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MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE ACTIVITY IN *ARACHIS HYPOGAEA* L. SEEDLINGS UNDER THE STRESS OF CHLORIDES AND CARBONATE OF SODIUM

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ABSTRACT

The effect of single salt solutions of chloride and carbonate of sodium on mitochondrial ATPase of 2, 4, 6 and 8 day old peanut seedlings was studied. A steady increase in ATPase activity levels of embryonicaxis and cotyledonary mitochondria was observed upto eight days both in control and in the treated; but the activity levels of the enzyme were more in control than in exposed seedlings. The decrease in the activity level was high in embryonic axis mitochondria when compared to that of cotyledonary, indicating high susceptibility of embryonicaxis mitochondria to metal ions. The possibility of greater decrease in ATPase activity followed by greater decay in tissues of carbonate treated seedlings is related to uncouplin g effect of oxidative metabolism with phosphorylation systems and inefficient energy production resulting in retarded growth of seedlings.

INTRODUCTION

THE growth of the most common oil yielding crop like peanut is suppiessed in arid and semiarid regions due to the large amounts of sodium chloride and carbonate inducing salinity and alkalinity in the soil; the crop plants grown in such environment show retarded growth[1]. Accumulation of cations in the cellular environment is known to alter the mitochonidral compactness and the functions of energy producing systems[2]. Mitochondrial activities were studied extensively in early stages of germination[3]; however the specific effect of the ions on the biochemical aspect of mitochondrial enzymes still remains obscure. Adenosine triphosphatase (ATPase) is closely associated with phosphorylation and utilisation of ATP[4] and its contents increase very rapidly during early stages of seed germination. Since ATPase is known to control a variety of metabolic pathways, it is programmed to study the effect of single salt solutions of chloride and carbonate of sodium on mitochondrial ATPase activity in order to assess the effects of these salts on energy releasing mechanisms of mitochondria.

MATERIALS AND METHODS

Peanut (*Arachis hypogaea* L., cultivar TMV_2) healthy and durable seeds of uniform size were surface sterilised with 0·1% mercuric chloride for a few minutes, washed in deionised water and transferred to petri dishes (15 cm dia.) with filter paper inside. Seedlings grown in deionised water are considered as control and others grown separately in solutions containing 68 meq/l of sodium chloride and 38 meq/l sodium carbonate as experimental ones. Petri dishes were kept in dark at 30° C and the media were changed daily to prevent microbial contamination. Cotyledons and embryonic axes were separated at convenient times and mitochondria were collected from the seedlings at 2-day intervals during the first eight days. The cotyledons and embryonicaxes were homogenised separately in *Tris* (*Tris*-(hydroxymethyl)-amino methane) HCl buffer of pH 7·2 at 0° C using Elvehjam–Potter homogeniser and the mitochondria were obtained as sediment by differential centrifugation[5].

Mitochondrial ATPase activity was estimated by the hydrolysis of ATP[6]. Assay was accomplished by incubating the enzyme (1·0 ml) together with ATP (1·5 mg/ml) made upto 3·0 ml with isolating medium at 30° C for one hour. Proteins were then precipitated and the supernatant was used to assay the inorganic phosphate. Phosphorus was estimated colorimetrically[7,8]. Digestion and distillation of ammonia was carried out for the determination of mitochondrial nitrogen[6]. ATPase activity was expressed as the release of inorganic phosphate, μ g per mg of mitochondrial nitrogen per hour.

RESULTS AND DISCUSSION

Mitochondrial ATPase activity levels in cotyledonary and embryonic-axis of 2, 4, 6 and 8 day old peanut seedlings (Table I) showed steady increase upto eight days. The increase in the enzyme activity was more pronounced in the embryonic axis than in the cotyledons. When seedlings were grown in chloride and carbonate of sodium, the mitochondrial ATPase activity in the cotyledons and embryonicaxis registered a continuous increase with the age of the seedlings but the activity levels in general were low in experimental seedlings. The per cent decrements in cotyledons of chloride and carbonate treated seedlings, over controls were 7, 14, 20 and 43 and 18, 23, 21 and 53 respectively on 2, 4, 6 and 8 days of growth, suggesting greater decrease in the activity levels of ATPase, under carbonate stress than under chloride. Similar inhibitory modulation was evinced even in the embryonicaxis of 2, 4, 6 and 8 days old seedlings by the salt solutions.

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TABLE I

Mitochondrial ATPase activity expressed as release of inorganic phosphate μg per mg of mitochondrilal nitrogen per hour

	Cotyledonary Mitochondria				Embryonicaxis Mitochondria			
	Age of the Seedlings in Days							
	2	4	6	8	2	4	6	8
Deionised water (Control)	26·72	40·13	46·16	92·00	36·56	61·45	74·16	85·46
S.E. ±	1·43	1·40	0·59	0·77	11·04	1·17	0·76	0·42
68 meq/1 NaCl	24·73	32·70	36·84	52·76	42·65	46·07	64·62	67·92
S.E. ±	1·44	1·37	1·17	0·96	1·22	0·67	1·36	1·51
% change over control	− 7·00	−19·00	−20·00	−43·00	+17·00	−25·00	−13·00	−20·00
38 meq/1 Na_2Co_3	22·03	31·00	36·32	43·06	41·33	53·11	63·09	70·50
S.E. ±	1·23	0·99	1·25	1·37	0·60	1·10	1·12	1·67
% change over control	−18·00	−27·00	−21·00	−53·00	+15·00	−14·00	−15·00	−18·00

The per cent decrease in the activity levels of the enzyme was more in embryonicaxis than in cotyledons of the treatments, indicating that the embryonicaxis mitochondria are more susceptible to the metal ions than the cotyledonary.

Several conflicting reports are available on the effect of monovalent cations on mitochondrial ATPase. Stimulatory effect was noticed by some workers[11–13]. Wheeler and Whittam[14] suggested that this enzyme may play a role in the transport of these cations across the cell membrane. Ulrich[15] reported a significant and constant decrease in enzyme activity when Na^+ and K^+ ions were added to mitochondrial suspensions. The data obtained in the present investigation add credence to the latter's findings.

The inhibition of mitochondrial ATPase by alkali metal ions appear to be more specific, and the quantitative changes in the ionic milieu of the mitochondria have marked effect on the coupling of oxidative phosphorylation. Our earlier studies[10] indicated high succinate oxidation and low cytochrome oxidation in the peanut seedlings treated with sodium carbonate. Hence, it can be presumed that the greater decay in the tissues of carbonate treated seedlings, may partly be due to the uncoupling effect of the oxidative metabolism with phosphorylating systems, as evinced by the decreased ATPase enzyme activity, resulting in an inefficient energy production and consequent retardation of the seedling growth.

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