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Improvement in hydroxy fatty acid seed oil content and other traits from interspecific hybrids of three *Lesquerella* species: *Lesquerella fendleri*, *L. pallida*, and *L. lindheimeri*

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Summary

Interspecific hybridization offers potential to improve the hydroxy fatty acid (HFA) content of lesquerella. *Lesquerella fendleri* is currently being developed for cultivation as a potential new industrial oilseed crop because of its seed productivity. However, it has lower HFA content compared to *L. pallida* and *L. lindheimeri*. The objectives of this research were to improve the HFA oil content of *L. fenderi* through interspecific hybridization and to characterize hybrids and successive generations for seed oil fatty acid profile, fertility, seed set and other morphological traits. In this work, three species were successfully hybridized, self-pollinated, and backcrossed. Ovule culture was used in some cases to produce progeny where interspecific hybrids did not produce viable embryos. The traits measured were petal length, ovules per silique, seeds per silique, and weight of 1000 seed. Patterns of leaf trichomes were used to distinguish between parents and hybrids. Seed per silique indicated that autofertility occurred in *L. pallida* but not in the other two species. HFA oil content of *L. fendleri* seed were 50.5% compared to 80 and 84% for *L. pallida* and *L. lindheimeri*, respectively. The HFA oil content of the hybrids ranged from 57 to 70% in A_2 and A_3 generations, and the range of values depended on the parents used in the crosses. These measurements will help predict the value of different interspecific crosses for breeding. Segregation for various yield related traits should allow selection for favorable improvements in the HFA trait and in seed yield.

Introduction

Lesquerella and *Physaria* species (Brassicaceae) are known for their seed oil, which contain hydroxy fatty acids (HFA) used for industrial purposes. There are approximately 87 North American species of *Lesquerella* and 24 species of *Physaria,* a closely related genus. *Lesquerella fendleri* is being developed for commercialization in preference to other species because of its productivity and adaptability to farm management practices (Dierig et al., 1993). The types of HFA consist of either lesquerolic (C20:1OH), auricolic (C20:2OH), or densipolic (C18:2OH) acids as the primary component of their seed oil.

Lesquerella species native to the western U.S. contain lesquerolic HFA which is similar to ricinoleic acid (C18:1OH) found in castor (*Ricinus communis* L.), except for two extra carbons in chain length. The longer chain length helps set lesquerella apart from castor, which is imported to the U.S. The potential applications of lesquerella oil include biodegradable lubricants, novel plastics, lithium greases, protective coatings, surfactants, drying agents, cosmetics, and pharmaceuticals (Roetheli et al., 1991). The seed meal contains antioxidants from glucosinolates that extend the oil stability (Abbott et al., 1997). Some species of *Lesquerella* have naturally occurring estolides that improve the pour points of vegetable oils, and have

been used as viscosity modifiers in lubricating oils (Hayes et al., 1995; Isbell & Cermak, 2002).

Seed of many of the *L. fendleri* accessions are from either wild populations or open pollinated bulk seed. Thus, this species appears to be very diverse phenotypically and genotypically. However, the range of natural variability for lesquerolic HFA is limited to between 45 and 55% of the total fatty acids (Dierig et al., 1996). Other species of *Lesquerella* have up to 85% of this acid but they lack other important yield related traits found in *L. fendleri* (Hayes et al., 1995; Dierig et al., 1996). Introgressing the high amounts of HFA, as well as other favorable traits, into *L. fendleri* from other species is one of the approaches used in our program to domesticate this species.

Attempts at an intertribal transfer of the HFA trait of *Lesquerella fendleri* into *Brassica napus* using somatic hybrids via protoplast fusion have been reported (Skarzhinskaya et al., 1996, 1998). Segregates were biased, favoring the *B. napus* cultivar for many morphological traits. Schröder-Pontoppidan et al. (1999) reported that variation occurred in succeeding generations in erucic and eicosaenoic acids. Only a few seeds contained any HFA by the F6 generation. These were in low amounts of either ricinoleic or densipolic HFA. None contained lesquerolic acid which is the predominant HFA found in *L. fendleri*.

