Studied on artificial resynthesis of *B . napus* from *B. olerecea* var. *aceaphala* and *B. camptris* through embryo rescue

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Abstract

At present, it is one of valid methods to widen rapeseed genetic resources and resolve the problem of lack of breeding materials via artificially resynthesized *Brassica napus*. In these experiments, *B. napus* were artificially resynthesized through culture of embryos which derive from interspecies hybrid of yellow-seed *B. oleracea var aceaphala(2n=C^oC^o=18) × yellow-seed B. campetris* (*2n=A^RA^R*=20). These embryos were cultivated in 9 different culture mediums. Results showed some interspecies embryos were obtained from these crosses (05K002×05K128, 05K002×05K121 and 05K25×05K128), however, there were big different suitable mediums during embryos culture; meanwhile, most embryos became death in other crosses (05K018×05K12 and 05K26×05K128) about 20 days after pollination. six newly newly formed hybrids (2n=A^RC^O=19)with cytoplasmic of *B. oleracea var aceaphala* were obtained via culture of empryo, and all clones were true hybrids with 19 chromosomes via cytology test. The hybrid plants were similar to female parent in morphology in seedling period and they grow quickly. These hybrids could benefit from further improving *B. napus* because of their some good agricultural traits. There were some fertile plants among four clones, and the other plants and clones were sterile. All the seeds of true hybrids were black

.**Key words:** Interspecies hybrid, Artificial Resynthesis, *B .napus*, Embryo culture

Introduction

Genus *Brassica* is widely cultivated in most parts of the world. It includes various important agronomical crops such as oilseed rape, cabbage, Chinese cabbage and so on. The *Brassica* genus includes several diploid and tetraploid cultivated species organized around three genomes named A, B, and C (U N, 1935). *B. napus* (*2n*=AACC=38) is an amphidiploid of *Brassica* from *B. olereaca* (*2n*=CC=18) and *B. rapa* (*2n*=AA=20), and widely cultivated to satisfy needs with oil-crop. In China, there were rare genetic source of *B. napus* for breeding and genetic studying because *B. napus* were imported from Europe and Japan before long. As such genetic and morphological variation of *B. napus* had occurred to a natural hybridization of species, it is valid method to widen rapeseed genetic resources and solve the problem of lack of breeding materials via resynthesized artificially *B. napus*. *B. rapa* and *B. oleracea* were different species in *Brassica* which lead to cross-incompatibility for them. How to improve the efficiency of resynthesized *B. napus* is important research goal. Some ways including embryo rescue and ovary culture can overcome cross-incompatibility (Inonata,N et al., 1976; Li Gen Yi et al., 1988; Liang Hong et al., 1990; Wen Yan Cheng et al., 1999; Yin Jia Ming et al., 2004; Zhang Guo Qing et al., 2003; Zhou Qing Yuan et al., 2003; Zhang Xiao Wei et al., 2001; Zhu Peng You et al., 2004).

B.oleracea var *aceaphala* (2n=2x=C^oC^o=18) is variation of *B. olecacea,* used for leafy ornamental and leafy vegetables. Some yellow-seed self-progenys with excellent agricultural features were obtained via domestication in Chongqing Rapseed Engineering and Technology Research Centre. Interspecies hybrids were obtained through *B. rapa*×*B. oleracea var aceaphala* (Ying Jia Ming et al., 2004; Zhou Qing Yuan et al., 2003)and *B.juncea*×*B. oleracea* var *aceaphala* (Zhou Qing Yuan et al., 2005), and some excellent agricultural traits had been transfer to *B. napus*. What`s more, all hybrids had no cytoplasm gene from *B. oleracea* var. *aceaphala*.

In this paper, interspecies hybrid embryo from *B. oleracea* var *aceaphala* as female were cultivated in nine different medium to study the influence of different cross for embryos rescue. Meanwhile, the genetic resource of *B. napus* for breeding woud be widen as more resynthesis *B. napus* were gained.

Material and method

Material and F1 Hybrid Production

Four yellow-seed *B. oleracea* var. *aceaphala* lines (05K002, 05K018, 05K26, 05K25) (2n=C^oC^o=18) and two yellow-seed *B. rapa* lines (05K121, 05K128) (2n=A^RA^R=20) were provided by Chongqing Rapseed Engineering and Biotechnology Reseach Centre. Seeds of the materials were planted in October ,2004, and were transplanted in experiment fields in Nov, 2004. Flower buds of four healthy plants of *B. oleracea* var *aceaphala* were emasculated and pistils were dusted repeatedly with the pollen of two *B. rapa* plants. After pollinated about 20 days, the pods were taken back and the embryos were cultured in the medium with different concentration of 6-BA and NAA (Table 1). Seedlings were multiplied in medium (MS+6-BA 3.0 mg/L +NAA 0.1 mg/L) after some embryos grown up. Six clones (F1, 06KH109, Kh110, KH111, KH112, KH113) and their parents were planted in experiment field of South West University in 2005.

