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METHYL JASMONATE INHIBITS ANTHOCYANIN SYNTHESIS IN SEEDLINGS OF COMMON BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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Exogenously applied jasmonic acid methyl ester (JA-Me) inhibited biosynthesis and accumulation of anthocyanins in hypocotyls of seedlings of etiolated common buckwheat (*Fagopyrum esculentum* Moench) exposed to light. The phenomenon was observed in experiments with various methods of JA-Me treatment, in whole seedlings and in excised hypocotyls. Even very low quantities of JA-Me taken by seeds during imbibition were enough to inhibit anthocyanin synthesis in buckwheat hypocotyls. This means that there are no significant barriers to the transport and action of JA-Me in buckwheat seedlings, as solute and in gaseous form. Although JA-Me inhibited accumulation of anthocyanins in buckwheat hypocotyls, it had no effect on phenylalanine and tyrosine ammonia-lyase activity. Such JA-Me action suggests that it can act not in the first but in later steps of anthocyanin biosynthesis. JA-Me had no effect on the level of anthocyanins in cotyledons or on hypocotyl growth, but clearly inhibited the growth of main roots of buckwheat seedlings.

Key words: Anthocyanins, *Fagopyrum esculentum*, common buckwheat, hypocotyls, cotyledons, methyl jasmonate.

INTRODUCTION

Anthocyanins are one of the most widespread classes of pigments in higher plants. They are important secondary metabolites produced through the flavonoid biosynthetic pathway in various plant organs (Winkel-Shirley, 2001). The anthocyanin biosynthetic pathway is controlled by environmental factors (light and temperature) and internal factors: plant hormones, other secondary metabolites and nutrients (Mol et al., 1996). Light acts as an essential stimulus and as a factor modulating the intensity of the pigment by affecting the regulatory and structural genes of anthocyanin biosynthesis. Although photoinduction of anthocyanin has been extensively studied in a variety of plants, the photoreceptors responsible have not been clearly defined (Sheoran et al., 2006).

Anthocyanin synthesis involves many steps, from the primary precursor (phenylalanine) to the final products – glycosides of anthocyanidines. Phenylalanine ammonia-lyase (PAL) is the first enzyme to catalyze the elimination of NH_3 from L-phenylalanine to give *trans*-cinnamate (Hanson and Havir, 1981). *Trans*-cinnamate can be a precursor of other secondary plant metabolites besides anthocyanins: phenolic acids, flavonols, lignins, proanthocyanidines, stilbenes, etc.

PAL activity has been reported to positively correlate with anthocyanin synthesis in grapes (Kataoka et al., 1983), strawberries (Given et al., 1988) and apples (Tan, 1979), but its role in regulating anthocyanin formation remains unclear. According to Wang et al. (2000), PAL is not the only factor regulating anthocyanin accumulation in apple fruit; they found that anthocyanin accumulation decreased when apples ripened, even though PAL activity was relatively high.

Anthocyanins play an important role in attracting insects or animals for pollination and seed dispersal. They also play a role as anti-oxidants and in protecting DNA and the photosynthetic apparatus from high radiation fluxes (Gould, 2004). Other possible functions of anthocyanins, such as protecting against cold stress or providing drought resistance, are likely to be associated with activities restricted to particular classes of plants (Chalker-Scott, 1999).

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Plant hormones such as auxins, gibberellins (GAs), cytokinins and abscisic acid (ABA) have also been implicated in anthocyanin biosynthesis and accumulation. In *Petunia* petals, for example, GAs promote anthocyanin accumulation and the expression of the anthocyanin biosynthetic chalcone synthase gene, whereas ABA reverses these processes (Weiss et al., 1995). In contrast, ABA stimulates anthocyanin production in maize kernels (Walbot et al., 1994) and in grape berries by affecting the expression of anthocyanin biosynthetic genes (Jeong et al., 2004).

