Development, cytological and molecular analysis and breeding potential of amphidiploids between *Raphanus Sativus* and *Brassica Alboglabra*

CHEN Honggao, WU Jiangsheng, LIU Chao, WANG Fang, CHENG Xiaomao, LI Yuan

National Key Laboratory of Crop Genetic Improvement, National Engineering Research Center of Rapeseed, Huazhong Agricultural University, Wuhan 430070, China Email: honggao_chen@webmail.hzau.edu.cn

Abstract

A fertile amphidiploid called Luobojielan (Raphanobrassica, RRCC, 2n=36) between *Raphanus sativus* cv. HQ-04 (2n=18, RR) and *Brassica alboglabra* (2n=18, CC) was synthesized and repeated selections for seed fertility were made from F_4 to F_{10}. F_{10} plants exhibited good fertility with 14.9 seeds per silique as well as 32.3 g per plant. GISH analysis indicated that 18 *R. sativus* chromosomes and 18 *B. alboglabra* chromosomes were entirely kept. Cytological observation revealed the regular meiotic behaviors and amplified fragment length polymorphism (AFLP) analysis showed the identical identity of F_{10} plants, which indicated the good genetic stability of F_{10} Luobojielan. The crossability of the Luobojielan with *R. sativus* and 5 *Brassica* species (13 cultivars) were investigated. Seeds were easy to be produced from crosses *B. napus*, *B. juncea* and *B. carinata* × Luobojielan as well as Luobojielan × *R. sativus*. Fewer seeds were also obtained from crosses Luobojielan × *B. napus*, *B. juncea* and *B. carinata*. Luobojielan might provide potential bridge material for gene introgression from *R. sativus* to *Brassica* cultivars.

Introduction

Intergeneric amphidiploids between *B. oleracea* and *R. sativus*, *Raphanobrassica* (2n=36, RRCC), have been synthesized with different breeding purpose for new vegetable, fodder or colored ornamental crops (Olesson and Ellerström 1980; Namai et al., 1980; Lange et al. 1989), for a genetic bridge of gene introgressions from *R. sativus* into *Brassica* cultivars, such as cytoplasmic male sterility (Paulman and Robbelen 1988) , club-root resistance (McNaughton 1973) and beet cyst nematode resistance (Hagimori et al. 1992; Lelivelt et al. 1993). However, most of these were not suitable for more extensive works because of the lower seed fertility (Olesson and Ellerström 1980; Namai et al. 1980; Matsuzawa et al. 2000). It is assumed that the seed fertility improvement may be achieved by mass selection for seed set (Tokumasu and Kato 1988; Lange et al. 1989).

In this study, one new amphidiploid *Raphanobrassica* between *R. sastivus* and *B. alboglabra* Bailey with high fertility was developed after successive selections, and its cytological and molecular characterization and crossability with *Brassica* species investigated.

Materials and methods

Plant: *R. sativus* cv. 'HQ-04' (a vegetable radish landrace in Wuhan) and *B. alboglabra* which were selfed for generations were used in the synthesis of amphidiploid *Raphanobrassica* 'Luobojielan'. The other materials (Table 1) were used in reciprocal crosses with *Raphanobrassica*.

Synthesis and fertility improvement of Raphanobrassica: The cross was made between *R. sativus* cv. HQ-04 and *B. alboglabra* with HQ-04 as the female parent in 1995 and F_1 plants were treated by 0.3% aqueous colchicines to double chromosome number. After two generations of open-pollinations, partially fertile putative amphidiploid plants with 2n = 36 and intermediate morphological characters were selected among 41 F_4 plants. Seeds harvested from these plants after open-pollination were planted and 385 F_5 plants were generated, which were permitted to pollinate each other within mosquito nylon net. Six F_5 plants with higher fertility were selected and their seeds were mixed to give 400 F_6 plants for the next cycle of fertility selection. By means of the similar method, selections for seed fertility were performed until F_{10}.

AFLP analysis: AFLP fingerprints were generated based on the protocol of Vos et al. (1995).

Cytological and GISH analysis: Ovaries and anthers separated from each plant were used for cytological study according to the procedure of Li et al. (1995). Slides were prepared for genomic *in situ* hybridization (GISH) according to the protocol of Snowdon et al. (1997).

Observation of crossability: Reciprocal crosses of *Raphanobrassica* with *R. sativus* and *Brassicas* species were made in field by hand in April of 2003 and 2004. The crossability was estimated based on the number of seeds obtained from 100 pollinated flowers.

Results

Fertility and Morphological character

Most of the morphological attributes of F_{10} plants were intermediate between those of their parent, by exhibiting vigorous vegetative growth and having large leaves and 10 to 13 primary branches. Flowers were white. The siliques of F_{10} were intermediate between the parent in shape and contained averagely 14.9 seeds (13 to 16), the body part consisted of a dehiscent bivalved part with a septum, containing about 10 to 12 seeds, the beak of silique was long and indehiscent, also containing 5 to 6 seeds. Seeds were more like *B. alboglabra* in shape and intermediate in size. About 80% visible ovules were full and the 1000-seeds weight reached 5.6 g. The seed yield per plant reached 32.3 g.

