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EFFECT OF CHITOSAN AND TULIP POLYSACCHARIDE GUM ON RED PIGMENT FORMATION IN WOUNDED BULBS OF *Hippeastrum* × *hybr.* hort.

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Introduction

Mechanically wounded, infected by *Phoma narcissi* or infested with mite *Steneotarsonemus laticeps* various organs of *Hippeastrum* induce synthesis of a red pigment in injured tissues [SANIEWSKA 1998]. The chemical nature of red pigment is not fully determined but it belongs to a class of oxidized flavan(s) [WINK, LEHMANN 1996; SANIEWSKA, BUDZIANOWSKI 1997; BUDZIANOWSKI, SANIEWSKA, unpublished]. Plant defense systems against biotic (pathogen and insect) and abiotic (mechanical wounding) stresses can be separated into two major categories: secondary metabolites and specific proteins. For a few years jasmonic acid (JA) is recognized as a key intercellular signal in mediating responses to the pathogen infection, insect attact, wounding, and elicitors such as chitosan, oligogalacturonides and systemin [DE BRUXELLES, ROBERTS 2001]. Thus, jasmonates represent an integral part of the signal transduction chain between stress signal(s) and stress responses.

It is well known that different kinds of oligosaccharides can function in plants as molecular signals (elicitors) that regulate growth, development and survival in the environment, through elicitation of various physiological and biochemical processes [EBEL, MITHÖFER 1998; CÔTE, HAHN 1994; ALDINGTON et al. 1991; DARVILL et al. 1992].

SANIEWSKA [2002a, 2002b] showed a strong stimulatory effect on mycelium growth and sporulation of *Fusarium oxysporum* f. sp. *tulipae* when tulip gums induced by this pathogen were added into some culture media such as potato dextrose agar (PDA), malt extract agar (MEA), and mineral, solid Czapek Dox Agar (CzDA). It seems that polysaccharides of tulip gums may act as elicitors, which control some processes connected or responsible for mycelium growth and sporulation, or partially can be used as a substrate for growth of cell wall of the pathogen.

WINK and LEHMANN [1996] showed that cell walls of *Saccharomyces cerevisiae* applied to wounded *Hippeastrum* scales bulb strongly increased biosynthesis of a red pigment in comparison to the control. On the other hand cell walls isolated from *Lactobacillus casei* had no such influence.

The aim of this work was to study the effect of chitosan and tulip gums, on a red pigment accumulation in wounded scales of *Hippeastrum* bulbs.

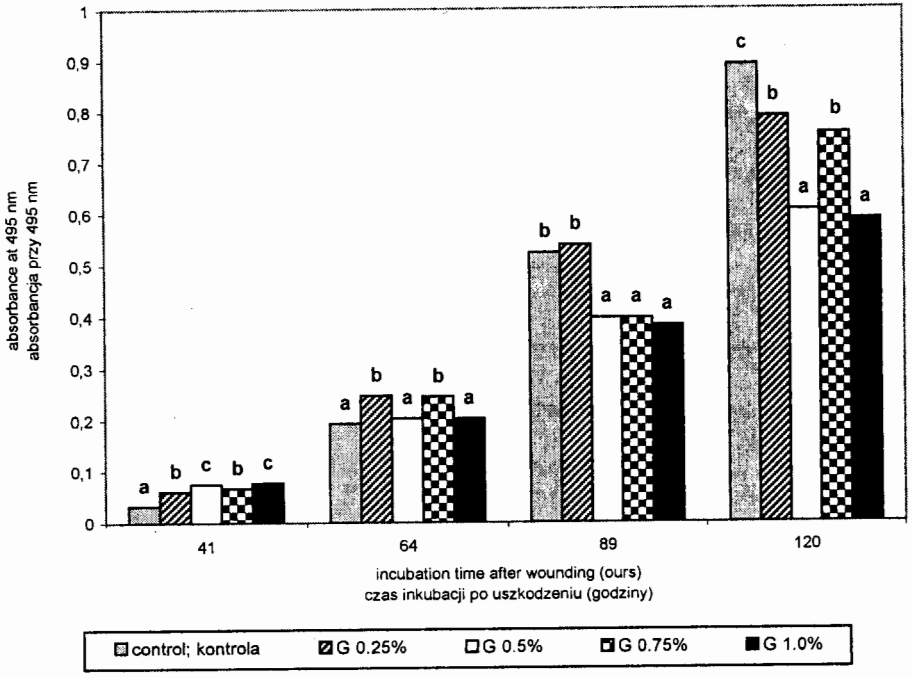
Material and methods

Hippeastrum x *hybr.* hort. 'Jan' was used in the experiments. The scales were cut into small pieces (dimension ca. 4 x 4 mm) and dipped for 3 h in water (control) or in 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5% chitosan dissolved in 0.3% glutamic acid, or in 0.25, 0.5, 0.75 and 1.0% water solution of tulip gum. Then the tissue samples were drained off and stored in closed Petri dishes at 20–25°C placed in a chamber with high humidity maintained. The pigment was extracted with 90% methanol and the absorbance of the extracts was measured at 495 nm.

