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COMPARATIVE STUDY OF THE EFFECTS OF METHYL JASMONATE AND ABSCISIC ACID ON RNA AND PROTEIN SYNTHESIS IN EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (*ZUCCHINI*)

Kalina Ananieva*, Evgueni D. Ananiev

Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria

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Summary. The effects of methyl jasmonate and abscisic acid on the polypeptide pattern of soluble proteins as well as *in vivo* protein and RNA synthesis and endogenous nuclear RNA polymerase activity were compared in excised marrow cotyledons grown in the light. Treatment of cotyledons with MeJA resulted in the induction of two abundant polypeptide bands with Mr 43 and 60 kDa and a significant accumulation of a polypeptide band with Mr 97.4 kDa as analysed by SDS–PAGE. In contrast to MeJA, ABA did not cause the formation of these specific polypeptide bands. Furthermore, the inhibitory effect of ABA on protein and RNA biosynthesis as well as the activity of total endogenous RNA polymerases was much stronger than the effect of MeJA. It is suggested that in excised marrow cotyledons MeJA and ABA act by different mechanisms in the regulation of cotyledon growth and development during the earlier stages of germination.

Key words: abscisic acid, excised cotyledons, endogenous RNA polymerases, *in vivo* protein and RNA synthesis, methyl jasmonate, polypeptide pattern

Abbreviations: MeJA – methyl jasmonate, ABA – abscisic acid, SDS-PAGE – SDS-polyacrylamide gel electrophoresis, AAM – amino acid mixture, PMSF – phenylmethylsulphonylfluoride, JIPs – jasmonate-induced polypeptides, LOX – lipoxygenase

* Corresponding author

Introduction

Methyl jasmonate and its related compounds are widespread in the plant kingdom and regarded as a new group of plant growth regulators (Sembdner and Parthier, 1993). Jasmonates play an important role in various physiological processes connected with plant growth and development (Sembdner and Parthier, 1993; Creelman and Mullet, 1997). On the other hand, jasmonic acid and its methyl ester are regarded as signalling substances, responsible for the activation of signal transduction pathways in response to different kinds of stress, such as water deficit (due to desiccation or osmoticum) (Lehmann et al., 1995), salt stress (Moons et al., 1997), wounding and pathogen attack (Creelman et al., 1992; Farmer and Ryan, 1992). Jasmonates induce the expression of genes encoding proteins with stress-related and protective functions: vegetative storage proteins in soybean (Staswick, 1990), proteinase inhibitors in tomato and potato (Farmer and Ryan, 1992), enzymes of phytoalexin synthesis (Gundlach et al., 1992), different isoforms of lipoxygenase in barley leaves (Feussner et al., 1995), as well as in soybean seedlings (Grimes et al., 1992).

It is well-known that other plant growth regulators, in particular ethylene and abscisic acid, are also involved as mediators in the defence programme of plants (Wasternack and Parthier, 1997). The similarities between jasmonic acid and abscisic acid in chemical structure and many physiological effects including plant stress responses are well documented in literature (Weidhase et al., 1987; Sembdner and Parthier, 1993; Melan et al., 1993). However, some evidence exist, that they could act by different mechanisms in the regulation of plant physiological processes (Wilen et al., 1992; Moons et al., 1997; Montague, 1997). It has been previously shown that ABA inhibits *in vivo* protein and RNA synthesis in excised pumpkin cotyledons (Klyachko et al., 1979). On the other hand, only a few data are available indicating either a slight stimulation of MeJA on the [^{35}S]-methionine incorporation into bulk proteins in barley leaf segments (Mueller-Uri et al., 1988) or a decrease in the incorporation rate of [^{14}C]-AAM after treatment of barley leaf discs with MeJA (Popova and Vaklinova, 1988; Metodiev, 1998). Furthermore, contradictory data have been reported that indicate either a close similarity (Weidhase et al., 1987) or a marked antagonism (Moons et al., 1997) between the effects of exogenously applied ABA and MeJA on the changes in the polypeptide pattern of soluble proteins.

