Chapter 18
New Crops Breeding: Lesquerella

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18.1 Introduction

Lesquerella species contain a seed oil which is approximately 55% lesquerolic acid, a 20-carbon long fatty acid with a single hydroxyl group and double bond, and has a similar hydroxy fatty acid (HFA) profile as castor oil. Large markets exist for hydroxylated oils as feedstocks for lithium greases, polymers in paints and coatings, base stocks for lubricants, nylon-11, hydraulic fluids, and applications in the personal care industry (Roetheli et al. 1992). The hydroxyl group of these oils makes them prime candidates as additives to diesel fuel to improve lubricity (Naughton 1992). Goodrum and Geller (2005) demonstrated that lesquerella oil has superior performance compared to castor, soybean, and rapeseed methyl esters at concentrations as low as 0.25% in reducing wear and damage to diesel engines, primarily with fuel injection systems. Castor oil also contains high amounts of HFAs, but the main HFA, ricinoleic acid, is two carbons shorter than lesquerolic acid, which imparts slightly different physico-chemical properties to the oil. Lesquerella could be established as a reliable, domestic oilseed supply of HFAs for a variety of industrial applications (Roetheli et al. 1992) and at the same time provide an alternative crop for farmers and increase local profits. Lesquerella will not replace current commodity crops but instead will be placed in a rotation with these crops, e.g., a 2-year, 3-crop rotation of lesquerella, grain sorghum, and cotton.

18.2 Origin and Domestication

Lesquerella is a genus of the Brassicaceae family with species ranging primarily from the arctic to southern Mexico, with another dozen species occurring in South America. The greatest concentration of taxa are in the southwestern United States and Mexico, where *L. fendleri* originates, and in the Rocky Mountain and

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intermontane basin region of the western U.S. (Rollins and Shaw 1973; Rollins 1993). *L. fendleri* (Fig. 18.1) contains the hydroxy fatty acid lesquerolic acid (14-hyroxy-cis-11-eicosenoic acid, C20:1-OH) as the main component of its seed oil. Other species with a second type of HFA in their seed oil, densipolic acid (12-hydroxy-cis-9-cis-15-octadecadienoic acid, C18:2-OH) originate from the eastern U.S. A third type of HFA called auricolic acid (14-hydroxy-cis-11,cis- 17-eicosadienoic acid, C20:2-OH) is found primarily in one species from Oklahoma.

Fig. 18.1 Flowers (*left*) of *Lesquerella fendleri* (Brassicaceae), and a lesquerella production field (*right*) in Arizona

The genus *Lesquerella* was named in honor of paleobotanist Leo Lesquereux (1806–1889), who came from Switzerland to the U.S. in 1848, and settled in Columbus, Ohio. His specialty was the collection of fossil plants, especially in connection with coal deposits. The taxonomy of *Lesquerell*a, commonly called bladderpod, was studied by Payson (1921) and later revised by Rollins (1955). O'Kane et al. (1999), Al-Shehbaz and O'Kane (2002), and O'Kane and Al-Shehbaz (2002) proposed that 75 species in the genus *Lesquerella* be transferred to the genus *Physaria* and all of the annual species of *Lesquerella* from the southeastern U.S. be placed into a new genus, *Paysonia*. Their new synonym for *L. fendleri* is *Physaria fendleri* (A. Gray). Public literature still refers to this plant as lesquerella or *L. fendleri* and Rollins and Shaw (1973) noted difficulties in the similarities between the two genera but also stated the problem of merging them because of similarities with *Lesquerella* and another genus *Alyssum*. The distinction for agronomic purposes and crop development is that *L. fendleri* is a short-lived perennial that can be grown as an annual. Some other species of *Lesquerella* and *Physaria* are perennial and do not flower until the second year of growth. *Physaria* species are not as adaptable for domestication because of this, even though they can be very productive seed yielders.

