Studies on rapeseed germplasm enhancement by use of cruciferous weed *Rorippa indica*

GUAN Rongzhan*, JIANG Shuhui, XIN Ruying, ZHANG Hongsheng

National Key Lab of crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, P.R. China Email: guanrzh@njau.edu.cn

Abstract

Cruciferous weed *Rorippa indica* has trait of excellent tolerance to soil wateriness and has trait with very short beak or without silique beak. To transfer traits of *R. indica* to *B. napus*, we used protoplast fusion and intergeneric hybridization technique for rapeseed germplasm enhancement. Using PEG-DMSO protoplast fusion approach and culturing with B_5 liquid medium, somatic fusion hybrids between *B. napus* and *R. indica* were obtained. The somatic hybrids were morphologically intermediate types between *B. napus* and *R. indica*, could seed. Three-way intergeneric hybridizations, (*B. napus*×*Orychopragmus violaceus*)F_1×*R. indica*, were successfully carried out by appropriate embryo rescue approach. Plants morphologically similar to that of *R. indica*, *B. napus, O. violaceus, B. campestris, B. juncea,* were observed in F_2 generation of the three-way cross, showing crazy segregations. In the population of 890 F_2 individuals, 20.9% was with short-beak less than 5mm. Morphological and cytogenetic identification showed that plants obtained by both ways were new rapeseed germplasm derived from *R. indica*.

Key words: *Rorippa indica, Brassica napus*, protoplast fusion, intergeneric hybridization

Introduction

Researches on intergeneric or interspecific crosses and somatic hybridization have demonstrated that transferring traits from one species to another in *Cruciferae*, is a efficient way to enrich rapeseed germplasm. By making use of genetic resources from cruciferous wild type, special traits such as fatty acid content unusual, cytoplasmic male sterility, etc., may be created in *B. napus*, and rapeseed germplasm bank may be enriched [1-11]. So many researches in these aspects have been carried out, and more researches may be needed for rapeseed improvement.

Rorippa indica (Linn.) Hiern of *Brassiceae*, is a kind of wild species distributing very broadly. Its traits excellent and distinctly different from *B. napus,* can be implications for rapeseed improvement. Its siliques with very short beak or without beak in *R. indica,* may be a property used for increasing economical coefficient of rapeseed. *R. indica* reaches usually height of around 1.9m in case of planting in lowland next to water, and grows to height of around 0.6m in case of planting in upland, showing its excellent wateriness tolerance which is necessary for canola variety planting in lowland after rice harvest, typically in Yangtze River Valley of China. The trait of flowers often apetalous in *R. indica*, is also a good traits for development of rapeseed germplasm with better disease resistances or potentially increasing photosynthetic utilizing efficiency.

Present researches were designed to utilize advantages of *R. indica* for rapeseed germplasm enhancement. By biotech way including intergeneric hybridizations and somatic fusion, we obtained a set of new germplasm with diverse traits.

Materials and methods

Plant materials: All plant materials were obtained from germplasm bank at Nanjing Agricultural University (NAU), and were planted in Jiangpu experimental station of NAU. NJ5280 is a canola line developed by authors. Wild species *O. violaceus* and *R. indica* were collected from rural area around Nanjing City, P.R. China.

Protoplast fusion: High-yield protoplast achieved from hypocotyls of NJ5280 and leaves of *R. indica* were fused by PEG-DMSO method, and then cultured with MS liquid medium. Enzyme combinations for isolating protoplast from *B. napus* L. and *R. indica* were 1% cellulase + 0.2% macerozyme+3 mmol/L MES and 0.25% cellulase +0.5% macerozyme+5 mmol/L MES, respectively. Fusion percentage of 10.4% was obtained on the condition of 30% PEG + 0.3mol/L glucose +50 mmol/L $CaCl_2 \cdot 2H_2O$ + 15% DMSO. Somatic fusion methods reported in another paper by authors may not be listed here.

