Chapter 6

Flax (*Linum usitatissimum* L.)

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INTRODUCTION

Flax (or linseed) is one of the predominant industrial oils seed crops grown in temperate climates. The seed oil of this oilseed crop is enriched in α-linolenic acid (ALA) (18:3$^{cis\Delta 9,12,15}$). Because of this, flax oil readily polymerizes on exposure to oxygen, making it useful for a variety of industrial products, including varnish and linoleum, while meal from pressed seed is useful as animal feed. Industrial demand declined after the 1960s due to a shift to acrylic paints and vinyl floor coverings, but now there is a renewed interest in using biomass-derived feedstocks. In addition to industrial applications, ω-3-enriched flaxseed oil is gaining importance in livestock feed and aqua-feed applications. The oil is also recognized as a good source of ALA for the human diet. Additional flax constituents, including fiber and lignans (Czemplik and Szopa, 2009; Vaisey-Genser and Morris, 2003), may provide human health benefits. "Solin"-type flax, which is enriched in linoleic acid (LA; 18:2$^{cis\Delta 9,12}$) and low in ALA content (<5%), has greater oxidative stability than regular flax. Solin was developed

Industrial Oil Crops. http://dx.doi.org/10.1016/B978-1-893997-98-1.00006-3

for widespread food use in place of sunflower and safflower seed oils (Vrinten et al., 2005; Ntiamoah and Rowland, 1997; Rowland, 1991). This chapter discusses the origin, production, agronomy, genetics and breeding, oil synthesis biochemistry, and biotechnology of flax, with a focus on industrial applications of the crop.

DIFFERENTIATION OF FIBER FLAX AND OILSEED FLAX

Flax cultivars have been selected for production of either fiber (fiber flax) or oil (oilseed flax). Location of production, climatic adaptation, and morphology of these types now differ considerably. Oilseed-type plants are usually shorter, have more branches, and produce more seeds, while fiber flax types are generally taller, have few branches, and have been selected for fiber (Gill, 1987). Bast fibers from flax, derived as part of the phloem, are long (4 cm), have high tensile strength, and have a high quality of cellulose (Deyholos, 2006). Fine flax fibers are used for linens and textiles, while coarser fibers are used for nonwoven textiles and twine. Both flax types have a short tap root system with fibrous branches. Flax is relatively shallow rooted, with only 4–7% of root mass deeper than 60 cm (Gan et al., 2009).

Production of both linen and oil is generally not compatible in the same crop. Bast fibers become more lignified and less flexible during flower and seed set, so high-quality linen fiber is harvested prior to seed ripening and harvest (Deyholos, 2006). Additionally, in many regions where flax is grown for oil, weather conditions do not permit postharvest fiber processing (retting), which requires a relatively wet fall climate. The 202,000 ha of fiber flax are mainly located in China, Russia, Egypt, and near the northwestern European coast (Vromans, 2006; Green et al., 2008). For oilseed production, the straw is considered an impediment during harvest and for subsequent seeding operations and until recently has been burned. However, the potential for alternative fiber uses such as industrial fibers for composites, paper, and nonwoven fiber increases the potential for whole plant utilization (Irvine et al., 2010). The majority of flax in North America is grown for the oil.

Flax Area and Production

Currently, oilseed flax is primarily grown in Canada, China, USA, India, and Russia (FAOSTAT, 2013). Canada is the world's largest producer and exporter of flaxseed, a position it has held since 1994 (Fig. 6.1). Canada's and Russia's role as a flaxseed producer has increased while that of India, Argentina, Europe, and the USA decreased since 1995 (Agriculture and Agri-Food Canada, 2010; FAOSTAT, 2013). In Canada, the annual production of flaxseed averaged 706,000 tons from 1999 to 2009. In 2009, flax was seeded on ~631,000 ha in Canada with production of ~861,000 ton (Agriculture and Agri-Food Canada, 2010). Canada exports 80–90% of its total flaxseed produced, mainly to Europe,

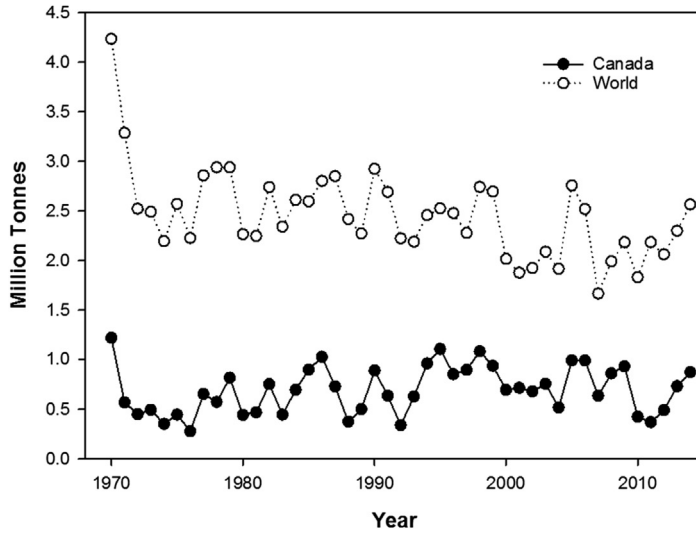

FIGURE 6.1 Global flaxseed production in million metric ton and the Canadian share of global production from 1997 to 2007. *Data adapted from the Food and Agriculture Organization (FAOSTAT data 2010).*

the USA, Japan, and South Korea (Flax Council of Canada, 2014a). The presence of transgene in Canadian shipments relates back to the mid-1990s introduction of GM flax variety CDC Triffid (McHughen et al., 1997). This variety was deregistered in 2001 due to concerns about the effect of its production on offshore markets. A decade after removal of transgenic flax from the commercial system in Canada, it was detected in grain shipments to Europe. This event resulted in disruption in trade between Canada and the European Union (EU). To demonstrate compliance the industry adopted a protocol testing grain samples (postharvest) using a real-time polymerase chain reaction (PCR) assay for the construct found in CDC Triffid. Since the inception of the testing protocol, the Flax Council of Canada has maintained a database of test results. Booker and Lamb (2012) evaluated GM presence in Canadian grain stocks and found the positive tests showed a downward trend, indicating removal of transgenic flax from the commercial system. However, low-level GM presence persists in Canadian grain stocks. A way forward for the industry in Canada includes renewal of seed stocks with reconstituted GM-free varieties (SaskFlax, 2013).

Origin and Domestication

The genus *Linum* is a large group with ~230 species (Heywood, 1993). The genus is divided into five sections, *Linum*, *Linastrum*, *Cathartolinum*, *Dasylinum*, and *Syllinum*, based on chromosome number, floral morphology, and interspecific compatibility (Gill, 1987). Cultivated flax, *Linum usitatissimum*, is placed in the section *Linum* and has 30 diploid chromosomes (Tammes, 1928;

Tutin et al., 1980) along with the proposed progenitors *L. angustifolium* and *L. bienne*, which share homostylous rather than heterosylous flowers.

The origin of flax lies in southern Europe, the Near East, or Central Asia (Tammes, 1925; Helbaek, 1959; Zeven, 1982). There is evidence of flax use in Neolithic cultures as a source of fiber. Flax fiber was identified in prehistoric sites in Israel and Syria (van Zeist and Bakker-Heeres, 1975). Flax was grown in Egypt between 4500 and 4000 BC and reached Switzerland by around 3000 BC; it was found somewhat later at Willium Hill in England (Smith, 1969). It is believed that Lois Hebert, the first farmer in Canada, introduced flax to Canada almost 400 years ago (Prairie Flax Products, 2007). By 1875, European settlers were growing flax on the Canadian prairies.

L. angustifolium, also known as pale flax, a closely related species of flax, is indigenous to the territory bordering the Mediterranean Sea, Iran, and the Canary Islands, and has a similarity to cultivated flax (Diederichsen and Hammer, 1995). Cultivated flax, *Linum usitatissimum*, and pale flax, *L. angustifolium*, are homostylous, inbreeding species and both species have similarity in chromosome number (Gill, 1987; Tammes, 1925). The genetically similar behavior of *L. angustifolium* and *L. usitatissimum* and the fact that they can easily hybridize with each other in any direction (male or female) and yield fertile hybrids (Gill, 1966) suggests that *L. angustifolium* is the wild progenitor of flax (Dillman and Goar, 1937). *L. usitatissimum* is differentiated from *L. angustifolium* by one translocation, while *L. africanum, L. corymbiferum*, and *L. decumbens* have departed from *L. angustifolium* in two translocations (Gill, 1966).