Excised Cucurbitaceae cotyledons have been well characterised as an useful tool for studying the effect of ABA on their growth and development during germination (Klyachko et al., 1979; Kulaeva, 1981). The objective of the present study was to compare the specific individual effects of exogenously applied MeJA and ABA in relation to their possible role as cell signalling substances based on the synthesis of specific polypeptides. This study was complemented by the investigation of *in vivo* protein and RNA biosynthesis in order to compare the physiological roles of MeJA and ABA in the regulation of developmental processes in excised cotyledons of *C. pepo* (*zucchini*) during germination.

Material and Methods

Plant material and cotyledon treatment

Seeds of *Cucurbita pepo* L. (*zucchini*), cv. Cocozelle, var. Tripolis were germinated on a moistened filter paper for 96 h in darkness at 28 °C. After excision of the embryonic axes, cotyledons were kept in darkness for further 24 h on distilled water in order to decrease endogenous cytokinins and ABA levels (Klyachko et al., 1979; Kusnetsov et al., 1994). Then the cotyledons were incubated on distilled water, or aqueous solutions containing either MeJA or ABA in equimolar concentrations (4.5×10^{-5} M) in the light (photon flux density 120 µmol.m^{-2}.s^{-1}, temperature 25 °C, humidity 70%) for 1–4 days. pH of MeJA solution was adjusted to 6.8–7.0 by addition of 0.1 N KOH in order to equalize it to the pH of ABA solution with the same concentration, while the ABA stock solution (1×10^{-3} M) was obtained by dissolving the (±) ABA racemate in alkali (0.1 N KOH) and diluted to the appropriate concentration with distilled water.

Protein extraction and SDS-PAGE

Soluble cell proteins were extracted in buffer containing 50 mM HEPES-NaOH, pH 8.0, 330 mM sorbitol, 2 mM KNO_3, 2 mM EDTA, 1 mM $MnCl_2$, 0.5 mM K_2HPO_4, 20 mM NaCl, 2 mM PMSF, dissolved in 2% SDS-sample buffer and electrophoretically separated in 12% polyacrylamide gels, containing 0.1% SDS (Laemmli, 1970). Polypeptides were stained with Coomassie R250. 40 µg proteins were charged per slot. Protein content was determined according to Lowry et al. (1951).

Labelling of proteins and total RNA

Protein synthesis in cotyledons was examined by *in vivo* labelling of the bulk proteins with [^{14}C]-(AAM) (Amersham, UK). Cotyledons with equal fresh weight were incubated with 5 µCi.ml^{-1} [^{14}C]-AAM for 4 h. [^{14}C]-incorporation in newly synthesised proteins was determined using the filter disc method as described by Shakirova et al. (1982).

In vivo RNA biosynthesis was determined by labelling cotyledons with [^{3}H]-uridine (UWVR, Praha, Czech Rep) for 4 h according to Osborne (1962) with modifications of Wollgiehn and Parthier (1964).

Endogenous RNA-polymerase activity

The total RNA polymerase activity was measured in isolated nuclei in a standard reaction mixture as described by Ananiev et al. (1987).

Results and Discussion

Effects of MeJA and ABA on the polypeptide profile of soluble proteins

Treatment of excised cotyledons of *Cucurbita pepo* L. (*zucchini*) with MeJA resulted in the accumulation of three abundant polypeptide bands with relative molecular masses of 97.4, 60 and 43 kDa already 24 h after MeJA addition (Fig. 1, lane 3) and

