REVIEW & INTERPRETATION

Genetically Engineered Flax: Potential Benefits, Risks, Regulations, and Mitigation of Transgene Movement

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ABSTRACT

Flax (*Linum usitatissimum* L.) has been grown for more than 6000 years, primarily for oil and fiber. Advances in plant biotechnology have resulted in flax cultivars with increased herbicides resistance and there is potential to produce transgenic flax with seed oil containing fatty acids with nutraceutical properties. Flax oil is a rich source of α-linolenic acid (ALA, $18{:}3^{cis\Delta 9,12,15}$), a precursor of the very long chain polyunsaturated fatty acids (VLCPUFA), eicosapentaenoic acid (EPA, $20{:}5^{cis\Delta 5,8,11,14,17}$), and docosahexaenoic acid (DHA, $22{:}6^{cis\Delta 4,7,10,13,16,19}$). Current research on medicinal applications of ω-3 fatty acids, especially to reduce the risk of cardiovascular diseases and cancer, suggests that genetic modification of flax may provide substantial health benefits. There are concerns, however, with the commercialization of genetically engineered (GE) flax (which includes the potential movement of transgenes by pollen and seed, and subsequent introgression with weedy and wild relatives, impact on non-target organisms, and changes in biodiversity). A prerequisite to the unconfined cultivation of transgenic flax is an environmental risk assessment analysis. In this paper, we discuss the history and current status of genetic transformations in flax, potential benefits and consequences of GE flax, and the government regulatory framework in Canada for regulating novel flax. Finally, we discuss the best management practices to mitigate transgene movement from transgenic flax. Our intent was to evaluate biology and agronomy to predict the environmental biosafety of GE flax before commercial cultivation.

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Abbreviations: ALA, alpha linolenic acid; AP, adventitious presence; ASCVD, atherosclerotic cardiovascular disease; APHIS, Animal and Plant Health Inspection Service; CFIA, Canadian Food Inspection Agency; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPSPS, *enolpyruvylshikimate-3-phosphate synthase;* EU, European Union; FDA, Food and Drug Administration; GRAS, generally recognized as safe; GE, genetically engineered; MCPA, 2-methyl-4-chlorophenoxyacetic acid; *npt*, neomycin phosphotransferase; KCS, *ketoacyl-CoA synthase*; PAT, *phosphonothricin acetyl transferase;* PBO, Plant Biosafety Office; PHB, polyhydroxybutyrate; PMI, phosphomannose isomerase; PNT, plant with novel trait; rDNA, recombinant deoxyribonucleic acid; RNAi, ribonucleic acid interference; TAG, triacylglycerol; USDA, United States Department of Agriculture; VLCPUFAs, very long chain polyunsaturated fatty acids.

Flax, *Linum usitatissimum* L., also known as linseed, is the third most important oilseed crop after canola (*Brassica napus* L.) and soybean [*Glycine max* (L.) Merr.] in Canada. Although the genus *Linum* is composed of approximately 230 species, cultivated flax is the only species of economic importance (Rowland et al., 1995). It is one of the oldest plants cultivated for fiber and oil (De Candolle, 1904). Archaeological evidence of flaxseed and flax-based products dates back to 5500–5000 BC at Tepe Sabz, Iran; 5800–5600 BC at Telles Sawwan, Iraq; and 8050–7542 BC at Tell Mureybat, Syria (Gill, 1987). The use of flax for various purposes including production of food, fiber, and medicine is well established (Lee, 2003).

Flax is an annual, dicotyledonous, inbreeding plant species grown on almost all continents. Flax varieties are distinguished as fiber flax (for bast fiber) and linseed (for seed oil). Flax fiber is

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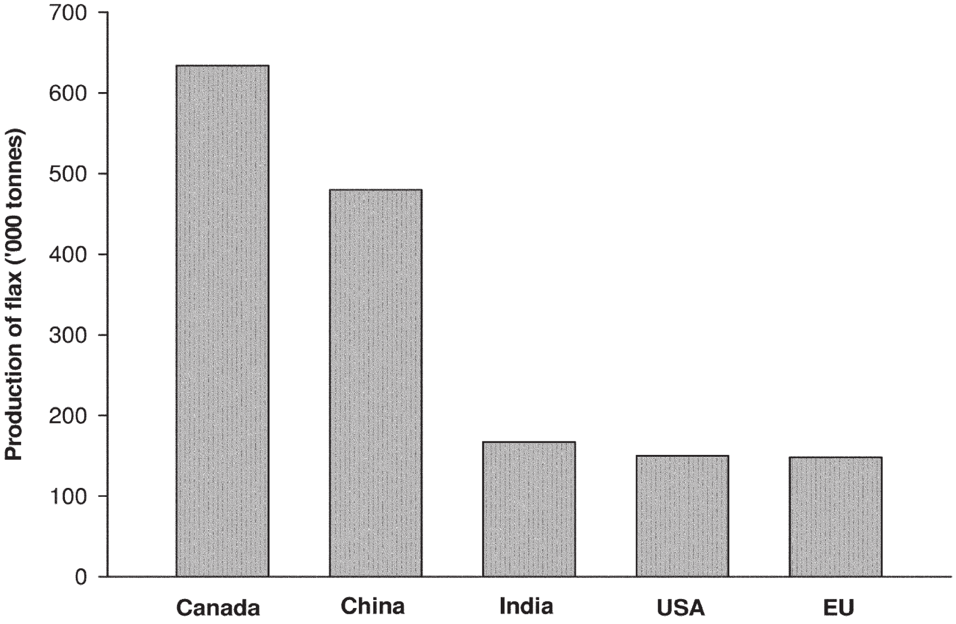

