EFFECT OF METHYL ESTER OF JASMONIC ACID, ABSCISIC ACID AND BENZYLADENINE ON CHLOROPHYLL SYNTHESIS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* (ZUCCHINI)

Evgueni D. Ananiev, Kalina Ananieva, Ivan Todorov

Institute of Plant Physiology "Acad. M. Popov", Bulg. Acad Sci., "Acad. G. Bonchev" str., block 21, Sofia 1113, BULGARIA

Received November 1, 2004

Summary. Except for the first stages of illumination, exogenous methyl ester of jasmonic acid (MeJA) inhibited much stronger than abscisic acid (ABA) the accumulation of chlorophyll in excised cotyledons of *C. pepo* L. (zucchini) exposed to controlled light conditions. Benzyladenine (BA) applied in a combination with MeJA or ABA at equimolar concentrations completely neutralized their inhibitory effect. In contrast to ABA, preincubation of cotyledons with MeJA in the dark increased its inhibitory effect on chlorophyll synthesis while the effect of ABA remained almost unchanged. Determination of δ-aminolevulinic acid (δ-ALA) in greening cotyledons revealed a close correlation between the effect of phytohormones on chlorophyll content and δ-ALA accumulation. MeJA inhibited to a greater extent than ABA the synthesis of δ-ALA. Our results suggest different mechanisms of inhibition of chlorophyll synthesis by both inhibitors. MeJA preferentially inhibitied chlorophyll accumulation at the level of chlorophyll precursors in the dark while ABA exerted its effect on the light-dependent reactions of chlorophyll synthesis.

Key words: abscisic acid, chlorophyll content, benzyladenine, excised cotyledons, δ-aminolevulinic acid, methyl ester of jasmonic acid,

Abbreviations: ABA – abscisic acid, BA – benzyladenine, δ-ALA – aminolevulinic acid, LA – levulinic acid, MeJA – methyl ester of jasmonic acid, TCA – trichloroacetic acid

Corresponding author, e-mail: ananiev@obzor.bio21.bas.bg

INTRODUCTION

Jasmonates (jasmonic acid (JA), its methyl ester (MeJA) and related compounds) are a group of ubiquitously occurring plant growth regulators which exhibit hormone-like properties in various physiological processes connected with plant growth and development such as germination and growth, flower development, leaf senescence, tuberization, fruit ripening (Creelman and Mullet, 1997; Wasternack and Hause, 2002). Promotion of leave senescence estimated by a decrease in chlorophyll content and depression of photosynthesis-related genes was one of the first reported physiological effects of jasmonates (Ueda et al., 1981; Creelman and Mullet, 1997; He et al., 2002). The data reported in literature so far refer mainly to excised or intact differentiated leaves after exogenous application of jasmonates (Miersch et al., 1986; Weidhase et al., 1987; He et al., 2002). However, very limited data exist on the effect of MeJA on chlorophyll accumulation during the initial stages of chlorophyll synthesis in greening leaves or cotyledons. Cotyledons of *Cucurbitaceae* represent storage organs, which undergo structural and functional differentiation during their transition into photosynthetic organ after emerging above the soil (Kulaeva, 1982). The genetic program of this transition is controlled by cytokinins and abscisic acid (ABA), the latter counteracting the positive effect of cytokinins (Kulaeva, 1982). Exogenous MeJA is considered as a chemical stress agent mimicking the effect that may also appear in response to external stress factors inducing senescence (Wasternack and Hause, 2002).

Our previous results showed that in excised greening zucchini cotyledons exogenous MeJA inhibited much stronger photosynthesis and chlorophyll accumulation than ABA (Ananiev and Ananieva, 2000). In the present study, we further analysed the inhibitory effect of MeJA and ABA on chlorophyll synthesis by determining the endogenous content of δ-ALA as a rate-limiting step in the biosynthesis of chlorophyll. We studied also the antagonistic effects of MeJA and ABA on the cytokinin-induced accumulation of chlorophyll by treating cotyledons with equimolar solutions of each inhibitor and BA.

MATERIALS AND METHODS:

Growth conditions and treatments

Seeds of *Cucurbita pepo* L. (zucchini), cv. *Cocozelle* were germinated in tap water in the dark at 28ºC. The cotyledons of 4-day-old plants were excised and transferred to Petri dishes with distilled water for another 24 h in the dark in order to reduce the levels of endogenous cytokinins and ABA. The cotyledons were then incubated for different periods of time on distilled water or aqueous solutions of 45 μM BA, 45 μM ABA or 45 μM MeJA as well as the equimolar mixtures of BA+MeJA and

BA+ABA (x 45 μM each, 1:1, v/v) in the light (photon flux density 100 μmol m^{-2} s^{-1}, t^0 25^0C, humidity 70% and photoperiod (12/12, light/dark).

