DEVELOPMENT OF CMS USING NUCLEO-CYTOPLASMIC INTERACTIONS BETWEEN RADISH (R.SATIVUS) AND RAPE (B.NAPUS)

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INTRODUCTION

Though most efforts in breeding of rape, Brassica napus, in the last two decades referred to the improvement of the quality of rapeseed a considerable increase of seed yield could also be obtained. Heterosis for seed yield in hybrid rapeseed has been reported by various authors (MORICE 1978, GRANT and BEVERSDORF 1983, RÖBBELEN 1985) and different systems of cross pollination control have been proposed for hybrid varieties. One of the CMS systems presently investigated is the alloplasmic system derived from male sterile radish (R.sativus) transferred to rape (ROUSSELLE 1978, MATHIAS 1983). The main problems of all male sterile rape lines with the radish cytoplasm are the total absence of restorer genes in rape and the fading of radish chloroplasts at low temperatures under the control of a rape nucleus. PELLETIER et al. (1983) removed the radish chloroplasts out of the cytoplasm of these lines by protoplast fusion and obtained normal green, male sterile rape. The present investigation was directed to produce new cross combinations between radish and rape and to eliminate the problems of chlorophyll deficiency and lack of restorer genes by additional transfer of the corresponding radish genes into the rape nucleus.

MATERIALS and METHODS

Plants of the male sterile radish line described by TOKUMASU (1951) were crossed to European radish varieties. F_2-progenies, male sterile or male fertile, were used as the female parent for the cross to transfer the male sterility and restorer genes, respectively. The male parent of the initial interspecific cross to radish was a spring rape variety. The hybrids were then backcrossed to one of the ancestral species of rape, i.e. Brassica campestris or B.oleracea. In the following backcrosses rape varieties were used again.

In the interspecific cross and the first backcross a sufficient number of progeny plants could only be obtained by application of in vitro embryo culture methods. A colchicine treatment was necessary for the hybrids between radish and rape in order to double the chromosome set of the ACR-individuals and to enable further crosses.

For mitotic investigations root tips of recently potted plants were investigated in Feulgen squash slides.

For selection of temperature stability of leaf colour in the radish alloplasmic CMS-plants, the young plants (8-10 leaves) were placed in a growth chamber at 8-10°C for at least 2 weeks. By using the plant colour atlas of BIESALSKI (1957) normal green plants could see identified at low temperatures.

RESULTS and DISCUSSIONS

Transfer of male sterility and radish genes for normal chloroplast development

The initial cross of male sterile radish and rape resulted in 34 hybrid plants out of 765 pollinated buds . After vegetative propagation 321 plants could be obtained. All plants had 28 chromosomes and pure white flowers indicating their hybrid character. After colchicine treatment about 50% of the plants showed female fertility on at least one branch. The successful colchicine application was visible by bigger petals and development of pods after cross pollination.

The chlorophyll deficiencies in the radish alloplasmic rape most probably reflect a disturbed interaction between radish cytoplasm and rape genome at low temperatures. Consequently in the radish genome genes must be assumed, which ensure a normal development of the plastids. Therefore, besides the transfer of male sterility, genes for normal chloroplast development have to be inserted from the radish into the rape genome in order to abolish the problems of chlorophyll deficiency in the alloplasmic rape lines.

Therefore the first backcross of the Raphanobrassica hybrids (AACCRR, $2n=56$) was not carried out with rape, but with its two monogenomic ancestral species B.campestris (AA, $2n=20$) and B.oleracea (CC, $2n=18$), respectively. In this way in the resulting offspring (AACR or ACCR) in each case one of the basic genomes of rape became coexistent with the radish genome in the haploid condition and allowed for allosyndesis between radish and rape chromosomes. This should secure a sufficient probability for transfer of the desired radish genes as shown in Fig.1.

