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EFFECTS OF SIMULTANEOUS USE OF METHYL JASMONATE WITH OTHER PLANT HORMONES ON THE LEVEL OF ANTHOCYANINS AND BIOGENIC AMINES IN SEEDLINGS OF COMMON BUCKWHEAT (*Fagopyrum esculentum* Moench)

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Abstract

The aim of the study was to assess the impact of auxin (IAA), gibberellin (GA_3) and cytokinin (kinetin), used solely and in combination with methyl jasmonate (MJ), on the accumulation of anthocyanins and biogenic amines in hypocotyls and cotyledons of common buckwheat (*Fagopyrum esculentum* Moench) seedlings. The obtained results indicate that accumulation of anthocyanins in buckwheat seedlings was dependent on the concentration of the phytohormone applied and the tissue studied. The combined use of MJ and IAA, GA_3 or kinetin partly reversed the effect of strong inhibition of anthocyanin synthesis by MJ. IAA used solely decreased the level of anthocyanins in de-etiolated buckwheat cotyledons. IAA also caused a reduction of putrescine content, both in hypocotyls and cotyledons of buckwheat seedlings. MJ used alone caused high accumulation of 2-phenylethylamine (PEA) in buckwheat cotyledons and hypocotyls. The simultaneous application of MJ and IAA, GA_3 or kinetin also stimulated PEA synthesis in buckwheat tissues, however this effect was significantly lower compared to the use of MJ only. A reverse significant correlation between PEA and anthocyanin contents occurred in buckwheat hypocotyls, but not in cotyledons. It was suggested that the deficiency of L-phenylalanine, a substrate for synthesis of 2-phenylethylamine, may be partly responsible for the decline in anthocyanin content in buckwheat hypocotyls under the influence of MJ.

Abbreviations: MJ – methyl jasmonate, IAA – indole-3-acetic acid, GA_3 – gibberellic acid, PEA – 2-phenylethylamine

Key words: methyl jasmonate, auxin, gibberellin, kinetin, buckwheat, anthocyanins, polyamines

INTRODUCTION

Anthocyanin accumulation in plants is dependent on many external and internal factors such as: light, mineral concentration, carbohydrate level, and phytohormones (Troyer, 1964; Loreti et al. 2008). In the non-chlorophyllous part of maize leaves, gibberellic acid and 6-benzylaminopurine inhibited anthocyanin biosynthesis (Kim et al. 2006). The effect of phytohormones on anthocyanin biosynthesis seems to be light dependent. In etiolated radish seedlings and seedlings grown under low light, the anthocyanin content of the hypocotyls was enhanced by cytokinins, whereas under high light intensity the accumulation decreased (Buschmann and Lichtenthaler, 1982). Light-induced anthocyanin synthesis in excised dark-grown internodes of *Sorghum* was depressed by the addition of IAA and 2,4-dichlorophenoxyacetic acid to the incubating medium (Vince, 1968). Also IAA markedly suppressed anthocyanin formation in maize seedlings exposed to light (Rengel and Kordan, 1987), however in other studies IAA did not affect anthocyanin biosynthesis in the plant (Kim et al. 2006). Inhibition of anthocyanin synthesis by IAA applied was also noted in cabbage seedlings (Kang and Burg, 1973). Gibberellic acid reduced anthocyanin accumulation in radish seedlings (Jain and Guruprasad, 1989), excised *Sorghum* internodes exposed to light (Vince, 1968), and maize seedlings (Kim et al. 2006; Rengel and Kordan, 1987). Jasmonates induce anthocyanin synthesis and accumulation in various tissues of many plant species (Saniewski et al. 1998, 2003, 2006). However, in light-exposed hypocotyls of buckwheat seedlings methyl jasmonate (MJ) applied as vapors or as water solution clearly reduced the anthocyanin content (Horbowicz et al. 2008). It was found that the decline was accompanied by increased accumulation of proanthocyanidins (Horbowicz et al. 2011 a).

