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Gum in apricot (*Prunus armeniaca* L.) shoots induced by methyl jasmonate

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Summary

It has been well known that some fungal pathogens (*Monilia laxa*, *M. fructigena, Cytospora cincta*), larvae of *Grapholita molesta* and plant hormone – ethylene, induce gummosis in apricot shoots. Methyl jasmonate (JA-Me) was also found to induce gummosis in apricot shoots as well as biotic and abiotic factors mentioned above. In order to know the mode of action of JA-Me on gum induction and/or formation, chemical composition of polysaccharides (after hydrolysis) in gums of apricot shoots induced by JA-Me compared with those by ethephon and their mixture, and naturally occurring ones was studied, resulted in the succesful identification of monosaccharides, and the similarity of a composition consisting of xylose, arabinose and galactose at molar ratio 1 : 10 : 14, respectively. These results suggest that beside different inducers of gum in apricot the mechanism of polysaccharides biosynthesis of gums is the same or similar. The physiological role for JA-Me on gum induction and/or formation in apricot shoots, and other species are also discussed.

Key words: apricot shoot, gum induction, methyl jasmonate, neutral sugar composition, polysaccharides, *Prunus armeniaca* L.

INTRODUCTION

Phenomena of gummosis are widely found in the plant kingdom. Gums in plants are induced by environmental stress factors such as pathogens infection, insect attack, mechanical and chemical injury, flooding stress and others. All of environmental factors stimulating gum exudation have been shown to promote ethylene production in plant tissues as well (B o o t h b y, 1983). Ethylene or ethylene-releasing compounds (i.e. ethephon; 2-chloroethylphosphonic acid) also stimulate gum formation in stonefruit trees and their fruits of the family *Rosaceae*, e.g. apricot (*Prunus armeniaca* L., *Prunus mume* L.) (B r a d l e y et al., 1969; L i et al., 1995), cherry (*Prunus cerasus* L.) (O l i e n and B u k o v a c, 1982a,b), peach (*Prunus persica* Batsch.) (B u c h a n a n and B i g g s, 1969; L i et al., 1995), plum (*Prunus domestica* L.) (B u k o v a c et al., 1969), and almond (*Prunus amygdalus* Batsch.) (R y u g o and L a b a v i t c h, 1978). It has been found that tulip bulbs infected by *Fusarium oxysporum* f. sp. *tulipae* produce considerable quantities of ethylene, being enough to cause gummosis in diseased and healthy bulbs stored in the same conditions (K a m e r b e e k and D e M u n k, 1976). K a m e r b e e k and D e M u n k (1976) have already reported that exogenous ethylene was also an inducer of gums formation in tulip bulbs and other bulbous ornamental plants.

On the other hand, it has recently been shown that jasmonates, jasmonic acid (JA) and methyl jasmonate (JA-Me), a new group of plant hormones (U e d a and K a t o, 1980; S a n i e w s k i, 1995; C r e e l m a n and M u l l e t, 1997; K o i w a et al., 1997; M u r o f u s h i et al., 1999), have a promoting effect on the induction and/or production of gums in tulips (S a n i e w s k i and P u c h a l s k i, 1988; S a n i e w s k i, 1989; S a n i e w s k i et al., 1998a, c; S a n i e w s k i et al., 2000b), peach (S a n i e w s k i et al., 1998b), plum and cherry (S a n i e w s k i et al., 1998d). Rapid increase of endogenous jasmonates occurred in plant tissues under stress conditions: pathogen infection, insect attack, mechanical wounding, and osmotic stress (S a n i e w s k i, 1997). Jasmonates has already been reported to control ethylene production in plant tissues (S a n i e w s k i, 1997). These observations strongly suggest that jasmonates are crucial compounds in induction and formation of gums in plants.

Chemical compositions of gums induced in several plants have already been reported.

Gums are a complex of polymeric structures contained mainly, as a monomers, pentoses, hexoses and uronic acids (B o o t h b y, 1983). Unfortunatelly, physiological role of gums in plants has not been clear yet but they seem to play important function in limiting the spread of pathogens and insects by isolating the infected tissues (B o o t h b y, 1983).

