# Studies on rapeseed germplasm enhancement by use of cruciferous weed *Descurainia sophia*

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**Abstract**

Cruciferous weed plant *Descurainia Sophia* has excellent resistance to stress, also has linolenic acid up to 40% in its seeds, may be special germplasm to *Brassica napus* L.. So we designed to enhance rapeseed germplasm with *D. Sophia* by protoplast fusion and intergenus hybridization. Using PEG-DMSO protoplast fusion approach and culturing with modified B5 liquid media, 19 somatic fusion hybrids between *B. napus* and *D. Sophia* were obtained. A intergeneric hybridization between *B. napus* and *D. sophia* was successfully carried out by appropriate embryo rescue approach. Morphological and cytogenetic identification showed that plants obtained from both protoplast fusion and intergeneric hybridization progenies were new rapeseed germplasm derived from *D. Sophia*.

**Key words:** *Descurainia sophia*, *Brassica napus*, protoplast fusion, intergenus hybridization

## Introduction

Herbaceous wild species *Descurainia Sophia* with much divided leaves, looks very different from *Brassica* species, but belong to *Cruciferae,* existing in North America, Asia, Africa and Europe. It was observed that *D. Sophia* can flourish even in the cold mountainous areas of altitude from 2300 to 3200 meters locating in or to Tibet, showing traits of tolerating to extremely low temperature. Its seed linolenic acid content, is around 40%, very high in comparison of other cruciferous wild species. Characteristics of high-content linolenic acid in its seeds may be basis of oil functional for prevention and cure of blood vessel diseases. Also, unlike most of other wild species with low seed oil content of about 20 –30%, *D. sophia* seeds is rich in oil, up to 40%.

To enhance rapeseed germplasm for special fatty acid constitution, *D. Sophia* as trait sources was used for transferring trait to rapeseed by way of somatic hybridization and intergeneric crosses.

## 1 Materials and methods

### *1.1 Plant materials*

All plant material were obtained from germplasm bank at Nanjing Agricultural University (NAU), and were planted in Jiangpu experimental station of NAU. NJ292 and NJ5280 are a canola line developed by authors. Huashuang No. 3 was from Huazhong Agricultural Univeristy. Wild species *D. sophia* was collected from Taixing county in Jiangsu province, P.R. China.

### *1.2 Somatic fusion*

Leaves of *Descurainia Sophia* and cotyledon of canola variety Huashuang No. 3 and NJ5280 in *B. napus* were used to isolate protoplast, and PEG-DMSO method was used to fuse protoplasts. Enzyme combination for isolating protoplast from *Descurainia Sophia* and *B. napus* were 4%Cellulase +0.5% Macerozyme +5 mmol/L MES and 1%Cellulase +0.2% Macerozyme+ 3 mmol/L MES, respectively. Improved B5 media was used for culturing fused protoplast with density $1\times10^5$. Media for regenerating from the calli was MS + 6-BA 4.0 mg/L + NAA 0.3mg/L + AgNO3 5.0mg/L. Rooting media was MS +IBA 0.1 mg/L+NAA 0.1mg/L. Somatic fusion methods reported in another paper by the authors [1], may not be listed in detail here.

### *1.3 Intergeneric hybridization*

A intergeneric cross (*B. napus*×D. sophia) was conducted by hand-cross and by embryo rescue technique. NJ292 and NJ5280 as maternal parent were pollinated with pollen carefully collected from *D. sphia* in large amounts, then intergeneric cross embryos were used for rescue. Seedlings derived from carefully cultured for about 2 months were transplanted into fields.

Embryo rescue technique may be described as following simplified procedure. Unmature green embryos got from the intergeneric crosses of 21 days-old, were taken out under asepsis conditions, transferred into culture media such as Ms media adding with 1mg/L 6-BA and 0.2mg/L NAA. For rooting, young seedling growing into height of 1.5-2.5 cm were transplanted into MS media adding with 0.5mg/L NAA and 0.5mg/L IBA. Seedling with 15-20 secondary roots may be carefully transplanted into sterilized fertile soil. Keeping moisture is a key measure for survival of the seedlings. When seedling grew larger, they were transplanted into field for yieiding more seeds.

*1.4 Morphology and cytogenetics*

Traits of all of progenies derived from our work, were observed and recorded.

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 according to Jaheir et al. (1989).

Seeds harvested from the hybridizations and parental species were germinated at 25°C. Root-tips were excised from these plants. The pre-treated 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.

