Studies of microspore culture and doubled haploid breeding on rapeseed: plant regeneration from microspore derived embryos of F_1 hybrids between *Brassica napus* and *B.juncea*

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ABSTRCT

Factors influencing embryogenesis from isolated microspores of F_1hybrids between *Brassica napus* and *B.juncea* were studied. The results showed: 1) the embryo yield was closely correlated with the genotype of F_1hybrids,but it was not correlated with the rate of pollen fertility; 2) donor plant age affected embryo yield of both medium responsive genotypes and low responsive genotypes. There were no obvious effects for high responsive genotypes; 3) anther color was a criterion for selecting suitable buds for the induction of embryogenesis, with yellowish green being the most suitable for the induction of embryogenesis; 4) better results were obtained from the embryos which were transferred from liquid NLN media to semisolid B_5 media for 3 days and then transferred to solid B_5 medium; 5) the optimal embryo age was 27 days. The small embryos from 0.2 cm to 0.3 cm in length were better than large embryos from 0.6 cm to 0.8 cm in length for induction of embryogenesis.

INTRODUCTION

Microspore culture for F_1 hybrids from *B.napus* × *B.juncea* is a particularly valuable tool in hybrid variety breeding programs. Isolated microspore culture of B.napus since Licher (1982) first reported, there have been much reports of embryo production from microspores. But there are few papers dealing with this subject that microspore culture of F_1 hybrid from reciprocal crosses between the *B.napus* and *B.juncea*.In this paper we report our findings of some factors beneficial to microspore embryogenesis in F_1 hybrid from *B.napus* ×*B.juncea*.

MATERIAKLS AND METHODS

The donor plant for microspore culture was used forty F_1 hybrid obtained from crosses between *B.napus* and *B.juncea*. Among forty F_1 hybrid tested, selected No30, No31, No32, No33, No34 as typical materials were analyzed Which the maternal materials included *B.napus* varieties:XLY2, XY11, XY13, XY15, GY5 and the paternal materials included *B.juncea* varieties: HLR, TJR, TYR, SDR, HYL. The microspore isolation and culture procedure was modified from Coventry et al(1988).

RESULTS

Effect of genotype and Pollen fertility Yield of microspore derived embryos was influenced by the genotypes. Among forty F_1 hybrids between *B.napus* × *B.juncea* tested, No30 was the most embryogenic line yielding on average 888 embryos per 100 buds. Yields of microspore-derived embryos of the selection five F_1 hybrids genotypes.

The results showed that according to different of embryo yield, it could be divided into three types:(1) high responsive material (No.30); (2) middle responsive material (No.32, No.34); (3) low responsive material (No.31, No. 33). In addition to results are also showed when F_1 pollen on *B.napus* × *B.juncea* usually range in fertility from about 30% 60% normal pollen, the pollen fertile is mot correlated with embryo yield. Such as No.30, which pollen fertility is 40%, the efficient embryos yield showed 888 embryos per 100 buds, whereas No.33, which pollen fertile is also 40%, the efficient embryo yield only 94 embryos per 100 buds etc. The above results showed embryo yield is closely correlated with genotype of F_1 hybrid, but is not correlated with rate of pollen fertility.

Donor age has obvious effect on efficient embryo yield of both middle responsive material (No.24) and low responsive material (No.31). Microspores from primary inflorescences (75 days old plant) did not form any embryos. If these microspores were from older plants (85 days old plant), more embryos can be produced. But donor age hasn't obvious effect on efficient

embryo yield of high responsive material. From 75 days to 95 days old plant, embryo yield was all high either younger plants or older plants. These results showed the embryo yield is closely correlated with age of donor plant both middle responsive material and low responsive material, but is not correlated with age of donor plant high responsive material.

Effect of anther color Anther color care is a very important aspect of a successful microspore culture system. Because anther color is related to microspore development stage.(Table 1). These results showed that selected bud for isolated culture should be selected anther color as yellowish green, which contain the microspores were most suitable for induction of embryogenetics.

Table 1 The relation between anther color and microspore embryogenesis from F_1 hybrid No.30 of *B.napus*×*B.juncea*

Anther color	Embryo yield(embryos/100buds)		
	Exp.	Exp.	Exp.
Yellow	0	0	0
Somewhat greenish yellow	0	0	0
Greenish yellow	80	50	52
Yellowish green	820	855	890
Somewhat yellowish green	720	702	730
Green	30	20	15

Effect of transfer embryo mode The experimental designs are used two transfer embryo mode. One is when embryos grow 21days old in the NLN media which are transferred to B_5 semisolid media (4g/L of agar). After 3 days, the embryos are transferred to B_5 solid media. Other mode is that embryos cultural 27 days old are transferred directly to B_5 solid media. Then, the plates with embryos are all placed in a 4 incubator for 10 days with 8 hr photoperiod. The results showed as table 4. Embryos are transferred from liquid media to B_5 semisolid media for 3 d, then transfer to B_5 solid media, which can promote regeneration and vegetation growth as leaves became deep green color as well as thickening. There are also plates with 17 embryos and all embryos develop into plantlets. The chromosome number of regeneration plants verity range from about 19 37. The chromosome number is 2n=37 are from one of regeneration plants. As above result showed that it is better transfer embryo mode then other one.

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 15.14%. It is the best of stage embryo transfer. About effect of embryo size, we observed that small embryos from 0.2cm to 0.3cm in length are better than large embryos from 0.6cm to 0.8cm in length. The regeneration rate (%) from small embryos are the highest in all experiment embryos. It is 36.3% regeneration rate.

DISCUSSION

Genotypes of donor plant have been found to play very important roles in embryos yield in a number of plant species. Recently, the loci on the genotype involved in regeneration ability have been identified using molecular markers in several plants. Results in this paper also confirm the importance of genotype.

Anther colour is related to microspore development stage, therefore anther color can become a very important criterion for selection suitable buds to induction of embryogenetics. This is an advantage. Because it is a qualitative character which is not easily influenced by both environment and genotype we can propose a view that anther color as criterion for selection suitable bud size for induction of embryogenetics, and further proposed, when anther color as yellowish green, which contain the microspores were most suitable for induction of embryogenetics. This viewpoint was not reported before.

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