect of Foliar Spray of ABA on Fresh weight and Dry Weight of *Withania somnifera*

Parameter	Treatment	Growth period (days)						
		75	130	145	160	175	190	205
Fresh weight g/plant								
Root	Control	0.821	1.01	1.12	1.92	2.75	3.1	3.7
	DW sprey	0.821	1.01	1.6	2.2	2.37	2.75	4.0
	ABA sprey	0.821	1.01	1.35	2.45	3.25	4.4	4.6
Stem	Control	1.006	1.3	2.0	2.33	3.65	4.45	5.9
	DW sprey	1.006	1.3	2.1	2.8	4.3	6.1	6.41
	ABA sprey	1.006	1.3	1.6	3.38	5.2	8.05	8.4
Leaf	Control	2.21	5.07	7.49	10.9	16.45	26.9	27.4
	DW sprey	2.21	5.07	7.49	10.9	16.45	26.9	27.4
	ABA sprey	2.21	5.07	7.49	10.9	16.45	26.9	27.4
Dry weight g/plant								
Root	Control	0.09	0.195	0.281	0.417	0.693	0.702	1.048
	DW sprey	0.09	0.195	0.48	0.493	0.715	0.764	1.562
	ABA sprey	0.09	0.195	0.39	0.634	0.765	1.562	2.374
Stem								
	Control	0.14	0.32	0.395	0.479	0.628	0.84	1.517
	DW sprey							
	ABA sprey	0.14	0.32	0.471	0.515	0.939	0.994	1.266
Leaf		0.14	0.32	0.335	0.763	0.921	1.698	1.962
	Control							
	DW sprey	0.58	0.689	0.768	1.446	2.044	2.192	4.212
	ABA sprey							
		0.58	0.689	1.034	1.355	2.145	2.512	2.669
		0.58	0.689	0.895	1.669	1.82	2.763	3.281

Table 3 represents the effect of foliar spray of ABA at vegetative stage on photosynthetic pigments in the leaf of early sown *Withania somnifera*. The chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids were generally more in ABA sprayed plants than that in control. DW treatment enhanced chlorophyll 'a', chlorophyll 'b' during 160 and 175 days. The higher amount of photosynthetic pigment may be considered as physiological adaptation against ABA spray. Generally ratio of chlorophyll 'a' : chlorophyll 'b' was lowered by spray treatment. DW and ABA spray more or less equaly effective. The ratio of total chlorophyll : carotenoids was higher in 160 days old treated plants in comparison to that in control. Reverse was found in 205 days old plants. The changes in ratio of photosynthetic pigments may be one of the cause of reduction in growth of treated plants. The higher total chlorophyll: carotenoids ratio indicates adaptation while lowering in ratio suggests inhibition in growth. Chlorophyll content in leaf tissues varies with age of the plant and growing season. The ratio of chlorophyll 'a' : 'b' in maize leaf varied with monsoon and winter season. The ratio was correlated with NAR (Kumar and Singh, 1996). The chlorophyll content is an estimate of the overall growth and productivity of plant. Change in total chlorophyll : carotenoids ratio is the sensitive indicator of oxidative damage (Hendry and Price, 1993).

Table – 3 Effect of foliar spray of ABA on photosynthetic pigments of *Withania Somnifera*

Treatment	Growth Period - d						
	75	130	145	160	175	190	205
	CHLOROPHYLL 'a' - mg/g fr wt						
C	1.650 ± 0.0070	1.699 ± 0.0028	3.383 ± 0.0079	2.600 ± 0.0070	3.760 ± 0.0050	3.813 ± 0.0033	3.694 ± 0.0064
DW			3.074 ± 0.0036	3.126 ± 0.0051	4.005 ± 0.0062	3.542 ± 0.0060	3.236 ± 0.063
ABA			2.898 ± 0.0042	3.641 ± 0.0105	4.845 ± 0.0035	3.975 ± 0.0147	4.120 ± 0.0109
	CHLOROPHYLL 'b' - mg/g fr wt						
C	1.724 ± 0.0063	1.714 ± 0.0076	2.795 ± 0.012	1.911 ± 0.062	3.467 ± 0.0049	2.965 ± 0.0061	3.819 ± 0.0035
DW			2.788	2.856	3.343	2.988	3.681

			± 0.0034	± 0.0060	± 0.0077	± 0.0061	± 0.0049
ABA			2.676 ± 0.0063	2.765 ± 0.0084	2.427 ± 0.0197	3.595 ± 0.0183	4.027 ± 0.0155
TOTAL CHLOROPHYLL - mg/g fr wt							
C	3.374 ± 0.077	3.413 ± 0.0035	6.178 ± 0.0056	4.511 ± 0.0099	7.227 ± 0.0035	6.778 ± 0.0085	7.513 ± 0.0042
DW			5.862 ± 0.0035	5.982 ± 0.010	7.348 ± 0.0049	6.530 ± 0.0070	6.917 ± 0.010
ABA			5.574 ± 0.0091	6.406 ± 0.0077	5.470 ± 0.0070	7.570 ± 0.072	8.047 ± 0.0091
CAROTENOIDS - mg/g fr wt							
C	3.328 ± 0.0084	3.319 ± 0.0040	5.824 ± 0.0072	4.738 ± 0.0077	5.958 ± 0.0084	5.682 ± 0.0084	6.978 ± 0.010
DW			5.558 ± 0.010	4.959 ± 0.0063	5.903 ± 0.0077	5.856 ± 0.0037	7.536 ± 0.0071
ABA			5.427 ± 0.0060	5.016 ± 0.0084	4.803 ± 0.0055	6.633 ± 0.0077	8126.000 ± 0.0063

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