

BRIEF COMMUNICATION

Cytokinin profile during early phase of germination of gram seeds after application of benzylaminopurine

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Qualitative and quantitative differences in cytokinin levels of gram seeds were observed during germination of control and 6-benzylaminopurine (BAP; 4.44×10^{-5} M) treated seeds. Zeatin riboside (ZR), zeatin (Z), dihydrozeatin (DHZ), isopentenyladenosine (iPA) and isopentenyladenine (iP) were determined using high pressure liquid chromatography. In the control seeds the ZR content increased markedly only after 24 h of germination. In BAP treated seeds, the increase in ZR content was stronger than in control seeds and it persisted during further germination. Z content increased during germination in both control and treated seeds. After 48 h of germination iP appeared in control and especially in treated seeds.

Additional key words: *Cicer arietinum*, dihydrozeatin, isopentenyladenine, isopentenyladenosine, zeatin.

Cytokinins are intimately involved in the physiological processes associated with seed and fruit development. The low levels of endogenous cytokinins in dry mature seeds (Davey and Van Staden 1979, Saha *et al.* 1984) and their rapid metabolism during imbibition suggest that germinating seeds may have the capacity to synthesize cytokinins. Cytokinins in the cotyledons of germinating seeds seem to regulate reserve mobilization (Gepstein and Ilan 1980). In germinating lupin seeds, the capacity for cytokinin biosynthesis is probably confined to the embryonic axis (Nandi *et al.* 1988). Furthermore, the reported control by axis of reserve mobilization

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Abbreviations: BAP - 6-benzylaminopurine; HPLC - high pressure liquid chromatography; ZR - zeatin riboside; Z - zeatin; DHZ - dihydrozeatin; iPA - isopentenyladenosine; iP - isopentenyladenine, f.m. - fresh mass.

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in dicot seeds (De Klerk 1986) and its replacement by applied cytokinins also suggest that germinating seeds synthesize cytokinins. The importance of endogenous cytokinins on reserve mobilization on germinating chick-pea seeds were studied by Munos *et al.* 1990.

This study reports comparison of changes in the levels of cytokinins in control and BAP-treated gram seeds during first three days of germination (24, 48 and 72 h after imbibitions).

Bengal gram (*Cicer arietinum* L.) cv. B-108 seeds (50 g) were collected, washed and evenly spread on Petri dishes without or with BAP (4.44×10^{-5} M, *i.e.* 10 ppm) and kept in dark at 24 °C. After 24, 48 and 72 h seeds were washed and samples were homogenized with chilled 80 % ethanol (1:5 m/v) in blender and re-extracted twice, each time at 4 °C with the same solvent. The extracts were centrifuged in cold at 5 800 g for 10 min. The supernatants were again extracted with *n*-butanol and then purified through Dowex 50 column (for detail see Saha and Sircar 1990). Further purification and quantification of cytokinins were performed by HPLC. The reverse phase column MCH-NCAP-5 [C_{18} (4.0 mm \times 15 cm)] was eluted at 0.5 cm³ min⁻¹ and UV absorbing compounds were monitored at 280 nm. The solvent used was acetonitrile:H₂O (30:70, v/v). The HPLC chromatograms of measured samples were compared to similar chromatogram of the authentic sample of different cytokinins.

The HPLC analysis of the semipurified extract of dry gram seeds showed the presence of ZR, Z, DHZ and iPA (iP was not detected). The content of ZR was much higher than that of Z. The amount of DHZ was also much more higher than that of Z, but less than ZR, the quantity of iPA was lower than that of other three cytokinins (Table 1).

Table 1. Cytokinin content [μ g g⁻¹(f.m)] during germination of control and BAP treated in gram seeds.

Treatment	ZR	Z	DHZ	iPA	iP
0 h	9.41	2.10	7.48	0.96	0
24 h control	21.53	3.06	4.80	0.73	0
24 h BAP	22.58	3.57	6.40	2.01	0
48 h control	11.45	3.06	5.78	4.68	1.84
48 h BAP	18.63	4.63	6.96	1.46	5.77
72 h control	9.76	4.59	3.69	0.48	0
72 h BAP	20.40	4.65	5.79	1.04	0.95

24 h after start of germination the quantities of ZR and Z in control and BAP treated seeds were more or less the same and much higher than in dry seeds. The content of DHZ was higher in BAP treated seeds than in control seeds but still less than in dry seeds. The content of iPA in BAP treated seeds was twice that in control seeds (Table 1). At 48 h of germination iP (which was not found earlier) appeared and much higher quantity was detected in treated seeds than in control ones. It may be assumed that it was due to presence of BAP or to the conversion of iPA to iP. In HPLC separation of ZR, Z, DHR, iPA were separated but iP and BAP were eluted in

the same region. At 48 and 72 h of germination ZR was gradually decreased in control seeds due to metabolism within the seeds or utilization during germination. Developing seeds have often been shown as an active site of cytokinin biosynthesis, but in BAP treated seeds the quantity of ZR was more or less the same during the whole period. Z was maintained on the similar level both in control and in treated material. In some plant tissues (e.g. radish roots, derooted radish seedlings and embryos) the metabolism of zeatin Z is simple and in the principal metabolites formed the unsaturated isoprenoid side chains. In plant tissues cleavage of side chains of Z and iP and their riboside is attributable to cytokinin oxidase (Guern and Peaud-Leonel 1981). The metabolic fate of externally applied cytokinin has been extensively studied and the various metabolites are well established (Letham and Palni 1983). BAP is degraded by cleavage of the N⁶ substituent (Doree and Guer 1973, Miernyk and Blades 1977). About 80 % of BAP taken up by soybean tissue appeared to be degraded by benzyl cleavage to yield adenine and an unidentified acid which resembled benzoic acid (Fox *et al.* 1972). Use of HPLC and thin layer chromatography provided evidence for the incorporation of adenine broken down from BAP, by isolated germinating embryos into trans-zeatin (Van Staden and Drewes 1993). The effect of BAP on reserve mobilization in yellow lupin seeds appears to be mediated at least in part, by a stimulus, probably a cytokinin, emanating from the embryonic axis (Nandi *et al.* 1995).

Some qualitative difference of endogenous cytokinins were also found in the germinating pea seeds during different period of germination (Saha 1992).

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