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Genotype effects of *Brassica napus* on its reproductive behavior after pollination with *B. juncea*

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Abstract. To investigate the cause of variation in the interspecific crossability of *Brassica napus*, three different genotypes were studied in respect of their reproductive behavior after pollination with *B. juncea*. There were great differences among maternal genotypes in allowing foreign pollen to germinate on and penetrate into their stigmas, leading to a wide diversity of interspecific fertilization. The division of the hybrid primary endosperm nucleus and zygote appeared normal in all combinations of crosses. While the abundant free nuclei of the endosperm developed properly and never became cellular, the embryos degenerated as early as 10 days after pollination when the cultivar Rucabo, which had the highest fertilization record with species of *B. juncea*, was involved. When 81007 was used as female parent, the endosperm grew a little but the embryo halted at the heart-torpedo stage. Lack of nourishment might be responsible for the observed embryo abortion. Among the sic hybrid combinations, the cross 84014A × Changyang hunagjie was the only one where endosperm tissue was observable and an abnormal embryo occurred prior to cellular endosperm formation. Apart from the three typical embryological features, significant variation was also demonstrated among each of the cross combinations. Genetic diversity appears to exist not only between varieties, but also within cultivars. In addition, methods for developing interspecific crossable lines are discussed.

Key words: *Brassica napus, B. juncea* – Hybridization – Genotype – Fertilization – Embryo development

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Introduction

It has proven difficult to obtain interspecific hybrid seeds between *Brassica napus* and *B. juncea* when the former was used as the female parent because of low crossability (Meng 1987). Meng and Yi (1988) reported that in the cross *B. napus* × *B. juncea*, the pollen tube of *B. juncea* was unable to penetrate the papillar cells of *B. napus* because of a heavy deposition of callose after pollination. Both the hybrid embryo and the endosperm developed slowly and ceased developing at the heart-shaped embryo stage and the free-nuclei endosperm phase respectively. Genetic divergence within a species might account for the variation in interspecific crossability. Genotype effects on pollen-pistil interaction after interspecific pollination have been reported in several genera including *Nicotiana*, *Trifolium* (Hadley and Openshaw 1980), *Triticum* (Sharma and Gill 1983), *Vigna* (Chen et al. 1983) and *Zinnia* (Boyle and Stimart 1986). In *Brassica*, Matsuzawa (1983) found that pollen germination indices among 31 cross combinations of *B. oleracea* × *B. campestris* varied from 0.7 to 3.8. Through morphological observation and a statistical analysis of pollen and papilla cells in the cross *B. napus* × *B. oleracea*, Meng and Zhou (1988) noticed a significant influence of the female genotype on pollen-stigma interaction.

Research relating to genotype influence on interspecific hybrid development is, however, limited. Oettler (1984) pointed out that the degree of differentiation of the hybrid embryo of *Triticum durum* × *Secale cereale* varied with the maternal parent. Boyle et al. (1987) analysed 60 cross combinations of *Zinnia angustifolia* × *Z. elegans*, and concluded that the percentage of emerging seeds and the percentage of normal hybrids varied greatly and was controlled by multigenes of *Z. angustifolia*, indicating that it is possible to explore the genetic variation within

species and so improve the interspecific crossability. How the parental genotype affect the specific development of the hybrid embryo and endosperm remains to be determined. There has been no report on the *Cruciferae* with respect to the influence of parental genetic differences on interspecific embryo development.

This study is concerned with genotype effects of *B. napus* on the reproductive biology of *B. napus* × *B. juncea* in terms of fertilization, embryo and endosperm development, and seed setting.

Materials and methods

Three varieties of *B. napus*, 81007, Rucabo, and 84014A, with a different seed set ability in crosses with *B. juncea*, were selected as female parents based on a previous screening (Wu and Meng 1992). The test cultivars of *B. juncea* were 113'68 and Changyang huangjie (a landrace from Central China, abbreviated to CH).

Crosses were made on buds 1 day after emasculating in the field. The pistils of five inflorescence from each cross were fixed in FPA (5% formalin, 5% propionic acid and 90% ethyl alcohol) 7 h and 24 h after interspecific pollination and stained by the aniline blue fluorescence (ABF) method (Martin 1959). Five to ten pistils of each inflorescence were examined for pollen germination and pollen tube penetration into the sigma and style. Seed-set scoring was on four cross-pollinated inflorescences of each combination after harvesting.