Biogenic amines are implicated in plant growth and developmental processes as well as in response to abiotic and biotic stress (Galston and Sawhney, 1990; Tabor and Tabor, 1984; Walters, 2003). Polyamines – major biogenic amines in plants, are synthesized by the decarboxylation of appropriate amino acids (Bagni and Tassoni, 2001). Aromatic monoamines in plants are the products of L-phenylalanine, L-tryptophan, and L-histidine decarboxylation to 2-phenylethylamine (PEA), tryptamine and histamine, respectively (Smith, 1977; Tieman et al. 2006). The presence of PEA was found in marine algae (Percot et al. 2009) and some terrestrial plants (Shabana et al. 2006). The role of PEA in plants remains unknown. Presumably, in the tissues of common buckwheat PEA can be used in synthesis of 2-phenylacetaldehyde which is an essential component of aroma composition (Janeš et al. 2009).

Exogenously applied phytohormones generally resulted in increased contents of polyamines in plant tissues. When dark-grown cucumber cotyledons were treated with cytokinin, a large increase in putrescine level was observed (Walker et al. 1988). In case of kinetin used in excised cucumber cotyledons incubated in the dark, the putrescine content markedly increased, whereas the level of spermidine and spermine decreased (Legocka and Żarnowska, 2000). Similarly, kinetin and 6-benzyladenine enhanced the putrescine content in cotyledons of lettuce seedlings (Cho, 1983). Contrary to the results of the cited papers, benzyladenine decreased the level of putrescine and did not affect spermidine and spermine content in senesced rice leaves (Chen and Kao, 1991).

The use of IAA increased polyamine levels in cultured tobacco cells (*Nicotiana tabacum* L) (Park and Lee, 1994) and the content of putrescine in tissues of *Glycine max* grown in vitro (Liu et al. 1998). When IAA and its precursors were applied to pepper plants cultivated in hydroponic conditions, an increase of spermine and a decrease of putrescine in leaves were observed (San-Francisco et al. 2005).

Gibberellic acid (GA_3) caused a marked increase of putrescine and spermidine content in light-grown pea seedlings (Dai et al. 1982) and of putrescine level in the epicotyl and leaf blade of barley seedlings (Asthir et al. 2004). The effect of methyl jasmonate on the biosynthesis of polyamines in plants is ambiguous. Treatment of barley seedlings with MJ led to the increase in the content of free polyamines and products of their metabolism – hydroxycinnamic acid amides (Walters et al. 2002). In hypocotyls and cotyledons of common buckwheat, MJ increased the content of putrescine and greatly stimulated accumulation of 2-phenylethylamine (Horbowicz et al. 2011a).

Data on the interaction of MJ and other phytohormones in plant tissues is limited. It has been shown that during induced senescence of barley leaf in the light conditions benzyladenine can eliminate the inhibitory effect of MJ on chlorophyll content and rubisco activity (Weidhase et al. 1987 a), however the cytokinin cannot prevent the formation of the so-called jasmonate-induced proteins (Weidhase et al. 1987b). Besides, the counteraction of cytokinins to the jasmonate inhibitory effect on the growth of different plants has also been reported (Ueda et al. 1981). Benzyladenine applied together with MJ neutralized the jasmonate action on the growth of excised cotyledons as well as on *in vivo* protein synthesis (Ananieva and Ananiev, 2000). Combined application of MJ and other phytohormones stimulated polyamine biosynthesis and accumulation of conjugated polyamines in tobacco leaf discs (Biondi et al. 2003). MJ induced accumulation of conjugated polyamines, which was further stimulated by auxin and counteracted by benzyladenine.

In our previously published paper it was suggested that the distinct inhibition of anthocyanin accumulation by MJ might be due to enhanced synthesis of PEA. Presumably, the potential reason for this phenomenon is that both metabolic routes use the same common substrate, which is L-phenylalanine (Horbowicz et al. 2011 a) (Fig.1). By changing the level of PEA and anthocyanins in tissues of buckwheat seedlings, we attempted to confirm this hypothesis. The aim of the present study was to evaluate the effect of auxin (IAA), gibberellin (GA_3), and cytokinin (kinetin) applied separately or in combination with MJ on the accumulation of anthocyanins and biogenic amines in hypocotyls and cotyledons of seedlings of common buckwheat (*Fagopyrum esculentum* Moench).