Chlorophyll content determination

Chlorophyll content was determined according to the method of Arnon (1949) and calculations were made using the coefficients of MacKinney (1941).

Determination of δ-aminolevulinic acis (ALA)

δ-ALA was determined by the method of Averina and Yaronskaya (1988). In these experiments the ALA-dehydrase inhibitor levulinic acid (LA) was used to induce the accumulation of d-ALA in greening cotyledons. This technique was first proposed by Beale (1971) in *Chlorella* and after that developed for higer plants (Harel and Klein, 1972). Briefly, after the period of exhaustion in the dark, cotyledons were treated for 12 h in the dark with the tested phytohormones and then exposed to the light in the presence of 20 mM LA and the same concentrations of phytohormones. Cotyledons (1 g) were ground with pestle in a mortar in 3 ml of 10% trichloroacetic acid (TCA) and the homogenate was heated in water bath at 100º C for 15 min. After centrifugation at 6000g for 10 min the supernatant was collected and the pellet was suspended in 3 ml of distilled water, heated again and centrifuged at the same conditions. The resulting pellet was resuspended in 3 ml of 1.0 M sodium acetate buffer, pH 4.6 and pelleted again. Acetylacetone (0.1 ml) and glacial acetic acid (0.1 ml) were added to 2 ml of the collected supernatants. The mixture was allowed to stay in tightly closed tubes at 100ºC for 15 min and 2 ml of 2% modified Ehrlich reagent was added after cooling. The absorbance of the pink color was determined after 15 min at 553 nm using a Carl Zeis- Iena spectrophotometer. The estimation of δ-ALA content was based on molar extinction coefficient of $6.8.10^4$ M^{-1} cm^{-1}.

Results and discussion

Exposure of dark grown excised cotyledons to controlled light conditions resulted in a very rapid increase of chlorophyll accumulation up to the 48^{th} h due to intensive biosynthesis of chlorophyll. After that the chlorophyll content reached steady-state values reflecting the equilibrium between the rates of synthesis and degradation (Fig.1). Both JA and MeJA inhibited strongly chlorophyll accumulation within all tested times of 24-72 h. Besides, the inhibitory effect of MeJA was much more pronounced compared to JA and in all further experiments we used MeJA. Except for the first 24 h, ABA inhibited chlorophyll accumulation to a much lesser extent than MeJA especially after prolonged treatment (48, 72 h). Incubation of cotyledons for 72 h with equimolar concentrations (45 μM) of ABA or MeJA decreased chlorophyll content in comparison with the control by 25% and 78%, respectively, thus indicating a stronger

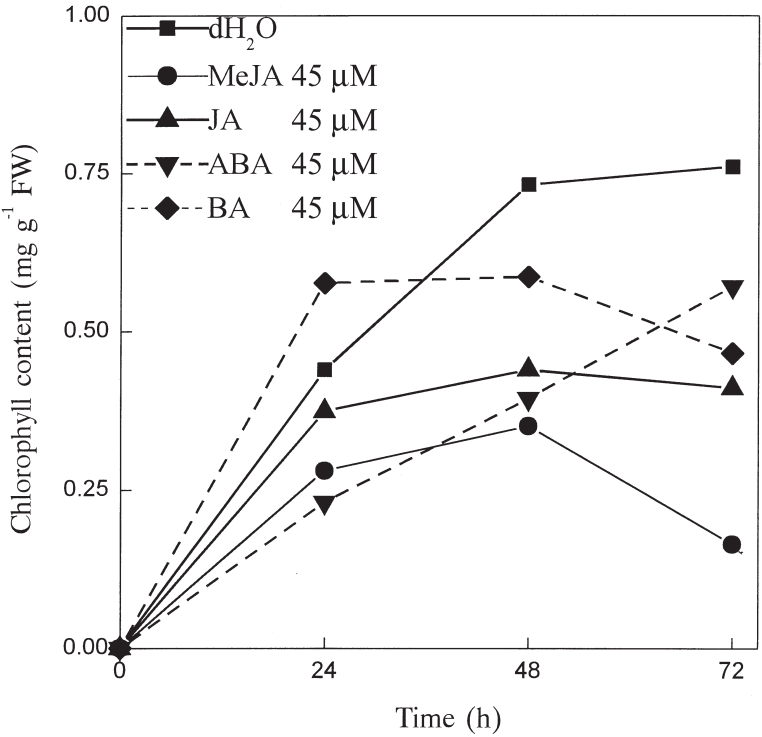

Fig. 1. Effects of MeJA, JA, ABA and BA on total chlorophyll content (chlorophyll *a+b*) in excised greening cotyledons of *C. pepo* (zucchini). Cotyledons were incubated in solutions of 45 µM each of MeJA, JA, ABA and BA in the light for different periods of time and chlorophyll content was determined as described in Materials and Methods. Each data point represents the mean value of five experiments. The SE values averaged 4% and did not exceed 7% of the means.