The data were subjected to an analysis of variance and Duncan's multiple range test at 5% of significance level it was used for means separation.

Results and discussion

Tulip bulbs infected by *Fusarium oxysporum* f. sp. *tulipae* were shown to produce considerable quantities of ethylene, enough for gums production in diseased and healthy bulbs stored in the same conditions [KAMERBEEK, DE MUNK 1976]. The gummosis in tulips was induced in healthy bulbs by exogenously applied ethylene or ethylene releasing compound, ethephon. Main constituents of tulip gum polysaccharides are xylose, arabinose and uronic acid(s) [SANIEWSKI et al. 2000].



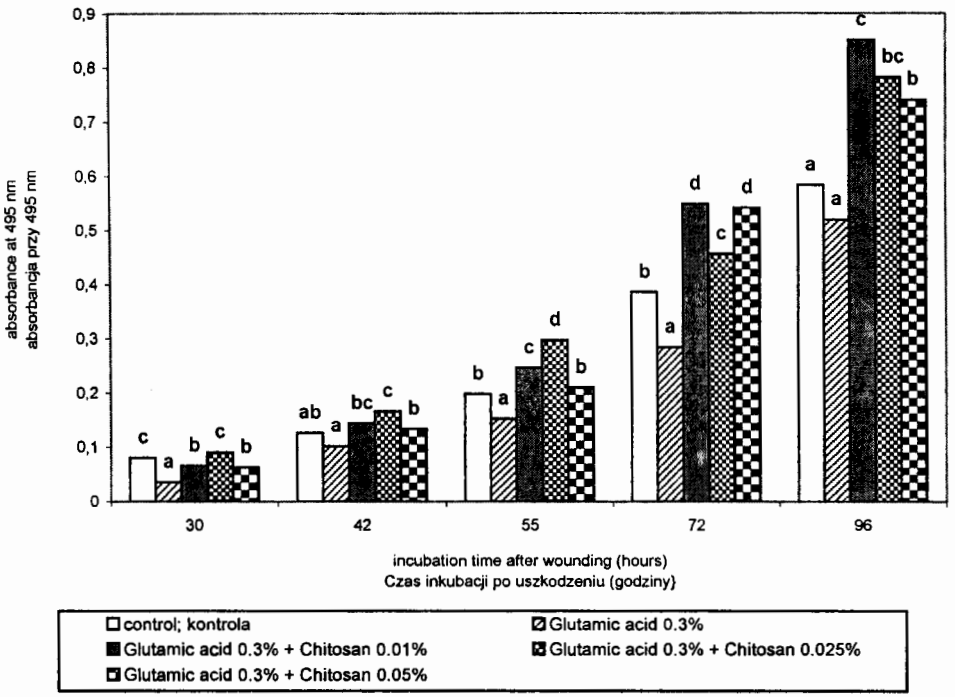
Note: in each of incubation time, means followed by the same letter are not significantly different at P = 0.05 according to Duncan's test; Objaśnienie: dla każdego czasu inkubacji średnie oznaczone tą samą literą nie różnią się istotnie (5%) według testu Duncana

Fig. 1. The effect of tulip gums (G), containing polysaccharides on red pigment formation in wounded scales of *Hippeastrum* bulbs

Fig. 1. Wpływ gum z tulipanów (G), zawierających polisaccharydy, na tworzenie się czerwonego barwnika w uszkodzonych łuskach cebul *Hippeastrum*

Tulip gums at a concentration from 0.25 to 1.0% slightly stimulated red pigment formation in wounded scales of *Hippeastrum* bulbs at the first part of incubation time (41 and 64 h), but later it had an inhibitory effect (Fig. 1). The effect of pure polysaccharides isolated from tulip gum on red pigment formation in wounded scales of *Hippeastrum* will be studied.