MATERIALS AND METHODS

Plant treatments

Seedlings of common buckwheat (*Fagopyrum esculentum* Moench) cv. 'Hruszowska' were used in this study. Germination was carried out in darkness at 24 ± 1°C during 4 days (Horbowicz et al. 2008). Four-day-old etiolated buckwheat seedlings were treated with various concentrations of IAA, GA_3 or kinetin added to the medium in which seedlings were placed or in combination with methyl jasmonate (MJ) vapors. In 2.5 L beakers with germinated seedlings, the water was replaced with water solutions of particular phytohormones, except for MJ which was applied as vapors. In case of MJ treatment, a strip of filter paper containing 50 µL of MJ was placed against the inner wall of the beaker with buckwheat seedlings, and the beaker was immediately closed tightly with silicon foam.

Seedlings treated with phytohormones were grown in an air-conditioned chamber in which the temperature was maintained at 22±2ºC/18±2ºC (day/night: 16h/8 h). Light (100 – 150 µMol x $m^{-2} \times s^{-1}$) was provided by high-pressure sodium lamps. After four days in such conditions, plants were subjected to analysis of anthocyanins and biogenic amines.

Determination of anthocyanins and amines

The determination of total anthocyanins was carried out using the method described by Mancinelli (1984) with small modifications (Horbowicz et al. 2008). Hypocotyl and cotyledon tissue was analyzed separately. Absorbance of the 1% HCl-methanol extracts was measured at 530 nm and 657 nm. The formula $A_{530} - 0.25A_{657}$ was used to compensate for the absorption of chlorophyll degradation products at 530 nm. Anthocyanins content was calculated as cyanidin-3-glucoside using 29600 as the molecular extinction coefficient.

Free amines were analyzed according to procedures described by Flores and Galston (1982) with slight modifications (Horbowicz et al. 2011a). Briefly, plant tissues were homogenized in 5% perchloric acid. Homogenates were centrifuged and amines were derivatised with benzoyl chloride. Benzoyl derivatives of amines were extracted with ethyl acetale, and pooled acetate layers were evaporated to dryness with a stream of air. The residue was dissolved in a mobile phase used for HPLC analysis. The mobile phase was a mixture of acetonitrile–water (45:55, v/v) at a flow rate of 1.0 mL min^{-1}. Benzoylated amines were eluted isocratically using an Eclipse XDB-C18 analytical column (4.6 × 150 mm, 5 µm particle size) and detected at 245 nm with a diode array detector (DAD). The amine contents were calculated from standard curves prepared from commercially available standards.

All the measurements were carried out in three (anthocyanins) or two (amines) replicates. The obtained results were subjected to statistical evaluation according to the Newman–Keuls test, $p \leq 0.05$.

RESULTS

Table 1 contains the results of anthocyanin content in hypocotyls and cotyledons of buckwheat seedlings treated with IAA, GA_3 and kinetin used alone or in combination with MJ. None of the hormones used, except MJ, affected anthocyanin content in hypocotyls. The combined application of IAA, GA_3 or kinetin (each at a concentration of 10^{-6} M), and MJ (10^{-4} M), reduced the level of anthocyanins in the buckwheat tissue (Table 1). A higher concentration of IAA, GA_3 or kinetin (10^{-4} M) and MJ (10^{-4} M) resulted in a greater reduction of pigment content in hypocotyls. However, the anthocyanin levels were significantly higher than in case of MJ vapors applied solely.

The application of GA_3 or kinetin used alone (each at a concentration of 10^{-8} M and 10^{-6} M), and combined with MJ (10^{-6} M + 10^{-4} M), did not affect the anthocyanin content in buckwheat cotyledons (Table 1). As in the case of hypocotyls, higher doses of these hormones (10^{-4} M), in combination with MJ (10^{-4} M), significantly decreased the level of pigments in buckwheat cotyledons.

IAA, used alone at a low concentration (10^{-8} M) or in combination with MJ vapors (10^{-6} M + 10^{-4} M, and 10^{-4} M + 10^{-4} M), did not affect the level of anthocyanins in cotyledons of buckwheat seedlings (Table 1). At higher doses of IAA (10^{-6} and 10^{-4} M), a significant reduction in anthocyanin content was observed.

Hypocotyls of buckwheat seedlings treated with the following phytohormones: IAA, kinetin and MJ (10^{-4} M each), accumulated a large content of 2-phenylethylamine (PEA), compared to control tissues (Fig.2). A particularly high increase in PEA levels occurred after MJ application, which resulted in about 50 times higher concentrations of the amine. Among the plant hormones studied, the application of GA_3 did not cause a significant increase of PEA level. The simultaneous use of IAA, GA_3 or kinetin and MJ resulted in a partial reduction (ca. 45–55%) in the content of PEA, as compared to tissues treated with MJ alone (Fig. 2).