Flowers of *Lesquerella* occur indeterminately on inflorescences, and following anthesis, seeds develop in siliques attached along the axis of the infructescence. A silique resulting from pollination of a single flower may contain up to 32 seeds. Seed yield is determined by the number of siliques, the number of seeds per silique, and seed weight.

Plants of *Lesquerella* species from the western U.S. are incompatible due to a sporophytic, multiple allele system that exists within the genus (Sampson, 1958). Natural hybridization does not occur between these western species, possibly due to identical SI alleles occurring between species. *Brassica campestris* and *B. oleracea* both possess effective sporophytic incompatibility systems and are the parental species of *B. napus,* which is self-fertile (Gómez-Campo, 1999). The incompatibility system in this case was weakened in polyploidy.

Eastern U.S. species of *Lesquerella* readily hybridize and are able to produce segregating generations (Rollins & Solbrig, 1973). This is the first report of the analysis of segregating generations of interspecific hybridization between western U.S. *Lesquerella* species, which in nature are incompatible.

Several *Lesquerella* species are on the federal endangered species list. *Lesquerella pallida,* used in this study, had not been relocated from an original collection in 1830 until 1981 when it was rediscovered in eastern Texas. It is estimated to occur over a two ha area (Nixon et al., 1983).

Our objective was to introgress the trait for high HFA content from two different species into *L. fendleri.* One high HFA species was *L. pallida*, the other was *L. lindheimeri.* We also sought to characterized hybrids and successive generations for seed oil fatty acid profile, fertility, seed set, and other morphological traits.

Materials and methods

Plant material

The parental plants used for pollinations included *L. fendleri*, *L. pallida*, and *L. lindheimeri*. Table 1 contains all the genotypes used and the ploidy level of each. These species were chosen because of the high HFA oil content of the latter two and because all three had the same haploid chromosome number of $n = 6$. Crosses included combinations of *L. fendleri* with one of the other two species, since *L. fendleri* is the most productive and best suited for domestication (Dierig et al., 1993). The plants of *L. fendleri* were from a released germplasm line selected for high oil content and seed yield (Dierig et al., 2001). *L. lindheimeri* seed was collected in Texas in 1995 and increased at the USDA, ARS, U.S. Water Conservation Laboratory (USWCL), Phoenix, Ariz. (Dierig et al., 1996). Seed of *L. pallida* were obtained in 1996 from a 1991 collection in Texas, maintained by San Antonio Botanical Gardens.

Greenhouse pollinations, ovule culture, and cytological examinations

Most crosses, except self pollinations of *L. pallida,* were made by bud pollination to overcome incompatibility. Flowers of interspecific crosses were emasculated and pollen was applied directly to the stigma. Pollinated flowers were then bagged with 5 × 18 cm glassine envelopes (Midco Enterprises, *St. Louis, MO*) adjusted in length for individual inflorescence. *Lesquerella pallida* produces seed through both auto- and allogamy and therefore plants used for self-pollinations were bagged but not bud pollinated. Pollinations were

Table 1. Genotypes, ploidy levels, and designations used for parents, interspecific hybrids, and successive generations

Genotype	Designation of genotype	Ploidy level	Colchicine treated	Generation
L. pallida	P	2x = 12	No	Parent
L. lindheimeri	L	2x = 12	No	Parent
L. fendleri	F	2x = 12	No	Parent
L. fendleri	F	4x = 24	Yes	Parent
L. pallida × *L. fendleri*	P × F	4x = 24	Yes	[a]A_1
L. fendleri × *L. pallida*	F × P	4x = 24	Yes	A_1
L. lindheimeri × *L. fendleri*	L × F	4x = 24	Yes	A_1
L. pallida × *L. fendleri*	P × F	4x = 24	Yes	A_2
L. pallida × *L. fendleri*	P × F	4x = 24	Yes	A_3
(*L. lindheimeri* × *L. fendleri*) × *L. fendleri*	L × F × F	3x = 18	Yes	BC_1A_1
(*L. lindheimeri* × *L. fendleri*) × *L. fendleri* × *L. fendleri*	L × F × F × F	3x or 4x	Yes	BC_2A_1
(*L. pallida* × *L. fendleri*) × *L. fendleri*	P × F × F	4x = 24	Yes	BC_1A_1
(*L. pallida* × *L. fendleri*) × *L. fendleri*	P × F × F	3x = 18	Yes	BC_1A_1
(*L. pallida* × *L. fendleri*) × *L. pallida*	P × F × P	3x = 18	Yes	BC_1A_1
(*L. fendleri* × *L. pallida*) × *L. fendleri*	F × P × F	3x = 18	Yes	BC_1A_1
(*L. fendleri* × *L. pallida*) × *L. pallida*	F × P × P	3x = 18	Yes	BC_1A_1

