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EFFECT OF ABA AND KINETIN ON GLUTAMATE DEHYDROGENASE AND GLUTAMATE OXALOACETATE TRANSAMINASE ACTIVITY IN GERMINATING GROUNDNUT SEEDS

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SUMMARY

Abscisic acid inhibited and kinetin promoted the germination of groundnut seeds. After 72 hours of imbibition, the dormant cultivar showed higher GDH activity as compared to non-dormant seeds. ABA induced dormancy in non-dormant seeds was accompanied by an increase in GDH and decrease in GOT activity. Kinetin treatment decreased the GDH activity in dormant seeds. Removal of dormancy by kinetin treatment was associated with an increase in GOT activity. The differential effects of ABA and kinetin on the activity of these enzymes therefore depend on the state of seed dormancy.

INTRODUCTION

It is well known that many of the steps in seed germination and plant growth are influenced by phytohormones. However, the molecular basis of physiological responses are not fully understood (Khan, 1967; Khan *et al.*, 1971; Tao and Khan, 1977). Growth regulating substances *e.g.* gibberellins and cytokinins have been shown to promote seed germination by regulating the synthesis of some hydrolytic enzymes (Crispeels and Varner, 1967a, 1967b; Jacobson and Varner, 1967). On the other hand, the growth inhibitors like ABA inhibits seed germination by inhibiting the gibberellic acid induced synthesis of these hydrolytic enzymes. It has been shown earlier that transamination of glycine from glutamic acid is enhanced by gibberellic acid in germinating wheat seed (Sengupta *et al.*, 1967). However, the influence of phytohormones on the enzymes of amino acid metabolism has not been studied adequately. The present paper reports the influence of ABA and kinetin on GDH and GOT enzymes during germination of dormant and non-dormant seeds of groundnut.

MATERIALS AND METHODS

Freshly harvested seeds of two groundnut cultivars viz. J-11 and M-13 were collected from the field. It has been observed earlier that the seed of

J-11 are non-dormant while the seeds of M-13 remain dormant for a period of about 60-70 days (Sengupta *et al.*, 1977). Experiments on the effects of growth regulators were made with (i) fresh seeds and (ii) stored seeds (100 days after harvest). The kernels of groundnut pods were used after removal of shells and soaked in 4×10^{-4}M ABA and 5×10^{-4}M kinet in before transferring them into petri dishes lined with moistened germinating paper. The seeds were pre-treated with 0.3% captan and the germination percentage was recorded according to ISTA regulation (Table I). For enzyme assay the seed coat and embryos of 72 hours imbibed seeds were removed and the cotyledons were washed with distilled water. Seeds were chilled and 5g materials were used for enzyme extraction. The procedure for enzyme extraction and partial purification of enzymes were followed as described earlier (Sengupta and Manglik, 1980). The activity of glutamate dehydrogenase (GDH) was assayed following measurement of oxidation of NADH spectrophotometrically at 340 nm (Bulen, 1956). The complete reaction mixture in the final volume of 3.0 ml contained 20 μ moles of α-ketoglutaric acid, 150 μ moles of ammonium sulphate, 125 μ moles of Tris-HCl buffer (pH-7.8) and 0.2 ml of enzyme extract. The reaction of the enzyme was initiated by adding 0.2 μ mole of NADH. The values of optical density were corrected against a blank where α-ketoglutarate was omitted. Specific activity of GDH is expressed as n moles of NADH oxidised per mg protein per minute. The activity of glutamate oxaloacetate transaminase (GOT) was assayed following the production of oxaloacetate measured at 280 nm (Green *et al.* 1945). The complete reaction mixture contained in a final volume of 3.0 ml, 100 μ moles of aspartate, 50 μ g of pyridoxal phosphate, 130 μ moles of Tris-HCl buffer (pH-7.8) and 0.2 ml of enzyme. The enzyme reaction was initiated by the addition of 20 μ moles of α-ketoglutarate. The specific activity of GOT was calculated as nano moles of oxaloacetic acid formed per mg protein per minute. The soluble protein content of the extract was estimated following the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Kinetin treatment enhanced the germination of dormant cultivar M-13 and the germination of non-dormant seeds of J-11 was inhibited by ABA as also reported earlier (Sengupta *et al.*, 1979). There was no dormancy present in the stored seeds of both the cultivars and their germination was inhibited by ABA treatment (Table I). No apparant change in germinability was observed when ABA was applied to dorment seeds or kinetin to non-dormant seeds. However, varietal differences in the level of enzyme activity in the seeds were observed. Fresh dormant seeds of M-13 showed higher GDH activtity as compared to non-darmant seeds and fresh J-11 seeds (Table II). Storage of seeds

