Comparison of isozyme electrophoresis and molecular methods in screening of near isogenic sunflower lines for resistance genes

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Summary

Downy mildew is one of the major diseases of cultivated sunflower. In order to screen for a marker, two pairs of "near isogenic lines" (NILs) which differ with respect to the presence or absence of a resistance gene were analysed by isozyme electrophoresis, DNA-fingerprinting, RFLP and RAPD technique. Although no marker could be identified, but the results gave information with regard to identification of lines and their pedigrees. The identification of one pair of NILs was even possible by esterase-isozyme-electrophoresis. The other techniques except the RFLP-technique revealed differences between NILs.

Introduction

During the last three years an increasing appearence of Downy mildew in Germany could be observed. This disease is caused by the fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni (ZIMMER and HOES, 1978). One of the known genes for resistance is the single dominant Pl2-gene which confers resistance to the races 1, 2 and 4 (VEAR and LECLERCQ, 1971; ZIMMER and KINMAN, 1971; ZIMMER and KINMAN, 1972). Due to the expansion of sunflower production in Germany from 20.000 ha in 1988 to 77.000 ha today (ZMP, 1992) the breeding for disease resistance became more and more important. Resistance breeding can be facilitated by the use of genetic or molecular markers. There are several techniques which have already been successfully used in screening for polymorphisms in other species. The oldest and most commonly applied method is the isozyme electrophoresis. For example the linkage map of tomato is based on both isozyme and RFLP-markers (BERNATZKY and TANKSLEY, 1986). A great number of resistance markers could be identified by the RFLP-technique. For example, markers for the Mla-locus for powdery mildew resistance in barley (SCHÜLLER et al., 1992) and a cyst nematode resistance gene in potato (BARONE et al.,1990). An alternative technique, which is less time consuming is the PCR (polymerase chain reaction). Depending on the length of the used primer the technique is named RAPD (random amplified polymorphic DNA, 9-10 bp) or AP-PCR (arbitrarily primed polymerase chain reaction, more than 10 bp). Both variants have been used to detect polymorphisms in several species (WELSH and McCLELLAND, 1990,1991; WILLIAMS et al., 1990). We examined two pairs of NILs (near isogenic lines) with the methods described above and with the DNA-fingerprint technique (WEISING and KAHL, 1990) in order to screen for a marker and to analyse the degree of conformity of the plant material.

Materials and Methods

Plant material:
Two pairs of NILs (S-1358-Pl2 / S-1358; AS-110-Pl2 / AS-110) which differ in the presence of the Pl2 resistance gene developed by VRANCEANU (pers. comm.) were used as plant material. The resistant lines were crossed with HA89 (cms), so that both F1 progenies and the line HA89 (cms and fertile analogue) could be examined.

Isozyme electrophoresis:
Isozyme analysis was carried out with the isozymes GPI (glucosephosphatisomerase), PGM (phosphoglucomutase) and esterase.

RFLP analysis:
DNA extraction, restriction digest of 10 µg DNA with the enzymes Bam HI, Eco RI, Eco RV and Hind III, agarosegelelectrophoresis, Southern-transfer and the radioactive hybridization were generally carried out as described in SAMBROOK et al. (1989) or by the respective producer protocols. For non-radioactive hybridization the Digoxigenin-system (Boehringer) was applied. As probes for RFLP analysis we cloned Eco RI or Pst I digested DNA of the sunflower line S-1358-Pl2 in the plasmidvector pBLUESCRIPT and hybridized to 35 Eco RI and 30 Pst I clones.

DNA-fingerprinting:
The same procedure as described above, except the enzymes (Alu I, Dra I, Hinf I, Nde II, Rsa I, Taq I) was applied. The Southern-blots were hybridized to the oligonucleotides $(GACA)_4$, $(GATA)_4$, $(GGAT)_4$ (Fresenius, Germany).

PCR-conditions:
PCR reactions were performed in 50µl of 1 x Taq buffer (NBL, adjusted to 3 mM $MgCl_2$), 0.4 µM dNTP, 0.5 µM primer, 40 ng template DNA and 1 U Taq-polymerase. 40 decamer primers (set B and T from Operon) for RAPD- analysis and 30 primers (12 up to 33 nucleotides long) were used. PCR amplification was carried out in 45 cycles of 1 min at 94°C, 1 min at 36°C (RAPDs) and 45°C-66°C (AP-PCR), 2 min at 72°C with an increasing extension time of 5 sec/cycle for AP-PCR.