The first interest in *Lesquerella* species for domestication came in the late 1950s. USDA-ARS began a massive screening of over 200 families of oilseed plants from the wild and described the new hydroxy fatty acids from this plant (Jones and Wolf 1960; Smith et al. 1961). Plant exploration trips to collect *Lesquerella* species were based on the fact that only occasionally does a plant with an unusual oil composition also have high crop potential with no real biological barriers for domestication. A series of articles *The Search for New Industrial Crops* and *The Search for New Industrial Oils* were published in Economic Botany and the Journal of the American Oil Chemists' Society between 1960 and 1984, and described lesquerella as well as other potential new crops. The second article in *The Search for New Industrial Crop* series was the first description of collections of *Lesquerella* made by USDA-ARS (Barclay et al. 1962). A breeding program by Dr. D. Rubis at the University of Arizona began in 1968 and continued until 1971, using the germplasm collected by USDA. Dr. Rubis generously contributed his germplasm to the USDA, ARS program in Arizona that began in 1986 by Dr. A.E. Thompson (Thompson and Dierig 1994). The breeding program is still ongoing at the ARS facility in Maricopa, Arizona.

L. fendleri is found in its native environment on calcareous soils in the southwestern states of Arizona, New Mexico, and Texas, with a few collections from southern Utah and Colorado by Rollins and Shaw (1973). Collections from the states of Coahuila, Chihuahua, Nuevo Leon, Zacatecas, and Durango, Mexico were made (Salywon et al. 2005; Rollins and Shaw 1973). These populations were usually associated with moisture availability in mixed, sparse vegetation, and were easily recognized by their glabrous siliques and fused trichomes which set *L. fendleri* apart from other *Lesquerella* species.

18.3 Genetic Resources

There are 233 *Lesquerella* accessions available in the National Plant Germplasm System (NPGS). One hundred and twenty of these are *L. fendleri*, and most of these accessions were collected during the period from 1993 until 2002 through trips supported by USDA-ARS (Dierig et al. 1996; Salywon et al. 2005). Prior to this there were only 17 species represented and 21 accessions of *L. fendleri* in NPGS (Thompson et al. 1992). In the USDA-ARS-ALARC working collection, there are now 413 accessions of 57 *Lesquerella* and 17 *Physaria* species. One hundred and thirty are *L. fendleri* accessions. A phenotypic evaluation of germplasm available in NPGS was completed by Jenderek et al. (2008). The curator within the NPGS for *Lesquerella* species is located at the USDA, ARS, National Arid Land Plant Genetic Resources Unit – Parlier, California.

In Rollins' review (Rollins 1993) of *Lesquerella* of North America, 83 species were included. Other species have since been discovered including four by Rollins (1997), Rollins et al. (1995) and Anderson et al. (1997) as well as others by O'Kane (1999) bringing the total number of North American species to about 90.

Some *Lesquerella* species are on federal or state lists as rare, endangered, or threatened species. One of these species, *L. pallida*, has been valuable to our breeding program because of its high lesquerolic HFA content. This species is also autofertile compared to the self incompatibility and open pollination of *L. fendleri*. No collections have been made of this species since the original collection in the 1800s, until a report by Nixon et al. (1983) on the rediscovery of the species.

A number of *Lesquerella* and *Physaria* species have traits of interest for genetic improvement, although none have the productivity of *L. fendleri*. Apart from plant accessions our database contains over 10,000 records of germplasm lines including various traits such as yellow seeds, non-shattering selections, salt tolerance, male sterility, five petal plants, multi-locule silique selections and other traits and crosses.

18.4 Major Breeding Achievements

An important accomplishment has been to provide a large collection of genetic diversity through plant germplasm collections. The numerous collection trips throughout the U.S. and Mexico and subsequent evaluations provided valuable diversity for the plant breeding program. Within these accessions, traits such as flower color, autofertility, plant architecture, seed coat color, male sterility, and seed oil characteristics have been identified. The inheritance of some of these traits has been determined to allow their use in the breeding program (Dierig et al. 2001).

The improvement of oil content has made a significant contribution to the commercialization process of lesquerella. Unimproved accessions of *L. fendleri* have oil contents around 24%. The last germplasm line publicly released had 32% (Dierig et al. 2006a), and another line ready for release has 36% oil content. The difference in oil yield between the unimproved and improved accessions is 86 liters/ha (56 gal per acre) oil versus 146 liters/ha (84 gal per acre) based on current yields of 1800 kg/ha, respectively.

