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C-genome chromosome substitution lines in *Brassica juncea* (L.) Coss

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Abstract

Spontaneously developed ‘C’ genome substitution lines of *Brassica juncea* (L.) Coss were identified in the F_7 generation of an interspecific cross between *B. juncea* (AABB; 2n = 36) and *B. napus* (AACC; 2n = 38). These lines were meiotically stable (2n = 36) and had normal male and female fertility. They were characterized by certain specific traits like bolting habit, leaf aberrations and variation for erucic acid content. Cytological evidence indicated that at least three lines has substitution for different chromosomes. The substitution stocks characterized in this study are important for their use in practical crop breeding and certain basic genetic studies.

Introduction

Introgression of alien genetic variation is often hampered by the lack of pairing between two well differentiated genomes. In such a situation individual chromosomes can be transferred intact from alien species, by developing disomic chromosome addition or substitution lines. The addition lines are often unstable due to low transmission frequency of the extra chromosome. The disomic substitution lines, on the other hand, have the same chromosome number as the recipient parent and are expected to be meiotically more stable. The disomic chromosome substitution can be produced by crossing monosomics with disomic addition lines. The chromosome substitutions are also known to occur spontaneously in interspecific crosses (Khush, 1973). Such disomic substitution stocks can be used for the study of the genetic effects of individual chromosomes on plant traits and for estimating the number of genes controlling a trait and their linkage relationships. Apart from theoretical interest, chromosome substitution lines help in exploitation of alien genetic variation for various agronomic and quality characteristics. So far, there is no report regarding the development of such a system in *Brassica*. In this communication, we report the identification of ‘C’ genome chromosome substitution lines in *Brassica juncea.* These lines arose spontaneously in an interspecific cross between *B. juncea* and *B. napus.*

Materials and methods

A high erucic acid commercial variety, RLM 198 of *Brassica juncea* (2n = 36; AABB) was crossed as seed parent with the low erucic acid exotic cultivar, Oro of *B. napus* (2n = 38; AACC). Nine partially fertile F_1 plants were selfed to produce the F_2 generation. The progeny obtained by open pollination of F_2 plants was bulked to raise the F_3 generation, where selection for *juncea* type segregants with improved pollen fertility was carried out. This was continued till F_6, where a total of 114 progenies with chromosome number 2n = 36 were retained. Out of these 114 stable progenies, thirty-two were crossed with the euploid recipient parent, RLM 198. Meiotic studies wherever necessary were carried out on young buds fixed in Carnoy’s solution containing ferric acetate as mordant and squashed in 2 per cent acetocarmine. A minimum of sixty cells were assayed for cytological studies.

Results and discussion

The 'C' genome chromosome substitution was confirmed in as many as ten progenies out of the thirty-two evaluated, as two univalents plus seventeen bivalents instead of regular 18II were observed in meiotic cells of the F_1 hybrid between euploid *B. juncea* and the suspected substitution lines (Fig. 1a). This behaviour was regular in all the substitution lines except in the F_1 of IS 86 × euploid, where 17II + 2I were observed in about 60% of the cells, while 18II were observed in the remaining 40% of cells. IS 86 is an interesting type because it resembles a centromere translocation as observed in wheat with triticale in its ancestry. The centromere translocations frequently observed between wheat and rye chromo-

