Pollen and Anther Proteins for Identification of the Somatic Hybrid of Potato and Tomato Plants

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Proteins extracted from pollen and anthers of tomato (*Lycopersicon esculentum* var. *cerasiforme* : mutant *"gilva"*), potato (*Solanum acaule*) and their somatic hybrids were quantitatively and qualitatively compared. The viability of pollen was high in both parental plants and extremely low in the somatic hybrid. The anthers of somatic hybrid were characterized by lower protein content and higher percentage of aborted micropollen than their parental plants. Either of parental plants had species-specific protein bands when their anther proteins were analysed with isoelectric focusing. The hybrid plants had an intermediate type of protein band patterns. The results show that pollen and anther proteins can be used for hybrid identification as well as for interspecific comparison.

Key words : Tomato, Potato, Somatic hybrid, Anther protein, Pollen protein.

Introduction

The technique of protoplast fusion has promoted progress in the plant sciences, since this technique offers a method of rapid reproduction with the advantages of omitting the sexual processes and of producing the hybrid plants from two sexually imcompatible species. The first success in somatic hybridization of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*[1]) was reported by Melchers *et al.* Later, another somatic hybrid of different species (*S. acaule* (4n=48) × *L. esculentum* var. *cerasiforme* : mutant *"gilva"* (chlorophyll-deficient)) was produced by Ninnemann and Melchers. The fused protoplasts from both partners were obtained by PEG-method.[2] For the identification of the somatic hybrids used in the present study, the methods using enzymes as well as satellite DNA have been applied with success.[3,4] Other methods using monoclonal antibodies[5] or morphological, cytological and biochemical attributes[6] had been applied to distinguish the somatic hybrid of other plants. In the present study, we attempted using the other character, the pollen grains, for identification of the somatic hybrids of

tomato and potato. Although the use of pollen and anther proteins to identify a somatic hybrid is conducted at a later developmental stage, it provides an alternative to the already-known methods.

Pollen grains, the male gametophytes of plants, have rather stable morphological and biochemical attributes. Such characteristics have been used as an important parameter for systematic study of different plant groups.[7] For instance, pollen proteins of *Typha* have been used to determine the interspecific relationship of this genus.[8] Gay *et al.*[9] have even shown that pollen proteins can be used to determine the genotype of pollen grain.

For the study of pollen proteins, it is necessary to use a micro-analytical method. The newly developed thin-layered IEF technique makes it possible to analyse the pollen proteins by using only a few grains.[10] In this study, this method was applied.

Materials and Methods

The somatic hybrids from tomato (*Lycopersicon esculentum* var. *cerasiforme* : mutant *"gilva"*, chrorophyll-deficient, 2n=24) and wild potato (*Solanum acaule*, 4n=48) were obtained by protoplast fusion performed by Ninnemann and Melchers. The protoplasts of *S. acaule* were treated with 0-6 mM iodoacetate prior to cell fusion. The hybrids and their parent plants were grown in the greenhouse of the Institute of Chemical Plant Physiology, University of Tübingen, FRG.

Viability test of pollen

Immediately after harvesting, the viability of pollen was determined by means of fluorochromatic reaction (FCR).[11,12] The percentage of fertile pollen was evaluated on the basis of a count of 500 pollen.

Extraction of pollen and anther proteins

Fresh pollen and anthers were harvested and immediately stored under refrigeration in order to avoid dehydration or rapid aging. The preservation of materials was done by plunging the pollen and anthers into liquid nitrogen, and then storing at −20℃.

Fresh and preserved materials of an anther were pressed in 20 μl buffer solution containing 20 mM tris-HCl (pH 6.85) and 1% mercaptoethanol. After three treatments of repeated freezing in liquid nitrogen and thawing at room temperature, the protein extracts were separated from the insoluble particulates by centrifugation at 15,000 g ×15min, 0℃. The extracts were then ready for further analysis.

Determination of protein concentrations

The protein content of pollen and anther was estimated by the modified Lowry method[13] with bovine serum albumin as the standard.