Intergeneric hybridization: A three-way cross ((*B. napus*×*O. violaceus*) F_1×*R. indica*) was conducted by hand-cross and by embryo rescue technique. NJ5280 as male parent was crossed with *O. violaceus* in large amounts, then inter-generic F_1's derived from rescued embryo were pollinated with pollen carefully collected from *R. indica*. Further rescued seedlings were transplanted into fields, and were amplified into population by selfing. Embryo rescue technique may be described as following simplified procedure. Premature green embryos got from the inter-generic crosses of 25 day-old, were taken out under asepsis conditions, transferred into culture media such as Ms medium added with 2mg/L 6-BA and 0.1mg/L NAA. For rooting, young seedling growing into height of 2-3cm were transplanted into MS medium added with 0.1mg/L NAA and 0.1mg/L IBA. Seedlings with 15-20 secondary roots were carefully transplanted into fertile soil. Keeping moisture is a key measure for survival of the seedlings. When seedlings grew larger, they were transplanted into field for yielding more seeds.

* Corresponding author: guanrzh@njau.edu.cn

Morphology and cytogenetics: Traits of all of intergeneric progenies were observed. For meiotic analysis, floral buds were fixed in Carnoy II solution (6:3:1 ethanol: chloroform: acetic acid) for 24 h and then transferred to 50% ethanol and stored at 4°C for at least 24 h or until use. Anthers containing PMCs at the metaphase-I were stained with acetocarmine and subsequently squashed.

Seeds harvested from the hybridizations and parental species were germinated at 25°C. Root-tips were excised from these plants. The root-tips were transferred to ice water and kept in a refrigerator for 24 h. The roots were subsequently fixed in Carnoy I solution (3:1) for 4 h at 4°C. The meristematic portion of the root-tips was squashed in 45% acetic acid after hydrolysis in 1mol HCl at 60°C for 8 min and stained.

Quality analysis: Quality traits were analyzed for content of oil, erucic acid and glucosinolates in seeds derived from progeny families.

Results

Morphology

By embryo rescue, we got 12 hybrids from crosses between *B. napus* and *O. violaceus*. The F_1 were morphologically more like rapeseed than *O. violaceus*, but they gave out less pollen than their parents. The F_1 plants were further crossed with *R. indica* through the rescue techniques. 15 seedlings of intergenric three-way cross F_1 were obtained from young embryo culture (Fig. 1). In the experimental field, 6 plants of the F_1 seedlings did not live through winter. At seedling stage, these three-way cross hybrids looked very similar to *R. indica*, but at maturing stage, the plants were more like cruciferous vegetable (*B. campestris*), which had more than 12 branches, long beaks, stems yellowish, intermediate harvesting time, earlier flowering time than rapeseed.

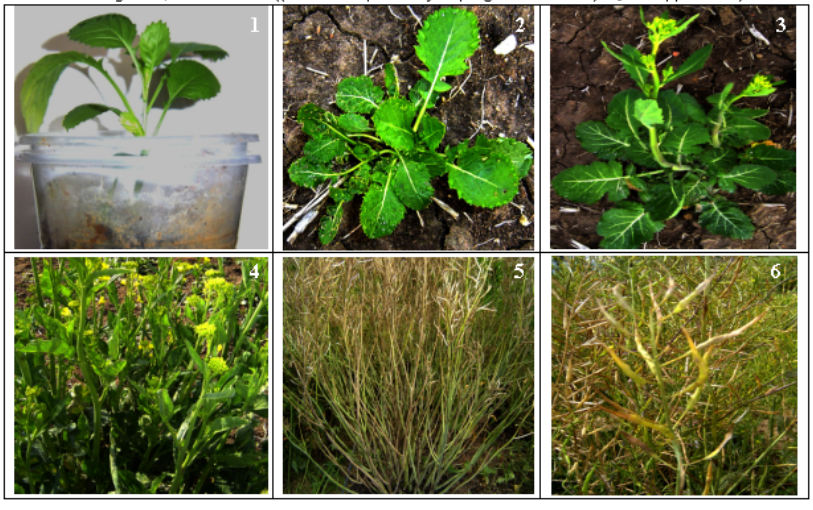


Fig.1 Experimental process of three-way cross ((*B. napus*×*O. violaceus*) F_1×*R. indica*) F_1 from seedling to maturing stage