In the tissue of buckwheat cotyledons, the PEA content was much higher than in hypocotyls (Fig. 3). Similarly to hypocotyls, under the influence of MJ buckwheat cotyledons accumulated large amounts of PEA. Other plant hormones (IAA, GA_3 or kinetin) applied individually, did not influence the levels of PEA. The simultaneous use of MJ and IAA, GA_3 or kinetin led to a partial reduction in the accumulation of PEA, compared to treatment only with MJ (Fig. 3).

Treatment of buckwheat seedlings with MJ vapors resulted in a small elevation of putrescine level in hypocotyls. The use of other plant hormones (IAA, GA_3 or kinetin) did not affect the content of putrescine in buckwheat hypocotyls, although its level was significantly lower than when MJ was applied alone (Table 2). The simultaneous use of MJ and GA_3 resulted in the decrease of putrescine content, in comparison to both the control and seedlings treated with MJ. When MJ was applied together with kinetin, a twofold increase in the level of polyamine was found. Cadaverine content in buckwheat hypocotyls was reduced to traces when IAA, GA_3 or kinetin were applied alone or together with MJ (Table 2). In the case of spermidine, the application of MJ alone, or together with IAA, kinetin or GA_3, reduced its content to traces. IAA, GA_3 or kinetin used together with MJ and IAA alone also decreased the content of tryptamine in hypocotyls of buckwheat seedlings. Among the tested hormones, GA_3 applied alone and with the addition of MJ vapors reduced the tryptamine content to traces. IAA and kinetin used alone or combined with MJ vapors did not affect the amine level in hypocotyl tissue (Table 2).

A much higher concentration of putrescine, cadaverine and spermidine occurred in cotyledons of buckwheat than in hypocotyls (Table 2). For instance, the content of putrescine and spermidine in the tissue was approximately tenfold higher than in the hypocotyls. The use of MJ, GA_3 or kinetin (10^{-4} M) caused a significant increase of putrescine levels in the cotyledons, while IAA decreased its content in comparison to the control plants and those treated with MJ alone. The simultaneous use of MJ and IAA, kinetin or GA_3 did not change the putrescine level compared to MJ--treated seedlings. However, the combination of MJ and IAA reversed the phenomenon of reduction of putrescine level under the influence of IAA (Table 2). In case of spermidine, MJ treatment decreased its level in cotyledons compared to the control tissue. Other phytohormones (IAA, GA_3 or kinetin) applied separately did not affect the level of this polyamine. The combination of IAA, GA_3 or kinetin and MJ did not change the situation, compared to the use of MJ alone. The application of MJ and IAA as well as their simultaneous use led to an increase in cadaverine content in buckwheat cotyledons. By contrast, the use of GA_3, and kinetin separately as well as with the addition of MJ did not affect the level of cadaverine in the tissue. The tested phytohormones used solely or simultaneously with MJ had no influence on the tryptamine level in buckwheat hypocotyls. In case of cotyledons, the tryptamine level was decreased to traces by MJ treatment, this trend was however reversed by simultaneous application of IAA, GA_3 and kinetin.

Table 1
Effect of IAA, GA_3 or kinetin used alone or simultaneously with vapors of methyl jasmonate (MJ) on the content of anthocyanins in hypocotyls and cotyledons of buckwheat seedlings (means ± SD). Means in columns followed by different letters are significantly different according to the Newman–Keuls test, $p \leq 0.05$

