Alcohol dehydrogenase isoenzymes from *Nicotiana tabacum* include ADH of both *N. sylvestris* and *N. tomentosiformis*

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Abstract

Electrophoretic separation of seed alcohol dehydrogenase (ADH) from *Nicotiana tabacum* on 12 % starch gels at pH 7.8 produced only one band with an apparent R_f of 0.65, which confirmed earlier reports. The same was found with pollen ADH. However, in polyacrylamide gel isoelectric focusing, seed ADH separated into three distinct bands with apparent pI of 5.33, 5.42 and 5.50. The pI 5.33 isoenzyme was found to be the essential form in *N. sylvestris* seeds. The analysis of charge properties of *N. tomentosiformis* seed ADH showed only one isoenzyme with pI of 5.56. These results present further evidence that *N. tabacum* has arisen from a cross between a *N. sylvestris* predecessor and an ancestral type of *N. tomentosiformis*. The presence of the pI 5.42 form in *N. tabacum* is consistent with the reported formation of heterodimeric ADH in tobacco hybrids.

Introduction

The activity of alcohol dehydrogenase (ADH, EC 1.1.1.1.) in plants is normally expressed in very specific organs, such as maturing seeds and pollen. It can also be induced in various tissues in response to anaerobic conditions allowing anaerobic glycolysis. The polymorphism of the enzyme has been studied in many species using electrophoretic separation on starch gels (Tanksley and Jones 1981, Kut and Evans 1984, Lefranc-Riandey 1984, Trick *et al.* 1988). Using this technique Kut and Evans (1984) found only a single band with an R_f of 0.66 in ADH extracts from seeds of *Nicotiana tabacum* which was rather unexpected in relation to the hypothesis that *N. tabacum* has arisen from a cross between predecessors of *N. sylvestris* and *N. tomentosiformis* (Smith 1979). The authors explained their findings in terms of identical ADH genes in the putative parental species. However, the results of ADH separation by isoelectric focusing presented here demonstrate multiple banding of *N. tabacum* ADH, and indicate that ADH in *N. sylvestris* and *N. tomentosiformis* are encoded by different genes.

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Abbreviations: ADH - alcohol dehydrogenase, IEF - isoelectric focusing.

Materials and methods

Dry seeds and pollen collected from greenhouse-grown plants of *Nicotiana tabacum* cv. Samsun, *N. sylvestris* and *N tomentosiformis* were crushed in a cooled mortar either in 0.1 M Tris-Cl pH 7.5, 1.5 % reduced glutathione for starch gel electrophoresis or in 0.05 M Tris-Cl pH 7.8 for isoelectric focusing (IEF).

Horizontal starch gel electrophoresis of ADH was assayed at 10, 12 and 14 % starch (*Sigma Chemical Company*, St. Louis, USA) concentrations and in three different buffer systems: sodium-borate/Tris-citrate pH 7.8, lithium-borate/Tris-citrate pH 8.3 and morpholine-citrate pH 6.1 (Shield *et al.* 1983). The gels were run at 45 mA, 4 °C for 4 h. ADH activity on the gels was detected by reaction with stain based on thiazolyl blue as described by Kut and Evans (1984).

IEF was performed in 0.8 mm thick 5 % polyacrylamide gels containing 2 % *Servalyte* (*Serva*, Heidelberg, Germany) pH 3 to 10. The gels were run on a flat bed apparatus at a constant power of 2 W at 25 °C for 2 h. At the end of the run, the pH gradient was measured by surface electrodes and the activity of ADH was visualized according to Brewer and Sing (1970). Stained gels were scanned at 633 nm on an *Ultroscan XL densitometer* (*LkB*, Uppsala, Sweden).

Results

In our IEF studies of *N. tabacum* pollen we observed multiple banding of ADH (not shown), in contrast to the reported (Kut and Evans 1984) single banding of seed ADH of this species in starch electrophoresis on 12 % gels at pH 7.8.

Examination of this discrepancy confirmed that in starch electrophoresis ADH activity extracted from *N. tabacum* seeds or pollen runs as only one band with an apparent R_f of 0.65 (Fig. 1A, lanes 1,2). Variation of starch concentration and buffer systems did not modify the separation significantly.

