Evaluation of the progeny produced by interspecific hybridization between *Camelina sativa* and *C. microcarpa*

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• **Background and aims** Camelina (*Camelina sativa*, Brassicaceae) has attracted interest in recent years as a novel oilseed crop, and an increasing number of studies have sought to enhance camelina's yield potential or to modify the composition of its oil. The ability of camelina to cross-hybridize with its wild relative, *C. microcarpa*, is of interest as a potential source of genetic variability for the crop.
• **Methods** Manual crosses were performed between the crop *C. sativa* and its wild relative *C. microcarpa*; F_1 and F_2 progenies were obtained. Cytology was used to study meiosis in the parents and F_1s and to evaluate pollen viability. Flow cytometry was used to estimate nuclear DNA amounts and fatty acid methyl ester analysis was used to evaluate the lipid composition of F_3 seeds.
• **Key Results** The F_1 plants obtained by interspecific crossing presented severe abnormalities at meiosis and low pollen viability, and produced very few F_2 seeds. The F_2s presented diverse phenotypes and in some cases severe developmental abnormalities. Many F_2s were aneuploid. The F_2s produced highly variable numbers of F_3 seeds, and certain F_3 seeds presented atypical lipid profiles.
• **Conclusions** Considering the meiotic abnormalities observed and the probability of aneuploidy in the F_2 plants, the *C. microcarpa* accessions used in this study would be difficult to use as sources of genetic variability for the crop.

Keywords: *Camelina sativa*, *Camelina microcarpa*, interspecific outcrossing, gene flow, aberrant meiosis, interspecific hybrid, aneuploidy.

INTRODUCTION

In recent decades, increased interest in developing more sustainable agriculture has led to renewed interest in novel crops. Among these, one of the most promising is camelina (*Camelina sativa*), an oilseed crop in the family Brassicaceae that is closely related to the model plant species *Arabidopsis thaliana*. Archaeological remains of camelina from 6000 BC have been found in Armenia (Hovsepyan and Willcox, 2008), and there is clear evidence of the cultivated species in France and elsewhere in Western Europe starting in the late Bronze Age (Toulemonde, 2010). Camelina continued to be an important oilseed crop in Northern Europe until the end of the 19th century, after which it was progressively replaced by others, such as oilseed rape.

As has been presented in depth in recent review articles (Eynck and Falk, 2013; Vollmann and Eynck, 2015; Faure and Tepfer, 2016; Berti *et al.*, 2016; Sainger *et al.*, 2017), camelina has numerous attractive features that explain the renewed interest: the oil extracted from its seeds has several potential uses that could grow in importance, either for food, for biofuel or for other industrial uses, and the residual seed-cake can be used in animal feed. Furthermore, the plant is remarkably robust, growing well on marginal lands with little or no inputs required, and is resistant to major pathogens and pests of other Brassicaceae crops, such as oilseed rape. Because camelina has been a neglected crop for the past century, the available cultivars have not undergone extensive improvement using modern plant breeding strategies. In recent years, several laboratories have presented evidence suggesting that the genetic diversity present in current cultivars is relatively narrow (Vollmann *et al.*, 2005; Ghamkhar *et al.*, 2010; Manca *et al.*, 2013; Galasso *et al.*, 2015; Singh *et al.*, 2015; Kurasiak-Popowska *et al.*, 2018; Luo *et al.*, 2019), which greatly diminishes the prospects for crop improvement.

There are (at least) two possible solutions for this concern. First, numerous laboratories have recently created camelina genotypes expressing transgenes that modify the composition of its oil reserves (for reviews see Berti *et al.*, 2016; Faure and Tepfer, 2016; Sainger *et al.*, 2017), and CRISPR-Cas9 genome-edited camelina lines with enhanced oil composition have also been created (Jiang *et al.*, 2017; Morineau *et al.*, 2017). The release of transgenic/edited camelina varieties faces regulatory restrictions, however.

As an alternative, the use of wild genetic resources is an interesting path to explore. Within the genus *Camelina*, *C. microcarpa* is of particular interest, since many accessions have a genome structure that is similar to that of the crop (Séguin-Swartz *et al.*, 2013; Martin *et al.*, 2017, 2019; Mandáková *et al.*, 2019). *Camelina sativa* and certain accessions of *C. microcarpa* have an allohexaploid genome (Kagale *et al.*, 2014), composed of three closely related sub-genomes ($2n = 6x = 40$). A very recent paper has demonstrated that *C. sativa* and *C. microcarpa* show the same global chromosome organization (Mandakova *et al.*, 2019), supporting the view that *C. microcarpa* could be the parental species of the crop (Brock

et al., 2018). It is also of note that crosses between Canadian accessions of hexaploid *C. microcarpa* can yield viable, partly fertile F_1 progeny in *C. microcarpa* × *C. sativa* crosses (Séguin-Swartz *et al.*, 2013; Martin *et al.*, 2019). Crosses between *C. sativa* and *C. microcarpa* are thus of interest from a plant breeder's perspective, as they provide the opportunity of using the crop's closest wild relatives as sources of diversity. This relies on the fitness of F_1 hybrids and succeeding generations, which has not been evaluated previously.