inhibitory effect of MeJA in this assay system. Treatment of cotyledons for 24 h with BA led to an increase in chlorophyll content by only 25-30%. Longer cytokinin treatment resulted in a decrease of chlorophyll accumulation. Although the applied concentration of BA (45 µM) was optimal for cotyledon growth (Kulaeva, 1982; Ananieva and Ananiev, 1999), it turned to be inhibitory for chlorophyll synthesis when chlorophyll was calculated on a fresh weight basis (Tabl. 1). The decrease in chlorophyll accumulation correlated closely with the increased fresh weight of cotyledons (Table 2). However, when determined on a dry weight basis, the cytokinin-stimulated chlorophyll accumulation was preserved at the same level (25-30%) within all tested times. This was obviously due to the decrease in cotyledons dry weight caused by cytokinin treatment (Table 2). The inhibitory effect of cytokinins on chlorophyll synthesis and chloroplast development accompanied by a positive effect on growth expansion has been previously reported for excised cotyledons of radish and cucumber (Thomas et al., 1981; Haru et al. 1982). This effect can be explained by the well-

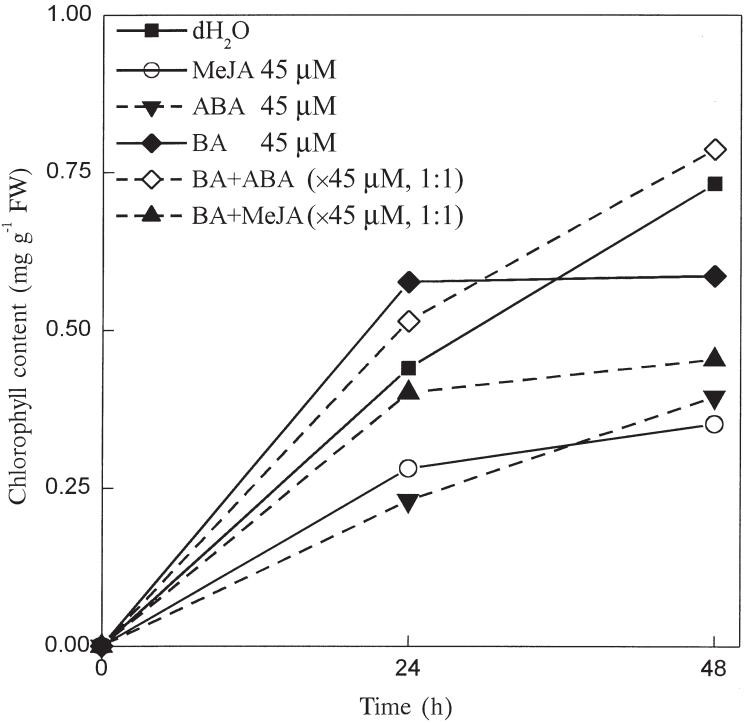

Fig. 2. Antagonistic effects of ABA and MeJA on BA-stimulated accumulation of total chlorophyll (chlorophyll *a*+*b*) in excised greening cotyledons of *C. pepo* (zucchini). Cotyledons were transferred to equimolar mixtures of BA+ABA and BA+MeJA (x 45 µM, 1:1, v/v) in the light for different periods of time and chlorophyll content was determined as described in Materials and Methods. Each data point represents the mean value of five experiments. The SE values averaged 4% and did not exceed 7% of the means.

known cytokinin-mediated stimulation of storage reserves degradation, which results in accumulation of osmotically active compounds and a subsequent increase in the water uptake (Kulaeva, 1982). Concerning the decrease in chlorophyll content after 48 h of BA treatment, it must be noted that this behavior closely correlated with

Table 1. Effect of BA (45 µM) on total chlorophyll content (chlorophyll *a*+*b*) in excised greening cotyledons of *Cucurbita pepo* L. (zucchini) calculated on fresh weight (FW) or dry weight (DW) basis

