Sex Plant Reprod (1997) 10:107–109

ORIGINAL PAPER

Gerben J. van Eldik · René K. Ruiter
Marinus M.A. van Herpen
Jan A.M. Schrauwen · George J. Wullems

Induced ADH gene expression and enzyme activity in pollinated pistils of *Solanum tuberosum*

Received: 13 November 1996 / Revision accepted: 8 January 1997

Abstract The regulation of alcohol dehydrogenase (ADH) in relation to in vivo pollen tube growth of *Solanum tuberosum* was investigated. *Adh* gene expression as well as ADH enzyme activity were induced in pollinated pistils. The induced ADH isozyme in pollinated pistils is not present in pollen or anthers. The same ADH isozyme is induced in leaves submerged in water. The significance of the induction of ADH activity for pollen tube growth is discussed.

Key words Pistil · Pollen tube growth · Pollen · Alcohol dehydrogenase · ADH

Introduction

The pistil plays an essential role during pollination and subsequent fertilisation in the nourishment, recognition, guidance and protection of the pollen tubes. Successful pollen tube growth depends on a variety of nutrients in the stylar transmitting tissue available to the pollen tubes (Herrero and Arbelo 1989; Kroh et al. 1970; Labarca and Loewus 1973; Vogt et al. 1994; Lind et al. 1996; Cheung et al. 1995; Wang et al. 1993; Wu et al. 1995). Among these substances are carbohydrates which are metabolised in the pistil after pollination and are assumed to supply energy for the growing pollen tubes (Herrero and Dickinson 1979; Ichimura and Yamamoto 1992; Knox 1984). Once pollen germinates after pollination, the pollen tubes grow very rapidly. Maize pollen tubes, for instance, can reach growth rates of approximately 1 cm/h (Bedinger et al. 1994). This fast pollen tube growth requires rapid formation of new cell wall material which represents a major metabolic load on the pollen tubes.

The internal metabolism of the pollen tubes seems to be partly anaerobic, because pollen tubes cultured in vitro produce ethanol. This suggests that respiration is insufficient to fulfill the pollen requirement for energy, and that fermentation is used as an accessory energy-generating pathway (Bucher et al. 1995). In pollinated pistils, the oxygen tension drops sharply in the region of the pollen tube tips. This indicates that pollen tube tips have a high oxygen consumption rate which results in local oxygen deprivation (Linskens and Schrauwen 1966). During oxygen deprivation, fermentation generates ATP and NAD^+ and results in the production of ethanol (Freeling and Bennett 1985). It is proposed that the ethanol synthesised in vivo by pollen tubes through the action of alcohol dehydrogenase (ADH) is remetabolised in the pistil by ADH, aldehyde dehydrogenase and acetyl-CoA synthetase (Bucher et al. 1995). Hence, ADH must already be present in unpollinated pistils or induced by pollination. To test this hypothesis, we investigated whether *adh* gene transcripts and subsequent ADH enzyme activity are induced by pollen tube growth or already present in unpollinated pistils of *Solanum tuberosum*.

Materials and methods

Plant material

Potato plants (*Solanum tuberosum*) were grown in a climate chamber at 20°C under a light/dark regime of 16/8 h. Characteristics of the plants have been described elsewhere (van Eldik et al. 1995). Mature pollen was collected from anthers at anthesis and applied to the stigma of flowers at the same stage. For anoxia studies, leaves were submerged in water for 18 h. All plant material was collected from mature flowers and plants.

Nucleic acid methods

Total RNA isolation and gel blot analyses were performed as described by van Eldik et al. (1995). For the detection of adh tran-

G.J. van Eldik[1] · R.K. Ruiter[2] · M.M.A. van Herpen
J.A.M. Schrauwen (✉) · G.J. Wullems
Department of Experimental Botany, University of Nijmegen,
Toernooiveld 1, 6525 ED Nijmegen, The Netherlands
Fax +31–24–3553450; e-mail schrauw@sci.kun.nl

Present addresses:
[1] Department of Genetics, University of Gent,
K.L. Ledeganckstraat 35, B-9000 Gent, Belgium
[2] Department of Brassica and Oilseeds Research,
John Innes Centre, Norwich Research Park, Colney,
Norwich NR4 7UH, England

scripts, sth-11 cDNA (Matton et al. 1990) from *S. tuberosum* was used. Enzyme activity of the ADH protein encoded by the sth-11 cDNA was demonstrated in vitro (Matton et al. 1990).

