# EXTRACTION OF DISOMIC ADDITION LINES B. NAPUS - B. NIGRA AND INTRODUCTION OF B. NIGRA TYPE PHOMA LINGAM RESISTANCE TO RAPESEED.

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U (1935) clearly showed that the species B. napus, (AACC, 2n = 38) B. juncea (AABB, 2n = 36) and B. carinata (BBCC, 2n = 34) are of allotetraploid origin. They have been derived from hybridisation between the diploid species B. campestris (AA, 2n = 20), B. oleracea (CC, 2n = 18) and B. nigra (BB, 2n = 16). On the other hand, the phylogenetic origins of these diploid ancestors are not well understood. They may be polyploid derivatives. From a study of secondary bivalent associations, Sikka (1940) suggested that the Brassica diploids might be derived from a common ancestor having five chromosomes. On the basis of chromosomal morphology, Röbbelen (1960) considered that the basic chromosome number in Brassica was six. Armstrong & Keller (1981 ; 1982) studied chromosome pairing in B. campestris and B. oleracea haploids, and suggested that the two species evolved from a common six chromosome genome. However, these authors doubted the obvious interpretation of meiotic configurations.

Fantes & Mackay (1979) and Quiros et al (1985) opened a new line of inquiry for studies of Brassica genome structure by creating addition lines. This approach has proved very productive in studies of other species such as the Triticineae (Law 1981). This study considers the production of disomic addition lines B. napus-B. nigra, in which single pairs of homologous chromosomes from black mustard are added to oilseed rape. It also attempts to determine 1) basic chromosome number in the genus Brassica 2) recombination possibilities between rapeseed and black mustard and notably potential for transfer of Phoma resistance.

## Materials and methods

The Tandem rapeseed line, 00, was crossed with the Junius german cultivar of black mustard, resistant to Phoma lingam. It was necessary to culture fertilised ovaries (Inomata 1979). Three backcrosses were established using Tandem as male. BC3 plants were fertilised and haplodiploidised (Fig.1) following the anther culture technique described by Renard & Dosba (1980).

Chromosome counts were made on root meristems from germinated seeds in petri dishes (25°C). Material was treated with a solution of 8-hydroxyquinoline (0.29 g/l) for 4 hours at room temperature, then fixed with (1/3) acetic ethanol for at least 30 min at 4°C. Meristems were hydrolysed in N HCl for 10 min at 60°C, then coloured with Schiff's reagent.

Floral buds were fixed in Carnoy solution with addition of chloral (0.5 % w/w) for 48 hrs, for observations on pollen-mother cells. MI stage anthers were lacerated and coloured in a drop of acetic carmine solution.

Phoma resistance was assessed at cotyledon stage, by inoculating the two lobes of each cotyledon with a suspension of pycniospores from a pluriconidian isolate ($10^7$ spores/ml), after damaging with a needle. Lesions were scored twelve days later (0 to 9) according to the size of the lesion, its aspect, and sporulation (Delwiche 1980).

## Results

An F1 plant 'Tandem o x Junius o with 27 chromosomes was obtained. Meiotic behaviour was recorded for 15 cells (8. 13' + 7. 26" + 0. 26''' + 0.8'''' + 0.06'''''). No amphidiploid could be obtained despite several attempts. To ensure the descendance, seven BC1 plants were produced by backcrossing with rapeseed. These all had 46 chromosomes. There was no significant difference in frequencies of the different meiotic configurations. Average meiotic behaviour was 9.41' + 16.23" + 0.21''' + 0.86''''. Only the descendance BC2 of a single BC1 plant was used. Sixty-seven plants with chromosome number between 36 to 43 were studied. The BC3 progenies, from BC2 plants of regular meiosis, with 19" plus one, two or three systematic univalents, were conserved : eight plants with 2n = 39 : 19" + 1' ; twelve plants with 2n = 40 : 19" + 2' ; seven plants with 2n = 41 : 19" + 3'. The twenty seven BC3 descendants studied, included ten plants for BC2 mother plants with 2n = 39, and fifteen plants for the others. Twenty-seven plants with 19" + 1' and three with 19" + 2' were selected from these.