	Chlorophyll content					
	mg g $^{-1}$ FW			mg g $^{-1}$DW		
Time	dH_2O	BA	% of control	dH_2O	BA	% of control
24 h	0,411±0,018	0,513±0,018	125	2,36±0,15	2,97±0,14	126
48 h	0,700±0,017	0,516±0,017	74	3,71±0,25	4,52±0,26	122
72 h	0,724±0,017	0,438±0,015	60	3,72±0,25	4,71±0,26	127

Table 2. Effect of BA (45 μM) on fresh weght (FW) or dry weight (DW) of excised greening cotyledons of *Cucurbita pepo* L. (zucchini)

	FW cotyledon $^{-1}$			DW cotyledon $^{-1}$		
Time	dH_2O	BA	% of control	dH_2O	BA	% of control
24 ч.	0,133±0,005	0,183±0,006	138	0,036±0,002	0,031±0,003	86
48 ч.	0,151±0,006	0,242±0,005	160	0,032±0,002	0,025±0,002	78
72 ч.	0,181±0,006	0,304±0,003	168	0,029±0,001	0,019±0,002	66

a similar decrease in the rate of photosynthesis (Ananiev and Ananieva, 2000). Obviously, in the detached cotyledons the normal "sink/source" relationship mechanisms might be seriously altered after prolonged cytokinin treatment due to a rapid exhaustion from essential metabolites, thus leading to a progressive decrease in the rate of chlorophyll synthesis and accumulation.

Much more interest deserves the relatively weak stimulation (only 26%) of chlorophyll synthesis after 24 h of BA treatment (Fig. 1). It is worth mentioning that under the experimental photoperiod (12 h/12 h dark/light), cotyledons were transferred to light in the presence of BA followed by treatment in the dark. Therefore, as reported earlier (Fletcher and McCullach, 1971; Fletcher et al., 1973; Lew and Tsuji, 1982; Reiss and Beale, 1995), in the assay conditions of concomitant BA and light treatment a lag period of chlorophyll synthesis occurred. On the other hand, it is well known that different optimal cytokinin concentrations exist for different physiological processes in plants (Leshem et al., 1994). Obviously, in excised zucchini cotyledons the concentration of 45 μM BA turned to be supraoptimal for chlorophyll synthesis when BA was applied together with light.

In many physiological systems ABA is manifested as a cytokinin antagonist and can neutralize the stimulatory effect of cytokinins (Kulaeva, 1982). The results presented in Fig. 2 showed that ABA neutralized the cytokinin effect on chlorophyll accumulation when applied in a mixture with BA and chlorophyll content was very similar to the control. Thus, at the level of chlorophyll synthesis and accumulation ABA acts as a typical cytokinin antagonist similarly to its neutralizing effect on cytokinin-stimulated growth, protein and RNA synthesis (Ananieva and Ananiev, 1999). Exogenous MeJA applied in a mixture BA+MeJA almost completely neutralized the stimulatory effect of BA on chlorophyll accumulation, especially during the first 24 h when the cytokinin effect was best pronounced.

In order to investigate further in details the effect of the phytohormones tested we compared the chlorophyll accumulation after preincubation of cotyledons with MeJA, ABA or BA (Fig. 3). The strongest cytokinin stimulation of chlorophyll synthesis (almost 2- fold) was observed when cotyledons were pretreated with BA for 12 h in the dark (Fig. 3,b). Cytokinin-induced stimulation in the dark was sustained even

when cotyledons were transferred to distilled water in the light (55% stimulation) (Fig. 3,c). These results indicated that the cytokinin effect was exerted at the stages of chlorophyll synthesis in the dark and it was most probably due to intensive accumulation of chlorophyll precursors. Among them a crucial role for chlorophyll synthesis plays δ-ALA. A lot of data has been accumulated showing that cytokinins stimulate its synthesis (Fletcher and McCullach, 1971; Fletcher et al., 1973; Lew and Tsuji, 1982). Special attention deserves the results obtained with ABA and MeJA using the approach of pretreatment in the dark. Preincubation of cotyledons with MeJA in the dark resulted in a much stronger inhibitory effect on chlorophyll accumulation in contrast to the effect of ABA which remained almost unchanged during both treatments (Fig. 3, a,b). Therefore, the stronger overall inhibitory effect of MeJA on chlorophyll accumulation could be due to its stronger effect on the dark reactions of

