Activity of cell wall degrading glycanases in methyl jasmonate-induced leaf abscission in *Kalanchoe blossfeldiana*

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A b s t r a c t

It was found previously that methyl jasmonate (JA-Me) induced leaf abscission in *Kalanchoe blossfeldiana*. In present studies it was shown that JA-Me markedly increased the total activities of cellulase, polygalacturonase, pectinase and xylanase in petioles, but did not affect activities of these enzymes in the blades and apical part of shoots of *K. blossfeldiana*. These results suggest that methyl jasmonate promotes the degradation of cell wall polysaccharides in the abscission zone and in this way induces leaf abscission in *Kalanchoe blossfeldiana*.

INTRODUCTION

Many studies concerned the role of jasmonic acid (JA) and methyl jasmonate (JA-Me) in senescence of different organs, mostly leaves (Ueda and Kato, 1980), but only a little attention has been paid to the role of hormones in abscission of leaves and other organs.

Curtis (1984) showed that methyl jasmonate slightly stimulated petiole abscission from debladed explants of *Vigna radiata* L. (Wilczek cv. Jumbo), whether applied proximally or distally, but did not induce leaf abscission from bladed explants.

Ueda et al. (1991) found that quantitative changes of abscisic acid and methyl jasmonate correlated with vernal leaf abscission of *Ficus superba* var. *japonica*, but the level of jasmonic acid was relatively low and remained constant in leaves during the growth cycle.

In further studies, Ueda et al. (1991 a) showed that JA and JA-Me promoted the abscission of bean petiole explants at concentration higher than 10 μM . These hormones had no effect on non-cellulosic polysaccharide contents in the pulvinus and the petiole sides of the abscission zone, but reduced the amount of cellulose in the petiole side.

Recently, Ueda et al. (1992, 1993) documented that jasmonic acid extremely increased the total acidity of cellulase in the pulvinus and the petiole of bean explants, and that JA-Me decreased the level of UDP-sugars in the abscission zone. The authors suggested that jasmonic acid promoted the degradation and also inhibited the synthesis of cell wall polysaccharides in the abscission zone, and as result promoted the abscission.

Also Hall and Horton (1994) investigated the effect of methyl jasmonate, both in solution and as a vapour, on the separation of pulvinar and petiolar tissues in explants containing the distal abscission zone of primary leaves of *Phaseolus vulgaris* cv. Contendor. These authors concluded that JA-Me had no apparent effect on abscission.

Recently, Saniewski and Węgrzynowicz-Lesiak (1994) showed that methyl jasmonate greatly promoted the leaf abscission in intact *Kalanchoe blossfeldiana* plants treated at different stages of growth and development. JA-Me evidently increased CO_2 evolution and slightly stimulated ethylene production. The leaf abscission induced by methyl jasmonate in *K. blossfeldiana* seemed not to be related to ethylene production. In further studies, Saniewski et al. (1994) documented that JA-Me did not affect or only slightly affected the content of free and bound fatty acids in petioles and blades. β -Sitosterol, campesterol and β -amyrin were identified in petioles and blades of *K. blossfeldiana*; JA-Me decreased the content of campesterol in petioles and increased the content of β -sitosterol in blades. It seems that leaf abscission induced by JA-Me is not connected with changes in content of free and bound fatty acids and sterols.

In this study the effect of methyl jasmonate on some cell wall degrading glycanases in petioles, blades and apical shoots of *Kalanchoe blossfeldiana* in relation to induced leaf abscission is presented.

MATERIAL AND METHODS

Kalanchoe blossfeldiana Poelln., a crassulacean acid metabolism (CAM) plant, is a short day plant. Cuttings (young shoots) were taken from intact *Kalanchoe* at the end of April and were rooted in the medium consisted of soil, peat moss and sand, under natural glasshouse conditions. After 1 month, the well rooted cuttings were planted individually in pots and cultivated according to standard conditions.

On September 25 and on November 23 the plants were sprayed with methyl jasmonate solution at the concentration of 800 mg/l in 0.05 % Tween 80. Plants treated with 0.05 % Tween 80 were used as a control. Six to ten plants were used per

treatment and 30 ml of solution per plant were used for spraying. One day after treatment the whole petioles, leaf blades and apical part of shoots (about 5 mm long) from 6 plants were collected and frozen at -20°C. Frozen samples were used for analysis of enzyme activities.

Enzyme activity assay. Enzyme extracts were prepared according to the method described earlier (Urbanek et al., 1991).

To determine pectinase, polygalacturonase, cellulase and xylanase activities, pectin NF, sodium polygalacturonate (Serva), CM-cellulose and xylan (Koch-Light) were used as the substrates, respectively.

Reaction mixture consisted of 0.1 ml of 1 % substrate solution, 0.2 ml of 0.1 M acetate buffer pH 5.0 and 0.2 ml of enzymatic solution. After 2 h of incubation at 30°C the amount of reducing groups released in the reaction mixtures was measured according to Nelson's method (Nelson, 1944). One unit of enzymatic activities was defined as the amount of enzyme liberating 1 μ mole of reducing groups per 2 hours. The results presented in the table 1 are the means of four measurements.

