

A COMPARISON OF ACTIVITIES OF VARIOUS CYTOKININS ON THE SENESCENCE OF SOYBEAN LEAF DISCS

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Abstract

The activities of seven cytokinins in retarding chlorophyll degradation were tested in two soybean varieties, 1039 and Shih-shih. The order of effectiveness of cytokinins with straight chains at the N⁶ position of adenine in 1039 variety was Z>DHZ>HAP>IPA, while that in Shih-shih variety, IPA>DHZ>Z>HAP. In both 1039 and Shih-shih varieties, BA was more effective than K in retarding chlorophyll degradation. BA, a synthetic cytokinin, was more effective than Z, a naturally occurring cytokinin, in 1039 variety. However, BA was less effective than IPA, a naturally occurring cytokinin, in Shih-shih variety. Structure-activity relationships for chlorophyll retention did not parallel the relationships found for tobacco callus growth stimulation.

Introduction

Ever since the discovery of the first cytokinin, kinetin (Miller *et al.*, 1955), various naturally occurring cytokinins have been isolated from higher plants, fungi and bacteria (Hall 1973; Horgan *et al.*, 1975; Skoog and Armstrong, 1970). Structure-activity relationships for the promotion of cell division in cytokinin-dependent tobacco callus tissue have been extensively investigated for the known naturally occurring cytokinins and for other structurally related cytokinins (Schmitz *et al.*, 1972; Skoog *et al.*, 1967; Vreman *et al.*, 1974). However, data for the ability of these cytokinins to retard chlorophyll degradation are much less extensive. In this paper, we report the influence of various cytokinins on chlorophyll degradation of soybean leaf discs.

Materials and Methods

Chemicals

Zeatin, isopentenyl adenine, dihydrozeatin, hexyladenine, benzyladenine and kinetin were obtained from Sigma Chemical Co., U. S. A. Benzimidazole was purchased from Tokyo Kasei Company, Japan.

Plant Materials and Incubation Conditions

Soybean (*Glycine max* L. Merr.) varieties 1039 and Shih-shih were used as plant materials. Soybean plants were grown as previously described (Hsia and Kao, 1978). On the 14th days after planting, leaf discs measuring 9 mm were punched from primary leaves and randomized. A group of 10 leaf discs was floated on 10 ml test solution in a 50 ml flask. Incubation was carried out in darkness at 30°C for 6 days. Chlorophyll was extracted and determined as before (Kao, 1980).

Results

Table 1 and Fig. 1 show the chemical structures and abbreviations of cytokinins used in this study. Cytokinins can be divided into three groups. (A) Cytokinins with straight chains attached at the N⁶ position of adenine, such as IPA, Z, DHZ and HAP; (B) cytokinins with ring structures at the N⁶ position of adenine, such as BA and K; (C) cytokinin without adenine moiety, such as BZI (Fig. 1). Cytokinin activity comparisons were based either on one curve being consistently above or below another over the 0.02 to 20 mg/l range or on actual differences at the lowest concentration where differences in chlorophyll retardation first occurred.

In 1039 variety, activity of the cytokinins with straight side chains decreased in the following order: Z>DHZ>HAP>IPA (Fig. 2). The effect

Table 1. Chemical structures and abbreviations of cytokinins tested

Cytokinin	Structure
Name (abbreviation)	<p>HNR</p> <p>R =</p>
Isopentenyladenine (IPA)	
Zeatin (Z)	
Dihydrozeatin (DHZ)	
Hexyladenine (HAP)	
Benzyladenine (BA)	
Kinetin (K)	

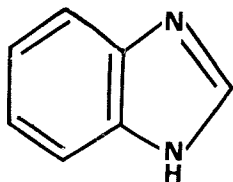


Fig. 1. Chemical structure of benzimidazole (BZI).