Treatment (concentration)		Anthocyanins (μg × g^{-1} fresh weight)	
		Hypocotyls	Cotyledons
1	Control	341.6 ± 37.9 a	242.5 ± 33.1 a
2	MJ (10^{-4} M)	97.8 ± 2.5 d	234.4 ± 47.5 a
3A	IAA (10^{-8} M)	270.5 ± 31.4 a	204.4 ± 69.4 a
3B	IAA (10^{-6} M)	396.4 ± 18.3 a	125.4 ± 19.0 b
3C	IAA (10^{-4} M)	339.5 ± 10.5 a	139.8 ± 7.5 b
4A	GA_3 (10^{-8} M)	305.0 ± 4.1 a	267.7 ± 28.7 a
4B	GA_3 (10^{-6} M)	342.2 ± 82.6 a	289.0 ± 80.9 a
4C	GA_3 (10^{-4} M)	332.9 ± 37.3 a	160.0 ± 46.0 ab
5A	Kinetin (10^{-8} M)	325.4 ± 27.5 a	241.8 ± 50.5 a
5B	Kinetin (10^{-6} M)	370.3 ± 41.8 a	401.0 ± 105.0 a
5C	Kinetin (10^{-4} M)	376.8 ± 85.8 a	235.8 ± 45.0 a
6A	MJ + IAA (10^{-4} M + 10^{-6} M)	229.1 ± 14.0 b	235.4 ± 22.9 a
6B	MJ + IAA (10^{-4} M + 10^{-4} M)	140.7 ± 22.4 cd	251.7 ± 18.0 a
7A	MJ + GA_3 (10^{-4} M + 10^{-6} M)	212.8 ± 25.9 b	272.7 ± 37.4 a
7B	MJ + GA_3 (10^{-4} M + 10^{-4} M)	158.5 ± 10.9 c	136.6 ± 16.6 b
8A	MJ + kinetin (10^{-4} M + 10^{-6} M)	232.5 ± 6.3 b	246.6 ± 18.8 a
8B	MJ + kinetin (10^{-4} M + 10^{-4} M)	143.7 ± 6.0 c	121.6 ± 18.8 b

Table 2
Effect of IAA, GA_3 and kinetin used alone or simultaneously with methyl jasmonate (MJ) vapors (each 10^{-4} M) on the content of polyamines in tissues of buckwheat seedlings.
Means in columns followed by different letters are significantly different according to the Newman–Keuls test, $p \leq 0.05$ tr (traces) < 5 μg × g^{-1} fresh weight

Treatment		Putrescine	Cadaverine	Spermidine	Tryptamine
		μg × g^{-1} fresh weight			
Hypocotyls					
1	Control	18.6 c	11.0 a	13.3 a	14.3 a
2	MJ	23.2 b	7.5 a	tr	14.5 a
3C	IAA	13.5 c	tr	15.1 a	8.8 a
4C	GA_3	13.8 c	tr	14.6 a	tr
5C	Kinetin	14.6 c	tr	7.5 a	10.6 a
6B	MJ + IAA	16.1 c	tr	tr	tr
7B	MJ + GA_3	9.7 d	tr	tr	tr
8B	MJ + kinetin	36.7 a	tr	tr	6.4 a
Cotyledons					
1	Control	182 b	30.1 b	149 a	10.6 a
2	MJ	330 a	40.7 a	109 b	tr
3C	IAA	93.1 c	37.7 a	141 a	9.8 a
4C	GA_3	265 a	29.6 b	158 a	7.5 a
5C	Kinetin	274 a	27.1 b	145 a	7.0 a
6B	MJ + IAA	273 a	39.5 a	113 b	8.5 a
7B	MJ + GA_3	265 a	26.2 b	85.2 b	5.5 a
8B	MJ + kinetin	268 a	30.6 b	96.3 b	7.5 a

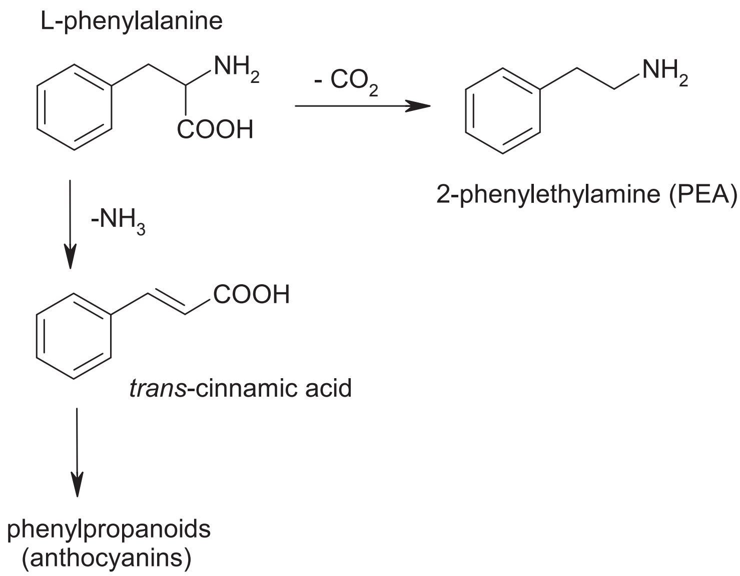

Fig. 1. Simplified scheme of L-phenylalanine transformation to phenylpropanoids and 2-phenylethylamine (PEA).

