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Methyl jasmonate is a more effective senescence-promoting factor in *Cucurbita pepo* (zucchini) cotyledons when compared with darkness at the early stage of senescence

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Summary

The effects of short-term darkening and methyl jasmonate (MeJA) on cotyledon senescence were studied 24 h after transfer of intact 7-day-old *Cucurbita pepo* (zucchini) seedlings to darkness or spraying with 100 μM MeJA. The jasmonate inhibitory effect on chlorophyll content and chloroplast transcriptional activity was stronger compared with darkness. Further, MeJA reduced the photosynthetic rate whereas darkness did not affect photosynthesis. Neither stress factor affected the photochemical quantum efficiency of photosystem II (PSII) estimated by the variable fluorescence (F_v)/maximal fluorescence (F_m) ratio, suggesting the existence of mechanisms protecting the functional activity of PSII at earlier stages of senescence, thus making this parameter more stable compared to others used to quantify senescence. Both stress factors caused a decrease in the content of physiologically active cytokinins, especially *trans*-zeatin (Z), with the jasmonate effect being much more pronounced when compared to darkness. Our results indicate that MeJA is a

Abbreviations: atpB, gene coding for the plastid ATP synthase beta subunit; CK, cytokinin; DHZ, dihydrozeatin; IP, N^6-(2-isopentenyl)adenine; iPR, N^6-(2-isopentenyl)adenine 9-riboside; JIPs, jasmonate-induced proteins; *lhb*Cl, gene coding for the type III light-harvesting chlorophyll-*a*/*b*-binding protein; LSU, the large subunit of Rubisco; MeJA, methyl jasmonate; *psaA* and *psaB*, genes coding for photosystem I P_{700} apoproteins A1 and A2, respectively; *rbcL*, gene coding for the large subunit of Rubisco; *rbcS*, gene coding for the small subunit of Rubisco; Rubisco, ribulose-1, 5-biphosphate carboxylase/oxygenase; SAGs, senescence-associated genes; Z, *trans*-zeatin; ZR, *trans*-zeatin 9-riboside.

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more potent inducer of senescence in zucchini cotyledons, at least within the relatively short period of the 24 h treatment. This is likely due to its stronger down-regulatory effect on the levels of physiologically active cytokinins.

Introduction

Leaf senescence is a highly regulated, ordered series of biochemical and physiological events comprising the final stage of leaf development (Noodén, 1988). During leaf senescence, cells undergo changes in metabolism and cellular structure. It is well known that the chloroplast is the first organelle that is broken down structurally during senescence. The process of chloroplast disassembly takes place in parallel with down-regulation of its functional activity (Smart, 1994; Gan, 2004). Chlorophyll degradation leads to a sharp decrease in leaf photosynthetic capacity. The anabolism of macromolecules such as proteins, lipids, and nucleic acids (DNA and RNA) is gradually replaced by their catabolism. In addition, the vast majority of genes that are expressed in young, photosynthetically active leaves and are related to photosynthesis and protein synthesis are down regulated at the onset of senescence, which is accompanied by the up-regulation of specific senescence-associated genes (SAGs) (Smart, 1994; Buchanan-Wollaston, 1997; Nam, 1997; Gan, 2004). Thus, senescence-specific molecular markers, e.g. *SAG12*, a gene encoding a cysteine proteinase in *Arabidopsis,* have been widely used to distinguish leaf senescence from other processes leading to cell death (Weaver et al., 1998; Pontier et al., 1999).