ADH activity assay by gel staining

The various potato tissues were separately ground in liquid nitrogen and then mixed with extraction buffer (100 mM TRIS-HCl pH 8.0, 5 mM DTT, 2% PVPP, 0.1% β-mercapto-ethanol) at 4°C. Native gels containing 7.5% polyacrylamide were prepared in ×1 TBE (90 mM TRIS-Borate, 1 mM EDTA pH 8.0) with each lane containing 10 µg of protein. Electrophoresis was performed at room temperature at 200 V in ×1 TBE. The gels were soaked in an ADH-activity staining solution of 300 µg/ml NAD^+, 200 µg/ml nitroblue tetrazolium, 100 µg/ml phenazine methanosulfonate, 1 mM TRIS-HCl pH 7.5 and 10% ethanol for 30 min at 37°C in the dark (Gregerson et al. 1991). After staining the gels were washed in distilled water.

Results

To study the possibility that ADH activity was present in unpollinated pistils we measured the ADH activity in flower organs and leaves of *Solanum tuberosum*. Enzyme activity does occur in mature pollen and complete anthers. ADH activity was not found in unpollinated pistils, nor in petals, ovaries and leaves (Fig. 1a).

To investigate whether ADH is induced in pistils after pollination, the ADH activity was determined in pistils 24 h after pollination using ADH-activity stain on polyacrylamide gels. As shown in Fig. 1b, two isozymes are present in pollinated pistils of which the faster-migrating form is predominant. The slower-migrating form corresponds to the isozyme present in anthers and pollen. In leaves subjected to anoxia by submersion in water, a treatment that induces ADH activity, an ADH isozyme is induced that comigrates with the predominant isozyme in pollinated pistils. This indicates that oxygen deprivation in leaves induces the same ADH isozyme that pollination induces in pistils.

To analyse whether the induction of ADH activity in pollinated pistils is preceded by induced *adh* gene expression, we determined the transcript levels of the ADH-encoding potato *sth-11* gene (Matton et al. 1990). The *sth-11* gene was predominantly expressed in pollinated pistils, anthers and roots, whereas in unpollinated pistils and all other organs tested only a faint hybridisation signal was present (Fig. 1c). This indicates that *adh* gene expression is induced after pollination in pistils.