*1.5 Quality analysis*

The crude fat was extracted by aether-petroleum ether mixed liquor and hydrolyzed by potassium hydroxide- methanol mixed liquor, analyzed by GC-MS with a Finnigan TRACE GC gas chromatograph coupled with a Finnigan TRACE DSQ mass spectrometer equipped with a DBwax fused silica capillary column(60m×0.25mm ID×0.25μm film thickness). The oven temperature programmed as from 175°C to 240°C at 3°C (10min isothermal). Carrier gas was purity helium (flow rate 1.0ml/min) and the split ratio was 1:50. The mass spectra was acquired with a source temperature of 200°C under a 70eV ionization potential. Full-scan analyses starting from 6.00min were in mass rang 40-600m/z.

Data was evaluated by Xcalibur 1.3 system software. Identification of the compounds was done by comparing the retention times and by the use of mass spectra database search (NIST MS search 2.0) and retention indices from accessible scientific literature as well as comparison of mass spectra from relevant literature.

## 2 Results

*2.1 Morphology*

By fusion approach, we got 19 somatic bybrid plants, of which 8 did not developed to flowering and died, 11 could flower, but 3 plants did not give out any pollens, showed complete sterility and could yield seeds by open-pollination. The somatic hybrids were slowly-developing, dwarf. Its leaves were divided more than *B. napus*, but more similar to *B. napus* than *D. sophia* (Fig. 1). The buds were wrinkled, and purple. The petals were wrinkled, the anthers were degenerated completely or partially in accordance to plants. Siliques were intermediate between rapeseed and flixweed. Their seed weights were 3.1- 3.8g per 1000 seeds.

Fig.1 The somatic hybrid at budding stage, showing buds and leaves

Due to smallness of *D. sophia* flower, rapeseeds canola line (NJ5280) were used as maternal parents, crossed with *D. sophia*. More than 2300 buds were castrated, and pollinated with flixweed pollens at 9 –10 a.m. of flowering time. 21 days later, unmature embryo were taken out for rescuing. We got 16 seedlings from more than 60 young embryoes. The young seedlings were carefully and gradually transplanted to field. Though carefully managed in the field,, there were also 9 seedlings died. Luckily, there were 7 seedlings survived though they developed retardedly, dwarfly. Appearances of $F_1$ plants were intermediate between their parents, but also more like rapeseed while their leaves were with more division. Their leaf colors were slightly yellowish, and stem colors slightly purple. The survived plants that were partially fertile, were selfed, and yielded about 1g seeds for every plants. Their siliques were about 3.1cm long, longer than *D. Sophia*. Size of the seeds were intermediate between their parents.

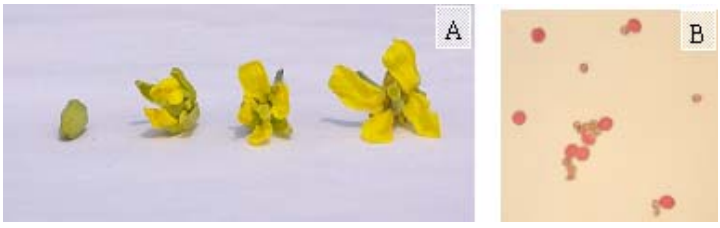


Fig 2. Flowers of the somatic hybrids from fertile parents.
A, flowers partial male sterile or complete sterile;
B, pollens fertile and dead

$F_2$ plants constituted a population with size of 273 plants of which one third could not live through the winter, and died. Viewing their morphological performances, though most of plants were like *Brassica* species, but diverse appearances could be observed as shown in Fig.3. Special leaf types which might be intermediate between the two parents, were observed in the population. $F_2$ plants were partially fertile or fertile, and could seed. We harvested 81 individual plants, got 1-18.5g seeds from different plants. Seeds from other plants were mixed and saved.

*2.2 Cytogenetics*

Chromosome of somatic hybrids and its selfing progenies were observed (Fig. 4). Antheral somatic chromosomes (2n=52) at mitosis metaphage were more than any parents (Fig. 4A). Chromosomes of progenies of somatic hybrids were unequal among plants, for example chromosomes of 23,24, 25 (Fig 4B,C,D) and 33, 34, etc, were observed in their somatic cell of root tips. This phenomena probably be results chromosome elimination of the somatic hybrids. There were less families with chromosomes surpassing parents (Fig.4E) (2n=38 for rapeseed and 2n=28 for *D. sophia*).