It is well known that cytokinins (CKs) are the major senescence-inhibiting hormones (Smart, 1994; Gan, 2004). The levels of some CKs in the leaf decrease with the progression of leaf senescence (van Staden et al., 1988; Gan and Amasino, 1996). Our previous results on endogenous CK levels during natural senescence of intact *Cucurbita pepo* (zucchini) cotyledons showed that the concentrations of the physiologically active CK bases, especially *trans*-zeatin, its riboside, nucleotide as well as *cis*-isomers of zeatin derivatives decreased between the 1st and 5th week of cultivation. In contrast, the levels of storage CK O-glucosides and physiologically inactive CK 7- and 9-glucosides increased with cotyledon senescence (Ananieva et al., 2004a). Further evidence confirming the regulatory role of CKs in leaf senescence is based on data showing that senescence can be delayed after exogenous application of CKs (van Staden et al., 1988; Noodén and Letham, 1993) or due to overproduction of CKs in transgenic plants transformed by the CK biosynthesis gene, isopentenyl transferase (*ipt*) (Smart et al., 1991; Gan and Amasino, 1995; Jordi et al., 2000).

Senescence is either genetically induced in an age-dependent manner or can be prematurely initiated in response to various sub-lethal environmental stresses caused by extreme temperatures, drought, shading, nutrient deficiency, pathogen infection, and wounding (Smart, 1994). Senescence can also be induced by different artificial methods such as detaching leaves, transfer of plants to darkness or application of senescence promoting plant growth substances. It has been shown previously that darkness can induce senescence in detached leaves (Biswal et al., 1983; Weaver et al., 1998) or intact leaves and cotyledons following transfer of whole plants to darkness (Biswal and Biswal, 1984; Oh et al., 1996; Weaver et al., 1998). Recently, evidence was presented that senescence is induced in individually darkened leaves, but delayed in whole darkened plants (Weaver and Amasino, 2001). It was suggested that the genes induced during both natural and whole plant dark-induced senescence may be primarily stress-responsive (Weaver et al., 1998; Weaver and Amasino, 2001). In contrast to true leaves, cotyledons do appear to senesce when whole seedlings are placed in darkness, thus implying differential regulation of senescence in true leaves and cotyledons (Weaver and Amasino, 2001). We have recently shown that similar to natural senescence, there was a progressive reduction in chlorophyll content accompanied by a decrease in physiologically active CKs and their nucleotides in *C. pepo* (zucchini) cotyledons following transfer of whole seedlings into darkness for 2 and 5 days (Ananieva et al., 2004a). In contrast with natural senescence, the storage CK O-glucosides decreased under dark conditions, suggesting differential metabolic regulation of endogenous CK levels during natural and dark-induced senescence of zucchini cotyledons.

Jasmonic acid (JA) and its derivative methyl jasmonate (MeJA) are powerful promoters of leaf senescence upon exogenous application (for reviews see Creelman and Mullet, 1997; Wasternack and Hause, 2002). The effectiveness of exogenous MeJA

in promoting leaf senescence has been shown in different plants, such as *Arabidopsis* wild-type and mutant leaves (Oh et al., 1996; Weaver et al., 1998; Woo et al., 2001) and barley leaf segments (Weidhase et al., 1987; Reinbothe et al., 1993a; Reinbothe et al., 1997). We have recently reported that the ability of MeJA to promote senescence in intact *C. pepo* (zucchini) cotyledons is at least partially due to down-regulation of endogenous CK levels and interconversions between their physiologically active and inactive forms (Ananieva et al., 2004b). Genes involved in JA biosynthesis were shown to be differentially up-regulated during leaf senescence of *Arabidopsis* resulting in a 4-fold increase in JA content in senescing leaves, thus supporting also a role of endogenous JA in *Arabidopsis* leaf senescence (He et al., 2002). In addition, the JA-induced leaf yellowing was accompanied by the expression of several senescence-associated genes including the senescence-specific molecular marker gene *SAG12*. On the other hand, evidence exists that transgenic potato plants expressing a flax allene oxide synthase gene (*AOS*) overproduce JA without visible symptoms of early senescence (Harms et al., 1995). Nevertheless, such data may not necessarily contradict the regulatory role of jasmonates in promoting senescence.

In this paper, we extend our earlier studies on the mechanisms of induced senescence in cotyledons by comparing the promoting effects of short-term darkening and MeJA treatment of intact 7-day-old *C. pepo* (zucchini) seedlings on cotyledon senescence. More specifically, we studied the ability of the two stress factors to promote some early symptoms of senescence in relation to their effects on endogenous CK levels.

