CONSTRUCTION OF DIFFERENT B-GENOME ADDITION LINES OF BRASSICA NAPUS L.

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INTRODUCTION

Addition lines have often been developed to allow for transfer of desirable agronomical traits into crop species. They were also used for dissection of the chromosomes of different genomes providing important information for phylogenetic studies, the characterization of genomes (e.g. Quiros et al. 1987) and the localization of individual genes. While identification of different chromosomes is easily possible in wheat and rye using C-banding technique, the cytological identification of Brassica chromosomes is very difficult because of their small size and missing chromomere structure. In order to facilitate the identification of the different chromosomes in Brassica, molecular markers have recently become available (Quiros et al. 1987; Chevre et al. 1991).

In this paper, we report on the construction of B. napus lines with extra chromosome additions derived from three different sources of the B genome, namely B. nigra, B. carinata and B. juncea. These addition lines were identified using phenotypic markers, such as resistance to Phoma lingam, erucic acid and glucosinolate composition of the seeds as well as genome specific markers, such as isozyme loci and RFLPs.

MATERIALS AND METHODS

Interspecific hybrids were raised from combinations of B. nigra (2558 = number of Brassica Collection Göttingen) with a 00-breeding line of Deutsche Saatveredelung, Lippstadt; B. oleracea (2708; variety 'Vitamina') with B. campestris (3220); and B. campestris (2909) with B. juncea (2084; variety 'Stoke'). The Canadian 00 rapeseed variety 'Andor' was used for recurrent backcrossing. Monosomic addition plants (AACC+1B, 2n=39) were selected by investigating meiotic pairing in metaphase I using the conventional acetocarmine squash technique. The selfed progenies of these plants segregated plants with 38 chromosomes as well as monosomic and disomic addition lines. About 30 monosomic and disomic addition lines from three sources of Brassica's B-genome and their euploid sister plants (2n=38) were used in the present study.

Fatty acid composition was determined by gaschromatography (GC) using the half seed technique according to Thies (1974). The glucosinolate profile was assessed using high performance liquid chromatography (HPLC) with samples of 200 mg defatted seed meal as described by Kräling et al. (1990). For testing of Phoma resistance each 10-12 plants were inoculated at the stem base by an aqueous pycnospore suspension at a concentration of 10^7 spores/ml. Plants then were kept in a plastic foil chamber and disease symptoms scored 5 to 7 weeks after inoculation using

a scale from one (no symptom) to 9 (collapse). The test was repeated twice.

Isozyme analysis was carried out using horizontal starch gel electrophoresis. Crude extract of young leaves, buds and seeds has been used and the following enzymes were assayed in different buffer systems: phosphoglucoisomerase (PGI), glutamate oxaloacetate (GOT) and triose phosphate isomerase (TPI) using a buffer system of pH 8.3; aconitase (ACO), phosphoglucomutase (PGM), alcohol dehydrogenase (ADH) and shikimic acid dehydrogenase (SDH) using a pH of 7.0 and malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGDH), isocitric dehydrogenase (IDH) and leucine amino peptidase (LAP) using a pH of 6.1.

For RFLP analysis, total genomic DNA was extracted from young leaves of monosomic addition lines. 2-5 µg DNA was digested with restriction endonucleases EcoR I and Hindi III; fractionation was conducted in 1% agarose gel and transferred onto nylon membranes. Membranes were hybridized with p32 oligolabelled probes from genomic and CDNA of B. oleracea and B. napus libraries (for details see Kianian 1990).

RESULTS

Trigenomic amphihaploid plants (ABC; 2n=27), were produced by reciprocal interspecific hybridizations within the Brassica triangle of U (1935) (cf. Bellin 1988). Frequently seed set was only achieved through open pollination of the trigenomic haploids in the greenhouse, which at that time was crowded with diverse B. napus materials, resulting in pentaploid plants in all combinations (AACCB, 2n=46).

The pentaploid AACCB plants exhibited a mean meiotic configuration of 7.97 I + 18.93 II + 0.002 III. In 85% of the studied PMCs a pairing of 8 I + 19 II was observed, whereas in 11% of the PMCs more than 8 univalents and in 4% one quadrivalent was shown. One cell exhibited a pairing of 7 I + 18 II + 1 III (Struss et al. 1991).