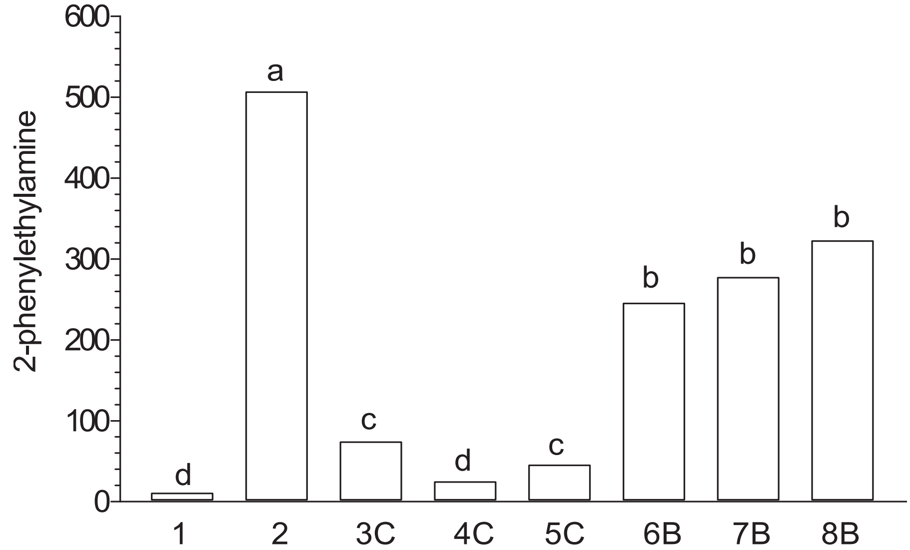

Fig.2. Effect of methyl jasmonate (MJ) used alone or simultaneously with IAA, GA_3 or kinetin on the content of 2-phenylethylamine (PEA) in buckwheat hypocotyls (μg × g^{-1} fresh weight). The description of bars is shown in Table 1. Bars marked by different letters are significantly different according to the Newman–Keuls test, $p \leq 0.05$.

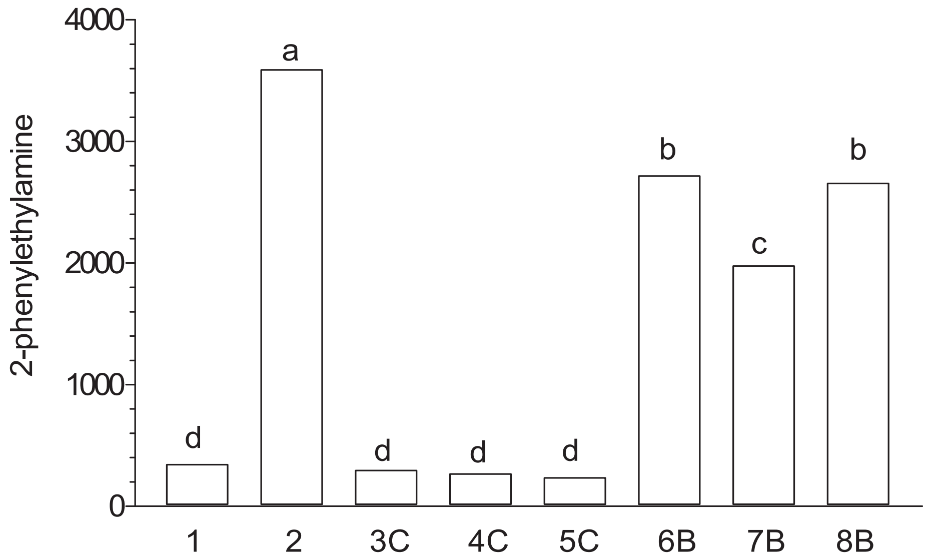

Fig.3. Effect of methyl jasmonate (MJ) used alone or simultaneously with IAA, GA_3 or kinetin on the content of 2-phenylethylamine (PEA) in buckwheat cotyledons (μg × g^{-1} fresh weight). The description of bars is shown in Table 1. Bars marked by different letters are significantly different according to the Newman–Keuls test, $p \leq 0.05$.