Materials and methods

Plant material and experimental scheme

Seeds of *Cucurbita pepo* L. (zucchini), cv. Cocozelle, were germinated on moistened filter paper for 96 h in darkness at 28 °C. The 4-day-old etiolated seedlings with a hypocotyl length of 4–6 cm were grown further on a nutrient solution (Yamagishi and Yamamoto, 1994) in a growth chamber at a photon flux density of 100 μmol m^{-2} s^{-1}, 26 $\pm$ 2 °C, relative humidity 70% and a 12 h day/night photoperiod. Seven-day-old seedlings grown in the light for three days were either transferred to darkness or sprayed with an aqueous solution of MeJA applied at a concentration of 100 μM. All measurements were done 24 h after treatment.

Chlorophyll determination

Chlorophyll was extracted in *N*, *N*-dimethylformamide as described by Moran and Porath (1980). Chlorophyll content was analyzed using a Cary 50 Conc UV-Visible spectrophotometer (Varian Pty Ltd, Victoria, Australia) and calculations were based on the extinction coefficients proposed by Inskeep and Bloom (1985). Each value represents the mean of three different experiments.

Net photosynthesis

Net photosynthetic rate was measured with a portable photosynthesis apparatus (LI-6400, Li Cor, Lincoln, NE, USA) at quantum flux density of 500 μmol m^{-2} s^{-1} PAR. Data were statistically processed using Systat 7.0. Each value represents the mean of three different experiments.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence emission from the adaxial side of the leaf was measured with a pulse amplitude modulation fluorometer (PAM 101-103, Walz, Effeltrich, Germany). The initial fluorescence yield (F_0) was excited by weak red modulated light (0.075 μmol m^{-2} s^{-1} PPFD), and the maximum total fluorescence yield (F_m) induced by a saturating white light pulse (1 s, over 3500 μmol m^{-2} s^{-1} PPFD, by Schott KL 1500 light source), were determined. Each value represents the mean of three different experiments.

Chloroplast isolation and run-on transcription assay

Intact chloroplasts were isolated from zucchini cotyledons using the discontinous gradient of Percoll (Amersham BiosciencesAB). Briefly, cotyledons were homogenized with grinding buffer containing 50 mM Hepes-KOH (pH 6.8), 0.33 M sorbitol, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 1.7 mM $Na_4P_2O_7$, 2 mM dithiothreitol (DTT) and 0.2 mM EDTA (Deng and Gruissem, 1995). The homogenate was filtered through two layers of Miracloth and centrifuged at 6000*g* for 30 s at 4 °C. The pellet containing chloroplasts was resuspended in grinding buffer and suspension was layered on a 40–80% Percoll gradient made after dissolving PCBF solution containing 100% Percoll, 3% polyethylene glycol (Mw 6000, w/v), 1% BSA (w/v) and 1% Ficoll (w/v). After centrifugation (6000*g*, 20 min, 4 °C) plastids banded on the 80% Percoll layer were collected, washed twice in the grinding buffer and resuspended in a buffer contain-

ing 50 mM Hepes-KOH (pH 8.0) and 0.33 M sorbitol. The intactness of the chloroplasts was verified by phase contrast microscopy (Axiovert 25, Carl Zeiss Iena, Germany) and the chloroplast number was determined using a hemocytometer according to Mullet and Klein (1987).

Run-on transcription was measured as described by Mullet and Klein (1987). All reactions were performed in a total volume of 25 μL containing 50 mM Hepes-KOH (pH 8.0), 25 mM potassium acetate, 10 mM dithiothreitol, 10 mM $MgCl_2$, 125 μM each of unlabeled ATP, GTP and CTP, 10 μM unlabeled UTP and 10 μCi [^{3}H] UTP (specific activity 43 Ci mmol^{-1}, Amersham) and 2.6×10^7 plastids. Transcription was carried out at 23 °C for 10 min and the reaction was stopped by spotting the reaction mixtures onto 50 μM EDTA-presoaked DE-81 Whatman paper. The filters were extensively washed in 5% Na_2HPO_4, followed by distilled water, 96% ethanol and finally in diethyl ether according to Hallick et al. (1976). Filters were air-dried and counted using a liquid scintillation counter (Beckman LS 1801, Irvine, USA). Each value represents the mean of three different experiments.