For embryological studies, six ovaries were dissected randomly from three pollinated inflorescences of each combination at 1, 3, 6, 10, 20 and 30 days after pollination respectively. Ovaries, and ovules that were dissected from ovaries older than 6 days, were fixed in Carnoy's fluid (3 parts ethyl alcohol: 1 part glacial-acetic acid). The staining solution, Ehrlich's hematoxylin, was prepared according to Zhen (1978). The whole ovary and ovule was stained in the solution for 1–2 weeks. The stained materials were embedded in paraffin and cut into sections for microscope observation.

Results

Numerous pollen grains of *B. juncea* adhered to each stigma of *B. napus* on most of the female parent individuals 7 h after pollination. However, only a few pollen grains could be seen on the stigmata of 81007, indicating, a very slow hydration process between the pollen of *B. juncea* and the stigma of this particular genotype of *B. napus* (Fig. 1, Table 1). Statistics showed that the number of pollen grains on the stigma were significantly different among maternal genotypes. Although callose deposition was routinely observed within the tip region of papillar cells, which should be an indication of rejection according to Dumas and Knox (1983), a considerable number of pollen tubes penetrated the stigma surface and entered the style (Fig. 2). The more pollen grains on the stigma, the greater the number of tubes in the style. The difference in allowing foreign pollen tubes to penetrate was attributable to the difference in pistil genotype and was most evident 24 h after pollination when the number of penetrated pollen tubes reached a peak. The number of pollen tubes of *B. juncea* in the style of Rucabo was on average twice that present in the style of other genotypes. It was found that the number of pollen tubes observed by ABF at 24 h after pollination was significantly correlated with the percentage of fertilized ovules counted from paraffin sections ($r = 0.997$). Whether, or how serious, a fertilization barrier existed between *B. napus* and *B. juncea* as the pollen donor depended on the compatibility between the pollen and the particular pistil genotypes of *B. napus*, since there was no unusual behaviour at double fertilization within the embryo sacs. Thus, there was no obvious difference with respect to double fertilization among different pollination combinations including self-pollinated *B. napus*. The egg elongated soon after it was fertilized by the sperm of *B. juncea*. The hybrid zygote underwent normal cell division 6 days after pollination, slightly later than after self-fertilization of *B. napus*. The primary endosperm nucleus divided immediately after the three-nuclei-fusion, leading to the formation of numerous free hybrid endosperm nuclei within the embryo sac of *B. napus* (Fig. 3). Ten days after pollination, however, abnormality appeared. One out of eleven globe embryos in the cross Rucabo × CH degenerated with a disordered endosperm surrounding the micropylar end. A pear-shaped embryo with only a few cells developed from 12 proembryos when 84014A was used as the female parent. A disintegrated endosperm around a degenerating globe embryo was also observed and the embryo sac became very narrow due to pressure from over-growth of the nucellar tissue, which implied somatoplastic sterility. However, the tissues of the hybrid embryo appeared normal when 81007 was involved.

At 20 days after self pollination in *B. napus*, most of the embryos differentiated beyond the heart-shaped stage and became torpedo-shaped. The endosperm nearly completed its transformation from free-nuclei to a cellular stage. In the meantime, the majority of hybrid embryos lagged at the globe or heart-shaped stage, so that development was obviously slower than that of selfed embryos. About 10% of hybrid embryos had a degenerated embryo proper. In the crosses of Rucabo × CH and 84014A × CH, it was discovered that all the suspensors, which usually disorganize at a late torpedo-shaped embryo stage in *Brassica* species, had deteriorated. The cells of the suspensor, became unusally swollen, vacuolized, and even broke down (Fig. 4). When the female parent was 81007, on the other hand, only one out of eight hybrid embryos appeared abnormal in the suspensor and most of the embryos degenerated relatively late. The most notable difference among the three maternal genotypes was the hybrid endosperm. The endosperm in the cross 81007 × CH grew only a little 10 days after pollination. Various types of abnormal behavior, such as a vacuolized