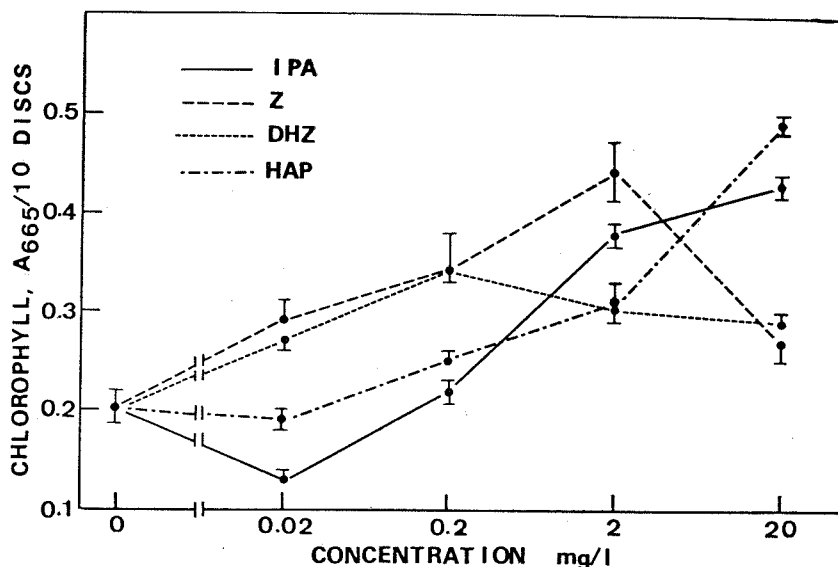


Fig. 2. Cytokinin activities of DHZ, IPA, HAP and Z in retarding chlorophyll degradation of leaf discs of soybean variety 1039.

in retarding chlorophyll degradation of cytokinins with ring structures at the N⁶ position of adenine was BA>K (Fig. 3). BZI at the concentration from 0.02 mg/l to 2 mg/l had no effect in retarding chlorophyll degradation. However, little effect of BZI was found at 20 mg/l (Fig. 3). BA, which has ring structure at the N⁶ position of adenine, was more effective than Z, which has isoprenoid side chain (Fig. 4).

In Shih-shih variety, the order of effectiveness in retarding chlorophyll degradation of cytokinins with straight side chains was IPA>DHZ>Z>HAP (Fig. 5). Senescence retardation effect of BA was also more active than that of K as in the case of 1039 variety (Fig. 6). BZI had no effect in delaying senescence at all concentrations tested (Fig. 6). When compared with IPA, BA was less effective in retarding senescence (Fig. 7).

Discussion

In tobacco bioassay system, the presence of the double bond in the N⁶ side

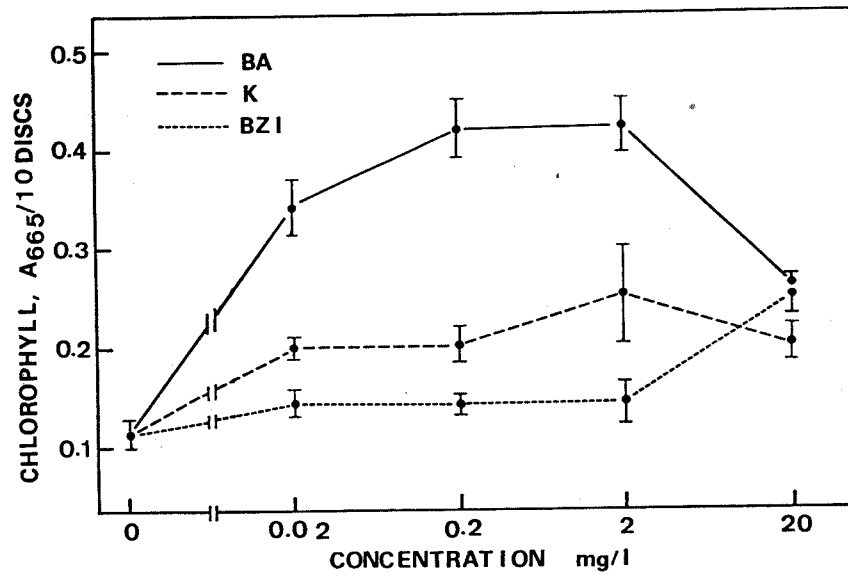


Fig. 3. Cytokinin activities of BA, K and BZI in retarding chlorophyll degradation of leaf discs of soybean variety 1039.