The seeds of $F_2$ plants of intergeneric cross were germinated for chromosome observation. Results showed that chromosome number differed among seeds. Though chromosome numbers of rootip generally were 21-30, there existed also roottips of which chromosome number surpassed parents (Fig. 5D).

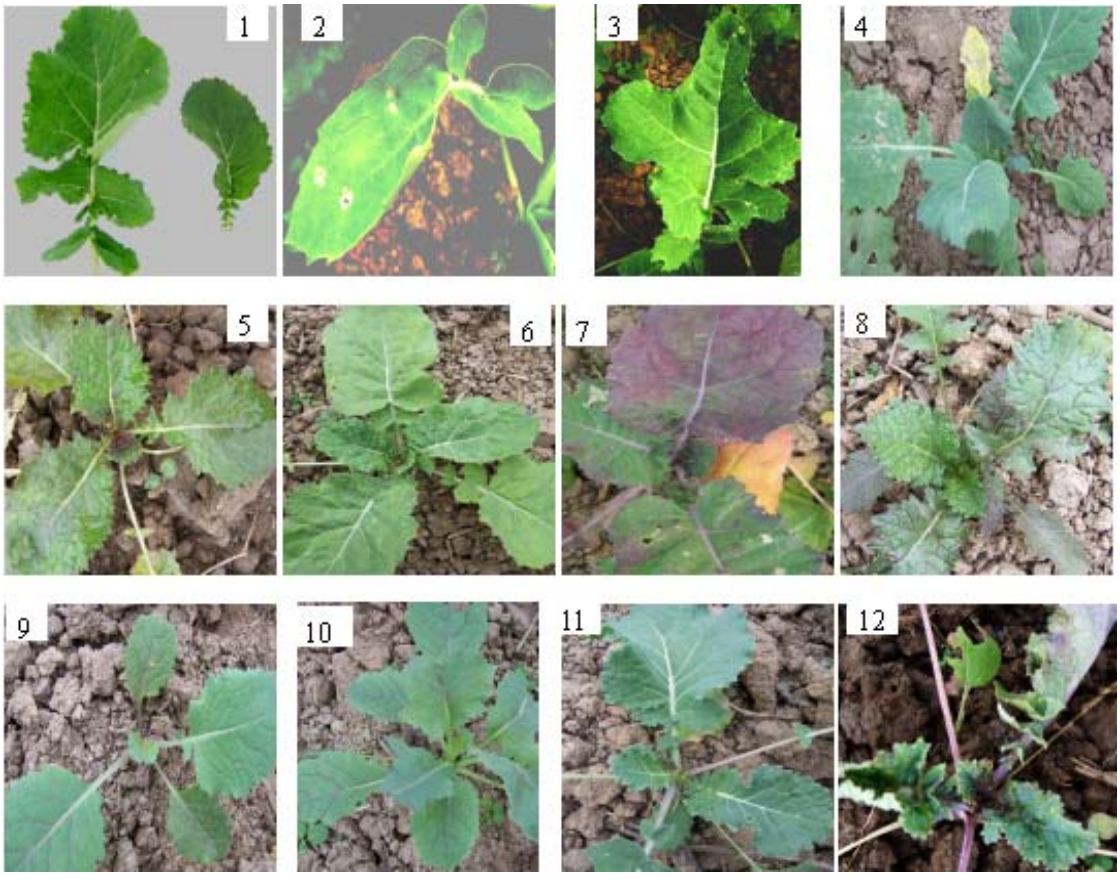


Fig. 3 Morphology of intergeneric cross porgenies derived from *B. napus*× *D.Sophia*

Fig.3.1, leaf comparison between rapeseed (left) and the intergeneric hybrid $F_1$ (right); Fig.3.2-3 indicated the special leaf type slightly like *D. Sophia* and with larger size; Fig.3.3-11 showed the diverse appearances in $F_2$ population; Fig.3.12 showed that $F_2$ plant that might be died thourgh winter.

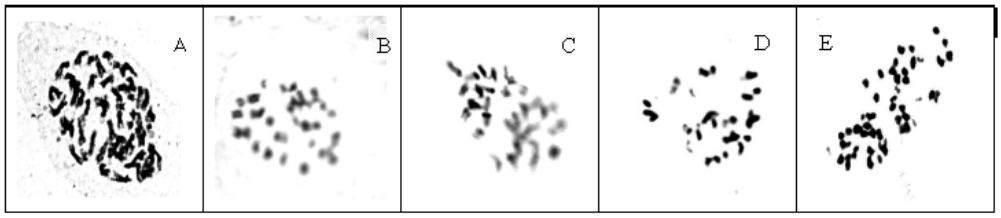


Fig.4 Chromosome observation of the somatic hybrids between D. Sophia and B.napus.