The objective of this study was to evaluate the viability of F_1 hybrids and succeeding generations. We first show that *C. sativa* × *C. microcarpa* F_1 plants had profoundly disturbed meiosis and low pollen viability, but that a few F_2 seeds could nonetheless be obtained. The F_2 individuals displayed a range of developmental abnormalities, and evaluation of nuclear DNA amounts suggests that many, if not all, were aneuploids. Nonetheless, certain F_2 individuals produced abundant F_3 seeds, demonstrating that the barriers to hybridization between *C. sativa* and hexaploid *C. microcarpa*, although strong, are not totally impermeable. As a proof of concept, we also show that certain F_3 seeds presented atypical lipid profiles, which opens potential avenues for plant breeding.

MATERIALS AND METHODS

Plant materials, growing conditions and interspecific crosses

DsRed F, a homozygous line of *Camelina sativa* 'Céline' expressing a DsRed transgene has been described (Julié-Galau *et al.*, 2014). *Camelina microcarpa* populations have been described across Europe and Western and Central Asia (Calasan *et al.*, 2019). In France, *C. microcarpa* is present in scattered populations primarily in the southern and eastern regions of the country (https://inpn.mnhn.fr/espece/cd_nom/87577). Seeds of two accessions of *C. microcarpa* were obtained from the Conservatoire Botanique du Bassin Parisien – Muséum National d'Histoire Naturelle (CBNP:MNHN): 03CF1063, referred to as Guillestre, and temporary accession 2487, referred to as Fleurey. Except when vernalization was applied, plants were grown in the greenhouse with a 16-h daylength, 25 °C day and 22 °C night temperature, and 40% relative humidity. Plants that were vernalized were first grown for 1 month as above, then transferred to a greenhouse maintained above 5 °C for 1 month, then returned to normal conditions.

For manual crosses, flowers of the female parent were emasculated before anthesis to avoid selfing, and were pollinated manually with the pollen of the male parent.

Cytology

Male meiotic spreads for 4′,6-diamidino-2-phenylindole (DAPI) staining were prepared as described for *Arabidopsis* in Chelysheva *et al.* (2013) from buds fixed in Carnoy's fixative (absolute ethanol:acetic acid, 3:1, v/v). Mature pollen grain viability was estimated as described by Alexander (1969).

Flow cytometry

Relative nuclear DNA amounts were determined with a CyFlo Space flow cytometer as described by Marie and Brown (1993). For each plant genotype, three young leaf samples were analysed. This entailed chopping with a fresh razor blade in Galbraith's chopping buffer (Galbraith *et al.*, 1983), addition of propidium iodide, and analysis according to the manufacturer's protocol. The position of the different ploidy peaks of hexaploid *C. sativa* 'Céline' had been determined previously relative to diploid *Arabidopsis thaliana* and tetraploid *Capsella bursa-pastoris* (Julié-Galau *et al.*, 2014), and thus served as internal reference.

Analysis of seed fatty acid composition

Fatty acid methyl esters (FAMEs) were extracted from single seeds, and then analysed by GC–MS according to Li *et al.* (2006). For each sample, a single seed was put in a glass tube to which was added methanol (1 mL), toluene (0.3 mL) and sulphuric acid (25 µL). The tubes were closed and warmed at 80 °C for 90 min. After cooling, hexane (0.5 mL) and water (1.5 mL) were added. The tubes were shaken and then centrifuged for 5 min at 1469 *g*. The hexane phase (50 µL) was transferred to a GC vial for injection. The samples were injected into an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer. The polar column was a BPX70 (SGE) (30 m). Oven temperature ramp was 16 °C min⁻¹ from 70 °C to 160 °C, then 4 °C min⁻¹ up to 240 °C and 10 °C min⁻¹ up to 260 °C for 4 min (run length 31.6 min). Helium constant flow rate was 1.52 mL min⁻¹. Temperatures were as follows: injector 250 °C, transfer line 290 °C, source 250 °C and quadrupole 150 °C.

RESULTS

Our work is based on previous observations that *C. sativa* and hexaploid *C. microcarpa* are cross-compatible (Séguin-Swartz *et al.*, 2013; Martin *et al.*, 2019); therefore, we have not re-assessed the frequency with which F_1 hybrids can be produced. In this study, we used the previously described line of *C. sativa* 'Céline' expressing a constitutive DsRed transgene (Julié-Galau *et al.*, 2014) as pollen donor to produce *C. microcarpa* × *C. sativa* hybrids. The presence of the DsRed marker gene facilitates confirmation that the F_1 seeds produced were genuine hybrids. Two French *C. microcarpa* accessions were used: CBNBP-MNHN accession 03CF1063 from Guillestre, originating ~50 km west of Gap, and CBNBP-MNHN temporary accession 2487, collected at Fleurey-sur-Ouche, ~10 km west of Dijon. Neither required stratification for germination, and both required vernalization to induce timely flowering, but would eventually flower in the greenhouse if maintained under non-vernalizing conditions for several months. Plants of the two *C. microcarpa* accessions were morphologically indistinguishable and behaved in the same manner in crosses with *C. sativa*, so further analyses of progeny were focused on those from crosses with the Guillestre accession, except for the meiosis studies, in which both accessions were used.

Similar to what has been reported with several Canadian hexaploid *C. microcarpa* accessions (Séguin-Swartz *et al.*, 2013; Martin *et al.*, 2019), for both French *C. microcarpa* accessions, manual pollination of emasculated flowers with pollen from DsRed *C. sativa* readily yielded DsRed-positive F_1 seeds,

and the reciprocal crosses were also successful, as judged by the phenotype of the putative *C. sativa* × *C. microcarpa* F_1 plants.

