BIOLOGIA PLANTARUM 38 (2): 289-292, 1996

BRIEF COMMUNICATION

Effect of irradiation and growth regulators on degradation processes in detached soybean leaves

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Abstract

Changes in soluble protein profile and chlorophyll (Chl) content in detached soybean leaves incubated in darkness or light were delayed by application of benzyladenine or indole-3-acetic acid and enhanced by abscisic acid. The degradation in light differed significantly from the degradation in darkness. Chl and proteins were lost at a higher rate in darkness than in light.

Additional key words: abscisic acid, benzyladenine, chlorophyll, *Glycine max* L., indole-3-acetic acid, soluble proteins.

One of the most characteristic features of leaf senescence is a progressive decline in photosynthetic capability accompanied by decline in contents of photosynthetic pigments and proteins and ribulose-1,5-bisphosphate carboxylase activity (Friedrick and Huffaker 1980). Senescence is sometimes modelled by incubating detached leaves in the dark. Darkness accelerates the degradation processes in leaves (Klerk *et al.* 1993), but in isolated chloroplasts light causes larger degradation (Choudhury *et al.* 1993). Cytokinins delay degradation processes, while abscisic acid (ABA) enhance them (Varga and Bruinsma 1973). Also ethylene is supposed to affect these processes. During dark degradation most of the photosynthetic membrane and enzyme proteins syntheses were reduced more drastically in detached than attached leaves (Thomas and Stoddart 1980). In detached barley leaves Miller and Huffaker (1985) found new endoproteases. We studied the contents of Chl and total proteins and the soluble protein profile of detached soybean leaves senescing either in darkness or light under application of three growth regulators.

Detached first leaves from 15 d-old plants of soybean (*Glycine max* L. cv. Co 1), were floated in sterile Petri plates on distilled water or solutions of growth

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Received 11 November 1994, *accepted* 15 September 1995.

regulators. The Petri plates were incubated under irradiance of 400 μmol m^{-2} s^{-1} or under dark in *Conviron* (*Controlled Environments Ltd.*, Canada) growth chamber at 25 °C for 7 d. Benzyladenine (BA), indole-3-acetic acid (IAA) and abscisic acid (ABA) were used at concentrations of 10, 10 and 100 μM, respectively.

For soluble protein analysis, the distal portion of the leaves was homogenised with 1.5 cm^3 of extraction buffer (100 mM Tris-HCl, 10 mM EDTA, 10 mM 2-mercapto-ethanol, 100 mM NaCl and 0.1 % ascorbic acid, pH 7.8) at 4 °C. The homogenate was centrifuged at 75 000 *g* (*Hitachi RPR 12-20* rotor) and proteins in the resulting supernatant were extracted with phenol, precipitated with acetone, centrifuged (1 000 *g*; *Hitachi RPR 18-3* rotor) and finally extracted with diethyl ether. The powdery pellets were recovered for gel electrophoresis analysis. The 15 % SDS-PAGE was performed as described by Laemmli (1970). In all cases 100 μg of protein was applied to each lane. The Coomassie Blue stained protein profiles were scanned by using a *Beckman DU64* spectrophotometer with a special gel scan apparatus. The contents of soluble and total proteins were determined according to Bradford (1976). Chl content was determined spectrophotometrically as described by Arnon (1949).

Incubation times required for 50 % loss of protein and Chl (Table 1) were delayed by irradiation and BA application. Under both light and dark, the ABA increased the rate of degradation processes.

Table 1. Incubation time [d] required for 50 % loss of chlorophyll and protein contents from leaf samples. Irradiance 400 μmol(photon) m^{-2} s^{-1}. Means of 3 independent experiments; SD - standard deviation of mean.

Incubation solution	Light chlorophyll	protein	Darkness chlorophyll	protein
Water	8	10	7	8
BA (10 μM)	13	14	11	13
IAA (10 μM)	10	8	7	8
ABA (100 μM)	5	6	5	6
SD	3.0	3.0	2.3	2.7

Chl degradation was highest in detached leaves incubated with ABA, followed by IAA and water (Table 2). BA retarded degradation of both the Chl and proteins under both light and dark. IAA was less effective. In ABA solution the rate of destruction of Chl and proteins was fast. Under darkness the rate of degradation of Chl and proteins was higher than under irradiation in all incubation conditions.