caused reduction in GDH activity irrespective of cultivar and state of dormancy. Treatment of ABA which caused inhibition of germination in J-11 and stored M-13 seeds also showed higher GDH activity as compared to untreated seeds. On the other hand, ABA treatment to fresh M-13 seeds did not show marked change in the level of enzyme activity. Similar results were also obtained when kinetin was treated to non-dormant J-11 and stored M-13 seeds. But in fresh M-13 seeds where kinetin was applied comparatively lower level of GDH was recorded even though there was enhanced rate of germination

Table I : Effect of ABA and kinetin on seed germination (%) in groundnut

Treatment	Fresh seed		Stored seed	
	J-11	M 13	J-11	M-13
Control	90	0	99	98
ABA	0	0	0	0
Kinetin	100	85	99	99

Table II : Effect of growth regulators on GDH activity (n moles NADH oxidised per mg protein per minute) in fresh and stored seeds during germination

Treatment	Fresh seed		Stored seed	
	J-11	M-13	J-11	M-13
Control	58.7	174.3	40.0	43.4
ABA	163.3	170.7	71.0	123.5
Kinetin	52.7	132.0	40.7	47.4

Table III : Effect of growth regulators on GOT activity (n moles oxaloacetate formed per mg protein per minnute) in fresh and stored seeds during germination

Treatment	Fresh seed		Stored seed	
	J-11	M-13	J-11	M-13
Control	100.6	82.7	112.0	117.7
ABA	67.0	84.7	56.0	50.7
Kinetin	118.5	165.7	115.0	122.0

A reverse trend in the level of GOT was observed during germination in these two groundnut cultivars (Table III). The fresh seeds of M-13 showed lower GOT activity as compared to J-11 and stored M-13 . Storage of seeds caused a slight increase in GOT activity in both the cultivars as compared to freshly harvested seeds. The treatment of ABA to J-11 and stored M-13 seeds caused a decreased GOT activity but did not alter the level of enzyme in fresh M-13. On the other hand, kinetin treatment to fresh dormant M-13 seeds, showed an enhanced GOT activity. Although some enhancement in GOT activity was noticed due to kinetin treatment in J-11 and stored M-13 seeds, the level of increase was much lower as compared to that of kinetin treated fresh M-13 seeds.

The results in the present investigation revealed that these two enzymes reacted differently to growth regulators applied during seed germination of groundnut. The levels of these enzymes were also different due to the presence or absence of seed dormancy. It is generally accepted that the action of ABA is inhibitory to both seed germination and activity of several enzymes (Khan *et al.*, 1971; Crispeels and Varner, 1967). The present study showed that although ABA inhibited the seed germination of groundnut, it was not inhibitory to GDH activity. It rather increased the activity of this enzyme when applied to non-dormant seeds. In dormant seeds ABA did neither cause enhancement in the activity of this enzyme nor caused any alteration in the germination of seeds. Moreover, when dormacy was removed through storage, a lower GDH activity compared to that in dormant seeds was observed. On the other hand, kinetin treatment, which caused enhanced germination of dormant M-13, decreased GDH activity. Kinetin treatment did not show any effect on GDH activity where no promotive effect on germination was noticed. Both the cultivars showed slightly higher GOT activity upon storage and low activity in dormant seeds. Breaking of dormancy by kinetin treatment was accompanied by an increase in GOT level and decreased by ABA imposed dormancy. These two growth regulators thus appears to act differently on GDH and GOT enzymes during seed germination and their action depends on the state of dormancy in groundnut seeds.

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