Create and broaden the germplasm pool of *B. napus* through interspecific hybridization between *B. carinata* and *B. rapa*

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Abstract

Interspecific hybridization is an important way to transfer desirable traits from one species to another, and has created new breeding resources in *Brassica* crops. *B. carinata* (BBCC, 2n=34) possesses lots of desirable agronomic characters which are rare in the other *Brassica* oilcrops. To make such good characters flow in the genus *Brassica*s, a great deal of crosses between *B. carinata* and *B. rapa* (AA, 2n=20) have been made. In total, 326 interspecific combinations were obtained involving 109 accessions of *B. carinata* and 29 accessions of *B.rapa*. In result, about five thousand true hybrid plants (ABC) were obtained after identified morphologically as well as molecularly. To restore the fertility of the triploid interspecific hybrids, the putative hybrids were treated with 0.1% colchicines and result in 276 combinations of hexaploid plants (AABBCC). These hexaploid plants can be used as a gene pool to cross the *B. napus* and broaden the geraplasm of *B. napus*. In the meanwhile, Great differences on the interspecific crossability were found among interspecific combinations and female parents. Sixteen accessions of *B. carinata* with high crossability were originally collected from the same place, southeast of the Great Rift Valley in Ethiopia. The significance of interspecific crossability is discussed.

Key words *B. napus B. carinata B. rapa* interspecific hybridization germplasm pool

Introduction

Brassica napus (AACC 2n=38) is now become the major rapeseed variety in China for its great potential high yield (Liu, 2000). But *B. napus* originally introduced from Europe with short planting history. Ethiopian mustard (*Brassica carinata*, BBCC, 2n=34) possesses lots of desirable agronomic characters which are rare in the other *Brassica* oilcrops: better heat and drought tolerance, less shattering, wide range of diseases and pests resistance, and yellow-seeded germplasm etc.(Cohen and Knowles 1984; Gugel et al. 1990; Bayeh and Medhin 1992; Yitbarek 1992). Meanwhile, China is one of the origins of *B. rapa* (AA 2n=20) and genetic basis of which is broad. *B. rapa* also owes some good traits such as: can grow well in barren soil, short growing period, drought resistance and cold resistance etc. Compared to *B. carinata* and *B. rapa,* genetic polymorphism of *B. napus* is more narrow. Interspecific hybridization is an important way to transfer desirable traits from one species to another, and has created new breeding resources in *Brassica* crops (Allard 1960; Raney et al. 1995; Inomata 1997; Prakash and Chopra 1998; Ripley and Beversdorf 2003). Therefore, to make good genes from *B. carinata* and *B. rapa* flow to *B. napus* through interspecific hybridization is a important way to create new germplasm for *B. napus.* (Rashid *et al.*1994; Saghai *et al*. 1996).

The crossability between *B. carinata* and other three basic species of *Brassica* and *B. napus* was very low (Stebbins 1958). Although several *B. carinata*-involved interspecific crosses were done and some desirable traits were transferred from *B. carinata* to other *Brassica* species (Meng et al. 1998; Choudhary *et al.* 2000; Rahman 2001; Tonguc and Griffiths 2004), exploring *B. carinata* genotypes with high interspecific crossability would promote such gene flow within the genus *Brassica.*

The objectives of this study were to create new bridge germplasm for introducing genic resources from *B. carinata* and *B. rapa* to *B. napus* and screen genotypes of *B. carinata* with high crossability to *B. rapa*.

Materials and methods

Plant materials**:** 107 accessions of *B. carinata* were provided by the Centre for Genetic Resources, the Netherlands (CGN, http://www.cgn.wageningen-ur.nl/pgr/), 3 accessions (88-216845, 80-139, 88-219790) of *B. carinata* originated from Germany (Table1). A total of 29 *B. rapa* accessions were collected from different regions of the world and have grown for several generations in Wuhan (Table 1).

Field trial: All accessions were sown in the field station of Huazhong Agricultural University (Wuhan) in autumn 2003. Each accession was planted in three lines and each line consisted of 10 plants. To ensure pollination by *B. rapa* throughout the flowering period of *B. carinata*, the seeds of *B. rapa were* sown 43 days and 55 days later than *B. carinata* respectively. When plants flowered in spring 2004, buds from 3 or 4 plants of each *B. carinata* accession were emasculated and randomly pollinated with pollen from *B. rapa*. To restore the fertility of the triploid interspecific hybrids, the putative hybrids were treated with 0.1% colchicine.