[a]"A" refers to the amphidiploid of F_1, F_2, andF_3 generations.

performed from December until June for three seasons (2000–2003) in a glass greenhouse at the USWCL.

Swollen siliques were excised 7 to 13 days after pollination (DAP) and aseptically dissected in the laboratory. Developing ovules (both healthy and shriveled) were placed into 60 × 15 mm plates containing 0.005 l of Murashige and Skoog (MS) medium (pH 5.7) supplemented with 0.5 g l^{-1} casein hydrolysate and 1.0 g l^{-1} gibberellic acid, at 25 °C under continuous illumination. After four weeks, germinated ovules were transferred to 100 × 25 mm plates containing 0.025 l of liquid MS medium with 0.1% colchicine and placed on a gyratory shaker at 60 rpm for 48 h to induce amphidiploidy in interspecific hybrids. After the colchicine treatment, ovules were rinsed in 0.025l of hormone-free liquid MS medium (pH 6.0) for 24 h and then plated into a solid MS medium supplemented with 1.0 mgl^{-1} kinetin and 4.25 mgl^{-1} silver nitrate ($AgNO_3$) to induce shoot initiation. Surviving cloned explants were rooted in GA7 vessels (Magenta Corp., *Chicago, IL*) containing 0.1l of liquid MS medium (pH 6.0) supplemented with 0.1 mgl^{-1} auxin (NAA) and transferred to peat pellets (Jiffy Products Ltd., *Shippagan, New Brunswick, Canada*) in the greenhouse. Five amphidiploid plants were cytologically confirmed by pollen mother cell (PMC) analysis.

Measurements

Ovules were counted from 10 siliques of each plant of the original species or hybrid crosses. Petal length was determined by measuring each of the four petals for 10 flowers per plant. Five leaf trichomes were chosen at random from both leaf surfaces and the number of rays counted. The trichomes were scored as fused or non-fused at the base of the tubercles, and forked or non-forked. These measurements were expressed as a percent of the five trichomes counted. Means and standard deviation of measurements were calculated.

Seed oil was analyzed for fatty acid composition on a HP 5890 gas chromatograph (GC) with a 25 m × 0.25 mm i.d. column (Hewlett-Packard, Palo Alto, CA) according to the method described by Dierig et al. (1996). Approximately 50 seeds were used for analysis except the A_3 seed, which were analyzed using only a half seed to conserve hybrids. The half seed analysis was carried out at the USDA, ARS, NCAUR, Peoria, IL, on a HP 5890 GC with a nonpolar 30 m × 0.22 mm i.d. column. A breeding line with seed from a homogeneous single plant was used as a check for both the half seed and bulk fatty acid analysis Seeds were cut in half so that distal ends of cotyledons were used for GC analysis while the remaining half with the proximal

portion of the cotyledons and the intact radicle were used for planting.

