CAN β-D-GLUCAN PROTECT OAT SEEDS AGAINST A HEAT STRESS?

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Abstract: Plants have evolved to live in environments where they are often exposed to different stress factors. Being sessile, they have developed specific mechanisms that allow them to detect precisely environmental changes and respond to complex conditions, minimizing damage while conserving valuable resources for growth and reproduction. The cell wall polysaccharide β-D-glucan observed in some species of *Poales* can determine responses to various environmental factors in specific plant developmental stages. It is located in the outer epidermal layer, at the place of stress attack and therefore its metabolism could relate to response of plant to environmental factors within moderate, physiological range. Putative protective role of β-D-glucan during heat stress was indicated through naked oats with higher content of β-D-glucan. It appeared that oats with higher β-D-glucan content are better adapted to stress conditions. The presented article discusses the β-D-glucan as a possible protective mechanism in oat during (heat) stress conditions.

Key words: barriers, β-D-glucan, cell wall, heat stress, oat, protective effect

1. Introduction

The (1→3)(1→4)-β-D-glucan is a linear homopolysaccharide of D-glucopyranose residues bonded with mixture of β-(1-3) and β-(1-4)-glycosidic linkages (MANTOVANI *et al.,* 2008) in cereals and some grass species of the order *Poales*. The most important sources of β-D-glucan among the main agricultural crops are seeds of barley and oat (HAVRLENTOVÁ and KRAIC, 2006). Naked oats contain usually higher amount of β-D-glucan in comparison with hulled ones (HAVRLENTOVÁ and KRAIC, 2006). Content of β-D-glucan content in oat is generally in the range of 0.27 – 0.58 (HOLTHAUS *et al.,* 1996), and is affected by both genetic and environmental factors (AMES *et al.,* 2006). Interactions of genotype with environment are also significant sources of variability in β-D-glucan content (YALCIN *et al.,* 2007), nevertheless genotype is superior to the environment (PETERSON *et al.*, 1995).

At the utmost extent β-D-glucan is located in the inner aleurone and subaleurone layer of the cell walls of endosperm and surrounding tissues (BROWN *et al.*, 1997), however it was observed in leaves and roots, too (INOUHE *et al*., 1997). CARPITA *et al*. (2001) found the polysaccharide to be distributed uniformly across the thin walls of the mesophyll cells and inner facing walls of the epidermis but concentrated primarily on the innermost portion of the outer facing walls of the epidermis.

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2. Function of β-glucans in plants

Beta-D-glucan plays an important role in cell wall architecture (VIRKKI *et al*., 2005) and plant development (BUCKERIDGE *et al*., 2004). Its primary role in plant cell wall is formation of thin surface layer on the cellulose microfibrils (VIRKKI *et al.,* 2005) which consequently interact with other cellulose-interlaced glycans during growth (CARPITA *et al*., 2001). The cell wall represents a key determinant of overall plants form, growth and development, and response of plant to abiotic and biotic stresses (FARROKHI *et al.,* 2006). Plant exposed to physical, chemical, or biological stress factors must be able to respond by hardening of cell surfaces and related tolerance or resistance (HOSON, 2002). Polysaccharides of the cell wall, including the β-D-glucan, determine responses to various environmental factors in specific plant developmental stages (HRMOVA and FINCHER, 2001). The β-D-glucan located in the outer epidermal layer within the family of the *Gramineae* is located at the place of stress attack (BUCKERIDGE *et al.,* 2004). Therefore metabolism of β-D-glucan could relate to response of plant to environmental factors within moderate, physiological range (HOSON, 1998) what accents its special role at least in cereals. Quick reinforcement of the cell wall may reduce the success of pathogen penetration. Such reinforcements occur through the accumulation of callose (beta-1,3-glucan) (PIRŠELOVÁ and MATUŠÍKOVÁ, 2013), β-D-glucan (BUCKERIDGE *et al*., 2004) and proteins in the area between the cell wall and the cell membrane.

