

Induction of anthocyanins accumulation by methyl jasmonate in shoots of *Crassula multica* Lam.

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S u m m a r y

In *Crassula multica* Lam. anthocyanins are formed naturally mostly in the stem near nodes and only traces in other parts of internodes. Methyl jasmonate (JA-Me) applied in lanolin paste at concentrations of 0.05, 0.1, 0.5 and 1.0% on the middle part of internodes greatly stimulated anthocyanins accumulation in the internodes and in the nodes of *Crassula multica*. The stimulatory effect was higher in younger tissues of the *Crassula multica* stem than in older ones, and depends on the used concentration of JA-Me. The possible role of jasmonates on anthocyanins formation in *Crassula multica* is discussed.

Key words: *Crassula multica*, methyl jasmonate, anthocyanins

INTRODUCTION

Jasmonic acid (JA), methyl jasmonate (JA-Me) and related jasmonates, are widely distributed in the plant kingdom and show various important biological activities in the regulation of plant growth and development, resulting in a consideration that they are putative new plant hormones. Levels of endogenous jasmonates, mainly JA, increase rapidly and transiently in plants or their organs under both abiotic and biotic stress conditions (Creelman and Mullet, 1995). Wounding induces an expression of defense-related genes whose products are mostly involved in defense responses against pathogen and insect attack (de Bruxelles and Roberts, 2001; Pieterse et al. 2001). Jasmonates are recognized as an integral part of the signal transduction chain between stress signal(s) and stress response(s). JA-Me can

act as an intracellular regulator, a diffusible intercellular signal transducer, or an airborne signal mediating intra- and interplant communications (Seo et al. 2001). Exogenously applied jasmonates had high stimulatory effect in the biosynthesis of a wide range of secondary metabolites in cell suspension cultures (Blechert et al. 1995; Gundlach et al. 1992) and intact plants (Aerts et al. 1994).

As was previously published, methyl jasmonate had stimulatory effect on anthocyanins accumulation in hypocotyl of light-grown soybean seedlings (Franceschi and Grimes 1991), in shoots of wild-type of *Arabidopsis thaliana* (Feys et al. 1994), in detached corollas of *Petunia* (Tamarit et al. 1995), in the stem and leaves of tulips (Saniewski et al. 1998a), in peach shoots (Saniewski et al. 1998b), in cell cultures of *Vaccinium pahalae* (Fang et al. 1999), in apple fruits (Kondo et al. 2001), in suspension cultures of *Vitis vinifera* (Zhang et al. 2002), and in shoots of *Kalanchoe blossfeldiana* (Saniewski et al. 2003).

In this paper we report results of the JA-Me influence on accumulation of anthocyanins in the stem of *Crassula multicava*.

MATERIALS AND METHODS

For the experiments cuttings from young plants of *Crassula multicava* Lam. grown in greenhouse were used. After rooting plants were grown in a mixture of soil, peat moss and sand in the same greenhouse conditions. Cuttings from 1 to 1.5-month-old plants were treated with 0.05, 0.1, 0.5 and 1.0% (w/w) of JA-Me in lanolin paste. Lanolin paste containing JA-Me was applied in the middle part of each internode of the plant stem. Control plants were treated with lanolin paste only. For each treatment, five plants were used. During the experiment carried out in January, samples for analyses were taken 23 and 39 days after treatment (DAT), and in the experiment carried out in June 22 DAT. In the June experiment, anthocyanins were analyzed separately in the lower, middle and upper part of the stem (results presented in Fig. 2). During the January experiment, pooled samples were analyzed, containing middle parts of three internodes (results on Fig. 3A), and internodes directly above nodes (results on Fig. 3B).

For the determination of anthocyanins content, parts of the plant stem from the middle part of the internode and from the internode directly above the node (ca. 1 g) were taken, cut into small pieces and macerated with 10 ml of 1.0% hydrochloric acid solution in 70% ethanol. Slurry was kept in tightly capped vials, and allowed to equilibrate overnight at +4°C in the dark. Absorption of the obtained anthocyanins solution was measured at 530 nm. Anthocyanins content calculation was based on a value of molecular absorption of cyanidin galactoside ($\epsilon = 44\ 800$) according to the Swain method (1965).