RESULTS AND DISCUSSION

Abscission control is important commercially for many agricultural crops; both the induction and prevention or delay of abscission are valuable measures to the grower (Nicklell, 1991). The mechanism of abscission of leaves and other organs, including hormonal control of the process (abscisic acid, ethylene, auxin), has been reviewed by many authors (Sexton and Roberts, 1982; Jankiewicz, 1985; Osborne, 1989; Reid and Goszczyńska, 1985). Abscission zone cells are produced in limited numbers only and usually constitute a plate of one or two rows across the base of the organ that is shed (Osborne, 1989). Immediately before and during cell separation hydrolases for cell wall weakening are produced and in some cases it has been shown that these enzymes are synthesized de novo (Sexton and Roberts, 1982).

Spraying of intact *Kalanchoe blossfeldiana* with methyl jasmonate at the concentration of 800 mg/l induced all leaves abscission two days after treatment (Fig. 1). All the analyses of enzyme activities were made one day after treatment with JA-Me when the leaves abscised easily after gentle shaking.

Activities of polygalacturonase and xylanase were difficult to detect in petioles of *Kalanchoe blossfeldiana* but methyl jasmonate slightly increased activities of these enzymes (Tab. 1). Also activity of cellulase was low in petioles and JA-Me increased it 2-3 times (Tab. 1). Activity of pectinase in petioles evidently increased after treatment with JA-Me (Tab. 1).

Generally, the observed changes in the activities of polygalacturonase, pectinase and xylanase in blades after treatment with JA-Me were very small and cellulase activity was not detected in blades (Tab. 1).

Fig. 1. The effect of methyl jasmonate at the concentration of 800 mg/l on leaf abscission of *Kalanchoe blossfeldiana*

Photograph taken on September 26: on the left – JA-Me 800 mg/l, on the right – control

Table 1

Activity of some glycanases in petioles, blades and apical shoots of *Kalanchoe blossfeldiana* after treatment on September 25 and November 23 with methyl jasmonate – inducing leaf abscission; activities (in units/g of fresh weight) measured 1 day after treatment

Enzyme	Petioles		Blades		Apical shoots	
	Control	JA-Me	Control	JA-Me	Control	JA-Me
Treatment on September 25						
Polygalacturonase	0	0.78	0.92	1.13	31.03	27.94
Pectinase	7.70	11.80	14.10	13.63	22.26	21.53
Xylanase	0	1.14	0.52	0.80	40.70	41.14
Cellulase	0.30	0.87	0	0	23.84	30.26
Treatment on November 23						
Polygalacturonase	0	0.05	0.28	0.37	4.71	6.20
Pectinase	3.73	6.51	6.00	7.45	16.92	18.70
Xylanase	0	0	1.10	1.35	11.47	10.57
Cellulase	0.44	0.89	0	0	7.79	8.82

High activities of all analysed enzymes, polygalacturonase, pectinase, xylanase and cellulase, were found in apical part of shoots but methyl jasmonate had no effect on activity of these enzymes (Tab. 1).

It should be mentioned that activities of all analysed enzymes in petioles, blades and apical shoots were higher in plants *K. blossfeldiana* taken for treatment and analysis on September 25 than on November 23.

As enzyme activation or biosynthesis is most probably localized in separation zone cells thus the results obtained from hydrolase analysis of the whole petioles might be lower than those which would be obtained only from separation zone.

These results suggest that methyl jasmonate promotes the degradation of cell wall polysaccharides in the abscission zone and in this way induces leaf abscission in *Kalanchoe blossfeldiana*; this hypothesis was previously formulated by Ueda et al. (1992, 1993).

Dörffling et al. (1978) found that stalks of young apple fruits cv. Golden Delicious taken off at the beginning of July, senescent petioles of *Coleus rehneltianus*, *Phaseolus vulgaris* and *Acer pseudoplatanus* contained at least three abscission-accelerating substances: two identified as abscisic acid (ABA) and xanthoxin and the third unidentified substance which had acidic properties and was less polar than ABA. It is possible that the third unidentified substance could be jasmonic acid or methyl jasmonate, since young apple fruits contain both JA and JA-Me (Meyer et al., 1984).

Jasmonic acid and its derivatives have a similar chemical structure and biosynthetic pathway to prostaglandins, which are important animal hormones and potent modulators of inflammatory responses. It should be mentioned that naturally occurring prostaglandins have been found also in plants, but reports on their existence and physiological role are limited (Saniowski, 1989). Prostaglandin $F_{2\alpha}$ was identified in flowering *Kalanchoe blossfeldiana*, but this compound could not be detected under long-day conditions (Janistyn, 1982).

The mechanism of leaf abscission induced by methyl jasmonate in *Kalanchoe blossfeldiana* will be the subject of further studies.

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Aktywność glukanaz rozkładających ściany komórkowe w czasie indukowanego odpadania liści u *Kalanchoe blossfeldiana* przez ester metylowy kwasu jasmonowego

Streszczenie

Poprzednio wykazano, że ester metylowy kwasu jasmonowego (JA-Me) indukuje odpadanie liści u *Kalanchoe blossfeldiana*. W obecnych badaniach wykazano, że JA-Me znacznie podwyższa całkowitą aktywność celulazy, polygalakturonazy, pektynazy i ksylanazy w ogonkach, ale nie wpływa na aktywność tych enzymów w blaszkach liściowych i pędach wierzchołkowych *Kalanchoe blossfeldiana*.

Otrzymane wyniki sugerują, że ester metylowy kwasu jasmonowego stymuluje degradację polisacharydów ścian komórkowych w warstwie odcinającej i w ten sposób indukuje odpadanie liści u *Kalanchoe blossfeldiana*.