A molecular study indicates that *L. bienne* is the sister species of *Linum usitatissimum* (Vromans, 2006), but some researchers consider *L. angustifolium* and *L. bienne* to be the same species (Tutin et al., 1980; Zohary and Hopf, 2000). A more detailed molecular study, including genome comparisons with molecular markers of these three species (*L. angustifolium*, *L. bienne*, and *L. usitatissimum*), has confirmed that they are very closely related genetically (Muravenko et al., 2003). *L. bienne* can be considered as a subspecies of *L. usitatissimum* rather than a separate species, and *L. angustifolium* can be considered as a wild progenitor of cultivated flax. According to Richharia (1962), evolution of flax might be a case of convergent evolution. The interspecific hybridization of flax with several other wild relatives with $n = 15$ reported to occur in North America has not been studied or reported yet (Jhala et al., 2008). The history of domestication of flax has not been clearly delineated and may have occurred at several places simultaneously (Harlan, 1965). It is believed that the usage as a fiber plant has provided the impetus for its domestication (Dillman, 1936). Various characters of the cultivated flax including annual habit, nonshattering capsules, self-fertilization, branching habits, and variability in fatty acid profile are some of the examples of the breeding efforts and result of interaction of many inherited and environmental factors. The early and rapid distribution of flax cultivation in the Old World resulted in a wide range of flax landraces adapted to different use and environmental conditions (Vavilov, 1926).

Agronomy of Flax Production

Climatic Adaptation

Flax thrives best in regions with a moist, moderately warm climate—free of late frosts in spring, with sufficient moisture during growing period and where long, continued rainy spells do not alternate with long, continued dryness (Dillman and Goar, 1937). Flax is well adapted to western Canada where temperatures range from 10 to 25°C during growing season (Braidek, 1975). High temperatures in the absence of drought decreases seed set and reduces yield (Cross et al., 2003). In the United States, flax is primarily grown in north central states including North Dakota, South Dakota, Montana, and Minnesota.

Soil and Fertility Requirements

Flax production is optimal on well-drained, medium heavy soils, especially silty loam, clay loam, and silty clays (Braidek, 1975). In Canada, flax is adapted to the mixed grasslands and moist mixed grassland agro-ecological regions, except on poorly drained or sandy soils. Because of its shallow rooting character, flax extracts 95% or more water from the top 71 cm of soil. Water stagnant conditions may result in chlorosis and stunting of flax. Though flax plants are tolerant to a wide range of pH, the best flax development was recorded at pH 6.0 in sand culture (Sinha and Saxena, 1965).

Flax responds to increased nitrogen availability, but less so than cereals or canola (Grant et al., 1999). The optimum nitrogen requirements for flax are 100–120 kg (soil residual nitrate-N and inorganic fertilizer) (Lafond et al., 2008), depending on available moisture. Increasing nitrogen fertilizer from 67% to 100% increased seed yield, bolls/plant, seed weight, and seed oil content. Flax had higher seed nitrogen concentrations than crops such as cereals, but because of the reduced yield, nitrogen removal from soil tends to be lower. Seed-placed fertilizers may injure flax seedlings. A low rate of phosphate, <20 kg/ha of P_2O_5, may be seed-placed, but alternatively it may be placed to the side at a depth of 25 mm to improve phosphorus nutrition in the flax plant (Flax Council of Canada, 2014c).

Seed Selection, Time of Sowing, and Seeding Rates

Flax cultivar choices are limited compared to other crops (<15 varieties in Canada in 2010). Cultivar choices are made on the basis of yield, rust resistance, maturity, and lodging. Most varieties are resistant to rust and Fusarium wilt, and cultivar selection, in combination with crop rotation, has reduced the impact of diseases. Canadian growers predominantly use farm grown seeds, but following the recent contamination of flaxseed with a genetically engineered (GE) trait unapproved in Europe, the use of certified seed is now recommended.

Because flax is sensitive to spring frosts, seeding must be delayed until the risk of frost is reduced. Later planting was favored at most northerly Canadian sites and early planting at the most southerly sites (Lafond et al., 2008).

A seeding rate of ~35–45 kg/ha was recommended, which resulted in a crop stand of 300–400 plants/m^2.

Crop Rotation

In western Canada, the majority of flax is grown in rotation with cereals to prevent disease; at least 3 years is recommended between flax crops (Flax Council of Canada, 2014c). Yields of spring wheat were higher following flax than following canola or field pea (Bourgeois and Entz, 1996) and flax yields were higher after mycorrhizal wheat than nonmycorrhizal canola (Khakbazan et al., 2009). Flax should not be grown after legumes or potato because of the potential for infection from *Rhizoctonia* spp. (Johnston et al., 2002).

Crop Protection

Flax is less competitive than most western Canadian crops with respect to weeds, and weeds result in significant yield decreases (Lafond et al., 2008). Grass weeds can be relatively easily controlled (Wall, 1994a) but a limited number of herbicides are available for broadleaf weed control (Wall and Kenaschuk, 1996; Wall, 1994b). The lack of competition and herbicide tolerance are the reasons herbicide resistance was the first trait chosen for genetic engineering of flax (see Market Constraints to Genetically Engineered Flax). Insect pests of flax in western Canada include flax bollworm, *Heliothis ononis* (Denis and Schiffermiller); several species of cutworms including redbacked cutworm, *Euxoa ochrogaster* (Guenee), pale western cutworm, *Agrotis orthogonia* (Morrison), early cutworm, *Euxoa tristicula* (Morrison) and army cutworm, *Euxoa auxiliaris* (Grote); the bertha armyworm, *Mamestra configurata* (Walker), the beet webworm, *Loxostege sticticalis* (L.); the potato aphid, *Macrosiphum euphorbiae* (Thomas); the aster leafhopper, *Macrosteles quadrilineatus* (Forbes); and the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). With the exception of the flax bollworm, flax insect pests are polyphagous.

Fungal diseases of flax include rust caused by *Melampsora lini* (Ehrenb.) Desmaz.; fusarium wilt caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. lini (Bolley) Snyder and Hansen; gray mold caused by *Botrytis cinerea* Fr.; alternaria blight caused by *Alternaria* spp.; powdery mildew caused by *Erysiphe polygoni* DC, *E. lini, E. cichracearum* DC, *Sphacelotheca lini, Leveillula taurica*, and *Oidum lini* Skoric; pasmo disease caused by *Septoria linicola* (Speg.) Garassini (telomorph). Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lini* (Bolley) Snyder and Hansen continues to be a problem in Canadian flax fields (Mpofu and Rashid, 2001), but several varieties are resistant to rust. Cultivar selection for resistance to pasmo, caused by *Septoria linicola*, has been less successful. *Septoria linicola*, attacks above-ground parts of flax and overwinters in the soil on infected flax stubble. Flax is most susceptible to pasmo in the ripening stage under moist conditions. Prevention may be achieved by early

seeding at the recommended rates to avoid high moisture conditions in the fall, following a rotation of at least 3 years between flax crops.

Flax is also susceptible to several viral diseases (Rashid, 2003). Viral diseases include crinkle, caused by oat blue dwarf virus (OBVD), and curly top, caused by the beet curly top virus (BCTV). Crinkle is transmitted by the six-spotted leafhopper, *Macrosteles fascifrons* (Uhl.), and results in stunting and reduced tillering. Curly top is transmitted by the leafhopper *Eutettix tenellus* (Baker) and results in general chlorosis and irregular leaf development where terminal leaves are grouped together.

Aster yellows is a disease caused by a phytoplasma, which is transmitted by the six-spotted leaf-hopper, *Macrosteles fascifrons* (Uhl.). Symptoms include stunted growth and abnormal flower development where floral parts are transformed into small leaves. It is beyond the scope of this chapter to discuss the control of insects and other pests of flax, and, thus, readers are directed to the Flax Council of Canada (2014c) and Rashid (2003).

Volunteer Flax

The small size of flaxseeds and difficult harvest conditions can result in high seed losses, as high as 6822 seeds/m^2 with the maximum flax yield loss of 331 kg/ha (Dexter et al., 2011). Volunteers emerge sooner following spring tillage than in direct seeding and are more numerous. Volunteer flax can persist and volunteers emerge for 2 years following flax harvest in western Canada (Dexter et al., 2010b).

Volunteer flax is considered a poor competitor, but it can interfere with harvesting operations, especially at high densities and can reduce seed quality (Knott, 1993); it may also contribute to the seed- and pollen-mediated gene flow (Dexter et al., 2010a,b; Jhala et al., 2008, 2011). Although the growing area of flax has not changed significantly over the past few years, the relative abundance of volunteer flax has increased from 2.0 to 15.3 m^{-2} in western Canada (Thomas et al., 1997). Volunteer flax was ranked as the 32nd most abundant weed on Canadian Prairies in the 1970s, but near the beginning of the 21st century it was the 26th (Leeson et al., 2005), indicating that it is becoming a problem in subsequent crops. In the 1997 Manitoba survey, volunteer flax was present in twice as many zero tillage fields, but at much lower average densities, compared to conventional tillage (Thomas et al., 1997), consistent with a recent small survey in Alberta (Dexter et al., 2010a). When volunteer flax is left uncontrolled, wheat yield may be reduced by 27% (Wall and Smith, 1999). In western Canada, a number of herbicides have been registered for volunteer flax control. Quinclorac provided good control of volunteer flax. Pre-emergence application of glyphosate reduced volunteer flax densities from 39 plants to <4 plants/m^2, while postemergence application of fluroxypyr plus MCPA or 2,4-D ester reduced volunteer flax densities to 2 plants/m^2 (Dexter et al., 2010a). In glufosinate-resistant canola, postemergence application of glufosinate effectively controlled volunteer flax (Jhala et al., 2010).