Table 1 Composition of culture medium for interspecific hybrid

medium	Embryo culture medium									Substitution culture medium	Rooting medium
	A	B	C	D	E	F	G	H	I		
6-BA(mg/L)	3.0	3.0	3.0	2.0	2.0	2.0	1.0	1.0	1.0	3.0	0
NAA(mg/L)	0.2	0.1	0.05	0.2	0.1	0.05	0.2	0.1	0.05	0.2	0

Note: Basal culture medium is MS.

Character Investigation

The characters of six clones (F1) and six self-progeny of parents were investigated and compared during per growing phase. Agricultural character of clones (F1) and their parents were studied during the harvest period in May, 2006

Cytology Identify

After root tips and young stem tips were picked from clones (F1), which were cultured about 15-20 days in rooting medium , thrown into 2.0×10^{-3}mol/L 8-Hydroxy Quinoline to pretreat for 3-5h at 20°C, following fixed in Farmer's solution (3:1, absolute alcohol : glacial acetic acid, v/v) for about 24 h. Fixed material were washed completely with distilled water and treated in 1mol/LHCl at 60°C for about 15 minutes. Chromosome sections were committed in Carbol fuchsia mordant and chromosome number was counted by microscope.

Results

The effect by different genitics for embryo rescue

In this experiment, ovary were taken back to rescue embryo. The rate of obtaining ovary was investigated and the quotiety of obtaining embryo were calculated (table 2). The result indicated that there was big difference for different combinations. Some crosses (05K002×05K128、05K002×05K121 and 05K25×05K128) had more embryos than others (05K018×05K128and 05K26×05K128) ,in which most embryos come to die. Embryos of three combinations were inoculated into nine mediums quickly, and the rate of obtaining seedling (rebirth seedling/culture embrryo×100%) was accounted after 45 days. The rate of regenerative seedling among the different crosses were different (table 3), therefore, it was efficient to improve embryo culture frequency through selecting suitable cross.

Table2 The effect by different cross combinations of efficiency for obtaining little embryo

Cross combination	No. of florets pollinated	No. of obtaining ovary	No. of obtaining embryo	Rate of ovary	Quotiety 1 of obtaining embryo	Quotiety 2 of obtaining embryo
05K002×05K128	215	110	323	51.16	1.503	2.9364
05K002×05K121	92	64	220	69.57	2.391	3.438
05K018×05K128	58	22	21	37.93	0.362	0.955
05K26×05K128	109	15	9	13.76	0.083	0.600
05K25×05K128	122	63	231	51.64	1.893	3.667

Note: Rate of ovary(%)= No. of obtaining ovary/ No. of florets pollinated×100%;
Quotiety 1 of obtaining embryo= No. of obtaining embryo/ No. of florets pollinated×100%;
Quotiety 2 of obtaining embryo= No. of obtaining embryo/ No. of obtaining ovary

Table3 The effect by different cross combinations of efficiency for cultivating embryo

Cross combination	No. of cultivating embryo	No. of seedling	Rate of obtaining seedling	No. of transplanting clone(serial number)
05K002×05K128	323	4	1.24	4(KH109,KH110,KH111,KH112)
05K002×05K121	220	1	0.45	0
05K25×05K128	231	1	0.43	1(KH114)

Table 4 The effect of efficiency of cultivating embryo with different hormone level in medium

Cross combination		Medium								
		A	B	C	D	E	F	G	H	I
05K002×05K128	No. of cultivating embryo	42	40	37	37	42	40	40	25	20
	No. of seedling	0	0	2	0	0	2	0	0	0
	Rate of obtaining seedling(%)	0	0	5.41	0	0	5.0	0	0	0
05K002×05K121	No. of cultivating embryo	32	29	30	31	20	20	20	19	19
	No. of seedling	0	0	0	0	0	0	0	1	0
	Rate of obtaining seedling(%)	0	0	0	0	0	0	0	5.26	0
05K25×05K128	No. of cultivating embryo	20	20	20	25	27	20	25	25	49
	No. of seedling	0	0	0	0	0	0	0		1
	Rate of obtaining seedling(%)	0	0	0	0	0	0	0		2.04

The Effect By Different Medium For Embryo Rescue:

Embryos of three crosses were inoculated in 9 medium and the rate of obtaining seedling was investigated after 45 days (Table 4). The results showed that suitable medium were obvious different among these crosses. The rate of differentiation is so low that only six clones were obtained though 778 embryos were cultured. Few seedling was obtained in A, B, D, E and G medium while a few plants were obtained in others medium. So, in order to increase the frequency of embryos rescue from interspecies hybridization, suitable medium should be selected for each cross.

Features of hybrid

Through cytological inspection, six clones (F1) from *B. oleracea* var aceaphala and *B. rapa* were true interspecies hybrids with 19 chromosomes. 20-50 seedling of these clones (F1; KH109, Kh110, KH111, KH112, KH113) and their parents (05K002, 05K018, 05K128) were planted in Nov., 2005. The abloom characteristic and leaf modality were observed in March, 2006(Fig 1) and agricultural features were investigated before harvest in Apr., 2006 (table 5). The result showed that most of characters of hybrids were similar to B.napus. The leaf modality and the leaf color of hybrids were intervenient with parent comparison. These hybrids plants grow quickly with big petals, deeper petal color and longer inflorescence than their parent, and number of available divarication of hybrids were similar to *B. napus*. Seeds of these hybrids (F1) were black. Among F1, some plants from four crosses(06KH109, 06KH111, 06KH112, 06KH114) had turned into amphidiploids ($2n=A^RA^RC^OC^O=38$) by chromosome naturally double, which had bigger petal, plumper anther, more pollen and elongate silk than diploid plants (Fig1, B-2), and they could produce self-progeny via assistant pollination while others plants were self-incompatibility.