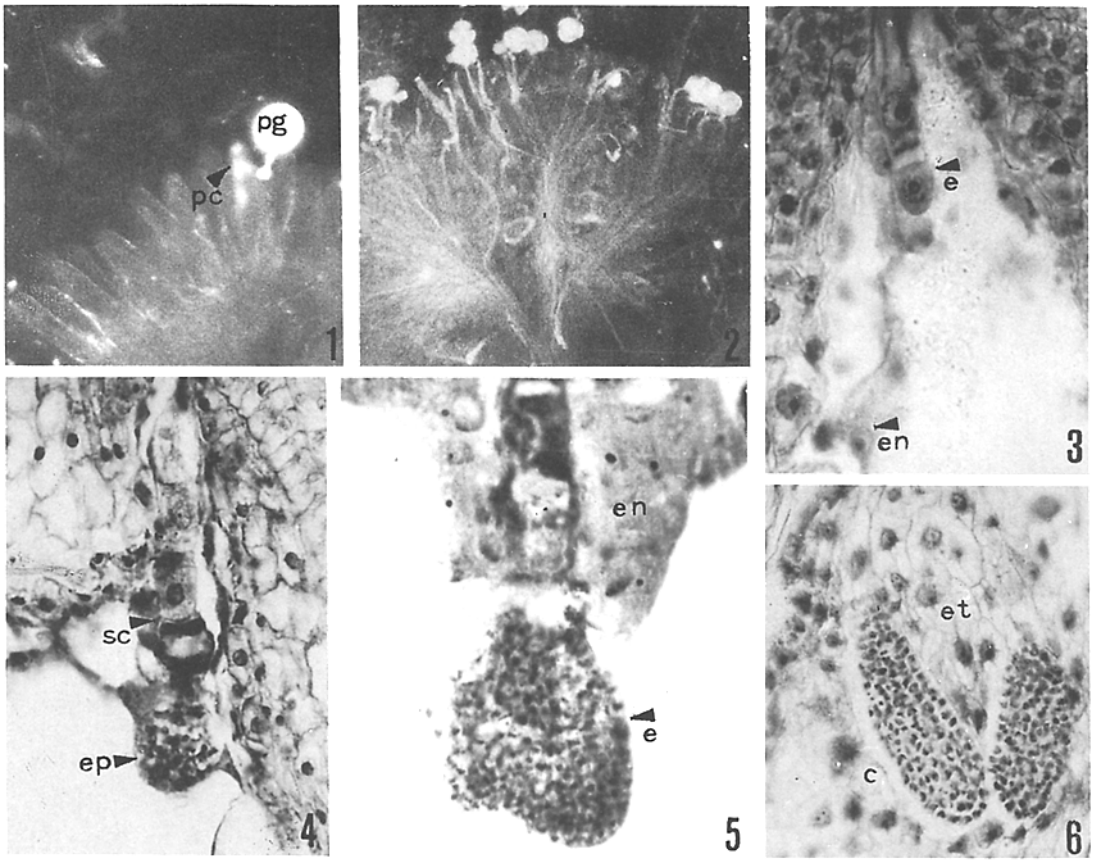

Fig. 1. 81007 × CH 7 h after pollination showing a pollen grain (*pg*) with a swollen tube tip adhering to a papilla cell (*pc*) which has deposited callose at its top, × 80

Fig. 2. Rucabo × CH at 24 h after pollination showing that plenty of pollen tubes had entered the style from the stigma surface, × 40

Fig. 3. 81007 × CH at 6 days after pollination showing normally developed endosperm nuclei (*en*) and proembryo (*e*) at this stage, × 100

Fig. 4. 84014A × CH at 20 days after pollination showing swollen and degenerated suspensor cells (*sc*) and a defunct embryo proper (*ep*), × 100

Fig. 5. 81007 × 113'68 at 30 days after pollination showing a collapsing heart-shaped embryo (*e*) with plenty of endosperm nuclei (*en*), surrounding its suspensor, × 150

Fig. 6. 84014A × CH at 30 days after pollination; two disintegrating cotyledens (*c*) and a torpedo-shaped embryo submerged in abundant endosperm tissue (*et*), × 150

Table 1. Effects of maternal genotypes of *B. napus* on interspecific fertilization pollinated with *B. juncea*

Female parent	7 h after pollination		24 h after pollination		3–6 days after pollination
	No. pollen grains per stigma	No. pollen tubes per pistil	No. pollen grains per stigma	No. pollen tubes per pistil	Fertilized ovules %
81007	1.27	0.04	45.67	13.33	11.5
84014A	12.77	9.51	24.67	12.33	8.0
Rucabo	20.60	10.87	48.43	25.29	31.1

cytoplasm, giant nuclei, clustered nuclei, and endosperm collapse, were observed in the endosperm at 20 days after pollination. By contrast, the endosperm of Rucabo × CH grew rapidly during a period from 10 days to 30 days after pollination and formed abundant free nuclei in the embryo sac. However, the free-nuclei-type endosperm never became cellular and did not show any serious abnormality until the hybrid embryo had disintegrated completely. On the other hand, a dramatic change occurred in the endosperm of 84014A × CH where cell walls formed in the endosperm cytoplasm. The endosperm tissue could be compared with that of selfed embryos in its degree of development at 30 days after pollination, although the heart- or torpedo-shaped hybrid embryos already appeared disordered or had collapsed completely (Fig. 6).