RESULTS AND DISCUSSION

In the stem internode of *Crassula multicava* plants, anthocyanins are formed in a much higher concentration than in other parts of the internode (Fig. 1). The reason for such phenomenon is unknown. Methyl jasmonate applied in lanolin paste in the



Fig. 1. The effect of JA Me applied in the middle part of the *Crassula multicava* internodes on anthocyanins accumulation; plants were treated in January and photographed 19 days after treatment (DAT)

A) intact plants:

left control, untreated or treated with lanolin only,

right JA Me 1.0%, the accumulation of anthocyanins can be seen



B) all leaves were excised just before taking the picture:

a) control,

b) control, treated with lanolin only,

c) JA Me 1.0%,

d) JA Me 0.5%,

e) JA Me 0.1%,

f) JA Me 0.05%

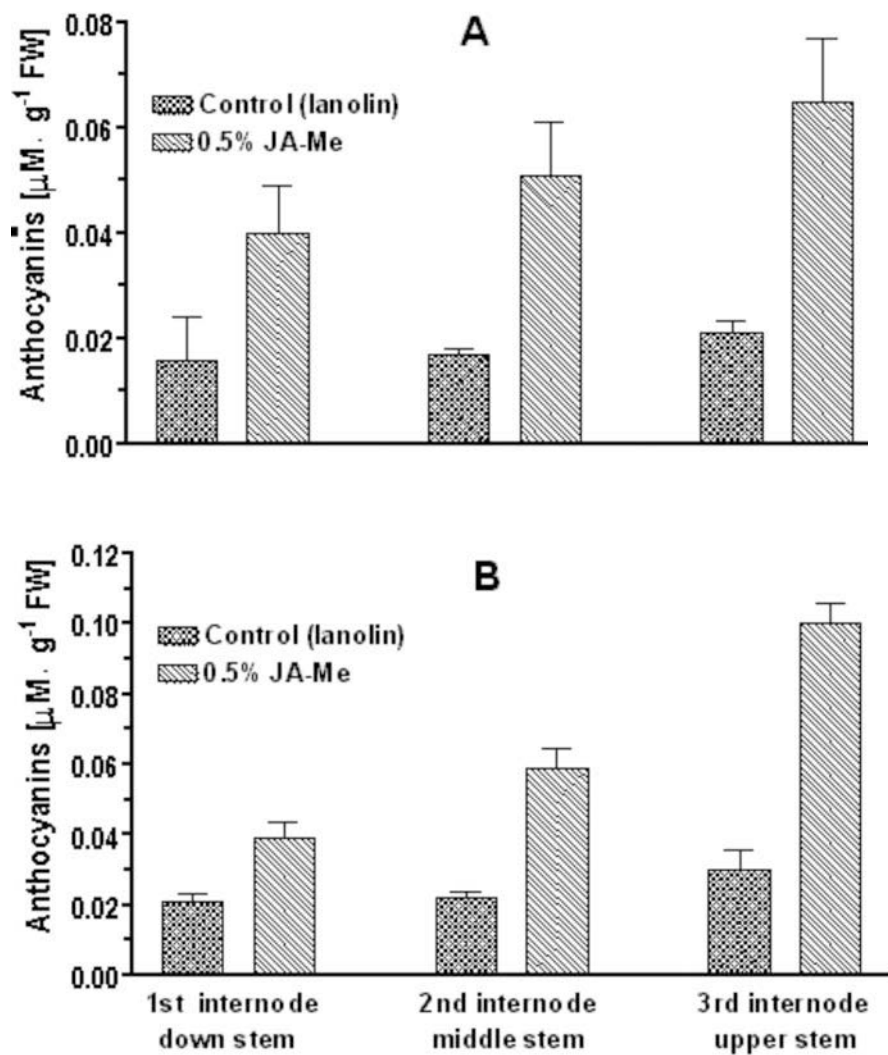


Fig. 2. The anthocyanins content in stem of *Crassula multicava* after treatment with JA Me 0.5% in lanolin paste (w/w) in the middle part of stem internodes (by means of two laboratory replicates \pm SD); treatments were carried out in June, and anthocyanins were analyzed 22 days after treatment (DAT):