Cytological and AFLP character

In F_{10} plants, a total of 1784 PMCs from 12 randomly selected plants were observed and majority (94%) showed regular chromosome pairing (18 bivalent) at diakinesis and the 18:18 segregation at anaphaseI.The frequency of multivalent was lower than 9.6%. Furthermore, GISH analysis indicated that 18 *R. sativus* chromosomes and 18 *B. alboglabra* chromosomes were entirely kept in the amphidiploids and all chromosomes only paired with their homologous chromosomes at diakinesis (Fig. 1A). These suggested that whole parent chromosome set were included and a diploid-like meiosis has been established in F10 plants (Fig. 1B).

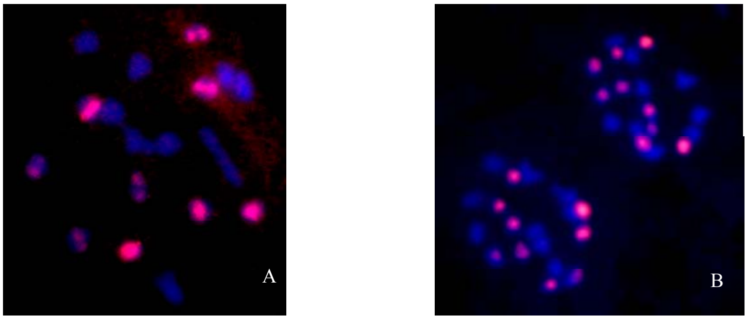

Fig. 1. Cytological character of F_{10} amphidiploid *Rapahnobrassica* 'Luobojielan'. A One PMC at diakinesis with 18 bivalent and 9 bivalent were labeled by *R. sativus* DNA probe (red); B One PMC at anaphase I with 18:18 segregation and with 9 *R. sativus* chromosomes (red) in each polar.

Twenty nine AFLP primer combinations were used and a total of 1346 fragments were generated among 30 F_{10} plants, ranging from 1261 to 1303, of which 155 were polymorphic bands. The number of polymorphic bands was only 4 to 17 between any two individuals, which indicated the identical genetic components of these plants.

Crossability of Raphanobrassica 'Luobojielan' with R. sativus and Brassica species

As showing in Table 1, Seeds were easy to be produced from crosses *B. napus*, *B. juncea* and *B. carinata* × Luobojielan as well as Luobojielan × *R. sativus*. Fewer seeds were also obtained from crosses Luobojielan × *B. napus*, *B. juncea* and *B. carinata*. but seeds formation were very difficult in *R. sativus* ×*Raphanobrassica* and both directions of *Raphanobrassica* × *B. oleracea* and *B. campestris*.

Table 1 Crossability of *Raphanobrassica* with *R. sativus* and *Brassica* species.

Tester		*Raphanobrassica* ×			× Raphanobrassica		
Species	Cultivars	P[a]	S[b]	S/P[c]	P	S	S/P
	Longju	1170	432	36.9	1220	0	0
R. sativus	HQ-04	1066	626	58.7	1451	3	0.2
	Huangzhou	913	355	38.9	1532	2	0.1
	Qixingjian	714	13	1.8	749	1	0.1
B. rapa	Xishuiyoucai	1228	2	0.2	1114	6	0.7
	Wanyou1	969	6	0.6	647	0	0
	Bailey	986	12	1.2	1170	54	4.6
B. oleracea	Jingfeng1	1013	0	0	1136	0	0
	Chunqiudapielan	1001	0	0	959	0	0
	Huashuang3	843	84	10	960	273	28.4
B. napus	Lanbao	1178	142	12.1	1023	497	48.6
	DH	1324	100	7.6	974	255	26.2
	Xinyou1	813	20	2.5	844	325	38.5
B. juncea	Xinyou2	891	16	2.9	905	364	40.2
	Dahuangjie	812	7	0.9	812	212	26.1
B.carinata	GO-7	791	74	9.5	865	578	66.8

[a]P and [b]S for total number of flowers pollinated and seeds seedling obtained in 2003 and 2004, [c]S/P for number of seeds obtained per 100 pollinated flowers.

Discussion

Raphanobrassica (RRCC) amphidiploids were numerously synthesized previously, however, most of them were lower fertility which restricted the really use of their agronomical potential. Many methods were tried to restore the fertility of hybrids such as different cross combinations, successive selfing and repeated selection, but the results were quite different in different experiments (Olesson and Ellerström 1980). In this study, we made a highly fertile *Raphanobrassica* 'Luobojielan' by *R. sativus* cv. HQ-04 × *B. alboglabra* and numerous selfing and selection, implies that the use of *B. alboglabra* leads to improved fertility of the hybrids, this was resulted from parental genotype or other genetic factors needed further study.

