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REGULAR ARTICLE

STUDY ON THE ROLE OF GLUCANASES IN DIGESTION OF CARNIVOROUS PLANT *DROSERA ROTUNDIFOLIA* L.

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ABSTRACT

Glucanases act as plant defense enzymes which are induced in plants under diverse stress but also non-stress conditions. They have many different roles in plants including normal physiological and developmental processes as well as stress response. Interestingly, in a few earlier studies they have also been detected in the digestive mucilage of traps of carnivorous plants. Here we show that glucanases are present and active in the digestive fluid of sundew. Their activity is upon induction of digestion processes significantly increased comparing to controls. Our data showed that laminarin, a polymeric β-glucan as artificial substrate for these enzymes, is gradually decomposed in the time period of plant digestion. At the same time,a temporal increase but subsequent decrease ofamount of reduced oligosaccharides was observed indicating to its assimilation by glands/leaves. Therefore we assume that plant glucanasesmight contribute to digestion processes of carnivorous sundew.

Keywords: β-1,3-glucanases, PR proteins, digestion, carnivorous plants, *Drosera*

INTRODUCTION

β-1,3-glucanases (EC 3.2.1.39) are abundant, highly regulated enzymes widely distributed in seed-plant species (**Meins et al., 1992; Høj et al., 1995; Simmons, 1994; Stone and Clarke, 1992**). In plants, they were reported to have various functions. They belong to pathogenesis-related proteins (PR-2 group) which are induced in response to infection of plants with microbial pathogens (**Leubner-Metzger and Meins, 1999**). Mainly in association with chitinases, another group of plant pathogenesis-related proteins, glucanases have been shown to have inhibitory effect on the growth of phytopathogenic fungi *in vitro* and *in planta* (**Mauch et al., 1988; Gonzáles-Teuber et al., 2010; Broglie et al., 1991; Jongedijk, 1995**). β-1,3-glucanases are also implicated in responses to abiotic stressors such as wounding, cold, ozone, UV-B and heavy metals (**Wu and Bradford, 2003; Ernst et al., 1996; Thalmair et al., 1996; Linthorst, 1991; Hincha et al., 1997; Brederode et al., 1991**).

Beside stress-related functions, β-1,3-glucanasesare implicated in diverse physiological and developmental processes in the uninfected plant including cell division (**Waterkeyn, 1967**), turnover of callose during plasmodesmatal opening (**Levy *et al*., 2007**), microsporogenesis (**Worrall et al., 1992**), pollen germination and tube growth (**Roggen and Stanley, 1969**), fertilization (**Lotan et al., 1989; Ori et al., 1990**), embryogenesis (**Dong and Dunstan, 1997; Helleboid et al, 1998**), fruit ripening **(Hinton and Pressey, 1980**), seed germination (**Vögeli-Lange et al., 1994**), mobilizationof storage reserves in the endosperm of cereal grains (**Fincher and Stone, 1993**) and bud dormancy (**Krabel et al., 1993**).

Previously it has been shown that some species of carnivorous plants can utilize nutrients from materials other than insects, e. g. pollen grains or fungal spores which are rich sources of β-glucans (**Juniper *et al*., 1989**). **Hatano and Hamada (2008)** found theglucanase in digestive fluid of pitcher plant *Nepenthes alata,*however,its microbial origin has not been excluded in this study. Furthermore, no enzyme activity studies with respect tothe plant digestion processitself were performed. **Matusikova et al. (2005)** studied the activity of plant chitinases in the digestive glands and fluid of sundew plantsand suggested their involvement in decomposition of captured prey.

In the present study we demonstrate, that glucanasesinleaf exudates of the carnivorous sundew plants can decompose β-glucan substrate into smaller molecules and that these are subsequently absorbed bysundew leaves. This is the first study bringing evidence on the contribution of glucanases to plant digestion.

MATERIAL AND METHODS

Plant material

Drosera rotundifolia plants were grown aseptically on agar medium (1/2 Murashige-Skoog, 10 % sucrose, 0,6 % plant agar, pH 5,2) in a 225 ml plastic boxes in growth chamber under 16/8 day/night period at 22 °C.

Induction of digestion and sample preparation

Digestive processes were induced by pipetting 10 µl of 20 % laminarin solution (corresponding to 2 mg of laminarin per leaf) onto the leaves. Non-induced plants served as a control.

Digestive fluids were collectedin different time points post induction (0 h, 8 h, 24 h, 48 h and 96 h) by immersing 10 leaves per plant in 250 µl of 0,5 M sodium acetate (pH 5,2) containing protease inhibitors (Complete Mini tabs, Roche), on ice. The leaves were immersedone by one, left to stay in the buffer for 20 seconds and then rinsed 6-7 times with the buffer using a pipette. For each time point, three replications were made.The collected eluates were frozenin liquid nitrogen and stored at -80 °C until use.