The first backcross by embryo culture techniques resulted in 48 BC_1-plants with the genome constitution AACR and one plant with the constitution ACCR from altogether 5278 pollinated buds. All 49 BC_1-plants had a dark green leaf colour even at low temperatures as was expected from the presence of the full radish chromosome number. Out of these 49 BC_1-plants 22 did not produce flowers or pods. In the second backcross, where rape was used as pollinator, 276 seeds could be obtained, which seemed to be enough to select for recombination between radish and rape chromosomes. The seedlings of this generation were first vegetatively propagated in vitro by stem segments. Each BC_2-genotype was devided into 5 clones and subjected to the different treatments simultanously. The temperature selection in the growth chamber at 10°C indicated, that 56 of the BC_2-plants had a leaf colour, comparable to normal rape varieties. Their chromosome numbers are summarized in Fig. 2. The chromosome numbers were not equally distributed between the possible limits of $2n=29-47$. Most of the plants had chromosome numbers of 38 and 39. 14 BC_2-plants could be selected having temperature tolerant chloroplasts and 38 chromosomes in their root tips as well.

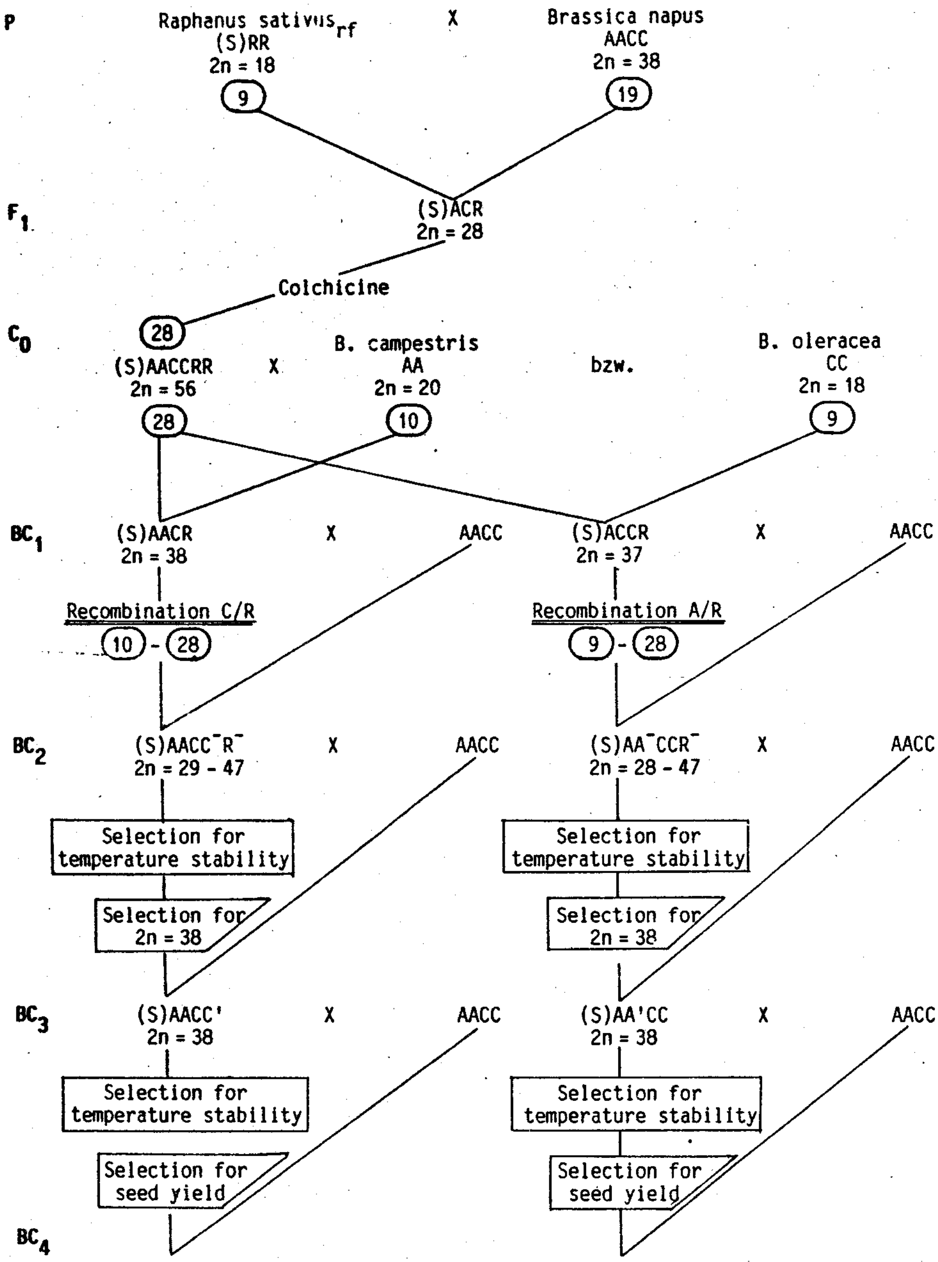

Fig. 1: Transfer of male sterility from radish to rape in consideration of a sufficient coolness tolerance of the chloroplasts.