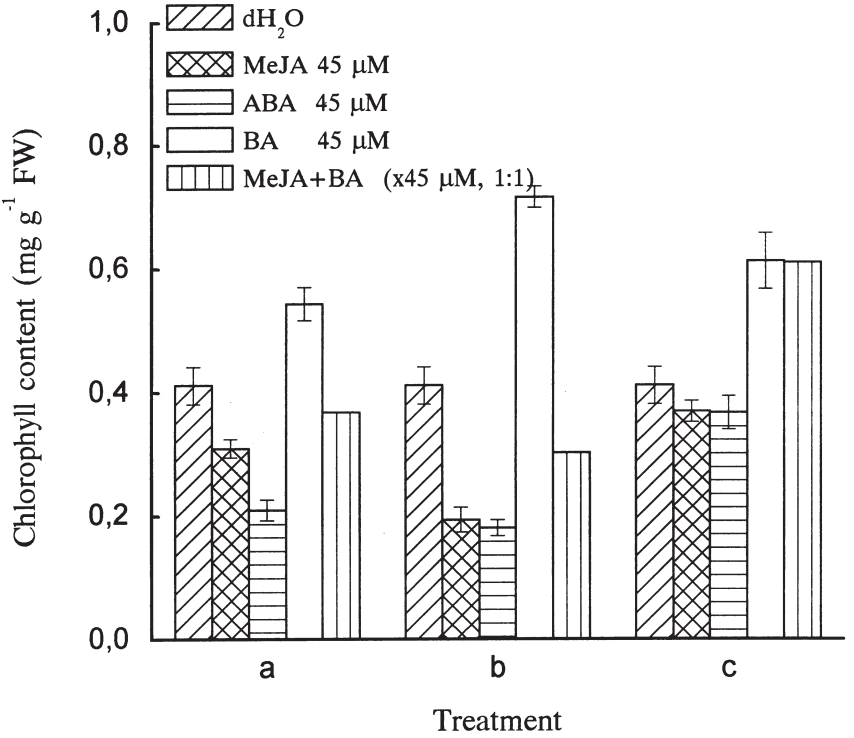

Fig. 3. Effect of different treatments of excised *C. pepo* (zucchini) cotyledons with MeJA, ABA and BA on total chlorophyll content (chlorophyll *a*+*b*). Cotyledon treatments were performed as follows: a/ transfer to solutions of 45 µM each of MeJA, ABA and BA in the light for 24 h; b/ preincubation with 45 µM each of MeJA, ABA and BA for 12 h in the dark followed by a transfer in the light in the presence of the same phytohormones. c/ preincubation with 45 µM each of MeJA, ABA and BA for 12 h in the dark followed by a transfer to the light in the absence of phytohormones. Each data point represents the mean value of three experiments.

chlorophyll synthesis pathway. This scheme of cotyledon pretreatment allowed also to study more precisely the antagonism between MeJA and BA on chlorophyll synthesis. Fig. 3, b shows that preincubation of cotyledons for 12 h in the dark with the mixture BA+MeJA resulted in mutual neutralization of their individual effects after their subsequent transfer to the light. On the other hand, the inhibitory effect of MeJA on cytokinin stimulation in the light was completely eliminated when cotyledons were transferred to distilled water (Fig. 3,c). These results showed that in contrast to MeJA, the cytokinin stimulatory effect was sustainable and that for the neutralization of its effect a continuous presence of MeJA was needed.

Now it is firmly established that the synthesis of d-ALA is the rate-limiting step in the biosynthesis of chlorophyll during the earliest stages of greening (Beale, 1978). It was found that the addition of δ-ALA abolished the specific lag phase in chlorophyll production after the onset of illumination (Castelfranco et al.,1974). LA has been extensively used for studying δ-ALA production since as a competitive inhibitor of δ-ALA dehydratase reaction LA causes accumulation of δ-ALA (Beale, 1978). Our results on δ-ALA production are presented in Fig. 4. In the presence of LA, δ-ALA accumulated very rapidly after all treatments due to the effect of light. In the control cotyledons endogenous δ-ALA levels reached values of about 200 nmoles per gram fresh weight for 12 h exposure in the light, which were similar to previously reported results (Fletcher et al., 1973; Lew and Tsuji, 1982). Exogenous MeJA inhibited δ-ALA production very rapidly (55% inhibition for the first 6h), while the inhibitory effect of ABA was less pronounced (18%). Treatment of cotyledons with BA in the dark followed by their transfer to the light doubled the amount of δ-ALA accumulation for the first 6 h. After that the δ-ALA production slightly decreased.