For transfer of the addition lines into the same genetic background the pentaploids were further backcrossed with the rapeseed variety 'Andor'. In each combination 3-5 pentaploid plants were used. The chromosome number the of BC1 progenies ranged from 38 to 46. Plants containing added chromosomes of the B-genome were backcrossed three more times with 'Andor'.

Finally, in the combination B. nigra x B. napus (and reciprocal) 9 monosomic plants (2n=39), in B. campestris x B. carinata 24 plants, and in B. oleracea x B. juncea 6 plants were identified. All monosomic addition lines showed a mean pairing at metaphase I of 19 II + 1 I. In all studied PMCs, at least one univalent was observed, even in those PMCs containing multivalents. Pollen viability was high in the monosomic addition lines and ranged from 85% to 92%. These plants also exhibited reasonable seed set.

To obtain disomic additions (2n=40), the monosomic plants were selfed. In the combination B. nigra x B. napus (and reciprocal) 4 disomic plants were obtained, although only after a second generation of selfing. In the other combinations B. campestris x B. carinata (and reciprocal) three and B. oleracea x B. juncea one disomic addition plants were obtained after one selfing. These disomic addition lines almost exclusively exhibited a meiotic pairing of 20 bivalents at M I.

Fatty acid analysis has been performed on nine monosomic addition lines of the combination B. nigra x B. napus (and

reciprocal) to identify the added chromosomes of B. nigra. B. nigra displayed a high content of erucic acid, whereas the recurrent parent 'Andor' is a 00-quality cultivar. Three monosomic addition lines exhibited erucic acid (Table 1). The addition line '48' in the disomic level showed about 35% erucic acid in seed. Sinigrin content of the monosomic addition line was determined in the reciprocal combination B. nigra x B. napus using HPLC analysis. Whereas the variety 'Andor' with a total amount of 10.23 µmol glucosinolates/g seed was free of sinigrin, B. nigra is characterized by its major glucosinolate sinigrin. Six addition lines contained sinigrin including its synthetic control by the added B. nigra-chromosome (Table 1).

The average Phoma-infection rate of the tested material is shown in Figure 1. B. nigra and the rapeseed variety 'Jet Neuf' exhibited high resistance, B. nigra being even more resistant than 'Jet Neuf'. The background parent 'Andor' on the other hand was highly susceptible. From the tested addition lines six expressed resistance. Particularly interesting were four other lines, which were derived from addition lines, but possessed 2n=38 chromosomes and nevertheless were definitely resistant (infection score about 5; see Fig. 1).

A series of isozyme and RFLP markers served to identify six different groups of addition lines having extra chromosomes from three B genome sources of Brassica (Table 2). Four isozyme loci were useful to confirm four groups of addition lines, previously identified by using phenotypic markers (cf. Table 1). Based on isozyme loci, two other groups of addition lines were evaluated additionally. One more line (No. 16903 from combination CC x AABB) was identified by RFLPs based on one probe-restriction endonuclease pBN33. Most of the probes disclosed B. nigra specific fragments.

DISCUSSION

Pentaploid plants (AACCB, 2n=46) were generated in our experiments after open pollination of the primary amphihaploids (ABC, n=27). Obviously these plants were derived from unreduced female gametes which were open pollinated by B. napus plants present in the same greenhouse. Heyn (1977) reported the occurrence of 0. I to 0.3% unreduced female gametes within the genus Brassica. Nuclear restitution was also observed in our BC_1 progenies, where 2.3% of the plants exhibited a genome constitution of AAACCCB resulting from unreduced female gametes of the pentaploid hybrids.

Chromosome pairing in the A, B, and C genomes of Brassica has been interpreted to be autosyndetic as well as allosyndetic in nature (Attia and Röbbelen 1986). From chromosome morphology at pachytene, Röbbelen (1960) assumed a basic genome of x=6 chromosomes in Brassica . He, thereby, confirmed the hypothesis of a secondary polyploid origin of the A, B, and C genomes. In our trigenomic haploids, the occurrence of bivalent and multivalent configurations supports this assumption. Among 242 PMCS studied in ABC plants, only one cell with 27 univalents was observed. But Attia and Röbbelen (1986) determined a relatively low amount of pairing between chromosomes of the B genome with those of the A and C genomes, respectively. Among 621 studied PMCS of AACCB pentaploids (2n=46) only one cell had formed 7 I + 18 II + 1 III, with the trivalent indicating pairing of B with A and C chromosomes.