Methyl jasmonate (JA-Me) vapor induced anthocyanin biosynthesis in light-grown soybean seedlings but inhibited anthocyanin accumulation in etiolated seedlings (Franceschi and Grimes, 1991); in that work, two distinct actions of JA-Me on anthocyanin biosynthesis were suggested: one in the light and another in the dark. Gaseous JA-Me may function as either an interplant messenger or a growth regulator (Farmer and Ryan, 1990). Anthocyanin accumulation in response to methyl jasmonate or jasmonic acid (JA) was greater in cooled than in uncooled tulip bulbs (Saniewski et al., 1998). Exogenously applied JA induced anthocyanin accumulation in various plants (Franceschi and Grimes, 1991; Tamari et al., 1995; Saniewski et al., 2003; Saniewski et al., 2006). Adding jasmonic acid to culture medium of sour cherry stimulated cyanidin 3-glucoside synthesis (Blando et al., 2005). The effect of JA could be reproduced by wounding (Tamari et al., 1995). In at least some host-pathogen interactions, anthocyanin induction may proceed via the jasmonate-wounding pathway (Feys et al., 1994).

Jasmonic acid is known as a wound-signal molecule. In *Arabidopsis thaliana* the level of JA in leaves increased more than 20-fold by 1 h after wounding (McConn et al., 1997). JA inhibits elicitor-induced PAL; thus it may function as an endogenous suppressor of the defense response (Ishiga et al., 2002). According to recently published data, exogenously applied methyl jasmonate is transported through phloem and xylem pathways (Thorpe et al., 2007). Through cell membranes JA-Me is probably transported by the same or a similar carrier as sucrose, due to enhancement of the energy of the plasma membrane (Thorpe et al., 2007).

Common buckwheat (*Fagopyrum esculentum* Moench) is a fast-growing dicotyledonous plant. Buckwheat seeds contain starch, high quality protein, and flavonoids – mainly rutin. The high level of rutin in buckwheat leaves and stem is accompanied by another member of the flavonoid family – anthocyanins, one of the final products of flavonoid biosynthetic pathways. They are accumulated in buckwheat mainly in stems, and in leaves where they are masked by chlorophylls. Among the anthocyanins present in buckwheat tissue are cyanidin and its glycosides (Troyer, 1958; 1964). Recently, four anthocyanins (cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, cyanidin 3-O-galactoside, cyanidin 3-O-galactopyranosyl-rhamnoside) were isolated from sprouts of common buckwheat (Kim et al., 2007). Among the studied Japanese cultivars, only one (Hokkai T10) accumulated small amounts of cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside in dark-grown sprouts, whereas other varieties and breeding lines accumulated traces of anthocyanins. Light exposure of dark-grown buckwheat seedlings caused quick anthocyanin synthesis. This formation of pigment is not accompanied by photosynthetic processes (Troyer, 1964). According to recently published data, in terms of quantity the major anthocyanin compound in buckwheat sprouts is cyanidin 3-O-rutinoside (Watanabe, 2007).

Hypocotyls provide a convenient model system for investigation of the role of anthocyanin because of their inherently low capacity to utilize light, due to their low level or lack of chlorophyll (Troyer, 1964; Sheroan et al., 2006; Kim et al., 2007). Seedlings typically attain maximum pigmentation a few days after germination or sprouting, so the experiments are easily repeated.

This study assesses the effect of methyl jasmonate applied in gaseous form and as water solutions on anthocyanin accumulation and on the activity of basic enzymes involved in anthocyanin biosynthesis in buckwheat seedlings exposed to light: phenyl ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL).

