THE EFFECTS OF SUCCESSIVE INBREEDING ON THE EMBRYO DEVELOPMENT AND THE ULTRASTRUCTURE OF SEEDLING COTYLEDON IN BRASSICA NAPUS

Meng Jinling

Institute of Crop Genetics and Breeding, Huazhong Agricultural University, Wuhan, Hupei, P.R.China

Introduction

Successive inbreeding in Brassica napus has been become a usual method in plant breeding. However, a series of studies have been showing that most economic characters and the yield of the rape got significant depression after several inbreeding generations (Wagner, 1954; Schuster etc., 1976; Grabiec etc., 1980; Liu Houli etc., 1983; Meng Jinling and Liu Houli, 1986). Moreover, there were yellowing-cotyledon seedlings in many lines of yellow-seeded Brassica napus (Liu Houli and Gao Yongton, 1985). The embryo or zygote is a germ of young generation so that the effects of inbreeding depression would be expressed firstly in the embryo development. Furthermore, it is easier to distinguish the genetic effects among different pollination treatments by studying the development of embryo and endosperm which undergo less environmental influence. This research program had been carried on from 1983 to 1985 for studying the effects of successive inbreeding of Brassica napus on the growth of pollen tube, the rate of fertilization, the development of embryo and endosperm as well as the ultrastructure of seedling cotyledons in order to study the mechanism of inbreeding depression and also try to find a way for overcoming inbreeding depression.

Materials and methods

The materials used were the yellow-seeded cousin inbred lines YSR-51 and YSR-52 which were inbred for 6 to 7 generations, and the brown-seeded cousin lines G314 and G105 that were inbred for 4 to 5 generations as well as their original open-pollinated populations. The self pollination (SP), the cousin pollination (CoP), and the cross pollination (pollination between YSR-51 and G314, CP) were carried on the same inflorescence of the inbred lines YSR-51 and G314 in the year 1983 and 1984. At the same time the two open-pollinated populations were still allowed to undergo open pollination (OP).

The ovaries and ovules were picked out at 1 to 72 hour or 1 to 50 days after pollination and fixed in Carnoy's fluid. The pollen germination and the pollen tube growth were examined by means of the aniline blue fluorescence staining technique. The development of embryo and endosperm was observed by using paraffin section method. The seedling

*This research program has been carried on under the direction of Prof. Liu Houli.

cotyledons were fixed with the osmian tetroside and the glutaraldehyde solution. The ultrastructure of cotyledon cells was observed on the transmitted electron microscope.

Results and discussion

1. Fertilization and embryo development.

At 72 hours after pollination, the number of pollen tubes per style in self and open pollination were 39 and 60 respectively, expressing the growth of selfing pollen tube slowly. The self-pollinated ovules were one-third times of fertilization frequency than that of open-pollinated ones during 2-5 days after pollination. It indicated that the self fertilization occurred latter than the control. There were less endosperm free nuclei and more slowly developed proembryos in the selfing ovules.But after the torpedo-embryo stage the open-pollinated embryo developed slowly and seed in pods were lighter and smaller than the selfing seeds. However, considering the whole nutrients stored in one pod, the selfing treatment was inferior to the open pollination for the less frequency of selfing fertilization, the reduced seed number per pod, and the lower oil content (Table 1).

Table 1. The level of embryo developments in different pollination treatments

Items	Days after pollination	Material groups	SP	CoP	CP	OP
Number of embryo cells	5 days	YSR	1.32±0.47	1.27±0.44	1.44±0.50	1.47±0.50
		G314	1.42±0.50	1.14±0.36	1.45±0.50	2.04±0.60
	10 days	YSR	4.92±1.94	5.44±1.76	6.43±3.03	6.17±2.12
		G314	5.85±2.42	6.00±2.72	8.12±3.30	10.93±3.15
Embryo diameter (μ)	50 days	YSR	2015±123	2088±113	2193±91	1996±98
		G314	2260±95	2204±121	2328±100	2063±70
1000 seeds weight (g)	Matured seed	YSR	3.51	3.39	3.72	3.12
		G314	3.64	3.86	4.48	3.52
Matured seeds number per pod	Matured seed	YSR	8.03±2.98	8.99±4.97	21.30±6.12	23.40±2.4

There were no notable differences between self and cross pollination on the speed of pollen germination, the rate and time of fertilization. However, 5 days after pollination, the division of embryo cells and endosperm nuclei in CP began to be ahead of SP so that the mean diameter of embryo and the weight of 1000 seeds in reciprocal crosses were larger and heavier than selfing ones, which showed strong heterosis. This implied that it may be possible to forerun the heterosis in the stage of embryo development.

There was no any remarkable difference between cousin pollination and self pollination on the fertilization and on the growth of embryo and endosperm as well as on the size of seed and the number of seed per silique. It may be suggested that the mating system between too close lines should be helpless to overcoming inbreeding depression in Brassica napus.

