*HELIA*, **15**, Nr. 17, p.p. 7-16 (1992)

# CONSTRUCTION OF A SUNFLOWER PEDIGREE MAP

M. Korell, G. Mösges and W. Friedt

*Institute of Agronomy and Plant Breeding I*
*Justus Liebig University, Ludwig str. 23, D–6300 Giessen, Germany*

SUMMARY

A pedigree map of sunflower (*Helianthus annuus*) was prepared which contains detailed information on North American sunflower materials, e.g., published in various issues of Crop Science. A catalogue provides futher information on the pedigree and important agronomic traits, like disease resistance, oil content and maturity of the released germplasm pools, germplasms, populations, lines and cultivars.

For verification of the relationships shown in the pedigree map, the restorer lines RHA 278 and RHA 279, which were derived from an $F_5$ sib–selection, were investigated by Southern analysis with 75 genomic probe/enzyme combinations. They differ in the $Pl_2$–gene conferring resistance to downy mildew, caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni. However, no restriction fragment length polymorphism (RFLP) could be detected. This result confirms the supposed close relationship between the two lines.

**Key words: Sunflower, pedigree map, RFLP analysis.**

## INTRODUCTION

*Helianthus* is one of the few crop genera which is native to North America (Seiler, 1988). In last 25 years, many germplasms and parental lines have been released by research workers of the United States Department of Agriculture (USDA) in California, North Dakota and Texas and the Agriculture Canada Research Station, Morden, Manitoba, respectively. Using wild relatives of sunflower to increase the genetic diversity in cultivated sunflower, more than 100 parental lines and more than 60 fertility restorer lines were released and most of them were described in Crop Science. This material was based on lines with a high oil content developed at VNIIMK, Krasnodar, Russia, and high–oil open–pollinated cultivars such as "Peredovik" bred in the former USSR.

It is difficult to obtain an overall view on this material because of the host of information, especially if one is looking only for a specific trait. Therefore, the aim of this work was to construct a sunflower pedigree map which should be helpful for obtaining information on genetic relationships in North America breeding material. A second goal of this investigation was to use molecular methods for verification of the supposed relationships by application of RFLP–analysis. The successful use of this technique has been demonstrated for many species (Helentjaris et al., 1985, Miller and Tanksley 1990, Song et al., 1990, Gebhardt et al., 1991, Graner et al., 1991. Wang et al., 1992; for review see Beckmann and Soller, 1986).

