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CHANGES IN ARABINOGALACTAN PROTEINS DURING SOMATIC EMBRYOGENESIS IN SUSPENSION *IN VITRO* CULTURES OF *DACTYLIS GLOMERATA* L.

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Abstract

ZAGORCHEV, L., R. STOINEVA and M. ODJAKOVA, 2013. Changes in arabinogalactan proteins during somatic embryogenesis in suspension *in vitro* cultures of *Dactylis glomerata* L. *Bulg. J. Agric. Sci.,* Supplement 2, 19: 35–38

Somatic embryogenesis is a remarkable process in plant cells in which somatic embryos are formed from a single somatic cell that could further regenerate into a whole plant. Arabinogalactan proteins (AGPs), a family of heavily glycosylated cell surface glycoproteins, are among the key regulators of somatic embryogenesis. The aim of the present study is to investigate the expression and localization of AGPs in embryogenic suspension cultures of *Dactylis glomerata* L., treated with NaCl. We have used two concentrations of NaCl – 0.085 M, which stimulates somatic embryogenesis and 0.17 M, which inhibits the process. The addition of $(\beta\text{-D-glucosyl})_3$ Yariv reagent to the growth medium, a synthetic phenylglycoside that specifically reacts with AGPs, inhibited both fresh mass accumulation and somatic embryogenesis. Many cells with abnormal shape were observed in cultures with Yariv reagent compared to controls showing the importance of AGPs for the development of plant cells. Salt stress showed differential effect on distribution of AGPs, recognized by LM2 and MAC207 antibodies as shown by immunoblot and immunofluorescence analyses. While LM2 antibody recognizes a glucuronic acid – containing epitop, MAC207 is directed against L-arabinose containing epitop. MAC207-recognized AGPs are found predominantly in the intracellular fraction in controls and migrates to the cell wall in salt-treated cultures. LM2-recognized AGPs almost disappeared from the cell wall at 0.17 M NaCl. The pattern of AGPs is differing according to the applied salt stress and at least some of them could be associated with the process of somatic embryogenesis.

Key words: arabinogalactan proteins, salt stress, somatic embryogenesis, Yariv reagent
Abbreviations: AGP – arabinogalactan proteins, BCA – bicinchoninic acid, FM – fresh mass, HRGP – hydroxyproline rich proteins, HRP – horseradish peroxidase, PEM – pro-embryogenic masses, SE – somatic embryogenesis

Introduction

AGPs comprise a major class of cell surface glycoproteins that are characteristic for most if not all plant species (review by Ellis et al., 2010). They belong to the superfamily of HRGPs and could be divided into several subclasses according to the composition of the carbohydrate part and the protein core. The great variability of AGPs is important for the broad spectrum of functions in plant growth and development. They are involved in cell growth and division, cell-to-cell contacts, programmed cell death etc. A role in abiotic stress response and somatic embryogenesis was also proposed. The role in somatic embryogenesis (SE) is assigned to AGPs containing β-1,4-linked N-acetylglucosamin residues in the carbohydrate part – a possible target for the enzymatic activity of chitinases. It is established that different endogenous plant chitinases could regulate the development of the plant embryo by generation of SE-stimulating factors and AGPs-derived oligosaccharides are the most probable ones (van. Hengel et al., 2002).

Investigation of AGPs most often involves the use of Yariv reagent, a synthetic phenylglycoside that specifically binds and precipitate those (Maurer et al., 2010). It is used for isolation, qualitative and quantitative analyses. When applied to the growth medium of *in vitro* cultures Yariv reagent blocks AGPs function and could be used for studying their role in

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cell growth and development. Another approach for studying AGPs is the use of monoclonal antibodies. Recently several large AGPs, HRGPs or oligosaccharide motives-directed antibodies collections have been developed that allow the study of a particular class of cell surface molecules in plant abiotic stress response and development (Namasivayam et al., 2010).

In the present study the possible role of AGPs in SE of salt-treated suspension *in vitro* cultures of the graminaceous Orchardgrass, *Dactylis glomerata* L. was investigated. Yariv reagent was supplemented to the growth medium to follow the changes in plant cell development in conditions of blocked AGPs. The distribution and cellular localization in stress conditions of AGPs, recognized by LM2 and MAC207 antibodies was also studied.

Material and Methods

Experimental object and salt treatment

In vitro suspension cultures of the highly embryogenic genotype Embryogen – P of *Dactylis glomerata* (Conger and Hanning, 1991) were used. Cultivation and salt treatment was according to Odjakova (Odjakova et al., 1992; 2001). $(\beta\text{-D-glucosyl})_3$ Yariv (Biosupplies) reagent was added to the growth medium in final concentration of 10.10^{-6} and 50.10^{-6} M (Maurer at al., 2010) in experiments for blockading AGPs.