Anther cultures were established from 18 BC3 plants with 2n = 39, and 3 plants with 2n = 40, from sixteen different descendances. This work is still in progress. So far, 26 plants with 20 or 40 chromosomes, from twelve mother plants, from eleven different descendances have been obtained.

Descendances from self-fertilisation of BC3 plants, are being currently analysed. Presently thirty-four, 40-chromosome plants have been selected in 19 descendances. This material must be regarded as addition lines.

Seven of the 27 BC3 selected plants, from six descendances, have proved to be Phoma resistant. Analysis of self-fertilisation descendance has not yet been completed. Initial results indicate the presence of two kinds of material. In some families there was no relationship between chromosome number and resistance. In the others, resistance is associated with presence of the, or the pair of additional chromosomes.

## Discussion

B. napus has often been crossed with most of the closely related species (Tsunoda et al. 1980, Prakash et Hinata 1980). However, the cross with B. nigra has only occasionnally succeeded. Eighty-five percent of hybrid plants obtained by Heyn (1977) had genomic composition ACBB. This is due to production of unreduced gametes

which may be more competitive, or have higher fertilising potential than haploid gametes. Only one 27 chromosome hybrid plant was found. Environmental conditions, and the genotype used, may have been unfavourable to production of unreduced gametes.

In the hybrid F1, 18 % of chromosomes paired as quadri- or penta-valents. This confirms the polyploidal nature of the genomes A, B, and C. Prakash (1973) and Mizushima (1950) showed that B genome chromosomes only paired to a slight extent with those of A and C type genomes, less than those of the latter with one another (Attia & Röbbelen 1986). These results were confirmed by chemotaxonomy studies (Vaughan 1966). The present study does not confirm a greater difference of B. nigra relative to the two other species. The frequency of paired chromosomes was 70 %, which is in the range (65 % and 78 %) found by Renard & Dosba (1980) for ten rapeseed haploids from ten different cultivars. It would be interesting to cross Junius with B. oleracea and B. campestris lines, to clarify the A-B(Junius ) and B(Junius)-C pairings. The hybrid obtained in this study was completely sterile, as for Heyn (1977), whereas Rousselle & Eber (1983) obtained four F2 plants from a single F1 individual.

The seven BC1 plants had fourty six chromosomes and it is likely that the female gametes involved were unreduced (n = 27, ACB). The eight B chromosomes would then have been transmitted in unrecombined state. The theoretical meiotic pairing behaviour of these plants is 19" + 8', supposing that B. nigra chromosomes do not pair, and ignoring possible effects of promotors or inhibitors. Mean number of univalents was 9.41. Attia & Röbbelen (1986) suggested that B. nigra might carry suppressors for homoeologous pairing in B. nigra hybrids, whose effects appear in crosses with either B. campestris or B. oleracea. This could then account for the higher than expected mean number of univalents. However, examination of basic data showed six or seven univalents in 8 % of cells. Thus, at least one or two B. nigra chromosomes can pair in BC1 plants. Could these then be involved in allosyndetic or autosyndetic associations ? The last hypothesis is plausible, since Prakash (1973) showed that 13 % of the PMC's for a haploid black mustard plant had two bivalents. However, the allosyndesis B-C and/or B-A is confirmed by the presence of 1) 38-chromosome genotypes resistant to blackleg 2) near invariable presence of a quadrivalent at meiosis of BC1 plants. The genetic system responsible for pairing in BC1 plants may be complex and presence of pairing promotors should be envisaged.

The use of anther cultures to obtain disomic addition lines in Brassica from monosomic ones is novel. A similar method allowed creation of disomic addition lines Wheat-Barley, (Islam & Shepherd 1981). Otherwise these only can be obtained with difficulty in descendances from self pollination of monosomic additions lines, because of poor transmission of the additional chromosome in pollen. Future studies will compare and assess the interest of the two methods (work in progress). First results suggest that both produce the required material.