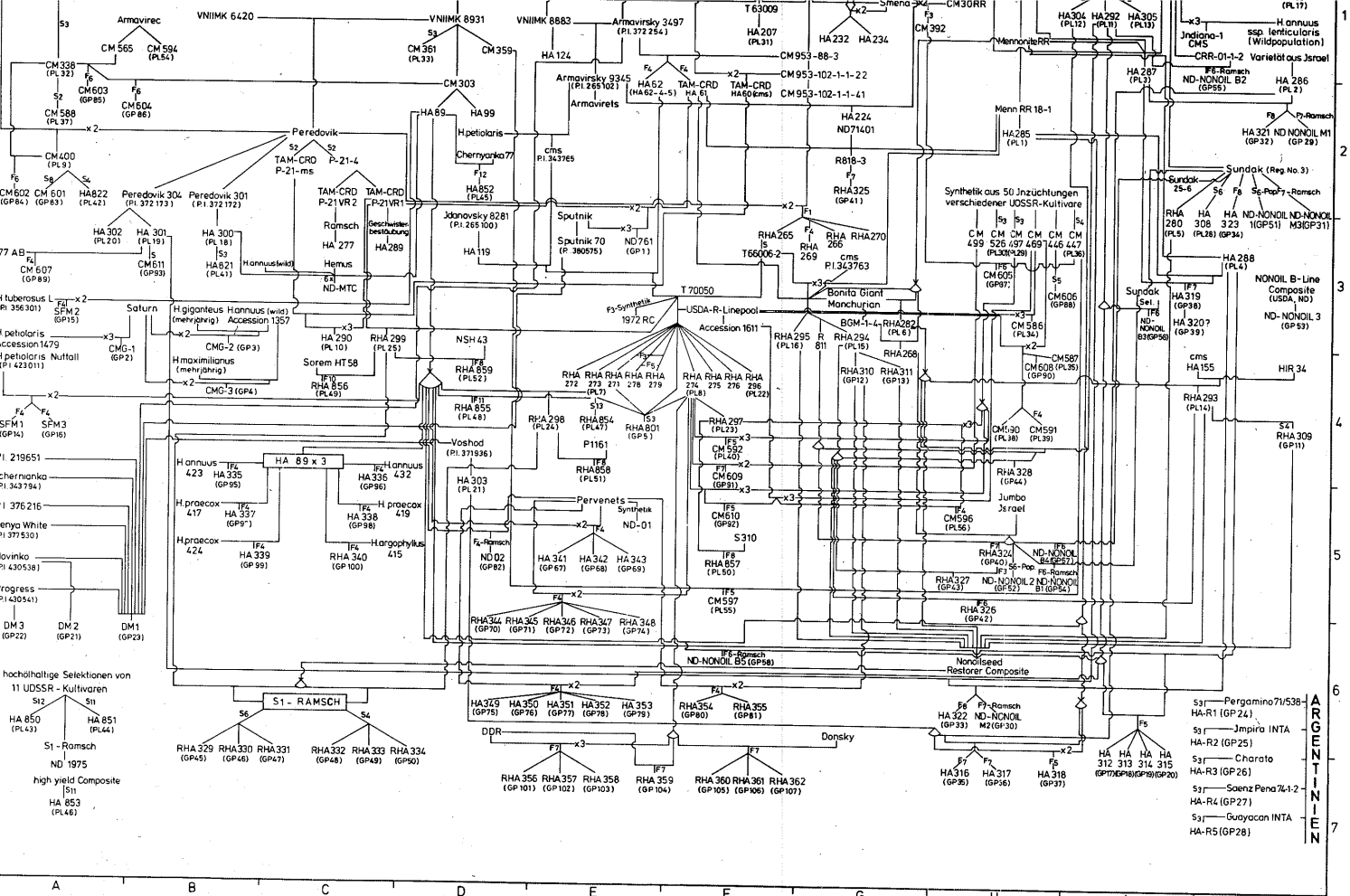



*Figure 1: Pedigree map of North American sunflower breeding material*



*HELIA*, **15**, Nr. 17, p.p. 7-16 (1992)

Table 1: Parts of the catalogue

| Line | Year Origin | Pedigree | Resistance | Oil | Maturity | Remarks (Crop Sci.) | Map |
|---|---|---|---|---|---|---|---|
| **Parental Lines and Germplasms** | | | | | | | |
| HA 89 | 1971 Texas | selected from CM 303 | Verticillium dahliae ($V_1$) | Oil | middle | | D2 |
| HA 99 | 1971 Texas | sib–selection from HA 89 | Verticillium dahliae ($V_1$) | Oil | middle | | D2 |
| HA 113 | 1971 Texas | isolated from VNIIMK 1646 | | Oil | middle | | F1 |
| **Restorer Lines and Populations** | | | | | | | |
| RHA 278 | 1975 Texas, North Dakota | cms PI 343765/HA 119//HA 62–4–5 and single plant selections from T 66006–2 (RHA265) | Plasmopara halstedii ($Pl_2$) | Oil | middle | recessively branched at the upper internodes | E4 |
| RHA 279 | 1975 Texas, North Dakota | cms PI 343765/HA 119//HA 62–4–5 and single plant selections from T 66006–2 (RHA 265), $F_5$ sib–selection from RHA 278 | Puccinia helianthi | Oil | middle | recessively branched at the upper internodes | E4 |
| RHA 269 | 1975 Texas, North Dakota | selfpollinated $F_1$ plant from the backcross Peredovik*2/CM 953–102–1–1–41, F4 sib–line from RHA 266 | Puccinia helianthi | Oil | middle | environmental sensitive form of recessive basal branching, taller and later than RHA 266 | G3 |
| **Germplasm Pools** | | | | | | | |
| Helianthus Germplasm Pool I (GP 7) | 1981 California | originated from 10000 lines from the Northrup King Company plus 100 lines from other sources | | Oil | | evaluated for seedling vigor, agronomic type (22:1276) | – |
| **Cultivars** | | | | | | | |
| Mingren (Reg.No.2) | 1964 Minnesota | five generations of mass selection in the Mennonite variety | | Nonoil | middle | single–headed (7:404) | H1 |

