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Short communication

Oligosaccharin and ABA synergistically affect the acquisition of freezing tolerance in winter wheat

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ABSTRACT

In this paper, we continue our studies of the previously discovered [O.A. Zabotina, D.A. Ayupova, O.N. Larskaya, O.N. Nikolaeva, G.I. Petrovicheva, A.I. Zabotin, Physiologically active oligosaccharides, accumulating in the roots of winter wheat during adaptation to low temperature, Russian Journal of Plant Physiology 45 (1998) 262] oligosaccharin (physiologically active oligosaccharide) GXAG, which stimulates the acquisition of freezing tolerance in winter varieties of *Triticum aestivum* L. The transient accumulation of GXAG in the tissues of winter wheat correlates with the temporal activation of cell wall glycosidases during the first hours of cold acclimation (2 °C). This finding suggests that the oligosaccharin is liberated as a result of the intensification of hemicellulose turnover. At low concentrations, GXAG initiates the acquisition of freezing tolerance in winter plants, in a manner similar to ABA, even at room temperature. The resultant effect of ABA and GXAG on the freezing tolerance of winter wheat depends on the sequence of pre-treatments with these two factors. When seedlings are pre-treated with GXAG a few hours before treatment with ABA, the effect is synergistic, and its impact depends on the duration of pre-treatment with GXAG. When ABA is applied first, the resultant effect on freezing tolerance is additive. The results obtained here lead to the conclusion that oligosaccharin, accumulating during the first hours of cold acclimation, functions as a partner of ABA during the initiation of freezing tolerance acquisition in winter plants. We hypothesize that GXAG increases cell receptivity to ABA signaling.

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1. Introduction

Bioactive oligosaccharides, termed "oligosaccharins", were first identified and characterized during studies of plant cell defensive reactions to pathogenic attacks [2]. Later, bioactive oligosaccharides that were obtained as products of acidic or enzymatic degradation of polysaccharides in vitro were shown to affect the growth and development of plants [3]. Oligosaccharins, as the products of acidic or enzymatic degradation in vitro, were then obtained from almost all major plant cell wall polysaccharides: xyloglucan [4,5], homogalacturonan [6,7], xylan [8], rhamnogalacturonan [9,10], galactoglucomannan [11,12], etc. Later, oligogalacturonides, structurally similar to those obtained from homogalacturonan in vitro, were demonstrated to be present in vivo. In this state, they are liberated from the cell walls as a result of pathogenic action and are involved in plant response reactions [13,14].

The accumulation of oligosaccharin in winter wheat during the first hours of cold acclimation [1] and a demonstration of its ability to stimulate freezing tolerance of winter plants [15,16] provided strong evidence that oligosaccharins exist in vivo. This oligosaccharin transiently accumulated in plant tissues; its endogenous concentration reached a maximum within 6 h after the plants were placed at 2 °C and then declined to an undetectable level within 12 h of treatment. The oligosaccharin that was discovered has a monosaccharide composition of Glc_5 Xyl_5 Ara Gal, abbreviated as GXAG. Pre-treatment of winter wheat seedlings with GXAG (1 μg/mL) before hardening for 7 days at 2 °C increased their freezing tolerance by 30% in comparison with that of non-pre-treated plants [15]. The effect of GXAG on freezing tolerance did not depend on the duration of pre-treatment (0.5–18 h) but drastically declined if the treatment was carried out as the seedlings were exposed to low temperatures [16]. Short-term accumulation of GXAG and its ability in low concentrations to stimulate freezing tolerance suggest that this oligosaccharin may serve as a messenger in a signaling

Abbreviations: ABA, abscisic acid; g_{DW}, gram of dry weight material; LT_{50}, temperature at which the efflux of 50% of the total electrolyte in the tissue occurs and is equivalent to a 50% lethality of the seedlings; GXAG, name of the oligosaccharin that is composed of 12 monosaccharides; *XTH*, endo-transglycosylase/hydrolase.

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pathway initiated by low temperatures [15]. To confirm this hypothesis, it is necessary to elucidate the site of action of GXAG during this process.

In this study, we continue to investigate the involvement of GXAG in the acquisition of freezing tolerance in winter plants and, in particular, the relation between oligosaccharin and ABA, a phytohormone that frequently plays a key role in plant adaptive responses to abiotic stresses.

2. Results and discussion

2.1. Effect of GXAG on the LT_{50} of winter wheat seedlings is structurally specific

The exogenous addition of GXAG to the media in which the roots of winter wheat seedlings were immersed decreased the LT_{50} of the seedlings by 30–35% (Table 1), measured at different time points during their growth at 20 °C. Parallel experiments with ABA treatment showed that the hormone had a similar effect on the LT_{50} of winter wheat seedlings (Table 1). ABA is well known to be involved in cold acclimation [17,18]. The results demonstrated that GXAG, similar to ABA, initiates the acquisition of freezing tolerance in winter wheat, even at room temperature. The effect of GXAG depends on its concentration (Fig. 1), with a maximum occurring at approximately 5 μg/mL. The estimated half-effective concentration is about 2.3 μg/mL, which is similar to typical effective hormonal concentrations [19].

Pre-treatment of winter wheat seedlings with other structurally and compositionally different oligosaccharides, such as hepta-β-1,6-glucan, oligo-laminarin (β-1,3-glucan), a mixture of oligogalacturonides (DP 8–15), cellobiose, oligo-galactoglucomannan, gentiobiose, oligopustulan, or a mixture of malto-oligosaccharides (DO 6–12), did not affect the LT_{50} values (Table 2). We also did not observe any effect when using either a mixture of oligosaccharides obtained after acid hydrolysis of cell wall hemicelluloses or purified octo-xyloglucan (XG8) (Table 2). These results demonstrated that the effect of GXAG is structurally specific, but a detailed structural characterization is required in order to understand structure–function correlations.