At present an immense information has been accumulated showing that light initiates the greening of etiolated cotyledons and cytokinin is the phytohormone promoting the effect of light, thus exerting a co-operative effect on the process of greening (Fletcher and McCullach, 1971; Fletcher et al., 1973; Lew and Tsuji, 1982; Reiss and Beale, 1995). Now it is firmly established that de-etiolation of cotyledons leads to a rapid conversion of the small amount of protochlorophyllide present in the etioplasts to chlorophyll (Koski et al., 1951) and this reaction is catalyzed by the light-dependent NADPH:protochlorophyllide oxidoreductase (POR, EC 1.6.99.1). During further illumination this photoconversion ceases and is followed by a lag phase for several hours. After that chlorophyll accumulation is restored till reaching its final content in the mature chloroplasts (Virgin, 1955). In the widely used model system of excised etiolated cotyledons of *Cucurbitaceae*, pretreatment with BA abolishes this lag period (Fletcher and McCullagh, 1971; Lew and Tsuji, 1982), due to increased synthesis of δ-ALA, which is the rate-limiting step in the biosynthesis of chlorophyll (Fletcher et al., 1973; Lew and Tsuji, 1982). During the last decade the molecular mechanisms of BA-induced stimulation of δ-ALA synthesis has been intensively studied and it was unequivocally shown that among the

three enzymes responsible for δ-ALA synthesis (glutamyl-tRNA synthetase, glutamyl-tRNA reductase and glutamate 1-semialdehyde (GSA) aminotransferase), cytokinins specifically stimulated the activity of glutamyl-tRNA reductase (Masuda et al., 1994; Masuda et al., 1995). Therefore, it is reasonable to assume that the 2-fold increase in δ-ALA content after BA preincubation of zucchini cotyledons prior to light treatment could be due to an increased activity of glutamyl-tRNA reductase (Fig. 4).

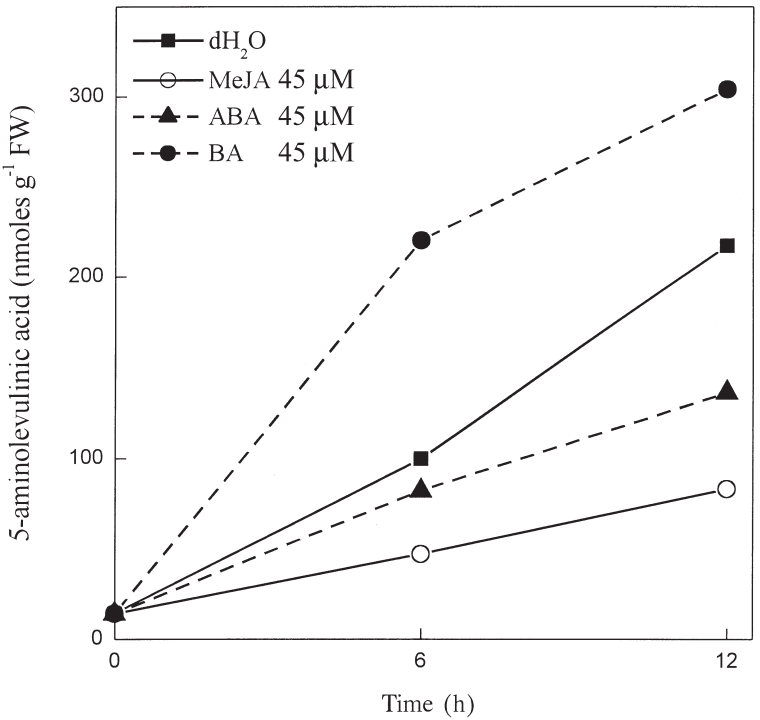

Fig. 4. Effects of MeJA, ABA and BA on the content of δ-ALA in excised greening cotyledons of *C.pepo* (zucchini). Cotyledons were pretreated for 12 h in the dark with 45 µM solutions of MeJA, ABA and BA. After preincubation cotyledons were transferred to the same phytohormone solutions for various times in the light in the presence of 20 mM levulinic acid (LA). δ-ALA accumulation was determined as described in Materials and Methods. Each data point represents the mean value of three experiments. The SE values averaged 12% and did not exceed 18% of the means.

ABA is widely known as a potent antagonist of cytokinins on photomorphogensis of dark-grown cotyledons including chloroplast development, maturation and chlorophyll synthesis (Kusnetsov et al., 1994; Kusnetsov et al., 1998). It has been previously shown that cytokinin stimulates and ABA inhibits greening of etiolated *Lupinus luteus* cotyledons by affecting the expression of the light-sensitive protochlorophyllide oxidoreductase (Kusnetsov et al., 1998). Our results confirm the antagonistic effect

of BA and ABA at the level of chlorophyll accumulation. Furthermore, the comparison of ABA-mediated inhibition of chlorophyll synthesis in the variants with and without ABA preincubation in the dark (Fig. 3 a,b) suggests that ABA affected mainly the light-dependent reactions, most probably the reduction of POR as shown earlier (Kusnetsov et al., 1998). This assumption is supported also by the relatively small inhibitory effect of ABA on δ-ALA synthesis occurring in the dark (Fig. 4).