Protein isolation

Protein isolation of cell wall bound, growth medium and intracellular fractions was as described by Odjakova (Odjakova et al., 2001). Protein concentrations were estimated by BCA (Thermo scientific) kit according to the producer's manual.

Protein separation and immunodetection

For separation of proteins SDS PAGE was performed according to Laemmli (1970) on T = 12.5 % and C = 3.3 % of the running gel and T = 4 % C = 3.3 % of the stacking gel. Semidry blot on nitrocellulose paper (0.45 μm) was performed on TE 70XP, Hoefer Scientific according to producer's manual. Western blot analysis of intracellular, cell wall – bound and growth medium protein fractions was performed with anti-AGPs antibodies LM2 and MAC207, 1:1000 (Plant probes, Leeds) as primary antibodies and anti-rat HRP conjugated antibody, 1:7500 (Sigma) as secondary antibody according to producer's protocol.

Results and Discussion

Effect of Yariv reagent treatment

Different salt concentrations showed differential effect on SE. While at 0.085 M NaCl SE was enhanced, at 0.17 M NaCl almost no somatic embryos were observed and organogenesis was observed rather than SE. The addition of Yariv reagent lead to significant inhibition of FM accumulation at all salt concentrations, but it is most profound in controls and cultures, treated with 0.085 M NaCl where the decrease in final fresh mass is around 50 % (Figure 1A) even at 10.10^{-6} M Yariv in the growth medium. This effect confirmed the general statement that AGPs are involved in cell division and that the addition of Yariv reagent leads to inhibition of growth of both cells in suspension cultures (Ben Amar et al., 2010; Maurer et al., 2010) and shoots and roots (Willats and Knox, 1996). At 50.10^{-6} M concentrations Yariv reagent bound to the somatic embryos, used for culture initiation (Figure 1B) and almost no

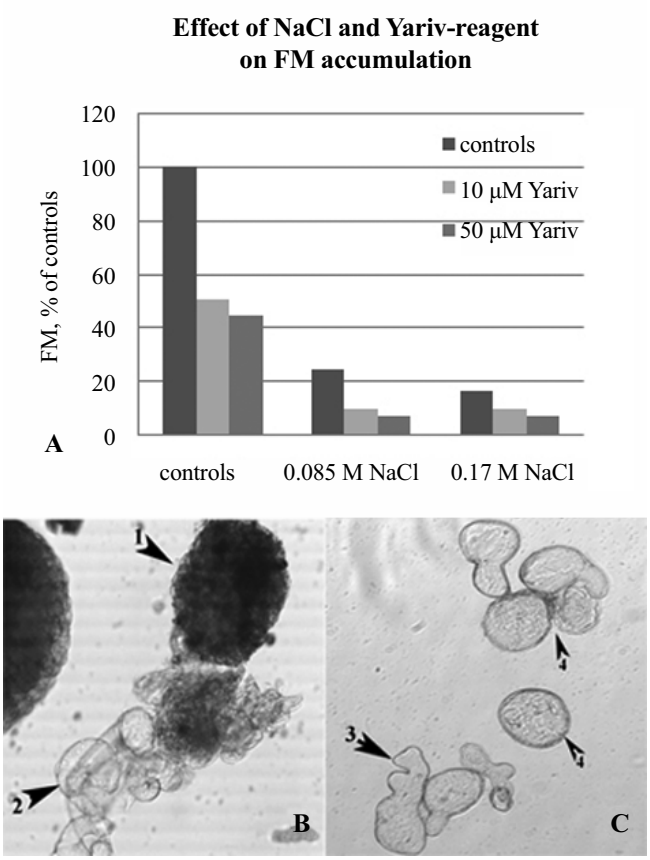

Fig. 1. Effect of NaCl and Yariv reagent on FM accumulation (A) and 10.10^{-6} M Yariv reagent on cell development and morphology (B and C). 1 – dense binding of Yariv reagent to somatic embryos, used for initiation of suspension cultures; 2 – newly developed single cells; 3 – cell with abnormal shape; 4 – distribution of the AGPs-Yariv reagent complexes on the cell surface and in cell-to-cell contacts

further cell division and growth were observed. At 10.10^{-6} M concentrations the red dye of AGP-Yariv reagent complexes were spread on the cell surface and in cell-to-cell contacts (Figure 1C), but most of the cells that were observed were single and with abnormal shape, differing from the previously reported round and banana-shaped cells (Somleva, 2000). The inability of Yariv-treated suspension cultures to form cell aggregates confirm the role of AGPs in cell-to-cell contact and aggregation that was previously reported (Capataz-Tafur et al., 2011). SE was also suppressed in Yariv-treated suspensions that could be explain by the inability of cells to aggregate in microclusters and PEMs and further developed into somatic embryos. Involvement of AGPs in SE is long known (Van Hengel et al., 2002) and recently confirmed (Poon et al., 2012). Steinmacher (Steinmacher et al., 2012) also reported inhibition of SE in peach palm by addition of Yariv reagent but interestingly no significant change in FM accumulation maybe due to the different role of AGPs in phylogenetically distant plant species. The abnormal shape of some of the cell should be attributed to incomplete cell division, caused by Yariv reagent. Similar effect – multinucleation and inability of cell plate formation were observed in *Marchantia polymorpha* protoplasts (Shibaya and Sugawara, 2009). Moreover, AGPs may be involved in primary cell wall architecture similarly to another class of HRGPs – extensins (Lamport et al., 2011) and Yariv-precipitation disrupts the proper deposition and orientation of cellulose microfibrils.