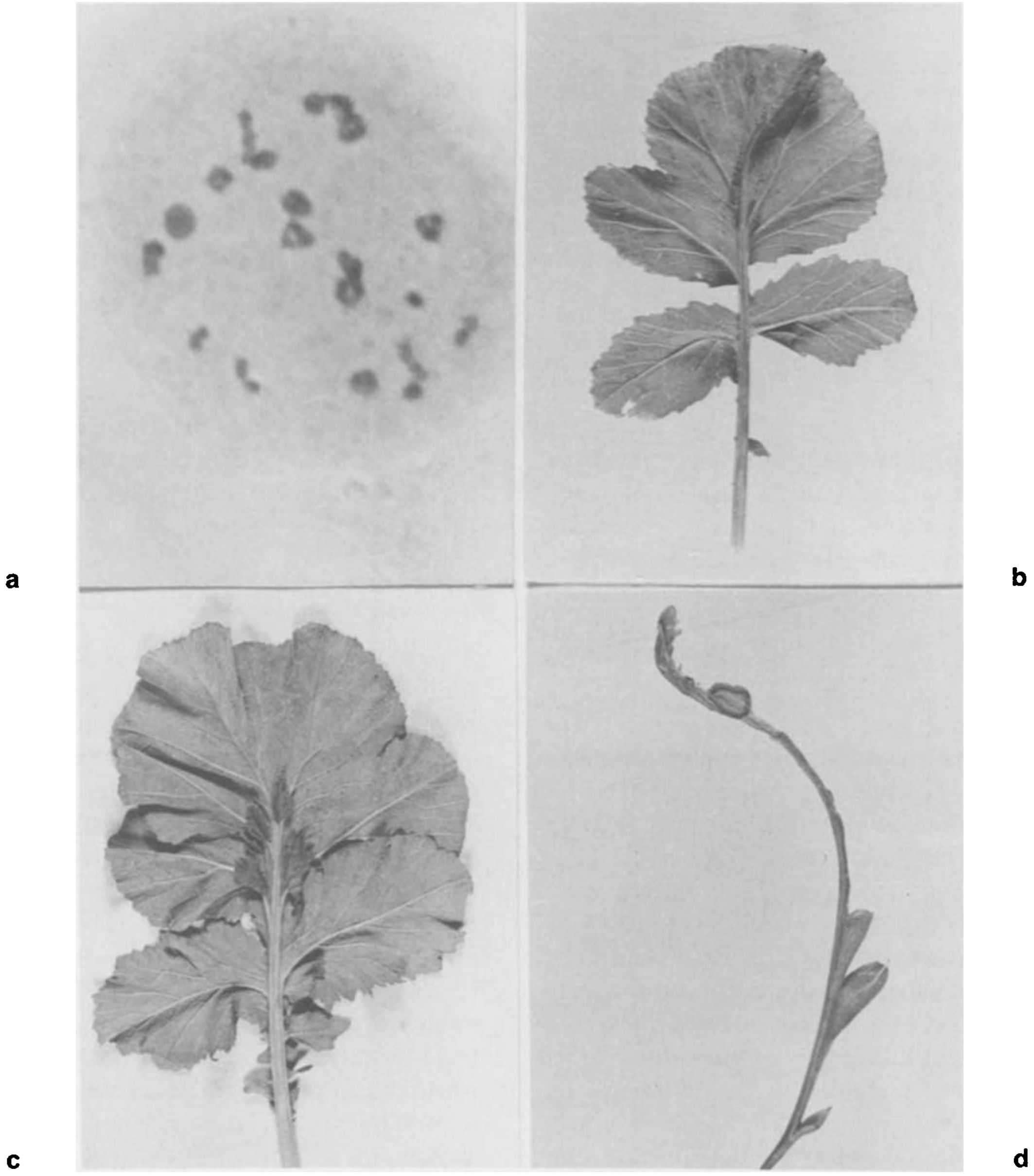

Fig. 1. a. Meiotic configuration in substitution line × euploid *juncea* (RLM 198), 17II + 2I at diakinesis; b. Leafy outgrowths on dorsal side of leaf; d. A modified flag leaf in IS 62.

somes, resulted from random fusion of the breaking univalents at MI (Lukaszewsky & Gustafson, 1983). When a long arm is translocated in a sub-acrocentric chromosome, only the short arm is homologous in the test cross, and univalents are observed in high frequency. Apart from its theoretical interest, IS 86 is also desirable from a breeding point of view as it is highly resistant to leaf miner and white rust. However, it is very tall, late in maturity, and has abnormal leaves. If it is confirmed to be a translocation homozygote, the hybridization between IS 86 and euploid *B. juncea* may throw up certain recombinants in which undesirable genes are eliminated through recombination between the translocated chromosome and the normal B-genome chromosome of *B. juncea.* Meiotic analysis in the substitution lines indicated 18II at metaphase I and a regular 18–18 chromosome distribution at anaphase in the majority of the cells studied. It is proposed that substitution of 'C' chromosomes may have occurred at the cost of chromosomes belonging to 'B' genome. This is due to the fact that *B. juncea* (AABB) and *B. napus* (AACC) have a common 'A' genome, and that the F_1 (AABC) between the two species will have regular pairing (10II) between the chromosomes belonging to the 'A' genome. Little or no pairing is expected between 'B' and 'C' genomes. This will result in 17 univalents (8B + 9C) or univalents accompanied by few allopairs. Mizushima (1968) has reported four bivalents following hybridization between *B. nigra* (BB) and *B. oleracea* (CC). Banga (1987) could observe as many as three bivalents in a spontaneous haploid of *B. carinata* (BC). This indicated that there are a few homeologous regions in some chromosomes belonging to the B and C genomes where pairing is possible. Selection for *juncea* type segregants followed by selfing and selection led to the development of stable disomic substitution lines having 'C' genome chromosomes in place of 'B' chromosomes. Such a spontaneous substitution occurs only at the cost of the chromosome phylogenetically related to the substituted chromosome. All the substitution stocks reported in this study carried substitutions for only one chromosome.