Fig. 4A showed the antheral somatic chromosomes more than the two parents. Fig.4B,C,D showed The somatic chromosomes of fusion-derived progeny plants (2n=23, 25, 24, etc), less than that of parents. Fig.4E showed. the somatic chromosomes of progeny surpassing parents.

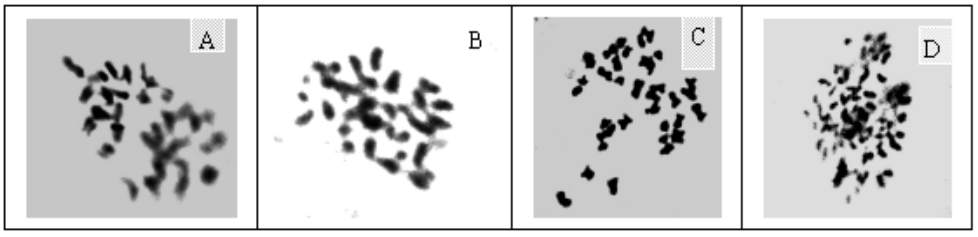


Fig.5 Chromosomes of $F_2$ progenies of the intergeneric cross between D. sophia and B. napus

Fig. 5A, B, C, D showed that seeds have different chromosme numbers

*2.3 Quality analysis*

Seed fatty acids were analyzed by GC-MS (Fig. 6). Screening the materials of 31 families with enough seeds derived from somatic fusion and intergeneric hybridization (F3), 3 families with high-content of linolenic acid, ranging from 22.34 to 26.11, were obtained, others were not high-content of linolinic acid, less than 12%. In a family named as BNDS7-39, fatty acid content (%) of palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, eicosenoic, and erucic acid, was 5.79, 0.24, 1.5, 25.51, 17.76, 25.35, 10.51, 8.2%, respectively. In this family inherited from canola parent and D. sophia, existence of erucic acid (8.2%) was probably because of erucic acid content (11.5%) of D. sophia.

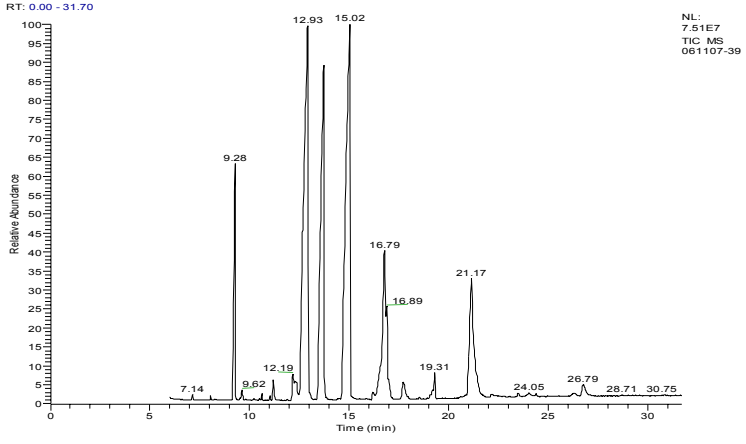


Fig. 6 Chromatographic profile of the fatty acids from line 7-39 by GC-MS

Note Retention time for palmitic, palmit, oleic, stearic, oleic, linoleic, linolenic, eicosenoic, erucic acid, was 9.28, 9.62, 12.19, 12.93, 13.73, 15.02, 16.79, 21.17, respectively.

## 3 Discussion

Linolenic acid as a trienoic fatty acid may play role in preventing and curing blood vessel diseases. The materials with high-content of linolenic acid may be beneficial to development of rapeseed as sources of function oil. A batch of other materials were obtained, included families with leaf type changed, chromosome number atypical, male sterility, etc. These materials reported may be useful in elucidation of genetic machanism and rapeseed breeding.

Many progenies from the protoplast fusions and intergeneric hybridization developed retardedly, partial progeny plants could not survive. Accroding to our researches (not listed here), genes in *D. Sophia* were less related to *B. napus* than *Arabidopsis thaliana*. The distant evolution relation between the two species may be considered as reasons for chromosome elimination of somatic hybrids and hybridization difficulties between the two species.

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