Results and discussion

Seed, flower, and trichome measurements

The three parental genotypes could be distinguished from each other based on a combination of the morphological traits listed in Table 2. *Lesquerella lindheimeri* plants had fewer ovules (9.8 ± 1.3) (mean ± standard deviation) and seeds per silique (7.8 ± 2.1) than the other two parents, *L. pallida* (18.1 ± 0.9 and 11.5 ± 1.85, respectively) and *L. fendleri* (24.9 ± 3.77 and 10.0 ± 4.69, respectively), but it had larger seed (1.09 ± 0.22 compared to 0.57±0.04 and 0.64 ± 0.11). *Lesquerella fendleri* has the potential for higher seed yields based on the number of ovules per silique, although the number of seeds per silique was similar to *L. pallida* and *L. fendleri*. Under normal irrigated field conditions, *L. fendleri* produces a greater number of seeds per silique because the plants are cross-pollinated rather than self-pollinated, as was required for this experiment. Outcrossed *L. fendleri* plants have much higher seed set than selfed plants (unpublished data). Self-pollinations in other taxa significantly reduced seed set due to either self-incompatibility (SI) preventing fertilization, or recessive deleterious alleles causing the zygotes to abort (Sage et al., 1999; Gueritaine et al., 2003). Although SI exists in most *Lesquerella* species, *L. pallida* appeared to be self-compatible. Self-incompatibility is overcome in *L. fendleri* by bud pollination indicating that either SI is late acting, or there is a prezygotic abortion of the ovules. *Lesquerella fendleri* varied more in number of ovules and seeds per silique than the other two species as indicated by the higher standard deviations. The petal lengths were also larger than in *L. lindheimeri* and *L. pallida* (10.4 ± 1.05 compared to 7.3 ± 0.77 and 6.4 ± 0.46).

The only differences between colchicine-treated *L. fendleri* amphidiploids and diploid plants were fewer seeds per silique, 3.8 ± 0.76 compared to 10.0 ± 4.69, and larger seed size, 0.87 ± 0.15 compared to 0.64 ± 0.11. Doubling of the chromosomes may have affected chromosomal pairing or caused a deleterious dosage effect which led to some aborted ovules, reducing seed set in the 4x plants. No difference in number of ovules per silique was found between the two. Flower sizes were only slightly, but not significantly larger.

Reciprocal interspecific A_1 hybrids between *L. fendleri* and *L. pallida* resulted in different flower colors. When the white-flowered *L. pallida* was used as the maternal parent (PXF), flowers were an intermediate pale yellow color and petal lengths were larger than either parent. In the reciprocal cross (FXP), flowers were

Table 2. Mean characteristics and standard deviations of parents, interspecific hybrids, and successive generations

Genotype	Number plants	Generation	Ovules/ silique	Seed /silique	1000 seed (g)	Petal length (mm)	Flower color	Pollen
P	11	Parent	18.1 ± 0.94	11.5 ± 1.85	0.57 ± 0.04	6.4 ± 0.46	White	Fertile
L	12	Parent	9.8 ± 1.30	7.8 ± 2.13	1.09 ± 0.22	7.3 ± 0.77	Yellow	Fertile
F	12	Parent	24.9 ± 3.77	10.0 ± 4.69	0.64 ± 0.11	10.4 ± 1.05	Yellow	Fertile
F [a]	6	Parent	25.2 ± 3.08	3.8 ± 0.76	0.87 ± 0.15	11.2 ± 1.42	Yellow	Fertile
P × F	5	A_1	19.2 ± 1.6	1.7 ± 0.82	1.03 ± 0.49	12.7 ± 1.16	Pale Yellow	Mixed
F × P	7	A_1	21.3 ± 1.28	NA[b]	NA	8.2 ± 0.55	White	Mixed
L × F	80	A_1	20.6 ± 3.6	NA	NA	7.7 ± 0.99	Yellow	Sterile
P × F	20	A_2	15.6 ± 2.0	5.0 ± 0.5[c]	1.23 ± 0.05	11.3 ± 1.07	Pale Yellow	Fertile
L × F × F	15	BC_1 A_1	22.0 ± 2.6	NA	NA	7.3 ± 0.82	Yellow	Sterile
L × F × F × F	5	BC_2 A_1	20.4 ± 3.8	NA	0.50 ± 0.06	10.0 ± 1.6	Yellow	Fertile
P × F × F	30	BC_1 A_1	19.8 ± 4.30	3.02 ± 0.5	0.75 ± 0.1	10.4 ± 1.18	Yellow	Fertile
P × F × P	5	BC_1 A_1	13.2 ± 1.6	NA	0.67 ± 0.08	10.3 ± 0.9	White	Fertile
F × P × F	10	BC_1 A_1	23.0 ± 2.4	NA	0.87 ± 0.11	11.3 ± 1.6	Yellow	Mixed
F × P × P	4	BC_1 A_1	15.5 ± 0.86	NA	NA	9.8 ± 0.37	White	Sterile