The ADH polymorphism in *N. tabacum* pollen observed by means of IEF suggested the possibility of using ADH isoenzymes for examination of the phylogenetic origin of this amphidiploid species. Thus, ADH of *N. sylvestris* and *N. tomentosiformis* was evaluated according to charge properties with polyacrylamide gel IEF using seed extracts and their mixtures. Seed ADH of *N. tabacum* separated into three distinct bands with apparent pI of 5.33, 5.42 and 5.50 (Fig. 1B). ADH of *N. sylvestris* produced only one strong band identical with the most acidic isoenzyme of *N. tabacum*. ADH from *N. tomentosiformis* seeds ran in the focusing system as a single band with very similar charge properties to the pI 5.50 of *N. tabacum*. The identity of the pI 5.33 isoenzymes from *N. tabacum* and *N. sylvestris* and the close similarity of the *N. tomentosiformis* ADH with the pI 5.50 form of *N. tabacum* could be confirmed by analysis of mixtures of seed extracts (Fig. 2).

Discussion

Various levels of evidence have been obtained indicating that ancestors of *N. sylvestris* and *N. tomentosiformis* are the most likely progenitors of *N. tabacum* (Smith 1979) and the results presented here provide further evidence for this hypothesis. *N. tabacum* ADH could be separated by IEF into three bands from which one was shown to be identical with the principal isoenzyme of *N. sylvestris* and one exhibited close pI similarity with the single ADH form of *N. tomentosiformis*. These findings are consistent with an analysis of artificially obtained hybrids according to which the present forms and the ancestral forms giving rise to *N. tabacum* may be identical in *N. sylvestris* but somewhat different in the case of *N. tomentosiformis* (Goodspeed and Clausen 1928).

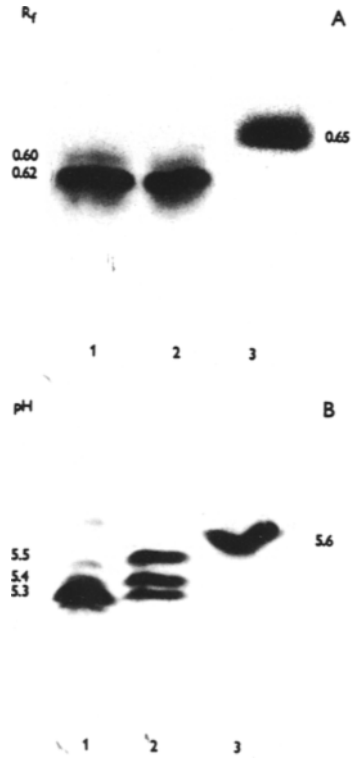

Fig. 1. Separation of ADH isoenzymes from *Nicotiana* pollen (lane A1) and seeds (lanes A2, A3 and B1 to B3) by starch gel electrophoresis (A) and isoelectric focusing (B). A1 to A3 - *N. tabacum*; B1 - *N. sylvestris*; B2 - *N. tabacum*; B3 - *N. tomentosiformis*. Starch concentration was 12 % (lanes A1, A2) or 14 % (lane A3) and the gels run at pH 7.8.

The active ADH enzyme in plants is dimeric and the isoenzymes are formed by homodimers and intergenic heterodimers. The formation of heterodimers has also been observed in *Nicotiana* hybrids (Kut and Evans 1984). The ADH isoenzyme of

N. tabacum with the intermediate mobility can thus be considered as an intergenic heterodimer formed by association of ADH polypeptides of *N. sylvestris* and *N. tomentosiformis*.

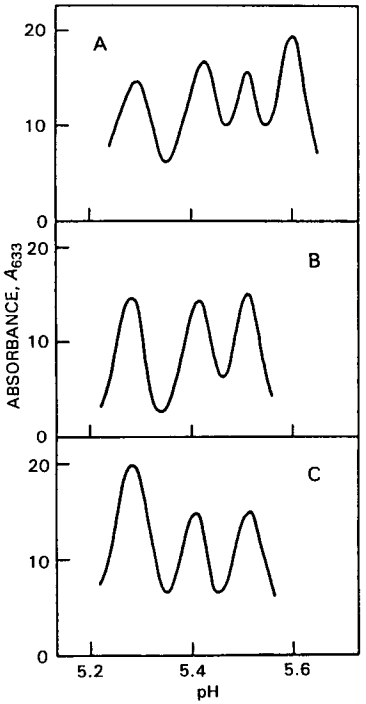

Fig. 2. Densitometry of seed ADH isoenzymes separated by polyacrylamide gel IEF. A - *N. tabacum* and *N. tomentosiformis* mixture; B - *N. tabacum*; C - *N. tabacum* and *N. sylvestris* mixture.

The results also point out the importance of isoenzyme analysis by both classical and IEF electrophoresis for classification of plant species and their phylogenetic relationships.

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