Journal of Plant Physiology 174 (2015) 1–4

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Short Communications

Hormonal regulation of gummosis and composition of gums from bulbs of hyacinth (*Hyacinthus orientalis*)

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ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 16 October 2014
Accepted 16 October 2014
Available online 27 October 2014

Keywords:
Ethylene
Gummosis
Hyacinth (*Hyacinthus orientalis*)
Methyl jasmonate
Sugar composition

ABSTRACT

Hyacinth (*Hyacinthus orientalis*) bulbs infected by *Fusarium oxysporum* showed the symptoms of gummosis. The purpose of this study was to clarify the hormonal regulation of gummosis and composition of gums from hyacinth bulbs. The application of ethephon (2-chloroethylphosphonic acid), an ethylene-releasing compound, at 2% (w/w, in lanolin) induced gummosis in hyacinth bulbs. Methyl jasmonate (JA-Me) at 1.5% (w/w, in lanolin) induced gummosis as well. Simultaneous application of JA-Me and ethephon further enhanced gummosis. Molecular mass distribution of hyacinth gums analyzed by gel permeation chromatography indicated that the gums were mainly homogenous polysaccharides with an average molecular weight of ca. 30 kDa. Analysis of the sugar composition of the gums after hydrolysis revealed that the majority were arabinose (ca. 35%) and galactose (ca. 40%) together with small amounts of fucose, rhamnose and uronic acids (ca. 5%, respectively), suggesting that the gums are pectic arabinogalactans. These results indicate that jasmonates (JAs) interact with ethylene to stimulate sugar metabolism, producing pectic arabinogalactans, and vice versa, leading to gummosis. These findings, together with those from our previous studies in tulips (*Tulipa gesneriana*) and grape hyacinth (*Muscari armeniacum*), revealed that sugar metabolism and hormonal regulation relating to gummosis are different among species of bulbous plants.

Introduction

Natural gums are organismic exudates mainly consisting of polysaccharides (Boothby, 1983). They are used in the food, pharmaceutical and chemical industries for emulsification, thickening, stabilization and other processes (Verbeken et al., 2003). Gummosis, the process of the induction, accumulation and exudation of gums, is found throughout the plant kingdom, especially in Rosaceae. Gummosis is induced by biotic and abiotic stress forms such as bacterial and fungal infections, insect attacks and mechanical injuries (Boothby, 1983). It is part of the plant defense system, since gums can impede the spread of diseases by isolating and sealing infected or infested tissues, thus preventing entry and movement of pathogens, the loss of water from the damaged tissues and other harmful occurrences.

Hormonal regulation of gummosis has been intensively studied in the trees and fruits of stone-fruit species of the Rosaceae family (Olien and Bukovac, 1982; Boothby, 1983; Morrison et al., 1987; Saniewski et al., 2006). Since all the abiotic and biotic environmental stress factors mentioned above are considered to be mediated by the action of ethylene produced in plant tissues, and exogenous ethylene or ethylene-releasing compounds (i.e. ethephon; 2-chloroethylphosphonic acid) induce gummosis, ethylene is considered to be the common factor for gummosis (Boothby, 1983). As well as ethylene, jasmonic acid (JA) and methyl jasmonate (JA-Me), known as jasmonates (JAs), also play important roles in the signal transduction pathways in response to stresses (Koiwa et al., 1997; Wasternack and Hause, 2013). JAs have also been reported to induce gummosis in various stone-fruit species of Rosaceae such as

Abbreviations: HPAEC-PAD, high performance anion-exchange chromatography with pulsed amperometric detection; JA, jasmonic acid; JA-Me, methyl jasmonate; JAs, jasmonates.

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http://dx.doi.org/10.1016/j.jplph.2014.10.007
0176-1617/

peach, plum and apricot (Saniewski et al., 1998a, 2004, 2006). The mechanism by which JAs and ethylene induce gummosis has not yet been adequately clarified.

Gummosis also takes place in bulbous plants such as tulip (*Tulipa gesneriana*), grape hyacinth (*Muscari armeniacum*) and *Narcissus*, especially in *Fusarium*-infected bulbs (Moore, 1949; Rees, 1972; Saniewski et al., 1998b). In tulip bulbs, infection with *Fusarium oxysporum* f. sp. *tulipae* or the application of ethylene (Kamerbeek and De Munk, 1976; De Hertogh et al., 1980; De Munk and Saniewski, 1989; De Wild et al., 2002) or JAs (Saniewski et al., 1998b; Skrzypek et al., 2005a,b) can produce large quantities of gums, suggesting that pathogen-induced or pathogen-produced ethylene or JAs in plant tissues are involved in gummosis (see review, Saniewski et al., 2007). In tulip shoots, JAs induced gummosis but ethylene did not, whereas ethylene synergistically stimulated gummosis induced by JAs, suggesting that JAs are essential or principal factors inducing gummosis in tulip shoots (Skrzypek et al., 2005a,b). On the contrary, in grape hyacinth bulbs, JA-Me alone did not induce gummosis, but significantly enhanced the ethylene-induced production of gums (Miyamoto et al., 2010). The sugar composition of gums from tulips and grape hyacinths were quite different (Skrzypek et al., 2005b; Miyamoto et al., 2010). These results suggest that the metabolism and the principal factor inducing gummosis are different among these plants, whereas the hormonal regulation of gummosis in other bulbous plants is much less studied.

