DEVELOPMENT OF NEMATODE-RESISTANT RAPESEED GENOTYPES VIA INTERSPECIFIC HYBRIDIZATION

A. Thierfelder (1), E. Hackenberg (2), K. Nichterlein (1), W. Friedt (1)

Institut für Pflanzenbau und Pflanzenzüchtung, (1) Pflanzenzüchtung, Ludwigstr. 23, (2) Biometrie und Populationsgenetik, Ludwigstr. 27, Justus-Liebig-Universität, D-6300 Giessen, Germany FR

INTRODUCTION

The white cyst nematode, Heterodera schachtii, is the causal agent of a serious pest in beet growing areas in Europe; e.g., many sugarbeet fields are still contaminated with this nematode. Commercial nematode resistant sugarbeet varieties are not yet available. Until now, the pest has been controlled by application of nematicides. Chemical control increases crop yield but a decline in nematode population does not always occur and this procedure must be avoided because of ecological reasons. Therefore, the most favourable measure at present is the cultivation of nematode resistant crops. Within Brassicaceae almost all species represent hosts for this nematode; particularly in Raphanus sativus and Sinapis alba resistance is well-known. Various resistant cultivars of yellow mustard (S. alba) and oil radish (R. sativus sp. oleifera) are used as green manure, because they prevent or limit the multiplication of the parasite. Within the same family rapeseed (Brassica napus) is also sucseptible to H. schachtii and resistance has not yet been observed. Although B. napus and R. sativus belong to different genera sexual hybridization is possible (Paulmann and Röbbelen 1988). Therefore, it should be feasible in a backcrossing programme to induce recombination in order to transfer the nematode resistance gene(s) to the genome of Brassica napus.

MATERIALS AND METHODS

Plant material

For wide hybridization six winter rapeseed genotypes and eight spring cultivars were crossed reciprocally with four radish genotypes which carry a high level of nematode resistance. The intergeneric hybrids (F_1) were backcrossed with three Brassica campestris cultivars and four Brassica oleracea genotypes, respectively (Fig. 1). Conventional bud pollination techniques were used for the production of intergeneric hybrids.

Ovule culture and plant regeneration

Developing siliqua were removed 20 to 30 days after pollination. The ovules were dissected under sterile conditions and