In comparison with ABA, the inhibitory effect of MeJA on chlorophyll synthesis and accumulation in our assay system was much stronger (Fig. 1; Fig. 3). There are a lot of data showing that jasmonates can inhibit the process of photosynthesis mainly by degradation of important photosynthetic enzymes (Muller-Uri et al., 1988; Wasternack et al., 1994; Ananieva and Ananiev, 1999). Molecular hybridization analyses with specific DNA probes revealed that MeJA down-regulated the synthesis of nuclear-encoded chloroplast proteins in barley like the small subunit (SSU) of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) and the light harvesting chlorophyll protein complex apoproteins (LHCPs) (Reinbothe et al., 1993; Reinbothe et al., 1997). Little is known about the effect of MeJA on the expression of genes involved in chlorophyll synthesis. To our knowledge the preferential inhibition of chlorophyll synthesis by MeJA in the dark including δ-ALA accumulation is reported for the first time. This effect was greater for MeJA and less pronounced in ABA-treated cotyledons. The comparative study of MeJA- and ABA-induced inhibition of chlorophyll synthesis and accumulation revealed distinct differences in their effects. Therefore, it is reasonable to assume possible different primary targets in chlorophyll synthesis pathway for the two inhibitors.

Conclusions

Our results showed a differential inhibitory effect of MeJA and ABA on chlorophyll synthesis and accumulation in excised greening zucchini cotyledons observed at the level of δ-ALA accumulation during the dark reactions of chlorophyll biosynthesis pathway. At that primary target the effect of MeJA was much more pronounced compared to ABA, which indicates that MeJA is a stronger senescence promoting factor in this assay system. The combined action of BA and MeJA or ABA restored total chlorophyll amount, thus indicating the counteraction between these phytohormones at the level of chlorophyll synthesis.

References

Ananiev, E.D., K.I. Ananieva, 2000. Comparative effects of methyl ester of jasmonic acid, abscisic acid and benzyladenine on chlorophyll content and photosynthesis in excised cotyledons of *Cucurbita pepo* L. (zucchini). Compt. Rend Acad. bulg. Sci., 53(5), 85-88.

Ananieva, K.I., E.D. Ananiev, 1999. Effect of methyl ester of jasmonic acid and benzylaminopurine on growth and protein profile in excised cotyledons of *Cucurbita pepo* L. (zucchini). Biol. Plantarum, 42, (4), 549-557.

Averina, N.G., E.B. Yaronskaya, 1988. Effect of 5-aminolevulinic acid on barley leaf growth. Plant Physiol, (Russ), 35, (5), 916-920.

Beale, S.I., 1971. The biosynthesis of d-aminolevulinic acid in *Chlorella*. Plant Physiol., 45, 504-506.

Beale, S.I., 1978. δ-aminolevulinic acid in plants: its biosynthesis, regulation and role in plastid development. Annu. Rev. Plant Physiol., 29, 95-120.

Castelfranco, P.A., P.M. Rich, S.I. Beale, 1974. The abolition of lag phase in greening cucumber cotyledons by exogenous δ-aminolevulinic acid. Plant Physiol., 53, 615-618.

Creelman , R.A., J.E. Mullet, 1997. Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 48, 355-358.

Fletcher, R.A., D. McCullagh, 1971. Benzyladenine as a regulator of chlorophyll synthesis in cucumber cotyledons. Can. J. Bot., 49, 2197-2201.

Fletcher, R.A., C. Teo, A. Ali, 1973. Stimulation of chlorophyll synthesis in cucumber cotyledons by benzyladenine. Can. J. Bot., 51, 937-939.

Harel, E., S. Klein, 1972. Light dependent formation of δ-aminolevulinic acid in etiolated leaves of higher plants. Biochem. Biophys. Res. Commun., 49, 364- -370.

Haru, K., K. Naito, Y. Suzuki, 1982. Differential effect of benzyladenine and potassium on DNA, RNA, protein and chlorophyll content and on expansion growth of detached cotyledons in the dark and light. Physiol. Plant., 55, 641-849.

He, Y., H. Fukushige, D.F. Hildebrand, S.S. Gan, 2002. Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. Plant Physiol., 128, 876-884.

Koski, V.M., C.S. French, J.H.C. Smith, 1951. The action spectrum for the transformation of protochlorophyll to chlorophyll a in normal and albino corn seedlings. Arch. Biochem. Biophys. 31, 1-7.