Isoelectric focusing (IEF) in polyacrylamide gel

Ten μl of protein extracts were applied onto ampholine polyacrylamide gels (240×100×1.0 ㎜) made by LKB at 2 ㎝ from the cathodic edge. The gel plates (gel composition : T=5%, C=3%) contained 3% (w / v) carrier ampholytes. The separation of protein was performed by IEF on LKB Ultrophor apparatus with 1,500 V, 50 mA and 25 W for 1.5 hours and 2,000 V, 25 mA and 25 W for 2.5 hours for pH 3.5-9.5 gel and pH 4.0-6.5 gel respectively. The cooling plate was maintained at 4℃. Immediately after focusing, the proteins were visualized by silver staining method.[10] All reagents used were of analytical grade.

Results

Viability of pollen

The number of pollen grains in an anther was definetely different in tomato and potato plants. The tomato plants had higher number of pollen in an anther than potato plants (Table 1). In comparing with the parental plants, somatic hybrid plants had very low number of pollen in an anther.

Table 1. Average number of pollen grains in an anther of *L. esculentum* var. *cerasiforme* : mutant *"gilva"*, *S. acaule* and different clones of their somatic hybrids

Plant	$\times 10^3$ Grains / anther
L. esculentum var. *cerasiforme* mutant *"gilva"*	132
S. acaule	13
Clone g+0* 457 II	2
Clone g+0* 433 II b 2 S 3	1
Clone g+2* 591 II aS 1	1.2
Clone g+6* 221 I S 1	1.5

* Symbols of clone : example : g+2, g=gilva, +2 represents treatment of 2 mM iodoacetate on *S. acaule*.

Most of the pollen grains from somatic hybrid plants were sterile. The sterile pollen might occupy over 99% of the total pollen grains in an anther (Table 2). These sterile pollen grains were easily recognized by their shrunken and mis-shaped form. In contrast to the hybrid plants, the percentages of fertile pollen in parental plants were relatively high, usually higher than 50% of the pollen in an anther.

Table 2. Average percentage of viable pollen in an anther of *L. esculentum* var. *cerasiforme* : mutant *"gilva"*, *S. acaule* and different clones of their somatic hybrids

Plant	Pollen viability (%)
L. esculentum var. *cerasiforme* mutant *"gilva"*	60.1
S. acaule	52.7
Clone g+0* 433 IIb2S3	0.3
Clone g+2* 591 IIa1	0.2
Clone g+2* 591 IIa12S1	0.6
Clone g+2* 283 IIb2bS4	0.1
Clone g+4* 465 IS15	0.0
Clone g+4* 473 IIbS4	0.0

* Symbols of clones are the same as noted in Table 1.

Protein content

Neither parental plant showed a large difference in the protein content of anthers (Table 3). Compared with the parental plants, the protein content of anthers in hybrid plants was very low ; it was only about 60% of that of their parent plants. There was no significant difference in the protein content of the anthers between different clones of hybrids.

In regard to the protein content of a pollen grain, neither parental plant differed from each other significantly. It was about 0.70 and 0.78 ng per pollen grain of *L. esculentum* var. *cerasiforme* : mutant *"gilva"* and *S. acaule*, respectively. Protein content of the hybrid pollen grain was not determined.

Protein band patterns analysed with IEF

Most of proteins extracted from pollen and anthers migrated to the acidic pH range when the proteins were separated by IEF of pH 3.5–9.0 gel and pH 4.0–6.5 gel. Due to the difficulty of obtaining adequate pollen proteins for the analysis of hybrid plants, the comparison between hybrid and parental plants was conducted by using the protein extract of the anthers. Figure 1 shows that there were about 32 distinguishable anther protein bands found in the pH range of 4.0–6.5. Either of the parental plants possessed about 3–5 species-specific protein bands in addition to the common protein bands (about 24 bands) within this pH range. In comparing with

Table 3. Average protein contents per mg anther (wet weight) of *L. esculentum* var. *cerasiforme* : mutant *"gilva"*, *S. acuale* and different clones of their somatic hybrids

Plant	μg Protein / mg anther
L. esculentum var. *cerasiforme* mutant *"gilva"*	42.83
S. acaule	38.61
Clone g+0* 457 II	23.10
Clone g+0* 433 IIb2S3	22.90
Clone g+2* 591 IIa1S1	24.75
Clone g+2* 591 IIa1S3	23.40
Clone g+2* 591 IIa2S1	24.20
Clone g+4* 465 IIaS1	24.00

* Symbols of clones are the same as noted in Table 1.

parental plants, the hybrid plants showed an intermediate type of protein band pattern. One new protein band other than those found in parental plants was detected in hybrid plants. The other results, shown in Fig. 2, indicate that the band pattern of pollen proteins separated with IEF were somewhat different from that of anther proteins. However, similar to the results obtained with anther proteins, both parental plants also showed significant differences in the band pattern of pollen proteins.

