Hybridization potential between the oilseed crucifer *Camelina sativa* and canola, mustard and related weeds

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Camelina sativa (false flax, gold-of-pleasure) is an ancient oilseed crop well-suited for production in western Canada (Gugel and Falk 2006). The species is amenable to genetic transformation (Lu and Kang 2008), making it an ideal platform crop for industrial oilseed production. The potential for hybridization between *C. sativa* and related crucifers was examined to evaluate the risk of gene flow between *C. sativa* and these species.

Materials and Methods

The plant materials used in the study are listed in Table 1. The genera *Camelina* and *Capsella* belong to the tribe Camelineae, the genus *Brassica* belongs to the tribe Brassiceae and the genus *Thlaspi* belongs to the tribe Thlaspideae. Plants of the annual species (*Brassica* spp., *C. alyssum*, *C. sativa*, *Capsella bursa-pastoris*, *Cap. rubella* and *T. arvense*) were raised under controlled environmental conditions (greenhouse or growth chamber). Plants of the biennial species (*C. microcarpa* and *C. rumelica*) were vernalized (4ºC, 12 h photoperiod) at the 5–6 leaf rosette stage in a growth chamber for up to six weeks prior to being returned to the greenhouse. Pollinations were made manually on emasculated pistils.

Pollinated pistils were examined for pollen germination and the presence of pollen tubes in ovaries using fluorescence microscopy. Inflorescences were excised at 6, 24 or 48 h post- pollination (hpp) and fixed in Carnoy's fluid I at room temperature (about 22ºC) for 24 h. The inflorescences were rinsed with deionized water, then placed in 1M NaOH and incubated at 60ºC for 50–60 min. The inflorescences were then cooled on ice, rinsed 3 times with deionized water and stained with 1% aniline blue prepared in 0.1 M K_2PO_4 buffer at 4ºC in the dark overnight. Flowers were dissected and the ovaries were examined using a Zeiss Axiovert 100 microscope equipped with ultraviolet light.

Seed set was observed at physiological maturity. DNA sequence data of the nuclear ribosomal internal transcribed spacer (ITS) was employed to verify the hybridity of progeny obtained from the crosses between *C. sativa* and the *Camelina* weed species.

Results and Discussion

Brassica nigra

One thousand pollinations were made on 28 plants of the *B. nigra* accessions. In most cases, pollinated pistils had shrivelled or dehisced within 15–20 days post-pollination (dpp) and seed set was nil. Pollination with *C. sativa* sometimes triggered pod development; however, no seed developed in these pods.

Eighty-seven pollinated pistils were examined for pollen germination and pollen tube development. There were few to no pollen grains on stigmas at 48 hpp, indicating that the pollen grains did not adhere to the stigmatic surface and were washed away by the fixation and staining process. In contrast, adherence of pollen grains in self-pollinated *C. sativa* was strong. The few pollen grains observed on the *B. nigra* stigmas were mostly un-germinated. The tubes of the pollen grains that had germinated failed to penetrate the stigmatic tissue. Thus, the probability of hybridization and gene flow from *C. sativa* to *B. nigra* is highly unlikely.

Brassica juncea, *B. napus* and *B. rapa*

A total of 1,372 pollinations with *C. sativa* pollen were made on 72 plants of the three *Brassica* species. Pollinated pistils shrivelled or dehisced within 15–21 dpp. As observed in the crosses with

B. nigra, pollination with *C. sativa* pollen sometimes triggered pod development but there was no seed development in these pods. Fifty plants of the *C. sativa* genotypes were pollinated with pollen of the three *Brassica* species (a total of 1,180 pollinations for the three *Brassica* species). Pollinated pistils also shrivelled or dehisced within 15–21 dpp. No intergeneric seed was recovered.

Cytological examination revealed that there were few to no pollen grains on the stigmas of the *Brassica* species pollinated with *C. sativa* at 48 hpp and that the few pollen grains that were observed had not germinated. In the reciprocal crosses, pollen of the *Brassica* species germinated on the stigmas of *C. sativa*, but there was no evidence of pollen growth in the ovaries.

The results confirm that hybridization between *C. sativa* and the *Brassica* species is unlikely, as observed by Salisbury (1991), who reported failure of seed formation in similar crosses.

Camelina alyssum

Pollen germination was observed on stigmas of *C. sativa* pollinated with *C. alyssum* at 6 hpp and pollen tubes were observed in styles and ovaries at 24 hpp. Pollen tubes were observed near ovules in a small number of samples. Seed set was 2.2 seeds per pollination (N=578 pollinations) in the cross *C. sativa* × *C. alyssum* and 1.4 seeds per pollination (N=639 pollinations) in the reciprocal cross. F_1 progeny plants (N=48) from the reciprocal cross *C. alyssum* × *C. sativa* were raised in the greenhouse. Of these plants, 46 were confirmed by ITS sequence analysis as F_1 interspecific hybrids. Average pollen fertility of the F_1 hybrids was 96.7%; F_2 seed was produced. *Camelina alyssum* has been reported as possibly being an interfertile relative of *C. sativa* (Tedin 1922), so hybridization between these species was not unexpected.