Gums in apricot (*Prunus armeniaca* L.) shoots have also been found after infection of some fungal pathogens (*Monilia laxa, M. fructigena, Cytospora cincta*), and by injury of larvae of *Grapholita molesta* (R o s i k et al., 1971; 1975). From the point of views described above, we want to know a possible involvement of JA-Me on gum formation in shoots of apricot. Neutral sugar composition of polysaccharides of apricot gum induced by JA-Me was also determined.

MATERIAL AND METHODS

Current growing shoots and 2-, 3-year old shoots of apricot (*Prunus armeniaca* L.) were treated with lanolin only (control), methyl jasmonate at concentrations of 0.5 and 1.0%, ethephon (2-chloroethylphosphonic acid) at a concentration of 1.0% and their mixture in lanolin paste as a ring about 4 mm width after gently abraded with a polishing cloth. Treatments were made at the end of July of 1998 and 2000. To analysis gums was collected from ten shoots per treatment.

Composition of polysaccharides in apricot gum formed by JA-Me, ethephon, their mixture, and naturally formed (possibly due to pathogen invasion) were determined separately. The analytical procedure was based on method described by B l a k e n e y et al. (1983) with minor modifications.

Gum was suspended in 72% sulphuric acid, and kept at room temperature overnight (16 hours). Afterwards samples were diluted with 5 volumes of distilled water, and heated at 100°C for 3 hours. After cooling solutions were adjusted to pH 12 with concentrated ammonia solution.

Monosaccharides contained in hydrolysate of gum were reduced to corresponded alditols with sodium borohydride dissolved in anhydrous dimethyl sulphoxide (DMSO) at temperature 40°C during 90 min. The excess of borohydride was decomposed by addition of acetic acid.

Such obtained alditols were acetylated in room temperature by freshly prepared mixture 1-methylimidazole-acetic anhydride (1:10). The excess of acetic anhydride was decomposed by adding 10 volumes of distilled water. Alditol acetates were extracted with chloroform and determined using a gas-liquid chromatograph. To prepare standard curves, mixtures of 10 to 500 micrograms of rhamnose, arabinose, xylose, mannose, galactose and glucose, and 100 micrograms of internal standard (*myo*-inositol) were reduced and acetylated by the method described above. Alditol acetates were separated on column filled with 10% Silar 10C on Chromosorb W, 80-100 mesh (200 cm x 2 mm i.d.) fitted to a Pye Unicam 204 chromatograph equipped with flame-ionization detector. The injection port and detector were kept in 250°C. The column oven temperature was kept for 1 min at 180°C following injection, and then raised at 4°C/min to 230°C, where it was maintained for 15 min.

RESULTS AND DISCUSSION

Methyl jasmonate at concentrations of 0.5 and 1.0% in lanolin paste, and ethephon at a concentration of 1.0% in lanolin paste, induced gum formation in one-year-old and older shoots of apricot (*Prunus armeniaca*). First symptoms of gummosis were observed 7 days after treatment, both in case of JA-Me and ethephon treatments. Largest amounts of gums were obtained at 12-day after treatments.