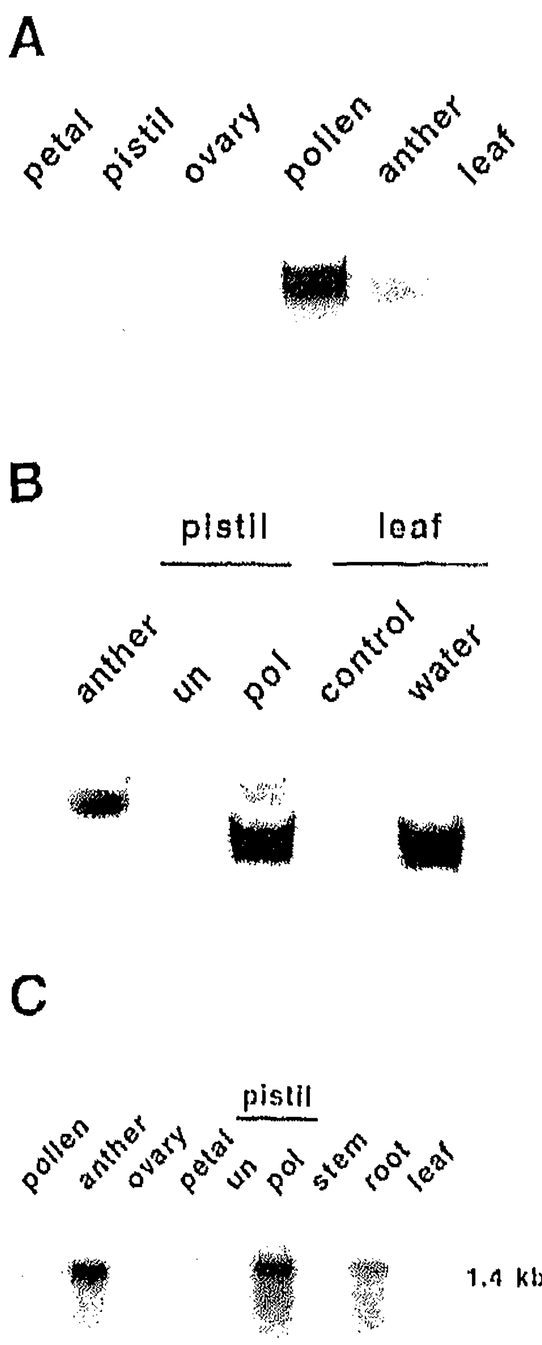

Fig. 1 ADH activity and gene expression in potato organs. **a** Organ-specific ADH activity. **b** ADH activity induced by pollen tube growth. Proteins (10 µg per lane) were isolated and fractionated by native polyacrylamide gel electrophoresis. The gels were stained for ADH activity. **c** Northern blot analysis of *sth-11* gene expression in different potato organs. Total RNA (10 µg per lane) was isolated, fractionated by agarose/formaldehyde gel electrophoresis and transferred to nylon membranes. After hybridisation to the sth-11 cDNA probe (Matton et al. 1990), the blots were washed in ×1 SSC, 0.1% SDS at 58°C and used for autoradiography. The size (*kb*) of the hybridising band is indicated; *un* unpollinated pistils; *pol* pollinated pistils

Discussion

Both *adh* gene expression and ADH enzyme activity are induced in pollinated pistils of *Solanum tuberosum*. Two different ADH isozymes are present in pollinated pistils. The faster-migrating form predominates and is induced by pollination (Fig. 1b). The slower-migrating form is less abundant and comparable to the ADH isozyme present in anthers and pollen, which suggests an origin from the pollen. Northern blot analysis revealed one single mRNA band (Fig. 1c). However, the presence of two different ADH mRNAs with the same size cannot be excluded; this situation has been described for rice seedlings (Xie and Wu 1989). Comparison between transcript levels and ADH activity (Fig. 1b,c) indicates that the ADH activity observed in pollinated pistils is presumably due to de novo transcription, as has also been described for ADH induction in other plant species (Gregerson et al. 1991; Kadowaki et al. 1988; Rousselin et al. 1994; Xie and Wu 1989).

Whether the induced levels of *adh* gene transcripts and ADH enzyme activity in pollinated pistils are localised in the growing pollen tubes or the surrounding tissues of the pistil could not be discerned with our approach. However, the induction of *adh* gene expression in pollen tubes is not likely, because *adh* is not expressed in pollen and no genes have been described which are transcribed only in pollen tubes.

In plants grown under normal, aerobic conditions, significant ADH enzyme activity is present only in pollen (Bucher et al. 1995; Schwartz 1971), whereas *adh* gene expression is induced in all tissues at high levels only af-

ter stress such as oxygen deprivation or elicitor treatment (Freeling and Bennett 1985; Matton et al. 1990; Xie and Wu 1989). The induction of the same ADH isozyme in pollinated pistils and in anoxic leaves suggests that during pollen tube growth oxygen deprivation is the inducing condition in pistils.

The induction of both *adh* gene expression and ADH enzyme activity in pistils after pollination indicates that in pollinated pistils fermentation is used as an energy-generating pathway. As a result of fermentation, ethanol is formed which may be produced by the pistil or the pollen tubes. However, it is likely that the growing pollen tubes produce ethanol because in vitro germinating pollen produce ethanol (Bucher et al. 1995). The ethanol formed in the pollen tubes would diffuse to the surrounding tissues and would be remetabolised by the pistil through the action of induced ADH enzyme activity, as has been proposed by Bucher et al. (1995). Ethanol can be toxic, and oxidation of the ethanol by the action of ADH in the pistil is an effective detoxification. However, ADH activity is not absolutely essential for plant reproduction. Mutant plants that do not express an *adh* gene are not sterile (Freeling and Bennett 1985; Wisman et al. 1993), although the absence of fermentation as an energy source pathway may reduce the growth rate of the pollen tubes.

ADH enzyme activity is present in complete anthers and mature pollen of potato (Fig. 1a), whereas *adh* gene expression is predominant only in complete anthers (Fig. 1c). This suggests that ADH proteins derived from sporophytic anther tissue are deposited on pollen, although *adh* gene expression and subsequent ADH accumulation in the pollen itself during early development cannot be excluded. The same discrepancy between *adh* gene expression and ADH enzyme activity has been described for *Petunia hybrida* (Gregerson et al. 1991).

Definite proof for the role of ADH enzyme activity in the metabolism of ethanol during pollen tube growth should be provided by experiments in which ADH enzyme activity is related to the production and accumulation of ethanol during in vivo pollen tube growth.

Acknowledgements We thank Theo Geurts and Gerard van der Weerden for culturing the plants. We are very grateful to Dr. N.M. Brisson (University of Montreal, Montreal, Canada) for providing the *Solanum tuberosum* sth-11 cDNA clone. Critical manuscript editing was done by Dr. A.E. Croes and Professor C. Mariani. This work was supported by the EC BRIDGE programme BIOT 900172.