The same developmental patterns of embryo and endosperm occurred when another cultivar of *B. juncea*, 113'68, was used as the male parent. The hybrid embryo of 81007 × 113'68 also degenerated later than that of other combinations (Fig. 5). An active torpedo-shaped embryo was found surrounded by a vigorous free-nuclei endosperm at 30 days after pollination whereas endosperms in most of the other embryo sacs had degenerated. The hybrid of Rucabo × 113'68 also degenerated very early and there was no embryo observable at 30 days after pollination although the endosperm was still alive in some of the embryo sacs. Cellular endosperm was never found in the cross of 84014A × 113'68 and most of the free-nuclei endosperm had collapsed with their embryos at 30 days after pollination. From the last cross, however, a true hybrid plant was obtained, which was the only one produced from hundreds of pollinated flowers involving all of the listed cross combinations. The hybrid plant showed a high degree of sterility and was intermediate between the male and female parents in respect of most morphological characters. At what stage the endosperm became cellular in the embryo from which the true hybrid plant was obtained remains unknown.

Discussion

The state of hybrid embryo and endosperm development usually determines the fate of interspecific hybrid seeds (Raghavan 1976). In a previous year, the three cultivars of *B. napus*, Rucabo, 84014A and 81007 (designated by the serial numbers 086, 031 and 099), gave sharply diverse performances when crossed as pistil parents with *B. juncea*. 84014A had the highest interspecific seed set with 4.02 hybrid seeds per pollinated flower among 116 maternal parents tested (Wu and Meng 1992). This was presumably due to the development of the endosperm, as state above. Rucabo showed a very high fertilization rate, 270% higher than the average, but a very low seed set. The degeneration of hybrid embryos at an early stage of development would explain the low seed set. The cultivar 81007 was the lowest in both interspecific fertilization rate and seed set. The results from two successive years were largely in agreement. They indicated that the maternal genotypes differed from each other in their reproductive behaviour after pollination with *B. juncea*. Why then was only one hybrid plant obtained from crosses when 84014A was involved in the experiment? Seed set is at the end of a sequence of events, including fertilization, zygote and embryo development, endosperm growth etc. Each event may result in the complete failure of a cross. Many independent genes would be involved in each of the different events. The larger the number of genes involved, the stronger the influence of environment on seed set. One way to create a relatively stable rapeseed line with high interspecific crossability is by recombination between genotypes of *B. napus* having respectively a high interspecific fertilization rate and a better hybrid embryo growing ability.

Not only did each cultivar of *B. napus* used show a specific interaction with *B. juncea* in terms of reproductive behavior after interspecific pollination, but variation within the same cross combination was also evident. In 84014A × CH, for example, only 8% of the ovules were fertilized 6 days after pollination, 1 out of 14 proembryos degenerated 10 days after pollination, and 3 out of 10 embryos reached the differentiation stage of embryo development. Similar variations were also described by other workers. Brink and Cooper (1941) reported that hybrid seeds between *Nicotiana rustica* and *N. tabacum*, and *N. rustica* and *N. glutinosa*, shrunk at different maturing stages due to somatoplastic sterility. Both embryo-free seeds with normal endosperm and endosperm-free seeds with small hybrid embryos were observed in the interspecific crosses of *Citrus* (Esen and Soost 1973) and *Gossypium* (Weaver 1957). Delayed fertilization might cause zygote or proembryo failure, in comparison with the fast-fertilized products of the intergeneric cross between *Oryza sativa* and *Sorghum vulgare* (Wu et al. 1965). Differences in the physiology and biochemistry among different maternal plants, ovaries, ovules, and pollen grains might well contribute to the variation seen within a given combination. However, genetic differences among male gametes or female gametes would be more desirable from a plant breeders point of view. Although the male and female parents we used had been self-pollinated for several generations, some genes involved in interspecific barriers would remain in a heterozygous state when the heterozygotes were selectively advantageous. Mutation might be another source of genetic variation for the gametes. Though the mutation rate is very low, 10^{-5} for higher plants (Nei 1975), the mutation frequency with respect to interspecific reproductive processes would be expected to be much higher than usual and for two rea-

sons. One could be the multigenic character of the process. The other would be the pollen selection taking place on the stigma and style of the female parent (Zamir 1983; Mulcahy and Sari-Gorla 1992), as a result of which only male gametes with a favourable mutation would be available for fertilisation. This implies that a breeder who wants to obtain highly interspecific crossable material should not only keep testing the crossability of the offspring of the crossable variety or plants selected, but should also test the crossability of interspecific hybrid plants, in which the mutated gene might exist. In fact, bridge lines for interspecific gene transfer between *L. esculentum* and *Lycopersicon peruvianum* have already been developed (Poysa 1990).

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