Figure 1. Production of flax in five major flax producing countries in 2007. (Source: FAOSTAT 2007).

used in the textile industry for linen cloth and also in the paper and pulp industry to make paper products including cigarette paper (Belonogova and Raldugina, 2006). Traditionally, linseed oil was obtained by extraction of seed with an organic solvent (Dillman, 1953). Currently, flax oil is used for manufacturing varnishes, paints, printing ink, linoleum, oilcloths, and plastics (Rowland et al., 1995). The fiber from these varieties may also be useful for bio-product applications such as geotextiles and insulation (Anonymous, 2006; Kymäläinen and Sjöberg, 2008).

The five major flax producing countries are Canada, China, India, the U.S., and the European Union (EU) (Fig. 1) (FAOSTAT, 2007). World linseed production has ranged from two to three million Mg per year for the last several years (AAFC, 2005). Canada is the world leader in the production and export of linseed (Flax Council of Canada, 2007). Canada produced ~39% of the world's total flax output in 1997 (AAFC, 1997). Production of flax in the last five years in Canada fluctuated annually depending on the markets (Fig. 2). In 2007, world linseed production was ~2.3 million Mg, with Canada accounting for ~29% (0.67 million Mg) of this production (FAOSTAT, 2007).

It is a challenge for flax breeders to develop flax cultivars to meet the requirements and demands of the changing markets and environment (Diederichsen, 2007). The introduction and development of recombinant deoxyribonucleic acid (rDNA) technology has provided an additional gene pool not previously attainable through conventional plant breeding methods such as hybridization, mutagenesis, and somaclonal variation to modify genomes and create genetic variability between and within species (McLaren, 2005). Many field crops containing single gene modifications conferring resistance to insects and herbicides have been commercialized (Miller and Conko, 2005). The next generation of transgenic crops will likely involve complex, multigene traits for industrial and pharmaceutical markets (Stewart and Knight, 2005; CFIA, 2005).

Flax was among the first commodity crop species to be genetically engineered by recombinant DNA technologies (McHughen, 2002). It was also among the first plant species to be genetically modified for imparting agronomic traits such as herbicide resistance (Jordan and McHughen, 1988; McHughen, 1989) and salt tolerance (McHughen, 1987). Flax has been transformed with resistance to several herbicides including glyphosate, glufosinate, and sulfonylurea (McHughen, 2002). The only GE flax cultivar in the world, 'CDC Triffid', resistant to sulfonylurea herbicide, was considered for commercial release in Canada in 1998 (McHughen, 2002), but after commercialization for six years, it was deregistered at the request of the flax industry because of the EU's concern with importing GE flaxseeds. Canada exports most of its flaxseed (>80%) to the EU, Japan, Korea, and the U.S. (Flax Council of Canada, 2007). This traditional linseed export market has remained GE free, essentially excluding the use of genetic engineering to further improve the crop for fiber and other non-food applications.

As a minor crop, flax has not benefited from intense breeding efforts and genetic engineering approaches to improve yield, increase competition with weeds, and decrease maturation time. In contrast, China, India, and the Ukraine have recently adopted large-scale flax production strategies (FAOSTAT, 2007) and may become global competitors to the export market currently dominated by Canada. Recently, the fiber-flax growing region in the EU has been faced with the problem of low quality fiber which has led to a rapid decrease in the demand of this product and the area committed to growing the crop (Wrobel-Kwiatkowska et al., 2007a). Governments and the flax industry in Canada and abroad have been slow to invest in genomics, molecular biology, and genetic engineering to enhance the performance of this multipurpose crop.

Before and during the introduction of GE crops, many scientists expressed grave concerns about ecological risks associated with GE crops (Rissler and Mellon, 1996; Snow and Palma, 1997; Ellstrand et al., 1999; Stewart et al., 2003). For conventional crop cultivars developed by conventional plant breeding methods, gene flow and the potential for non-target effects were not a concern (Stewart and McLean, 2004) or was limited to maintain the genetic purity of breeder seeds (Hucl, 1996). In contrast, GE technology, especially after its commercialization in 1996, began to bring about concern with the general public and government regulatory agencies regarding possible environmental effects of these crops (Poppy, 2000; Wilkinson et al., 2003).

The issues surrounding GE crops can be divided into environmental, food and feed safety concerns, and issues of market access. While the former two issues can be addressed using science-based risk assessment tools, the latter issue of market access can be intractable. Information on the potential benefits, risks, and regulations of GE flax may be useful to the flax industry for the development and commercialization of GE flax for bio-industrial and nutraceutical markets. The objectives of this paper are to:

- discuss the history and current status of genetic engineering of flax,
- discuss the potential benefits and risks of growing GE flax commercially,
- provide information on the registration and regulation of GE flax in Canada, and
- describe the best management practices to mitigate transgene movement from GE flax to conventional, organic flax, and wild or weedy species and their potential impact on the environment.