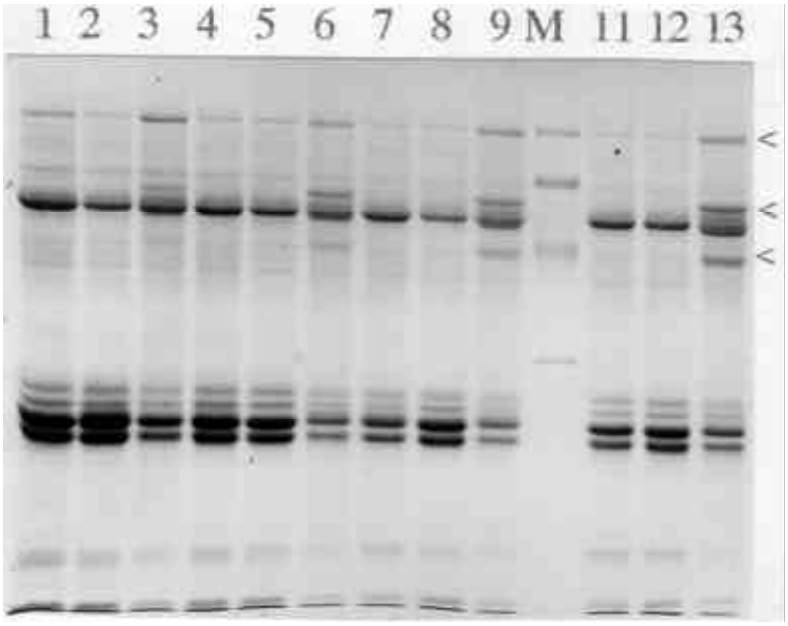

Fig.1. Polypeptide profiles of total soluble proteins extracted from detached marrow cotyledons floated on water, MeJA (4.5×10^{-5} M) and ABA (4.5×10^{-5} M) under light conditions. Key to lane numbers: 24 h incubation on: water (1), ABA (2), MeJA (3); 48 h incubation on: water (4), ABA (5), MeJA (6); 72 h incubation on: water (7); ABA (8), MeJA (9); 96 h incubation on: water (11), ABA (12), MeJA (13). Arrowheads indicate the positions of MeJA-affected polypeptides with Mr 97.4, 60 and 43 kDa, respectively. M – marker proteins: phosphorilase B (92.5 kDa), albumin bovine (BSA) (67 kDa), albumin egg (45 kDa) and carboanhydrase (29 kDa).

their accumulation increased with duration of treatment (Fig.1, lanes 6, 9, 13). Two of the bands – 60 and 43 kDa – were absent in the polypeptide profile of the water control. Experiments with the inhibitors of protein synthesis (cycloheximide) and mRNA synthesis (cordycepin) showed that these polypeptide bands were induced by MeJA and their induction was regulated at the level of transcription, as we have previously shown (Ananieva and Ananiev, 1998). The accumulation of 97.4 kDa polypep-

tide was only strongly stimulated by MeJA. The molecular mass of this polypeptide was determined by separation in 8% SDS-PAGE using high molecular markers (data not presented). Our results are consistent with results of other authors showing that MeJA-treatment can trigger a rapid shift in the spectrum of protein metabolism resulting in *de novo* synthesis of abundant polypeptides (JIPs) in different plant tissues, such as barley leaves (Weidhase et al., 1987; Mueller-Uri et al., 1988), excised cotton cotyledons (Reinbothe et al., 1992), rice roots (Moons et al., 1997). This dramatic alteration of gene expression in jasmonate-exposed plant tissues has been proved to be species- and tissue-specific (Herrmann et al., 1989). Most probably, the induction of abundant polypeptides in the excised marrow cotyledons is a specific response of cotyledon cells to the exogenously applied MeJA which acts as a chemical stress agent. A certain analogy could be supposed between the polypeptide with Mr 60 kDa induced by MeJA in excised *zucchini* cotyledons and JIP60 found in barley leaves. It has been shown that JIP60 is a ribosome-inactivating protein responsible for the down-regulation of the overall protein synthesis leading to cell death (apopthosis) after a long-term MeJA treatment (Reinbothe et al., 1994). Besides, the polypeptide band with Mr 97.4 kDa in the polypeptide profile of the excised cotyledons, found to be considerably stimulated by MeJA, could be considered as a putative candidate for the LOX protein (EC 1.13.11.12). Our assumption is based on its molecular mass, pronounced expression during germination, tissue-specific localisation and its induction by exogenous MeJA or different stress conditions in other plants (Siedow, 1991; Melan et al., 1993).