Characterization of C. microcarpa × C. sativa F_1 hybrids

Six F_1 hybrids were grown in the greenhouse under long-day conditions. In the early vegetative phase, they remained in a rosette, as did the *C. microcarpa* parent, whereas the stems of the *C. sativa* elongated without a rosette phase (Fig. 1A). Later, after 2 months of growth, the F_1 hybrids had bolted and began to flower, but the *C. microcarpa* parents remained in a rosette (Fig. 1B). Induction of flowering in the hybrids was therefore intermediate between the two parents.

Although in the early phases of flowering the F_1 hybrids appeared to be entirely sterile, at later stages a few seeds were produced. When pollen viability was evaluated by Alexander staining, nearly all pollen grains of the parental species were viable, as expected, but only a small percentage of the pollen of the F_1 hybrids (<3 %) was viable (Table 1). These values are in the low range relative to those reported by Séguin-Swartz *et al.*

(2013) and Martin *et al.* (2019), who reported pollen viabilities in F_1 hybrids that ranged from 1.3 to 45 %. The low pollen viability was likely the cause of the very low ability of the F_1 plants to produce self-progeny, since they produced only 30–50 seeds per plant. This low seed productivity was also reflected in the weight of the seeds per plant (Table 2).

Meiotic defects in F_1 hybrids between C. sativa and C. microcarpa

The strong sterility observed in the F_1 hybrids prompted us to investigate male meiosis by staining pollen mother-cell chromosome spreads with DAPI. As a prerequisite, we first characterized male meiosis in *C. sativa*, which had not been assessed previously. We observed all the conspicuous landmarks of canonical meiotic progression. At pachytene, pairs of homologous chromosomes formed a ribbon-like structure that resulted from their intimate association via the synaptonemal complex (Fig. 2A). The synaptonemal complex then disassembled, and chromosomes condensed until discrete separate bivalents became visible at diakinesis (Fig. 2B). In all cells ($n = 12$), about 20 bivalents were discernible, while no obvious multivalents (association of more than two chromosomes) were observed. At metaphase I, bivalents were under tension, with homologous centromeres directed towards opposite poles; at this stage, the homologues were still connected with one another by chiasmata, the cytological manifestation of meiotic crossovers ($n = 10$; Fig. 2C). At anaphase I the homologues separated, leading to the formation of dyads corresponding to two groups of 20 chromosomes (Fig. 2D). The chromosomes then partially decondensed before the second meiotic division took place. At metaphase II, the individual chromosomes, each consisting of two chromatids, aligned on the metaphase II spindle (Fig. 2E). At anaphase II, individual chromatids migrated to opposite spindle poles, forming four groups of 20 chromatids at telophase II (Fig. 2F), which ultimately gave rise to a tetrad of four microspores ($n = 4$). All these results indicate that meiosis is very regular in *C. sativa*, which, despite being a hexaploid, displays diploid meiotic behaviour, at least cytologically.

Male meiosis in *C. microcarpa* was very similar, although a few meiotic defects were observed (Fig. 2G–I; Supplementary Data Fig. S1A–C). Briefly, as in *C. sativa*, chromosomes first condensed, synapsed and recombined during the first meiotic division. Again, about 20 bivalents could be visualized at metaphase I (Fig. 2G; $n = 15$ in *C. microcarpa* Fleurey). Occasional
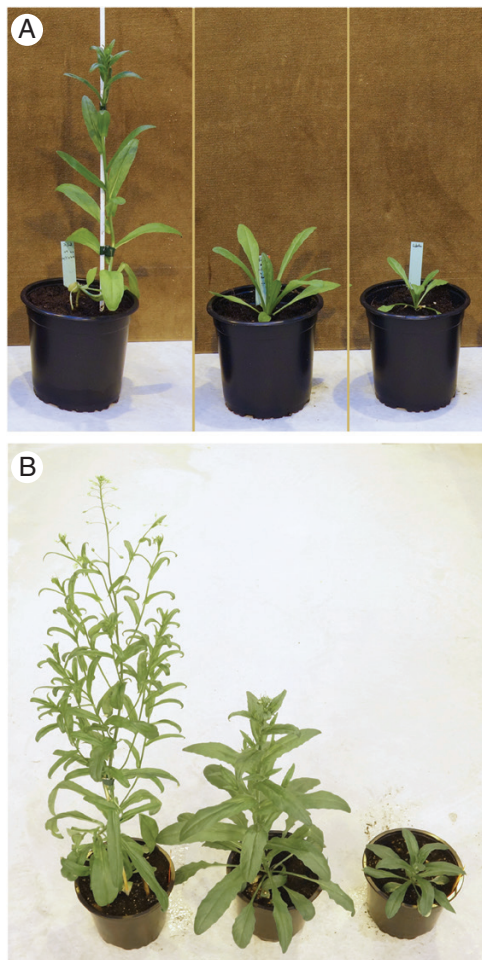
Fig. 1. Plants of cultivated camelina, its wild relative *C. microcarpa* and the F_1 hybrid. (Left to right) *C. sativa* 'Céline', F_1 hybrid *C. microcarpa* × *C. sativa* and *C. microcarpa* Guillestre, 1 (A) and 2 (B) months after sowing. Plants were grown in 14-cm pots.

TABLE 1. *Alexander staining of pollen of* C. sativa, C. microcarpa *and* F_1 *hybrids*

	Stain positive	Stain negative	Percentage stain positive
C. sativa DsRed F	245	2	99.2
C. microcarpa Guillestre	122	0	100
C. microcarpa Guillestre × *C. sativa* DsRed F	6	255	2.3
C. sativa DsRed F × *C. microcarpa* Guillestre	8	426	1.8

Viability of freshly harvested pollen grains was evaluated by Alexander staining.