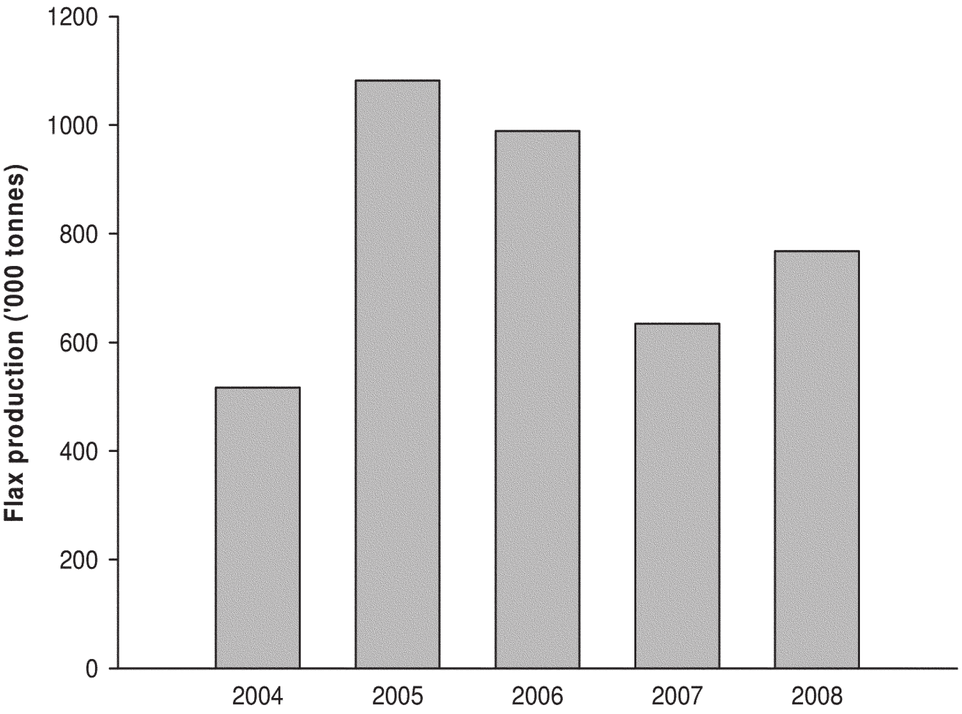

Figure 2. Production of flax in the last five years in Canada. (Source: Flax Council of Canada, 2008).

HISTORY AND CURRENT STATUS OF GENETIC ENGINEERING OF FLAX

An *Agrobacterium*-mediated transfer system has been successfully used as a vector to transfer desired genes to flax (Hepburn et al., 1983; Mlynarova et al., 1994). Hepburn et al. (1983) reported that flax was susceptible to *Agrobacterium tumefaciens*-induced crown gall infection. Herbicide resistance was the first transgenic agronomic trait in flax (Jordan and McHughen, 1988). A glyphosate-resistant plant was derived by delivering the 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene to flax hypocotyl tissue (Jordan and McHughen, 1988). In another study, *Agrobacterium*-mediated gene transfer successfully incorporated chlorsulfuron resistance in flax (McHughen, 1989). When the transgenic flax progeny were grown in soil containing chlorsulfuron in the greenhouse the plants survived (McHughen, 1989). There was no significant difference in the overall agronomic performance of transgenic lines when grown in sulfonylurea-treated versus untreated soils in the field (McHughen and Holm, 1991; McSheffrey et al., 1992).

The first transgenic flax cultivar, "CDC Triffid", received regulatory clearance in 1996 in Canada (McHughen, 2002). It was expected that CDC Triffid would provide a broadleaf cropping option to summer fallowing or continuous cropping to cereals in soils previously treated with sulfonylurea herbicides (McHughen et al., 1997). CDC Triffid was deregistered, however, in 2001 on the request of the Flax Council of Canada and the Saskatchewan Flax Development Commission. Much of the discussion surrounding this cultivar probably conjured up recollections of the science fiction novel, *"The Day of the Triffids"* by Wyndham (1951) where man-eating plants wreak havoc and attempt to take over the world!

The transformation of flax with a phosphonothricin acetyltransferase *(PAT*) gene conferring tolerance to the nonselective herbicide glufosinate was attempted and field tested (McHughen and Holm, 1995). The particle gun bombardment system was also used for genetic engineering of flax (Wijayanto, 1998; Wijayanto and McHughen, 1999). The *GUS* (β-glucuronidase) reporter gene was used to test different seed specific promoters in flax (Drexler et al., 2003). The results suggested that the β-ketoacyl-CoA synthase (*KCS*) gene and the gene encoding napin (a major storage protein in *Brassica napus* L.) would not be expressed at high enough levels to be useful promoters, but *USP* (encoding an unknown seed protein from *Vicia faba* L.) and *LeB4* (encoding a legumin protein from *Vicia faba*) promoters could be successfully used for heterologous gene expression in flax (Drexler et al., 2003).

To find an alternate source of antibiotic resistance genes, Lamblin et al. (2007) introduced the phosphomannose isomerase gene (*PMI*) as an alternative selectable marker for *Agrobacterium*-mediated transformation in flax.

The results indicated that the PMI/mannose selection system could be successfully used for isolation of transgenic flax plants. Finally, expression of a cDNA encoding potato β-1,3-glucanase in flax improved the resistance of transgenic lines to *Fusarium oxysporum* and *Fusarium culmorum* (three-fold higher) compared to nontransformed plants (Wrobel-Kwiatkowska et al., 2004).