DISCUSSION

The transformation of plants from dark-grown (etiolated) to light-grown (de-etiolated) is accompanied by a number of important phenotypic changes such as reduced shoot elongation, quick expansion of true leaves, and development of chloroplasts. Many of these processes are regulated by plant hormones (S y m o n s and R e i d , 2003). A characteristic element of de-etiolation of buckwheat seedlings is fast accumulation of anthocyanins in their hypocotyls and cotyledons (H o r b o w i c z et al. 2008). However, methyl jasmonate (MJ) applied during the de-etiolation process reduced the level of anthocyanins in hypocotyls, although it did not affect pigment content in cotyledons (H o r b o w i c z et al. 2008). The inhibitory effect of MJ on the accumulation of anthocyanins in hypocotyls of buckwheat and the absence of such an effect in the case of cotyledons were confirmed in the present study (Table 1). The simultaneous use of MJ and IAA, GA_3 or kinetin partly reversed the effect of strong inhibition of anthocyanin synthesis by MJ in buckwheat hypocotyls. This was more evident when IAA, GA_3 and kinetin were applied at a concentration of 10^{-6} M, compared to a concentration of 10^{-4}M.

The observed reduction in anthocyanin content in buckwheat cotyledons under the influence of IAA is a confirmation of the results of similar studies on tissues of maize (R e n g e l and K o r d a n , 1987). Although the use of GA_3 and kinetin alone did not affect the level of anthocyanins, but the application of these phytohormones and MJ inhibited the accumulation of anthocyanins in buckwheat cotyledons. The synergistic influence of combined application of GA_3 or kinetin and MJ was not previously described.

The possible reason for the reduction of anthocyanins in hypocotyls of buckwheat seedlings is the use of L-phenylalanine to produce of PEA (Fig. 1). The synthesis of PEA leads to a deficiency of L-phenylalanine and reduced synthesis of *trans*-cinnamic acid – the starting substrate for all phenylpropanoids. The accumulation of large amounts of PEA in buckwheat hypocotyls under the influence of MJ was accompanied by a significant decrease of *trans*-cinnamic acid, as described by us earlier (H o r b o w i c z et al. 2011b). Looking for further explanation of these metabolic changes, we studied the hypothesis that the change in the levels of anthocyanins is the result of L-phenylalanine deficiency. We assumed that the change in PEA content through the use of other plant hormones may increase the anthocyanin content. Among the phytohormones applied, MJ, IAA and kinetin used alone stimulated the production of PEA in buckwheat hypocotyls, however in cotyledons only MJ had a definitely stimulant effect on the synthesis of the amine (Figs 2 and 3). Of these plant hormones, MJ showed the greatest stimulatory effect. The simultaneous use of MJ and IAA, GA_3 or kinetin partially reduced the stimulating effect of PEA synthesis by treatment with MJ. This phenomenon was observed in both studied tissues of buckwheat seedlings: hypocotyls and cotyledons. It is probably the result of increased activity of L-phenylalanine decarboxylase by MJ. The results of studies on the effect of IAA, GA_3 and kinetin on the content of PEA or the effect of their combined use with MJ have not yet been described.

In the present work, by changing the amount of anthocyanins and PEA we could evaluate the potential effects of the accumulation of large amounts of PEA on the level of anthocyanins by calculating the significance of the correlation between the content of both compounds. The calculation was done for cotyledons and hypocotyls of buckwheat seedlings separately. A significant reverse correlation between PEA and anthocyanin content occurred in buckwheat hypocotyls, but not in cotyledons. In buckwheat hypocotyls, the anthocyanin content was significantly dependent on the PEA level (goodness of fit = 0.8802; F=44.06; P= 0.0006; n=8). Hypocotyls had higher levels of PEA but a lower content of anthocyanins. The obtained different results for hypocotyls and cotyledons indicate that in the tissues carrying out photosynthesis the deficiency of L-phenylalanine is quickly complemented. Moreover, in case of cotyledons MJ significantly enhanced *trans*-cinnamic acid synthesis (H o r b o w i c z et al. 2011 b).