TABLE 2. *Weight of seeds produced by* individual C. microcarpa × C. sativa F_1 *and* F_2 *plants*

Sample	Seed weight (g)
C. sativa DsRed F	12.00
	15.21
C. microcarpa Guillestre	3.95
	5.90
F_1 of *C. microcarpa* Guillestre × *C. sativa* DsRed F	
F_1-A	0.03
F_1-B	0.01
F_2 of *C. microcarpa* Guillestre × *C. sativa* DsRed F	
F_2-1	< 0.01
F_2-2	0
F_2-3	2.68
F_2-4	0.47
F_2-5	0.54
F_2-6	0.03
F_2-7	0.05
F_2-8	0
F_2-9	0
F_2-10	0.01
F_2-11	0.07
F_2-12	0
F_2-13	2.14
F_2-14	0.13
F_2-15	0
F_2-16	< 0.01
F_2-17	3.88
F_2-18	< 0.01
F_2-19	0
F_2-20	0.30
F_2-21	< 0.01
F_2-22	0
F_2-23	0
F_2-24	0
F_2-25	0
F_2-26	0.19
F_2-27	0.26
F_2-28	2.01
F_2-29	0.39
F_2-30	0
F_2-31	0
F_2-32	0
F_2-33	0
F_2-34	0
F_2-35	0
F_2-36	0
F_2-37	0
F_2-38	1.07
F_2-39	1.04
F_2-40	0.66
F_2-41	< 0.01

chromosomal abnormalities appeared at anaphase I, with some laggards and/or fragments in some cells of *C. microcarpa* Fleurey ($n = 6$; Fig. 2H). The second division then separated sister chromatids and resulted in four pools of chromosomes, each consisting of a single chromatid (Fig. 2I; Supplementary Data Fig. S1C).

The situation in *C. sativa* × *C. microcarpa* Fleurey F_1 hybrids was very different, illustrating a wide range of meiotic defects. Numerous univalents (i.e. chromosomes that failed to form crossovers) were observed in all metaphase I plates ($n = 41$), scattered around the spindle equator (Fig. 2J). Anaphase I ($n = 24$) and telophase I were also very irregular, showing the presence of numerous laggards (Fig. 2K) and

bridges (Fig. 2L) in some cells. In particular, we observed at least one clear instance in which the bridge was very close to one or two fragments (Fig. 2K), suggesting, if confirmed, that a crossover may have occurred within a paracentric inversion. The second division followed in the usual way, except for the presence of fragments in some 'dyads' and (individual) chromatids unaligned on the metaphase II plate or lagging between the two groups of separating chromatids (Fig. 2M). Bridges connecting different groups of individualized chromatids were also observed at anaphase II/telophase II, suggesting sister-chromatid breakage and rejoining at an earlier stage ($n = 7$; Fig. 2N, O). Similar, although less numerous, defects were observed during meiosis of *C. microcarpa* Guillestre × *C. sativa*: univalents at diakinesis (Supplementary Data Fig. S1D) and metaphase I ($n = 93$; Supplementary Data Fig. S1E, F), laggards and bridges at anaphase I and anaphase II ($n = 4$; Supplementary Data Fig. S1G, H) and unbalanced tetrads (Supplementary Data Fig. S1I).

Characterization of F_2 *generation plants*

As stated above, and despite the conspicuous abnormalities observed during male meiosis of F_1 hybrids between *C. sativa* and *C. microcarpa*, 20–50 F_2 seeds were eventually produced by self-fertilization of the F_1s. The F_2 progeny of two *C. microcarpa* Guillestre × *C. sativa* F_1 hybrids were grown in the greenhouse, the first (42 seeds) without vernalization, the second (41 seeds) with a 1-month vernalization period. As shown in Fig. 3A, B, in the non-vernalized group the F_2 plants displayed highly diverse phenotypes, with marked differences in leaf morphology, stem elongation, and the ability to enter reproductive phase. In some F_2 individuals, flowering was only moderately delayed relative to *C. sativa*, whereas others did not flower after several months in the absence of vernalization. Leaf shape and the presence/absence of teeth on the leaf margin were also extremely variable. Since there was no evident correlation between the diverse morphological parameters, no attempt to quantify them was made. Two F_2 individuals displayed remarkably abnormal floral development: in plant F_2-B5 the sepals were not shed, and developed into elongated leaf-like appendages (Fig. 3D); in plant F_2-E7 all floral organs were leaflike (Fig. 3E). Both plants were entirely sterile.

Considering the abnormal meiosis observed in pollen formation and the transgressive segregation of developmental features, it was of interest to evaluate the nuclear DNA amounts of F_2 progeny. This was carried out on the second series of F_2 plants, which had been vernalized. As was expected, since it was already known that *C. sativa* and *C. microcarpa* Guillestre are both hexaploid, the DNA amounts of the two parental species and their F_1 progeny were equivalent (Fig. 4). By contrast, the DNA amounts of F_2 individuals were quite diverse, and several had distinctly higher nuclear DNA amounts than the parental species or the F_1 hybrids, the extreme case being F_2-9, in which the nuclear DNA amount was more than 10% greater than the highest parental value; these results strongly suggest that many F_2s were aneuploid to varying degrees.