Chitosan, a β-1,4-D-glucosamine polymer, is a natural compound of the cell wall of several fungi. Chitosan at a concentration of 0.01, 0.025 and 0.05%, dissolved in 0.3% of glutamic acid, substantially stimulated the red pigment formation in wounded scales of *Hippeastrum* bulbs (Fig. 2), but used at higher concentrations inhibited the red pigment accumulation proportionally to its concentration (Fig. 3).



Note: see Fig. 1; Objaśnienie: patrz Fig. 1

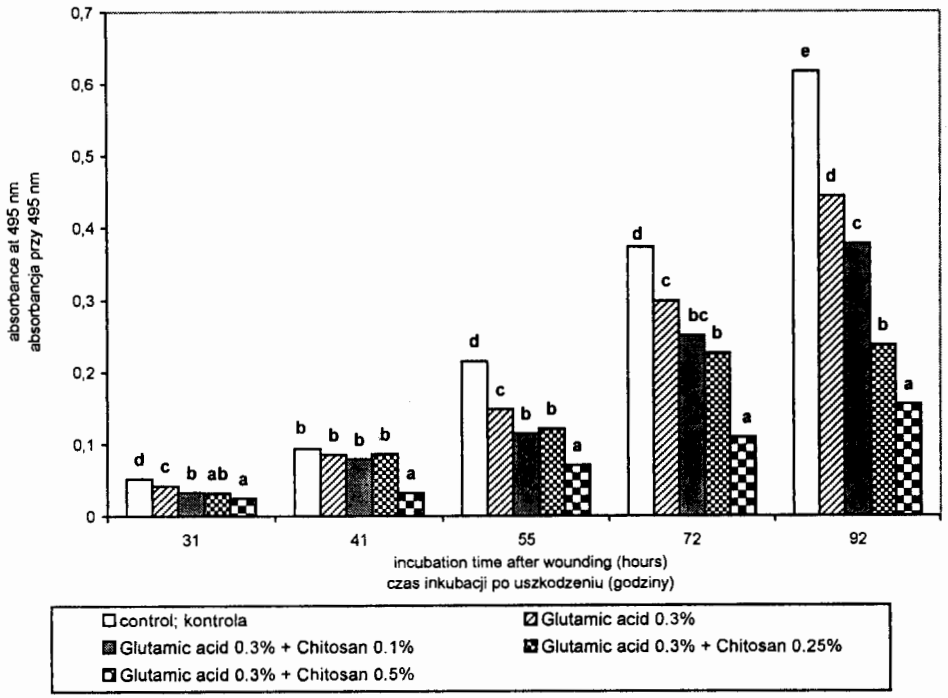
Fig. 2. The effect of chitosan at 0.01–0.05% concentrations on red pigment formation in wounded scales of *Hippeastrum* bulbs

Fig. 2. Wpływ chitozanu w stężeniach 0,01–0,05% na tworzenie się czerwonego barwnika w uszkodzonych łuskach cebul *Hippeastrum*

Glutamic acid alone at a concentration of 0.3%, used as dissolvent for chitosan, slightly inhibited red pigment formation in wounded scales of *Hippeastrum* (Fig. 2, 3).

It is known that oligogalacturonides and chitosan can induce the expression the genes involved in plant defense systems, and take part in biosynthesis of the secondary metabolites, probably through the octadecanoic pathway. Chitosan increased the synthesis of secondary metabolites in various species, for example: phytoalexin pisatin in pea pods [WALKER-SIMMONS et al. 1983], phytoalexins saku-

ranetin and momilactone A in the leaves of rice seedlings [AGRAWAL et al. 2002], rosmarinic acid and eugenol in *Ocimum basilicum* [KIM et al. 2005], betacyanin in suspension cultured cells of *Portulaca* sp. [BHUIYAN, ADACHI 2003], isoflavonoids in species of family *Fabaceae* [COSIO et al. 1996], lignin in suspension culture of *Pinus elliotii* [LESNEY 1989], coumarin derivatives in cell suspension culture in parsley [CONRATH et al. 1989], antraquinone in *Morinda citrifolia* and hernandulcinin in *Lippia ducis* [DÖRNENBURG, KNORR 1995], anthraquinones in *Rubia tinctorum* [VASCONSUELO et al. 2004] and anthocyanin in cell cultures in *Vaccinium pahalae* [FANG et al. 1999]. Moreover, yeast elicitor (70–80% v/v ethanol-insoluble oligosaccharide fraction prepared from yeast extract) stimulated biosynthesis of β-thujaplicin in *Cupressus lusitanica* cell cultures [ZHAO, SAKAI 2003].