2.2. Accumulation of GXAG results from cell wall polysaccharide turnover

GXAG is liberated and accumulates only during the first hours of cold acclimation, possibly due to activation of glycosidases localized in the cell walls. We studied the activities of several glycosidases extracted from the cell walls of wheat roots during the first 24 h of hardening at 2 °C (Fig. 2). All the glycosidases that were studied showed increasing activities during the first few hours after the initiation of hardening. The activities reached a maximum within 4 h of cold treatment and returned to their initial levels after 24 h. These observations are in agreement with previously reported results [20], in which the alteration of cell wall polysaccharide metabolism during the first 24 h of cold acclimation of winter wheat seedlings has been studied using pulse-chase experiments. The most intense turnover of the hemicellulosic fraction in comparison with pectins and celluloses was observed during the first 6 h. Using size-exclusion chromatography, the accumulation of relatively small molecular-sized carbohydrates was demonstrated in the buffer soluble fraction obtained from the chilled seedlings [20]. The liberation of xyloglucan fragments as a result of xyloglucan metabolism in a *Rose* cell suspension culture has also been demonstrated by Mcdougall and Fry [21]. In addition, gene expression profiling in *Arabidopsis* under cold conditions [22] demonstrated an intensification of hemicellulose metabolic activity in cold conditions, as did profiling of carbohydrate-active enzymes from cold-treated Poplar tissues [23]. In both cases, up-regulation of endotransglucosylase/hydrolase (*XTH*) genes involved in the xyloglucan rearrangements was observed. The sequence of events: activation of glycosidases within 4 h, intensification of

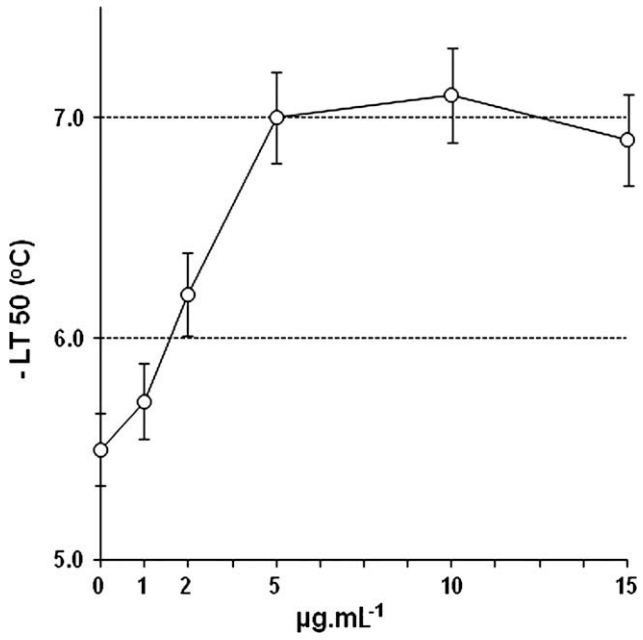

Fig. 1. Concentration dependence of GXAG freezing tolerance-inducing activity. Freezing tolerance values (LT_{50}) of the winter wheat seedlings treated for 15 h with different concentrations of GXAG (μg L^{-1}) were determined after they grew for 7 days at 20 °C. $-LT_{50}$ indicates the negative temperature at which 50% of the electrolyte efflux occurs. The bars represent the means ± SE ($n = 3$).

Table 1
Effect of GXAG and ABA on the freezing tolerance (LT_{50}) of winter wheat seedlings grown at 20 °C.

	LT_{50}, °C		
	Day 0	Day 3	Day 7
No treatment	−5.7 ± 0.2	−5.6 ± 0.3	−5.8 ± 0.2
+GXAG (5 μg/mL)	−5.7 ± 0.2	−7.0 ± 0.2	−7.8 ± 0.4
+ABA (1 μM)	−5.7 ± 0.2	−7.3 ± 0.2	−8.2 ± 0.3

All values represent average means ± SE ($n = 5$).

Table 2
Effect of GXAG and other structurally different oligosaccharides on the freezing tolerance of winter wheat seedlings (LT_{50}) determined after 7 days of hardening at 2 °C.

	LT_{50}, °C		
Control at 20 °C for 7 days	−5.7 ± 0.4		
Hardening at 2 °C for 7 days	−9.4 ± 0.3		
Concentration of oligosaccharide	0.5 μg/mL	5 μg/mL	50 μg/mL
+GXAG	−9.9 ± 0.4	−12.8 ± 0.3*	−11.5 ± 0.3*
+Xyloglucan digest (DP 6–15)	−9.7 ± 0.4	−9.8 ± 0.5	−9.6 ± 0,3
+XG8	−8.2 ± 0.4	−7.5 ± 0.3*	−8.0 ± 0.3*
+hepta-β-1,6-glucan	–	−9.9 ± 0.2	–
+oligopustulans	–	−10.1 ± 0.4	–
+cellobiose	–	−10.2 ± 0.3	–
+oligogalacturonides	–	−10.0 ± 0.5	–
+oligoglucogalactomannan	–	−10.3 ± 0.3	–
+malto-oligosaccharides	–	−10.2 ± 0.2	–

All values represent average means ± SE ($n = 3$); *$p < 0.05$; "–" indicates not tested.