Tissue culture–derived systems have played a critical role in the incorporation of genetic engineering approaches into germplasm improvement programs. Flax has a long history of being transformed by tissue culture techniques including regeneration from protoplasts, and hypocotyl-, cotyledon- and leaf-derived callus (Barakat and Cockling, 1983; 1985). Haploid plants of flax have been derived through microspore-derived culture (McHughen, 2002) and anther culture (Obert et al., 2004). These approaches and their applications in flax have been discussed in detail (McHughen, 2002; Millam et al., 2005).

Quality traits in flax were improved utilizing new information on gene identification and molecular expression. Several methods have been compared for genetic transformation in fiber flax (Polyakov et al., 1998). An alternate antibiotic selection method involving the use of hygromycin B has been developed (Rakousky et al., 1999). In addition, non-transgenic methods including somaclonal variation (O'Conner et al., 1991) and mutagenesis to develop "Solin" [the name given by the Flax Council of Canada to describe flax cultivars with less than 5.0% alpha linolenic acid (ALA) for use in the food industry] flax cultivars have been developed and successfully implemented in flax breeding programs (Dribnenki et al., 1999; Green and Marshall, 1984; Green, 1986).

The market value of flax fibers strongly depends on their mechanical properties. To reduce lignin content, gene silencing, ribonucleic acid interference (RNAi) technologies were successfully employed to produce plants with modified elastic properties. A significant increase in the lignin precursor content and a reduction in the pectin and hemicellulose were obtained in transgenic lines (Wrobel-Kwiatkowska et al., 2007a), which may increase the extractability of fibers (Wrobel-Kwiatkowska et al., 2007a).

The biotechnological production of bio-plastics, including polyhydroxybutyrate (PHB) and polyhydroxyalkanoate (PHA), has been explored in both microorganisms and plants (Suriyamonglok et al., 2007). To produce biodegradable composites, flax was transformed with bacterial genes that encoded enzymes catalyzing the formation of PHB (Wrobel-Kwiatkowska et al., 2007b). The protocol resulted in a modification of the mechanical properties of the stem wherein PHB accumulated in growing fiber cells. This study has paved the way for the large-scale production of biodegradable composites in the future. Recent developments in plant genomics, the availability of microarray technology, and development of metabolomics technology will soon make it possible to understand the complex relationship between genes and flax fiber quality (Kymäläinen and Sjöberg, 2008).

Although several genetic modifications have been attempted in agronomic and other value-added traits in flax, no transgenic cultivars of flax are currently available for commercial production.

POTENTIAL BENEFITS OF GE FLAX

With unique oil and fiber properties, flax is considered to be a model plant species for multipurpose use with whole plant utilization (Jhala and Hall, 2009). In addition to the traditional industrial and non-food uses of flax, with the increasing information on molecular biology derived from identification and expression of genes, the potential for the production of transgenic flax for quality traits has been developed (Ebskamp, 2002; Kymäläinen and Sjöberg, 2008). Agronomic limitations of flax production may also be minimized by developing GE flax cultivars. The potential benefits of flax are discussed in detail below.

Increased Agronomic Performance

Flax is a poor competitor with weeds (McHughen, 2002) and flax yield can be reduced in the presence of weeds (Anonymous, 2006), in part because flax has a comparatively small photosynthetic surface (Mani and Bhardwaj, 1965). Weeds can also adversely affect the quality of flaxseed oil, and decrease the flaxseed oil content and iodine value (Bell and Nalewaja, 1968). Although herbicides are registered for controlling weeds in flax, they are limited in their utility. The development of herbicide-resistant flax (especially glyphosate- or glufosinate-resistant cultivars) would result in enhanced weed control.

Flax is a relatively long season crop, taking about 100 to 120 d to mature depending on the cultivar (Anonymous, 2006). To avoid damage by early spring frosts, early-maturing cultivars are preferred by Canadian farmers. Genetic engineering may be useful in developing flax cultivars with early maturity, which would reduce the risk of crop damage by spring or fall frosts or lodging from snowfall. Flax is also sensitive to salt, which is the major limitation in some flax growing areas of North America and Egypt (McHughen, 2002). An attempt was made to develop a flax cultivar tolerant to salinity by somaclonal variation with cellular selection (Rowland et al., 1995). Other abiotic stress-tolerant traits being developed in crops such as cold tolerance and improved nitrogen use efficiency could benefit flax (Warwick et al., 2008). Recently, Monsanto developed the first transgenic drought-tolerant maize (*Zea mays* L.) and has applied to the United States Department of Agriculture (USDA) and Food and Drug Administration (FDA) for regulatory approvals (Anonymous, 2009). The development of drought-tolerant flax also has enormous potential to increase yield where moisture is a limiting factor.

Linseed Oil for Industrial Applications

Flaxseed oil has several industrial applications because of higher levels of ALA compared to other oilseed species including canola (*Brassica napus* L. and *B. rapa* L.), safflower (*Carthamus tinctorius* L.) or sunflower (*Helianthus annuus* L.). Currently available flaxseed oil for industrial applications has a maximum 70% of ALA which was achieved by a traditional plant breeding germplasm development program (Ntiamoah and Rowland, 1997). The drying quality of flaxseed oil is useful for industrial applications in manufacturing paints, varnishes, linoleum, printer's ink, and other coatings (Rowland et al., 1995). Although the use of traditional plant breeding methods like natural or induced mutations for increasing the level of ALA within the genus *Linum* remains a valid option, the transgenic approach may play an important role. The manipulation of the fatty acid composition of oil to produce oils with >70% ALA by genetic engineering may be possible. This would increase the drying quality of linseed oil and thus extend its industrial applications (Rowland et al., 1995).

