



## Short communication

## The level of flavonoids and amines in de-etiolated and methyl jasmonate treated seedling of common buckwheat



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## ABSTRACT

The effects of atmospheric methyl jasmonate on the level of flavonoids and biogenic amines in de-etiolated seedlings of common buckwheat (*Fagopyrum esculentum* Moench) were investigated. In cotyledons and hypocotyls of etiolated seedlings, some traces of anthocyanins were found, with no flavones and flavonols identified. A measurable content of flavones and flavonols was, however, determined in roots. De-etiolation process stimulated the accumulation of all flavonoid types. Methyl jasmonate clearly decreased the content of anthocyanins in the hypocotyl, not affecting their level in cotyledons. In case of roots, the content of anthocyanins increased after a 4-day treatment. In general, reduction in the level of flavones and flavonols was recorded only in the hypocotyl, however it was not always significant. Cotyledons of the seedlings treated with methyl jasmonate responded by a slight increase in flavonoids level. Methyl jasmonate considerably induced the accumulation of 2-phenylethylamine in all the seedling organs, increasing the content of putrescine and tryptamine in cotyledons, and decreasing the level of tryptamine in roots.

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## 1. Introduction

Jasmonates play a crucial role in enhancing plant resistance against various biotic stresses and have been, therefore, extensively studied (reviewed by Wasternack and Hause, 2013). Jasmonates accumulate in wounded plants and in plants which are treated with elicitors of pathogen defense (Creelman et al., 1992; Gundlach et al., 1992). Jasmonates also activate biosynthesis of protease inhibitors that increase the protection of plants against insect attack (Farmer and Ryan, 1990) and induce production of various secondary metabolites. Additionally, they stimulate the biosynthesis of defense proteins responsible for improving plants resistance to stresses (Aerts et al., 1994).

Methyl jasmonate (MeJA) stimulates the senescence processes in plants and inhibits their growth (Hung and Kao, 1996; Ueda et al., 1996). Jasmonates have also been noted to induce expression

of genes encoding enzymes involved in flavonoid biosynthesis (Dittrich et al., 1992; Gundlach et al., 1992). In the etiolated sprouts of common buckwheat, MeJA significantly increased the content of chlorogenic acid, catechin, iso-orientin, orientin, rutin, vitexin, and quercitrin (Kim et al., 2011). In addition, MeJA was also noted to stimulate the accumulation of anthocyanins in many plants (Franceschi and Grimes, 1991; Saniewski et al., 2003; Horbowicz et al., 2014). However, contrary to many results published so far, MeJA has also been observed to reduce the level of anthocyanins in the hypocotyl of common buckwheat, (*Fagopyrum esculentum* Moench) (Horbowicz et al., 2008).

Jasmonates have also been shown to stimulate the production of free polyamines and its conjugates (Biondi et al., 2000, 2001). MeJA led to significant increases in the levels of free and conjugated putrescine and spermine in barley and wheat (Walters et al., 2002; Haggag and Abd-El-Kareem, 2009).

Previously, we discovered that MeJA caused a considerable increase of 2-phenylethylamine (PEA) in cotyledons and hypocotyls of common buckwheat seedlings (Horbowicz et al., 2011a). Recently, it has also been found that MeJA slightly induced the biosynthesis of PEA in the leaves of the giant knotweed, *Fallopia sachalinensis* (Noge and Tamogami, 2013). PEA is an alkaloid synthesized by decarboxylation of L-phenylalanine (Smith, 1977).

**Abbreviations:** MeJA, methyl jasmonate; PEA, 2-phenylethylamine; Cy-Glu-Rha, cyanidin 3-glucorhamnoside; Cy-Gal-Rha, cyanidin 3-galactorhamnoside; Cy-Gal, cyanidin 3-galactoside; Cy-Glu, cyanidin 3-glucoside; Q-Gal-Rha, quercetin 3-galactorhamnoside; Q-Glu-Rha, quercetin 3-glucorhamnoside.

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Basing on our previous studies, we decided to examine the impact of the de-etiolation process and atmospheric methyl jasmonate on the accumulation of flavonoids and amines in the organs of common buckwheat seedlings: roots, hypocotyl and cotyledons.

## 2. Material and methods

Seeds of common buckwheat (*F. esculentum* Moench; cv. Hruszowska) were germinated in darkness at  $24 \pm 1^\circ\text{C}$  within 4 days. Subsequently, the seedlings obtained were transferred to a growth chamber with a 16/8 h day/night photo period,  $65 \pm 5\%$  relative humidity; temperature  $22 \pm 2^\circ\text{C}/18 \pm 2^\circ\text{C}$ . Light ( $100 \pm 10 \mu\text{M} \times \text{m}^{-2} \times \text{s}^{-1}$ ) was provided by 400 W high-pressure sodium lamps. In the above conditions seedlings were treated for 4 or 7 days with MeJA vapors, as described earlier Horbowicz et al. (2008). The concentration of atmospheric MeJA was calculated as  $0.01 \text{ mM} \times \text{dm}^{-3}$  (assuming its complete evaporation). The number of seedlings per one jar was in the range of 50–60 (one replicate), with the experiments being carried out in three replicates. Etiolated and de-etiolated seedlings, after four or seven days of MeJA treatment, were divided into roots, cotyledons and hypocotyls, and freeze-dried separately. Such samples were subsequently subjected to chemical analyses.

