ARABINOGALACTAN PROTEINS IN SALT-ADAPTED SUSPENSION CULTURES OF *DACTYLIS GLOMERATA* L.

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Summary. Arabinogalactan-proteins (AGPs) are widely distributed in higher plants and play multiple roles in various processes associated with plant growth and development, including cell expansion, cell proliferation and embryogenesis. Soluble extracellular AGPs are also abundant in the growth medium of suspension. Levels of cell wall bound and extracellular AGPs were measured spectrophotometrically using Yariv reagent and were compared between salt-adapted and control (unadapted) embryogenic suspension cultures of *Dactylis glomerata* L. While mild salt stress enhanced somatic embryogenesis, higher salt concentrations affected negatively the regeneration capacity. No significant quantitative differences of AGPs in the cell wall bound fractions were found while AGPs in the growth medium showed a dramatic increase in cultures, treated with high salt concentrations. Most of the Yariv reagent precipitated a fraction consisting of high molecular weight proteins. Isolated and partially fractioned extracellular AGPs from salt-adapted and control cultures showed a great difference in the electrophoretic patterns.

***Key words*:** salt stress, arabinogalactan proteins (AGPs), Yariv reagent.

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INTRODUCTION

Arabinogalactan proteins (AGPs) are a large group of abundant in the plant cell surface proteoglycans with a vital role in plant growth and development (Gaspar *et al*., 2001). They constitute part of the hydroxiproline-rich glycoproteins (HRGPs) superfamily (Majewska-Sawka and Nothnagel, 2000) which are restricted to plants and green algae. AGPs are typically composed of over 90 % carbohydrates, predominantly galactose and arabinose which form a heterogeneous and highly branched glycan chains and short protein core.

There are contradictory data about their exact functions and possible involvement in a broad variety of processes as plant growth and development, plant defence (Showalter and Varner, 1989; Guan and Nothnagel, 2004), cell proliferation (Thompson and Knox, 1998), cell expansion (Willats and Knox, 1996; Ding and Zhu, 1997), cell differentiation (Knox et al., 1991), cell extension (Lee et. al., 2005) and somatic embryogenesis (Thompson and Knox, 1998; Chapman et al., 2000, van Hengel et. al., 2001).

The group of "classical" AGPs consists of hyperglycosylated polypeptides with a C-terminal hydrophobic sequence that direct the addition of a glycosylphosphatidylinositol (GPI) anchor (Schultz *et al*., 2000) and tethere them to the plasma membrane. Different signals lead to the cleavage of the anchor and release of soluble AGP monomers through the cell wall into the growth medium in suspension cultures or into the middle lamella and the intercellular space of cells. The continuous release of AGPs is characteristic of the rapid growing plant cells.

Another feature of AGPs is their selective binding by β-D-glucosyl Yariv reagent (β-D-glcY) (Yariv et al., 1962). The addition of β-D-glcY to cell cultures leads to a wide variety of biological effects, but in general it suppresses cell division and growth. Recent studies have shown that salt stress highly upregulates the release of AGPs in the culture medium in tobacco BY-2 cells (Lamport et. al., 2006) thus supporting the prediction that they may be involved in plant response and adaptation to different stresses.

In the present study, the changes in the growth medium and cell wall bound AGPs fractions of salt-adapted embryogenic suspension cultures

of orchardgrass (*Dactylis glomerata* L.) were investigated in order to monitor the involvement of AGPs in salt stress response and somatic embryogenesis.

MATERIALS AND METHODS

Plant material

Embryogenic suspension cultures were initiated from embryoids from 28 days callus of *Dactylis glomerata L.*, genotype Embryogen-P (Conger and Hanning, 1992) in liquid SH media (Shenk and Hildebrandt, 1972) supplemented with 30 μM Dicamba (Duchefa). For salt stress treatments concentrations of 0.085, 0.170 and 0.255 M NaCl were applied. All cultures were maintained in dark on a rotary shaker (105 rpm) at 25 ^{o}C.

Protein isolation

Suspension cultures at day 28 were centrifuged at 500 x *g* for 5 min. For the isolation of extracellular proteins, culture media was subjected to dialysis overnight against distilled water and lyophilized.

Cell wall bound proteins were isolated by grounding the cells with liquid nitrogen, washing the pellets three times with distilled water and extraction for one 1h at 4 ^{o}C with 0.2 M TRIS.HCl buffer pH 7.4, containing 1 M NaCl. After centrifugation at 10 000 g for 15 min the supernatants were subjected to dialysis against ddH_2O and lyophilized.

AGPs isolation and colorimetric assay

Carbohydrate content in the growth medium and cell wall bound protein fractions was estimated by phenol-sulphuric method (Dubois et. al., 1956). For AGPs isolation β-glcY was added to a final concentration of 1:45 to the total carbohydrate content and incubated overnight at 4 ^{o}C. After centrifugation at 10 000 x *g* for 15 min the pellets were washed twice with 0.15 M NaCl. For quantification pellets were dissolved in 0.02 M NaOH and absorbance at wavelength 457 was measured (Lamport et. al., 2006).

Gum Arabic was used as a standard.

For electrophoretic separation the β-D-glcY – AGPs complexes were dissolved in DMSO and 10 % sodium dithionite and subsequently desalted and partially separated on PD 10 columns (Sigma). Fractions of 125 µl were collected.

SDS PAGE

SDS PAGE was conducted according to Laemli (1970) using 5 % acrylamide for the separating gel and supplemented with 0.4% agarose. Electrophoreses were run at 40 mA on minigel system (Cleaver Scientific). All gels were silver stained (Nesterenko, 1994).