References

Bedinger PA, Hardeman KJ, Loukides CA (1994) Travelling in style: the cell biology of pollen. Trends Cell Biol 4:132–138

Bucher M, Brander KA, Sbicego S, Mandel T, Kuhlemeier C (1995) Aerobic fermentation in tobacco pollen. Plant Mol Biol 28:739–750

Cheung AY, Wang H, Wu HM (1995) A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. Cell 82:383–393

Eldik GJ van, Vriezen WH, Wingens M, Ruiter RK, Van Herpen MMA, Schrauwen JAM, Wullems GJ (1995) A pistil-specific gene of *Solanum tuberosum* is predominantly expressed in the stylar cortex. Sex Plant Reprod 8:173–179

Freeling M, Bennett DC (1985) Maize Adh1. Annu Rev Plant Physiol 19:297–323

Gregerson R, McLean M, Beld M, Gerats AGM, Strommer J (1991) Structure, expression, chromosomal location and product of the gene encoding *adh1* in *Petunia*. Plant Mol Biol 17:37–48

Herrero M, Arbelo A (1989) Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). Am J Bot 76(10):1441–1447

Herrero M, Dickinson HG (1979) Pollen-pistil incompatibility in *Petunia hybrida*: changes in the pistil following compatible and incompatible intraspecific crosses. J Cell Sci 36:1–18

Ichimura K, Yamamoto Y (1992) Changes in the amount and composition of stylar canal exudate after self-pollination or cross-pollination in self-incompatible *Lilium longiflorum* Thunb. J Jpn Soc Hort Sci 61:609–617

Kadowaki KI, Matsuoka M, Murai N, Harada K (1988) Induction of two alcohol dehydrogenase polypeptides in rice roots during anaerobiosis. Plant Sci 54:29–36

Knox RB (1984) Pollen-pistil interactions. In: Linskens HF, Heslop-Harrison J (eds) Encyclopedia of plant physiology, vol 17. Springer, Berlin Heidelberg New York, pp 508–608

Kroh M, Miki-Hirosige H, Rosen W, Loewus F (1970) Incorporation of label into pollen tube walls from myoinositol-labeled *Lilium longiflorum* pistils. Plant Physiol 45:92–94

Labarca C, Loewus F (1973) The nutritional role of the pistil exudate in pollen tube wall formation in *Lilium longiflorum*. Plant Physiol 52:87–92

Lind JL, Bonig I, Clarke AE, Anderson MA (1996) A style-specific 120-kDa glycoprotein enters pollen tubes of Nicotiana alata in vivo. Sex Plant Reprod 9:75–86

Linskens HF, Schrauwen J (1966) Measurement of oxygen tension changes in the style during pollen tube growth. Planta 71:98–106

Matton DP, Constabel CP, Brisson N (1990) Alcohol dehydrogenase gene expression in potato following elicitor and stress treatment. Plant Mol Biol 14:775–783

Rousselin P, Toro Perea N, Doferus R, Tahar B, Caboche M, Jacobs M (1994) Complementation of an alcohol dehydrogenase-deficient *Nicotiana plumbaginifolia* mutant by transformation with the *Arabidopsis thaliana* Adh gene. Trans Res 3:376–387

Schwartz D (1971) Genetic control of alcohol dehydrogenase. A competition model for regulation of gene action. Genetics 67:411–425

Vogt T, Pollak P, Tarlyn N, Taylor LP (1994) Pollination- or wound-induced kaempferol accumulation in petunia stigmas enhances seed production. Plant Cell 6:11–23

Wang H, Wu HM, Cheung AY (1993) Development and pollination regulated accumulation and glycosylation of a stylar transmitting tissue-specific proline-rich protein. Plant Cell 5:1639–1650

Wisman E, Ramanna MS, Zabel P (1993) Isolation of two independent allyl alcohol resistant *Adh-1* null mutants in tomato following selection of pollen and seeds. Plant Sci 95:79–88

Wu HM, Wang H, Cheung AY (1995) A pollen tube growth stimulatory glycoprotein is deglycosylated by pollen tubes and displays a glycosylation gradient in the flower. Cell 82:395–403

Xie Y, Wu R (1989) Rice alcohol dehydrogenase genes: anaerobic induction, organ specific expression and characterization of cDNA clones. Plant Mol Biol 13:53–68