When vernalized, all F_2 plants were able to flower, but their fertility was highly variable. As shown in Table 2, approximately half of them were sterile or nearly so (fewer than ten
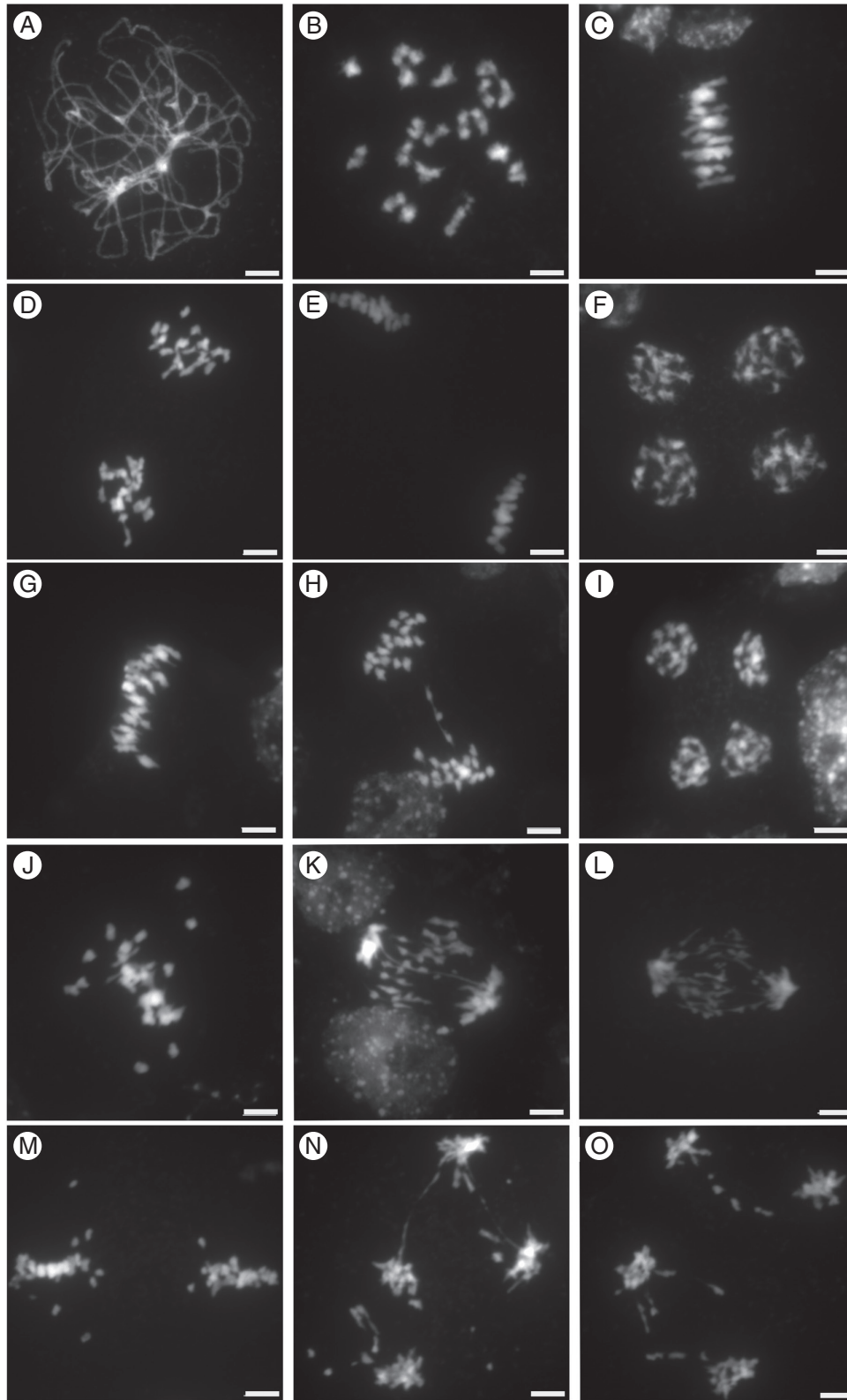
FIG. 2. Meiosis in *C. sativa*, *C. microcarpa* and their *F*₁ hybrid. DAPI staining of male meiotic cells in *C. sativa* 'Céline' (A–F), *C. microcarpa* Fleurey (G–I) and *C. microcarpa* Fleurey × *C. sativa* 'Céline' *F*₁ hybrids (J–O). Pachytene (A); diakinesis (B); metaphase I (C,G, J); anaphase I (D, H, K, L); metaphase II (E, M), early anaphase II (N,O); telophase II (F, I). Scale bar = 5 μm.