[a]Colchicine treated $n = 4x = 24$.
[b] Data not available.
[c]Mix of shriveled and normal seed.

white. When either PXF or FXP was backcrossed to *L. fendleri* as the male parent, flower color was yellow. Flowers were white when either was backcrossed to *L. pallida* as the male parent. This could be an indication of paternal inheritance for flower color possibly caused by a mitochondrially-associated plasmid (Erickson & Kemble, 1990). However, more segregating progeny are needed to determine the exact mode of inheritance.

The ovules per silique in both reciprocal A_1 PF hybrids were about the same number as *L. pallida*. However, the seed weight in the PXF crosses was 1.03 ± 0.49, almost twice the size of either *L. pallida* or *L. fendleri*, even though seed set was low. Of the five A_1 plants measured, three were sterile and two produced pollen. In the reciprocal cross (FXP) with the yellow flowered *L. fendleri* as the maternal parent, A_1 flowers were white and petal lengths were 8.2 ± 0.55 which were intermediate between the *L. fendleri* (10.4 ± 1.05) and *L. pallida* (6.4 ± 0.046) parents. The A_1 plants had mixed pollen fertility but no seed was produced from these five plants. It is not yet known if flower color could affect insect preference for pollination of the hybrids compared to the parents. The differences between reciprocal crosses for seed set could be attributed to the self incompatibility system that regulates pollen/pistil interactions (Gueritaine et al., 2003).

In the backcross generations of both the PXF and FXP plants, there was a dosage effect on the number of ovules per silique. The more *L. fendleri* genome was represented, the higher the number of ovules per silique. However, when backcrossed to *L. pallida* the number of ovules per silique decreased to 13.2 ± 1.6 compared to 19.2 ± 1.6 from the PXF, and 21.3 ± 1.28 from the FXP hybrids.

In the *L. lindheimeri* × *L. fendleri* (LXF) A_1 plants, the number of ovules per silique was 20.6 ± 3.6 which was similar to the *L. fendleri* parent of 24.9 ± 3.77. No seed were produced because the hybrid plants measured were sterile. Petal lengths of LXF A_1 plants were 7.7 ± 0.99, which were similar to the maternal parent (7.3 ± 0.77), and 7.3 ± 0.82 in LXFXF BC_1A_1 generation. The reason for the smaller petal lengths could be that the hybrids did not double in chromosome number and were an F_1 rather than an A_1 generation. Hybridity was not in question because trichome morphology was intermediate. Only after the second generation of backcrossing were the LXF plants fertile. The number of ovules per silique in these generations was similar.

The only plants which produced A_2generations for this study were from the PXF hybrids. The number of ovules per silique in the A_2generation was 15.6 ± 2.0, which was less than either original parent (18.1 ± 0.94 and 24.9 ± 3.77) or the A_1 generation (19.2 ± 1.6). The seed weight for this sample was double that of either parent but seed set was still poor.