A) in the middle part of internode,

B) in internode directly above node

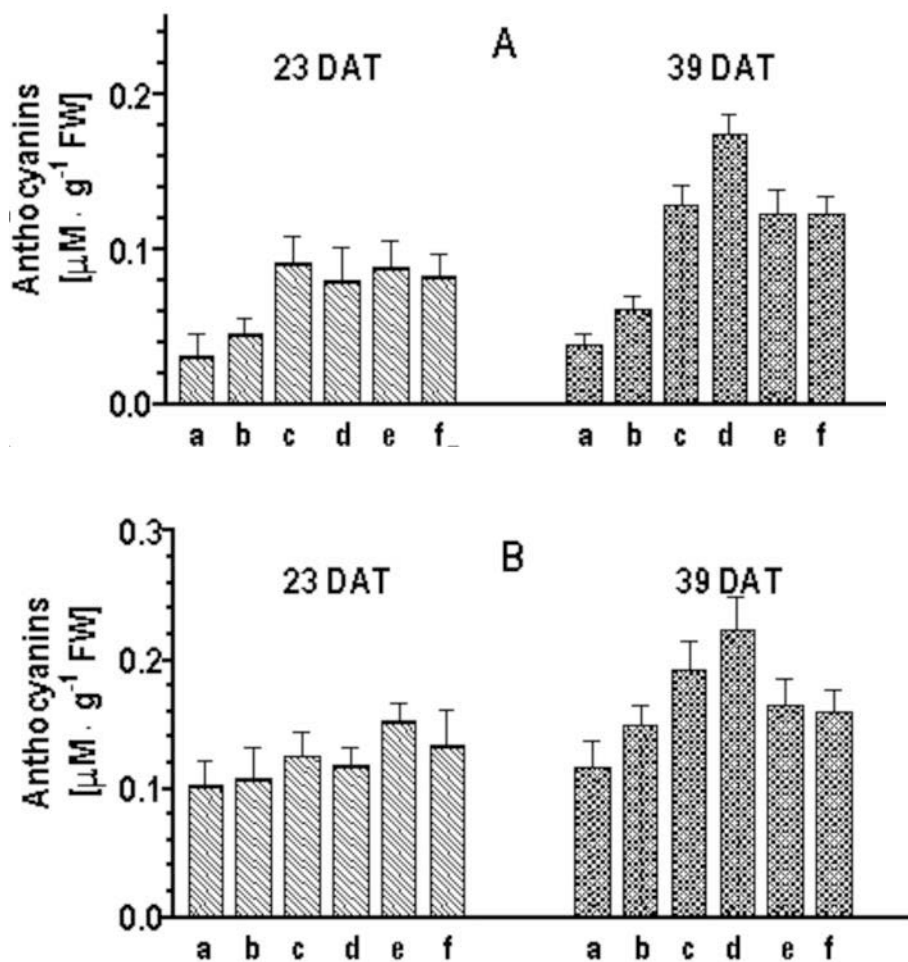


Fig. 3. The anthocyanins content in stem of *Crassula multicava* after treatment with JA Me applied in the middle part of internodes (by means of two laboratory replicates \pm SD); treatments were carried out in January, and anthocyanins were analyzed 23 and 39 days after treatment (DAT):