CK analysis

Cytokinins were extracted and purified using dual mode solid phase extraction (Dobrev and Kamínek, 2002) and determined by HPLC-MS as described elsewhere (Ananieva et al., 2004a). Briefly, frozen cotyledons (1.5 g) were homogenized and extracted overnight in cold methanol:water:formic acid (15:4:1,v/v/v). Following centrifugation (15 000 g, 4 °C, 20 min) parts of lipids were removed from the supernatant by filtration through Sep-Pack †C_{18} cartridge. After evaporation to near dryness, the residue was dissolved in 5 mL 1 M formic acid and applied to an Oasis MCX column (150 mg reverse-phase cation-exchange sorbent). After washing the column with 5 ml of formic acid and 5 mL of methanol CK nucleotides were eluted with 5 mL 0.35 M NH_4OH. CK bases, ribosides, and glucosides were eluted with 5 mL 0.35 M NH_4OH in 60% (v/v) methanol. The eluate containing CK bases, ribosides and glucosides was evaporated to dryness using a Speed-Vac. The eluate containing CK nucleotides was evaporated in the same way to 2–3 mL to remove the ammonia. The CK nucleotides were dephosphorylated to nucleosides by incubation with calf-intestine alkaline phosphatase (Sigma).

The CKs were quantified by HPLC linked to an Ion Trap Mass Spectrometer Finnigan LCQ-equipped with an electrospray interface using a RP column (Phenomenex, AQUA, 2 × 250 mm). Linear gradient of acetonitrile (B) in 0.0005%, v/v, acetic acid in water (A): 10% B for 5 min, to 17% B in 15 min and to 50% B in 35 min was used at a flow rate of 0.2 mL min^{-1}. Detection and quantification were carried out using a Finnigan LCQ operated in the positive ion, full-scan MS/Ms mode using a multi-level calibration graph with [^{2}H] labeled CKs as internal standards. Each analysis was repeated twice. The results presented here are the mean values of two different experiments.

Results

Chlorophyll content, net photosynthetic rate and photochemical quantum efficiency of photosystem II

To investigate the potential roles of darkness and MeJA in cotyledon senescence, 7-day-old zucchini seedlings grown in the light for 3 days were either transferred to darkness or sprayed with 100 μM MeJA. At this stage, the seedlings had only cotyledons and the primary leaves had just emerged. The progress of senescence acceleration was monitored by the loss of chlorophyll and inhibition of both photosynthetic activity and PSII functional activity. Although no visible yellowing of cotyledons was observed 24 h after treatment, total chlorophyll content was decreased following dark and MeJA treatments by 11% and 17%, respectively (Fig. 1). On the other hand, net photosynthetic rate remained unchanged after 24 h dark treatment, whereas it was lowered by 27% upon MeJA treatment (Fig. 2). Changes in the F_v/F_m ratio ($F_v = F_m - F_0$) indicate variations in the photochemical quantum efficiency of PSII (Maxwell and Johnson, 2000). Our results showed that neither of the senescence promoting factors investigated induced any changes in PSII functional activity (Fig. 3).

Chloroplast run-on transcription

The senescence promoting effects of both short-term darkening and MeJA treatment were further studied through the changes in total chloroplast RNA synthesis using intact isolated plastids placed into a hypotonic transcription reaction mixture which caused plastids to lyse (Mullet and Klein, 1987).