Generally, Chl and proteins are more readily lost in darkness than in light; this indicates that synthesis of specific mRNAs and subsequent translation process are light-regulated (Thompson and White 1991). The extensive changes observed during the incubation under darkness are partially reversed upon return to light (Malik 1987).

The spectrophotometric scan data obtained for the protein profile of the SDS-PAGE (Table 3) indicated a major loss of polypeptides of subunits of ribulose-1,5-

bisphosphate carboxylase (55 and 15 kDa) and in other cytosolic proteins in the molecular range of 61, 39, 32, 19 and 17 kDa during light and even more under dark incubation of detached leaves. Incubation with BA showed best protection of the above proteins against degradation under both light and dark. BA inhibited the

Table 2. Changes in chlorophyll and protein contents [g kg^{-1}(d.m.)] in detached leaves of 15-d-old plants incubated on water or BA, IAA or ABA solutions (10, 10 and 100 μM, respectively) for 7d. Irradiance 400 μmol m^{-2} s^{-1}. Figures in parentheses indicate % reduction over value prior to incubation.

Treatment		Chlorophyll	Total proteins
Prior incubation		1.99 (0)	36.5 (0)
Water	light	1.55 (42)	24.0 (34)
	dark	1.01 (49)	21.0 (42)
BA	light	1.70 (15)	34.0 (7)
	dark	1.37 (31)	33.7 (8)
IAA	light	1.29 (35)	18.5 (49)
	dark	1.00 (50)	21.0 (43)
ABA	light	0.60 (70)	17.5 (52)
	dark	0.59 (70)	17.2 (53)
SD		0.46	7.75

Table 3. Changes in protein contents [μg] after incubation of detached leaves on water or solutions of growth regulators for 7 d. Spectrophotometric scan data of specific protein bands were resolved in 15 % SDS-PAGE. Values were deduced from peak area of each bond. For each lane 100 μg of protein was loaded. ND - not detected.

Treatment		M_r [kDa] 61	55	39	32	26	19	17	15
Prior		5.5	8.2	12.1	20.5	ND	17.2	13.2	3.5
Water	light	ND	ND	4.1	8.6	ND	8.2	7.6	2.0
	dark	ND	ND	4.9	8.6	ND	8.5	7.8	2.1
BA	light	4.5	7.3	11.6	19.8	ND	12.5	11.8	2.8
	dark	3.2	5.5	10.2	15.7	ND	14.9	10.4	2.5
IAA	light	ND	2.5	6.8	14.4	ND	12.5	7.4	2.8
	dark	ND	ND	3.2	11.0	ND	8.1	4.4	1.8
ABA	light	ND	ND	ND	0.4	ND	0.3	ND	2.1
	dark	ND	ND	ND	2.7	1.2	0.2	ND	0.7
SD		2.1	3.3	4.4	6.6	0.4	5.6	4.5	0.8

turnover of senescence promoting inzymes there by protecting major proteins against the degradative process (Varga and Bruinsma 1973). Incubation with IAA under both light and dark protected only the polypeptides of 39, 32, 19 and 17 kDa. IAA and NAA interact with the protein synthetic apparatus (De Leo and Sacher 1970). Incubation of detached leaves with ABA resulted in total degradation of soluble proteins; only the proteins of 32, 19 and 15 kDa were retained (Table 3). ABA

increases acid protease formation in light but perhaps decreases it in darkness (Zhi-Yi *et al.* 1988). ABA also inhibits the formation of ethylene and thus may reduce degradation of protein in darkness. In the dark, ABA induced formation of a polypeptide of 26 kDa. Induction of such ABA specific proteins was reported by Lin and Ho (1986). These proteins were active against gibberellic acid and affected also synthesis of other proteins.

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