RESULTS AND DISCUSSION

AGPs were isolated from lyophilized cell wall bound and growth medium protein fractions from salt-adapted embryogenic suspension cultures of *Dactylis glomerata* L. using the ability of β-D-glcY to specifically bind them. Quantified spectrophotometrically at A_{457}, AGPs from salt treated cultures showed no significant differences in the cell wall fraction (Fig. 1A) while in the growth medium there was a dramatic increase in AGPs in cultures treated with 0.170 M and 0.255 M NaCl (Fig. 1B). In these

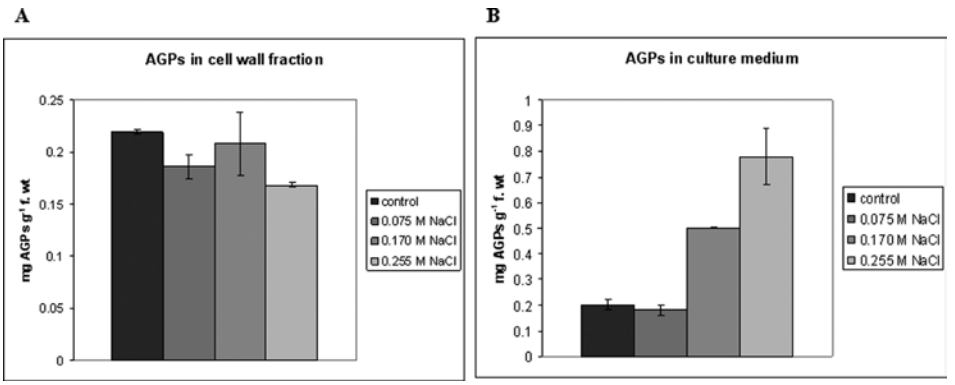
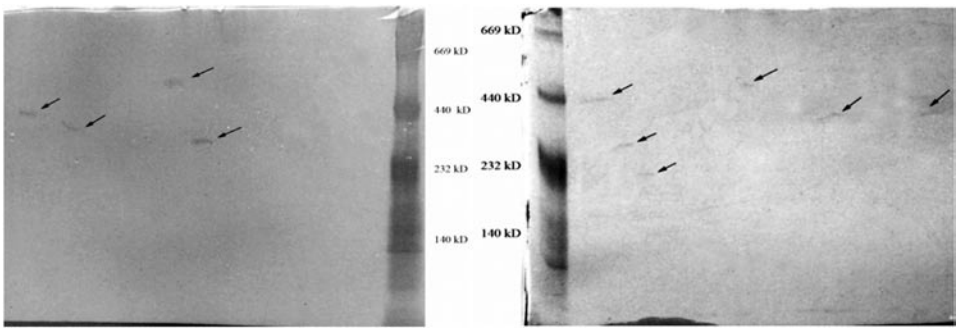


Fig. 1. AGPs in the cell wall fractions and culture media in control and salt-treated suspension cultures of *Dactylis glomerata* L.

treatments somatic embryogenesis was severely suppressed, while in control and treated with 0.085 M NaCl cultures the rate of formation of somatic embryos was significant (Odjakova, 1992).

While previous reports indicated that salt stress adaptation decreased the levels of AGPs in the growth medium in cell cultures from tobacco (Zhu et. al., 1993), in recent experiments the opposite effect has been shown (Lamport et. al., 2006). AGPs released in the growth medium in higher salt concentrations could be related to stress response and stress adaptation rather than somatic embryogenesis as expected. Extracellular



Molecular weight, kD	control cultures	0.085 M NaCl	0.170 M NaCl	0.255 M NaCl
531	-	+ (3)	-	-
527	-	-	-	+ (8)
441	+ (1)	-	+ (5)	+ (10')
356	+ (2)	-	-	+ (9, 10'')
317	-	+ (4)	+ (6)	-
229	-	-	+ (7)	-

Fig. 2. Electrophoregrams of AGPs from the growth medium of control cultures (1,2) and cultures treated with 0.075 M (3,4), 0.170 M (5,6 and 7) and 0.255 M (8, 9 and 10) NaCl. Numbers correspond to the peaks in flow on PD10 desalting columns.

AGPs were established as a major factor in restoring and increasing somatic embryogenesis in tissue cultures (Kreuger and van Holst, 1993), being a target for endochitinase cleavage (van Hengel et. al., 2001). The dramatic increase of growth medium AGPs fraction in 0.170 M and 0.255 M NaCl when formation of somatic embryos was severely suppressed indicated their involvement in stress response.

Recent publications suggest the involvement of AGPs in various processes of adaptation to stress and triggering signalling pathways in pathogen response (Mashigushi et. al., 2008). Moreover, a possible relation between AGPs quantity and roots formations typical for the suspensions treated with higher salt stress could be suggested. The role of AGPs in root cell elongation was previously reported (Ding and Zhu, 1997).

The identified fractions from the growth medium of control and salt treated cultures showed vast differences between the control and salt treated cultures both on peaks from chromatographic columns and SDS PAGE (Fig. 2).

Extracellular AGPs appeared to be a broad proteoglycan family of molecules with varying molecular weights. It has been established that the protein backbone of these molecules is coded by multigene families in various species like Arabidopsis (Shultz et. al., 2000) with highly regulated expression which is an indication for their significance in plant development. The release of different AGPs in response to the variations of NaCl concentration suggests their involvement in multiple processes related to stress adaptation, cell-cell signalling, etc. It is not clear whether this variety is due to *de novo* synthesis or processing of the existing carbohydrate moieties, but AGPs seemed to be regulated nonequally in response to the salt stress.

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