FIG. 3. Phenotype of F_2 plants. (A) A control *C. sativa* plant (left) and four representative F_2 individuals are shown 2 months after sowing, without vernalization. (B) Two-month-old F_2 plants seen from above. (C) A normal inflorescence is shown in comparison with abnormal floral development of (D) plant F_2-B5 and (E) plant F_2-E7, shown 3 months after sowing.
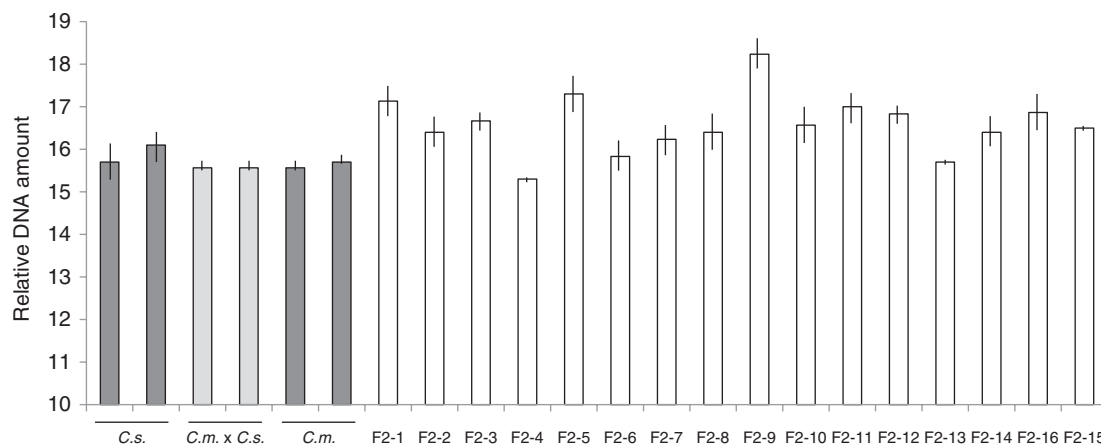

FIG. 4. Flow cytometry of nuclear DNA amounts. Two individuals of the parental *C. sativa* and *C. microcarpa* parents (dark grey bars), two F_1 *C. microcarpa* × *C. sativa* hybrids (light grey bars) and 16 F_2 individuals (white bars) are shown. Relative DNA amount refers to the position of peaks on the flow cytometer readout. The analysis was carried out in triplicate; mean ± s.e.m. values are shown. *C.s.*, *C. sativa*; *C.m.* × *C.s.*, F_1 hybrid; *C.m.*, *C. microcarpa* Guillestre.

seeds were harvested from plants indicated <0.01 g). However, four F_2 individuals produced abundant seeds (F_2-3, F_2-13, F_2-17 and F_2-28), but F_3 seed quality was highly heterogeneous,

with many seeds showing diverse abnormalities, such as abortion, incomplete filling and premature germination (Fig. 5). The viability of F_3 seeds was not evaluated.
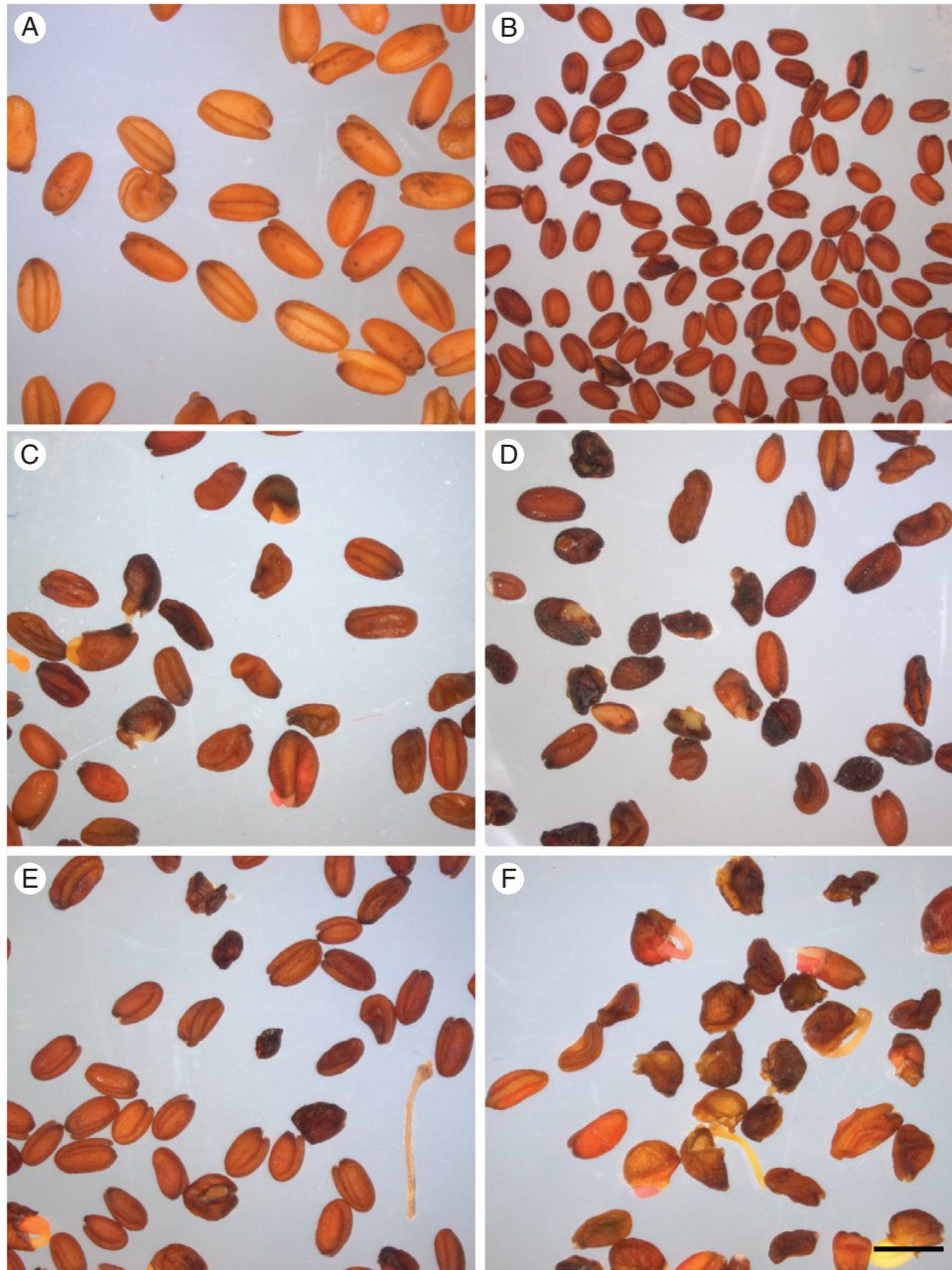
FIG. 5. Seeds produced by *C. sativa*, *C. microcarpa* Guillestre and F_1 and F_2 progeny. Seeds produced by self-pollinated *C. sativa* (A), *C. microcarpa* (B), F_1 hybrids F_1-A (C) and F_1-B (D), and F_2 hybrids F_2-3 (E) and F_2-13 (F). Scale bar = 2 mm.