## PEDIGREE MAP

The almost complete information on the North American sunflower material documented in various issues of Crop Science is a powerful tool for sunflower breeders. This material was originated from old open pollinated populations like Mammoth Russian, Armavirsky, Mennonite, Smena, Armavirec, Jdanovsky, Peredovik, Pervenets of the institute VNIIMK at Krasnodar, Russia. Since a compilation of the vast information will be helpful for every sunflower breeder, a map was generated by using various articles on registration of sunflower germplasms and lines published in Crop Science until 1989. Information not documented in Crop Science was provided by Jerry F. Miller, Fargo, North Dakota.

The complete pedigree consists of the following four parts:

* a map showing genetic relationships (Figure 1),
* a catalogue which contains the original pedigree data and important agronomic traits, like disease resistances, oil content, maturity, as well as further items like the registration number and the reference (parts of this catalogue are shown in Table 1),
* a list of abbreviations,
* a reference index for rapid orientation.

The catalogue, the list of abbreviations, and the reference index are also available on IBM compatible EDP. If the user is interested in further information on sunflower releases in the USA and Canada, the catalogue and the reference index will lead directly to the respective articles in Crop Science, where detailed information on the individual material can be found.

### Genetic Relationship between Selected Lines

For RFLP–analysis the restorer liner RHA 278 and RHA 279 were used. The two lines are derived from an $F_5$ sib–selection differing in the $Pl_2$–gene conferring resistance against the Red River Race of *Plasmopara halstedii* (Farl.) Berl. et de Toni. This gene was first described in the line HA 61 (Zimmer and Kinman, 1972), a sib–line of the donor parent HA 62–4–5 (HA 62) from which the resistance gene of RHA 278 is derived (Figure 2).

According to the pedigree, a close relationship between these two lines is supposed. However, they differ with regard to the $Pl_2$–gene which is further investigated in our institute by sreening near isogenic lines (Mösges and Friedt, 1992). Therefore, an attempt was made to find a difference on the molecular level using several randomly selected genomic clones as probes in Southern analysis.

## MATERIALS AND METHODS

Plants of the restorer lines RHA 278 and RHA 279 were grown in the greenhouse and the young leaves were harvested on ice after two days incubation in a dark room to avoid an accumulation of polysaccharides and stored at –80°C.

DNA from leaves was extracted according to Murray and Thompson (1980) and purified with a method for a large scale plasmid minipreparation (Wilson, 1987).

The genomic DNA (ca. 10$\mu$g) was digested with the restriction enzymes *Bam HI*, *Eco RV*, *Hind* III, *Eco RI*, and *Bst EII*, using 2.5 units per $\mu$g DNA for 5 h or overnight,