Meiotic pairing in our monosomic B genome addition lines in 97% of the investigated PMCS resulted in 19 II + 1 I. The selfed

progenies of these monosomic plants were cytologically stable and essentially displayed 19 II + 1 I. This proves a considerable tolerance against aneuploidy in our material as well as a regular pairing of the homologues of the A and C genomes. Obviously the added B genome chromosome did not affect the meiotic pairing within the A and C genomes. Fan et al. (1985) also reported on meiotic stability and aneuploidy tolerance in B. campestris /B. oleracea and B. napus/Raphanus sativus addition lines.

Erucic acid content in the seed is controlled by two genes with additive effect (Jönsson 1977). In the amphidiploid B. campestris also two gene loci for erucic acid were reported (Fernandez-Escobar et al. 1988). In B. campestris and B. oleracea the erucic acid is controlled by one gene only. We thus assume that in B. napus also one gene controls erucic acid production in the seeds. Therefore, the addition lines with erucic acid in the seeds have been placed into one group. According to the presence of erucic acid and sinigrin in the seeds and of plant resistance to P. lingam, the nine monosomic addition lines from the reciprocal cross-combination B. nigra x B. napus were adjoined into four groups (Table 1, see A-D).

Our addition lines provide a good source for the characterization of different chromosomes of the Brassica B genomes and for the localization of agronomical interesting traits which they contain. Using phenotypic markers (i.e., erucic acid and glucosinolate content of the seed and plant resistance to P. lingam) four groups of addition lines from the combination B. nigra x B. napus (and reciprocal) were identified. On the base of isozyme loci, i.e., TPI, ADH, SDH, and PGM, the four groups were confirmed. In addition using the isozyme markers for PGI-2 and Aco, two more groups of addition lines were identified. More investigations are on the way to establish the complete set of all eight B genome addition lines and to reveal evolutionary differences in the different B genome origins present in our material.

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Table 1: Grouping of monosomic B. nigra addition lines of B. napus based on seed content of erucic acid and sinigrin in % of total glucosinolates (GLS) and plant resistance to Phoma lingam (+ = resistant, - = susceptible)

Addition line No.	Erucic acid (%)	Total GLS µmol/g seed	Sinigrin (%)	Resistance to Phoma	Group of line
11	0.0	14.61	9.47	+	C
15	0.3	12.42	--	-	B
26	0.3	8.95	--	+	D
29	0.3	9.70	7.96	+	C
34	17.8	14.73	4.30	-	A
37	0.2	23.63	3.36	+	C
46	15.1	24.35	4.68	-	A
48	16.8	24.55	8.91	+	A
55	0.6	13.21	--	+	D
B. nigra	37.1	130	97.0	+	
'Andor'	0.0	10.23	--	-	

Table 2: Grouping of B genome chromosomes addition lines based on isozyme loci

Addition line No.	Isozyme loci	Group
48[1], 34[1], 46[1], 1442 [2]	TPI-1	A
15[1]	ADH-1	B
11[1], 29[1], 37,[1]	PGM-3	C
26[1],55[1], 16947[2]	SDH-1	D
1401[3]	PGI-2	E
16923[2]	6PGDH-2	F

[1]= BB x AACC, [2] = BBCC x AA, [3]= CC x AABB

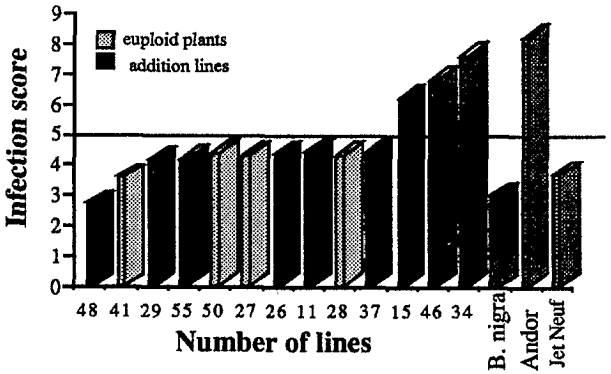

Fig. 1 : Phoma resistance of monosomic B. nigra addition lines of B. napus, euploid sister plants (2n=38), the parents and the rapeseed variety "Jet Neuf"