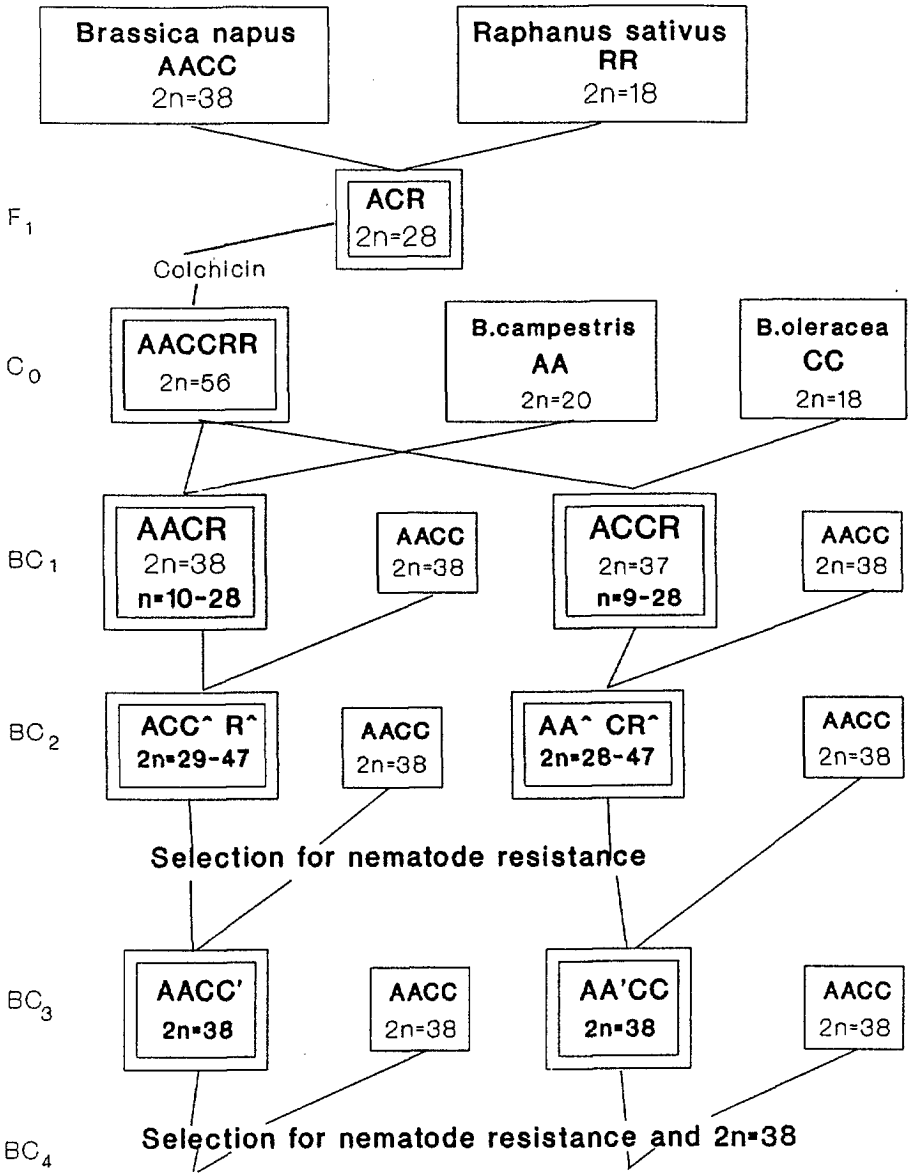

Fig. 1. Transfer of nematode resistance from radish to rapeseed with selection for nematode resistance (A^, C^ and R^ = hypoploid genomes, A' and C' = reconstructed genomes after genetic recombination)

placed on modified M&S-medium (Murashige and Skoog 1962), modified N&N-medium (Nitsch and Nitsch 1969), modified White-medium (White 1943) and E12-medium (Delourme et al. 1989).

Identification of hybrids

Chromosome numbers of the hybrids were determinated in root tips of plantlets 14 days after transfer to soil. Therefore, the root tips were treated with 2mM 8-hydroxyquinoline for 2.5h, fixed in propanol/acetic acid (3/1) and stained with orcein-HCl.
In most of the cases the hybrids could be easily identified on the basis of their morphology. In doubtful cases the hybrid nature could be confirmed by application of starch-gel-electrophoresis (cf. Hackenberg et al. 1990).

Chromosome doubling

The intergeneric hybrids are expected to have an amphihaploid genomic constitution (ACR). For chromosome doubling, 4-week-old plants were dipped reversely into a colchicine solution (0.05% and 0.2%) for 24h at 5°C.

RESULTS AND DISCUSSION

From a total of 12,376 pollinated buds 2,000 pods were obtained. They contained 5,376 ovules. The efficiency of hybrid recovery varied greatly depending on the cross direction. In combinations with B. napus as a female, the germination rate was three-times higher than in the reciprocal cross direction. The average germination rate in vitro was 3.6% (Tab. 1).

Table 1. Effect of cross direction on the germination rate of ovules plated on solid media after hybridization of different B. napus cultivars with nematode resistant R. sativus cultivars

Cross direction	Plated ovules	Germinated ovules	Germination rate (%)
B. napus x R. sativus	680	61	9.0
R. sativus x B. napus	4,696	132	2.8
Total	5,376	193	3.6

Finally, after micropropagation by axillary branching 261 individuals out of 50 cross combinations could be transferred into soil. All of them showed 28 chromosomes, an intermediate between the two parents, R. sativus (RR, 2n=18) and B. napus (AACC, 2n=38). The majority of the hybrids had pure white

flowers, only three exhibited a pale yellow colour. A wide range of morphological characteristics, concerning height, size and shape could be observed. Hybrids which included wintertypes of rapeseed required a vernalization period of 8 weeks at 5°C to induce generative development.