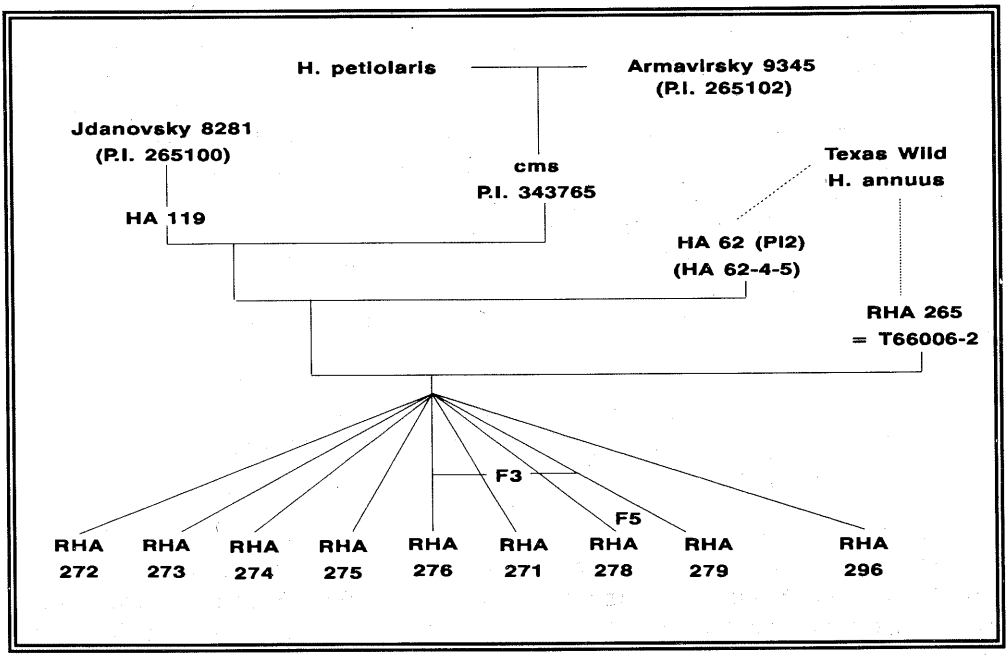


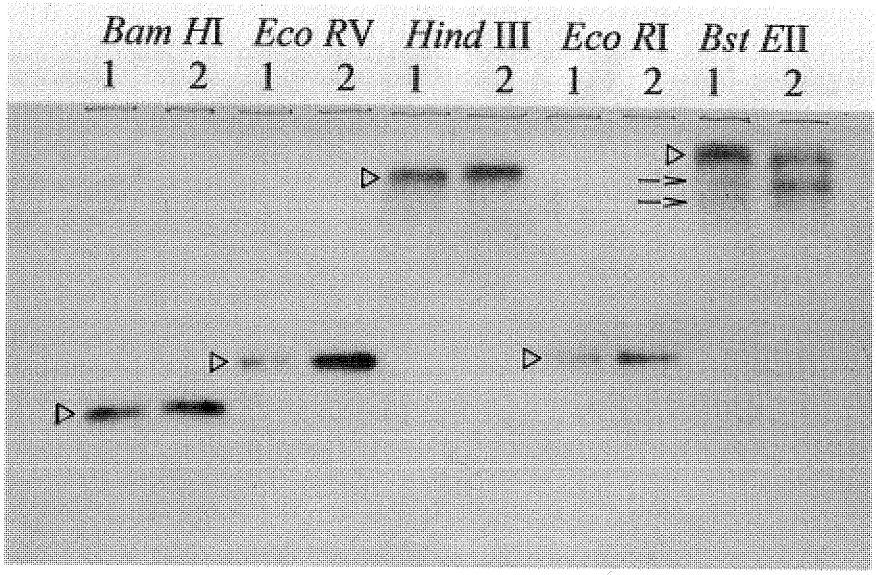

*Figure 2: Pedigree of the F5–siblines RHA 278 and RHA 279*

according to the supplier's instructions (Boehringer). The restriction fragments were size–separated on a 0.8% agarose gel (Maniatis et al., 1989) and transferred to a nylon membrane (GSP, Du Pont).

