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Metabolic Aspects in the Germinating Seeds of *Cicer arietinum*, Supplemented with Auxin and/or Cations

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ABSTRACT

Phytohormones possibly utilize ionic channel for the amplification and signal transduction in generating an impact on the cellular metabolism. With this goal, the seeds of *Cicer arietinum* (L) cv. BG-256 were imbibed, for six hours, in the aqueous solution of indole-3-acetic acid and/or Ca^{++}/K^{+} and then transferred to distilled water for the rest of the period. The germinating seeds were sampled at 12, 24 and 36 h of the soaking and analysed for the activity of nitrate reductase and the contents of total protein, nitrate, calcium and potassium. All the parameters had significantly higher values in the seeds, soaked in auxin only and excelled over that fed with auxin and/or cation. Control seeds possessed minimum values for all the characteristics, except nitrate.

Key Words: Metabolic; Germinating; Seeds; Supplemented; Cation

INTRODUCTION

Seeds contain sufficient quantity of food reserves which support the growth and development of the seedling, unless a photosynthesizing autotrophic plant is established. These reserves represent the discret, intracellular bodies of lipid, protein, carbohydrate, organic phosphate and/or various inorganic compounds (Bewley & Black, 1985). On hydration, they act as a source of energy (ATP); reducing power (NADH & NADPH) and carbon skeleton. These germinating seeds, on imbibition, consume an elevated level of oxygen, which may partly be attributed to the activation/hydration of mitochondrial enzymes, involved in the Krebs cycle and electron transport chain (Salisbury & Ross, 1991). Moreover, enzymes, like lipases, proteinases, phosphatases, hydrolases (Bewley & Black, 1985; Mayer & Poljakoff-Mayber, 1989; Coccuni & Negrini, 1991; Washio & Ishikawa, 1992; Bernier & Ballance, 1993; Bernhardt *et al*., 1993) are either activated or synthesized a fresh. The simpler substances, released by the enzymatic action on the storage bodies are carried from the endosperm/cotyledons to the embryonic axis and are consumed in the synthesis of new required material (Davies & Slack, 1981; Mayer & Poljakoff Mayber, 1989).

Each stage of seed germination requires a definite ratio of various plant hormones. Among them, gibberellic acid is acclaimed to be implicated in the early process of germination of the seeds (Mohanty & Sahoo, 1992; Sharma & Saran, 1992; Patel & Saxena, 1994). However, the involvement of auxins cannot be denied but the knowledge about pinpoint action is lacking (Bewley & Black, 1985).

The mature seeds are also loaded with a sufficient quantity of cations and anions where calcium is not only the structural units of the membranes but the two regulate the functioning of the membranes and the cellular metabolism (Bewley & Black, 1985). However, their availability in the cell may be altered by the hormonal action on the ionic channels.

Certain important aspects of the seed metabolism, during germination, in association with the elevated level of the cations (Ca^{++} and/or K^{+}) and/or auxin, at varied intervals, were studied. It may help in understanding the aspects of interdependence of these factors in the regulation of the early process of seed germination.

MATERIALS AND METHODS

The seeds of *Cicer arietinum* L. cv. BG-256 were purchased from National Seed Corporation Ltd., New Delhi. Healthy seeds of uniform size were surface sterilized (0.01% aqueous solution of mercuric chloride) followed by repeated washings with sterilized, double distilled water (DDW). Twenty-five seeds were soaked for a total period of 6 h in: (a) DDW or aqueous solution of IAA (10^{-8}M); Ca^{++} (4 /8 μM); K^{+} (6 /12 μM), (b) aqueous solution of Ca^{++} (4 /8 μM) or K^{+} (6 /12 μM) in the first half (3 h) and IAA (10^{-8}M) in the second half (3 h), (c) aqueous solution of IAA (10^{-8}M) in the first half (3 h) and Ca^{++} (4/8 μM) or K^{+} (6/12 μM) in the second half (3 h).