MATERIALS AND METHODS

PLANT MATERIAL

Seedlings of common buckwheat (*Fagopyrum esculentum* Moench) cv. Hruszowska were used in this study. Germination was carried out by placing buckwheat seeds between two layers of wet filter paper (20 × 10 cm) which were then rolled up and inserted in a 2 l beaker containing ~200 ml tap water. Ten seeds were germinated in each roll. Germination was carried out in darkness at 24±1°C. During standardization of the protocol, anthocyanin accumulation was studied in buckwheat seedlings germinated 4 days in darkness and then exposed to artificial light for 0, 1, 2 and 3 days. Seedlings were grown with a 16 h photoperiod and 65±5% relative humidity. Chamber temperature was maintained at 24±2°C/16±2°C (day/night). Light (100 μmol $m^{-2} \cdot s^{-1}$) was provided by cool white and daylight fluorescent tubes in equal numbers.

EXPERIMENTAL PROCEDURES FOR JA-ME TREATMENT

(1) After four days, buckwheat seedlings were taken for experiments with JA-Me. In beakers with seedlings the water was replaced with water solutions of methyl jasmonate (JA-Me) at 10^{-8}, 10^{-6} or 10^{-4} M concentration. In the control sample the water was freshened. Because the JA-Me was added in a small volume (0.4 cm^3) of ethyl alcohol, an additional control with the same amount of ethyl alcohol was prepared as well. After 8 h pre-incubation in darkness, the seedlings in beakers were exposed to light under a 16 h photoperiod for 3 days, with temperature and humidity as described above.

(2) Four-day-old buckwheat seedlings were treated with methyl jasmonate vapor in a 0.9 l jar. Filter paper containing JA-Me was placed against the inner wall of the jar containing the rolls with buckwheat seedlings, and the jar was immediately closed tightly. The ethyl alcohol used for prepare JA-Me solutions was evaporated at ambient temperature for 5 min before the filter paper was put in the jar. A preliminary experiment showed that the loss of JA-Me during that time is negligible (data not shown). The amounts of atmospheric JA-Me in these treatments were calculated as 10^{-6}, 10^{-5} or 10^{-4} M per l (assuming its complete evaporation). After 8 h pre-incubation in darkness, the seedlings were exposed to light for 3 days as described above.

(3) The buckwheat seedlings were treated with water solutions of JA-Me (10^{-8}, 10^{-6} or 10^{-4} M) after excision of their roots. Because JA-Me added to the water was in a small volume (0.4 cm^3) of ethyl alcohol, an additional control with the same amount of ethyl alcohol was prepared as well. After 8 h pre-incubation in darkness, the seedlings were exposed to light for 3 days as described above.

(4) Buckwheat seedlings were exposed to JA-Me vapor (10^{-6}, 10^{-5} or 10^{-4} M) as described above after excision of their roots. After 8 h pre-incubation in darkness, the seedlings were exposed to light for 3 days as described above (1–3).

(5) Seeds of buckwheat were imbibed for 24 h with water solutions of JA-Me (10^{-8}, 10^{-6}, 10^{-4} or 10^{-2} M), germinated for 3 days in darkness as described earlier, and exposed to light to light for 3 days as described above (1–4).

DETERMINATION OF ANTHOCYANINS

Anthocyanin was extracted and measured as described by Mancinelli (1984). Eight to ten seedlings per replicate were taken for analysis. Hypocotyl and cotyledon tissues (2–3 mm hand-cut pieces, 100–200 mg) were extracted separately with acidified (1% HCl, w/v) methanol for 24 h at room temperature in darkness with occasional shaking. The extracts were carefully decanted and their absorbance was measured at 530 nm (peak absorption of anthocyanin) and 657 nm (peak absorption of chlorophyll degradation products). The formula A530 – 0.25A657 was used to compensate for the absorption of chlorophyll degradation products at 530 nm. Anthocyanin content was calculated as cyanidin-3-glucoside using 29,600 as molecular extinction coefficient and 445 as molecular weight. Cyanidin-3-glucoside is one of the main anthocyanins of buckwheat tissue (Kim et al., 2007). Three or four independent replicates were analyzed, for hypocotyls and cotyledons separately.

