STUDIES ON IN VIVO INTERSPECIFIC HYBRIDISATION IN BRASSICA

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SUMMARY

Studies were undertaken to explore the possibility of exploiting interspecific variability by species hybridisation in the genus *Brassica*. Six divergent species, viz. *B. juncea*, *B. napus*, *B. campestris*, *B. chinensis*, *B. nigra* and *B. japonica* were used in an attempt to produce interspecific hybrids by following conventional (*in vivo*) method of hybridisation. The resultant F_1 hybrids showed considerable variations for morphological traits contributing to yield. The meiotic analysis of F_1 hybrids revealed the presence of bivalents and univalents which can be attributed to the difference in the genetic constitution of the parental species. The pollen fertility of various cross combinations ranged from 57.00% (*B. campestris* x *B. chinensis*) to 16.00% (*B. chinensis* x *B. juncea*). The variation for pod setting was from 4.76% (*B. chinensis* x *B. napus*) to 71.05% (*B. juncea* x *B. napus*). The hybrid of cross, *B. juncea* x *B. napus* was recorded for maximum seed set (74.69%), whereas the hybrid of cross, *B. chinensis* x *B. napus* was recorded for minimum seed set (50.00%). The per cent germination of hybrid seed was maximum (54.54) again in the cross, *B. juncea* x *B. napus* and minimum (9.09) in the cross, *B. napus* x *B. juncea*. The present investigation thus showed the potential of using *in vivo* method of interspecific hybridisation for induction of genetic variability in *Brassica* species and introgression of desirable genes from related/ non cultivated species to commercially cultivated species.

INTRODUCTION

The oilseed crops have an important role in country's economy. In the world oliferous *Brassica* ranks fifth in importance among oilseeds. There are 159 species in the genus *Brassica*, which includes a number of wild species also. The study of chromosome number and meiotic behaviour indicated polyploid nature of *Brassica* species. The elementary species are *B. nigra*, *B. oleracea* and *B. campestris* with 2n = 16, 18 and 20 respectively, whereas the natural alloploids B. *carinata*, B. *juncea* and B. *napus* have 2n = 34, 36 and 38 respectively. A-genome was assigned to B. *campestris*, B to B. *nigra* and C to B. *oleracea*. The genomes of higher chromosomal species were designated AB for B. *juncea*, BC for B. *carinata* and AC for B. *napus*. A new species of B. *tournefortii* with 2n = 20 and D genome has also been identified. All these species are chromosomally balanced and show regular bivalent formation.

MATERIAL AND METHODS

The experimental material consisted of different cultivars of following species :

1. B. *nigra*
2. B. *campestris* cv. TL-15
3. B. *chinensis*
4. B. *japonica*
5. B. *juncea* cv. RLM 198
6. B. *napus* cv. Tower

In each case, hand pollinations were made in the field. The emasculations were done generally in the evening and pollinations in the morning at about 10 o'clock. Observations on different morphological traits like plant height, length of main shoot, number of primary and secondary branches, days to first flowering, number of pods on main shoot, pod length and number of seeds per pod were carried out. The crossability of different crosses was observed by recording per cent pod set, per cent seed set and per cent germination of hybrid (F_1) seeds. For cytological studies, young buds were collected in the morning and fixed in freshly prepared 3:1 alcohol & acetic acid fixative. Anthers were macerated in 2 per cent acetocarmine and studied under microscope to determine different meiotic stages (diakinesis, metaphase-I, anaphase-I). Pollen fertility was recorded by using acetocarmine staining technique.

RESULTS

The differences for various morphological characters, viz. plant height, length of main shoot, number of primary and secondary branches, days to first flowering, number of pods on main shoot, pod length and number of seeds per pod were recorded in case of *Brassica* species and interspecific crosses. Highly significant differences for most of the morphological traits were observed between different species and their crosses, except for number of primary and secondary branches.

1. Per cent pod set : Percentage of pod set ranged from 4.76 (B. *chinensis* x B. *napus*) to 71.05 (B. *juncea* x B. *napus*), whereas it was 14.54 and 23.80 in the crosses of B. *napus* x B. *juncea* and B. *campestris* x B. *chinensis* respectively (Table 1).

2. Per cent seed set : It was highest in cross of B. *juncea* x B. *napus* (74.69%) and lowest in case of B. *chinensis* x B. *napus* cross (50.00%). Except for the first cross, percentage was almost same in other three crosses (Table 1).

3. Per cent germination of hybrid seeds : It ranged from 9.09% (B. *napus* x B. *juncea* and B. *chinensis* and B. *napus*) to 54.54%

(B. juncea x B. napus). It was 45.45% in cross of B. campestris x B. chinensis).

4. Per cent pollen fertility : Pollen fertility ranged from 57% in the cross of B. campestris x B. chinensis to 16% in the cross of B. napus x B. juncea. The pollen fertility in the crosses, B. juncea x B. napus and B. chinensis x B. napus was 39% and 28% respectively.