Expression and localization of AGPs

Immunoblot analyses showed high molecular weight (>100 kDa) bands, recognized by LM2 antibody both in growth medium and cell wall – bound (Figure 2) protein fractions. In growth medium, the fraction was more intense in salt-treated cultures. Generally, AGPs are found to be predominantly higher molecular weight cell surface or extracellular glycoproteins with a high heterogeneity of the carbohydrate residues (Hijazi et al., 2012). Unlike previous results for *Dactylis* from Western blot analyses with antibodies from the JIM series (Zagorchev and Odjakova, 2011) the LM2-recognized AGPs are visualized as distinct sharp bands and not diffused fractions in both cell wall and growth medium. The highest presence of LM2-recognized AGPs in the growth medium of salt-treated cultures is in agreement with several studies, showing that salt stress increase the concentration of AGPs in the growth medium in *Dactylis* (Zagorchev et al., 2008) and several other plant species (Lamport et al., 2006; Lin et al., 2011). Additionally three lower molecular weight bands were recognized by LM2 antibody in cell wall – bound protein fraction that almost disappeared in cultures, treated with 0.17 M NaCl. LM2 antibody was

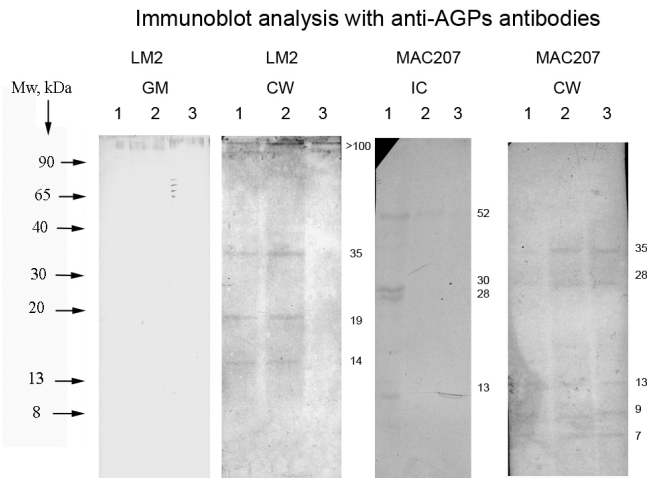

Fig. 2. Immunoblot analysis of different protein fractions from *Dactylis glomerata* suspension cultures with anti-AGPs antibodies. GM – growth medium; CW – cell wall; IC – intracellular; 1 – controls; 2 – cultures, treated with 0.085 M NaCl; 3 – cultures, treated with 0.17 M NaCl; The Mw of Color Burst electrophoresis markers (Sigma) in kDa are shown on the left. The apparent Mw in kDa of the AGPs fractions, recognized by the antibodies are shown on the right

selected against AGPs from rice containing β-linked glucuronic acid epitop. As confirmed by the fractions, recognized in the growth medium it is generally considered an antibody that recognizes predominantly secretory AGPs (Samaj et al., 2000).

AGPs, recognized by MAC207 antibody were with comparatively low molecular weight, presented only in the intracellular protein fraction of control cultures, and in cell wall – bound fraction in salt-treated cultures. Intracellular AGPs, recognized by MAC207 antibody were reported before (Abreu and Oliveira, 2004) and their migration from the cytoplasm in controls to the cell wall in salt-treated cultures should be related to the salt stress response. As the two antibodies are directed against different epitopes (L-arabinose containing for MAC207), they recognizes different classes of AGPs. LM2-recognized AGPs diminished in the cell wall and increased in the growth medium while MAC207-recognized AGPs migrated from the cytoplasm to the cell wall in salt treated cultures.

Conclusions

The addition of β-D-glucosyl Yariv reagent to the growth medium of embryogenic suspension cultures of *Dactylis*

glomerata L. lead to a variety of effects, showing the important role of AGPs for cell growth and morphology, cell-to-cell contacts and somatic embryogenesis. Salt stress differentially affected the presence and localization of some of the AGPs, recognized by LM2 and MAC207 antibodies. While LM2-recognized AGPs were cell wall and extracellular glycoproteins and disappeared in high salt concentration, MAC207-recognized antibodies were found to migrate from the intracellular fraction to the cell wall in salt-treated cultures indicating that both of them were involved in salt stress response and the different embryogenic response at 0.085 M and 0.17 M NaCl.

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