Camelina microcarpa

Two accessions of *C. microcarpa* collected near Saskatoon, Saskatchewan, were used in these crosses. At 6 hpp, pollen had germinated on most stigmas regardless of the direction of the cross. Pollen tubes were observed in most ovaries at 24 hpp, and often near the ovules and/or the micropylar end of the ovules. In the cross *C. sativa* × *C. microcarpa*, seed set was 0.3 seeds per pollination (N=308 pollinations) with accession 36009 and 2.2 seeds per pollination (N=282 pollinations) with accession 36010. In the reciprocal cross, seed set was 0.9 seeds per pollination (N=325 pollinations) with accession 36009 and 3.3 seeds per pollination (N=283 pollinations) with accession 36010. Twenty F_1 progeny plants from the cross *C. microcarpa* accession 36010 × *C. sativa* were raised in the greenhouse. All 20 plants were confirmed to be interspecific hybrids by ITS sequence analysis. Pollen fertility of 13 F_1 plants ranged from 3.8–26.6% viable pollen (ave. 11.8%); a few F_2 seeds were produced on selfed F_1 plants.

Camelina rumelica

Seed set was 1.1 seeds per pollination (N=646 pollinations) in the cross *C. sativa* × *C. rumelica* and 0.6 seeds per pollination (N=521 pollinations) in the reciprocal cross. Three F_1 hybrid plants were detected by ITS sequence analysis in the cross *C. sativa* × *C. rumelica*. The F_1 hybrid plants had very low pollen fertility (1.2%) and did not produce F_2 seed.

Capsella bursa-pastoris

Fifty pistils of *C. sativa* were pollinated with *Cap. bursa-pastoris* pollen. Germinated pollen grains were found on stigmas in 68.0% of the pistils. There was no evidence of pollen tube growth, suggesting that F_1 hybrid formation in this cross is unlikely. A total of 49 pistils were examined in the reciprocal cross, *Cap. bursa-pastoris* × *C. sativa*. Germinated pollen was observed on stigmas of 81.6% of the pistils and pollen tubes were observed in 55.1% of the pistils. Of these pistils, 30.6% had tubes near ovules. Seed set was not determined in these crosses.

Capsella rubella

Fifty-two pistils of *C. sativa* were pollinated with *Cap. rubella* pollen. Germinated pollen grains were found in 63.4% of the pistils. There was evidence of pollen tube growth in four pistils and a few tubes were observed near ovules in two pistils. In the reciprocal cross, *Cap. rubella* × *C. sativa*, germinated pollen grains were observed on 50% of the pistils (N=52); pollen tubes were observed in the ovary in 27.0% of the pistils and near the ovules. Seed set was not determined in these crosses.

Thlaspi arvense

A total of 47 pistils of *C. sativa* were pollinated with *T. arvense*. Over half of the pistils (59.6%) had germinated pollen grains on the stigmas. One pollen tube was observed in stylar tissue in two pistils.

Pollen tubes were not observed in the ovaries. In the reciprocal cross, *T. arvense* × *C. sativa*, 20.0% of the pistils (N=45) had germinated pollen grains on the stigmas. There was no evidence of pollen tube growth in the stylar tissue in this cross. Seed set was not determined in these crosses.

Conclusions

The results of the study confirmed interspecific hybridization between *C. sativa* and *Camelina* weedy relatives, particularly *C. alyssum*. The results also indicated that hybridization between *C. sativa* and distant relatives such as *Brassica* species and *T. arvense* is unlikely. Further studies with *Capsella* species, which belong to the same tribe as *Camelina*, are warranted.

Literature cited

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Table 1. Germplasm used in hybridization studies between *Camelina sativa* and related crucifers.

Species	Common name	Genotypes
Brassica juncea (L.) Czern.	Mustard	cv. AC Vulcan (oriental mustard, Canada) cv. Arid (canola-quality mustard, Canada) cv. Centennial Brown (brown mustard, Canada) PI 179850 (Pakistan)
B. napus L.	Oilseed rape	cv. Scoop (canola, Australia) cv. Westar (canola, Canada) line DH27303 (canola, Canada)
B. nigra L.	Black mustard	PI 131512 (The Netherlands) PI 173860 (India) PI 175071 (India) PI 194902 (Ethiopia)
B. rapa L.	Turnip rape	cv. AC Sunbeam (canola, Canada) cv. AC Parkland (canola, Canada) cv. R500 (yellow sarson rapeseed, Canada)
Camelina alyssum (Mill.) Thell.	Flat-seeded false flax	PI 650132 (Germany)
C. microcarpa Andrz. ex DC.	Small-seeded false flax	36009 (Canada) 36010 (Canada)
C. rumelica Velen.		PI 650138 (Iran)
C. sativa (L.) Crantz	False flax	cv. Calena (Austria) cv. Céline (France) cv. Kirgizskij 1 (former Soviet Union) cv. Voronezskij 339 (former Soviet Union) PI 311735 (Poland) PI 650151 (Sweden) SRS 933 (former Soviet Union)
Capsella bursa-pastoris (L.) Medik.	Shepherd's purse	CN 101991 (Canada)
Cap. rubella Reut.	Pink shepherd's purse	CS22561 (Italy)
Thlaspi arvense L.	Stinkweed	Ames 15736 (Germany)