FLAX GENETICS AND BREEDING

Flax is a diploid ($2n=30$) autogamous (primarily self-pollinating) crop plant. Improvement in flax has lagged behind other oilseeds, including canola and soybean, in part because flax occupies a smaller niche as an oilseed and, consequently, received reduced resources for development. Genetic diversity within the crop is low, and cannot be readily supplemented by intraspecific hybridization. Finally, methods of hybrid seed production have not been developed.

Inheritance of crop characteristics can range for one major gene not influenced by environment (qualitative traits) to control by many genes, much influenced by the environment (quantitative traits). Many important traits in flax including days to flowering, days to maturity, plant height, plant branching and seed yield are quantitative, and demonstrate additive gene effects that are predominant, as in other autogamous crops (Patel and Chopde, 1981; Salej et al., 2007; Bhateria et al., 2006; Mohammadi et al., 2010). Selection of parents and in early generations is therefore critical in flax breeding programs.

Breeding Objectives

Conventional breeding of flax has focused on stabilizing yield across diverse environments, enhancing oil production and quality, discovering durable resistance to major wilt and rust diseases (Kenaschuk and Rowland, 1993; Mpofu and Rashid, 2001), improving lodging resistance, and adopting crop phenology for regional climatic limitations.

Seed Yield

Seed yield is a quantitative trait that is the most important in an oilseed flax breeding program. Numerous crop characteristics and environmental factors may influence seed yield, but little is published on basic crop characteristics of flax that affect yield, such as canopy expansion and light interception, dry matter production, and partitioning. During the reproductive phase, light use efficiency and harvest index are correlated with grain production under favorable growing conditions (D'Antuono and Rossini, 1995). Selection for seed yield is normally delayed until later generations when selection for complex traits is more effective.

Maturity

Early maturity is an important trait that protects crops from stresses such as disease, heat, drought, and frost. Early maturity aids harvest as immature flax remains green, which entangles machinery and reduces grain quality. The time from flax seeding to harvest can vary between 90 and 150 days (Diederichsen and Richards, 2003). Flax is considered a long day plant, where lengthening days hasten the reproductive phase of development, but the degree of photosensitivity in flax varies greatly.

Maturity is quantitatively inherited and little is known about the genetic basis of early maturity in flax. There is evidence, however, that major genes under epigenetic control contribute to the expression of early flowering. Gene methylation silences gene expression (reviewed in Suzuki and Bird, 2008 and Gehring and Henikoff, 2007) so that methlyated genes are transcriptionally inactive, while genes that are not methylated remain active. Early flowering flax genotypes from the *Royal* (*R*) genotype (an oil seed cultivar) and *Large* (*L*) genotroph derived from Stormont cirrus, a fiber cultivar (Durrant, 1971), were generated when germinating seedlings were treated with 5-azacytidine (Fieldes, 1994). The stable, heritable changes in early flowering lines resulted in shorter plants with fewer leaves that flower 7–13 days earlier than untreated control lines. Early flowering lines have a reduced late-vegetative phase (Fieldes and Harvey, 2004) and their DNA is hypomethylated compared to the untreated control lines (Fieldes et al., 2005; Brown et al., 2008). Fieldes and Amyot (1999) proposed a three loci genetic model to explain the phenotypic ratios observed in segregating generations of crosses between early flowering lines RE1 and LE1 and their corresponding control lines (RC and LC). For the early flowering phenotype to be observed, they proposed that all three loci must be demethylated. Furthermore, because all three loci are demethylated as a result of heritable effects of 5-azacytidine, only a critical threshold of floral stimulus is required to induce flowering.

Disease Resistance

Most early efforts in flax improvement in North America were aimed at breeding disease resistance (Kenaschuk and Rowland, 1993), and the most practical method of controlling disease remains the development and use of resistant varieties, combined with effective crop rotations.

The gene-for-gene theory of host–pathogen interactions was formulated by Harold Flor (1942) in flax using flax rust, *Melampsora lini*, as a model. For each host gene conferring resistance, he demonstrated the presence of a complementary gene in the pathogen determining avirulence. The disadvantage of this type of specific resistance/immunity is that the new pathogen races may evolve to overcome host resistance. However, there are a number of genes (*K*, *L*, *M*, and *P*) that may confer rust resistance in flax. Canadian varieties have alleles of rust resistance genes singly or in combination (S. Duguid, K. Rashid, personal communication). Hausner et al. (1999a,b) identified DNA molecular markers for specific alleles. All Canadian flax varieties are immune to local rust races and immunity to race specific rust has held up since the 1970s, and there has been no reoccurrence of flax rust in Canadian flax fields.

Fusarium wilt caused by *Fusarium oxysporum* f.sp. lini (Bolley) Snyder and Hansen is a limiting factor in flax production. Seedlings may be killed by the disease shortly after emergence, while delayed infections causes yellowing and wilting of leaves (Bailey et al., 2009). The fungus persists in the soil and crop debris for many years and wind-blown or run-off soil, or spores on seed may

spread the fungus from one field to another. Canadian flax varieties for release in Canada must be resistant/moderately resistant to fusarium wilt; however, it has been very difficult to breed for in yellow seeded flax varieties (P. Dribnenki, personal communication).

Further characterization of flax (host) wilt (pathogen) interactions with a wide diversity of flax lines and fungal isolates and development of DNA markers for host resistance genes would speed progress for breeding for resistance to fusarium wilt in flax. However, screening varieties for fusarium wilt resistance in flax wilt nurseries is problematic. There are substantial differences in disease development and severity between years and fields due to differences in pathogen population structure, resulting variable results (Mpofu and Rashid, 2001). Diversity in tolerance to fusarium wilt has been reported (Diederichsen et al., 2008) with higher than average resistance for in flax accessions from East Asia, average resistance in those from North America and South America, and lower than average resistance in those from Europe (mostly fiber flax) and the Indian subcontinent. Spielmeyer et al. (1998a) demonstrated that two major quantitative trait loci (QTL) with additive effects accounted for 38% and 26% of the genetic variability for fusarium wilt resistance. Using amplified fragment length polymorphism (AFLP), they mapped the QTL to two linkage groups (Spielmeyer et al., 1998b). Early generation screening is most effective to incorporate major wilt resistance.

Pasmo disease caused by *Septoria linicola* (Speg.) Garassini is prevalent and widespread on flax in Western Canada (Rashid and Duguid, 2010). Most Canadian flax cultivars are susceptible and little is known about the pathogenicity of different *S. linicola* isolates. Rapid disease development is exhibited in areas with high humidity and temperature and yield of susceptible varieties infected during flowering can be reduced by 75% (Saskston, 1959). *S. linicola* survives from one season to the next on infected crop residues left in the field (Bailey et al., 2009). Field sanitation methods, such as tillage, are not advisable due to conflict with soil conservation (no till) practices.

Powdery mildew was first observed in Western Canada in 1997 (Bailey et al., 2009). Powdery mildew can spread quickly and its incidence and severity have increased sharply in Manitoba and Saskatchewan. The causal agent is the fungus *Oidium lini*. Little is known about the overwintering and host range of this fungus in western Canada. Early infections may cause severe defoliation of the flax plant and reduce the yield and quality of seed (Bailey et al., 2009). Certain Canadian flax varieties have demonstrated a good level of resistance to this disease and flax breeders are encouraged to incorporate and strive to maintain resistance to powdery mildew in new flax varieties.

Agronomic Traits

Lodging resistance and plant height are important traits in cultivar development. Lodging reduces seed yield in flax and may hinder harvesting of the crop.

Lodging in flax may be associated with incidence of pasmo (Vera et al., 2011). Plant height at maturity and lodging resistance are considered quantitative characters and are selected for using field trials in the target environment.

Seed Size

Seed size is an important trait in flax. Large seeds offer advantages such as superior vigor in germination and seedling establishment that translate into greater capacity for yield. Larger seeded varieties are less prone to losses during harvest and seed cleaning. There is potential to accumulate more oil in a larger seed as the embryo is relatively larger. This is a particularly important characteristic for flax, where the seed coat constitutes nearly 40% of the seed mass, but contributes very little to the oil content. Global flax collections (eg, Plant Gene Resources Center) (PGRC, 2011) contain flax lines with large seed size, and genetic crossing results in large seed progeny. Inheritance of seed size is quantitative and selecting from the extremes of a population distribution may result in lines with the targeted seed size (Mohammadi et al., 2010). However, producing a large seed cultivar that retains the field performance of elite varieties is challenging, as the genetic basis that underpins the yield is multifaceted. Until DNA markers for seed size are available for introgressing large seed loci into elite varieties, the only feasible way to select for large seed size together with yield is in later generations using field trials.

Seed Constituents

Seed oil and protein are highly heritable traits in flax and can be selected for in early generations (see Oil Composition and Properties). Canadian flax breeders aim for 50% oil and 25% protein content on a dry matter basis.