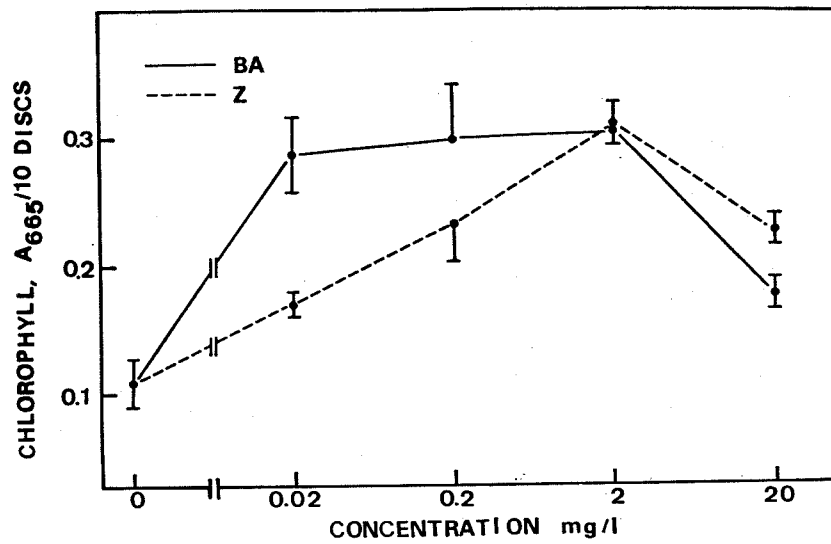


Fig. 4. Cytokinin activities of BA and Z in retarding chlorophyll degradation of leaf discs of soybean variety 1039.

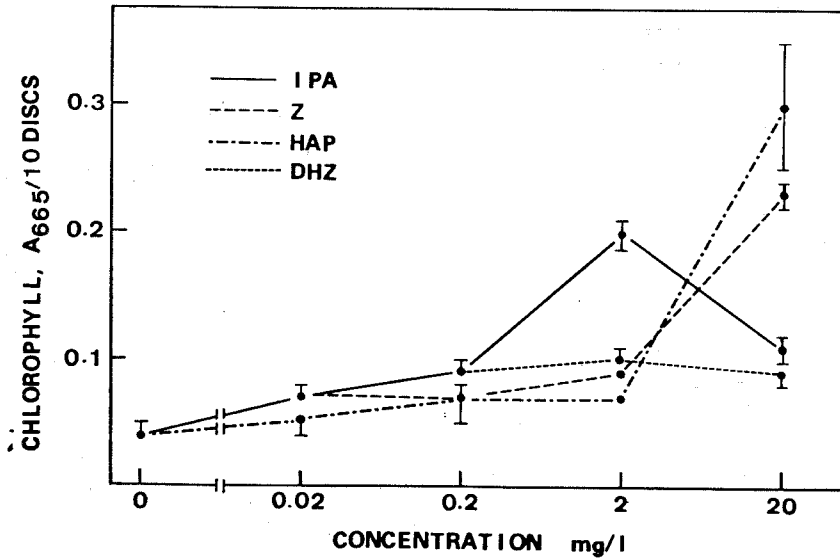


Fig. 5. Cytokinin activities of DHZ, IPA, HAP and Z in retarding chlorophyll degradation of leaf discs of soybean variety Shih-shih.

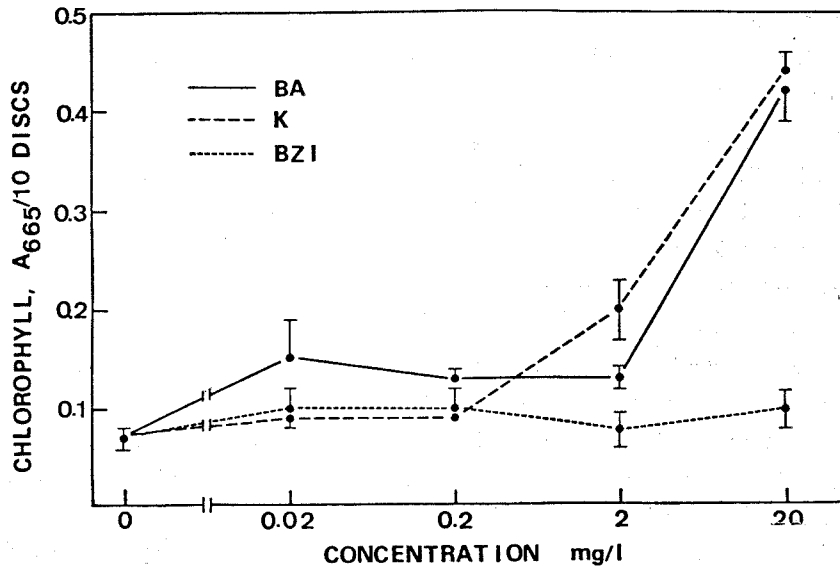


Fig. 6. Cytokinin activities of BA, K and BZI in retarding chlorophyll degradation of leaf discs of soybean variety Shih-shih.