Lipid composition of F_3 *seeds*

A major objective of research in camelina is modification of the seed oil composition, so it was of interest to evaluate whether the lipid composition of the seeds produced by hybrid progenies was as profoundly modified as the F_2 plant phenotypes. Since each seed within an F_3 population is likely to be genetically distinct, in particular since many F_2s were probably aneuploid, the fatty acid composition of three seeds produced by each F_2 plant was analysed individually; representative results are shown in Fig. 6A. The fatty acid profiles of the parental species and the F_1 hybrids were essentially identical, with C18:3 >

C18:2 > C18:1 = 20:1 > C16:0. As expected, the fatty acid composition within many F_3 populations was quite heterogeneous, suggesting that genes controlling fatty acid composition were segregating within these populations. One F_3 population, F_3-18, was clearly different, with all three seeds showing greatly increased levels of C18:2, and with C18:3 correspondingly reduced to near zero. Since the F_2-18 plant produced only three seeds, its progeny could not be analysed further. The three F_3-6 seeds displayed a similar but less drastic change in C18:2 and C18:3, and since F_2-6 produced sufficient seed, more seeds of the F_3-6 population were analysed (Fig. 6B). These results
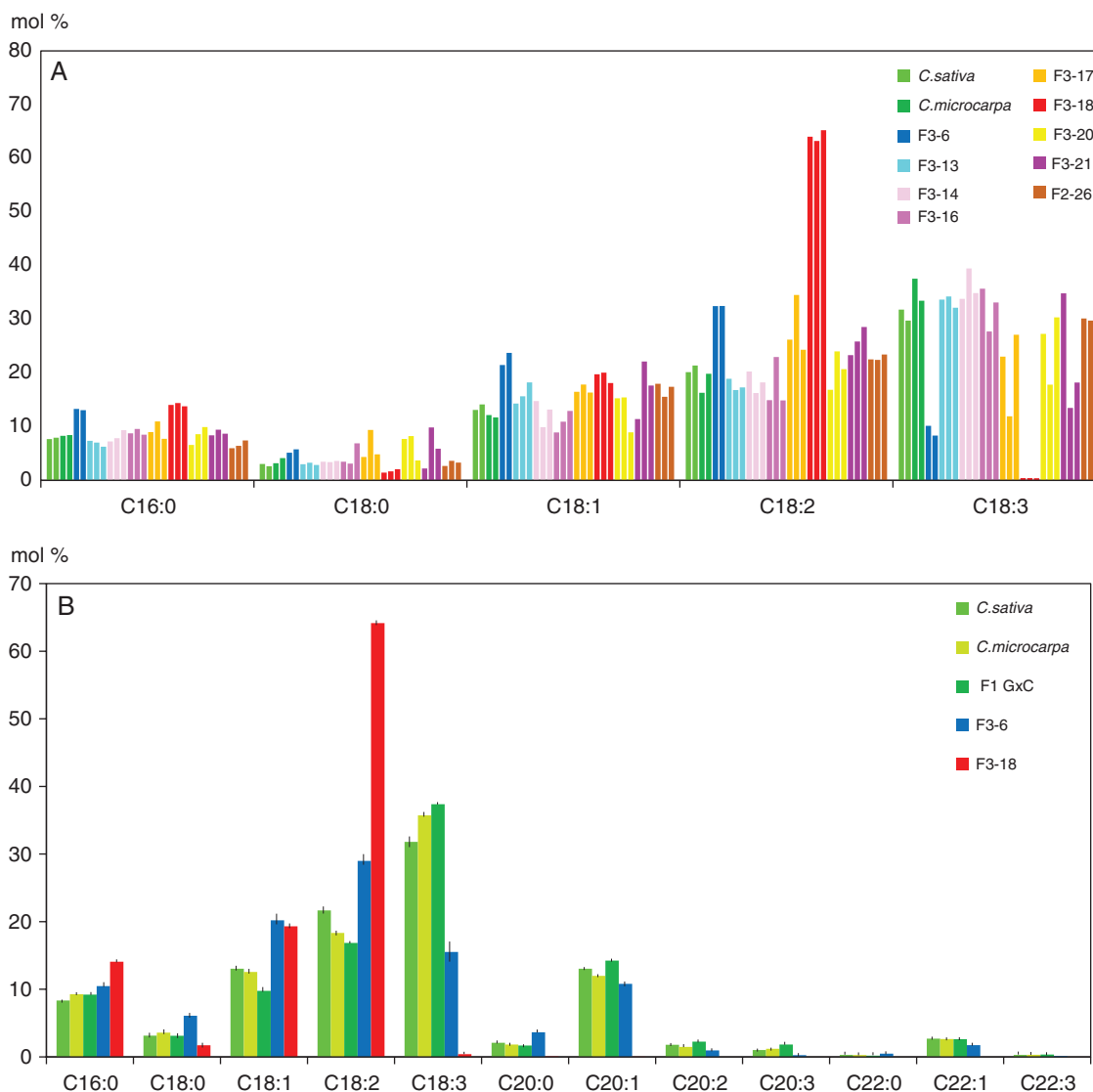
FIG. 6. Relative fatty acid profiles of F_2 and F_3 hybrid seeds. (A) Major fatty acid profile of individual seeds of nine F_3 populations and controls was analysed by GC–MS. For each population, two or three seeds were analysed. Each bar represents a single seed. (B) Mean values ($\pm$ s.e.m.) obtained with a greater number of individual seeds. F_1 G×C = F_2 seeds produced by the cross *C. microcarpa* × *C. sativa.* For all samples $n = 9$, except F_1 G×C ($n = 4$) and F_3-18 ($n = 3$).