Seed color in flax was used in Canada to identify traditional high-ALA acid flax (brown seed) and low-ALA flax (yellow seed) Solin market types (see Mutation Breeding). The Canadian Grain Commission (2013) has removed the requirement for yellow seed coat to be a phenotypic marker for the Solin oil profile. This requirement can be removed because the two traits are not linked and randomly assort, allowing the yellow seed trait to be used for other oil profiles in flax. Mittapalli and Rowland (2003) elucidated the allelic–gene relationship of the dominant yellow gene, the variegated recessive gene, and unknown spontaneous recessive yellow genes in flax. Segregation analysis indicated seed color is governed by four independently inherited loci, designated as the dominant *Y1* allele and recessive *g*, *d*, and *b1* alleles. The variegated seed color is conditioned by a second recessive allele of the *b1* locus, designated as *b1vg*. The recessive genes (*g*, *d*, and *b1vg*), when homozygous recessive, are epistatic to the other loci carrying dominant alleles. Palmitic and variegated seed loci segregated independently (Saeidi and Rowland, 1997) and thus Solin varieties could be developed for both high palmitic acid and variegated seed color.

Conventional Breeding Methods

Breeding in autogamous crop plants involves creating genetic variability, selecting the best recombinants and fixing the genes by making them homozygous. Homozygous varieties are called pure-line varieties and are true breeding. They are productive in the environments in which they were bred, but may not perform well outside these environments. Breeding in flax predominantly involves producing pure-line varieties through pedigree breeding.

Selection of Parents

Selection of parental material is of paramount importance in a flax breeding program. Selection of parental germplasm begins with an understanding of the goal of the breeding program (see Breeding Objectives) and the plant breeder must consider many characters for improvement and assign priorities to the traits considered for improvement. The way parents are selected depends on the trait(s) of interest, the purpose of the cross, the relative importance of characters other than yield, the pedigree of the pure-lines, and the resources and time available. Greater genetic diversity between the parents, results in greater genetic variability and likelihood of transgressive segregants, progeny that combine the genes of parental genotypes and have a phenotypic expression level superior to either parent.

Genetically diverse parents with complementary characteristics are chosen to create a segregating population. The parental lines can be from many different sources including existing cultivars, adapted elite breeding lines, or new introductions from global flax collections. The Plant Gene Resources Canada (PGRC, 2011), Saskatchewan, provides access to more than 3300 accessions of *Linum* L. germplasm, one of the larger collections of flax in the world (Diederichsen and Fu, 2008). A core world collection of 500 distinct flax accessions has been selected, based on genetic diversity studies (Fu, 2006), phenology, and morphological and agronomic traits (Diederichsen and Raney, 2006; Diederichsen et al., 2006, 2008; Diederichsen and Ulrich, 2009), as well as importance to Canadian agriculture (Kenaschuk and Rowland, 1993).

Inbreeding Selection and Line Evaluation

Pure-Line Method

Commercial flax cultivars are pure-lines developed via hybridization of inbred lines with complementary traits followed by pedigree selection of recombinant lines from segregating progeny (Culbertson, 1954; Kenaschuk, 1975). The aim is to obtain a pure-line cultivar that combines all the genes and meets the breeding objectives.

Pedigree Method of Breeding

Pedigree selection method begins with the intercrossing of inbred lines, followed by selfing of the F_1 hybrid plants to produce a large F_2 seed population.

F_2 seed from individual crosses is sown the following growing season and single plants are harvested from F_2 plots. Seed harvested from single plants planted in F_3 progeny rows. Progeny row selections within F_3 families based on stand maturity and vigor are made and single plants are harvested, along with bulk seed from the row. Seed quality traits such as oil quality, oil content, and protein content are determined using near infrared resonance or gas chromatography from samples taken from the bulk seed lots, and data used to further scale down the population of desirable lines. Seed harvested from F_3 single plants is used to establish F_4 rows and the selection cycle of between sister-lines and within-family selection continues and is repeated in the F_5 generation. In the F_5 generation, the bulk seed is used to establish replicated small yield plots to facilitate between line selections for seed yield, and the single plant selections are grown as progeny rows. Superior F_5 lines are identified on the basis of yield and seed quality attributes in the replicated plots, and sub-lines of these are used to establish the subsequent F_6 generation. Selected pure-lines are advanced into multilocation yield trials to compare their performance and adaptability with that of existing cultivars. In the F_6 generation, the bulk seed from F_6 rows is used to establish small increase plots for the development of breeder seed.

Green et al. (2008) describe a disease resistance screening program for flax wilt where large populations of F_2 plants are grown in a field on soil that is heavily infected with *Fusarium oxysporum*, the causal organism of flax wilt. The selected F_2 plants are then retested as F_3 progeny rows to identify homozygous wilt-resistant lines. In parallel, F_3 plants may be inoculated with specific strains of flax rust (*Melamspora lini*) under glasshouse conditions to identify rust-resistant lines. This method is limited by available space and time, and therefore disease-resistance screening generally consists of F_6 rows planted in wilt nurseries. Later-generation pure-line plants under glasshouse conditions may also be inoculated with specific races of flax rust (*Melampsora lini*) to identify and eliminate compatible lines. Disease-resistance screening in later generations is less than ideal as segregants with major resistance genes may not be carried forward in the breeding program, resulting in advanced breeding lines without the major wilt and rust resistance genes. The further development of DNA markers for major wilt and rust resistance genes will allow breeders to screen in the F_2 and F_3 generation for homozygous wilt- and rust-resistant lines, thus circumventing the reliance on disease-resistance phenotyping in the fieldhouse or glasshouse.

Bulk Population Method of Breeding

The bulk population method of selection is amenable to small grain crops that cannot be harvested singly, which is a necessary requirement of the pedigree selection method. Selection is delayed until later generations when selection for more complex characters (eg, seed yield) is more effective. In the bulk method, a segregating population is harvested together and a sample of the seed is used to plant the next generation. For example, the F_2 population is planted

and seed harvested from the entire population and bulked. Seed is randomly obtained from the bulked seed population and a plot of F_3 is seeded. The F_3 plot is similarly harvested and a plot of F_4 planted. This is continued until the F_6 generation. Selection begins in the F_6 generation, which is largely homozygous. Large populations of F_6 need to be planted for selection to be effective at this stage. Two methods of selection can be practiced at this stage: either pure-line selection (single plant selections, plant to row progenies, selections among homozygous lines) or mass selection (large number of individual plants selected and bulked and carried forward). The bulk method can produce lines with the desired traits if the environment where the method is conducted favors those characteristics (Orf, 2008).

Single-Seed Descent

Goulden (1939) first proposed single-seed descent as an alternative to pedigree and bulk breeding methods. He noted that breeding in self-pollinated crops involves two processes: achieving homozygosity and selection of traits with high and low heritability. He noted that in the pedigree method both processes are conducted simultaneously, resulting in characters with low heritability (such as seed yield) not selected for efficiently. Single plant selection (plant to row progenies), however, is an ideal way to identify transgressive segregants and achieve homozygosity, whereas in the bulk breeding method, selecting for rare transgressive segregants is not possible and bulking seeds is not an efficient way of achieving homozygosity.

In single-seed descent, attaining homozygosity and selection are separated into two different phases. Brim (1966) described single-seed descent as a modification of the pedigree selection method that allows accelerated development of recombinant inbred lines (RILs). RIL populations are derived from single-seed descent of large F_2 populations involving crosses of diverse flax lines to a high yielding and adapted cultivar. Single bolls/seeds are retained from each plant to produce subsequent generations, which were similarly advanced by single-seed descent to an advanced generation (eg, F_6). The generation cycle time can be reduced by growing the single-seed descent generation in the growth chamber under conditions of high plant density and restricted nutrition, which allows for three generations per year. This selection technique allows homozygosity to be achieved in a short time and the fixing of traits within the RILs. No selection is conducted within a population, so the RIL populations segregate (differ) for the traits in the contrasting parental lines. Selection can begin in the F_7 generation when homozygousity is achieved and additive gene effects are high. Furthermore, the variation in the F_2 is represented in the F_7 through single-seed descent. The F_7 can be grown as plant to row progenies. Undesirable lines can be removed. Selected F_8 lines can be grown in multirow plots and preliminary yield tests conducted.

RIL populations are ideal for the genetic study of traits and the genetic mapping of DNA markers of differing traits in the RIL population. Estimation

of the genetic and environmental components of traits is possible when the RIL population is grown in different environments, across years, when genotype by environment interactions are integrated in QTL analysis, thus resulting in DNA markers reflecting the amount and direction of reaction of the plant to environment (Van Bueren Lammerts et al., 2010).

Backcross Breeding

Backcross breeding is used to incorporate simply inherited traits from unadapted donor parents into recipient lines and involves repeated cycles of crossing to the recipient line (recurrent parent), followed by selection of the trait being transferred (Kenaschuk, 1975). Backcross breeding was first used in flax to develop a set of rust differentials by transferring individual rust resistance alleles into the cv. Bison (Flor, 1955). These rust differentials have been extensively utilized in flax rust research and as a source of resistance genes for backcrossing into flax varieties to overcome their susceptibility to new races of flax rust (Green et al., 2008). Backcross breeding plays an important role in transfer of novel alleles from mutant populations into elite recipient lines. Backcross breeding was used to intogress the (*ln1* and *ln2*) and a yellow seed color marker gene into Canadian flax cultivars McGregor and Norlin, to develop Linola cultivars (Dribnenki and Green, 1995; Dribnenki et al., 1996) and CDC Gold.