Table 3. Leaf trichome characteristics and standard deviations of three *Lesquerella* species, interspecific hybrids, and backcross generations

			Tubercles	
Genotype	Generation	Rays/trichome	Fused (%)	Forked (%)
P	Parent	6.08 ± 0.41	0	64
L	Parent	5.64 ± 0.43	0	96
F	Parent	16.24 ± 1.7	100	0
P × F	A_1	10.0 ± 0.92	0	80
L × F	A_1	10.2 ± 1.54	28	14
P × F × F	BC_1 A_1	15.1 ± 2.77	44	23
P × F × P	BC_1 A_1	11.84 ± 1.3	48	68
F × P × F	BC_1 A_1	15.42 ± 1.9	58	32
F × P × P	BC_1 A_1	13.95 ± 1.3	40	100
L × F × F	BC_1 A_1	11.5 ± 1.29	37	23
L × F × F × F	BC_2 A_1	17.6 ± 1.92	61	27

Trichome morphology was useful in distinguishing *L. fendleri* from the other two species and the parents from hybrids (Table 3). The trichomes of *L. fendleri* are always fused for half or more of their length. The high density of trichomes on leaves of *L. fendleri* gives them a silvery-gray appearance compared to the other two species. Rollins & Shaw (1973) noted that this characteristic sets *L. fendleri* apart from the majority of other North American species. The number of rays per trichome and lack of forking of the tubercles also distinguished *L. fendleri* from *L. lindheimeri* and *L. pallida*. These traits appear stable enough to environmental influences to confirm hybridity among the three species in this study. The rays per trichome of the A_1 hybrids were intermediate between the parents. None of the tubercles were fused when *L. pallida* was used as the maternal parent. However, in the BC_1A_1 and BC_2A_1 generations, the proportion of fused and/or forked tubercles varied considerably. The number of rays per trichomes for the most part corresponded with the number of doses that *L. fendleri* contributed. The LXFXF cross was an exception to this.

Seed oil characteristics

The lesquerolic acid content of the *L. pallida* parent line in 2003 was surprisingly 30% lower than when tested the previous year (Table 4). There were adequate

Table 4. Means and standard deviations of fatty acid seed oil profile of parents and interspecific backcross generations

Genotype	Num Plts.	C16:0 Palmitic (%)	C16:1 Palmitoleic (%)	C18:0 Stearic (%)	C18:1 oleic (%)	C:18:2 linoleic (%)	C18:3 linolenic (%)	C18:2-OH densipolic (%)	C20:1-OH lesquerolic (%)	C20:2-OH auricolic (%)
P (2003)	10	2.8 ± 0.5	2.1 ± 0.4	3.4 ± 0.4	17.2 ± 1.3	19.8 ± 3.7	6.5 ± 1.0	0.0	49.4 ± 3.6	0.0
P (2002)	5	1.8 ± 0.2	0.0	1.1 ± 0.5	6.1 ± 2.5	5.3 ± 0.5	4.2 ± 0.7	1.0 ± 0.5	80.1 ± 1.2	0.0
L[a]	12/5	1.2 ± 0.1	0.02 ± 0.1	1.9 ± 0.2	5.4 ± 1.2	4.1 ± 0.5	1.9 ± 0.2	1.4 ± 0.1	84.0 ± 1.5	0.0
F[a]	12/5	1.3 ± 0.3	0.2 ± 0.1	1.9 ± 0.9	18.0 ± 1.2	7.8 ± 0.7	14.7 ± 1.0	0.4 ± 0.1	50.4 ± 1.4	3.3 ± 0.8
F[b]	5	1.7 ± 0.1	0.0	2.5 ± 0.4	19.4 ± 2.5	8.1 ± 0.6	15.2 ± 0.9	1.0 ± 0.1	50.1 ± 2.4	2.3 ± 1.2
P × F × F	47	2.6 ± 0.2	1.8 ± 0.3	3.2 ± 0.4	42.8 ± 5.8	7.0 ± 0.8	7.6 ± 0.7	1.2 ± 0.03	29.6 ± 5.0	2.0 ± 0.2
P × F × F[c]	1	2.2	0.0	2.4	25.3	9.5	7.7	1.4	50.1	0.0
P × F × P	5	1.6 ± 0.1	0.7 ± 0.1	1.7 ± 0.1	13.3 ± 1.1	8.1 ± 0.4	11.2 ± 1.5	1.1 ± 0.2	58.2 ± 2.1	2.4 ± 0.7
F × P × F	18	1.5 ± 0.2	0.6 ± 0.04	1.6 ± 0.1	13.6 ± 1	8.1 ± 0.5	11.5 ± 0.6	0.6 ± 0.3	57.4 ± 1.9	3.8 ± 0.3
L × F × F × F	4	1.8 ± 0.2	0.9 ± 0.2	2.5 ± 0.2	16.9 ± 2.0	9.6 ± 1.2	11.7 ± 0.8	1.0 ± 0.1	52.1 ± 3.0	2.4 ± 0.4
A_2 P × F-7	1	2.37	0.0	2.01	8.30	7.12	8.70	0.0	69.9	0.0
A_3 P × F-7.24	4	3.0 ± 0.4	0.6 ± 0.4	2.0 ± 0.2	5.6 ± 0.3	6.1 ± 1.2	11.4 ± 1.9	0.0	66.4 ± 3.3	0.6 ± 0.1
A_3 P × F-7.19	2	4.4 ± 0.4	0.6 ± 0.6	2.8 ± 0.2	5.4 ± 0.5	7.3 ± 0.3	13.4 ± 0.1	0.0	58.4 ± 0.6	0.6 ± 0.1
A_3 P × F-7.22	5	4.1 ± 0.7	1.3 ± 0.3	2.6 ± 0.3	6.9 ± 0.6	9.9 ± 1.9	11.4 ± 1.3	0.0	56.6 ± 2.7	0.3 ± 0.2
A_3 P × F-11	4	3.0 ± 0.4	1.2 ± 0.2	2.0 ± 0.3	8.4 ± 1.8	6.0 ± 0.5	13.0 ± 1.2	0.0	59.5 ± 0.3	0.7 ± 0.4