DETERMINATION OF ENZYME ACTIVITY

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was determined by monitoring *trans*-cinnamic acid (*trans*-CA) formation at 290 nm (ε = 9,300 $M^{-1}cm^{-1}$) in 1 cm quartz cuvettes. Tyrosine ammonia-lyase (TAL) activity was determined by detecting 4-hydroxycoumaric acid (p-HCA) at 315 nm (ε = 9,500 $M^{-1}cm^{-1}$). Due to possible hydroxylation of *trans*-CA to p-HCA by cinnamate 4-hydroxylase, besides the extinction at 290 nm (for *trans*-cinnamic acid), additionally the extinction values at 315 nm were measured (to check the level of 4-hydroxycoumaric acid) in the reaction mixture for determination of PAL activity.

Hypocotyl tissues (1 g fresh weight) were homogenized in 4 cm^3 ice-cold 100 mM Tris-HCl buffer (pH 8.8) + 2 mM mercaptoethanol with a mortar and pestle and centrifuged at 16,000 g for 10 min at 2°C. After centrifugation the pellet was discarded and the supernatant (crude enzyme extract) was used for PAL and TAL assays. All assays were carried out by 2 h incubation of 1.0 ml enzyme extract and 0.5 ml L-Phe or L-Tre in a final volume of 3 ml with 100 mM Tris-HCl buffer, at 35°C. PAL activity in buckwheat seedling hypocotyls is expressed as the sum of *trans*-CA and p-HCA (nmoles) produced during 1 h incubation by 1 g fresh plant tissue. TAL activity is expressed as nmoles of p-HCA formed during 1 h incubation per g fresh plant tissue. Analyses were carried out in four independent replicates.

STATISTICS

The statistical significance of the differences between results was evaluated with the Student *t*-test. The criterion of significance is taken as $p \leq 0.05$.

RESULTS AND DISCUSSION

Figure 1 (Experiment 1) shows anthocyanin (ACN) accumulation in buckwheat hypocotyls and cotyle-

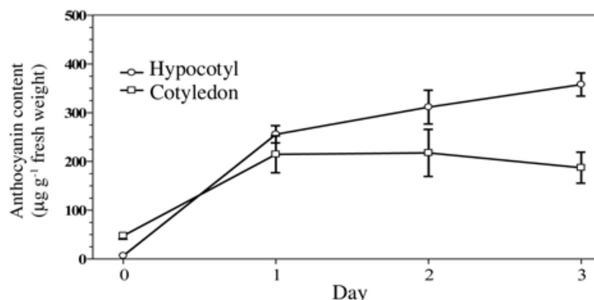

Fig 1. Accumulation of anthocyanins in hypocotyls and cotyledons of etiolated buckwheat seedlings during 3-day exposure to a day/night regime. Results are means ±SD.

