

Microspore Culture of F₁ Hybrid between *Brassica napus* and *Brassica juncea* is the Effective way on Breeding

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Microspore culture for F₁ hybrids *B. napus* × *B. juncea* is a particularly valuable tool in hybrid breeding programs (Chame *et al.* 1988, Guan 1999). Microspore culture has been successful in several *Brassica* species. The main objectives of the present investigations are as follows: (1) we used *B. napus* as female, as it has good yield and higher oil content. *B. juncea* as male has many desirable agronomic traits including higher tolerance to drought and high temperatures, resistance to shattering and disease. The F₁ hybrids *B. napus* × *B. juncea* can combine the valuable traits of both the species, which can produce doubled haploid lines in isolated microspore culture. The main advantage of using doubled haploids are (1) the rapid and theoretically complete homozygosity of the offspring, resulting in a time saving of several years in cultivar development; (2) to study relationship between microspore culture and pollen fertility of F₁ hybrid, or anther colour; (3) to determine the mode of embryo transfer, age of donor plant, embryo age and size effect on isolated microspore culture of F₁ hybrid. In this paper, we report our research results about this subject.

MATERIAL AND METHODS

Plant Material

The donor plants for microspore culture were F₁ hybrids *Brassica napus* × *Brassica juncea*. The numbering of F₁ hybrids are No.27, No.28, No.29, No.30 and No. 31. Their parents were *B. napus* as female including XL220, XY230, XL250, and GY5. *B. juncea* as male parent including HLR, TJR, TYR and SDR.

Donor Plant Condition

Optimal growth conditions produce healthy plants and enhance embryogenic responses. Light, temperature, water and nutrients are all necessary to produce healthy plants (Coventry 1988, Guan 1995, 1999). Donor plants were planted in 24±2°C 21±2°C light/dark periods. At flowering these plants were transferred to a conditioned growth room with a 10°C/5°C (day/night) temperature region, and a 16h photoperiod at an intensity of 2600 - 3000Lux. Because cold treatment (10°C/ 5°C) can enhance embryogenesis. Plants were watered and fertilized daily.

Cytological Observations

The relationship between anther colour and microspore development stage was observed, according to the method described by Li (1984, 1991). Fertility was assessed as the percentage of pollen stained by the aceto-carmin solution. At least 30 buds from different plants and 1000 pollen grains were observed per bud following the method used by Li (1986).

Isolation and Culture of Microspores

Flower buds 3.5~7mm in length and green with a little yellow anther colour (containing microspores at late uninucleate or mid uninucleate stages) were used. Selected buds were excised and surface-sterilized with a 7% (W/V) solution of calcium hypochlorite for 10 min and rinsed three times with sterile distilled water. The microspore isolation and culture procedure was modified from Coventry *et al.* (1988). Buds were gently squashed in a washing solution of B₅ medium supplemented with 13% (W/V) sucrose and the resulting suspension filtered through 63µm and 44µm nylon screens into graduated 50ml conical tubes. The microspore were collected by centrifugation at 1000 rpm for 4 min. Decant under sterile conditions, add more B₅ wash and repeat for a total of 4 spins and then resuspended in NLN at a ratio of 1 bud to 1ml medium. Microspores were cultured in the dark in 50 mm Petri dishes containing 10 ml volumes of NLN medium. After an initial incubation of the microspores for 14 days at 30°C. When the culture is 14 days old, put plates in paper box on a slow shaker (60 rpm) at 25°C for 7~10 days to ensure proper development of the embryos.

Plant Regeneration

When the embryos are 21-28 days or older they are transferred to B₅ solid media and are placed in a 4°C incubator, for 10 days with an 8 hr photoperiod. After 10 days in the cold incubator, plates are transferred to a 27°C incubator with a 12 hr/12 hr (day/night) cycle. Fully germinated embryos were transplanted, 4~6 weeks from the time of transfer, to soil-free mix in the greenhouse.

RESULTS

Pollen fertility and Genotype

The pollen fertility of F₁ hybrid centred around 30-60% by pollen stainability (Fig. 6). The results show pollen fertility is not correlated with embryo yield. such as No.31 has 60% normal pollen and embryo yield is 154 embryos per 100 buds. Whereas No.27 only has 40% normal pollen and embryo yield is 888 embryo per 100 buds. The embryo yield of No.27 more than of No.31; the No. 27 responded to embryogenesis better than others.

Genotype is a very important factor in the embryogenic frequency of F₁ hybrids of No.27, No.28, No.29, No.30 and No.31. The genotypic differences within these genotypes produced variable embryogenic results. According to differences in embryo yield, it can be divided into three types: (1) high responsive material (No.27); (2) medium responsive material (No.29, No.31); and (3) low responsive material (No.28, No.30)

The above results show that embryo yield is closely correlated with genotype of F₁ hybrid, but is not correlated with rate of pollen fertility.

Age of Donor Plants

Donor plant age has obvious effect on efficient embryo yield of both medium responsive material (No.29) and low responsive material (No.28). Microspores from primary inflorescences (75~80 days old plant) did not produce any embryos, If these microspores were from older plants (90~95 days old plant), more embryos can be produced. However, donor plant age has no obvious effect on efficient embryo yield of high responsive material (No.27). From 75 days to 95 days old plant, embryo yield was all high either from younger plants of older plants. These results show that embryo yield is closely correlated with the age of donor plant in both medium responsive and low responsive material, but is not correlated with the age of high responsive donor plant.