Rapeseed has good tolerance to aneuploidy. Fan & Tai (1985) found that male and female fertility of two monosomic lines were as high as that of the corresponding disomic line, and suggested that it might be possible to obtain a whole set of monosomics in B. napus. Sernyk & Stefansson (1982) and Fan et al. (1985) showed

that chromosome pairs of closely related species, could be added to those of rapeseed, and that the result was stable. Chromosome addition plants BC3 (2n = 39) are invariably in univalent condition at metaphase 1. It is likely that it is the B. nigra chromosomes that are not recombined with those of rapeseed over succeeding generations.

Extraction of all B. nigra pairs as additionals, would have been possible if anther culture had been undertaken on BC1 plants (2n = 46 : AACCB). Doubling for the plants obtained with n = 20 (AC + 1 chromosome B) would have given plants with 2n = 40, likely to be stable. This because an additional pair, even given a high degree of homology with a rapeseed pair, would have little chance of being involved in allosyndetic pairing in a disomic genomic context. (19" AA and CC and 1" BB). Pairings are preferentially between homologues. Three backcrosses were undertaken to ensure that genetic information carried by genomes A and C of the BC3 plants, would be as close as possible to that of Tandem. In this way it is likely that most B. nigra genes introduced by recombination, would have been eliminated. Consequently, comparison of Tandem with addition lines, and between addition lines, should facilitate characterisation of each additional pair. Other methods will be used in this respect in the future, namely : cytological (stability, transmission levels), biochemical (marking with storage proteins), molecular (in collaboration with M. Delseny, Perpignan, for studies using RFLP).

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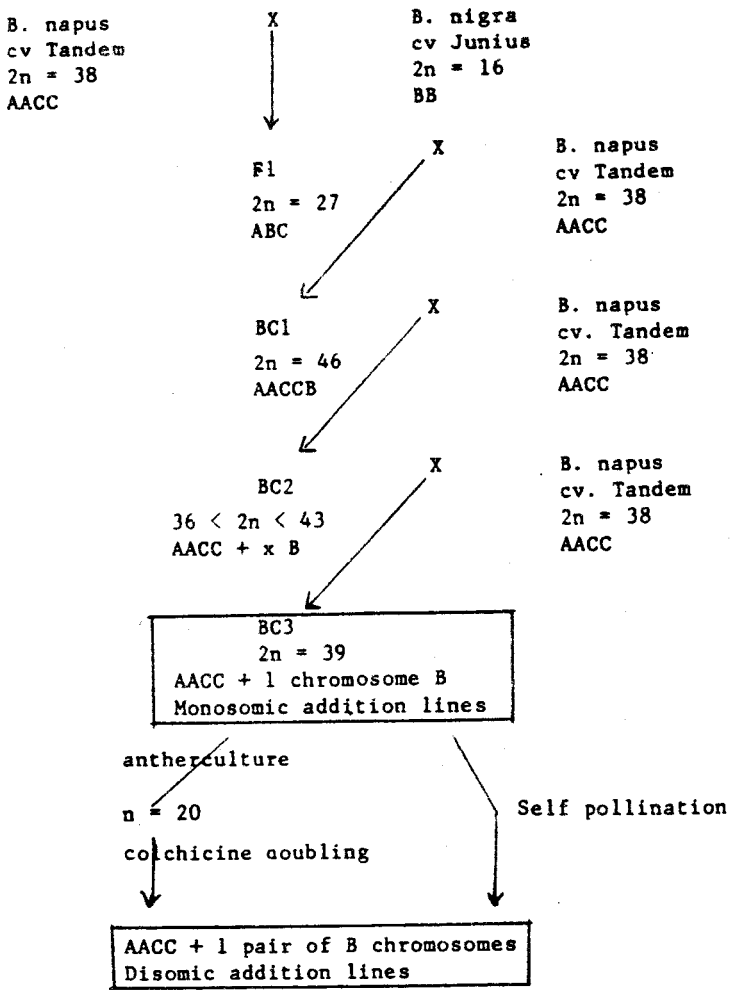


Fig 1 : Scheme of obtaining of disomic addition lines
B. napus - B. nigra