Determination of laminarin and glucose content in leaf eluates

Laminarin content was detected fluorimetricallyin 20 µl aliquots of leaf eluatesfollowing the protocol of**Kauss (1989)**. Glucose levels were detected spectrophotometrically in 100 µl aliquots of leaf exudates using DNS method (**Miller, 1959**).

Determination of glucanase activity in polyacrylamide gels

Glucanase activity of leaf eluates was determined after separation of 30 µl aliquots of leaf eluates in SDS-polyacrylamide gels (**Sambrook, 1989**) containing 2,5 $g.l^{-1}$of laminarin. After renaturation of proteins, gels were incubated for 2 hours at 37 °C and visualized using 2,3,5-triphenyltetrazolium chloride (Sigma) according to **Pan et al. (1991)**.

Evaluation of results

Data were processed using the table editor MS Excel 2007.

RESULTS AND DISCUSSION

Determination of laminarin and glucose content in leaf eluates

After collection, leaf eluates were analyzed for presence of residual laminarin. Results have shown, that after mild increase at the beginning of experiment, the amount of this substrate on leaves gradually decreased with progress of digestion process(Figure 1B).

At the same time, the content of reducing sugars (measured as glucose) increasedwith time andreached maximum at 48 h. Further on their level decreased to the end of the experiment (Figure 1A).

The substrate turnover data appear to coincide with the profile of glucanases in the analyzed samples (Figure 2). The single glucanase isoform of ~40 kDa is present in both induced and non-induced samples, while its activity is significantly higher after 48 hours post induction with laminarin. This indicates to gradual activation of glucanases upon induction of digestion and subsequent assimilation of reducing oligosaccharides by the plant.

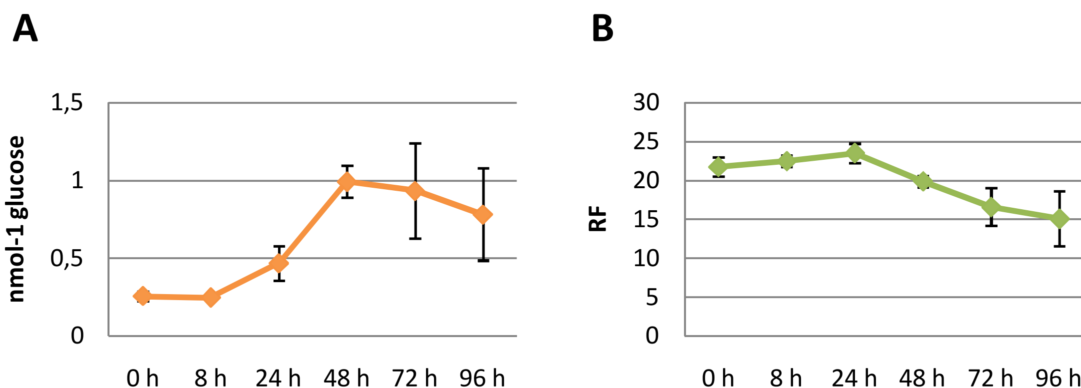

Figure 1 A) glucose levels in leaf eluatescollected in different time points after addition of laminarin on leaves; B) laminarin levels in leaf eluatescollected in different time points after its addition on sundew leaves (laminarin levels are directly proportional to values of fluorescence RF)

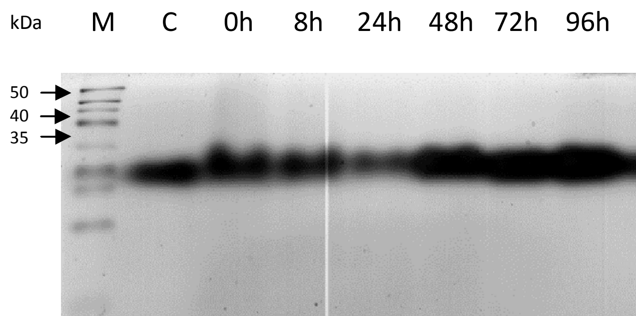

Figure 2 Glucanase activities in leaf eluates collected in different time points after addition of laminarin on sundew leaves. One isoform with size of ~40 kDa was detected.

CONCLUSION

Glucanases play a role in digestion of *Drosera rotundifolia* plants. It is not sure, however, if this is the primary role of glucanases present in digestive fluids or just a side effect besides its defense activity against pathogenic microorganisms.

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