Each treatment was replicated five times and the whole experiment was repeated once. Treated seeds, after being rinsed with DDW, were transferred to the petriplates containing absorbent cotton, moistened with 50 cm^3 of DDW only and were incubated in a growth chamber at a temperature of 25±2°C, in dark. The seeds were sampled after 12, 24 and 36 h of soaking (this included the duration of the treatment) and analysed for the activity of nitrate reductase (NR) and the contents of nitrate, protein, calcium

and potassium. The NR activity was estimated according to the method of Jaworski (1971), nitrate content by Johnson and Ulrich (1950), protein by Lowry *et al*. (1951); whereas, calcium and potassium contents were estimated flame photometrically. All the data were analysed statistically and critical difference (CD) at 5% level of probability (P = 0.05) was calculated as described by Gomez and Gomez (1984).

RESULTS

Nitrate reductase activity. It is evident that the activity of nitrate reductase increased significantly as the germination progressed, upto 36 h of imbibition (Table I). Irrespective of the treatment, the values, at all the three stages of sampling, were higher over, the respective control. The treatment of the seeds with indole-3-acetic acid (10^{-8}M) alone proved best in elevating the activity of the enzyme. The values, at 12, 24 and 36 h of sampling, were 50, 95 and 77% higher, over the water soaked control. Treating the seeds with either of the ions (Ca^{++}/K^{+}) alone did not prove as effective as in association with auxin. However, the activity of the enzyme was comparatively higher where auxin treatment was immediately followed by ionic treatment and that too with calcium (8 μM) where the values were second highest.

Nitrate content. The nitrate content decreased significantly with the progress of the germination (Table II). The seeds soaked in water (control) possessed highest values, throughout the germination period, which were significantly higher over most of the other treatments. Least quantities were recorded in the seeds, treated with auxin alone and were closely followed by the treatments employing ionic association before or after auxin treatment.

Protein content. As the germination advanced from 12 to 36 h, the total protein content exhibited a progressive increase (Table III). The values were significantly higher in all the treatments, over the control. Throughout the study, it was noted that the seeds pre-treated with auxin gave maximum response to calcium, therefore, possessed largest quantities of the proteins. The respective increase, at 12, 24, 36 h of sampling, was 23, 16 and 17% over the control.

Calcium content. There was no significant change in the calcium content with the state of seed germination (Table IV). However, all the treatments increased its values, over the control. It is quite prominent that the content of the calcium in the seed was in proportion to its bathing medium. Moreover, the presence of potassium in the soaking medium also favoured an increase in calcium content of the germinating seed and most effectively at a higher concentration (12 μM).

Table I. The interaction of IAA (10^{-8}M) with Ca^{2+} (4 and 8μM) or K^{+} (6 and 12 μM) on the nitrate reductase activity (nmol$NO_3H^{-1}g^{-1}$FW) in the seeds of *Cicer arietinum* L.cv.BG-256, at 12, 24 and 36 hours of the sampling

Treatments	Sampling time (Hours)		
	12	24	36
Control	200.11	288.40	359.03
IAA	300.17	565.03	635.66
Ca 4 μM	247.20	341.37	405.10
Ca 8 μM	268.35	383.45	441.49
K 6 μM	229.54	305.45	369.25
K 12 μM	258.97	349.30	410.15
Ca 4 μM + IAA	260.35	396.65	471.30
Ca 8 μM + IAA	274.13	443.45	510.25
K 6 μM + IAA	251.45	370.13	440.35
K 12 μM + IAA	263.66	405.10	460.29
IAA + Ca 4 μM	279.35	419.30	479.36
IAA + Ca 8 μM	284.68	461.25	525.14
IAA + K 6 μM	263.45	386.10	451.39
IAA + K 12 μM	269.38	429.35	485.25
C.D. at 5 %	020.26	018.65	031.25

Table II. The interaction of IAA (10^{-8}M) with Ca^{2+} (4 and 8μM) or K^{+} (6 and 12 μM) on the nitrate content (ppm) in the seeds of *Cicer arietinum* L.cv.BG-256, at 12, 24 and 36 hours of the sampling