Note: see Fig. 1; Objaśnienie: patrz Fig. 1

Fig. 3. The effect of chitosan at 0.1–0.5% concentrations on red pigment formation in wounded scales of *Hippeastrum*

Fig. 3. Wpływ chitozanu w stężeniach 0,1–0,5% na tworzenie się czerwonego barwnika w uszkodzonych łuskach cebul *Hippeastrum*

Transcriptional activation, induced by both chitosan and jasmonic acid, of genes encoding phenylalanine ammonia lyase and protease inhibitors, suggests that chitosan may influence the pathways involving JA [WALKER-SIMMONS et al. 1983; DOARES et al. 1995]. RAKWAL et al. [2002] found that chitosan caused a rapid increase of 12-oxo-phytodienoic acid (OPDA) and JA level in the intact rice seedlings leaves; similar increase of OPDA and JA level was observed in the wounded leaves of rice seedlings.

It should be mentioned that after mechanical injuries, when an intensive reddish colouration developed on scales or basal plate of *Hippeastrum*, the main pathogen of the plant, *Phoma narcissi* does not induce the disease symptoms [SANIEWSKA 1998]. This implies that the red pigment or other compounds (colourless) induced by tissue injury may become a defense barrier against the penetration and development of the pathogen. Fraction containing red pigments formed after mechanical wounding of tissues of *Hippeastrum* also inhibited the mycelium growth of other pathogens, not pathogenic for *Hippeastrum* [SANIEWSKA 2002c]. Thus, the red pigment from wounded *Hippeastrum* organs may be used in future as a natural fungicide.

Conclusions

1. Flavonoid origin, red pigment formation in the wounded scales of *Hippeastrum* bulbs is partly stimulated by tulip gums, containing polysaccharides, at the first part of incubation time (2–3 days), but later this phenomenon was inhibited.
2. The red pigment formation is substantially stimulated by low concentrations of chitosan (0.01–0.05%), and inhibited by its higher concentrations, proportionally to the applied chitosan level in the wounded scales of *Hippeastrum*.

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Key words: bulbs, chitosan, *Hippeastrum*, jasmonates, red pigment, tulip gums, wounding

Summary

Various oligosaccharides and chitosan can function in plants as elicitors of various physiological and biochemical processes. They can induce gene expression involved in plant defense systems, and take part in biosynthesis of secondary metabolites, probably through the octadecanoic pathway. In this work the effect of chitosan and tulip gum on red pigment formation of flavan nature in the wounded scales of *Hippeastrum* bulbs was studied. It is known that red pigment indicates the antifungal activities. It was found that tulip gums stimulated the red pigment formation at the first part of incubation time (2–3 days), but later an inhibitory effect was observed. Chitosan at low concentrations (0.01–0.05%) stimulated the red pigment accumulation, however its higher concentrations in wounded scales of *Hippeastrum* had a contrary effect.

WPŁYW CHITOZANU I POLISACHARYDÓW GUM TULIPANA NA TWORZENIE SIĘ CZERWONEGO BARWNIKA W USZKODZONYCH CEBULACH *Hippeastrum* × *hybr.* hort.

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Słowa kluczowe: cebule, chitozan, czerwony barwnik, gumy tulipana, *Hippeastrum*, jasmoniany, uszkodzenie

Streszczenie

Różne rodzaje oligosacharydów i chitozan oddziałują na rośliny jako elicytory wielu procesów fizjologicznych i biochemicznych. Indukują ekspresję genów

biorących udział w systemie obronnym i biorą udział w biosyntezie metabolitów wtórnych, prawdopodobnie poprzez szlak oktadekanowy. Podjęto badania nad wpływem chitozanu i gum tulipana na tworzenie się czerwonego barwnika, natury flawanowej, w uszkodzonych łuskach cebul *Hippeastrum*. Już wcześniej stwierdzono, że ten czerwony barwnik wykazuje antygrzybowe właściwości. Gumy tulipana stymulowały tworzenie się czerwonego barwnika w pierwszych 2–3 dniach po traktowaniu uszkodzonych łusek, a w dalszym okresie następowało hamowanie akumulacji tego barwnika. Chitozan zastosowany w uszkodzonych łuskach *Hippeastrum* w niskich stężeniach (0,01–0,05%) stymulował akumulację czerwonego barwnika, jednakże wyższe stężenia chitozanu miały działanie hamujące, proporcjonalnie do ich wartości.

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