Results

The comparison of isozyme and DNA-patterns of both pairs of NILs did not reveal a band which could be used as a marker. Either the pattern of NILs were identical or one pair differed in the pattern but not the other one. In some cases the bands are totally different but the pattern did not correlate with the presence of the resistance gene. With regard to isozymes it was possible to obtain banding patterns with all of the three enzymes used (Fig.1); however, the quality and

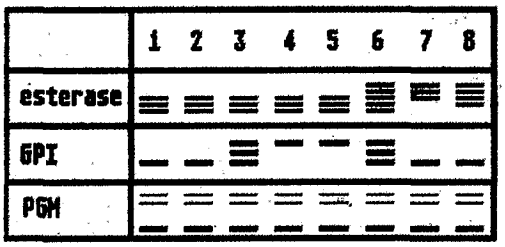

Fig. 1. Isozyme analysis of NILs [S-1358-Pl2 (2) / S-1358 (1), AS-110-Pl2 (7) / AS-110 (8)] and F1s [HA89 (cms) x S-1358-Pl2 (3), HA89 (cms) x AS-110-Pl2 (6)] and HA89 [cms (5) and fertile analogue (4)]with the isozymes GPI, PGM and esterase.

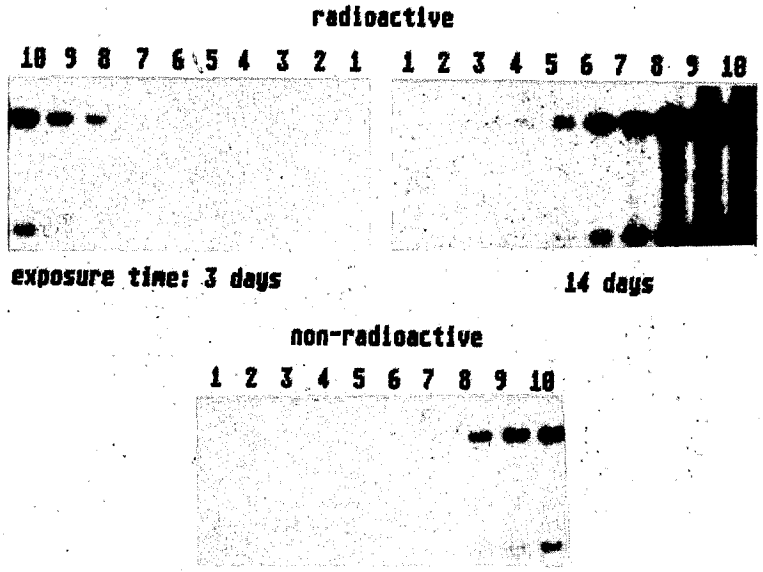

Fig. 2. Comparison of non-radioactive (Digoxigenin system, Boehringer) and radioactive ([^{32}P]dCTP) hybridization. The same southern-blot with different amounts of Eco RI digested genomic DNA (lane 1-10: 5 ng, 10 ng, 25 ng, 50 ng, 100 ng, 250 ng, 500 ng, 1 µg, 2.5 µg, 5 µg) was hybridized first non-radioactively and later radioactively.

degree of information differed. PGM showed no differences in the pattern with very weak bands. The very clearly visible GPI bands allowed the separation of plant material in three groups of patterns: both pairs of NILs, both F1 progenies and HA89 (cms), HA89 (fertile). Esterase showed specific patterns for AS-110-Pl2, AS-110 and HA89 (cms) x AS-110-Pl2.

Both pairs of NILs and HA89 (cms) which were hybridized to 65 probes for RFLP-analysis the same pattern in all cases. The comparison of non-radioactive and radioactive hybridizations demonstrated that the radioactive method is 25 x more sensitive (Fig.2).

For identification of the most informative enzyme/probe combination for DNA-fingerprinting both pairs of NILs were examined. The probe $(GATA)_4$ gave the best signals both qualitatively and quantitatively (Fig.3, Fig.4). Therefore, the F1s and HA89 (cms/fertile) were analyzed by the combination Eco RI, Hinf I/$(GATA)_4$. Each DNA gave a specific pattern except HA89 (cms) and HA89 (fertile).