Table 1 the cultivars of *B. carinata* and *B. rapa* used in trail

B. carinata						*B. rapa*	
Accession No. of CGN	Interspecific Crossability index	Accession No. of CGN	Interspecific Crossability index	Accession No. of CGN	Interspecific Crossability index	Name	Origin
CGN03930	44	CGN03968	17	CGN04006	0	3907	
CGN03931	6	CGN03969	0	CGN04007	10	Maverick	Sweden
CGN03932	6	CGN03970	19	CGN04008	3	Baiguotianyoucai	Hubei
CGN03933	4	CGN03971	8	CGN04009	15	Baijian13hao	Jiangsu
CGN03934	11	CGN03972	1	CGN04010	1	Denglongzhong	zhejiang
CGN03935	28	CGN03973	5	CGN04011	6	Dongkoutianyoucai	
CGN03936	18	CGN03974	5	CGN04012	8	Fenyangyoucai	Shanxi
CGN03937	12	CGN03975	26	CGN04013	12	Qixingjian	Sichuan
CGN03938	9	CGN03976	1	CGN04014	1	Shangdangyoucai	Shanxi
CGN03939	8	CGN03977	29	CGN04015	12	Shiqianyoucaibai	Guizhou
CGN03940	19	CGN03978	19	CGN04016	3	Tianmendayeyoucai	Hubei
CGN03941	40	CGN03979	2	CGN04017	1	Tianmenyoucaibai	Hubei
CGN03942	4	CGN03980	0	CGN04018	3	Wulitianyoucai	
CGN03943	19	CGN03981	0	CGN04019	3	Xinghuayoucai	Jiangsu
CGN03944	9	CGN03982	1	CGN04020	18	Yangyou2hao	Zhejiang
CGN03945	8	CGN03983	2	CGN04021	5	Zhejiang1hao	Zhejiang
CGN03947	3	CGN03984	15	CGN04022	4	Xishuiyoucaibai	Hubei
CGN03948	1	CGN03985	25	CGN04023	4	Guidingtianyoucai	
CGN03949	28	CGN03986	5	CGN04024	39	Shanxiyoucai	Shaixi
CGN03950	8	CGN03988	1	CGN04025	5	03caudle	Gansu
CGN03951	1	CGN03989	42	CGN04026	2	2001b2	Gansu
CGN03952	0	CGN03990	3	CGN04027	4	2002b30	Gansu
CGN03953	11	CGN03991	5	CGN04028	10	2002b1	Gansu
CGN03954	5	CGN03993	23	CGN04029	6	2002b8	Gansu
CGN03955	8	CGN03994	15	CGN04030	16	2002b11	Gansu
CGN03956	11	CGN03995	80	CGN04031	20	2002b20	Gansu
CGN03957	13	CGN03996	42	CGN04032	7	97q-3	Gansu
CGN03958	18	CGN03997	2	CGN04033	12	Longyou3hao	Gansu
CGN03959	4	CGN03998	43	CGN04034	11	Longyou4hao	Gansu
CGN03960	10	CGN03999	59	CGN04035	0		
CGN03961	12	CGN04000	32	CGN04036	19		
CGN03962	10	CGN04001	14	CGN04037	16		
CGN03963	11	CGN04002	18	CGN06985			
CGN03964	43	CGN04003	3	88-216845	12		
CGN03965	29	CGN04004	47	80-139	1		
CGN03966	2	CGN04005	44	88-219790	2		
CGN03967	1						

Results

A number 109 *B. carinata* accessions were randomly crossed as females with 29 accessions of *B. rapa* resulting in 409 interspecific combinations. Eventually, 13,000 seeds were obtained from 40,000 cross-pollinated buds involving 375 combinations. Since *B. carinata* and *B. rapa* can be easily distinguished from each other by morphology, it is not difficult to identify true hybrid plants by their intermediate characters. Besides the morphologically differences, most of interspecific hybrids showed hybrid vigor at the period of vegetative growth. Some of the hybrids were verified by molecular marker analysis and chromosome counting for they resembled their maternal parents (data not show). Eventually, more than 5000 hybrid plants were produced from 326 interspecific combinations. However, only 23.3% of the hybrid plants had their chromosome doubled due to the low efficiency of our colchicine treatment leading to 276 combinations of hexaploid plants obtained. The fertility of pollen from those hexaploids is partly restored and fecundity is near normal (fig.1).There are 4 to 13 seeds in every single silique according to different siliques. So, lots of AABBCC hexaploids are obtained.