By contrast, application of ABA did not cause the accumulation of these specific polypeptide bands even after prolonged treatment (96 h), (Fig.1, lane 12). Furthermore, the MeJA-induced bands lacked in the polypeptide profiles of ABA-treated cotyledons when higher concentrations of ABA were used (1.10^{-4} M) (data not shown here). The differences in the polypeptide spectra triggered by MeJA and ABA concerned also the polypeptide bands in the region of 20–25 kDa and the low molecular polypeptides representing cotyledons reserve proteins (globulins). MeJA caused a gradual decrease of these polypeptides especially after prolonged treatment (96 h) (Fig. 1, lane 13) whereas ABA inhibited the degradation of these polypeptides (Fig. 1, lane 12). Therefore, in excised *zucchini* cotyledons MeJA can significantly alter gene expression as determined by the changes in the polypeptide pattern of soluble proteins. On the other hand, our results show differential effects of MeJA and ABA on the polypeptide spectra thus suggesting different mechanisms of action of the two plant growth regulators.

In vivo protein synthesis

The incorporation experiments with labelled amino acids (Fig. 2) showed that both ABA and MeJA inhibited *de novo* protein biosynthesis. However, ABA decreased

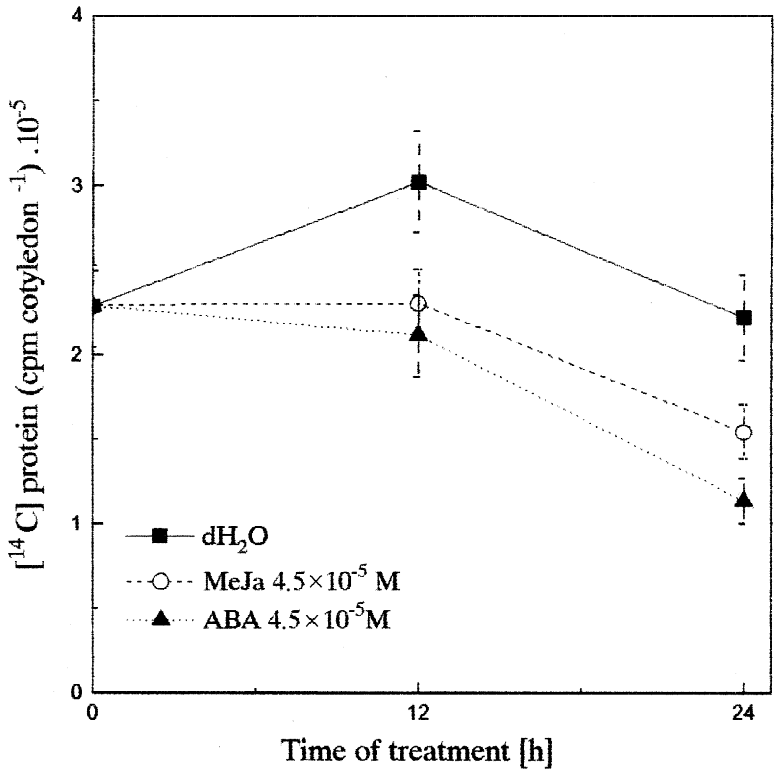

Fig. 2. Effects of MeJA and ABA on incorporation of [^{14}C]-labelled amino acids in total soluble proteins. Means are presented with S.E.

the rate of incorporation to a greater extent (50% at 24th h of treatment as compared to the control) than MeJA (30%). On the other hand, jasmonate effect did not differ from the control when the incorporation values were expressed as % of total uptake (Table 1). Therefore, the inhibition of [^{14}C]-incorporation rate in MeJA-treated cotyledons was accompanied by a similar decrease in the uptake of the radioactive label. The effect of ABA on this parameter (Table 1) was more pronounced than the MeJA effect (about 10% inhibition compared to the control) suggesting that ABA was more effective in inhibiting [^{14}C]-incorporation rate than the uptake capacity of isolated cotyledons. These results were in agreement with the data on the uptake of [^{14}C]-amino acids (data not shown).