Our results from the run-on transcription analyses showed that dark treatment decreased total chloroplast RNA synthesis by 23% whereas MeJA led to a 55% inhibition (Fig. 4). Experiments with

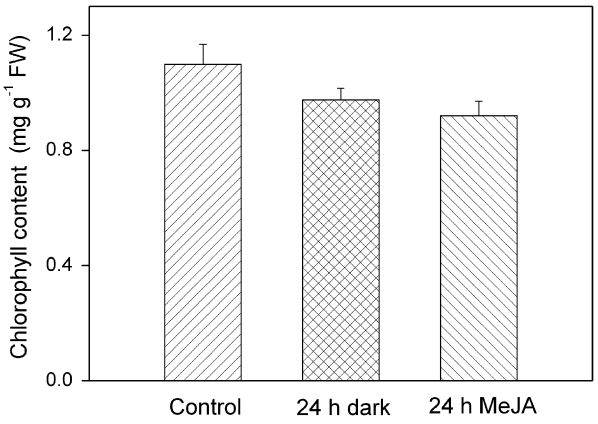

Figure 1. Chlorophyll content in *Cucurbita pepo* (zucchini) cotyledons. Seven-day-old seedlings grown at a 12 h dark/light photoperiod were either transferred to darkness or sprayed with 100 μM MeJA. Measurements were performed 24 h after treatment. Vertical bars indicate SE. FW, fresh weight.

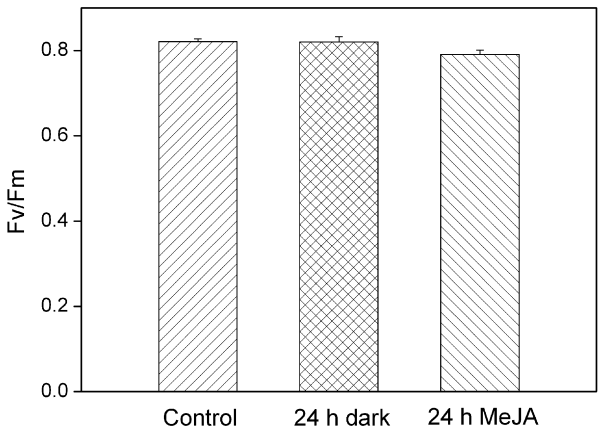

Figure 3. Changes in the maximum quantum efficiency of PSII (variable fluorescence (F_v)/maximal fluorescence (F_m) ratio) in *Cucurbita pepo* (zucchini) cotyledons of 7-day-old seedlings after dark and MeJA treatments. All measurements were performed 24 h after treatment. Vertical bars indicate SE.

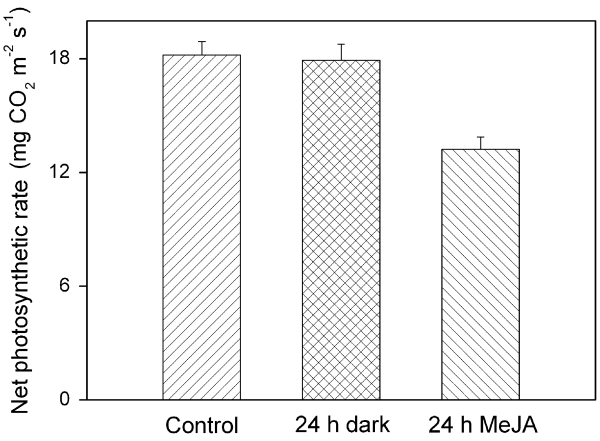

Figure 2. Net photosynthetic rate in *Cucurbita pepo* (zucchini) cotyledons. Seven-day-old seedlings grown at a 12 h dark/light photoperiod were either transferred to darkness or sprayed with 100 μM MeJA. Measurements were done 24 h after treatment. Net photosynthetic rate was measured with a portable photosynthesis apparatus LI-6400 at quantum flux density of 500 $\mu mol\, m^{-2}\, s^{-1}$ PAR. Vertical bars indicate SE.