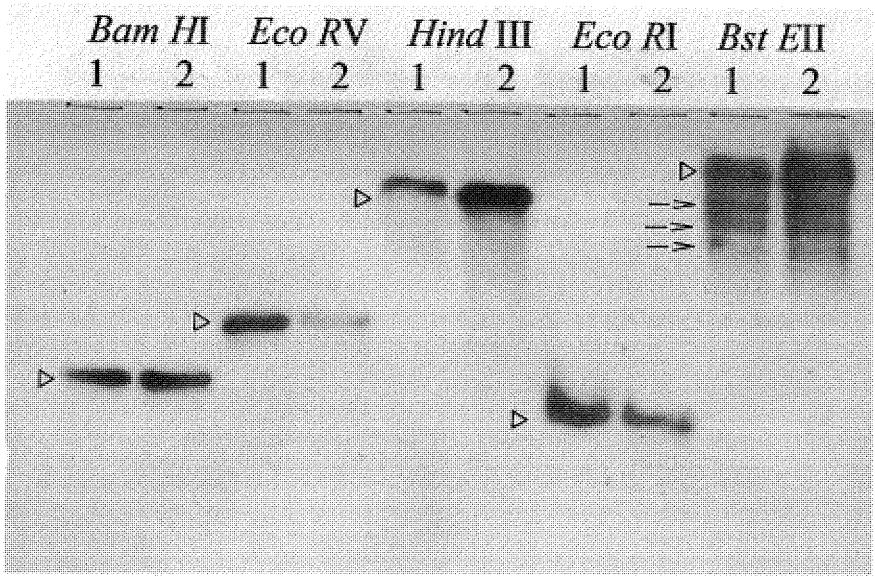
Inserts of 15 randomly selected genomic sunflower clones from RHA 278 with sizes between 0.4 and 3.2 kb were used as probes. These clones were generated by digesting total DNA with *Eco RI* and *Bam HI*. The fragments were separated on 1% agarose gel. The low molecular fragments (<5kb) were electroeluted, ligated into *Eco RI*– and *Bam HI*–digested pUC 18 vector and transformed into *E. coli* strain JM 83. Recombinant plasmids were preparated according to Birnboim and Doly (1979). The inserts were isolated by electroelution from agarose gels and randomly labelled with $^{32}$p– $\alpha$ dCTP (Amersham, Feinberg and Vogelstein, 1983). Prehybridization, hybridization, posthybridization washes and autoradiography were performed as described by the manufacturer (Du Pont).

## RESULTS

Total genomic DNA of the restorer lines was digested with the restriction enzymes *Bam HI*, *Eco RV*, *Hind* III, *Eco RI*, and *Bst EII* and hybridized against 15 random genomic sunflower clones with sizes between 0.4 and 3.2 kb. Figure 3 shows the hybridization




*Hybridization pattern obtained after probing the digested DNA of RHA 278 (1) and RHA 279 (2) with the genomic clone 1 (0.9 kb)*




*Hybridization pattern obtained after probing the digested DNA of RHA 278 (1) and RHA 279 (2) with the genomic clone 10 (1.4kb)*

*Figure 3: Autoradiographs*

patterns after probing the digested DNA with the clones 1 (0.9 kb) and 10 (1.4 kb). No differences in the patterns of the two restorer lines could be found in all cases, either in the major or the minor signals (Figure 3). Therefore, no RFLP could be detected with the 75 genomic probe/enzyme combinations applied. This result confirms the supposed close relationship between these two lines. However, due to the limited number of probe/enzyme combinations further experiments will be necessary in order to finally verify the close relationship of these lines.

## CONCLUSIONS

A pedigree map of American sunflower lines is considered as a useful tool for sunflower breeders, especially those in Europe. At the first glance, the complex network of the pedigree map may cause a certain degree of confusion. However, the map provides a general view on the genetic relationships and shows the 'genetic network' of the selected line(s). A successful way to work with this map is to start with the catalogue (Table 1) to receive information on the material appropriate for the respective breeding aim. If additional information are needed, the registration articles published in Crop Science contain detailed information on the selected line(s). Further details can be obtained through the authors; the catalogue and the reference index are available on IBM compatible EDP.

RFLP–analysis is a highly specific tool for the investigation of genetic relationships. Besides this technique, 2 different 'fingerprinting' methods have been developed recently, also providing information for phylogenetic studies. Differences in banding patterns are either generated by hybridization of digested genomic DNA with simple repeated sequences (e.g., $(GATA)_4$) or hypervariable minisatellite probes. This so–called 'DNA or oligo–fingerprinting has already found a wide range of applications in plants (Weising and Kahl, 1990, Beyermann et al., 1992, Dallas 1988), also in the genus *Helianthus* (Dehmer et al., 1992, Dehmer and Friedt, 1992b). The second possibility to obtain fingerprint patterns is to employ PCR amplifications with AP–PCR or RAPD primers (Williams et al., 1990, Welsh et al., 1992, Klein–Lankhorst et al., 1992, Dehmer and Friedt, 1992a). These techniques allow plant breeders to fill in some gaps with respect to genetic information available for the respective plant species.