In vivo RNA synthesis and total RNA-polymerase activity in isolated nuclei

The data presented in Fig. 3. show the effects of MeJA and ABA on the *in vivo* RNA synthesis. As total RNA in the plant cell consists mainly of ribosomal RNA, the results obtained reflect the effects of the two growth regulators predominantly on rRNA

Table 1. Effects of MeJA and ABA on the rates of incorporation of [^{14}C]-labelled amino acids into proteins and [^{3}H]-uridine in total RNAs in excised marrow cotyledons. The incorporation values are expressed as % of total radioactivity uptake.

Cotyledon treatment	Time of treatment (h)	Incorporation as % of uptake	
		[^{14}C]-AAM incorporation	[^{3}H]-uridine incorporation
Water	12	88	85
	24	71	84
MeJA	12	85	81
	24	72	82
ABA	12	79	82
	24	65	86

synthesis. In contrast to protein synthesis, MeJA stimulated the incorporation rate of [^{3}H]-uridine into newly synthesized cell RNAs within the first 12 h of incubation.

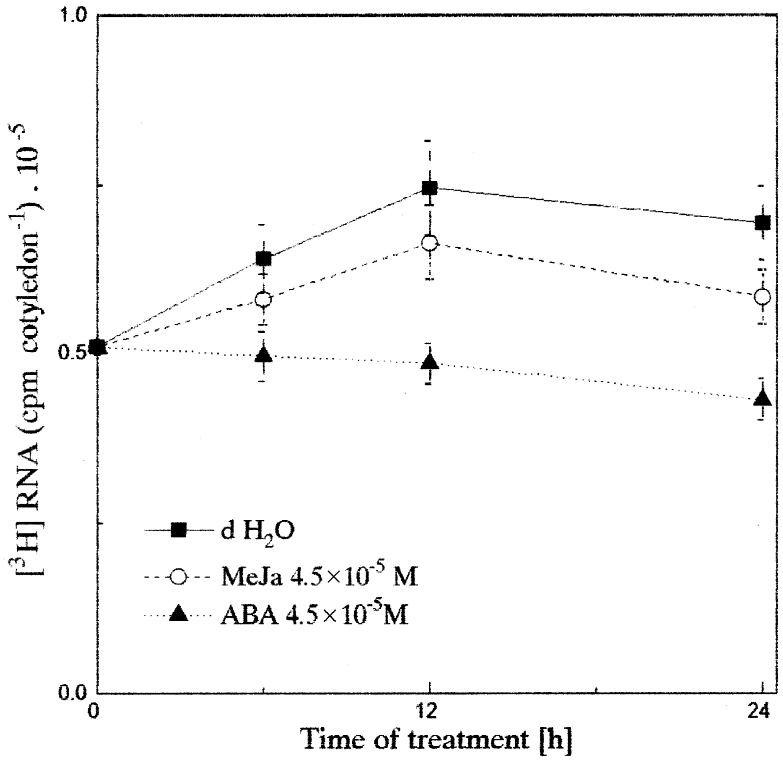

Fig. 3. Effects of MeJA and ABA on [^{3}H]-uridine incorporation into total cell RNAs. Means are presented with S.E.

The maximum registered at 12th h was followed by a slight decrease so that the incorporation values measured at 24th h of MeJA-treatment were higher than the initial value (incorporation rate in the isolated cotyledons in darkness, prior to treatment). In addition, the inhibitory effect of MeJA on RNA biosynthesis (15% at 24th h as compared to the control) was about 2-fold lower than the effect on *in vivo* protein synthesis (see Fig. 2). These results show that MeJA was more effective in suppressing protein synthesis than total RNA synthesis in the isolated marrow cotyledons. Similarly to *de novo* protein synthesis, application of ABA decreased the incorporation rate of [^{3}H]-uridine to a much greater extent (38% at 24th h of incubation) than MeJA (15%). By contrast, no differences between MeJA and ABA were observed when the incorporation values were expressed as % of total uptake (Table 1). These results indicate that the rate of incorporation upon MeJA and ABA-treatments decreased proportionally to the rate of uptake of the radioactive precursor by the cotyledons (data not shown). Therefore, it could be suggested that the uptake capacity of the cotyledons and the redistribution of radioactive precursors of RNA synthesis between intracellular pools were also affected by both plant growth substances.