The oil profile of this species has been surveyed by Dierig et al. (2006b) and lesquerolic HFA was highly variable. *L. fendleri* appears to have a limit of around 67%. Hayes et al. (1995) reported that *L. fendleri* fills only 2 of the 3 triglyceride positions with HFA. The *sn* 2 position is left unfilled, which explains the upper limit of two-thirds or 67% for HFA content. A few other species of *Lesquerella* are able to fill all 3 positions and have HFA content of up to 89%, which is near the level of castor. Current lines contain 60% lesquerolic acid. A single gene mutation with high oleic and 0% lesquerolic acid was recently discovered (Dierig et al. 2008, in prep.). This germplasm provides a platform for examining a molecular approach for transforming lesquerella with genes in the HFA biosynthetic pathway to increase the HFA content.

Interspecific hybrids between *L. fendleri* and two other species have been developed, introgressing the high lesquerolic trait into *L. fendleri*. The hybrids are not ready for public release until further improvements are made but have over 75% lesquerolic acid (Dierig et al. 2004). Seeds per pod (silique) are fewer than *L. fendleri*, but this is as expected since the other parent species had larger but fewer seed, and the hybrid expressed mid parent values. The hybrids hold promise for providing non-genetically modified traits not currently available in *L. fendleri* such as high lesquerolic acid, autofertility, and an expanded geographical area for commercial production.

18.5 Current Breeding Goals

Lesquerella fendleri plants are open-pollinated, highly genetically diverse, and have no biological barriers to being a commercial crop. *L. fendleri* is highly productive and it is felt that by exploring other areas of research the crop has the potential to double in yields. A few traits that could be further exploited will be discussed below, although this is not a comprehensive list.

18.5.1 Oil Content and Fatty Acid Profile

There have been two plant germplasm releases with improved total oil and lesquerolic acid contents (Dierig et al. 1998, 2006a). Results from a recurrent selection program have consistently produced plants with oil content between 40 and 45%. This appears to be the upper limit for the oil content of this plant. However, this is the average value of seeds from a single plant. There has yet to be a screening of single seeds for this trait; a technique has just been developed, but will still be difficult because of the small seed size (Isbell et al. 2008). A single seed weighs approximately 0.0006 g. Screening germplasm via single seeds for this trait should allow faster progress in increasing oil content.

The upper limit for lesquerolic acid HFA content in *L. fendleri* appears to be about 67% (described above). Some market applications may require this to be kept at the limit if the material, such as nylon 11, requires a difunctional triglyceride. Lines are being developed through the development of interspecific hybrids with greater than 80% lesquerolic acid utilizing species with all three positions of the triglyceride (trifunctional) and introgressing that trait into the hybrids. An improved line with these traits would fit most of the same markets as imported castor. The other desirable goals would be to lower the unsaturated fatty acids linoleic and linolenic acids.

18.5.2 Seed Yield

The current lesquerella seed yields are approximately 1800 kg/ha, but it is felt that the plant has the potential of yielding 2500–3000 kg/ha. This increase will come through a combination of improved agronomic practice and breeding. Some agronomic issues include more precise plant spacing, better irrigation management, and more efficient harvesting. Developing more productive varieties will include selection based on harvest index and for specific environments. *L. fendleri* is the most productive of all species so far tested because of its extensive branching and subsequent flowering along each branch. Selection for branching at warmer or cooler temperatures will identify plants better adapted for growth in different climates.

Plants that reach maturity in a shorter time period will save production costs. This will require selection based on seed germination at cooler temperatures, or plants that will branch at lower temperatures.

18.5.3 Wider Adaptation and Shorter Growing Period

Since these two goals are related, they will be discussed together. These issues are also tied into the discussion above on improved seed yield. If plants are able to be developed that branch at lower or cooler temperatures, the planting date could be moved from October to February. Planting later than February in Arizona is not desirable because of the probability of summer rains that may occur during the dry down period causing seed shatter. Lesquerella does not normally shatter; however, when irrigation is terminated and a desiccant is applied 7–10 days before combining, the crop exposed to hard rains could lose its seed yield.

18.5.4 Autofertility

A few *Lesquerella* species are autofertile such as *L. pallida* and *L. mcvaughiana*. Both have the same chromosome number as *L. fendleri* with potential for use as a source of germplasm to introgress this desirable trait. Within *L. fendleri* there may also be potential for variation in autofertility, but it has not been looked for in a thorough manner. The advantage of autofertility would be that pollinators would not be required to increase seed yield. Plants may begin flowering in February or earlier in warmer years but these temperatures are still too cool for pollinators to be present in the field which causes a yield reduction. There are also years when feral bee populations are not as abundant resulting in lower yields, unless managed bees are used. The cost of bees for pollination obviously results in higher production costs and can be significant due to problems beekeepers are experiencing such as colony collapse disorder. Self pollination might prove to be detrimental due to inbreeding depression, but the trait still warrants investigation.