Treatments	Sampling time (Hours)		
	12	24	36
Control	2.49	2.20	1.66
IAA	2.11	1.72	1.09
Ca 4 μM	2.40	2.14	1.56
Ca 8 μM	2.31	2.05	1.43
K 6 μM	2.45	1.98	1.36
K 12 μM	2.42	2.08	1.46
Ca 4 μM + IAA	2.29	1.96	1.28
Ca 8 μM + IAA	2.25	1.89	1.19
K 6 μM + IAA	2.30	1.92	1.23
K 12 μM + IAA	2.24	1.83	1.16
IAA + Ca 4 μM	2.22	1.79	1.21
IAA + Ca 8 μM	2.20	1.88	1.13
IAA + K 6 μM	2.18	1.83	1.18
IAA + K 12 μM	2.24	1.93	1.28
C.D. at 5 %	0.16	0.21	0.29

Table III. The interaction of IAA (10^{-8}M) with Ca^{2+} (4 and 8μM) or K^{+} (6 and 12 μM) on the protein content (%) in the seeds of *Cicer arietinum* L.cv.BG-256, at 12, 24 and 36 hours of the sampling

Treatments	Sampling time (Hours)		
	12	24	36
Control	18.53	20.24	21.43
IAA	22.79	25.69	27.65
Ca 4 μM	21.58	24.48	26.23
Ca 8 μM	21.19	24.36	26.20
K 6 μM	20.68	23.80	25.10
K 12 μM	22.44	25.60	29.30
Ca 4 μM + IAA	21.45	23.85	24.93
Ca 8 μM + IAA	21.68	24.15	25.90
K 6 μM + IAA	21.25	23.90	25.35
K 12 μM + IAA	22.13	25.03	27.02
IAA + Ca 4 μM	23.00	25.85	27.80
IAA + Ca 8 μM	23.09	26.25	29.89
IAA + K 6 μM	21.68	24.68	26.45
IAA + K 12 μM	22.68	25.32	27.25
C.D. at 5 %	00.98	00.79	01.35

Potassium content. Like the other cation, the potassium content also did not change significantly with time, in the germinating seeds (Table V). The concentration of the cation in the soaking medium determined its values in the seed. It was highest in the seeds, pre-treated with auxin followed with potassium (12 μM), at all the samplings. Soaking the seeds in calcium solution also elevated the level of potassium in the seed.

DISCUSSION

The water uptake by the seeds is followed with *de novo* synthesis of hydrolytic enzymes (Bewley & Black, 1985). This facilitates a shift in metabolic activity by speeding up the solubilization of stored food material.

Table IV. The interaction of IAA (10^{-8}M) with Ca^{2+} (4 and 8μM) or K^+ (6 and 12 μM) on the calcium content (%) in the seeds of *Cicer arietinum* L.cv.BG-256, at 12, 24 and 36 hours of the sampling

Treatments	Sampling time (Hours)		
	12	24	36
Control	0.125	0.132	0.137
IAA	0.139	0.143	0.147
Ca 4 μM	0.156	0.159	0.163
Ca 8 μM	0.169	0.174	0.179
K 6 μM	0.143	0.149	0.154
K 12 μM	0.145	0.151	0.156
Ca 4 μM + IAA	0.168	0.174	0.178
Ca 8 μM + IAA	0.170	0.188	0.191
K 6 μM + IAA	0.148	0.151	0.156
K 12 μM + IAA	0.147	0.153	0.162
IAA + Ca 4 μM	0.158	0.170	0.178
IAA + Ca 8 μM	0.180	0.181	0.196
IAA + K 6 μM	0.153	0.159	0.163
IAA + K 12 μM	0.152	0.157	0.166
C.D. at 5 %	0.011	0.013	0.009

Table V. The interaction of IAA (10^{-8}M) with Ca^{2+} (4 and 8μM) or K^+ (6 and 12 μM) on the potassium content (%) in the seeds of *Cicer arietinum* L.cv.BG-256, at 12, 24 and 36 hours of the sampling