The likely effect of plant hormones on the decarboxylation of L-phenylalanine to PEA was an important reason to check whether it also applied to the synthesis of other amines formed by decarboxylation of relevant amino acids: putrescine, tryptamine, and cadaverine. MJ vapors enhanced the level of putrescine in cotyledons and hypocotyls as well as the level of cadaverine in cotyledons (Table 2). The results are similar to those found in the previously described studies on the effects of MJ applied to the root zone as solutions (H o r b o w i c z et al. 2011a). The other plant hormones studied, IAA, GA_3 and kinetin, applied solely and combined use of IAA and MJ, did not affect the content of putrescine in hypocotyls, however the simultaneous treatment with MJ and GA_3 caused a significant decline, while MJ + kinetin resulted in a doubling of the putrescine level (Table 2)

Unlike the other plant hormones, IAA caused a reduction in putrescine content, both in hypocotyls and cotyledons of buckwheat seedlings. Our results confirm earlier data in which IAA and its precursors reduced the level of putrescine in tissues of pepper (S a n - F r a n c i s c o et al. 2005). However, the obtained results are contrary to other published data (P a r k and L e e , 1994; L i u et al. 1998). According to these results, IAA enhanced activities of arginine decarboxylase, ornithine decarboxylase, and *S*-adenosylmethionine decarboxylase – the key enzymes of polyamine biosynthesis, therefore the use of the plant hormone increased polyamine levels (P a r k and L e e , 1994). Also, exogenously applied indole-3-butyric acid (IBA) and naphatalene acetic acid (NAA) induced synthesis of putrescine in tissues of *Glycine max* grown *in vitro* (L i u et al. 1998).

The increase of putrescine in buckwheat cotyledons by GA_3 confirms a similar phenomenon observed in pea seedlings. In pea tissue, the use of the plant hormone caused markedly increased activity of arginine decarboxylase as well as an increase in the level of putrescine and spermidine (D a i et al. 1982). Also, the application of gibberellin significantly increased putrescine levels in both the epicotyl and leaf blade of barley seedlings (A s t h i r et al. 2004).

Spermidine is not synthesized by simple decarboxylation but by alkylation of putrescine. MJ alone or combined with IAA, GA_3 or kinetin caused a decline of spermidine in hypocotyls and cotyledons of buckwheat seedlings (Table 2). There was no change in the polyamine level under the influence of GA_3, IAA and kinetin used alone. The obtained results confirm earlier published data for rice leaves (Chen and Kao, 1991), but are inconsistent with the results obtained for tissues of light-grown pea seedlings treated with GA_3 (Dai et al. 1982).

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Wpływ jednoczesnego użycia jasmonianu metylu z innymi hormonami roślinnymi na poziom antocyjanów i amin biogennych w siewkach gryki zwyczajnej (*Fagopyrum esculentum* Moench)

Streszczenie

Celem pracy była ocena wpływu auksyny (IAA), gibereliny (GA_3) i cytokininy (kinetyna) stosowanych wyłącznie, oraz jednocześnie z parami jasmonianu metylu (MJ), na akumulację antocyjanów i amin biogennych w hipokotylach i liścieniach siewek gryki zwyczajnej (*Fagopyrum esculentum* Moench). Uzyskane wyniki wskazują, że nagromadzanie antocyjanów w siewkach gryki było zależne od stężenia zastosowanego fitohormonu i badanej tkanki. Łączne stosowanie MJ i IAA, GA_3 lub kinetyny częściowo odwracało efekt silnego hamowania syntezy antocyjanów przez MJ. IAA zastosowany samodzielnie obniżał poziom antocyjanów w liścieniach oraz powodował zmniejszenie zawartości putrescyny w hipokotylach i liścieniach gryki. MJ stosowany samodzielnie powodował duże nagromadzanie 2-fenyloetyloaminy (PEA) w liścieniach i hipokotylach gryki. Jednoczesne stosowanie MJ i IAA, GA_3 lub kinetyny stymulowało również wzmożoną syntezę PEA w tkankach gryki, jednak wpływ ten był znacznie niższy w porównaniu do użycia jedynie MJ. Wystąpiła odwrotna korelacja między zawartością PEA i antocyjanów w hipokotylach gryki, ale nie w liścieniach. Zasugerowano, że za spadek zawartości antocyjanów w hipokotylach gryki pod wpływem MJ może być częściowo odpowiedzialny niedobór L-fenyloalaniny będącej substratem do syntezy 2-fenyloetyloaminy (PEA).