The evaluation of the electrophoretic pattern of several isozymes - i.e. glucosephosphate isomerase (GPI), leucine aminopeptidase (LAP), peroxidase (PER), shikimate dehydrogenase (SDH) - proved to be a useful tool for the identification of hybrids. All of the investigated individual cross progeny could be identified as hybrids by at least one isozyme (e.g. GPI, Fig. 2).

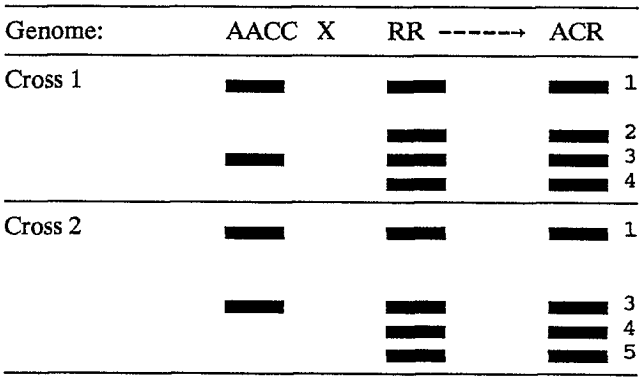

Fig. 2. GPI pattern of intergeneric hybrids (genome ACR) and their ancestors B. napus (genome AACC) and R. sativus (genome RR); Crosses 1 & 2 are examples.

Until now the colchicine treatment resulted in several partly fertile amphidiploid hybrids (AACCRR, 2n=56). Successfully diploidized individuals could be easily identified. These plants had a normal anther development with fertile pollen. Besides, they produced several fertilized pods after cross pollination.
The first backcrosses were carried out with the monogenomic ancestors of rapeseed, B. campestris (AA, 2n=20) and B. oleracea (CC, 2n=18). Again, in this generation ovule culture was applied to overcome incompatibility mechanisms and to increase the seed yield of plants.
The crosses were made reciprocally. The aim is to achieve progenies with the genome constitution ACCR and AACR, respectively. Besides highly sterile gametes , these BC_1 plants are assumed to produce gametes with recombination between chromosomes of the R genome and those of the A- and C-genomes, respectively (Paulmann and Röbbelen 1988). These progenies will be crossed with high yielding rapeseed genotypes. Based on the assumption that the genes controlling nematode resistance are included in such homoeologous recombination it should be possible to select progenies which exhibit resistance against the white cyst nematode.

ACKNOWLEDGEMENTS: The present research is supported by the Gemeinschaft zur Förderung der privaten deutschen landwirtschaftlichen Pflanzenzüchtung (GFP), Bonn. The authors gratefully acknowlegde gifts of seed samples by the Seed Company P.H. Petersen, Lundsgaard, Germany FR.

REFERENCES

DELOURME R.F., F.EBER, and A.M. CHEVRE, 1989. Intergeneric hybridization of Diplotaxis erucoides with Brassica napus. I. Cytogenetic analysis of F_1 and BC_1 progenies. Euphytica 41: 123-128

HACKENBERG E.M., H. MÜNDGES-CHRISTMANN and W. KÖHLER, 1990. Enzymmarker als Selektionshilfe in der Rapszüchtung. Vorträge für Pflanzenzüchtg. 18: 158-172

MURASHIGE T. and F. SKOOG, 1962. A revised medium for rapid growth of bioassys wih tobacco tissue culture. Physiol. Plant. 15: 473-497

NITSCH J.P. and C. NITSCH, 1969. Haploid plants from pollen grains. Science 16: 85-87

PAULMANN W. and G. RÖBBELEN, 1988. Effective transfer of cytoplasmatic male sterility from radish (Raphanus sativus L.) to rape (Brassica napus L.). Plant Breeding 100: 299-309

WHITE, P.R., 1943. The cultivation of animal and plant cells. Ronald Press, New York.