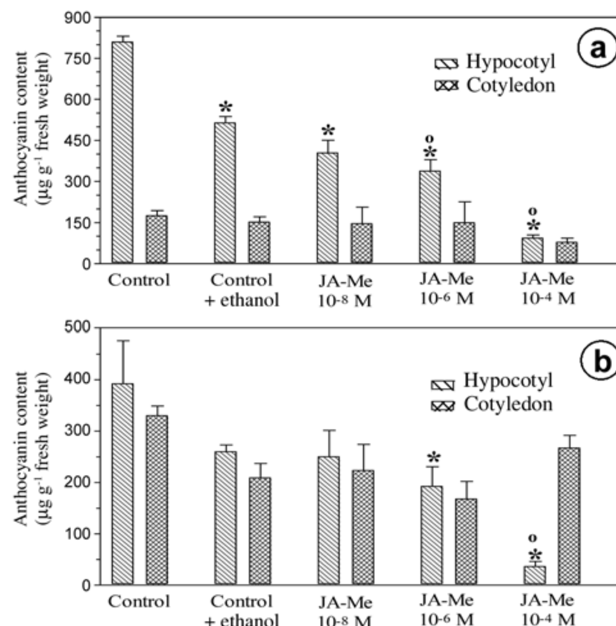

Fig. 2. Effect of concentration of jasmonic acid methyl ester (JA-Me) in solution on anthocyanin accumulation in hypocotyls and cotyledons of etiolated buckwheat seedlings exposed for 3 days to a day/night regime. (**a**) Whole seedlings, (**b**) Excised upper part (hypocotyl + cotyledon). Results are means ±SD. Means marked (*) differ significantly from control; Means marked (o) differ significantly from control containing ethyl alcohol; Student's *t*-test ($p \leq 0.05$).

dons during 3 days of growth with a 16 h photoperiod. After completing etiolation, the buckwheat seedlings contained very low levels of anthocyanins. ACN was quickly synthesized in buckwheat cotyledons and hypocotyls already after the first 16 h of light exposure. During the next 2 days, further accumulation of ANC was observed in hypocotyls but not cotyledons (Fig. 1). After 3 days, ANC content reached 360 $\mu g \cdot g^{-1}$ fresh weight in buckwheat hypocotyls and 190 $\mu g \cdot g^{-1}$ in cotyledons.

Watanabe (2007) recently described differential accumulation of ANC in buckwheat cotyledons and hypocotyls: cotyledons of commercially obtained buckwheat sprouts from three different sources contained 3 to 15 times less ANC than hypocotyls, and ANC content in the sprouts increased with light 72 h after 2-day germination in dark. Those results are in accord with our data shown in Figure 1.

Generally, induction of anthocyanin biosynthesis requires light, and ANC content in plant tissues varies with light exposure duration and levels (Rabino and Mancinelli, 1986; Mol et al., 1996; Chalker-Scott, 1999). Light influences anthocyanin accumulation primarily through activation of the transcription factors that regulate the flavonoid biosynthetic pathway, although some plant tissues have a mechanism for light-independent synthesis of ANC (Irani and Grotewold, 2005).

Lower ANC in green cotyledons than in hypocotyls is probably due to suppression of ANC synthesis as chlorophyll starts to accumulate (Steyn et al., 2002). The prolonged presence of anthocyanins is usually restricted to tissues in which photosynthesis is not a primary function: petioles, stems, and lower layers of shade leaves. It is suggested that anthocyanin accumulation is part of the general plant response to light stress (Steyn et al., 2002).

Another suggestion is that response to light can be indirect, because enhanced sugar supply increases the level of anthocyanin, as found in *Petunia* plants (Weiss et al., 1995) and carob (*Ceratonia siliqua* L.) (Vinterhalter et al., 2007). The idea is that the higher level of sugars – the first effect of photosynthesis (and light) – could be the main reason for increased ANC in some plants exposed to light.

Figure 2 summarizes the results of experiments on treatment of whole buckwheat seedlings and excised hypocotyls with JA-Me in water solution. Increased JA-Me concentration inhibited synthesis and accumulation of anthocyanins in buckwheat hypocotyls. Anthocyanin levels also declined in control samples containing 0.4 cm^3 ethyl alcohol (EtOH control) in 200 cm^3 water. According to Mancinelli et al. (1975), ethanol and propanol were inhibitors of anthocyanin biosynthesis in cabbage seedlings at concentrations higher than 0.1%. In our control the concentration of alcohol was 0.2%.