Table 5 Some agriculture trait of synthesized *B. napus* and their parents

No.	**Cross combination**	Seed color	Height (cm)	Position of branch(cm)	Length of main anthotaxy (cm)	No.of branches	Total length of availability branch (cm)	Average length of availability branch (cm)
05K002	*B. olerecea.Var aceaphala*	yellow	111.5	23.9	42.1	16.6	698.7	42.10
05K025	*B.olerecea.Var aceaphala*	yellow	127.3	31.7	45	12.7	734.5	57.83
05K121	*B.rapa*	yellow	119.1	20.61	38.5	7.4	578.0	78.11
05K128	*B. rapa*	yellow	112.1	16.4	32.6	9.7	624.0	64.3
06KH109	05K002×05K128	black	190.2	40.8	95.2	7.8	740.4	94.60
06KH110	05K002×05K128	black	236.6	22.0	145.0	10.6	1286.8	120.88
06KH111	05K002×05K128	black	220.2	31.8	126.1	9.1	1026.4	110.5535
06KH112	05K002×05K128	black	203.8	41.6	107.2	7.6	766.0	100.23
06KH113	05K0002×05K121	black	219.4	37.6	127.8	8.0	937.6	117.26
06KH114	05K025×05K128	black	170.0	47.0	81.0	8. 7	778.3	90.72

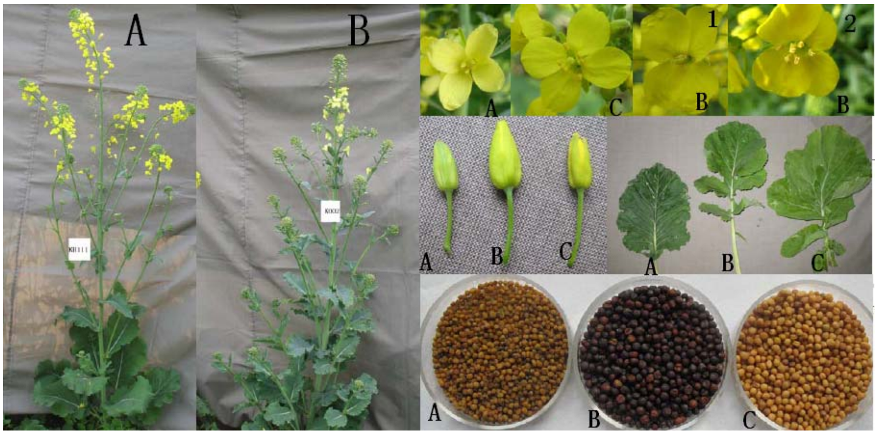

Fig. 1 Morphology of hybrids and their parents

A, *B. oleracea var.aceaphala* (05K002); B, hybrid (06KH111); C , *B. rapa*(05K128)

Discussion

Interspecies hybridization with *B. olerecea* and *B. rapa* is an available way to create new *B. napus*. There is highly cross-incompatibility between *B .olerecea* and *B. rapa*, so some scholars has done plenty of experiments to find a suitable way to resolve the problem, hereby, embryo resuce and ovary culture has been proved to be efficacious ways. No seed has been obtained via ovary culture from *B. olerecea*.var *acaphacea* and *B. rapa* while *B. olerecea* var *acaphacea* was female (Zhou Qing Yuan, et al., 2003). In this article, embryo culture was suitable ways for interspecies hybridization between *B. olerecea* var *acephalea* and *B. rapa*, the result is similar to what another report had done(Li Gen Yi et al., 1988).

B.olerecea.var *acaphacea* is ideal relative for improving present *B. napus*. Since 2000, some hybrids from *B. rapa* (female) and *B. olerecea var acaphacea* (male)hand been obtained ,and their plants and the beginning of available divarication were higher (Zhou Qing Yuan, et al.,2006) though *B. olerecea.*var *acaphacea* is shorter and beginning of availability divarication are lower, and the number of availability divarication is more than *B. napus*. In this article, six interspecies hybridization clones (F1) were created with *B. olerecea.*var *acaphacea's* cytoplasm, and their main anthotaxy and availability divarition were long and positon of availability divarication was low. So, these hybrids benefit from further improving *B. napus*.

In this paper, chromosome was naturally doubled in some crosses with the rates doubling is 3.33%, 8.33%, 2.86, 3.33%, respectively. Polyploidy has played a major role in the evolution of *B. napus*. Chromosomes of whole plant were completely doubled, it was showed that natural doubling of chromosomes in *B. napus* could happen during seed and embryo germination phase or clones manifold process.

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