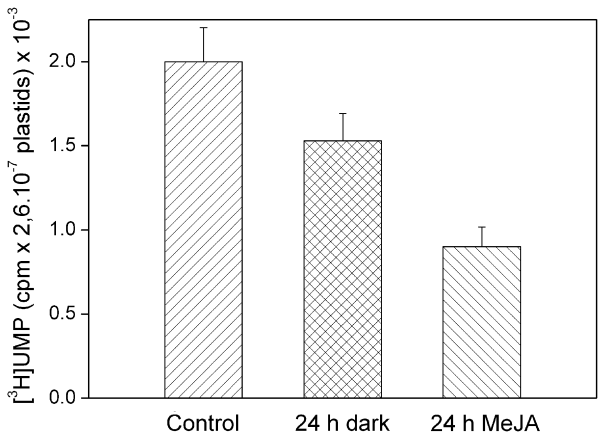

Figure 4. Transcriptional activity in chloroplasts isolated from *Cucurbita pepo* (zucchini) cotyledons. Seven-day-old seedlings grown at a 12 h dark/light photoperiod were either transferred to darkness or sprayed with 100 μM MeJA. Chloroplasts were isolated 24 h after treatment as described in Materials and Methods. The amount of incorporated [^{3}H] UTP in a standard run-on assay with lysed plastids was determined using 2.6×10^7 chloroplasts and 10 min incubation time. Vertical bars indicate SE.

excised cotyledons floated for 24 h on a MeJA solution showed a much more pronounced inhibition of chloroplast RNA synthesis (70%) (data not shown).

Analysis of physiologically active CKs

Using HPLC/MS/MS, we determined the endogenous levels of physiologically active CKs [*trans*-zeatin (Z), dihydrozeatin (DHZ), N^6-(2-isopentenyl)adenine (iP), *trans*-zeatin 9-riboside (ZR) and N^6-(2-isopentenyl) adenine 9-riboside (iPR)] as well as of much less active *cis*-zeatin (*cis*-Z) and *cis*-zeatin 9-riboside (*cis*-ZR), (Kamínek et al., 1979) (Table 1). The content of total *trans*-zeatins (Z and ZR) decreased after transfer to darkness and MeJA treatment by almost 30% and 80%, respectively. Furthermore, there was a decrease in the total content of physiologically active CKs upon dark and

Table 1. Endogenous levels of physiologically active CKs and *cis*-zeatins (pmol g^{-1} FW) in cotyledons of intact *Cucurbita pepo* (zucchini) seedlings grown under 12 h-photoperiod

Cytokinin	8-day-old control	1 d after transfer to darkness	1 d after MeJA treatment
iP	1.2	0.9	0.8
iPR	0.5	ND	0.5
Z	2.9	1.7	0.7
ZR	0.4	0.9	ND
DHZ	0.7	0.8	0.7
cis-Z	0.5	1.6	ND
cis-ZR	1.9	1.9	0.9
Total *trans*-zeatins	3.3	2.6	0.7
Total *cis*-zeatins	2.4	3.5	0.9
Trans:cis-zeatins	1.4	0.7	0.8

CKs were determined in cotyledons of 8-d untreated plants (controls) and 1 d after spraying of 7-day-old plants with MeJA (100 μM) or transfer of 7-day-old plants to darkness. CK contents were determined using HPLC/MS/MS as described in Materials and methods. The standard errors (SE) varied within the range of 23–37%. ND, not detected.

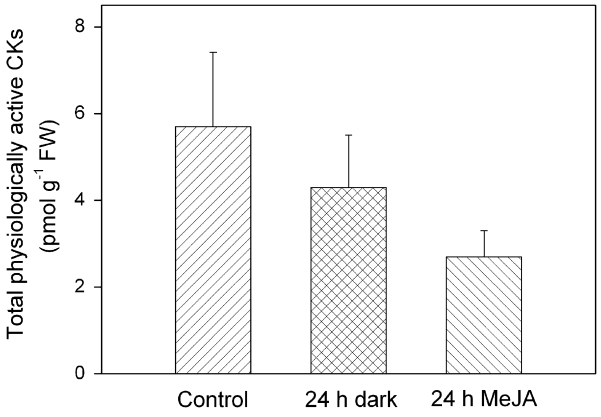

Figure 5. Changes in the content of total physiologically active cytokinins (pmol g^{-1} FW) in *Cucurbita pepo* (zucchini) cotyledons of 7-day-old seedlings after dark and MeJA treatments. All measurements were done 24 h after treatment. Cytokinins were determined using HPLC/MS/MS. Vertical bars indicate SE.