Novel Oil Products

Flax can be modified to produce oil for human consumption and manufacturing margarines. The first strategy was to replace ALA in flax with palmitic acid (Rowland et al., 1995). The level of ALA (<3.0% ALA) in linseed oil by traditional mutation breeding methods has been reduced to produce Solin varieties (Rowland, 1991; Ntiamoah and Rowland, 1997). Zero percent ALA cultivars of flax could not be obtained by traditional plant breeding methods, but may be achieved through genetic engineering methods by reducing the activity of delta-15 desaturase (Jain et al., 1999). Linseed oil modification can be achieved by adopting transgenic approaches including elucidation of the basic biochemical pathways of oil synthesis, availability of promoter elements that restrict expression of introduced genes, and silencing or cloning of the genes encoding enzymes involved in flaxseed oil synthesis.

Flax for the Nutraceutical Market

Modern North American diet is high in total and saturated fats, ω-6 fatty acids, and *trans*-fatty acids; and low in ω-3 fatty acids (Burdge and Calder, 2005). This nutritional imbalance has led nutrition experts to recommend increasing ω-3 fatty acid intake (Fitzpatrick, 2007). Flax is valuable for the nutraceutical market because it is rich in fat, protein, and dietary fiber (Jenkins et al., 1999). Flax is considered the richest plant source of ALA, an ω-3 fatty acid. It has been estimated that the oil of common flax cultivars in Canada contains about 55% ALA (Rowland et al., 1995). ALA can, however, serve as the precursor of ω-3 very long chain polyunsaturated fatty acids (VLCPUFAs), eicosapentaenoic acid (EPA or $20{:}5^{cis\Delta5,8,11,14,17}$), and docosahexaenoic acid (DHA or $22{:}6^{cis\Delta4,7,10,13,16,19}$). VLCPUFAs are the subject of interest because of their roles in human health and nutrition; in particular DHA is an important constituent of the brain and retina (Hoffman et al., 2009; Neuringer and Connor, 1986). Special GE flax can also be engineered for enhanced use in animal feed, especially for poultry, to increase the level of ω-3 fatty acids in eggs.

Triacylglycerol (TAG) biosynthesis has been studied extensively in a number of oilseed crops (Weselake, 2005). Research in this area in flax, however, is limited (Abbadi et al., 2004; Stymne et al., 1992). On the basis of experiments on storage lipid accumulation and acyltransferase action in developing flax seed, Sorensen et al. (2005) have suggested that if the appropriate acyl-CoA-dependent desaturation/elongation pathways are introduced and expressed in flax, ALA-CoA may be converted into EPA-CoA through a pathway that uses enzymes that only work on acyl-CoA. This has now been demonstrated by Hoffman et al. (2009) in the seeds of *Arabidopsis thaliana*. Modifying the biosynthesis of VLCPUFAs in oilseed crops by genetic engineering has been a major objective among plant biotechnologists hoping to provide novel ω-3 oils for the nutraceutical market (Abbadi et al., 2004). For example, GE soybean with elevated levels of ω-3 fatty acids is undergoing field testing in the U.S. (Anonymous, 2008). The accumulation of ω-3 VLCPUFAs in transgenic flax seed could someday represent a breakthrough in the search for an alternative vegetarian source of fish oil.

Flax for Functional Foods

Functional food can be defined as food claimed to have health-promoting or disease-prevention properties in addition to basic nutritional properties. Many health claims have been made for whole flax seed, flax meal, and milled flax. Studies have shown that consumption of flax seed in the daily diet can modestly reduce serum total and low-density lipoprotein cholesterol, decrease inflammation, and raise the level of ω-3 fatty acids, especially ALA and EPA (Jenkins et al., 1999; Thompson et al., 2005). Flaxseed has recently gained attention in the area of atherosclerotic cardiovascular disease (ASCVD) prevention because it contains ALA, lignan, phytoestrogen, and soluble fiber. Daily consumption of 15 to 50 g of flaxseed can improve cardiovascular risk factors, primarily by modestly improving blood lipid profiles (Bloedon and Szapary, 2004). In addition to ALA, flax is also one of the richest plant sources of lignans, which play a role in plant growth and act as antioxidants in human metabolism (Morris, 2007). Lignan metabolism is a complex process and can be studied by adopting transgenic approaches, which may increase the use of flax-based lignans (Muir and Westcott, 2003). Thus, because flax has functional food properties, it may fit well as a plant source for the development of drugs and therapeutics in the future, especially to reduce the risk of cardiovascular diseases (Paschos et al., 2007).

Recent research also indicates that flax seed is useful in controlling inflammation and reducing the risk of diabetes and cancer (Thompson et al., 2005). It is beyond the scope of this manuscript, however, to discuss the medical applications of flax; however, readers are directed to Tarpila et al. (2002); Vaisey-Genser and Morris (2003); Bloedon and Szapary (2004); and Morris (2007).