A^-, C^- and R^- = hypoploid genomes
A' and C' = genomes with recombinations

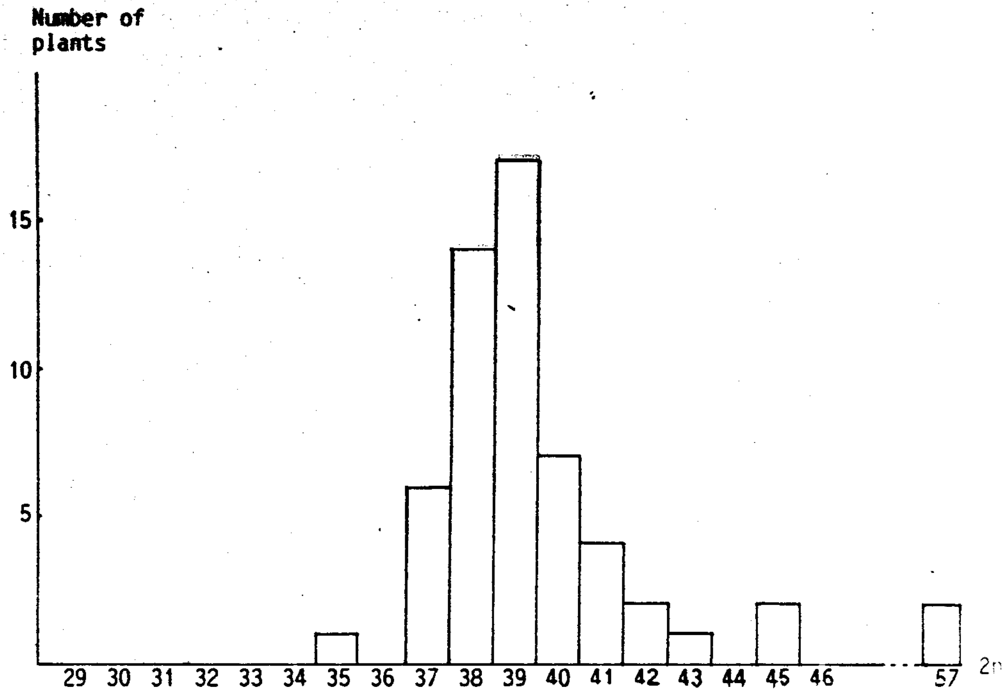

Fig. 2: Distribution of chromosome numbers of the selected BC_2-plants

Further backcrosses, carried out with spring and winter rape cultivars, resulted in increasing female fertility of the CMS-plants with more than 10 seeds per pod. In each backcross generation a portion of temperature tolerant normal green plants could be obtained, having the corresponding radish genes incorporated.

Transfer of restorer genes

In a parallel backcross programme restorer genes, which are lacking in rape, have been transferred from radish to rape, too. The female parent of this interspecific crosses was the male fertile F_2-progeny of a radish line, carrying the Rf genes heterozygous. The first backcrosses were similar to the transfer of CMS (Fig.3). After combination of radish and rape the radish chromosomes hat to be eliminated with the exception of radish genes responsible for fertility restoration.

The hybrids of radish Rf and rape and the first backcross generation showed no male fertility, probably because of general disturbances of vitality after interspecific crosses. In the second backcross generation 2 partially male fertile plants were observed. These 2 plants were used as female parents in crosses with rape in order to increase the performance of seed yield. Selfing of these plants was not successful. In the next generation 42 BC_2-plants could be grown, of which 6 plants were partially male fertile again.