MeJA treatments by 25% and 53%, respectively (Fig. 5). This was due mainly to the significant decrease in the content of Z (1.7- and 4-fold decrease following dark and MeJA treatments, respectively). Total *cis*-zeatins increased after dark treatment, whereas their content decreased due to MeJA application. However, total *cis*-zeatins (*cis*-Z and *cis*-ZR) remained at a higher level (30% higher) compared with *trans*-zeatins upon both treatments. Thus, both stress factors lowered the *trans:cis* zeatins ratio by approximately 50% compared with controls (Table 1). A similar trend of decrease in the *trans:cis* zeatins ratio was observed following incubation of whole zucchini seedlings in the dark for 2 and 5 days (Ananieva et al. 2004a).

Discussion

Photosynthetic parameters such as chlorophyll content, photosynthetic rate and the F_v/F_m ratio are typical senescence-associated physiological markers (Smart, 1994; Oh et al., 1996; Woo et al., 2001; Gan, 2004). Many studies have shown that leaf senescence correlates with a progressive decline in the rate of photosynthesis paralleled by a decrease in Rubisco activity and content (Crafts-Brander et al., 1990; Jiang et al., 1993). The F_v/F_m ratio in fully expanded leaves was found to decrease with progression of leaf senescence (Gan, 2004). On the other hand, no changes were registered in the photochemical efficiency of PSII after 1-d dark treatment of detached leaves of stay-green *Arabidopsis* mutants, which was followed by a rapid drop observed thereafter till the 5th day of dark incubation (Oh et al., 2003). In the wild type leaves, this decrease did not begin even after 2-d dark treatment. In addition, no changes in the F_v/F_m ratio were observed in young sunflower leaves during senescence induced by moderate KCl stress (Vieira Santos et al., 2001). Our results showing lack of effect of 24 h dark treatment on the F_v/F_m ratio in zucchini cotyledons (Fig. 3) are in agreement with the above studies, suggesting that short-term dark treatment is not sufficient to inhibit significantly the functional activity of PSII. Further, darkness did not affect the photosynthetic rate of cotyledons (Fig. 2). Thus, the above two parameters used in our study to quantify senescence could be considered as more stable compared with chlorophyll content, which was found to decrease already after 24 h dark treatment of 7-day-old zucchini cotyledons (Fig. 1).