Though the disomic substitution lines could be easily marked as *juncea* type, there were some striking differences (Table 1). The most prominent feature of the majority of these lines was their bolting habit. This is in total contrast to the flowering behaviour in *Brassica juncea. Brassica napus*(AACC) as well the donor of the 'C' genome i.e. *B. oleracea* is a quantitatively long day plant. These have a basic vegetative period (BVP) i.e. a period of juvenility

Table 1. Distinguishing features of the disomic substitution lines of *Brassica juncea.*

Entry	Pollen fertility (%)	Female fertility (%)	Days to flowering/ bolting	Flowering habit	Leaf type	Flower type	Pod type	Yield per plant (gm)	Oil content (%)	Erucic acid (%)
IS 10	78	73	90	NB	N	N	N	13.7	34.9	45.4
IS 20	83	81	97	B	N	N	N	10.1	37.3	44.3
IS 36	54	58	92	B	Ab	Ap	N	10.5	33.8	47.2
IS 62	62	78	89	B	Ab	Ap	N	5.7	35.0	48.8
IS 64	75	80	95	B	Ab	Ap	N	10.6	35.1	43.5
IS 83	59	67	87	B	Ab	N	N	8.1	36.2	47.1
IS 86	61	74	103	B	Ab	N	N	7.1	40.0	40.8
IS 68*	76	75	83	B	Ab	Ap	N	8.7	32.4	16.8
IS 98*	85	82	70	NB	N	Ap	N	9.5	36.8	35.9
IS 106L*	85	78	73	NB	Ab	Ap	S	8.1	32.4	49.5
RLM 198	93	82	83	NB	N	N	N	11.7	39.5	50.0
Oro	87	89	116	B	N	N	N	15.9	43.0	2.0

B = Bolting, NB = Non-bolting, N = Normal, Ab = Abnormal, Ap = Apetalous, S = Spinous.

* Cytologically confirmed to be different substitutions.

through which a plant must pass before flowering occurs. Due to the influence of the 'C' genome, the initial growth rate in *B. napus* is very slow until bolting, after which growth is very fast. *B. juncea* has a short basic vegetative phase and as such there is not much difference between growth rates observed during BVP and after initiation of the flowering shoot. The *napus*-like flowering behaviour in certain substitution stocks can be attributed to the influence of chromosomes belonging to the 'C' genome. Apart from changed flowering behaviour, other morphological/biochemical features of substitution stocks included:

1. sudden appearance of numerous leaf-like projections around the mid rib on the dorsal side of the leaf
2. lower level of erucic acid i.e. 16.8% in IS 68 and 35% in IS 98 as compared to 50% in the recipient RLM 198

In the leaves the development of leafy outgrowths around the dorsal mid rib seemed to be closely correlated with bolting. Apparently, the genes located on the substituted 'C' chromosomes become activated by floral stimulus.

The identification of the substitution 'C' chromosomes and the substituted 'B' chromosomes requries a complete monosomic series in the recipient which at present is not available in *Brassica*. Depending on the morphological diversity, it is proposed that entries IS 10, IS 20, IS 36, IS 68, IS 86, IS 98 and IS 106L (Table 1) are substitutions for different chromosomes. The meiotic analysis of F_1 hybrids, Is 68 × IS 98, IS 98 × IS 106L and IS 68 × IS 106L indicated the presence of four univalents and 16 bivalents in each case. This confirms these three lines to be substitution for different chromosomes.

The majority of the disomic substitution lines produced in this study are meiotically stable and have normal vigour and fertility . This suggests that the genetic activity of the substituted B-chromosome is being compensated by a 'C' chromosome, which is possible only if the substituted chromosome is phylogenetically related to the replaced chromosome. The lines are significant for their potential use in crop improvement, the study of gene expression and the effect of background genetic information on the introgressed alien variation.

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