JA-Me significantly affected synthesis of anthocyanins in buckwheat hypocotyls, but only at the highest concentration (10^{-4} M; Fig. 2a); it decreased by more than 6-fold versus the control after 3 days of treatment. In the low-concentration JA-Me treatments (10^{-6}, 10^{-8} M) the declines were 25% and 4%, respectively; these differences were not significant. Anthocyanin levels in buckwheat seedling cotyledons did not significantly differ from the control in

TABLE 1. Effect of concentration of jasmonic acid methyl ester (JA-Me) in solution on hypocotyl and primary root length (mm) in etiolated buckwheat seedlings exposed for 3 days to a day/night regime. Data are means ±SD of three independent replicates (30 to 40 seedlings measured per replicate). Means followed by (*) differ significantly from control; ns – no significant difference from control. Student's *t*-test ($p \leq 0.05$)

Treatment	Length at day 0	Length at day 3	Length increase between days 0 and 3
		Hypocotyl	
Control	49.4 ± 2.1	70.7 ± 2.4	21.4 ± 1.0
10^{-8} M JA-Me	47.5 ± 1.9 ns	73.1 ± 2.3 ns	26.0 ± 1.3 *
10^{-6} M JA-Me	47.0 ± 2.3 ns	70.3 ± 2.3 ns	23.3 ± 1.3 ns
10^{-4} M JA-Me	47.7 ± 2.3 ns	69.2 ± 2.6 ns	21.5 ± 1.3 ns
		Primary root	
Control	47.8 ± 2.4 -	85.8 ± 2.9 -	38.0 ± 2.1 -
10^{-8} M JA-Me	53.7 ± 2.3 ns	75.8 ± 2.7 ns	22.1 ± 1.7 *
10^{-6} M JA-Me	54.0 ± 2.5 ns	77.7 ± 2.6 ns	23.7 ± 1.7 *
10^{-4} M JA-Me	48.6 ± 2.5 ns	56.7 ± 2.3 *	8.2 ± 1.0 *

any of the JA-Me treatments, although a slight increase was noted in seedlings treated with the highest dose (Fig. 2a).

The anthocyanin determinations from excised hypocotyls after JA-Me treatment were similar to those from seedlings with roots (Fig. 2b). ANC content in buckwheat hypocotyls treated with 10^{-4} M JA-Me was almost 6-fold lower than the ethanol control, 34% lower for the 10^{-6} M dose, and lower for the 10^{-8} M dose. ANC levels in buckwheat seedling cotyledons did not significantly differ between the treatments and control. In general, anthocyanin levels in cotyledons of hypocotyls without roots were lower than in cotyledons of whole seedlings (Fig. 2a,b).

Treatment of buckwheat seedlings with 10^{-8} M JA-Me solution slightly stimulated hypocotyl elongation, but JA-Me at higher concentrations did not (Tab. 1). In contrast, JA-Me had a great influence on the growth of the main root. Even low concentrations of JA-Me (10^{-8} M and 10^{-6} M) slowed root growth by 40%. The highest concentration of JA-Me (10^{-4} M) in solution almost halted main root growth: it was almost 5 times slower than in the control seedlings. In wild-type *Arabidopsis thaliana*, Staswick et al. (1992) reported ~50% inhibition of growth of the primary root when seedlings were placed on agar medium containing 0.1 M JA-Me. Methyl jasmonate inhibits root and hypocotyls growth of sunflower seedlings (Corbineau et al., 1988). Similarly, jasmonic acid (10^{-7} – 10^{-5} M concentrations) rapidly inhibited root growth and decreased cell elongation in isolated tomato roots cultured in vitro (Tung et al., 1996).