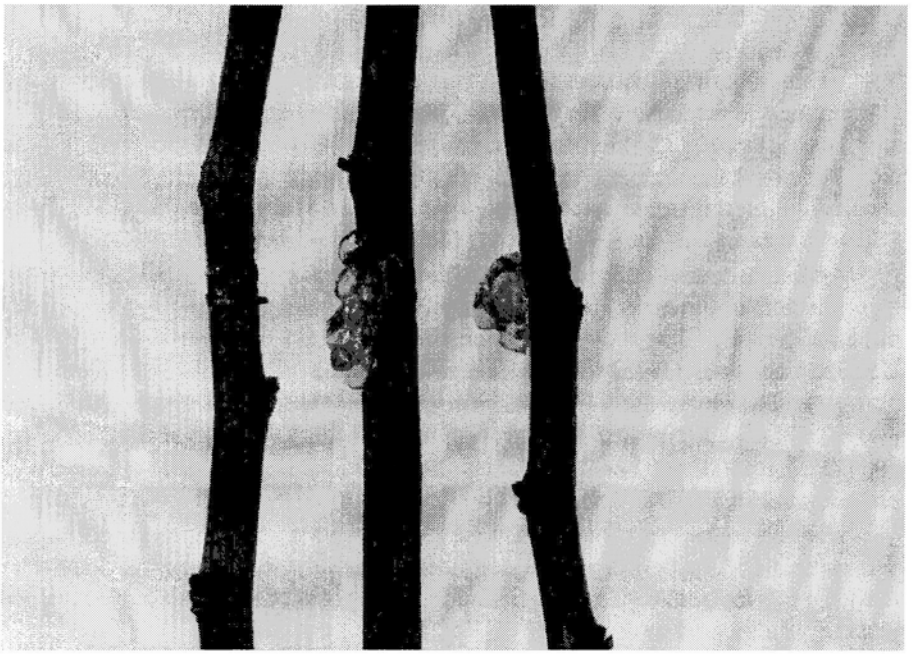

Fig. 1. Gum formation on the apricot shoots induced by JA-Me and ethephon; on left - control, lanolin only; in middle – JA-Me 1.0% in lanolin; on right ethephon 1.0% in lanolin

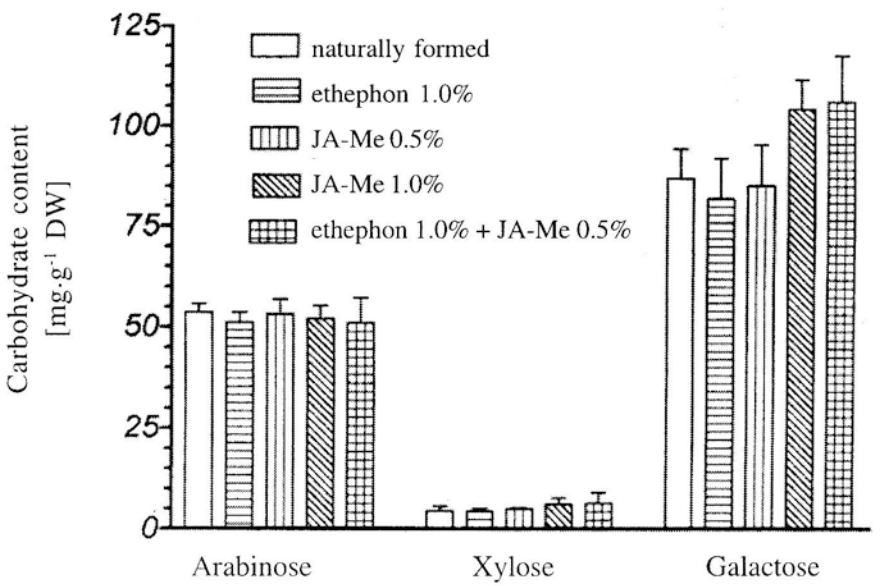

Fig. 2. Neutral sugar compositions of gums in apricot shoots induced by JA-Me, ethephon, their mixture and formed under influence unknown factor (possibly by pathogens)

Compositions of neutral sugars in polysaccharides of apricot gums induced by different chemicals of JA-Me, ethephon, mixture JA-Me and ethephon as well as naturally formed one were almost similar. The molar ratio of galactose, arabinose and xylose found in gums was 14 : 10 : 1, respectively. Uronic acids contents, however, have not been determined yet.