It was not possible to identify a marker band by PCR with 70 primers, but some primers showed an interesting pattern. Therefore, the F1 progenies, HA89 (cms)

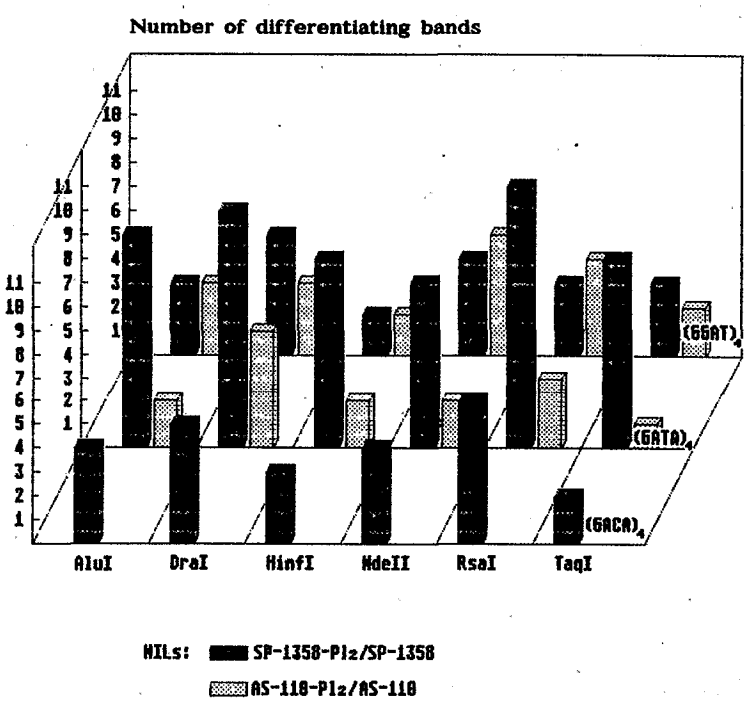

Fig. 3. Number of bands that differentiate between the NILs (S-1358-Pl2 / S-1358, AS-110-Pl2 / AS-110)using DNA fingerprinting technique.

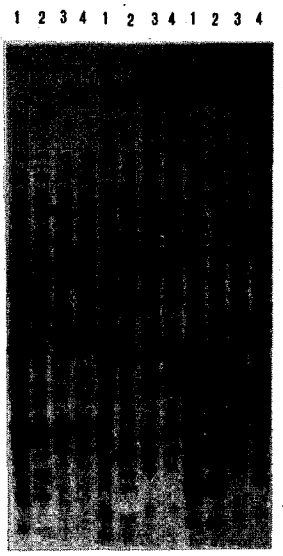

Fig.4. DNA-fingerprinting of NILs [S-1358-Pl2 (1) / S-1358 (2), AS-110-Pl2 (3) / AS-110 (4)] by using restriction enzymes Alu I (a), Dra I (b), Hinf I (c) and $(GATA)_4$ as hybridization probe.

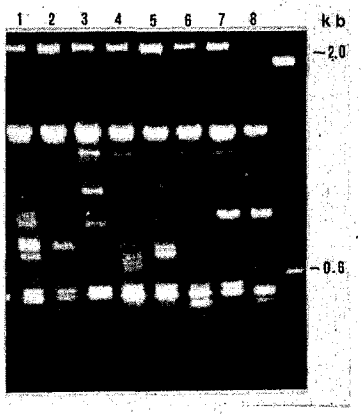

Fig. 5. PCR-amplification of NILs [S-1358-Pl2 (2) / S-1358 (1), AS-110-Pl2 (7) / AS-110 (8)] and F1s [HA89 (cms) x S-1358-Pl2 (3), HA89 (cms) x AS-110-Pl2 (6)] and HA89 [cms (5) and fertile analogue (4)], with AP-PCR-primer AP17.

and HA89 (fertile) were analyzed with these primers, too. In several cases the pattern of each DNA proved to be specific, so that an identification is possible. However, a clear identification of progeny and pedigrees remains difficult because of non-parental bands (Fig. 5).

Discussion

Although a molecular marker for the resistance gene Pl2 could not be identified, the results show that in some cases - for example identification of NILs and hybrids - the isozyme analysis gave the same information than molecular techniques. The PCR technique allows a differentiation of unrelated DNAs. However, in the present case several non-parental bands made a clear pedigree assessment difficult. The same phenomenon was also described by RIEDY et al. (1992). Consequently, the DNA-fingerprinting is the best method for identification and progeny analysis today. But the PCR technique will probably be the method of choice for marker selection as well as for identification and pedigree analysis in the future after optimizing the technique.

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