[a]Mean of years 2002 and 2003 data.
[b]4x amphidiploid.
[c]Backcross pollen parent was 4x amphidiploid.

numbers of samples analyzed to ensure that this was not attributed to immature seed or errors associated with the GC. In both years the seed analyzed was obtained from controlled self pollinations. Plants from the previous year were those used in developing the backcross and segregating hybrid generations presented herein. The plants used in the backcrosses were derived from the same seed as those used for oil measurements.

Hayes et al. (1995) reported differences in the proportion of lesquerolic acid bonded to the *sn*-2 position of the triacylglycerol (TG) positions between *L. fendleri* and *L. lindheimeri*. All of the lesquerolic acyl groups are located at the *sn*-1 and *sn*-3 positions in *L. fendleri* and excluded from the *sn*-2 position, whereas lesquerolic acid in *L. lindheimeri* is present in significant amounts in all three positions. The inability of *L. fendleri* to include lesquerolic acid at the *sn*-2 position limits the theoretical upper limit of lesquerolic acid to 66% of the total oil content. This difference in the ability to have significant amounts of lesquerolic acid in the *sn*-2 position accounts for the higher lesquerolic content (84.0 ± 1.5%) in *L. lindheimeri* (Table 4). This is the first report of the fatty acid profile of *L. pallida,* and therefore, the structure of the TG has never been reported. *Lesquerella pallida* must theoretically be able to incorporate lesquerolic acid at all three*sn* positions of the TG molecule because in year 2002 of this study the lesquerolic content of the oil was greater than 66% (Table 4). Since this species is rare and on the federal endangered list, no reports have been published characterizing its oil. It could be that some environmental or genetic factor, such as a maternal inheritance effect, triggers whether all three positions contain lesquerolic acyl groups leading to higher amounts of the TG. Engeseth & Stymne (1996) showed that linseed microsomal membranes desaturate rincinoleic acid at the *sn*-3 TG position without a loss of activity. The desaturation activity could be occurring in *L. pallida* but the initiation of the enzyme is not known. Maternal effects on fatty acid profiles are known to occur in other oilseed crops such as soybeans (Brim et al., 1968) and rapeseed (Downey & Craig, 1964).