## ACKNOWLEDGEMENTS

*We wish to thank Dr. Jerry F. Miller, USDA–ARS, North Dakota State University, Fargo, ND, 58105, USA, for providing detailed information on sunflower germplasms and for various advice. Thanks are also due to Mr. Fritz Mühlhans for excellent drawings.*

## REFERENCES


Beckmann, J.S. and M. Soller, 1986. Restriction Fragment Length Polymorphisms in Plant Genetic Improvement. Oxford Surveys of Plant Molecular & Cell Biology, Vol. 3.

Beyermann, B., P. Nürnberg, A. Weihe, M. Meixner, J.T. Epplen and T. Börner, 1992. Fingerprinting plant genomes with oligonucleotide probes specific for simple repetitive DNA sequences. Theor. Appl. Genet. 83, 691–694.

Birnboim, H.C. and J. Doly, 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res. 7, 481–489.

Dallas, J.F., 1988. Detection of DNA 'fingerprints' of cultivated rice by hybridization with a human minisatellite DNA probe. Proc. Natl. Acad. Sci., USA, 85, 6831–6835.

Dehmer, K.J., G. Mösges and W. Friedt, 1992. DNA–Fingerprinting bei der Sonnenblume. Vortr. Pflanzenzüchtg. 22, 365–366.

Dehmer, K.J. and W. Friedt, 1992a. PCR fingerprinting in the genus *Helianthus*. XIII EUCARPIA–Congress, Angers, France, Book of Posters Abstracts, 623–624.

Dehmer, K.J. and W. Friedt, 1992b. Species–specific clones versus DNA fingerprinting; Differentiation in the genus *Helianthus* on a molecular level. 13th International Sunflower Conference Pisa (Italy), in press.

Feinberg, A.P. and Vogelstein, 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132, 6.

Gebhardt, Ch., E. Ritter, A. Barone, T. Debener, B. Walkemeier, U. Schachtschabel, H. Kaufmann, R.D. Thompson, M.W. Bonierbale, M.W. Ganal, S.D. Tanksley and F. Salamini, 1991. RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor. Appl. Genet. 83, 49–57.

Graner, A., A. Jahoor, J. Schondelmaier, H. Siedler, K. Pillen, G. Fischbeck, G. Wenzel and R.G. Herrmann, 1991. Construction of an RFLP map of barley. Theor. Appl. Genet. 83, 250–256.

Helentjaris, T., G. King, M. Slocum, C. Siedenstrang and S. Wegman, 1985. Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol. Biol. 5, 109–118.

Klein–Lankhorst, R.M., A. Vermunt, R. Weide, T. Liharska and P. Zabel, 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet. 83, 108–114.

Maniatis, F., E.F. Fritsch and J. Sambrook , 1989. Molecular cloning (A LABORATORY MANUAL). Cold Spring Harbour Laboratory Press.

Miller, J.C. and S.D. Tanksley, 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet. 80, 437–448.

Mösges, G. and W. Friedt, 1992. Comparison of isozyme electrophoresis and molecular methods in screening near isogenic lines for resistance genes. 13th International Sunflower Conference Pisa (Italy), in press.

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res. 8, 4321.

Seiler, G.J., 1988. The genus *Helianthus* as a source of genetic variability for cultivated sunflower. In: Proc. 12th Int. Sunflower Conf., Vol. 1 (Novi Sad, Yugoslavia). Int. Sunflower Assoc., Toowoomba, Australia, 17–58.

Song, K., T.C. Osborn and P.H. Williams, 1990. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor. Appl. Genet. 79, 497–506.

Wang, Z.Y., G. Second and S.D. Tanksley, 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor. Appl. Genet. 83, 565–581.

Welsh, J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral, 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP–PCR). Theor. Appl. Genet. 82, 473–476.

Weising, K. and G.Kahl, 1990. DNA fingerprinting in plants – the potential of a new method. BFE, Vol. 7, No. 3, 230–235.

Wilson, K., 1987. Preparation of genomic DNA from bacteria. In: Current Protocols in Molecular Biology, J. Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res., Vol. 18, No. 22, 6531–6535.

Zimmer, D.E. and M.L. Kinman, 1972. Downy mildew resistance in cultivated sunflower and its inheritance. Crop Sci. 12, 749.