Value-Added Products

Genetic engineering of flax may lead to new opportunities for fiber production, with several applications in the textile industry. Bast fibers of flax are used as insulation due to their thermal properties and eco-friendly features (Smeder and Liljedahl, 1996). Recent developments in plant genomics, proteomics, molecular biology, and microarray technology have made it possible to understand the relationship between genes and fiber quality (Wrobel-Kwiatkowska, 2007a). High yielding fiber flax cultivars can be produced by genetic mapping and recombinant DNA technologies (Ebskamp, 2002). Biocomposites made up from flax fiber based on polyhydroxybutyrate (PHB) polymers may be an eco-friendly and biodegradable alternative to conventional plastics (Wrobel-Kwiatkowska et al., 2007b). For more information on the uses of flax fibers see Foulk et al. (2002); Rennebaum et al. (2002) and Moryganov et al. (2008). The secondary cell wall of flax stem contains high levels of cellulose, hemicellulose, and smaller amounts of lignins and pectins. Research on flax fiber cultivar development by genetic engineering is necessary to promote the use of flax for fibers (Rennebaum et al., 2002).

The fine fraction obtained as a byproduct of dehulling (a process of preparing flaxseed for value-added industrial products) could be a potential ingredient in pet food, whereas the medium and mix fractions can be blended into poultry feed formulations (Oomah and Mazza, 1998).

POTENTIAL RISKS OF GE FLAX

Potential risks of GE crops to food, feed, and environmental systems have been delineated by the Government of Canada. Before commercial cultivation, plants with novel traits (PNTs) are subject to environmental biosafety regulations administered by the Plant Biosafety Office of the Canadian Food Inspection Agency (CFIA) in Canada (CFIA, 2007b), and by USDA–Animal and Plant Health Inspection Service (APHIS) in the U.S. (ERS-USDA, 2008). The risks of GE crops depend on both the biology of the species and construction, insertion, and function of the transgenic trait. Transformation of unknown genes into crops may have potential to create new food allergens in humans and animals (Andow and Zwahlen, 2006). Conventional flax has no inherent food and feed safety concerns and recently a panel of experts from the United States FDA has given Generally Recognized As Safe (GRAS) status to whole and milled flax seeds (Flax Council of Canada, 2009). Health risks of GE flax (like other GE crops) will be compared to conventional flax to determine if they are substantially equivalent through testing in animal and human systems, before release (Chassy, 2007; Kok et al., 2008).

Environmental risks include both those associated with the crop (the trait) and effects of changes in conventional agronomic practices. Environmental concerns of GE crops raised by stakeholders include the potential of transgenic crops to become weeds (Ellstrand and Schierenbeck, 2006), which may become invasive in nature and require a costly management strategy (Gaines et al., 2007; Knispel et al., 2008). Flax is a highly domesticated species and volunteer flax populations are ephemeral under agronomic conditions, diminishing over three years. There is no evidence that volunteer flax is invasive of ruderal or natural areas. Unless a transgenic trait confers a significant fitness advantage flax is unlikely to be invasive. GE flax may also need to be examined for its potential to become a plant pest and for impacts on non-target organisms and biodiversity. GE crops may cross-pollinate with wild and weedy species (Beckie et al., 2003; Hall et al., 2003; Ellstrand, 2005; Warwick and Stewart, 2005). If transgenes introgress with the genomes of wild or weedy relatives, they may cause changes to those populations. Flax has the ability to hybridize with at least nine species of *Linum* occurring in Asia and Europe with the same chromosome number as cultivated flax ($n = 15$) (Jhala et al., 2008). While there are eight *Linum* species identified in Canada, only *L. rigidum* Pursh var. rigidum and *L. sulcatum* Riddell have the same chromosome number, indicating a potential for transgenic introgression. Their inter-specific hybridization with flax needs to be quantified.

In Canada and the U.S., approved GE crops are assumed to be as safe as conventional and are commingled (Brookes and Barfoot, 2004). Segregation and product labeling are not required in North America, but they are required in Europe and other countries (Devos et al., 2009). Because of non-uniform GE acceptance and legislation regulating GE-labeling in food and feed, market disruptions continue to be a major risk for developers, producers, and commodity traders (Ramessar et al., 2008). For example, the first transgenic flax resistant to sulfonylurea (Millam et al., 2005), and more recently, glyphosate-resistant wheat (*Triticum aestivum* L.), were not commercialized because of market issues (Stokstad, 2004). Coexistence between GE and conventional flax production systems will depend on the limiting seed- and pollen-mediated gene flow through good management and identity preservation practices. Flax is an inbreeding species and the rate of outcrossing has been described in the range of 1 to 4% (Robinson, 1937; Dillman, 1938). Recent studies conducted in western Canada, however, suggest that intra-specific pollen-mediated gene flow was less than 2.0%, when two different cultivars of flax were grown 0.1 m apart. Some rare outcrossing events were recorded up to 35 m, but at a very low frequency (Jhala et al., unpublished data). Gene flow beyond 1.0 m was less than 0.9%. Thus, pollen-mediated gene flow from transgenic flax to non-transgenic or organic flax may be mitigated through best management practices to reduce market risk.