According to R o s i k et al. (1971) apricot gums formed by *Cytospora cincta* Sacc. contained galactose, arabinose, xylose, mannose, glucuronic acid and 4-O-methyl-glucuronic acid. Similary to our results data obtained by R o s i k et al. (1971) indicate that polysaccharides of apricot gums contained two main carbohydrates: galactose and arabinose, with xylose as minor component. Different result between R o s i k ' s and present investigations is the presence of mannose in polysaccharides of apricot gums, being probably due to differences in cultivated conditions of plants. It has been known that cultivated conditions affect chemical compositions of gums (R o s i k et al., 1971).

Results obtained in this study suggest that a similar sugar metabolism operates to induce gums in apricot shoots besides of different inducers since sugar compositions of polysaccharides of gums induced by different inducers are quite similar (or the same). It is possible that gum is synthesized de novo from sucrose or glucose via nucleoside diphosphate derivatives. Some papers reported that such gum formation is accompanied by decline of starch granules, and/or by breakdown of cell wall polysaccharides (B o o t h b y, 1983; N a i r et al., 1980, M o r r i s o n et al., 1987a, b; M o r r i s o n and P o l i t o, 1985; S a n i e w s k i and D y k i, 1997).

Methyl jasmonate applied exogenously caused gummosis in apricot shoots as well as tulip bulbs, stem and basal part of leaves, in opposite to action of ACC which not formed gum in stem and leaves of tulips (S a n i e w s k i and W ę g r z y n o w i c z - L e s i a k, 1994, 1995). On the other hand, simultaneous application of JA-Me and ACC greatly accelerates gum formation in stem and basal part of leaves of tulips in comparison to JA-Me used alone (S a n i e w s k i et al., 1998c; S a n i e w s k i et al., 2000b). This result was true in peach shoot (S a n i e w s k i et al., 1998b). Judging from the fact described above, the process of gum induction in plants may be regulated directly by ethylene, JA-Me or by a signal network in which individual are signals mediated by ethylene and jasmonates „cross-talk" (S a n i e w s k i et al., 1999). It is possible that in the case of exogenously applied ethylene (or ethylene releasing compounds) or JA-Me, interaction with endogenous jasmonate or ethylene, gene(s) expression responsible for gum biosynthesis take place. As physiological role of JA-Me for gum induction and/or formation in apricot shoots as well as other species has not been clear yet, further investigations in relation to molecular analyses for this kind of „cross-talk" are required in future.

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Indukcja gum przez jasmonian metylu w pędach moreli (*Prunus armeniaca* L.)

Streszczenie

W pędach moreli (*Prunus armeniaca* L.) gumy mogą być indukowane pod wpływem infekcji przez patogeny grzybowe (*Monilia laxa, M. fructigena, Cytospora cincta*), larwy *Grapholita molesta* i egzogennie podany etylen. W badaniach własnych stwierdzono, że jasmonian metylu (JA-Me) w stężeniu 0.5 i 1.0% w paście lanolinowej podany na jednoroczne i starsze pędy moreli indukuje (podobnie jak etylen) tworzenie się gum po 7 dniach od traktowania. Skład cukrów gum moreli analizowano przy użyciu chromatografii gazowej, po uprzedniej hydrolizie kwasowej polisacharydów, redukcji monocukrów do odpowiednich alkoholi cukrowych i po przekształceniu ich w pochodne acetylowe. Oddzielnie analizowano gumy indukowane przez etefon, jasmonian metylu i mieszaninę etefonu z JA-Me oraz gumy naturalnie wytworzone na pędzie (nie określono pod wpływem jakiego czynnika). We wszystkich gumach, niezależnie od czynnika indukującego, stwierdzono zbliżony jakościowy i ilościowy skład cukrów. Gumy te zawierają ksylozę, arabinozę i galaktozę w proporcji molowej odpowiednio: 1 : 10 : 14. Nie określano zawartości kwasów uronowych, które również występują w gumach moreli. Można przypuszczać, że biosynteza polisacharydów gum w pędach moreli, bez względu na czynnik indukujący, przebiega podobnie, jeśli nie identycznie. Fizjologiczna rola jasmonianu metylu w indukcji i tworzeniu gum w pędach moreli i w innych gatunkach roślin jest w pracy dyskutowana.