JA-Me is relatively volatile at room temperature. This volatility may be a factor in the physiological activity of this compound, in that it may act as a gaseous hormone in plants, analogous to ethylene (Farmer and Ryan, 1990). Our results of JA-Me vapor treatment of whole buckwheat seedlings and excised hypocotyls are shown in Figure 3. In the experiments, buckwheat seedlings were treated with 10^{-6}, 10^{-5} and 10^{-4} M JA-Me. The ambient JA-Me concentrations in the closed jars were calculated on the assumption that the applied amounts were evaporated. JA-Me strongly and significantly inhibited anthocyanin accumulation at all vapor concentrations applied in seedlings with roots (Fig. 3a) and seedlings without roots (Fig. 3b). The effect was much stronger in excised seedlings: 10^{-4} M JA-Me reduced ANC by 11-fold versus the control. A hundred-fold less concentrated JA-Me vapor (10^{-6} M) reduced ANC by 30%, and 10^{-5} M more than halved it (Fig. 3b). As with the solution treatments, JA-Me vapor had no effect on ANC levels in buckwheat cotyledons (Fig. 3a,b). These results do not accord with previously published work in which JA application induced anthocyanin accumulation in various tissues (Franceschi and Grimes, 1991; Tamari et al., 1995). According to Franceschi and Grimes (1991), atmospheric JA-Me induced a 5- to 7-fold increase in anthocyanin accumulation in light-grown soybean seedlings, but inhibited anthocyanin biosynthesis in etiolated seedlings. In our study, anthocyanin biosynthesis in buckwheat seedlings was strongly inhibited when JA-Me vapor or solution were applied before they were exposed to light. We suggest that JA-Me blocked ANC formation because the buckwheat seedlings did not recognize the light conditions, or else that the anthocyanin synthesis inhibitors that they had formed during darkness were still active in light conditions, due to the presence of JA-Me. Another reason might be that JA-Me inhibits the catalytic effect of light in ANC

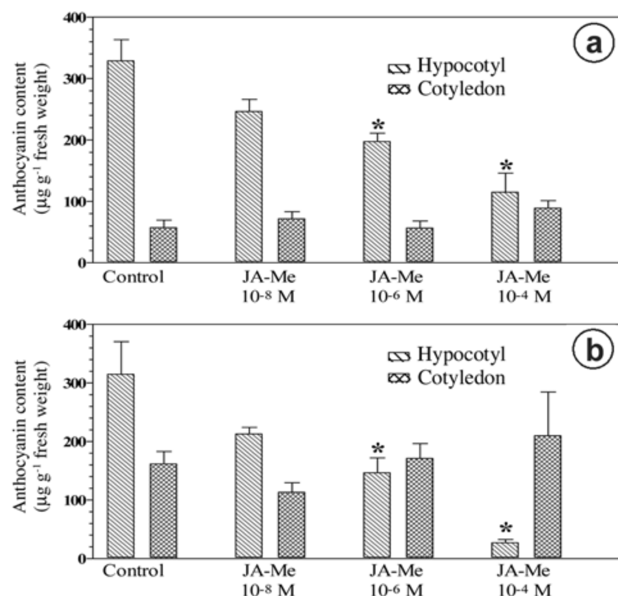

Fig. 3. Effect of concentration of jasmonic acid methyl ester (JA-Me) applied as vapors on anthocyanin accumulation in hypocotyls and cotyledons of etiolated buckwheat seedlings exposed for 3 days to a day/night regime. (**a**) Whole seedlings; (**b**) Excised upper part (hypocotyl + cotyledon). Results are means ±SD. Means marked (*) differ significantly from control; Student's *t*-test ($p \leq 0.05$).

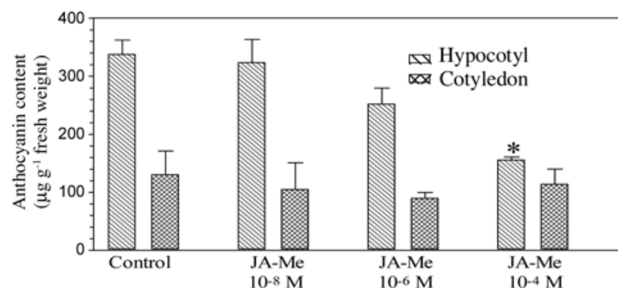

Fig. 4. Effect of concentration of jasmonic acid methyl ester (JA-Me) in solution for seed imbibition on anthocyanin accumulation in hypocotyls and cotyledons of etiolated buckwheat seedlings exposed for 3 days to a day/night regime. Results are means ±SD. Means marked (*) differ significantly from control; Student's *t*-test ($p \leq 0.05$).