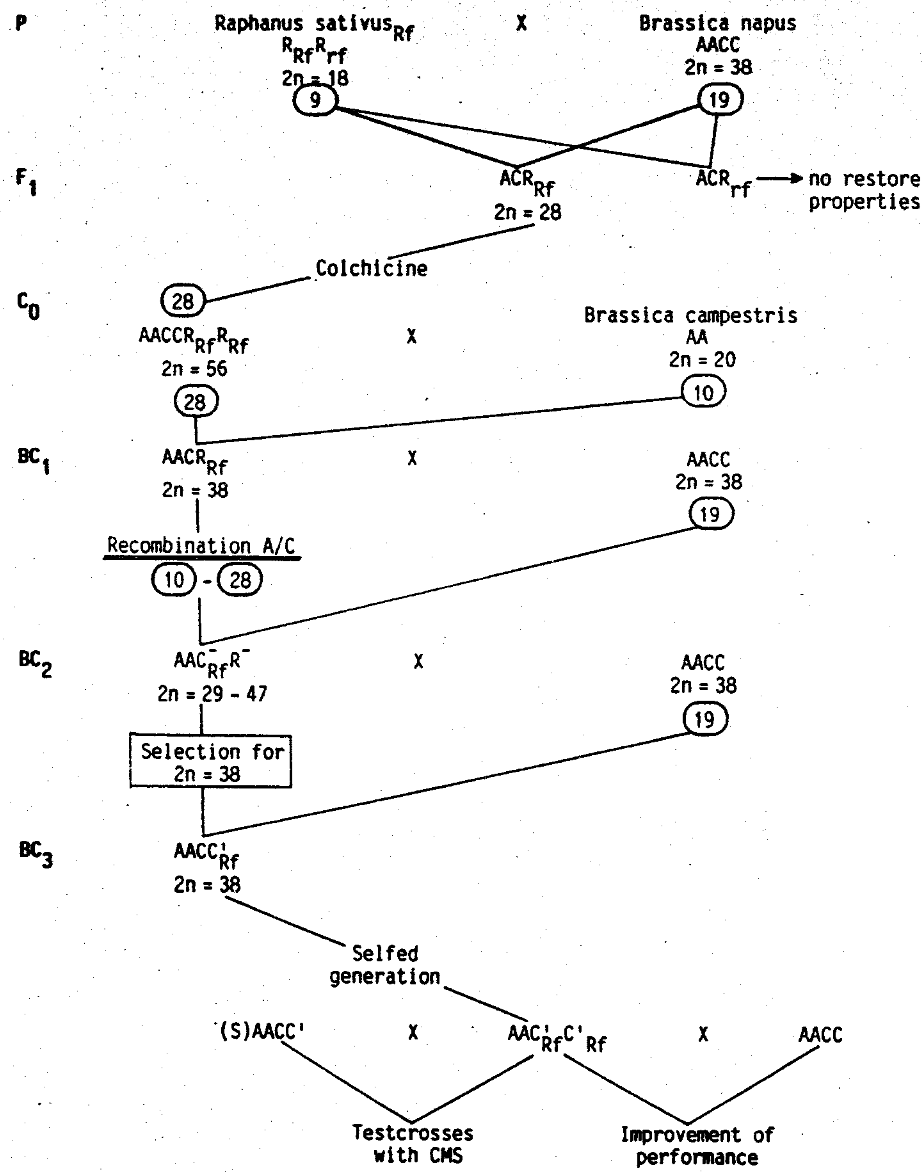

Fig. 3: Transfer of restorer genes from radish to rape.

A^-, C^- and R^- = hypoploid genomes
A' and C' = genomes with recombinations

Table 1: Fourth cross generation of the 6 male fertile BC_3-plants

Selection Number	x rape seeds/ pod	x rape seeds total	selfed seeds/ pod	selfed seeds total	male parent for CMS seeds/ pod	male parent for CMS seeds total
11120	1.5	12	1	4	-	-
11121	-	-	-	-	3.3	30
11128	1	11	1	1	4.8	67
11129	1.2	18	1	1	2.3	85
11143	2.7	19	-	-	3.4	58
11147	1	5	1	2	2.0	10
		65		8		250

Different crosses were carried out with the 6 male fertile BC_3-plant (Tab. 1). The relatively high seed yield in crosses with CMS-plants showed, that with increasing backcrosses the success of pollination was improved. First seeds from elfing could also be obtained, opening the chance to get the restorer genes homozygous.

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