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The Effect of Plant Growth Regulators and Temperature Shock on 1AA-oxidase Activity of Roots and Endogenous ABA Level of Leaves

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bstract

e effect of plant growth regulators. Indole acetic acid (1AA), Abscisic acid (ABA) and Kinetin each at 10^{-6}M were used seed soaking treatment on *Glycine max* (L.) NARC 1. Plants were subjected to 3h temperature shock at 42°C continued r a period of 10 days. The plants were harvested 46 days after sowing and analyzed for 1AA oxidase activity and for dogenous level of ABA. IAA oxidase activity of roots from temperature shocked plants were found significantly lower an that of control. The maximum values were recorded in IAA and kinetin treated plants and the minimum value was corded in ABA treated plants. Whereas, the plants maintained without temperature shock was found significantly higher an that of control. The maximum values were recorded in IAA and ABA treated plants as compared to kinetin. The mperature shocked plants increased the endogenous level of ABA as compared to without heat shocked. This increase the endogenous ABA level in the leaves might be the result of de novo synthesis of ABA in response to increase in mperature.

troduction

ybean is perhaps one of the oldest food crops of the rld due to its good quality oil, protein contents and soil riching properties. The seed contain about 20 per cent and good quality protein, 23 per cent corbohydrates ,5 r cent minerals, 3 per cent crude fibre, 9 precent isture and reasonable amount of vitamins and nerals(Gandhi *et al.*,1985).

A is a natural growth inhibitor and well known as "stress rmone".It is a sesquiterpenoid in nature.The regulatory e for ABA is best documented for response to stress, ecially in water deficiency. Abscisic acid accumulates in nts during water stress (Hsiao, 1973) and prevents the s of water by closing stomata and increasing the draulic conductivity of roots. Kinetin is a synthetic okinin which does not occur naturally in plants. Due to property of actively promoting cell division (in njunction with auxin) it was given the name of kinetin. sent attempt has been undertaken to evaluate the ects of growth regulators and temperature shock on A-oxidase activity of roots and endogenous ABA level eaves of soybean.

aterials and Methods

face sterilized seeds of soybean were soaked in distilled ter (Treated as control) and aqueous solution of 1AA $^{-6}$M), ABA (10^{-6}M) and kinetin (10^{-6}M) for 6h were wn in earthen pots measuring 24 X 30 cm^2, filled with xture of sand and soil in 1:3, organic manure and DAP ammenium phosphate) were also added. The seed in the s were allowed to grow during mid August under trolled conditions. Plant were placed in growth room at 30°C night/day and the relative humidity varied from 85 per cent with photo period of 16 h. Four weeks r sowing, half of the plants in the pots were subjected to temperature shock. The temperature was increased at the rate of 2°C h and maintained at 42°C for 3 h for 10 d; during that period the relative humidity varied from 62-75 per cent .

IAA oxidase activity: 1AA oxidase activity of roots was measured by the estimation of residual 1AA using Salkowskis reagent, (Malik and Sing, 1980).

Endogenous level of ABA: Extraction and purification for endogenous ABA was made from leaves of the control and treated plants according to the method of Hillman (1978). Leaves (7 gm) were homogenized in 80 per cent methanol with butylated hydroxy toluene (BHT) added as an antioxidant. The extraction was made for 72h with frequent change of solvent after every 24h. The extract was filtered through Buchner funnel, and reduced to aqueous phase using rotary thin film evaporator at 35°C. The aqueous phase was adjusted to pH 9 and partitioned 3 (with 1/3rd volume of ethyl acetate to remove basic and natural compounds. The organic phase was discarded. The aqueous phase was readjusted to pH 2.5-3 using 0.1 N HCl and partitioned 3 (with 1/3rd volume of ethyl acetate. The sample was dried on RFE at 35°C. The residue was dissolved in methanol (100%), dried under oxygen-free nitrogen, and then re-dissolved in 100 per cent MeOH (100 ul). HPLC (Model 1C -, A-Shimadzu Ltd. Japan, Detector SPD-6AV (Shimadzu) Column: C-18. Time Flow = 1 ml/minute, OVEN T = 25 C, Att: = 6, Mobile phase = Acetonytrite.

Results and Discussion

The result of ANOVA and DMRT of 1AA-oxidase activity of roots of temperature shocked plants (Table 1 and 2) showed that 1AA-oxidase activity was significantly lower than that of control ($P<0.05$). Among the treatments, the maximum value was recorded in 1AA followed by Kinetin-treated plants as compared to ABA. The result of

Table 1: ANOVA of 1AA (ug)g^{-1} fresh weight of roots (Temperature shocked plants) in *Glycine max* L. Following four treatments with plant growth regulators.

Source	DF	SS	MS	FC	Table value of F
Replication	3	4218.35	1406.117	4.990	9.78
Treatment	2	634.28	317.14	1.124	10.92
Error	6	1692.3	282.05		
Total	11	6544.93			

$P < 0.01$

Table 2: DMRT of four treatment means of 1AA (ug) g^{-1} fresh weight of roots (temperature shocked plant).