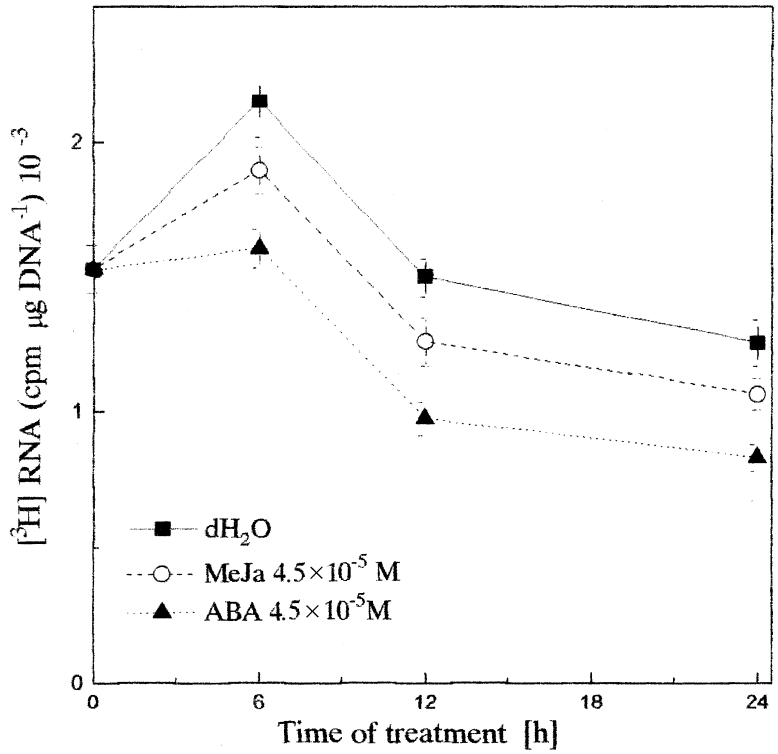

Fig. 4. Endogenous RNA-polymerase activity in nuclei from excised marrow cotyledons treated with MeJA and ABA. Means are presented with S.E.

In order to eliminate the interference effect of endogenous intracellular pools of precursors of RNA biosynthesis we investigated further the *in vitro* activity of total endogenous RNA polymerases in nuclei isolated from excised marrow cotyledons after incubation with either MeJA or ABA. The results showed a close similarity between MeJA and ABA inhibitory action on total nuclear RNA polymerase activity and their effects on total RNA synthesis (Fig. 4). Therefore, the inhibition of *in vivo* RNA synthesis could be considered as a consequence of decreased activity of the endogenous nuclear RNA polymerases rather than a lowered rate of uptake of the radioactive precursor. In comparison to *in vivo* RNA synthesis, a shift (at 6th h) in the maximum of the RNA polymerase activity was registered not only after MeJA-treatment but also in the presence of ABA. This stimulation in the activity of endogenous RNA polymerases reflects the well-known positive effect of light on the process of transcription and indicates that light could mimic the inhibitory effect of ABA within the first 6 h of incubation. Furthermore, the shift of the maximum in RNA polymerase activity (6th h) compared with the maximum of *in vivo* RNA synthesis (12th h) can be accounted for by the increase in the elongation rate of transcription of nascent RNA chains which takes place in the nuclei and precedes the appearance of matured RNA-molecules in the cytoplasm as determined by the *in vivo* incorporation of [^{3}H]-uridine.

In conclusion, the comparative study of the effects of MeJA and ABA in excised cotyledons of *C. pepo* (*zucchini*) suggests a specific signalling function of MeJA resulting in a marked accumulation of a set of specific proteins presumably with stress related functions. In contrast to MeJA, the exogenous application of ABA does not significantly alter cotyledon gene expression as none of these MeJA-specific polypeptides were detected. Furthermore, the stronger inhibitory effect of ABA as compared to MeJA on protein and RNA synthesis indicates that it is rather involved in the down-regulation of the developmental processes in cotyledons during the earlier stages of germination.

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