The F_1 plants of the three-way crosses were selfed, selfing and open-pollinating seeds were harvested completely. Carefully planting the progenies, we got a F_2 population of 890 individual plants in which morphological varieations was observed (Fig. 2). Various morphological types such as appearances similar to *B. campestris, B. napus, B. juncea, O. violaceus*, etc, were all observed, but 32.2% plants were more similar to *R. indica* than other *Brassica* species by leaf type identification. Leaf color ranged from yellow, dark green to purple. Leafs and stems of 51.5% plants were covered with trichomes. Fertilities of F_2 individuals ranged from male sterile to complete fertile, but every plant could yield seeds of which weights were from less than 1g to more than 20g. Colors of seeds harvested, included yellow, brown, black and other middle colors. Their length of silique beak varied greatly, were from 0.2mm to 15mm. Percentage of beaks less than 5mm was 20.9%. Flowering time and other traits also showed great variation. From morphological observation, it can be inferred that this population had big selection potentials of traits such as seed and leaf color, leaf shape, silique beak, and many other traits.

Somatic hybrids were intermediate between the two parents (Fig.3), but more like the parent NJ5280. Their siliques were with beak of around 0.6cm shorter than that of rapeseeds. By transplanting seedlings of parents at the same time as controls, the flowering times of hybrids were about 10 days earlier than the rapeseed line, but about 8 days later than *R. indica*. Somatic plants were partially fertile, could seed through selfing or open-pollinating way. The branch numbers of somatic hybrids were 10-13 less than *R. indica*. The mean weight per 1000 seeds was around 2.2g, different from the two parents.

Cytogenetics

In the F_2 population, chromosome observations at meiotic metaphase I stage showed unequal number of chromosomes among individual plants (Fig.4). Chromosomes of 15-20 bivalents were observed. Chromosome paired well, indicating that *R. indica* possibly had close evolution relation to rapeseed and *O. violaceus*. The fact that PMCs with more chromosomes were not observed, possibly was caused by observations of limited samples fixed.

Fig.2 Several appearances in F_2 population derived from ((*B. napus*×*O. violaceus*) F_1×*R. indica*)

Fig. 3 Somatic hybrid and their parents NJ5280 and *R. indica* at budding stage

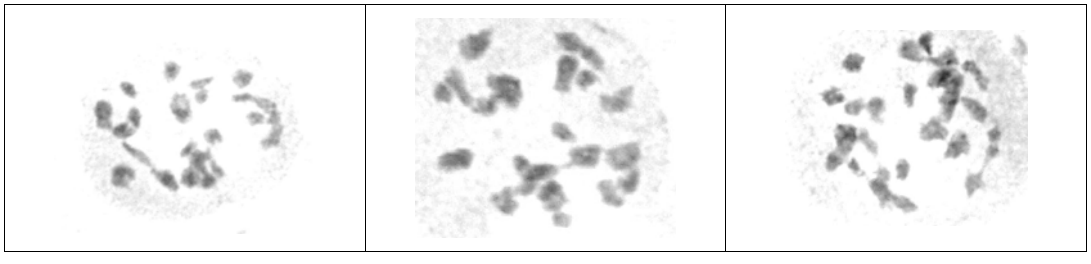
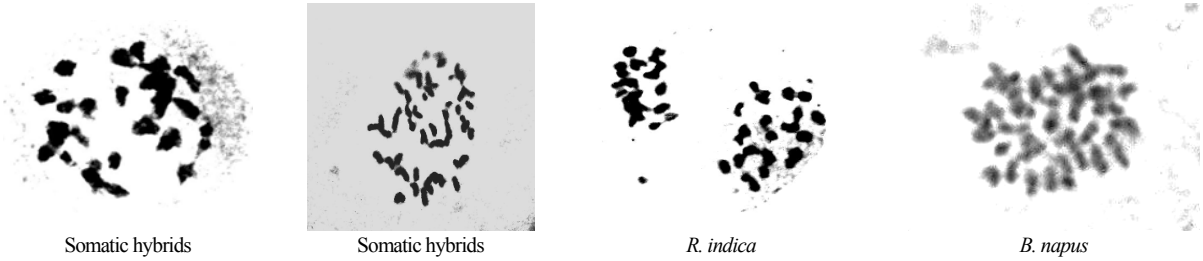