Discussion

The pollen wall is composed of exine and intine. It has been found that these two components developed from different origins, the exine from diploid tapetum and the intine from haploid gametophyte.[14,15] The proteins found in the exine and intine are therefore sporophytic and gametophytic in origin respectively. The pollen proteins extracted with buffer, as used in this study, cover both parts because these proteins are readily desolved from the pollen wall when the pollen were soaked in aqueous solutions.[16]

By analysis with IEF, it was found that most pollen and anther proteins from *L. esculentum* var. *cerasiforme* : mutant *"gilva"* and *S. acaule* as well as their somatic hybrid plants were located in the acidic pH range, especially between pH 4.5 and 6.0. In this range, both the tomato and potato plants had some species-specific protein bands. This indicates that the qualitative comparison between plants can be performed at this range.

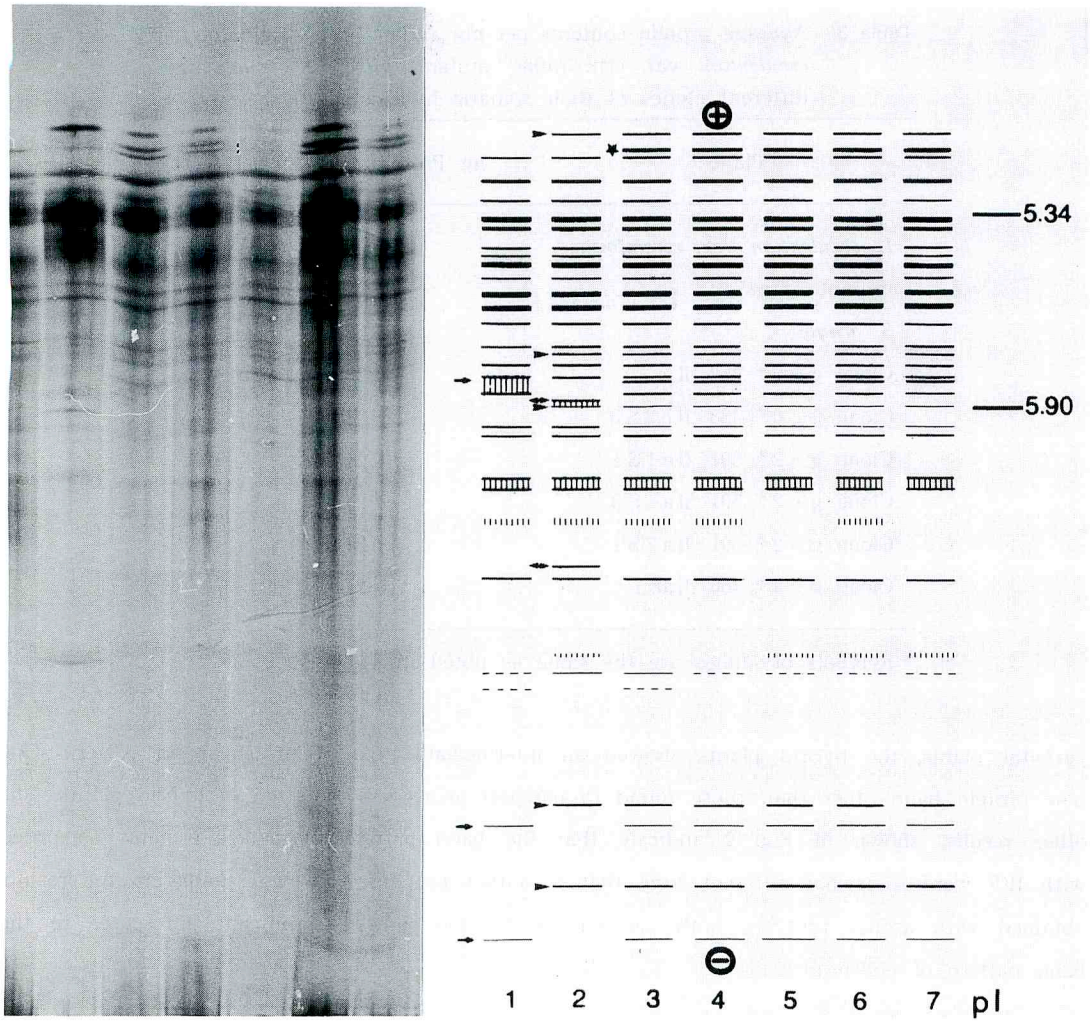

Fig. 1. Band patterns of anther proteins of *S. acaule* (1), *L. esculentum* var. *cerasiforme* : mutant *"gilva"* (2) and different clones of their somatic hybrids : g+2 591(3), g+4 465 (4), g+4 465 (5), g+6 221 (6), and g+6 288 (7) separated with the non-linear pH gradient IEF between pH range of 4.0 and 6.5. →, specific band of *S. acaule* ; ➤, specific band of *L. esculentum* var. *cerasiforme* mutant *"gilva"*; ★, new band of somatic hybrid ; ⟷, specific bands of *L. esculentum* var. *cerasiforme* mutant *"gilva"* but not found in somatic hybrids.