Recurrent Selection

Recurrent selection is a variation of backcross breeding, where selection for performance is practiced within consecutive segregating progeny generations after the population has undergone selection for the major trait being transferred (Green et al., 2008). A number of high-performing lines are crossed back to the recurrent parent to initiate another cycle with up to five cycles of hybridization back to the recurrent parent. This approach allows for the transmission of the major trait, while providing the possibility of increasing overall performance above that of the recurrent parent through contribution of favorable minor genes from the donor parent.

Early-Generation Testing

Early-generation testing is designed to identify bulk hybrid populations so that only families that have the greatest potential are carried forward in the breeding program. For the pedigree method to be effective, it is necessary that selection be confined to visible characteristics with high heritability in early generations. A large number of F_2 progeny rows must be carried forward to the F_6 generation, so that poorly heritable characters, for example, seed yield, can be selected at a later generation. Early-generation testing and a modified form of recurrent selection are being tested to improve seed yield in flax. Using field trials, bulk F_3 populations are tested. $F_{3:4}$ plants from the highest yielding families are selected from increase plots and grown in an off-season nursery as single-plant

progeny rows. F_5 seed from harvested rows is used to grow preliminary yield trials. The superior lines are further evaluated using field trials and the best lines are intercrossed to begin another breeding cycle. This method of selection is designed to improve the frequency of favorable alleles (for the trait in this example, seed yield undergoing selection) in a population.

Pollen-Mediated Gene Flow

While flax is considered primarily autogamous, pollen-mediated gene flow may occur and allow gene movement between pure breeding lines (Jhala et al., 2009). Pollen-mediated gene flow was quantified between two cultivars of flax in western Canada using the xenia effect of dominant alleles of high-ALA to the low-ALA trait as a marker (Jhala et al., 2011). Frequency of gene flow was highest near the source: 0.0185 at 0.1 m but declined rapidly with distance. Outcrossing was reduced by 50% (O_{50}) and 90% (O_{90}) between 0.85 and 2.64 m and 5.68–17.56 m, respectively. No gene flow was detected at any site or year >35 m distance from the pollen source. Low levels of gene flow are unlikely to be problematic for seed growers but must be considered when isolation distances are established within breeding programs.

Male Sterility

Male sterility is the inability of the plant to produce functional anthers or pollen, through chromosomal aberrations, gene action, or cytoplasmic influences. Plant breeders make use of cytoplasmic male sterility and pollen fertility restorer genes to take advantage of hybrid vigor and superior performance of the hybrid over the parental lines due to heterosis. Many components critical for commercial hybrid production (Palmer et al., 2001) are lacking for flax: a stable male-sterile, female-fertile sterility system; a selection system to obtain 100% female plants that set seed normally and can be mechanically harvested; and an efficient pollen transfer mechanism from pollen parent to female parent. Considerable research is needed before an efficient, productive, and cost-effective system of hybrid seed production could be available in flax (Green et al., 2008).

Mutation Breeding

The primary strategy in mutation-based breeding has been to upgrade elite lines by altering one or two production-limiting or quality traits (Ahloowalia et al., 2004; Rowland and Bhatty, 1990) using radiation or chemical mutagenesis. Mutation breeding in flax led to the development of a new type of edible flaxseed oil that has nearly eliminated ALA (Green and Marshall, 1984; Rowland, 1991). The deficient ALA trait is known to be controlled by two recessive genes (*ln1* and *ln2*) at independent loci (Green et al., 1984; Green, 1986c; Rowland, 1991; Ntiamoah and Rowland, 1997). Vrinten et al. (2005) reported the cloning

of two members of the *FATTY ACID DESATURASE* (*FAD3*) family of microsomal Δ15-FADs, designated *LuFAD3A* and *LuFAD3B* (to be discussed further under Flaxseed Oil). Mutation breeding has a much greater potential to enhance the available traits in flax, including further modification to fatty acid profiles and the reduction of bast fiber content (McKenzie, 2011). Mutation breeding may have potential in the future to remove undesirable compounds from flaxseed, such as cyanogenic glycosides and linatine (Green et al., 2008).

Use of Genetic Markers in Flax Breeding

The development of genetic markers in flax has not kept pace with other oil seed crops. Rapid amplification of polymorphic DNA (RAPD) markers has been used to assess the genetic diversity of North American flax lines (Fu et al., 2003a) and to test for the presence of off-types in breeding lines (Fu et al., 2003b; McKenzie, 2011). The number of available RAPD was limited and their usefulness was limited. Recently expressed sequence tags-single sequence repeats (EST-SSR) markers for flax have been developed (Roose-Amsaleg et al., 2006). Cloutier et al. (2009) reported the development of 275 polymorphic EST-SSRs which can be used to develop physical maps, QTL mapping, and potential to decrease the time for cultivar development. Following development of these markers, a consensus genetic and physical map using DNA markers in combination with the recent sequencing of the flax genome (http://www.phytozome.org/flax.php; Wang et al., 2012) was reported (Cloutier et al., 2012). This map was generated using three different mapping populations (CDC Bethune/MacBeth, E1474/Viking, and SP2047/UGG5-5). The consensus map has 670 DNA markers anchored to 204 of the 416 fingerprinted contigs of the physical map and the overall map density is 2.0 cM for markers arranged on 15 linkage groups. Genome-wide SNP discovery in flax is now possible using genotyping-by-sequencing approach that can be used to identify QTLs/regions of the genome linked to novel genes in this crop plant for disease resistance, seed and fiber yield, and traits of importance to human and animal health (Kumar et al., 2012). These genomic resources will be available for research purposes.

Market Constraints to Genetically Engineered Flax

Flax was among the first crop species to be genetically engineered to insert genes of potential agronomic value. Herbicide resistant traits have been expressed in flax including chlorsulfuron and metsulfuron methyl resistance (McSheffrey et al., 1992), glufosinate-ammonium (McHughen and Holm, 1995), and glyphosate resistance (Jordan and McHughen, 1988). The first GE flax cultivar "CDC Triffid" was registered in Canada in 1997–98 and was approved for food and feed and environmental release (McHughen et al., 1997). In 2001, prior to broad-scale release, this cultivar was deregistered at the request of the flax industry to protect the Canadian flaxseed export market to European countries (McSheffrey

et al., 1992). In 2009, traces of CDC Triffid were found in Germany (Bedard, 2009). The Flax Council of Canada (2014b) has detected widespread, low-level presence of GE flax in commercial flax stocks and by 2014 industry stewardships programs were successful in clearing most of the CDC Triffid from the Canadian flax system. (Reference Flax Council of Canada: http://flaxcouncil.ca/flax-council-of-canada-stewardship-program-update/). The source(s) of contamination have not yet been determined but are not associated with a single point source. Consumer and political concerns about GE crops for food and feed continue to be pervasive in the EU, and this incident temporarily halted exports of Canadian flaxseeds to key markets including the EU and Brazil (Flax Council of Canada, 2010). Since identification of CDC Triffid, extensive flaxseed testing has been instituted in Canada: prior to planting, post-harvest, at initial receptor sites (elevators), and at grain terminals prior to export. The majority (>80%) of grain exports to the EU take place from Thunder Bay, and in 2011, 5% of flax samples were positive for the presence of CDC Triffid. Testing of adventitious presence, a critical element of regulatory compliance, is confounded by the practical level of detection of PCR assays (0.01% or 1 GE seed in 9999 conventional seeds) and the large sources of error inherent in taking representative and random samples in large seed lots. The testing protocol implemented requires collection of a 2-kg sample of any flax entering the handling system, and testing of four 60-g subsamples for the presence of GE flax (Canadian Grain Commission, 2010). The sampling protocol presumably gives a 95% probability (or 5% error) of detecting 1 GE seed in 9999 non-GE flaxseeds (Remund et al., 2001; Whitaker et al., 2001). Lamb and Booker (2011) demonstrated, however, that low levels of presumed contamination are indistinguishable from the number of positive tests expected from a clean seed lot given the potential rates of false positives. This finding has significant implications for the testing of flaxseed lots for the presence of GE seed. While testing will be required for the foreseeable future to reduce the risk of product rejection, zero tolerance in the bulk grain handling system is neither verifiable nor achievable technically. The reader is referred to Jhala et al. (2009) for a more in-depth discussion of the potential benefits and risks, regulatory aspects, and gene-flow issues associated with GE flax.

FLAXSEED OIL

The vegetable oil obtained from flaxseed has been used for many centuries, and flax cultivation dates back to a few millennia (Henriksen and Robinson, 1996). Phylogenetic analysis has indicated that flax was initially domesticated for oil instead of fiber (Allaby et al., 2005). This ALA-rich oil has unique properties that are useful for food and non-food applications. Food-grade oil can be obtained by cold-pressing and commercialized as "flaxseed oil." Alternatively, extraction with solvents can be performed when the oil is used for nonfood industrial applications. In this case the oil is often referred to as "linseed oil"

and can be used in the production of paints, varnishes, and linoleum flooring. Recently, considerable attention has also been devoted to flaxseed as a functional food ingredient due to reported health benefits (Goyal et al., 2014). The specific components, which contribute mostly to these benefits, have not been completely elucidated, but most of the research has focused on oil components, lignans, and soluble polysaccharides.