2. Variations and aberrations at embryology and cytology.

From Table 1,it showed not only the differences of average values among different pollinating treatments but also revealed the various variations with different treatments. Therefore, the variation coefficients in the frequency of fertilization, in the number of endoperm free nuclei, and in the number of embryo cells were statistically analysed (Table 2). The result showed no matter which pollinating way being used in the successive inbreeding progenies, the population was much less uniform at the fertilized ability and at the early embryo development, because each variation coefficient of self, cousin, and cross pollination was significantly larger than that of open pollinated population. In addition, in the three formers, there were more single fertilized embryo sacs, abnormal even disintegrated endosperm nuclei, degenerated zygotes, and corrupted proembryos at the early stage of embryo growth and more malformedly differentiate embryos during the latter stage. According to the collected data from 1358 ovules of yellow-seeded rape at 2-5 days after pollination, the frequencies of ovules appeared abnormal embryo development in the self, cousin, and cross pollination treatments were 6.48%, 4.43%, and 3.51% respectively, which were markedly higher than 0.95%, the frequency of abnormal ovules in open-pollinated population. The higher frequency of the abortion was coincide with the larger variation coefficient of embryo development in pollinating to inbred offsprings.

The higher frequencies of univalents, chromosome bridges and fragments during pollen mother cell meiosis of inbred lines were found in rye and maize (Krik, 1974; Balint, 1974). In this experiment, it was also found that the yellow-seeded inbred line ƒSR-51 had more rate of univalents and bridges in its meiosis of PMC than that of open pollinated population. These cytological studies suggested that the more abnormal ovules appeared, the larger variation cofficients of embryo development in successive inbreeding progenies, and the chromosome aberrations in structure or in number were increased by selfing itself, followed by forming defective gametes. The increasing of genetic variations would add to the genetic load and multiply the degree of inbreeding degeneration. On the other hand, a few of outstanding varied individuals might also come from the variations. These outstanding individuals may be treated as new breeding materials for further studies.

3. The ultrastructural changes of the chloroplast in yellowish cotyledons.

The yellow-seeded inbred line ƒSR-51 expressed yellowing cotyledons at early seedling stage with sharply reducing of carotinoid and chlorophyll. The chloroplast of yellowing

cotyledons occurred marked change in shape under the observation of ultrathin sections. It had a flat edge and its outer membrane facing the central vacuole of the cell could not normally project so that the external form of the chloroplast was not a single-convex lens shaped but a disc shaped, and the section form was not an olive but a bar in shape. The width of the chloroplast became very narrow when observed from the bar-shaped section (Table 3). Both the failure of outer membrane project, which reduced the contact face between the chloroplast and the cytoplasm, and the factors, which were the cause for the outer membrane failing to project, would decrease the efficiency of the photosynthesis. It may be another reason for the depression of successive inbreeding offsprings of Brassia napus.

Table 2 The variation coefficients of different pollination treatments during fertilization and proembryo development in yellow-seeded rape

Items	Content	SP	CoP	CP	OP
Rate of fertilization	Mean value of C.V.	41.47	40.18	30.99	14.18
	Significance of differece	a	a	a	b
Number of endosperm free nuclei	Mean value of C.V.	41.83	51.02	47.41	30.94
	Significance of differece	ab	a	a	b
Number of embryo cells	Mean value of C.V.	36.35	33.53	37.99	27.71
	Significance of difference	a	a	a	b

Table 3. The change of some indexes of chloroplast structure in cells of seedling cotyledons

Pollination treatment	Lenth of Choronlast section(μ)	Width of chloroplast section(μ)	Number of chloroplast lamelae	Number of chloroplast granum
SP	4.90±1.20	1.49±0.35	17.13±2.79	20.29±5.06
OP	5.18±0.97	2.38±0.34	23.86±2.70	31.43±5.71

On the internal structure of the chloroplast in yellowing cotyledons, the number of stroma lamelae and grana strikngly reduced. There were slowly fat metabolizing, extremly reduced ribosomes and endoplasm reticulam, vary spare lamelae, and much low density of chloroplast ribosomes in some of seriously yellowing cotyledons. The electron density of the stroma droped down much and even appeared vesicle showing complete destruction of the chloroplast structure.

On the contrary, there were no any yellowing phenomenon and any unusual changes of chloroplast structure in the hybrid seedling of YSR-51XG314. This indicated that the seedling yellowing was the recessive character controlled by nuclear genes and suggested that the seedling yellowing and even the inbreeding depression should be reversed by some suitable interline hybridization and forced selection in the hybrid offsprings.

References

1. Grabiec, B. & Bolcerak, K. 1980 Hodowla Roslin, Aklimatyzacja i Nasiennictwo 24(3): 235-247.
2. Liu Houli etc. 1983 J. Huazhong Agriculture College 2(3): 82-86.
3. Kirk, D.J. and Jones, R.N. 1974 Heredity 32(3): 357-373.
4. Meng Jinling and Liu Houli, 1986 Acta Agronomic Sinica (China) 12(2): 79-85.
5. Schuster, W. and Michael, J. 1976 Z Pflanzenzütung 77:56-66
6. Wagner, M. 1954 Z. Pflanzenzütung 33: 237-266.