Although a direct comparison of pollen proteins between hybrid and parental plants was not performed due to the difficulty of obtaining enough pollen grains, the comparison by IEF analysis of pollen proteins between both parental plants showed that there were some species-specific protein bands.

Some methods, including the use of isozymes[3] and satellite DNA,[4] have been applied for

the identification of the somatic hybrids of tomato and potato plants. These methods permit hybrid identification at earlier developmental stage of plants, such as at callus or seedling stage. The use pollen and anther proteins, however, provides an alternative to the already-known methods, even though it is conducted at a later developmental stage. The pollen and anther proteins are rather stable. They did not change qualitatively during storage in the refrigerater for several days (data not shown). By studying *Vitis vinifera*,[17] it has shown that the expression of clone-specific characters of pollen proteins was rather stable and was independent of change of environmental factors. This research indicates that pollen and anther proteins are suitable for species identificaton or interspecific comparison.

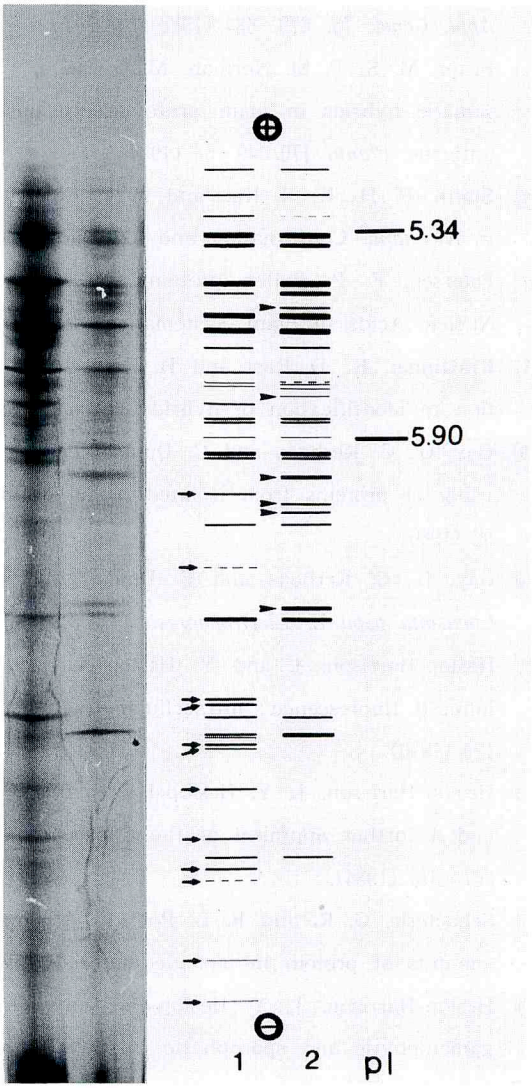

Fig. 2. Band patterns of pollen proteins from *S. acaule* (1) and *L. esculentum* var. *cerasiforme* : mutant *"gilva"* (2) separated with the non-linear pH gradient IEF between pH range of 4.0 and 6.5. →, specific band of *S. acuale* ; ➤, specific band of *L. escuientum* var. *cerasiforme* : mutant *"gilva"*.

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