Significant differences between the two years for *L. pallida* were also found in C18:1 (6.1 ± 2.5% and 17.2 ± 1.3%) and C18:2 contents (5.3 ± 0.5 and 19.8 ± 3.7). Differences between this *L. pallida* profile and *L. fendleri* were more pronounced with C18:2 and C18:3 contents. Reed et al. (1997) confirmed that *L. fendleri* accumulates lesquerolic acid as a result of hydroxylation of oleic acid followed by conversion via

elongation. It appears the higher amount of C18:1 and C18:2 are a result of low hydroxylase or elongase activation, or more generally, a reduction in the efficiency of the hydroxylation pathway.

All parent plants were diploids $2n = 12$. The colchicine-treated *L. fendleri* amphidiploids were similar in oil profile to the diploid *L. fendleri* plants.

The two types of PXFXF backcross hybrids differed from each other in oil profiles. One was a result of an amphidiploid plant crossed to a diploid *L. fendleri* plant, resulting in triploid offspring, the other was backcrossed to a colchicine treated tetraploid *L. fendleri* plant resulting in a tetraploid offspring. The lesquerolic acid content with a balanced chromosome set ($4x \times 4x = 4x$) was 50.1% compared to $29.6 \pm 5.0\%$ in plants with unbalanced sets ($4x \times 2x = 3x$). It is unclear if the proportion of lesquerolic acid in the other putative triploids was low compared to the tetraploid. The PXFXP and FXPXF were also triploid and had significantly greater amounts of lesquerolic acid ($57.4 \pm 1.9\%$ compared to $50.4 \pm 1.4\%$) and significant lower amounts of oleic ($13.3 \pm 1.1\%$ compared to $18.0 \pm 1.2\%$) than the *L. fendleri* parent. The oil profiles of these two backcrosses were similar even though the ratios of parental genome doses were different. The predicted mid-parent values for lesquerolic acid of PXFXP and FXPXF were 72.5 and 57.5%, respectively (using data from 2001). The FXPXF backcross value was almost exactly as predicted; however, the PXFXP differed, although was not highly significant ($\chi^2 = 3.1$, $df = 1$, $p = 0.07$). The predicted mid-parent value for lesquerolic acid content was 54% compared to the actual value of $52.1 \pm 3.0\%$ for the LXFXFXF cross.

None of the A_1 generation plants and only one A_2 PXF plant set enough seed for destructive analysis. The LXF plants were not as successful as the PXF in ovule culture and no A_2 plants resulted. The PXF A_2 had a lesquerolic acid value of nearly 70%. This analysis was done before we were able to analyze half seeds. The A_2 plants (A_3 seed) produced similar results with segregating values for lesquerolic acid content between 56 and 66%.

The introgression of higher lesquerolic acid content from other *Lesquerella* species into *L. fendleri* has provided a new direction for improving the commercialization potential of this crop. The increase in lesquerolic acid from 50% in *L. fendleri* to 70% in at least one of the hybrids, adds significantly higher values for growers and producers. Further improvements should be possible as more plants from segregating generations become available for selecting plants with high lesquerolic content as well as other favorable traits. *Lesquerella pallida* may be valuable as a donor parent for the autofertility trait. This is the only species we have observed with this trait and could be valuable in eliminating the need for pollinators for seed production. Hybrids containing this species also seem to respond better to ovule culture. A problem we encountered in this study was the differences in the oil profile between the two years. If this obstacle is manageable by finding an inducible environmental trigger or by knowing the genetic inheritance, *L. pallida,* an endangered species could have a major impact on the commercialization of this new alternative crop. This information highlights the critical value of these and other endangered plants have on agriculture.

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Interpretive Summary

We found that there is not enough natural variability to improve an important oil characteristic, hydroxy fatty acids (HFA), in plants from lesquerella a species being commercialized. Much higher amounts are present in the oil of related species, even though they do not produce as much seed. The species are prevented from producing seed (they are sterile) when crossed together because of a biological barrier. Hybrids were produced between species that do not normally cross using novel methods. We found different combinations of traits that confirmed they did not occur from either crossing with another plant of the same species or with itself. There was a substantial increase in the HFA amount in the seed oil in these hybrids above the range of variability normally found. These hybrids provide a new basis for opportunities in commercialization of this potential new crop, since oil will be cheaper and products will be more profitable.