confirm that the fatty acid composition of the F_3-6 seeds is distinct from that of the controls, with an increased level of C18:2 and decreased C18:3.

DISCUSSION

Camelina is a re-emerging crop that shows limited genetic diversity (Vollmann *et al.*, 2005; Ghamkhar *et al.*, 2010; Manca *et al.*, 2013; Galasso *et al.*, 2015; Singh *et al.*, 2015; Kurasiak-Popowska *et al.*, 2018; Luo *et al.*, 2019). Our aim in this study was to explore whether *C. microcarpa* could be used as a source of useful genes to widen the genetic diversity of *C. sativa*. Our results suggest that this approach is not as simple as it looked.

Our results first confirmed previous findings that *C. sativa* and *C. microcarpa* are sexually compatible (Séguin-Swartz

et al., 2013; Martin *et al.*, 2019). We observed that the F_1 hybrids, unremarkably, displayed a morphological phenotype intermediate between that of the two parental species during the vegetative phase, and the lipid composition of the F_1 seeds was equivalent to that of the parents. Transgressive phenotypes were also observed for some traits, such as the concentrations of certain primary metabolites following virus infections, which were markedly higher in the F_1 hybrids than in both parental species (Chesnais *et al.*, 2019).

We also observed that F_1 *C. microcarpa* × *C. sativa* hybrids showed very reduced fertility. This is very likely due to the frequent and diverse meiotic abnormalities we observed in these plants, in particular the presence of univalents, bridges and fragments in many cells. Likewise, observation of bridges at anaphase I/telophase I or anaphase II/telophase II may be indicative of chromosome and/or sister-chromatid breakage and rejoining

([Fig. 2](); Supplementary Data [Fig. S1]()). Although further studies are required to analyse the exact nature and mechanisms associated with the observed meiotic defects (e.g. [Friebe *et al.*, 2005]()), their wide occurrence does not support the assumption that *C. sativa* was domesticated from *C. microcarpa* ([Brock *et al.*, 2018]()), or that it was from *C. microcarpa* genotypes that would be quite different from those used in this study. Considering the meiotic abnormalities we observed in the *C. microcarpa* × *C. sativa* F_1s, it is also surprising that [Mandáková *et al.* (2019)]() observed no differences in chromosome structure between *C. sativa* and the *C. microcarpa* accession they studied. This apparent discrepancy raises the question whether different *C. microcarpa* accessions may in fact have different chromosome organizations, some that are *sativa*-like (e.g. ones in which the F_1s have higher fertility), and others with more divergent structure. In this connection, we observed varying degrees of meiotic defects in the two hybrids we characterized. It was also observed that certain Canadian *C. microcarpa* accessions yielded F_1s with distinctly higher levels of pollen viability (up to 45 %) in crosses with *C. sativa* ([Séguin-Swartz *et al.*, 2013](); [Martin *et al.*, 2019]()). We do not know whether these hybrids show a more regular meiosis compared with those characterized here, but these genotypes might be better candidates for introgressing genes from *C. microcarpa* into the crop. Furthermore, these results suggest that the likelihood of introgression of transgenes from genetically modified camelina would be lower with the *C. microcarpa* accessions studied here compared with accessions with higher F_1 fertility ([Séguin-Swartz *et al.*, 2013](); [Martin *et al.*, 2019]()).

Surprisingly, and in spite of the low F_1 fertility, some F_2 plants can be obtained, which makes it possible move on to the next generations. Notably, we identified four F_2 individuals (out of 41) that produced a significant number of seeds, which suggests, rather surprisingly, that later generations of self-progeny could be readily obtainable. We also observed lipid profiles that were unlike those of the parental species in the progeny of certain F_2s. This suggests that interspecific hybrids could indeed be a useful source of novel traits in camelina. However, considering the number of meiotic abnormalities observed in the F_1 progenies and the aneuploidy of many F_2 individuals, returning to the normal genome structure of cultivated camelina through a series of backcrosses may remain an arduous process.

Altogether our results demonstrate that *C. sativa* and *C. microcarpa* may be less related to one another than previously thought, or that there is a great diversity of genome structure within *C. microcarpa*. In terms of pre-breeding (gene introgression from *C. microcarpa* into cultivated camelina), our results suggest that, at least for *C. microcarpa* genotypes similar to those studied here, this would not be a project to be engaged in lightly.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: meiosis in *C. microcarpa* Guillestre and its F_1 hybrid with *C. sativa* 'Céline'.

FUNDING

ACKNOWLEDGMENTS

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