Oil Composition and Properties

The oil content of flaxseed ranges between 35% and 45% (w/w), although higher values have been reported (Western Canadian flaxseed, 2010). The main component of most vegetable oils is triacylglycerol (TAG), which accumulates during the maturation stage of seed development and is used by the plant as a source of reduced carbon to support seedling growth after germination. The chemical composition of TAGs from flaxseed oil is well characterized (Vereshchagin and Novitskaya, 1965). The proportions of the five major fatty acids, namely ALA, palmitic, stearic, oleic, and LA, found in flaxseed oil are shown in Table 6.1. ALA can accumulate to levels as high as 64% depending on the cultivar and growth conditions (Green and Marshall, 1981). It has been well documented that environmental factors such as soil and climate can influence fatty acid composition of seed oils. Oleaginous crops cultivated at lower temperatures usually accumulate higher levels of polyunsaturated fatty acids (PUFAs) at the expense of saturated and monounsaturated fatty acids (Green, 1986b; Deng and Scarth, 1998). This effect is evident when comparing flaxseed cultivated in North Dakota with similar varieties grown in western Canada (Hettiarachy et al., 1990).

The high content of ALA influences some physicochemical properties of vegetable oils. For example, flaxseed oil with high ALA content has a higher specific gravity and lower melting and flash points than the oil of a determined flax cultivar with low-ALA content (Przybylski, 2005). The unsaponifiable matter composes a minor fraction of vegetable oils. However, it contains compounds that play an important role in preventing lipid oxidation, which affects the flavor and nutritional quality of the oil (Rathjen and Steinhart, 1997). Among such compounds are phytosterols and antioxidants (tocopherols, tocotrienols, and plastochromanol-8) (Table 6.1). Flaxseed oil contains a similar amount of unsaponifiable components compared to other vegetable oils. It is characterized, however, by relatively low amounts of total tocopherols and high contents of Δ5-avenasterol, tocotrienols, and plastochromanol-8 (Velasco and Goffman, 2000; Ahmed et al., 2005). Plastochromanol-8 is a potent antioxidant (Olejnik et al., 1997) and might play a role in the stability of flaxseed oil.

Processing of Flax Oil for Industrial Applications

A number of vegetable oils have been traditionally used in industrial coating formulations for their unique fatty acid compositions. These include oils from

TABLE 6.1 Properties of Flax and Linola™ Oils

Parameter	Flax Oil	Linola™ Oil[a]
Relative density (20°C/water at 4°C)	0.93–0.94	0.92
Refractive index (n_D 20°C)	1.48	1.47
Viscosity (cp)	–	46.4
Iodine value	182–203	144
Unsaponifiable matter (%)	0.1–1.7	0.6
Saponification value (mg HOH/g)	187–195	185
Free fatty acids (% oleic)	0.1–2.0	<0.02
Triacylglycerol (%)	94–98	96–98
Fatty Acid	**%, wt**	
14:0	Trace	Trace
16:0	5.0–7.0	5.6–6.1
16:1	Trace-0.2	–
18:0	2.0–6.0	3.8–4.0
18:1 (*cis*Δ9)	17.9–19.0	15.5–15.9
18:2 (*cis*Δ9,12)	14.7–24.1	71.3–71.8
α-18:3 (*cis*Δ9,12,15)	47.4–58.7	2.0
20:0	Trace-0.4	–
Sterols	**%, wt**	
Brassicasterol	1	1
Campesterol	27	23
Stigmasterol	8	4
β-Sitosterol	50	54
Δ^5-Avenasterol	10	18
Total sterols (g/kg)	2.3	2.2
Tocopherols	**ppm**	
α-Tocopherol	20	15
γ-Tocopherol	200	200
δ-Tocopherol	7	5
Plastochromanol-8	120	110
Total tocopherols	347	330

[a]*Refined, bleached and deodorized.*
Adapted from Przybylski, R., 2005 Flax oil and high linolenic oils. In: Shahidi, F. (Ed.), Bailey's Industrial Oil and Fat Products. John Wiley & Sons, Inc., Hoboken, NJ, pp. 281–298.

tung, perilla, oiticica, castor, and flaxseed (Derksen et al., 1995). The high degree of desaturation of flax oil makes it highly susceptible for oxidation at high temperatures, leading to cross-linking and polymerization reactions. As a result, it forms a soft and durable film upon air exposure, a property that is advantageous for paints and varnishes. In addition, flax oil turn paints more fluid, transparent, and glossy. To increase its binder properties, flax oil is refined to remove lecithins and free fatty acids. The refining process provides partial oxidation of double bonds and initiates polymerization, resulting in high viscosity and decreasing the time required for drying. Depending on the application, different refining processes can be used to achieve the desired balance of viscosity and drying time. For example, to improve flow of oil based paints, flax oil is "boiled" at temperature of 137.5°C in the presence of zirconium, manganese, or cobalt until the specific gravity reaches 0.942 at 15.6°C. Using this process, flax oil viscosity increases from 40 cP to 80–120 cP, while the drying time decreases from few days to 12–20 h. To improve some physicochemical properties of highly pigmented paints (eg, for undercoats and wall paints), "blown oil" is prepared by increasing the degree of polymerization, removing the metal components previously mentioned, thus increasing the viscosity to >3 P and reducing the drying time to 24–36 h. Processed flax oil can be also be used as an ingredient of ink formulations. In this case, "stand oil" is prepared by increasing the temperature to 290°C under air exposure until the viscosity reaches ~200 P. Flax oil is also used for the production of linoleum flooring, leather-imitation, among other products (Jhala and Hall, 2010; Rowland et al., 1995).

Development and Uses of Low–α-Linolenic Flaxseed Varieties

Despite of many uses of flaxseed oil, the high ALA content renders low oxidative stability at high temperatures, making this oil unsuitable for cooking. To expand the potential markets for flaxseed, low-ALA flax varieties were developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia in partnership with the United Grain Growers Ltd. (currently Viterra, Canada) using traditional breeding techniques (Green, 1986a). These lines, which are known as Linola™, or Solin in Canada, have a yellow seed color that helps distinguish them from the traditionally brown flaxseed. Low-ALA varieties are deficient in the enzyme that converts LA to ALA (to be discussed in the next section), with ALA composing not more than 2% of total seed fatty acids. Consequently, LA accumulates at high levels of ~70% (Table 6.1), which is comparable to other high-LA oils such as sunflower and safflower. This vegetable oil has increased oxidative stability and low-ALA varieties can be used in conventional oilseed processing for seed crushing and oil refinement (Green and Dribnenki, 1994). Deodorized Linola™ oil has comparable flavor quality to canola oil and has been approved by the Food and Drug Administration for use as cooking and salad oil and as an ingredient in food products. Moreover, Linola™ can be cultivated in the same regions as traditional flaxseed

(Weiss, 1993), representing an alternative for a high-LA oil seed production in climate conditions from the Canadian Prairies and northern Europe. Further improvement of low-ALA flax varieties include enhancing the feeding quality of the meal and reducing saturated fatty acid content.

Potential for Seed Oil Modification

The value and industrial usefulness of an oil increase if it is highly enriched in a particular fatty acid (Taylor et al., 2011). For example, castor bean contains ~90% ricinoleic acid, which has contributed to its use numerous industrial applications including the production polymers such as nylon 6, 10 (Somerville and Bonetta, 2001). Although flax is highly enriched in ALA, increasing the content of this PUFA further could increase the industrial usefulness of flax oil and also render it more useful for genetic engineering to produce other industrial fatty acids from ALA. The same may apply to Solin that contains high levels of LA.

Seed Oil Formation

The process of seed oil formation has been reviewed in considerable detail elsewhere (eg, Weselake et al., 2009; Taylor et al., 2011; Bates and Browse, 2012). Here, we present a brief overview of fatty acid and TAG biosynthesis with a focus on aspects relevant to flax. A generalized schematic representation of this process in developing oleaginous seeds is depicted in Fig. 6.2. After de novo synthesis in plastids, fatty acids are exported as saturated (without double bonds) or monounsaturated (single double bond) entities to the cytoplasm. Several enzymes involved in the biosynthesis of fatty acids including β-ketoacyl CoA synthase, fatty acid elongase, stearoyl-ACP desaturase (SAD), and FAD have been previously identified in flaxseed (Fofana et al., 2004). SAD is a soluble enzyme that catalyzes the production of monounsaturated fatty acids, while attached to acyl carrier protein, in plastid. When fatty acids are released in the cytoplasm, they become esterified with coenzyme A (CoA), forming the main substrate for the acyl-CoA–dependent assembly of phospholipids and TAGs.