The well-known ability of jasmonates to promote or enhance senescence upon exogenous application is often characterized by loss of chlorophyll, degradation of Rubisco and inhibition of its biosynthesis, decreased rates of transpiration and photosynthesis, up-regulation of several SAGs shown for different plants (Weidhase et al., 1987; Beltrano et al., 1998; Oh et al., 1996; Weaver et al., 1998; He et al., 2001, 2002). In addition, jasmonates can induce disorganization of the thylakoid membrane system of chloroplasts (Ananieva et al., 2004b). Jasmonates can also affect the functional activity of PSII (Woo et al., 2001; He et al., 2002). Consistent with the visible yellowing, the F_v/F_m ratio was shown to decrease markedly in detached young *Arabidopsis* leaves treated with jasmonic acid (JA) for 4 days under darkness (He et al., 2002). In the present work, we found that spraying of intact 7-day-old zucchini cotyledons with MeJA did not affect the F_v/F_m ratio measured 24 h after treatment (Fig. 3). However, photosynthetic rate was found to decrease significantly (Fig. 2). These results indicate that photosynthetic capacity is more susceptible to the MeJA action compared with the functional activity of PSII, thus suggesting a stronger inhibitory effect of jasmonates on Calvin cycle compared with the photochemical electron transport reactions of photosynthesis. Recently, we have shown that similar to the F_v/F_m ratio, the actual quantum yield of PSII electron transport in the light-adapted state ($\Phi PSII = (F_{m'}-F)/F_{m'}$) remained also unchanged after both dark and MeJA treatments (Ananieva et al., 2005). Furthermore, no effect was observed in the above parameters even after more acute MeJA treatment when zucchini cotyledons excised from 7-day-old seedlings were incubated for another 24 h on MeJA solution (data not shown). The lack of effect of both stress factors on the photochemical efficiency of PSII although chlorophyll content was found to decrease implies the existence of mechanisms protecting the functional activity of PSII at least within the 24 h period of treatment. A study of the senescence processes in attached first foliage leaves of barley has revealed that, in spite of the early decrease in total protein and chlorophyll content, efficiency of remaining photosystem II units stays high for about 3 weeks, followed by a rapid decrease, reaching values close to zero 2 weeks later (Miersch et al., 2000). Therefore, the decreases in chlorophyll content and the functional activity of PSII do not occur simultaneously during senescence which is in agreement with our data. Moreover, our results refer to the relatively short period of 24 h treatment and most probably, the expected decrease in PSII efficiency could occur at later stages of the senescence process. Thus, the photochemical efficiency of PSII could be considered as a more stable parameter when compared to others used to quantify senescence.

A study of the responses of the chloroplast transcriptional apparatus in barley primary leaves to 2-d dark incubation has revealed a dramatic decline in the overall chloroplast transcription due mainly to reduced transcript accumulation of a definite group of genes related to photosynthesis (*rbcL*, *psaA*,*B*, *atpB*) (Krause et al., 1998). Consistent with the above study our results showed that as in leaves, a decline in chloroplast overall transcriptional activity (23%) was observed also in cotyledons of intact zucchini seedlings already after a 24 h dark period (Fig. 4). Much stronger was the decline in chloroplast transcription sdetected in the MeJA-treated cotyledons (55%) (Fig. 4). Furthermore, our experiments with excised cotyledons showing even more drastic inhibition of chloroplast RNA synthesis (70%) (data not shown) indicate that chloroplast transcription is a more sensitive senescence-related parameter compared with those related to photosynthesis used in this study to quantify senescence acceleration in response to MeJA treatment. This strong inhibitory effect on chloroplast transcription could be due to the selective inhibition of a definite group of mRNAs related to photosynthesis, thus leading to the decreased photosynthetic rate registered already 24 h after MeJA treatment (Fig. 2). The suppression of photosynthesis in the MeJA-treated cotyledons could be also due to the rapid degradation of Rubisco reported for barley leaf segments (Reinbothe et al., 1993a), as well as to the down-regulation of genes for nuclear- and plastid-encoded chloroplast photosynthetic proteins (e.g. *rbc*S, *lhbCl*), thus contributing to the senescence-promoting effect of jasmonates (Reinbothe et al., 1993a, b, 1997).

The inverse correlation between CK levels and the progression of senescence shown in a variety of tissues and plant species represents one of the major lines of evidence supporting an inhibitory role of CKs in leaf senescence (Gan, 2004). Both senescence-promoting factors used in the present study caused a decrease in the content of physiologically active CKs, especially *trans*-Z, the jasmonate effect being much more pronounced when compared with darkness (Table 1, Fig. 5). Thus, based on the results on chlorophyll content changes, net photosynthetic rate and chloroplast transcriptional activity, a conclusion could be drawn that MeJA is a more effective inducer of cotyledon senescence compared with darkness

within the relatively short period of 24 h treatment. The higher capacity of MeJA to promote cotyledon senescence could be partly due to the stronger reduction in the levels of physiologically active CKs, especially *trans*-Z.

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