In the meanwhile, Great differences on the interspecific crossability were found among different accessions of *B. carinata*. Some accessions of *B. carinata* could yield more than 80 hybrid seeds per 100 interspecific pollinated buds, and some produced zero (Table 1). We defined the number of hybrid plants obtained from 100 cross-pollinated buds as the interspecific crossability index (ICI)**.** ICI higher than 20 was considered as accessions with high crossability and resulted in 20 accessions. Interestingly, sixteen of the 20 high ICI accessions originate from southeast of the Great Rift Valley, Ethiopia. Furthermore, thirteen of the 16 Great Rift Valley accessions were geographically distributed at a narrow region. If these variations on the interspecific crossability in *B. carinata* were determined by genetic, further work will do in following.

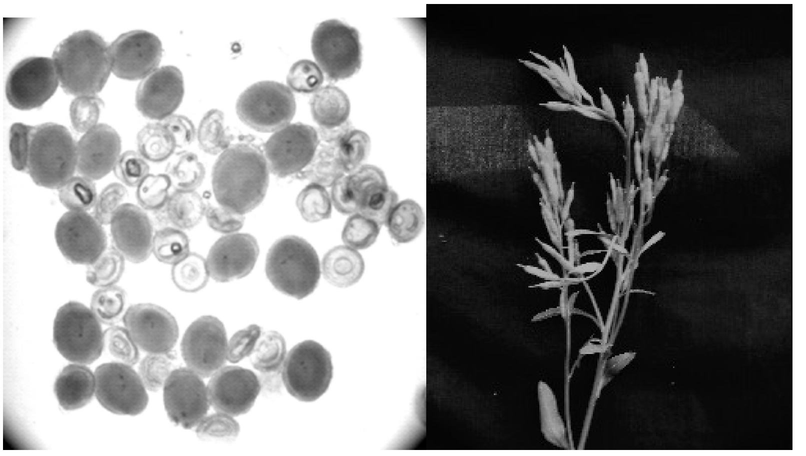

Fig.1 The pollens come from one hexaploid, of which big and solid were fertile and which little and transparent were sterile (left); the fecundity of one hexaploid plant (right).

Discussion

In recent decades, many efforts have been done to increase the genetic basis of *B. napus* which is narrow as compared to *B. rapa* and *B. carinata*. Previous studies on cytogenetics and molecular genetics revealed that there are significant differences in *Brassica*ceae between the A, B, and C genomes from three dipoid species and three amphidipoid species respectively (McGrath and Quiros, 1990). Our previous research indicated that the hexaploid hybrid (AABBCC) derived from interspecific crossing between *B. carinata* and *B. rapa* (AA) could be used as bridge hybrid by crossing it to *B. napus* (AACC), and then selfing the pentaploid (AABCC) hybrid to form a new-typed *B. napus* with half of the A genome coming from *B. rapa* and half of the C genome coming from *B. carinata*. Our results show that this kind of new-typed *B. napus* is very different from the original one and show strong heterosis which we named as sub-genomic heterosis (Li *et al*. 2004; 2006). Breeding bridge materials of hexaploids (AABBCC) combined the good characters of *B. rapa* and *B. carinata* together, are essential to create such new-typed *B. napus*. In our trail, great genetic diversity were detected between different accessions of *B. carinata* from morphologic and molecular markers (data not show) and these variations have brought to ABC hybrids through crossing, so, the hexaploids also rich in genetic basis. In actually, these materials have broadened the available genetic basis of *B. napus* and can improve the *B. napus* substantially. In the same way, which hexaploids can also improve *B. juncea*.

Interspecific reproductive isolation is the main mechanism for speciation and specific maintenance (Griffiths *et al*. 2002). However, genetic leaks on the isolation system have occasionally enabled interspecific gene flow and promoted evolution of species. Previous studies indicated that the interspecific crossability was mainly controlled by the maternal genotype in *Brassicas* (Meng *et al*. 1992; Meng and Lu 1993; Liu and Meng 1995). The crossability between *B. carinata* and *B. rapa* is very low especially when the latter is used as female parent (Choudhary *et al*. 2000). In the present experiment, diversity with respect to interspecific crossability was obvious among 109 *B. carinata* accessions and some accessions with high crossability to *B. rapa* were selected. These lines could be used as bridge material for transferring desirable characters to other *Brassica* species and vice versa. In contrast to the most interspecific combinations where cross-pollinated siliques developed only 15-20d after pollination, the compatible interspecific combinations usually produced fully developed seeds within each cross-pollinated silique. This implied that accessions of *B. carinata* had an ability to allow foreign pollen to germinate on their stigmas and pollen tubes to penetrate their styles, but only the cultivar with high crossability had a mechanism to enable the hybrid embryos to fully develop. The genetic basis for the interspecific crossability would be further investigated.

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