In the endoplasmic reticulum (ER), additional double bonds are produced by the catalytic action of membrane-bound desaturases. The FAD2 catalyzes the conversion of oleic acid ($18{:}1^{cis\Delta 9}$) to LA ($18{:}2^{cis\Delta 9,12}$), while the fatty acid Δ15 desaturase (FAD3) catalyzes the conversion of LA to ALA ($18{:}3^{cis\Delta 9,12,15}$). These reactions utilize the acyl chains at the *sn*-2 position of phosphatidylcholine (PC) as substrates in a catalytic process that requires an associated electron transport chain containing cytochrome b5 (Smith et al., 1990). In flaxseed, two genes encoding FAD3 have been characterized (*LuFAD3A* and *LuFAD3B*) (Vrinten et al., 2005). Point mutations in these genes lead to inactive polypeptides, co-segregating with low levels of unsaturation in the low-ALA flaxseed varieties. This indicates that *LuFAD3A* and *LuFAD3B* encode the main enzymes responsible for FAD3 enzyme activity in flaxseed, accounting for the key genetic factors responsible for the uniqueness of this vegetable oil. Banik et al. (2011)

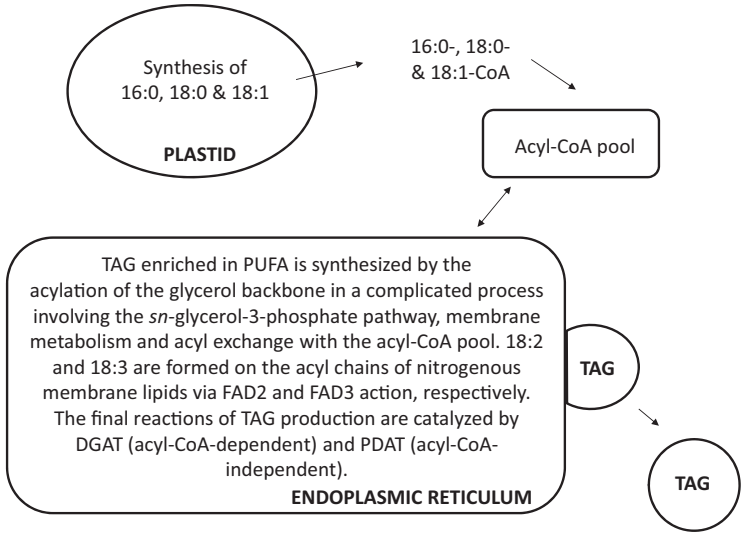

FIGURE 6.2 Simplification of triacylglycerol (TAG) synthesis in a developing seeds of oleaginous plants. *CoA*, coenzyme A; *FAD*, fatty acid desaturase; *PUFA*, polyunsaturated fatty acids; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid (*cis*Δ9); 18:2, linoleic acid (*cis*Δ9,12); 18:3, α-linolenic acid (*cis*Δ9,12,15). *Adapted from Weselake, R.J., Shah, S., Taylor, D.C., Rahman, H., Shah, S., Laroche, A., McVetty, P.B.E., Harwood, J.L., 2009. Increasing the flow of carbon into seed oil. Biotechnol. Adv. 27, 866–878 and Bates, P.D., Browse, J. 2012. The significance of different diacylglycerol synthesis pathways on plant oil composition and bioengineering. Front. Plant Sci. 3, 147.*

investigated the expression of *FAD3A*, *FAD3B*, and a novel *FAD3C* during seed development in high-, moderate-, and low-ALA genotypes of flax. Their results demonstrated that FAD3A and FAD3B were responsible for ALA accumulation in flaxseeds but the novel FAD3C did not appear to have a major role in this process. Recently, Khadake et al. (2011) reported that variants of FAD3 in flax differed in the rate at which they could catalyze the conversion of LA to ALA. Subtle changes in amino acid sequence appeared to account for the variation in enzyme activity. Expression of *SAD* and *FAD2* has been shown to be differentially modulated during flaxseed development (Fofana et al., 2006). *SAD* was maximally expressed at very early stages of seed development whereas *FAD2* showed maximal expression 16 days after anthesis. Production of LA did not positively correlate with *FAD2* expression. Instead, ALA levels steadily rose, which suggested that LA was a transient substrate that was rapidly utilized by FAD3. Khadake et al. (2009) have cloned a second *FAD2* from flax genomic DNA, which was designated *LuFAD2-2*. The enzyme was encoded by an intronless gene and exhibited 85% similarity in amino acid sequence to the previously reported FAD2. Functional expression of LuFAD2-2 in yeast suggested that the recombinant enzyme had a similar substrate specificity to LuFAD2.

The process of TAG formation is intricately associated with membrane metabolism. Various mechanisms are operative in moving PUFA from

PUFA-enriched PC into TAG (Weselake et al., 2009; Bates and Browse, 2012). Although TAG can be produced via the *sn*-glycerol-3-phosphate pathway, most of the TAG formation in PUFA-enriched crops involves modifications at the level of PC. Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA–dependent acylation of *sn*-1,2 diacylglycerol (DAG) to produce TAG (Lung and Weselake, 2006; Liu et al., 2012). At least two distinct membrane-bound polypeptides, referred to as DGAT1 and DGAT2, have been identified in plants (Hobbs et al., 1999; Shockey et al., 2006). A soluble polypeptide displaying DGAT activity has been reported although its role for seed oil formation is yet to be determined (Saha et al., 2006). In some oilseed species, the level of DGAT activity during seed development has a major influence in the flow of carbon into TAG (Perry and Harwood, 1993; Zou et al., 1999; Cahoon et al., 2007; Zheng et al., 2008). It has been previously reported that DGAT activity from microsomal membranes of flaxseed displays high specificity for ALA (Sorensen et al., 2005). Another pathway leading to TAG formation is catalyzed by phospholipid:diacylglycerol transferase (PDAT), which transfers the acyl moiety from the *sn*-2 position of PC to *sn*-1,2-DAG (Dahlqvist et al., 2000). This enzyme might have a substantial influence on the incorporation of ALA into TAG since it directly utilizes the product of FAD3 as a substrate. It has been demonstrated that PDAT and DGAT reactions have overlapping functions in oil formation in Arabidopsis (Zhang et al., 2009). Rao et al. (2008) investigated the fatty acid composition of PC and TAG during seed development in flax in relation to *FAD3* expression. Although FAD3 appeared to have a major role in producing ALA-enriched TAG, there also appeared be selective transfer of ALA into TAG from PC presumably catalyzed by acyltransferase(s). Recently, several *DGAT* and *PDAT* genes were cloned from developing flaxseed and functionally characterized in yeast and Arabidopsis (Pan et al., 2013). This study indicated that developing flaxseed produced a pair of PDAT homologs exhibiting a preference for substrates containing ALA. Thus, the encoding genes may be useful in genetic engineering or molecular breeding strategies aimed at further increasing the ALA content of flax oil. It is anticipated that the recently developed genomic resources for flax (Venglat et al., 2011; Wang et al., 2012) will further accelerate research on deciphering the details of oil biosynthesis in this crop.

TAG is stored in organelles known as oilbodies, which are a core of neutral lipids surrounded by a monolayer of phospholipids and coated by proteins known as oleosins (Huang, 1996). Oleosins function as structural proteins, preventing the coalescence of oilbodies during seed maturation, and preserving these organelles as small entities, which may facilitate TAG mobilization during seedling growth (Siloto et al., 2006). Flax oil bodies have an average diameter of 1.34 μm, corresponding to twice the average size of canola oilbodies as a result of lower oleosin to TAG ratio (Tzen et al., 1993). The *OLEOSIN* gene family in flaxseed is composed of two genes encoding a high molecular mass isoform (18.6 kDa) and four genes encoding a low molecular mass isoform (16.03 kDa) (Chaudhary, 1975).

High-Palmitic Acid Flaxseed

While low levels of saturated fatty acids are generally desired for cooking oils, small-chain saturated fatty acids such as palmitic acid are used for the production of margarines, shortening and soaps. A flaxseed cultivar with double content of palmitic acid was developed by Rowland et al. (2002) to target this market niche. Heterologous expression of a medium-chain thioestearase from *Cuphea wrightii* in this variety further increased the level of palmitic acid to 39%, resulting also in the production of lauric (C12:0) and myristic (C14:0) acids that have other industrial applications (Rowland et al., 2002). For example, lauric acid–enriched oils are useful for production of detergents (Taylor et al., 2011).

Potential for Producing Oils Containing Conjugated Octadecatrienoic Acids

PUFAs are usually synthesized having two single bonds separating each double bond. Some plant species deviate from this, producing linolenic acids with at least one pair of double bonds separated by one single bond. Plant oils enriched in conjugated octadecatrienoic acids are useful raw materials for the production of organic coatings and polymers (Derksen et al., 1995; www.cyberlipid.org/fa/acid0003.htm). For example, the oil from tung tree (*Aleurites fordii*) seeds, which is rich in α-eleostearic acid (18:3$^{\Delta cis9,trans11,13}$), is a very effective drying oil, requiring lower levels of oxygen for hardening and drying more quickly than flax oil (Gunstone and Harwood, 2007). Other industrially useful octadecatrienoic acids include calendic acid (18:3$^{\Delta trans8,10,cis12}$) from marigold (*Calendula officinalis*) seed, licanic acid (18:3$^{4\text{-oxo}\Delta cis9,trans11,13}$) from the oiticica tree (*Lieania rigida*), and punicic acid (18:3$^{\Delta cis9,trans11,cis13}$) from pomegranate (*Punica granatum*) seed (www.lipidlibrary.aocs.org/plantbio/unusualfa/index.htm).