TABLE 2. Effect of concentration of jasmonic acid methyl ester (JA-Me) in solution on phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) activity in hypocotyls of etiolated buckwheat seedlings exposed for 3 days to a day/night regime. Data are means ±SD of three independent replicates. ns – no significant difference from control. Student's *t*-test ($p \leq 0.05$)

JA-Me concentration	PAL activity nmoles *trans*-CA·g^{-1}·h^{-1}	TAL activity nmoles p-HCA·g^{-1}·h^{-1}
0 (control)	171.4 ± 32.2	85.3 ± 0.9
10^{-8} M	250.2 ± 61.1 ns	84.6 ± 3.6 ns
10^{-6} M	192.3 ± 35.3 ns	89.7 ± 5.3 ns
10^{-4} M	179.6 ± 9.7 ns	105.4 ± 2.6 ns

biosynthesis by blocking light receptors present in buckwheat tissue. These hypotheses on the role of JA-Me need further study.

In cultures of *Vaccinium pahalae* (Fang et al., 1999), *Tulipa gesneriana* (Saniewski et al., 1998), *Kalanchoe blossfeldiana* (Saniewski et al., 2003), *Crassula multicava* (Saniewski et al., 2006) and suspension cultures of *Vitis vinifera* (Zhang et al., 2002), treatment with liquid or atmospheric JA-Me led to enhanced anthocyanin biosynthesis.

In the next experiment, buckwheat seeds were imbibed for 24 h in water solutions of JA-Me (10^{-4}, 10^{-6} and 10^{-8} M). During that time the seeds increased their mean weight by ~10 mg, meaning that each seed could absorb ~10^{-9}, 10^{-11} and 10^{-13} M JA-Me, respectively. Such low quantities of JA-Me also affected anthocyanin accumulation in buckwheat seedlings grown from them (Fig. 4). The 10^{-6} and 10^{-8} M JA-Me imbibition solutions slightly decreased the ANC level, but the 10^{-4} M concentration significantly inhibited accumulation of anthocyanins in buckwheat hypocotyls, reducing it by more than 50% (Fig. 4). It also inhibited root growth but had no influence on hypocotyl length (data not shown). Higher concentrations of JA-Me (10^{-3} and 10^{-2} M) used for imbibition totally suppressed buckwheat seed germination (data not shown).

We analyzed the activity of the enzymes phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) in order to learn why ANC declined in hypocotyls of JA-Me-treated buckwheat seedling. Surprisingly, JA-Me had no influence on the activity of these enzymes (Tab. 2). The results contrast with those from earlier work in which exogenously added methyl jasmonate initiated *de novo* transcription of PAL genes (Gundlach et al., 1992). Treatment of the first leaves of barley seedlings with methyl jasmonate led to significant increases in levels of free polyamines. These changes were accompanied by increased activity of phenylalanine ammonia-lyase and other defense-related enzymes (Walters et al., 2002).

CONCLUSIONS

Our results indicate that the way in which biosynthesis of anthocyanins is regulated by JA-Me in seedlings of common buckwheat probably differs from its mode in other plants. In contrast to many

earlier published results from various studied plants, methyl ester of jasmonic acid inhibited synthesis and accumulation of anthocyanins in buckwheat hypocotyls exposed to light. To our knowledge this is the first description of this phenomenon in higher plants in experiments employing JA-Me water solutions and vapors applied to whole seedlings and excised hypocotyls. The very low quantities of JA-Me taken by seeds during imbibition were enough to inhibit anthocyanin synthesis in buckwheat hypocotyls. This means that there are no significant barriers to the transport and action of solute and gaseous JA-Me in buckwheat plants.

JA-Me inhibited accumulation of anthocyanins in buckwheat hypocotyls, but had no effect on phenylalanine and tyrosine ammonia-lyase activity, suggesting that it can act not in the first but in later steps of ANC biosynthesis.

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