3. β-glucans under heat stress

Abiotic, or biotic forms of stress can induce changes in the plant metabolism, as in the context of production of primary metabolites, so in the secondary metabolites production, too. As one of the first are generated reactive oxygen species (hydrogen peroxide, singlet oxygen, superoxide radical, etc.), or nitrogen oxide, which triggers a cascade of defense reactions such as lipid peroxidation and synthesis of specific proteins (HÉMATY *et al*., 2009). In the category of defensive molecules (defensive proteins) – active especially during fungal pathogenesis - the important are so called PR (pathogenesis-related) proteins. Their antimicrobial properties have previously been detected *in vitro* as well as *in planta* (HAMMOND-KOSACK and JONES, 1996). The subgroup of these proteins, beta-1,3-glucanases (PR-3), are enzymes that hydrolyze polysaccharides of fungal cell walls while their role in plant defense has been proven in transgenic plants (SUNDARESHA *et al*., 2010). Typical symptoms of pathogens toxicity are also reduction of photosynthetic pigments, changes in chlorophyll fluorescence and also changes in the activity of antioxidant active enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) (REDONDO-GÓMEZ, 2013).

In one of our experiments, the objective was to expose oat seeds with high and low content of β-D-glucan to high temperature. Temperature was used as a stress factor and relations between stress expression in the seed and the content of β-D-glucan as a potentially protective tool was monitored. Mature seeds of eight oats

of Slovak provenience (with the content 2.55 – 5.63 % of β-D-glucan) were exposed to temperature 20 ºC, 30 ºC, 40 ºC, 50 ºC, and 60 ºC for 7 and 14 days, respectively. The amount of β-D-glucan was determined using the method of MCCLEARY and CODD (1991) (AOAC 995.16) *via* an assay kit (Megazyme Inc., Bray, Ireland). Viability of oat seeds, as an indicator of plant damage, was determined according to relevant national technical norm (STN 461011-14, 2005).

The results showed that heat treatment did not influence significantly the content of β-D-glucan in oat seeds, neither after 7 nor 14 days of heat treatment. Evaluation of heat stress impact to seed viability revealed statistically significant negative correlation between seed viability and heat treatment for both the naked (r = -0.98**, $P < 0.05$) and hulled oats (r = -0.79**, $P < 0.05$). Analysis showed also that time of exposition to elevated temperature, as well as oat genotype, significantly ($P \leq 0.01$) influenced viability of hulled oat seeds. Statistically significant source of variability in viability of naked oat seeds was only genotype represented in our study by the content of β-D-glucan. Despite the fact that correlation between content of β-D-glucan and seed viability after heat treatment was not statistically confirmed (moderate correlation in naked oats, r = 0.55 and low correlation in hulled oats, r = 0.10) it could be speculated on putative protective role of the cell wall polysaccharide β-D-glucan against heat stress, at least in naked oats. Environmental signals, including elevated temperature, are able to elicit responses of plant such as changes in the cell walls where the β-D-glucan is an essential substance, especially in oat. The β-D-glucan is a highly hygroscopic cell wall component. Thus, seeds with higher β-D-glucan content should retain more water, water loses have mild expression due to higher mobility of water, seeds show higher rate of drying, and due to strong desiccation the cracking of seeds is absenting (FAST SEEFELDT *et al.,* 2007).

High temperature during growing season and drought before grain harvest (SAVIN and MOLINA-CANO, 2001) could increase the content of β-D-glucan in oat grains. The same effect has been observed in barley EHRENBERGEROVÁ *et al.* (2003). The authors concluded that the contents of β-D-glucan and arabinoxylans were significantly affected by genotype, environmental conditions during the vegetation period and by their interactions. Moreover, higher temperature increased the β-D-glucan content in barley seeds. On the other side, short exposition of barley plants to very high temperature may partially reduce the content of β-D-glucan (SAVIN *et al.,* 1997). Relationship between higher content of β-D-glucan and elevated temperature could relate in grasses to pre-transport of some substances within the plant body in direction to grains (DUPONT and ALTENBACH, 2003) where the β-D-glucan accumulates in cells not only as structural element of walls but also as endosperm storage material (BUCKERIDGE *et al.,* 2004). Very important is timing of stress factors such as heat, drought, or humidity (MACNICOL *et al.,* 1993). Other conditions such as nitrogen content in soil and precipitation also influence content of β-D-glucan in cereal grains (GÜLER, 2003). Generally, the plant cell wall containing polysaccharides, including the β-D-glucan as its major component, is a dynamic structure responding to external stimuli such as

abiotic and biotic stresses (FARROKHI *et al.,* 2006). Subsequently, either the breakdown or the accumulation of these polysaccharides determine the composition and properties of cell walls (HOSON, 1998) and hereby response to external stimuli.