Seed-mediated gene flow, the movement of GE flax seeds during harvest, handling, grading, and transportation may increase the volunteerism and ferality and may also lead to adventitious presence (AP) in conventional crops. A recent study on post-harvest gene escape in four important GE crops, canola, maize, sugar beet (*Beta vulgaris* L.), and wheat suggest that seed-mediated gene flow cause seed dissemination and volunteerism (Gruber et al., 2008). The pathways of seed-mediated gene flow are numerous and stochastic. One of the keys to reduce seed-mediated gene flow is to control volunteer flax populations in subsequent crops.

REGULATION OF GE FLAX IN CANADA

Canada has a unique, science-based regulatory system administered by the Plant Biosafety Office (PBO) of the CFIA for the import and environmental release of PNTs. CFIA has defined *PNTs as those plants containing a trait that is not present in plants of the same species already existing in Canada, or is present at a level outside the range of that trait in stable, cultivated populations of that plant species in Canada* (CFIA, 2007a). PNTs can be produced by many techniques including conventional breeding, mutagenesis, or by modern genetic engineering. The CFIA regulates the PNTs, regardless of method of breeding, requiring data, especially to document environmental, food, and feed safety. The PBO of the CFIA is responsible for regulating the agronomic and horticultural PNTs, including post-commercialization monitoring and inspection (CFIA, 2007b). Health Canada is responsible for food derived from biotech crops (Health Canada, 1994). Environment Canada is also the authority for regulating other products derived from PNTs. The CFIA has successfully approved >70 PNTs irrespective of their production method in Canada.

In Canada, GE flax requires registration before seed can be sold commercially in the market under the statutory authority of the Seeds Act. On the basis of a positive recommendation by an independent body, the federal Minister of Agriculture issues a certificate of registration for a new flax cultivar. In addition to this, there are five assessment criteria for determining environmental biosafety of GE flax as described in CFIA Directive 94-08 (CFIA, 2007b). The applicant is responsible for providing the appropriate data and relevant scientific information to the CFIA including the environmental risk of the new GE flax cultivar to its counterpart(s) already present in Canada. The PBO compares this information with the available biology document of flax, which contains information on reproductive biology, origin, occurrence, and distribution of closely related species of flax, breeding history, interaction of flax with different species, and other relevant information (CFIA, 2001). For several plant species including flax, however, the biology documents available on CFIA website were prepared before experience with GE cultivars. Thus, there is need to update CFIA's crop biology documents including the biology of flax, on the basis of current scientific data.

Confined field trials of any new GE flax cultivar are required to minimize the potential environmental impact of the new trait while conducting environmental biosafety experiments at various locations in Canada. CFIA officers are responsible for inspection of the applicant's confined field trials to ensure they meet the conditions of the confinement. For unconfined release of GE flax, the CFIA relies on the report of the CFIA officer responsible for the inspection of the confined trials, confidential research reports and also on the peer-reviewed published research (if any) developed from the testing of new PNTs (Corbet et al., 2007).

The Canadian regulatory framework is flexible to address the regulation of the PNTs through the ongoing development of clear and transparent criteria before commercial production of transgenic crops. For more details on the Canadian regulatory framework for PNTs see Demeke et al. (2006); Corbet et al. (2007); Smyth and McHughen (2008) and the U.S. regulatory framework see McHughen and Smyth (2008); USDA/APHIS (2008).

BEST MANAGEMENT PRACTICES TO MITIGATE TRANSGENE MOVEMENT

Canadian flax seed is being exported mainly to Europe, Japan, the U.S. and South Korea (Flax Council of Canada, 2007). The EU has established a labeling threshold level of 0.9% of AP defined as " the percentage of GM-DNA copy numbers in relations to target taxon specific DNA copy numbers calculated in terms of haploid genomes" (Council of the European Parliament, 2003). The EU threshold and wide applicability of analysis methods have been challenged. Weighardt (2006) has described the EU labeling thresholds as impractical and unscientific and suggested that caution should be exercised when analyzing GE content in processed food and feed (Weighardt, 2007). Japan and South Korea have 5 and 3% tolerance limits, respectively (Demeke et al., 2006). To preserve the export market for conventional flax seed, it must be segregated from transgenic material. Gene flow, either by pollen and/or seed during the production and transport process are major routes of transgene movement. For effective coexistence of biotech, conventional, and organic flax, Canadian flax growers will be required to mitigate the pollen- and seed-mediated gene flow.

Mitigating Seed-Mediated Gene flow

In the case of an autogamous species like flax, seed-mediated gene flow will be more significant and stochastic than pollen-mediated gene flow. 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 in western Canada (Thomas et al., 1997). Volunteer flax was ranked as the 32nd most abundant weed in the Canadian Prairies in the 1970s, but in the 1990s and 2000s it was replaced as the 26th most abundant weed (Leeson et al., 2005), indicating that seed-mediated gene flow may become a problem if transgenic volunteer flax is not effectively controlled. Left uncontrolled, volunteer flax seeds can be harvested with subsequent crops, usually cereals. In the year following flax production, volunteers are numerous.