Treatment	Mean	S.E	Duncan Test
Kinetin	45.1667	11.9175	B
ABA	12.0333	5.2346	C
IAA	52.700	13.4990	A
Control	61.6667	6.0093	D

All such means, which share a common English letter, are nonsignificantly different, otherwise they differ significantly at least $P < 0.05$.

Table 3: ANOVA of 1AA (ug) g^{-1} fresh weight of roots (without temperature shock plants) in *Glycine max* L. following four Treatments.

Source	DF	SS	MS	FC	Table value of F
Replication	3	104.01	34.67	2.89	9.78
Treatment	2	20.65	10.325	1.163	99.33
Error	6	72.05	12.01		
Total	11	196.71			

P , 0.01

Table 4: DMRT of four Treatment means of 1AA (ug) g^{-1} fresh weight of roots without temperature shock plants).

Treatment	Mean	S.E	Duncan Test
Kinetin	5.000	0.0577	B
ABA	10.1667	3.4197	A
IAA	2.7000	0.5508	D
Control	3.2667	0.0822	C

All such means, which share a common English letter, are non-significantly different, otherwise they differ significantly at least at $P < 0.05$.

ANOVA and DMRT (Table 3 and 4) of plants without temperature shock showed significant difference ($P<0.05$) among the treatments. The maximum value was recorded in ABA treated plants than that of 1AA and kinetin.

The maximum decrease in 1AA-oxidase activity was recorded due to ABA treatment. Previous reports indicated ABA-induced increase in resistance to temperature shock (Boussiba, *et al.*, 1975). There is some evidence suggesting that the activity of this enzyme is inversely correlated to 1AA level. Markhart (1984) suggested that ABA might act by protecting membrane from damage by temperature shock conditions. ABA is stress hormone and the endogenous levels of ABA have shown to be increased in heat shock (Letham *et al.*, 1978).

In plants without temperature shock 1AA oxidase activity of roots was significantly different than that of control. It has been shown that kinetin inhibited root tips also contain more peroxidase and destroy more 1AA (Jacob Levitt, 1974) because 1AA-oxidase is an isoenzyme of peroxidase, the similar mechanism of IAA, destruction might operate. Letham (1978) also reported that ABA increase the production of IAA.

Analysis of the data showed that temperature shock increased the endogenous level of ABA in leaves of the plant by 47 per cent (729 ng g^{-1} fwt ABA in treated plants vs. 494 ng g^{-1} Fwt in control). This increase in the endogenous ABA level in the leaves might be the result of de novo synthesis of ABA in response to increase in temperature. This was generally considered as an adaptive response to the stress conditions e-g salinity, heat, cold stress and moisture stress etc. (Topcuoglu *et al.*, 1990). Corbineau *et al.* (1991) observed that dormant embryos were one to ten times more responsive to ABA at 30°C than at 10°C.

According to Kiyosue, *et al.* (1994) accumulation of abscisic acid (ABA) in *Arabidopsis thaliana* L. began to increase 2 hours after plants had been subjected to dehydration stress and reached maximum levels after 10 hourse.

References

Boussiba, S. A. Rinkin and A.E.Richment, 1975. The role of ABA in cross adoptation of tobacco plants. Plant Physiol., 56: 337-339

Corbineau, F. A. Poljakoff-Maybar, and D.Corne, 1991. Responsiveness to abscisic acid of embryos of dormant oat (Avena sativa) seeds. Involvement of ABA-induceable proteins, Phisiologia Plantarum, 83: 1-6

Gandhi, A.P., M.M. Nenwant and A. Nawab , 1985. Some physio-chemical characteristics of soybean. Food Chemistry, 17: 71-74.

Hillman, J.R., 1978. The identification and quantitative analysis of Abscisic acid in plant extracts.Isolation of plant growth substances. Seminar series 4: 115-13

Hsiao, T.C., 1973. Plant response to water stress. Ann Rev. Plant Physiol., 24: 519-570.

Kiyosue, T.K., Yamaguchi-Shinozaki and K, Shinozaki 1994. Cloning of cDNAs for genes that are early responsive to dehydration strees (ERDS) in Arabidopsis thaliana L,: Odemtofocatopm pf tjree ERDS as H congnate genes, Plant Mol. Biol., 25: 791-798.

tham, D.S., P.B. Goodwin and T.J.V. Higgens,1978. Phytohormones and Related compounds. A comprehensive Treatire Elsevier/North Holland Biomedical Press, Amstersdam, 11: 575-576.

vitt, J., 1974. Introduction to plant physiology (2nd ed.) The C.V. Mosby company, U.S.A., 19: 303-344.

lik, C.D. and M.B. Singh, 1980. Plant enzymology. Plant enzymology and Histo. enzymology. Karachi. Kayani Publisher, New Delhi, Ludhianam pp: 51-53.

Markhart, A.I., 1984. Amelioration of chilling induced water stress by ABA induced changes in root hydraulic conductance. Plant Physiol., 74: 81-83.

Topcuoglu, S. F and Husnu Cakirlar., 1990. Changes in the levels of abscisic acid (ABA) depending on the salt concentration and the leaf age of sunflower plant *Helianthus anus* L.) grown under the salt stressed conditions. DOGA BIYOL SERISI, 14: 79-95.