- A) in the middle part of internode,
- B) in internode directly above node,
- a) control, untreated,
- b) control, treated with lanolin only,
- c) JA Me 1.0%,
- d) JA Me 0.5%,
- e) JA Me 0.1%,
- f) JA Me 0.05%

middle part of each internode substantially stimulated anthocyanins accumulation in the stem of *Crassula multica* (Figs. 1-3). The highest level of anthocyanins was found in the stem treated with JA-Me at a concentration of 0.5%. In younger, upper parts of the stem, stimulation of anthocyanins biosynthesis was higher than in older, lower parts (Fig. 2). In control plants anthocyanins content ranged from 0.02 to 0.03 $\mu\text{M}\cdot\text{g}^{-1}$ fresh weight, and in treated plant stems from 0.04 to 0.10 $\mu\text{M}\cdot\text{g}^{-1}$. The experiment with various doses of JA-Me (started in January) has shown that accumulation of anthocyanins is continuing in time (Fig. 3). After 23 days from treatment (DAT), the level of anthocyanins was practically independent of the used JA-Me concentration, but 39 DAT the JA-Me at 0.5% concentration had higher effect on anthocyanins accumulation than the doses: 1.0, 0.1 or 0.05%. It seems that 1.0% JA-Me in lanolin paste can be phytotoxic for *Crassula multica* plants. Due to such a possibility, in the next experiment, carried out in June, the effect of 0.5% JA-Me on anthocyanins biosynthesis was only studied.

The mechanism of the stimulatory effect of JA-Me on anthocyanins biosynthesis in *Crassula multica* is unknown. One of the possible explanations for this is that JA-Me functions as a stress second messenger in plants. Support for this hypothesis was found by Farmer and Ryan (1990) who demonstrated that JA-Me induced the expression of proteinase inhibitor genes, known to be involved in resistance to herbivory. Finally, accumulation of high amounts of anthocyanins was the plant response to JA-Me signal.

It is already known that low concentrations of jasmonates induce expression of genes encoding enzymes of flavonoid biosynthesis: phenylalanine ammonia lyase, chalcone synthase, 4-coumarate CoA ligase, dihydroflavonol-4-reductase (Creelman et al. 1992; Gundlach et al. 1992; Tamari et al. 1995; Dittrich et al. 1992). Because the mentioned enzymes are involved in anthocyanins pathways, as well, the induction of genes responsible for their biosynthesis can have an indirect effect on anthocyanins accumulation, too.

Anthocyanins are one of the main classes of flavonoids which play important roles in the biology of plants by affecting several developmental processes (Taylor and Grote, 2005; Winkel-Shirley, 2002). Flavonoids, mainly quercetin, kaempferol and apigenin, are endogenous auxin transport inhibitors, inhibitors of lipid peroxidation, strong antioxidants, substrates for peroxidases, and play an important role in defense against pathogens and insects, as well as protect plants against stresses during vegetation and are involved in many other processes.

Studies on the identification of anthocyanin types induced by methyl jasmonate in *Crassula multica* and pathways of their biosynthesis are in progress.

CONCLUSIONS

1. Methyl jasmonate substantially stimulates anthocyanin accumulation in stem of *Crassula multica*.

2. Stimulatory effect of methyl jasmonate on anthocyanins accumulation depends on dose JA-Me, and age of plant tissue of *Crassula multica*.

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Indukcja akumulacji antocyjanów przez jasmonian metylu w pędach *Crassula multicava* Lam.

Streszczenie

W pędach *Crassula multicava* Lam. w naturalnych warunkach antocyjany tworzą się głównie w łodydze w pobliżu węzłów i tylko śladowe ilości w innych częściach międzywęzli. Jasmonian metylu (JA-Me) podany w paście lanolinowej w stężeniach 0,05, 0,1, 0,5 i 1,0% w środku poszczególnych międzywęzli silnie stymulował akumulację antocyjanów w międzywęzłach i węzłach *Crassula multicava*. Stymulacja ta była większa w młodszych, górnych tkankach pędu niż w starszych i zależna była od stężenia użytego JA-Me. Możliwa rola jasmonianów w akumulacji antocyjanów w pędach *Crassula multicava* jest dyskutowana.