There are some herbicides registered for the control of volunteer flax. Quinclorac has been registered for control of volunteer flax in spring wheat providing consistently good control at 100 or 200 g a.i. ha^{-1} (Wall and Smith, 1999). Pre-emergence application of glyphosate or glyphosate plus tribenuron-methyl reduced volunteer flax densities from 39 plants to <4 plants m^{-2} (Dexter et al., unpublished data). Post-emergence application of either fluroxypyr plus 2-methyl-4-chlorophenoxyacetic acid (MCPA) or 2,4-D ester reduced the volunteer flax density to 2 plants m^{-2}. Experiments to mitigate volunteer flax in glufosinate- and imidazolinone-resistant canola indicate that the combination of glyphosate applied pre-emergence and glufosinate applied post-emergence at recommended rates can control volunteer flax in glufosinate-resistant canola (personal observation). Pre-emergence application of glyphosate was effective to reduce AP of volunteer flax in both the canola traits. Without recharge, flax volunteer populations are ephemeral, and do not persist more than three years. Sound agronomic practices for controlling volunteer populations, including cultivation of competitive crops in rotation, timely and effective weed control practices, and pre- and post-harvest monitoring and mitigation will reduce populations of volunteer transgenic flax and the risk of seed-mediated gene flow.

Mitigating Pollen-Mediated Gene Flow

The frequency of gene flow in flax declines rapidly with increasing distance from the donor field. Since maximum out-crossing has been observed within an area of 3 m of the pollen donor, an isolation distance of 3 m between fields would likely reduce pollen-mediated gene flow significantly (<0.14%). Alternatively, if the area of 3 m around the transgenic flax is removed after flowering, but before seed set, it can reduce the out-crossing significantly between GE and conventional flax cultivars. If the buffer zone around the transgenic flax field is strictly followed, the EU standard (0.9%) for adventitious presence of GE flax in non-GE or organic flax production systems can be achieved. Border or trap rows (non-GE crop borders grown around a GE crop) of non-transgenic crop and barren zone both have been tested for efficacy in transgene containment. The experiments conducted by Morris et al. (1994) in canola suggest that the trap rows were effective in reducing gene flow to distant populations, but their effectiveness was highly dependent on the width of the trap. In comparison to canola, flax has a lower outcrossing frequency, and thus may need less area (1–3 m) for trap rows.

In the event that GE flax is grown beside organic flax, with no established thresholds for presence of AP, an increased level of pollen-mediated gene flow reduction may be required, including the following practices.

- Flowering synchrony can be reduced by sowing organic flax at different dates than biotech flax to reduce the pollen-mediated gene flow between GE and organic flax cultivars.
- In addition to isolation distance, a trap crop may be required for organic growers. The removal of 1 m of the crop adjacent to other flax crops after flowering may reduce the risk of transgene dissemination.
- Harvest loss should be reduced to minimize the volunteer flax population in subsequent years.
- For organic growers, without the application of pre-seeding herbicides, the seeding of subsequent crops should be delayed to allow pre-seeding tillage, which may reduce the presence of volunteers.
- Diversification of crops in rotation should be adopted.

A better understanding of crop-to-wild gene flow and the best management practices to mitigate the transgene movement from transgenic flax to its wild and weedy species is required as a part of the ecological risk assessment to assess potential negative consequences to biodiversity. The majority of flax wild relatives that are likely to hybridize with transgenic flax are distributed in the Mediterranean and Southeast Asia, the probable centers of the origin of flax (Jhala et al., 2008). The Canadian Prairie is the largest flax growing region in the world, and only three wild relatives of flax (*Linum lewisii, Linum rigidum,* and *Linum sulcatum*) are found in this region (Scoggan, 1993). It is important to note, however, that the presence of wild relatives of flax does not imply that successful hybridization will occur. There is no evidence to date for inter-specific hybridization of these three species with cultivated flax. None of these species has been reported as weeds on the Canadian Prairie in agronomic weed surveys conducted during the 1970s to the 2000s (Leeson et al., 2005). The limited number of flax wild relatives and their limited occurrence and distribution in Canada make it less likely for inter-specific transgene movement. Several integrated weed management practices during crop season, including herbicide applications, may also reduce the populations of closely related species, and thus the chances of gene flow from transgenic flax to wild relatives.

Transgene movement cannot be prevented, but can be reduced by adopting best management practices to below threshold levels in conventional flax. If transgenic flax were cultivated in western Canada, volunteer flax could be effectively controlled by herbicides in cereals and canola. If herbicide-resistant flax is introduced into market, extension agronomists should play a vital role in advising growers to adopt integrated weed-management systems, crop rotation, spot application of herbicides, and use of herbicide mixtures for reducing herbicide-resistant weeds and for controlling herbicide resistant flax volunteers (Beckie, 2007; Blackshaw et al., 2008; Devos et al., 2004).

CONCLUSIONS

The utility of transgenic modifications for flax have been apparent to flax breeders for many years. The resources available for development of flax have been relatively small compared to the resources applied to the development of Canada's other oilseed crops, including canola and soybean. Advances in cell and molecular biology now allow plant breeders to respond more quickly to agronomic limitations of flax and fiber production, and increasing consumer demands for functional food. Transgenic technologies could increase the utility and value of oils and fiber products, and reduce production risk to growers through the improvements in agronomic performance discussed in this paper. As one of the first genetically modified crops, the tools for rapid improvement of flax germplasm are available.

Without the consent of society at large, GE crops will fail in the market place. While questions remain to be answered about the risk of each proposed trait on a case by case basis, there is little evidence that domestic flax itself poses an inherent risk to food, feed, or the environment. While market harm remains the primary concern for developers and growers, coexistence between conventional and GE flax can be achieved with reasonable AP thresholds and with appropriate mitigation and testing measures in place.

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