Previously published studies were focused to synthesis, mobility, and accumulation of the β-D-glucan during plant growth and seed development (reference). Our study was concerned to relations between content of β-D-glucan and viability of mature oat seeds stressed by high temperature. Heat stress parameters, i.e. temperatures between 40 °C to 60 °C should simulate a condition in field during the summer when the final stage of oat seed maturation occurs. The mean content of β-D-glucan analyzed in mature seeds before heat treatment was 4.54 % in naked and 3.50 % in hulled oats. Content of the β-D-glucan in seeds exposed to high temperatures for 7 and 14 days, respectively, was more balanced in naked in comparison to hulled oats and has been changed (increased or decreased) only slightly in both types (hulled and naked) oats in comparison to non-treated control seeds. OLIVEIRA *et al.* (2012) have reported that content of the β-D-glucan in mature oat seeds was affected by air drying at temperature above 50 °C, and the extracted β-D-glucan physically changed after drying at above 75 °C. Simultaneously, water holding capacity of the β-D-glucan was decreased at temperature above 50 °C and water retention capacity decreased at more than 75 °C. High temperature may have result to degradation of β-glucosidic linkages in β-D-glucan and its disintegration to low molecular weight fragments or depolymerization of the linear structure, thus changing the content and properties of the β-D-glucan (BUTT *et al.,* 2008). On the other hand, high temperatures (50 °C – 60 °C) can counteract against decreasing of β-D-glucan content as detected by FASTNAUGHT (2001) during process of β-D-glucan extraction where these temperatures may not be sufficient to inactivate endogenous β-glucanases.

Content of β-D-glucan could be related to thickness and consequently to insulation capacity of cell walls. Correspondence between higher content of β-D-glucan and greater cell walls thickness has been reported in barley by BHATTY *et al.* (1991). Similar attribute of β-D-glucan in stress response to low temperature has been reported by TAKEDA *et al.* (2011) in barley in mutants lacking (1,3;1,4)-β-D-glucan. They were more sensitive to chilling, probably due to thinner cell walls providing weaker temperature protection. Specific association between the role of β-D-glucan and cell protection during heat stress (58 °C) has been demonstrated in bacteria (STACK *et al.,* 2010). Genetically modified strain of probiotic *Lactobacillus paracasei* NFBC 338 endogenously produced β-D-glucan. Heat stress assays revealed that production of this polysaccharide was associated with significantly increased protection during heat stress (60-fold). Another factor affecting stability and content of β-D-glucan in heat stressed seeds is inhibition of hydrolytic enzymes such as xyloglucan transglycosylases/hydrolases abundant in the apoplastic space (ROSE *et al.*, 2002). Further examples include hydrolysing xyloglucan backbones, β-glucanases, and other endo-type enzymes with β-glucanase activity (HRMOVA and FINCHER, 2001) breaking β-glucosidic linkages in β-glucans.

4. Conclusions

The cell wall polysaccharide β-D-glucan plays an important role in the cell wall architecture and plant development, too. According to some studies its metabolism could relate to response of plant to environmental factors within moderate, physiological range. This fact accents a special role of the β-D-glucan at least in cereals. Exposure to temperatures of 20 ºC, 30 ºC, 40 ºC, 50 ºC, and 6 0ºC for 7 and 14 days, respectively, did not influence the content of β-D-glucan in seeds. However, significant negative correlation was observed between seed viability and heat treatment for both naked (r = -0.98**, $P < 0.05$) and hulled oats (r = -0.79**, $P < 0.05$). This indicates heat treatment as a stress factor. Time of exposition and oat genotype significantly ($P \leq 0.01$) influenced life cycle in hulled oats. In naked seeds, only genotype was the source of variability. The putative protective role of the cell wall polysaccharide β-D-glucan against heat stress, at least in naked oats, however, requires further investigations.

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