For the analysis of anthocyanins, flavonols and flavones, a HPLC–DAD–MS–ESI system (LC10, MS detector QP8000 $\alpha$ ; Shimadzu) equipped with a  $250 \times 2.0 \text{ mm}$  i.d. Cadenza CD–C18 column was applied, as described earlier (Horbowicz et al., 2011b). The amines were analyzed by the HPLC–DAD (Agilent Technologies, model 1200 Series) after derivatization with benzoyl chloride (Horbowicz et al., 2011a). Student's *t*-test was used for statistical evaluation of the differences between control and treated plants.

## 3. Results and discussion

### 3.1. Anthocyanins

No anthocyanins were detected in etiolated roots of buckwheat seedling. Cotyledons were identified with a low concentration of cyanidin 3-glucorhamnoside (Cy–Glu–Rha) and cyanidin 3-galactorhamnoside (Cy–Gal–Rha), whereas the hypocotyl with traces of Cy–Gal–Rha (Table 1). The reason why the compounds occur in the cotyledons is not known. It may be speculated that immediate activation of photosynthesis provides a substrate(s) for the production of anthocyanins in cotyledons. The time required to

prepare samples for the freeze-drying process may have been sufficient to activate the anthocyanin biosynthetic pathway.

After 4 days of de-etiolation process, all tissues of buckwheat seedlings examined were determined to contain measurable amounts of anthocyanins. The highest content of anthocyanins was found in the hypocotyl, lower in cotyledons and the lowest in roots (Table 1). In the tissues of buckwheat seedlings only glycosides of cyanidin occurred: 3-galactoside (Cy–Gal), 3-glucoside (Cy–Glu), Cy–Glu–Rha and Cy–Gal–Rha. All the anthocyanins listed occur in the hypocotyl, but the cotyledon and roots contain a measurable content of Cy–Gal–Rha and Cy–Glu–Rha only. Earlier, a rapid accumulation of anthocyanins in buckwheat tissues upon exposure to light was found by Watanabe and Ito (2003).

The use of MeJA vapors resulted in a marked reduction of all anthocyanins present in the hypocotyl. In case of cotyledons, the decrease in anthocyanin content was determined insignificant, however an increase was noted in the level of Cy–Gal–Rha in roots (Table 1). The results obtained confirm our previous studies, where MeJA was applied as solution (Horbowicz et al., 2008). However, the increase of Cy–Gal–Rha triggered by MeJA in root tissues constitutes a new finding. Advanced process of senescence (7 days) caused only a slight decrease in anthocyanin content in cotyledons, in contrast to the hypocotyl, where the decline was more significant. At the same time only trace amounts of anthocyanins were found in root tissues (Table 1).

### 3.2. Flavones and flavonols

Etiolated cotyledons and hypocotyl did not contain measurable amounts of flavones and flavonols, but root tissues were identified with a small, yet measurable, amount of these compounds (Table 2). They may have originated from the seeds, since accumulation of flavones and flavonols takes place during seeds maturation. If the content of these compounds had resulted from *de novo* synthesis, some traces of anthocyanins, the other flavonoid pathway metabolites, would have been noted. The following six flavonoids have been identified in buckwheat grain: rutin, orientin, vitexin, quercetin, *iso*-vitexin (*i*-vitexin), and *iso*-orientin (*i*-orientin) (Dietrych-Szóstak and Oleszek, 1999; Koyama et al., 2013). Rutin and *i*-vitexin were the only flavonoid components of buckwheat seeds, while hulls contained all of these compounds. Rutin is mainly concentrated in the husk of common buckwheat seed, with its level in the inner part of the seed being considered low (Steadman et al., 2001).

Our results of cotyledons and hypocotyl analysis differ from those published by Tsurunaga et al. (2013). They reported that

**Table 1**  
Effect of de-etiolation and methyl jasmonate (MeJA) treatment on accumulation of anthocyanins ( $\text{mg} \times \text{g}^{-1}$  DW; means  $\pm$  SD) in tissues of common buckwheat seedlings (tr – traces, below  $0.01 \text{ mg} \times \text{g}^{-1}$  DW; nd – not detected). Significant differences between control and MJ treated are indicated with asterisks \* ( $n=3$ ;  $p < 0.05$ ). Lack of asterisks means no significant differences.

Analysed anthocyanin	Etiolated seedlings	After 4 days in day/night conditions		After 7 days in day/night conditions	
		Control	MeJA treated	Control	MeJA treated
<b>Cotyledons</b>					
Cy–Gal–Rha	$0.05 \pm 0.02$	$0.56 \pm 0.05$	$0.46 \pm 0.09$	$0.55 \pm 0.06$	$0.36 \pm 0.07$
Cy–Glu–Rha	$0.01 \pm 0.01$	$0.10 \pm 0.02$	$0.08 \pm 0.01$	$0.08 \pm 0.02$	$0.05 \pm 0.02$
<b>Hypocotyl</b>					
Cy–Gal–Rha	nd	$1.35 \pm 0.02$	$0.15 \pm 0.04^*$	$0.81 \pm 0.02$	$0.11 \pm 0.03^*$
Cy–Glu–Rha	tr	$0.12 \pm 0.02$	$0.03 \pm 0.01^*$	$0.07 \pm 0.02$	$0.02 \pm 0.01$
Cy–Gal	nd	$0.11 \pm 0.02$	$0.01 \pm 0.01^*$	$0.06 \pm 0.02$	$0.01 \pm 0.01$
Cy–Glu	nd	$0.03 \pm 0.01$	tr	$0.01 \pm 0.01$	tr
<b>Roots</b>					
Cy–Gal–Rha	nd	$0.05 \pm 0.01$	$0.17 \pm 0.02^*$	$0.03 \pm 0.01$	$0.01 \pm 0.01$
Cy–Glu–Rha	nd	tr	$0.03 \pm 0.01$	tr	tr

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