Conjugated double bonds can be introduced through the catalytic action of a homolog of FAD2 known as fatty acid conjugase (FADX), which utilizes LA as substrate (Rawat et al., 2012). Examples of linolenic acids that are derived from LA, including some conjugated trienes, are shown in Fig. 6.3. An FADX from pomegranate catalyzes the conversion of the Δ12 double bond of LA into two conjugated double bonds (*cis*Δ11 and *cis*Δ13), forming punicic acid (Hornung et al., 2002; Iwabuchi et al., 2003). In the case of calendic acid, an FADX from marigold catalyzes the modification of the Δ9 double bond of LA, producing conjugated *trans*Δ8 and *trans*Δ10 double bonds (Cahoon et al., 2001). Formation of α-eleostearic acid in tung tree seed is catalyzed by an FADX that catalyzes the conversion of the Δ12 double bond of LA into conjugated *trans*Δ11 and *trans*Δ13 double bonds (Dyer et al., 2002). Expression of FADX from these species in oilseed crops has the potential to be used as a biotechnological tool for large-scale production of seed oil containing conjugated fatty acids. High-LA flaxseed (ie, Solin or Linola™) logically represents an attractive target for production of oil containing conjugated octadecatrienoic acids. A combination of ALA, α-eleostearic, and other conjugated octadecatrienoic acids could potentially produce unique oils with novel properties and industrial applications.

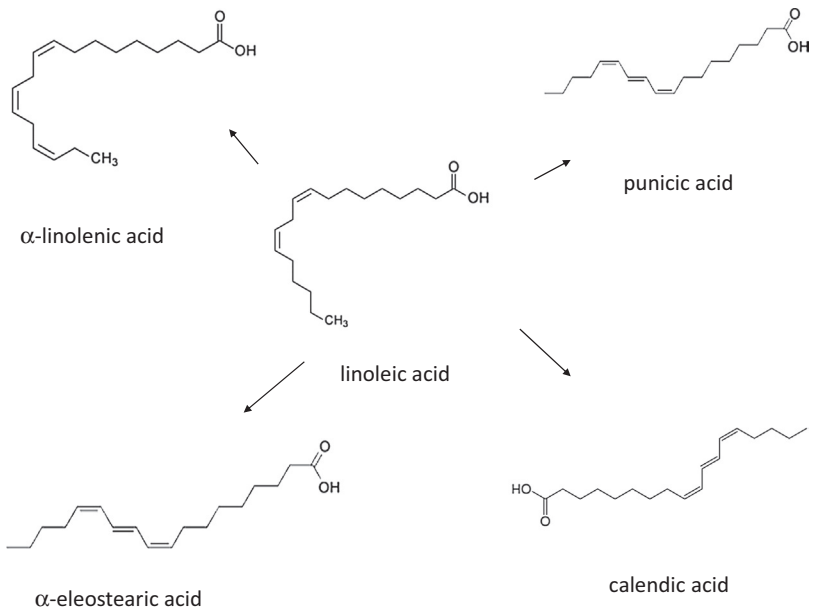

FIGURE 6.3 Examples of linolenic acids derived from linoleic acid. The formation of α-linolenic acid, which contains methylene-interrupted double bonds, is catalyzed by fatty acid desaturase (FAD) 2. The formation of punicic acid, α-eleostearic acid and calendic acid, which contain conjugated or non–methylene-interrupted double bonds, are catalyzed by divergent forms of FAD2 (FADX). The substrates of these FAD2 or FADX are nitrogenous phospholipids (eg, phosphatidylcholine). *See Cahoon, E.B., Dietrich, C.R., Meyer, K., Damude, H.G., Dyer, J.M., Kinney, A.J., 2006. Conjugated fatty acids accumulate to high levels in phospholipids of metabolically engineered soybean and Arabidopsis seeds. Phytochemistry, 67, 1166–1176 and* www.lipidlibrary.aocs.org/plantbio/unusualfa/index.htm.

Engineering the Antioxidant Capacity of Flax

Although Europe has traditionally shown strong resistance to the development of GE crops, in the past decade there has been intensive research on transgenic flax conducted by investigators from Wroclaw University in Poland. One area of their investigations has focused on increasing the antioxidant capacity of flax, which has the potential to protect PUFA from oxidation (Lorenc-Kukula et al., 2005; Żuk et al., 2011, 2012). In addition to decreasing the potential for PUFA rancidity, the antioxidants themselves may also provide health benefits in the human diet. In a recent study, expression of the gene encoding chalcone synthase was co-suppressed in developing Linola-type flax in order to partially redirect substrates from flavonoid biosynthesis to other routes in the phenylpropanoid pathway (Zuk et al., 2012). This modification resulted in overproduction of hydrolyzable tannins in the transgenic plants, which changed seeds from light yellow to brown. The antioxidant capacity was significantly increased by 40–50% in extracts of seeds from transgenic lines and the stability of the oil

was increased as indicated by a decrease in peroxide value by ~72%. Interestingly, the proportion of ALA increased by ~25-fold in the seed oil transgenic plants compared to the control. The high accumulation of ALA was difficult to explain, but the authors speculated that FAD3 may have somehow been activated. Transgenic plants also showed increased resistance to *Fusarium* spp. infection. In related work, Fujisawa et al. (2008) expressed a bacterial phytoene synthase gene, *crtB*, in developing flaxseed which resulted in a 7.8- to 18.6-fold increase in total carotenoids in seeds. Lutein was the only carotenoid that was detected in control seeds. β-Carotene and α-carotene were present in a ratio of ~3:1. β-Carotene is the main source of dietary vitamin A. The various approaches to increase the antioxidant capacity of flaxseed could prove useful in the context of further increasing the ALA content of flax oil or in engineering the production of oils containing conjuagated ocatdecatrienoic acids, which are even more prone to oxidation than ALA.

Engineering Flax Fiber

Flax fibers can be separated from other parts of the stem by the process of retting providing raw material for the textile industry (Easson et al., 1996). Overproduction of flavonoids in flaxseed through genetic engineering has been shown to increase the antioxidative capacity of flax fiber, suggesting the modified fibers may be useful for biomedical applications such as wound dressings (Żuk et al., 2011). FT-IR analysis of fibers from transgenic plants indicated a change in the arrangement of cellulose polymer in the fibers and there was also a significant decrease in the quantity of hydrogen bonds. Recently, the introduction into flax of a cDNA encoding a potato β-1,3-glucanse resulted in fibers with increased levels of pectins and phenolics and decreased levels of lignin (Wojtasik et al., 2013). The mechanical properties of the resulting fibers were improved by their biochemical composition and the increased antioxidant capacity of the fibers suggested they would be useful in biomedical applications.

Researchers at the University of Wroclaw have also engineered flax to accumulate bioplastic in the growing fibers (Wróbel et al., 2004). Three bacterial genes were expressed in developing flax stems so as to produce poly-β-hydroxybutyrate, a biodegradable plastic. There was a substantial improvement in the elastic properties of the fibers from the GE plants and there was a decrease in lignin, pectin, and hemicellulose content, which improved retting efficiency (Wróbel-Kwiatkowska et al., 2007). In addition, there was an increased resistance to pathogen infection in transgenic plants which was attributable to an increase in phenolic acid content. In more recent work, the investigators have explored the properties of composites prepared by combining bioplastic flax fibers with polylactic acid or poly-ε-caprolactone, which served as the matrix while the bioplastic flax fibers served as reinforcement (Wróbel-Kwiatkowska et al., 2012). Composites of polylactic acid and bioplastic flax fibers appeared to display bacteriostatic, platelet antiaggregation and noncytotoxic effects. The investigators suggested that the composites might find applications as implants to replace damaged human tissues.

CLOSING COMMENTS

In addition to its traditional industrial uses in the production of linoleum and paints and varnishes, flax has the potential to become a platform crop for even more powerful drying agents, antioxidants, and high performance fibers. Flax oil has the potential to be part of a growing specialty oils market that could drive a substantial increase in production of the crop. To compete for the potentially large modified oils markets, however, flax must overcome major challenges. Although the crop is amenable to genetic transformation, it has yet to benefit, like other oilseeds crops have, from enhanced agronomic traits such as herbicide-resistance, enhanced yield from hybrid breeding and increased breeding efficiency from the routine use of marker-assisted breeding for trait development. Flax has not yet received major investments in trait and cultivar development. Seed size, yield, yield stability, disease resistance, and weed control remain significant barriers for flax production. The recent sequencing of the flax genome (/www.phytozome.org/flax.php; Wang et al., 2012), however, represents a major milestone in advancement of this crop and is sure to provide valuable tools for genomics-assisted breeding of this crop in addition to identifying targets for biotechnological modification of the crop.

ACKNOWLEDGMENTS

R.J.W. thanks Alberta Enterprise and Advanced Education, AVAC Ltd., Genome Canada, Genome Prairie, the Canada Research Chairs Program, the Canada Foundation for Innovation, and Genome Canada and Genome Prairie for supporting his research on flax. H.B. would like to thank University of Saskatchewan, Saskatchewan Ministry of Agriculture, SeCan, SaskFlax Development Commission, and Genome Canada and Genome Prairie for research support. L.M.H. would like to acknowledge the contribution of Alberta Ingenuity, Alberta Advanced Education, the University of Alberta, Alberta Agriculture and Rural Development, Vitera, Alberta Crop Industrial Development Fund, and Natural Science and Engineering Research Council. The authors thank Crystal Snyder for her critical assessment of the manuscript.

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