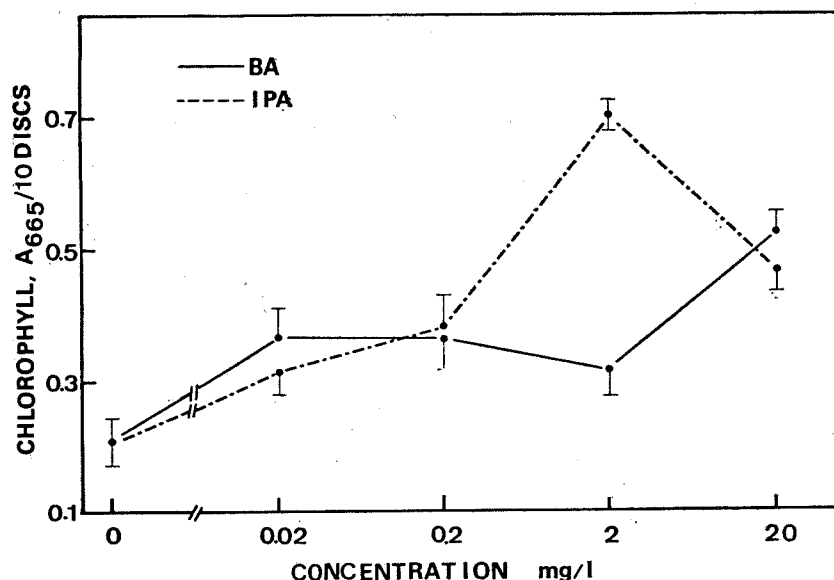


Fig. 7. Cytokinin activities of BA and IPA in retarding chlorophyll degradation of leaf discs of soybean variety Shih-shih,

chain of Z and IPA resulted in a dramatic increase in activity relative to DHZ (Leonard *et al.*, 1969; Leonard *et al.*, 1971; Schmitz *et al.*, 1972). However, cytokinins bearing unsaturated isoprenoid side chains do not seem to be necessarily required to retard chlorophyll degradation (Fig. 2 and 5). It has been reported that hydroxylation of isoprenoid side chain at position 4 generally enhances cytokinin activity in tobacco callus growth (Leonard *et al.*, 1969; Letham, 1967). The presence of hydroxyl group at the position 4 of isoprenoid side chain indeed resulted in an increase of activity in retarding chlorophyll degradation in 1039 variety (Fig. 2), but not in Shih-shih variety (Fig. 5). It appears that structure-activity relationships for chlorophyll retention did not parallel the relationships found for tobacco callus growth stimulation.

Varga and Bruinsma (1973) reported that K was more effective than BA in retarding chlorophyll degradation of detached oat leaves. However, using the same plant material, Biddington and Thomas (1978) found that K was less effective than BA in chlorophyll retention. Our results (Fig. 3 and 6) showed that in both 1039 and Shih-shih varieties BA was more effective than K. Whitty and Hall (1974) found that BA was resistant to a cytokinin oxidase isolated from maize. Therefore, it is conceivable that the observed BA activity may be related, at least in part, to its resistance to cytokinin oxidase in soybean leaf discs.

It has been observed that synthetic cytokinins, K and BA, were highly

active in delaying chlorophyll degradation of detached oat leaves while naturally occurring cytokinins, Z and IPA, were either without effect or less effective (Biddington and Thomas 1977; Dumbroff and Walker, 1979; Varga and Bruinsma, 1973). However, in soybean Shih-shih variety BA is less effective than IPA in chlorophyll retardation (Fig. 7). By using leaf segments of seven-week-old rice plants, Mishra and Pradhan (1973) found that K was relatively ineffective but BZI was effective in retarding senescence. In contrast, we found that BZI was inactive or slightly active at very high concentration (20 mg/l, Fig. 3 and 6). Apparently, the sensitivity of cytokinins in retarding senescence is species or variety specific; its sensitivity increases in some plants or some varieties and decreases in other.

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Cytokinins 化學結構之變化對大豆葉片老化之影響

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本研究主要在瞭解七種不同化學結構之 Cytokinins 對大豆 1039 與十石品種葉片老化之影響。就 1039 品種而言，具有鏈狀側鏈之 Cytokinins 對延緩葉片老化之效果依序為 Z>DHZ>HAP>IPA。而環狀側鏈之 Cytokinins 其效果為 B>K。BZI 則需高濃度 (20 mg/l) 始有延緩效果。環狀側鏈之 Cytokinins (BA) 其延緩效果大於鏈狀側鏈之 Cytokinins (Z)。就十石品種而言，具有鏈狀側鏈之 Cytokinins 對延緩葉片老化之效果則為 IPA>DHZ>Z>HAP。而環狀側鏈之 Cytokinins 其效果亦如 1039 品種為 B>K。BZI 無延緩葉片老化之效果。鏈狀側鏈之 IPA 其延緩效果大於環狀側鏈之 BA。不同化學結構之 Cytokinins 對葉片老化延緩效果似乎與 Cytokinins 促進菸草癒傷組織之生長效應不同。