Hyacinth bulbs are damaged by several bacterial and fungal diseases. Some pathogens such as *Pectobacterium carotovorum* subsp. *carotovorum* (formerly known as *Erwinia carotovora* subsp. carotovora) and *Dickeya* spp. (formerly *Erwinia chrysanthemi*) are known to induce gummosis when they infect hyacinth bulbs (Kamerbeek and De Munk, 1976; Van Doorn et al., 2011). We recently found that some infected hyacinth plants showed the symptoms of gummosis in the Polish plantations. The pathogen was identified as *F. oxysporum*, based on the key morphological and cultural characteristics given for *Fusarium* (Booth, 1971). *F. oxysporum* is known to cause not only gray-brown lesions scattered over the bulb scales of hyacinths but also basal rot. Little is known about gummosis in hyacinth bulbs. The purpose of this study is to clarify the hormonal regulation of gummosis and the chemical composition of gums in hyacinth.

Material and methods

Plant materials and hormone treatment

Hyacinth (*Hyacinthus orientalis* L.) bulbs were dug out from an experimental field in Poland. After lifting, the bulbs were stored at room temperature (17–22 °C) until use. Intact and wounded (where the basal plate is scooped out) bulbs were treated with ca. 350 mg of lanolin (control), or the ethylene-releasing substance ethephon (2-chloroethylphosphonic acid, 2%, w/w), methyl jasmonate (JA-Me) (1.5%, w/w), or a mixture of ethephon and JA-Me in lanolin paste. These chemical treatments were applied to the basal plate of intact bulbs or on the cut surface of wounded bulbs during the period of July to August. The treated bulbs were stored at room temperature (17–22 °C). The doses of JA-Me and ethephon were based on our previous experiments with tulip and grape hyacinth bulbs (Skrzypek et al., 2005b; Miyamoto et al., 2010).

After incubation, gummosis was observed by eye and photographed. Gums were collected, dried at room temperature and kept in the refrigerator until analysis. A sample of 10–12 bulbs was used in each treatment. A significant amount of gum was formed by the simultaneous application of ethephon and JA-Me, so these gums were subjected to sugar analysis.

Sugar analyses of hyacinth bulb gum

Hyacinth gum was dissolved in hot water and the solution was centrifuged at 3000 × *g* for 10 min. Almost all gum was recovered in the supernatant. Total sugar and uronic acid contents were determined by the phenol-sulfuric acid method (Dubois et al., 1956) using glucose (Glc) as a standard, and by the carbazole-sulfuric acid method using glucuronic acid (GlcA) as a standard (Galambos, 1967), respectively.

The molecular mass of hyacinth gum was estimated by gel permeation chromatography with a gel-permeation column (TSK-gel G5000PW, Tosoh Co. Ltd., Tokyo, Japan) according to the method of Wakabayashi et al. (1997). A portion of gum dissolved in hot distilled water was subjected to a HPLC (LC-6A, Shimadzu Co. Ltd., Kyoto, Japan) equipped with a refractive index detector (RID-6A, Shimadzu Co. Ltd., Kyoto, Japan). The sample was eluted with the potassium phosphate buffer (50 mM, pH 7.2) at a flow rate of 1 mL/min, and fractions (0.5 mL volume) of the sample were collected. Total sugar contents in each fraction were determined by the phenol-sulfuric acid method. The average molecular mass of gum was estimated according to the equation reported by Nishitani and Masuda (1981). Dextrans (Sigma) of 10, 40, 70, 120 and 500 kDa were used as molecular mass markers.

Sugar composition of gum was analyzed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using a Dionex DX-500 liquid chromatograph fitted with a CarboPac PA-1 column (4 mm × 250 mm, Dionex Japan, Osaka, Japan) as described by Ishikawa et al. (2000) and Konishi et al. (2008). A portion of gum solution dissolved in distilled hot water was air-dried, then hydrolyzed with 2 N trifluoroacetic acid at 121 °C for 1 h. The samples were air-dried, then subjected to HPAEC-PAD analysis.

Results and discussion

Hormonal regulation of gummosis in hyacinth bulbs

Infection by *P. carotovorum* subsp. *carotovorum* substantially induces ethylene production in hyacinth bulbs (Van Doorn et al., 2011). Exogenosly applied ethylene has been reported to induce gummosis in hyacinth bulbs (Kamerbeek and De Munk, 1976). The infection of *F. oxysporum* f. sp. *tulipae*, and the application of ethylene or JAs to tulip bulbs have been reported to produce large quantities of gum (see review, Saniewski et al., 2007). Thus pathogen-induced ethylene and/or JAs in plant tissues, or these compounds produced by pathogens, are possibly involved in gummosis.

As shown in Fig. 1A, the application of ethephon at 2% (w/w in lanolin) to the basal plate of intact bulbs induced gummosis within several days, with gums being exuded around the basal plate application site and also being observed on the top of bulbs through the inside of the bulbs, whereas lanolin alone had no effect. JA-Me at 1.5% (w/w in lanolin) also induced gummosis. Simultaneous application of JA-Me and ethephon led to enhanced induction of gummosis. These facts suggest that both JAs and ethylene are principal factors in the induction of gummosis in hyacinth bulbs.

Fig. 1B showed the effect of ethephon and/or JA-Me on gummosis in wounded bulbs whose basal part was scooped out. Only slight gummosis was observed in the wounded bulbs induced solely by the scooping out of the basal part of the bulbs. The application of ethephon or JA-Me substantially induced gummosis. It is possible that the gummosis in wounded bulbs is due partly to the accumulation of the phytohormones ethylene and/or JAs in response to wounding.