Effect of Anther Colour

Anther colour is a very important aspect of a successful microspore culture system, as it is related to microspore development stage and presented in (Table 4). Yellow (Fig.1 a, b, c), the microspore development stage is trinucleate (Fig.2). When anther colour is somewhat greenish yellow (Fig.1 d, e) the microspore development stage is trinucleate - binucleate (Fig.2,3). When anther colour is greenish yellow (Fig.1f), the microspore development stage is binucleate – uninucleate (Fig. 3, 4). When anther colour is yellowish green (Fig.1 g), the microspore development stage is late uninucleate (Fig.4), mid uninucleate (Fig.5). When anther colour shows somewhat green (Fig.1 h) the microspore development stage is mid uninucleate (Fig.5).When anther colour is green (Fig.1i,j), the microspore development stage shows early uninucleate-tetrad and meiosis stage. For the initial isolations, the terminal are removed from the donor plants just as they begin to bolt and usually before any visible flower development. At this development stage, there are about 40% buds in a floral disc with yellowish green anther colour which contain the responding microspores with the highest embryo yield. These results show that selected bud for isolated culture should have anther colour as yellowish green, which contain the microspores and are most suitable for induction of embryogenesis.

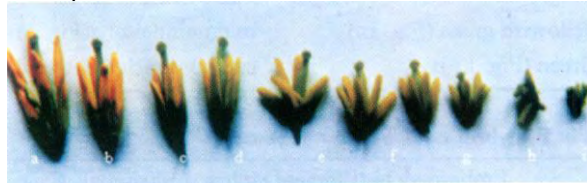


Fig 1. The variation regularity of anther color of one disc of F₁ hybrid from *B. napus*×*B. juncea*

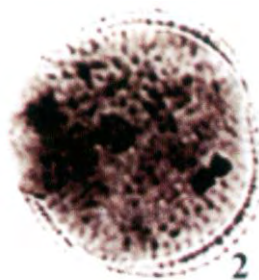


Fig.2. The microspore development stage is trinucleate

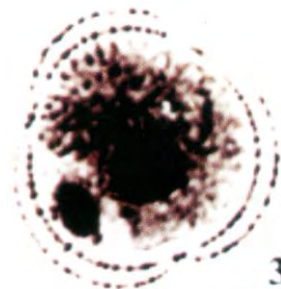


Fig.3. The microspore development stage is binucleate

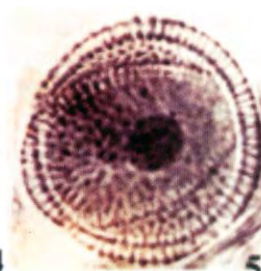
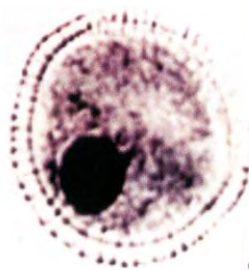


Fig.4-5. The microspore development stage is late-uninucleate and mid-uninucleate

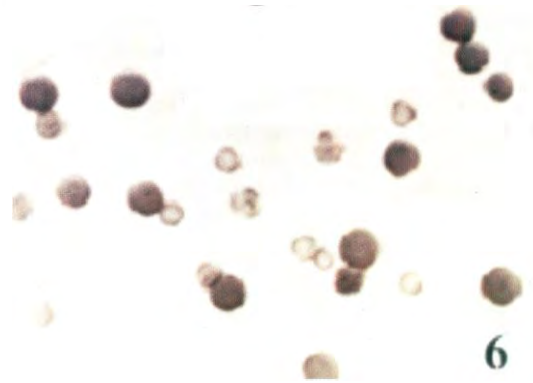


Fig.6. F₁ pollen from *B.napus*×*B.juncea*, showing fertilizable pollens and sterile pollens

Effect of Transfer Embryo Mode

Embryos were transferred in two ways. One is when embryos grow 21 days old in the NLN media and are transferred to B₅ semisolid media (4g/L of agar). After 3 days, the embryos are transferred to B₅ solid media. Other mode is that embryos 27 days old are transferred directly to B₅ solid media. Then, the plates with embryos are placed in a 4°C incubator for 10 days with 8 hr photoperiod. The results are shown in Table 5. Embryos are transferred from liquid media to B₅ semisolid media for 3 days, then transfer to B₅ solid media, which can promote regeneration and vegetation growth as leaves become deep green and also thickened. There is also a plate with 17 embryos and all embryos develop into plantlets (Fig 7, 8). The chromosome number of regeneration plants ranges from 19-37. As above result indicate it, is a better transfer embryo mode then the other one.

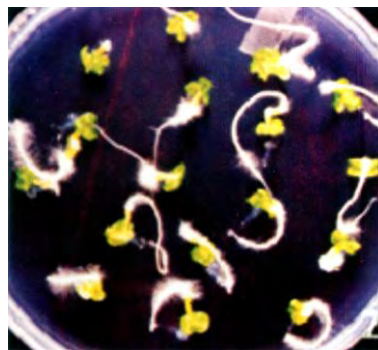


Fig.7. Regeneration of plantlets from embryogenic of No.27 of *B. napus*×*B. juncea*



Fig.8. In regenerated plants from microspore culture of No.27 of *B. napus*×*B. juncea*

Effect of Embryo Age and Size

Embryo age means the culture time from microspore isolation culture until embryos are transferred to solid medium as embryo age. When embryo age is 27 days old, the plantlet regeneration rate raised to 15%. It is the best stage for embryo transfer. .

Regarding the effect of embryo size, we observed that small embryos from 0.2cm to 0.3cm in length are better than big embryos from 0.6cm to 0.8cm in length. The regeneration rate (%) from small embryos is the highest in all experiments which is around 36.3 percent.