18.6 Breeding Methods and Techniques

Lesquerella, as well as other oilseeds in the Brassicaceae family, are generally cross pollinated and self incompatible. A single flower produces up to 30 seeds inside a silique (pod) and each seed has the potential to originate from a different pollen source causing each seed to be genetically different. Although more genetic variability for selection is generated, a desired trait is more difficult to select because the seeds from a single plant are half instead of full siblings, as self pollination rarely occurs (Dierig et al. 2004). Half seed selections and inheritance studies have allowed selection of mutants in the fatty acid profile instead of through half sib selection. The ability to analyze a half or a single seed of *L. fendleri* for fatty acid and oil content has greatly improved breeding of this crop (Isbell et al. 2008). Since the seed is very small, this has proved to be a greater challenge compared to other oilseed crops where it has been accomplished. The seed of *L. fendleri* weighs about 0.6 mg, which is less than half the size of alfalfa seed.

Interspecific hybrids have been a focus of our program to utilize traits not found in *L. fendleri* but that occur in other *Lesquerella* species. Eastern species hybridize in nature but species native to the western U.S. do not hybridize readily. The current method used for producing these hybrids is described in Dierig et al. (2004). This includes use of ovule culture and colchicine treatment. Cytological information has also been essential to determining the behavior of parents and progeny in these crosses. One example of this was in a cross of another species with *L. fenderi* that was thought to have the same chromosome number. When some of the progeny failed to have the high HFA trait of this parent, cytological examination, using pollen mother cells, found that the seed which had originated from an exploration trip and was thought to be a single species, was actually a mix of two different species (D.T. Ray, personal data). Bud pollinations, where buds are manually opened before anthesis to pollinate, are routinely used in both inter- and intra-specific crosses to overcome incompatibility. Other breeding methods include those used for cross pollinated crops such as recurrent selection.

Mutation breeding has been utilized in our program using ethyl methane sulfonate (EMS). We have found that *L. fendleri* has a tremendous amount of variability and many of the mutants we have in our collection such as fatty acid mutants (0% lesquerolic acid + high oleic; 0% linolenic; 0% linoleic), cream flower color, male sterility, yellow seed coat; a 5-petal flower, and multi-locule silique, also occurred without EMS treatment.

18.7 Integration of Information Technology and New Biotechnologies

Information technology has aided the progress of lesquerella breeding by integrating data management and creating a more efficient use of plant germplasm. White et al. (2007) described the importance of this especially with new crop development where the scientific input is much reduced compared to established, traditional crops. New crop development is especially prone to fluctuating efforts over time due to the limited available financial resources. Often times research results do not get published because funding ends or a researcher retires. Intellectual Property Rights can also be an issue for exclusive licensing of products from new crops or Plant Variety Protection and germplasm used in developing the variety along with breeding methods. The goal of developing an information technology system for lesquerella has been the documentation of germplasm and collection information, maintaining careful records of genealogy and population structures, and developing a network available to interested users. This information system keeps careful documentation of all the available phenotypic and molecular documentation. The system we have utilized is the International Crop Information System (ICIS, www.icis.org).

SSR markers have been developed for lesquerella (Salywon and Dierig 2006). These SSRs along with other molecular markers have value in association mapping of traits, such as lower linolenic acid content. The association mapping methodology calculates linkage disequilibrium and is superior to quantitative trait loci (QTLs) mapping in determining genetic variances (Zhao et al. 2007). Bioinformatics approaches such as searching the EST libraries for DNA sequences with similarity to important genes are now being used in lesquerella. Necessary genes to incorporate HFA at the *sn*-2 position of *L. fendleri* seed oil are being examined (Dyer and Mullen 2005).

18.8 Seed Production

There is currently no lesquerella commercial seed production. Technology Crops International (TCI, www.techcrops.com) is currently working on a marketing strategy to begin production. They plan beginning commercial production of lesquerella seed in areas of the southwestern U.S.

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