Kulaeva O.N., 1982 Hormonal regulation of plant physiological processes at the level of RNA and protein synthesis, Nauka, Moskva (In Russ.)

Kusnetsov ,V.V., R. Oelmuller, M.I. Sarwat, S.A. Porfirova, G.N. Cherepnova, R. Herrmann, O.N. Kulaeva, 1994. Cytokinins, abscisic acid and light affect accumulation of chlo-

roplast proteins in *Lupinus luteus* L. cotyledons without notable effect on steady-state mRNA levels. Planta, 194, 318-327.

Kusnetsov ,V.V., R.G. Herrmann, O.N. Kulaeva, R. Oelmuller 1998. Cytokinin stimulates and abscisic acid inhibits greening of etiolated *Lupinus luteus* L. cotyledons by affecting the expression of the light-sensitive protochlorophyllide oxidoreductase. Mol. Gen. Gen., 259, 21-28.

Leshem, B., R. Ronen, E. Soudry, S. Lurie, S. Gepstein, 1994. Cytokinin at a large range of concentrations determines rates of polypeptide metabolism in cultured melon cotyledons. J. Plant Physiol., 143, 330-336.

Lew, R., H. Tsuji, 1982. Effect of benzyladenine treatment duration on d-aminolevulinic acid accumulation in the dark, chlorophyll lag phase abolition, and long-term chlorophyll production in excised cotyledons of dark-grown seedlings. Plant Physiol., 69, 663-667.

MacKinney, G.,1941. Absorption of light by chlorophyll solutions. J. Biol. Chem., 140, 315-322.

Masuda, T., R. Tanaka, Yu. Shioi, K. Takamiya, C.G. Kannangara, H. Tsuji, 1994. Mechanism of benzyladenine-induced stimulation of the synthesis of δ-aminolevulinic acid in greening cucumber cotyledons: benzyladenine increases levels of plastid $tRNA^{Glu}$. Plant Cell Physiol., 35, 183-188.

Masuda, T., H. Ohta, Yu. Shioi, H. Tsuji, K. Takamiya, 1995. Stimulation of glutamyl- tRNA reductase activity in greening cucumber cotyledons. Plant Cell Physiol., 36, 1237-1243.

Miersch, O., A. Meyer, S. Vorkfeld, G. Sembdner, 1986. Occurrence of (+)-7- isojasmonic acid in *Vicia faba* L. and its biological activity. J. Plant Growth Regul., 5, 91-100.

Muller-Uri, F., B. Parthier, L. Nover, 1988. Jasmonate-induced alteration of gene expression in barley leaf segments analyzed by *in vivo* and *in vitro* protein synthesis. Planta, 176, 241-247.

Reinbothe, S., C. Reinbothe, B. Parthier, 1993. Methyl jasmonate-regulated translation of nuclear-encoded chloroplast proteins in barley (*Hordeum vulgare* L. cv. salome). J. Biol. Chem., 268, 10606-10611.

Reinbothe, S., B. Parthier, C. Reinbothe, 1997. Temporal pattern of jasmonate-induced alterations in gene expression of barley leaves. Planta, 201, 281-287.

Reiss, C., S.I. Beale, 1995. External calcium requirements for light induction of chlorophyll accumulation and its enhancement by red light and cytokinin pretreatment in excised etiolated cucumber cotyledons. Planta, 196, 635-641.

Thomas, J., C. Ross, C. Chastain, N. Koomanoff, J. Hendrix, 1981. Cytokinin-induced wall extensibility in excised cotyledons of radish and cucumber. Plant Physiol, 68, 107-110.

Ueda, J., J. Kato, H. YamaneN. Takahashi, 1981. Inhibitory effect of methyl jasmonate and its related compounds on kinetin-induced retardation of oat leaf senescence. Physiol. Plant., 52, 305-309.

Virgin, H.I., 1955. Protochlorophyll formation and greening in etiolated barley leaves. Physiol. Plant., 8, 630-643.

Wasternack, C., B. Hause, 2002. Jasmonates and octadecanoids: signals in plant stress responses and development. Prog. Nucleic Acid Res. Mol. Biol., 72, 165-221

Wasternack, C., R. Atzorn, B. Blume, J. Leopold, B. Parthier, 1994. Ursolic acid inhibits synthesis of jasmonate-induced proteins in barley leaves. Phytochemistry, 35, 49-54.

Weidhase R.A., H.-M. Kramell, J. Lehmann, W. Liebisch, W. Lerbs, B. Parthier, 1987. Degradation of ribulose-1,5-biphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic methyl ester, and countaraction by cytokinin. Physiol. Plant., 69, 161-166.