Treatments	Sampling time (Hours)		
	12	24	36
Control	0.532	0.546	0.553
IAA	0.565	0.573	0.580
Ca 4 μM	0.568	0.577	0.583
Ca 8 μM	0.564	0.573	0.580
K 6 μM	0.603	0.613	0.624
K 12 μM	0.629	0.636	0.641
Ca 4 μM + IAA	0.570	0.583	0.596
Ca 8 μM + IAA	0.569	0.579	0.586
K 6 μM + IAA	0.613	0.626	0.633
K 12 μM + IAA	0.639	0.647	0.656
IAA + Ca 4 μM	0.579	0.589	0.597
IAA + Ca 8 μM	0.583	0.593	0.604
IAA + K 6 μM	0.636	0.651	0.663
IAA + K 12 μM	0.660	0.673	0.677
C.D. at 5 %	0.021	0.018	0.023

Embryonic axis receives these simpler substances in sufficient quantity to sustain its growth (Beevers, 1968). However, a number of internal and external factors, including the level of phytohormones (Davies, 1987) and inorganic elements (Marschner, 1986), have a significant impact on the whole process of seed germination.

A perusal of the present observations (Table I) revealed that with the progress of the seed germination its level of nitrate reductase (NR) activity increased but that of nitrate exhibited a reverse trend (Table II). It is in conformity with Tahir and Farooq (1989), Hayat (1997) and Hussain (2001). As NR is an inducible enzyme (Afridi & Hewitt, 1964), therefore, its level is largely determined by the nitrate content of the tissue. Moreover, phytohormones also elevate its level (Ahmad, 1994; Ahmad & Hayat, 1999; Ahmad *et al*., 2001; Hayat *et al*., 2001). The germinating seeds, in this case, totally depended on the reserves for the supply of nitrate, as it was not made available in the soaking medium or thereafter. Its level should have, therefore, been somewhat the same in all the treatments. Under this uniform condition, treating the seeds, for a short duration, with aqueous solution of indole-3-acetic acid and/or cations (Ca^{++}/K^+) significantly increased the activity of NR (Table I). Among the treatments, auxin alone proved most effective in inducing the activity of the enzyme by possibly involving the genes directly (Jones & Prasad, 1992) and/or through the use of cations whose involvement in such cases is acclaimed by Ching and Choe (1998). The other enzymes, α-amylase (Ying *et al*., 1996) and NAD^+ kinase (Lixia *et al*., 1999) are known to have a close association with the cellular level of the Ca^{++}. Likewise, potassium is involved, as a co-factor, in regulating the level of various enzymes (Evans & Sorger, 1966). In the present case, the increase in the level of either Ca^{++} (Table IV) or K^+ (Table V) is associated with the elevation of the enzyme activity (Table I). However, the combination of ions (Ca^{++}/K^+) with auxin was more effective than ions alone, in regulating a higher level of NR activity. To maintain a higher level of the enzyme activity a continuous presence of the auxin for a longer duration (i.e. 6 h) seems to be essential than for a shorter duration (i.e. 3 h) before /after the ionic treatment.

The germinating seeds totally depended on the reserves for the supply of the nitrate (Table II) as it was not given from outside. Nitrate content, therefore, exhibited an increase relationship with NRA. Its maximum quantity was observed in the seeds soaked in water (control) together with the lowest activity of NR. Thus, it may be derived that the treatment of the seeds with auxin or even with ions (Ca^{++}/K^+) activates the initial, major rate limiting steps (Hopkins, 1995) in the process of nitrate reduction. It will naturally facilitate the availability of organic nitrogen and in turn that of amino acids, needed in the synthesis of the required proteins. A parallel relationship of the total proteins (Table III) with that of the activity of NR (Table I) exists. This comparability has also been reported earlier by Singh and Singh (1985). The regulation of protein synthesis by the

hormones (Bhatia *et al*., 1998; Qui *et al*., 1998), the elements (Arora & Dua, 1994) or hormone induced secretion of proteins by activating Ca^{++} channel (Mendvedev *et al*., 1999) is acclaimed. Moreover, a relationship between the intercellular level of Ca^{++}, calmodulin, protein phosphorylation and dephosphorylation with GA_3 (Yael & Weiss, 1999) or an interaction effect of GA_3 + $CaCl_2$ on the level of calmodulin protein (Schurink *et al*., 1996) was also noticed.

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