Meiotic behaviour of the interspecific hybrids of the cross of B. juncea x B. napus revealed the presence of 37 chromosomes in the form of bivalents and univalents. At anaphase-I, they were unequally distributed to both the poles with some laggards. The cross of B. campestris x B. chinensis showed the presence of 10 bivalents, whereas in case of cross B. chinensis x B. napus 10 bivalents and 9 univalents were present.

DISCUSSION

Existing variability in a specis can be supplemented with new variability by wide hybridisation. The tribe Brassicae offers a great potential to incorporate genes from one species to another. Variability for morphological characters can be utilized in increasing yield. Variability for different morphological characters was also recorded by many other workers. However, several crossability barriers are encountered in wide hybridisation, so it is essential to know about crossability and genomic relations between different species of Brassica. The findings indicated substantial variability for various economic characters. The observed differences in crossability may be due to the differences in the genetic constitution of the parental species. Variability for this character has also been reported by Khanna and Chowdhury (1974 and 1981) while working with B. juncea, B. campestris, B. chinensis and B. carinata and their crosses. The differences in per cent pod set and seed set may be attributed to the differences in pollen fertility, rate of pollen tube growth and penetration and abortion rate of embryos. Low germination percentage was mainly due to seed sterility of F_1's.

Meiotic studies also are in conformity with the studies of Morinaga (1934), Sikka (1940), Ramanujam and Srinivasachar (1943) and Nwankit (1970 and 1971). They recorded the presence of bivalents and univalents. The presence of bivalents and univalents depends upon the genetic constitution of the parental species. The formation of bivalents in crosses shows the possibilities of transferring the desirable characters from one species to other, by the means of crossing over that takes place during the meiosis. So, desirable lines could be developed by extensive crossings. It will lead to the improvement in the agronomically desirable varieties of rapeseed and mustard.

Various aspects of crossability studied in F_1 hybrids of crosses involving different *Brassica* species (Tables 1, 2 and 3) are :

BIBLIOGRAPHY

Catcheside, G.D. 1934. The chromosome relationships in the swede and turnip groups of *Brassica*. Ann. Bot. 48 : 601-633.

Inomata, N. 1980. Hybrid progenies of the cross *B. campestris* x *B. oleracea*. I. Cytological studies on F_1 hybrids. Japan J. Genet., 55 (3): 189-202.

Johanston, T.D. 1974. Transfer of disease resistance from *B. campestris* to rape (*B. napus*). Euphytica, 23 : 681-683.

Khanna, V.K. and Chowdhury, J.B. 1974. Studies on pollen tube growth and seed set in some interspecific crosses in *Brassica*. HAU J Res., 4 (3): 231-235.

Khanna, V.K. and Chowdhury, J.B. 1981. Studies on meiosis, pollen tube growth and seed set in some interspecific crosses in *Brassica*. HAU J. Ras. II (2) : 162-168.

Labana, K.S. 1980. Interspecific hybridisation in *Brassica*. In. Breeding Oilseed Crops, ed. Khem Singh Gill. pp. 267, PAU.

McNaugheten, H. 1972. *B. napocampestris* L. (2n = 58). I. Synthesis, cytology, fertility and general considerations. Euphytica, 22 : 301-309.

Morigana, T. 1929. Interspecific hybridisation in *Brassica*. The cytology of F_1s of *B. napella* and various other species with 10 chromosomes. Cytologia, 1 : 16-27.

Morinaga, T. 1934. Interspecific hybridisation in *Brassica*. The cytology of F_1 hybrids of *B. juncea* x *B. nigra*. Cytologia, 6 : 62-67.

Nwankiti, O. 1970. Cytogenetic and breeding studies with *Brassica*. I. Cytogenetic experiments with *B. napocampestris*. Hereditas, 66 : 109-126.

Table 1 : Per cent pod and seed set in different crosses of *Brassica* species.

Cross	No. of buds pollinated	No. of pods formed	Total No. of seeds	Pod set (%)	Seed set (%)
B. juncea x *B. napus*	950	675	3025	71.05	74.69
B. napus x *B. juncea*	495	72	245	14.54	58.05
B. campestris x *B. chinensis*	1050	250	785	23.80	52.33
B. chinensis x *B. napus*	840	40	100	4.76	50.00

Table 2 : Germination percentage of hybrid seeds of different crosses

Cross	No. of seeds sown	No. of seeds germinated	Per cent germination
B. juncea x *B. napus*	44	24	54.54
B. napus x *B. juncea*	11	1	9.09
B. campestris x *B. chinensis*	22	10	45.45
B. chinensis x *B. napus*	11	1	9.09

Table 3 : Per cent pollen fertility of F_1 hybrids

Cross	% pollen fertility
B. juncea x *B. napus*	39
B. napus x *B. juncea*....................	16
B. campestris x *B. chinensis*	57
B. chinensis x *B. napus*	28