R. sativus is a potential resource for *Brassica* breeding (Paulman and Robbelen 1988; McNaughton 1973; Hagimori et al. 1992; Lelivelt et al. 1993). Sexual hybridization for gene transfer between *B. napus* and *R. sativus* has been reported, but only very few hybrid plants have been obtained due to the poor crossability between the two species (Chopinet 1944; Takeshita et al. 1980; Lelivelt et al. 1993). The present *Raphanobrassica* exhibited high fertility and good crossability with *R. sativus*, *B. napus*, *B. juncea* and *B. carinata*. Homoeologous synapsis between R genome and A genome of *B. campestris*, C genome of *B. oleracea*, AC genome of *B. napus* as well as BC genome of *B. carinata* at meiosis have been reported (Namai 1976; Mizushima 1980; Harberd 1980) which hints the possibility of transferring valuable traits from R genome into *Brassica* genomes. In this sense, the present *Raphanobrassica* might provide potential bridge material for gene introgression from *R. sativus* to *Brassica* cultivars.

Conclusions

The new amphidiploid *Raohanobrassica* "Luobojielan" exhibited highly fertile, good genetic stability and good crossability with *Raphanus* and *Brassica* crops, which hint the potential as a crop in its owner right and a bridge material.

References

Chopinet R.(1944) Hybrids intergénériques *Raphano-Brassica.* Rev. Hortic. **116**: 98–100.

Hagimori M., Nagaoka M., Kato N. and Yoshikawa, H.(1992) Production and characterization of somatic hybrids between the Japanese radish and cauliflower. Theor. Appl. Genet. **84**: 819–824.

Harberd D.J. and McArthur E.D.(1980) Meiotic analysis of some species and genus hybrids in the Brassicaeas. In: Tsunoda S., Hinata K. and Gómez-Campo C. (eds), Brassica crops and wild allies. Japan Science Society Press, Tokyo, pp 65–87.

Lange W., Toxopeus H., Lubberts J.H., Dolstra O. and Harrewijn J.L.(1989) The development of *Raparadish* (×*Brassicoraphanus*, 2n=38), a new crop in agriculture. Euphytica. **40**: 1–14.

Lelivelt C.L.C., Lang W., Dolstra O.(1993) Intergeneric crosses for the transfer of resistance to the beet cyst nematode from *Raphanus sativus* to *Brassica napus*. Euphytica. **68**: 111–120.

Li Z., Liu H.L. and Luo P.(1995) Production and cytogenetics of intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus.* Theor. Appl. Genet. **91**: 131–136.

Matsuzawa Y., Funayama T., Kamibayashi M., Konnai M., Bang S.W. and Kaneko Y.(2000) Synthetic *Brassica rapa*-*Raphanus sativus* amphidiploid lines developed by reciprocal hybridization. Plant Breeding **119**: 357–359.

McNaughton I.H.(1973) Resistance of *Raphanobrassica* to clubroot disease. Nature 243: 547–548.

Mizushima V.(1980) Genome analysis in *Brassica* and allied genera. In: Tsunoda S., Hinata K. and Gómez-Campo C. (eds), *Brassica* crops and wild allies. Japan Scientific Societies Press, Tokyo, pp 89–105.

Namai H.(1976) Cytogenetic and breeding studies on transfer of economic characters by menas of interspecific and intergeneric crossing in the tribe *Brassiceae* of *Cruciferae*. Mem. Fac. Agric. Tokyo Univ. Edu. **22**: 101–107.

Namai H., Sarashima M. and Hosoda T.(1980) Interspecific and intergeneric hybridization breeding in Japan. In: Tsunoda S., Hinata K. and Gomez-Campo C. (eds), *Brassica* crops and wild allies. Japan Science Society Press, Tokyo, pp191–202.

Olesson G. and Ellerstrom S.(1980) Polyploidy breeding in Europe. In: Tsunoda S., Hinata K. and Gomez-Campo C. (eds), *Brassica* crops and wild allies. Japan Science Societies Press, Tokyo, pp 167–190.

Snowdon R.J., Köhler W., Friedt W. and Köhler A.(1997) Genome in situ hybridization in Brassica amphidiploids and interspecific hybrids. Theor. Appl. Genet. **95**: 1320–1324.

Takeshita M., Kato M. and Tokumasu S.(1980) Application of ovule culture to the production of intergeneric hybrids in *Brassica* and *Raphanus*. Jpn. J. Genet. **55**: 573–587.

Tokumasu S. and Kato M.(1988) Chromosomal and genic structure of *Brassicoraphanus* related to seed fertility and the presentation of an instance of improvement of its fertility. Euphytica **39**: 145–151.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J. and Kuiper M.(1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.