Fig. 4 Chromosome observation of partial plants from the F_2 population derived from the three-way crosses (*B. napus*×*O. violaceus*) F_1×*R indica*) at meiotic matephase-I

In the selfing progenies of somatic hybrids, chromosomes in root cells were unequal (not listed here). Seed with Chromosomes more than parents were also observed in root-tips from selfing seed (Fig. 5).



Somatic hybrids Somatic hybrids *R. indica* *B. napus*

Fig. 5 Chromosomes of somatic hybrids, selfed pr0genies and their parents

Seed quality analysis

Quality traits of mixed seeds of F_3 families of the intergeneric population were analyzed. Fequency distributions (Fig 6) showed that in the three-way cross-derived population, seed oil, erucic acid and glucosinolates content varied greatly. There existed materials with low erucic acid content and low glucosinolates content families. This was in accordance with their parents in which NJ5280 is double-low, O. *violaceus* is a plant with low-erucic acid in its seeds (0.63%), *R. indica* has seed erucic acid content of 21.7%. These results indicated that this population had big selection potentials of seed quality.

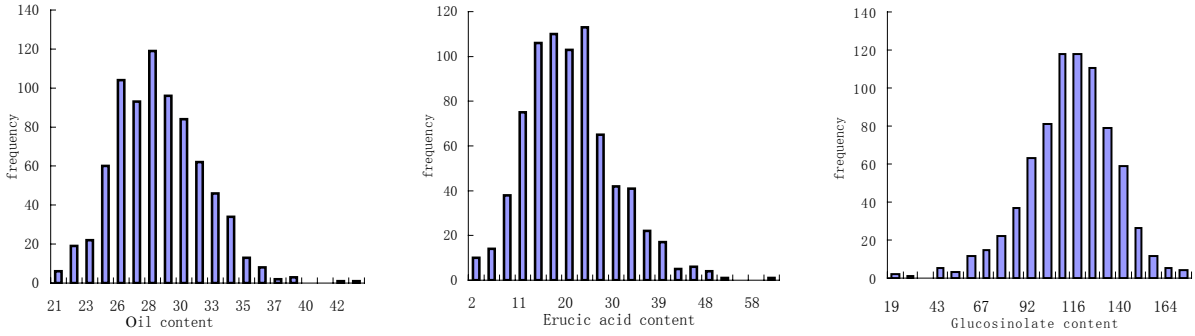


Fig. 6 Frequency distribution of seed quality traits in the F_2 population of integeneric three-way cross

Discussion

Unlike most of rapeseed-related interspecific hybrids with growing appearance similar to rapeseed, plants derived from the three-way cross were more like that of *R. indica*, probably because of genome of *R. indica* dominated in the population. This phenomenon might be beneficial to gene transfer from *R. indica* to other *Brassica* species. *R. indica* is also a potherb with function of curing asthma, its enriched germplasm from the three-way cross will be undoubtedly a better basis of domesticating *R. indica* for fast growing and easily cultivating.

Genetic materials created by this research, showed very rich variations which may be used for development of rapeseed with shortened silique beak, yellow seed and wateriness-tolerance, male sterility, etc. Researches provided by this paper were preliminary. Further researches may be continued in future. However, the materials were saved in germplasm bank at NAU, may be exchangeable only for research.

Acknowledgement

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