

# Potential Hybridization of Flax with Weedy and Wild Relatives: An Avenue for Movement of Engineered Genes?

Amit J. Jhala, Linda M. Hall,\* and Jocelyn C. Hall

## ABSTRACT

Flax (*Linum usitatissimum* L.) is being evaluated as a crop platform for the production of bio-industrial and nutraceutical products. An important consideration for the release of any novel trait is the potential for gene flow to wild or weedy relatives and the impact it may have on their populations. The potential for gene introgression from transgenic flax to wild relatives, the occurrence, the phylogeny of flax wild relatives and reported interspecific hybridization was reviewed to initiate the evaluation of environmental risk of novel flax in Canada. The genus *Linum* contains approximately 230 species which are distributed in many parts of the world and may grow in sympatry with cultivated flax. Interspecific hybridization and cytogenetic studies between flax and congeneric species demonstrated that cultivated flax has the ability to hybridize and form viable  $F_1$  plants with at least nine species of *Linum* (*L. africanum*, *L. angustifolium*, *L. corymbiferum*, *L. decumbens*, *L. floccosum*, *L. hirsutum*, *L. nervosum*, *L. pallescens*, and *L. tenue*). Hybridization of flax with many other wild relatives has either not been studied or reported. However, based on the evidence of reported hybridization with wild or weedy relatives, gene flow from flax to wild or weedy relatives is possible in several species native to North America, depending on species distribution, sympatry, concurrent flowering, ploidy level, and sexual compatibility.

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**Abbreviations:** AFLP, amplified fragment length polymorphism; APG, Angiosperm Phylogeny Group; CFIA, Canadian Food Inspection Agency; EMBO, European Molecular Biology Organization; GM, genetically modified; ITS, internal transcribed spacer region; NCRPIS, North Central Regional Plant Introduction Station; PGRG, Plant Gene Resources of Canada; RAPD, random amplification of polymorphic DNA; *sad2*, stearyl-ACP desaturase II.

**F**LAX (*Linum usitatissimum* L.) is the sixth largest oilseed crop in the world and is one of the oldest cultivated plants (Bhatty and Rowland, 1990). It is grown for linen fiber, the earliest vegetable fiber domesticated by mankind, and as an oilseed (Dillman, 1938; Richharia, 1962). The center of origin of flax has not been identified (Lay and Dybing, 1989), but it was reportedly disseminated from Egypt (Cooke, 1903) where it was in use during the time of the Pharaohs. Flax fabrics from Egyptian mummy-cloths were dated at > 4500 years (De Candolle, 1904; Matthews, 1908). It is believed that Phoenicians were responsible for transporting flax into Europe from the Near East (Rosberg, 1996; Stephens, 1997) during the period from 2500 to 1200 B.C. Flax cultivation by Aryans extended north to Russia and Finland (De Candolle, 1904). During the Colonial era, European colonists transported flax to North America, New Zealand, and Australia (Rosberg, 1996).

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Fiber flax prospered in North America for many years as production followed settlers westward (Hammond and Miller, 1994). Fiber flax has been cultivated in the Netherlands and probably in Belgium and northern France since ancient times. Today, fiber flax is grown primarily in China, Russia, Egypt, and near the northwestern European coast for the production of high quality linen (Vromans, 2006). In 2004, linseed was grown in 47 countries, and seed production was 1.903 million metric tonnes (Smith and Jimmerson, 2005). Canada, China, and the United States together are responsible for 64% of the total world flax seed output. Canada is currently the world's leader in the production and export of flax seed, a position it has held since 1994. In 2006, Canada produced 1.041 million tonnes of flax seed (Statistics Canada, 2006) and exported 80 to 90% of the total production, mainly to Europe, the U.S., Japan, and South Korea (Flax Council of Canada, 2007a).

Flax was among the first crop species to be both genetically engineered with *Agrobacterium* mediated transformation and transformed with genes of potential agronomic value (McHughen, 2002). Several novel traits have been expressed in flax including chlorsulfuron and metsulfuron methyl resistance (McSheffrey et al., 1992), glufosinate-ammonium resistance (McHughen and Holm, 1995), and glyphosate resistance (Jordan and McHughen, 1988). Only one transgenic flax cultivar, CDC Triffid (McHughen et al., 1997), was released in Canada in 1998 for unconfined use in fields with persistent herbicide residues (CFIA, 2004b), but it was deregistered almost immediately at the request of the flax industry (Flax Council of Canada, 2007b). Although transgenic flax may have been a solution to significant agronomic issues such as weed control (McHughen, 1989) or disease resistance (Polyakov et al., 1998), a concern over the market's reaction to the import of genetically modified material halted all transgenic development of the crop, even for primary use in paint and flooring industries and animal feed as a co-product. Since then, the EU is moving toward being more open to bioproducts and transgenic crops (Hricova, 2002; Breithaupt, 2004; see also Millam et al., 2005).

GM crops are now grown worldwide, and the number of species and the area under production continues to increase (James, 2003; Nap et al., 2003). One of the critical concerns that must be addressed before the release of a novel crop is the potential movement of transgenes from GM crops to wild populations (Raybould and Gray, 1993; CFIA, 2004a). A better understanding of crop-to-wild gene flow is essential for ecological risk assessment of the potential for transgene spread (Dale, 1993; Conner et al., 2003). In addition, the potential impact on biodiversity (Wilkinson et al., 2003) and genetic resources must be evaluated (Ellstrand, 1988; Andow and Alstad, 1998). Risk assessment of transgenic flax including transgene

movement from transgenic flax to its weedy relatives is in progress (Hall et al., 2006).

We hypothesize that *L. usitatissimum* is more likely to hybridize with closely related species having a similar ploidy level, genome, and chromosome pairing. Our objective is to establish the potential risk of gene flow from transgenic flax before experimental testing, based on (i) biology, distribution, and flowering phenology of closely related species to flax, (ii) relatedness and crossability, and (iii) probability of interspecific hybridization and introgression between transgenic flax and its wild or weedy species.

## TAXONOMY AND PHYLOGENY

Flax is a member of the family Linaceae which is composed of 22 genera (Vromans, 2006) and approximately 300 species (Hickey, 1988; Heywood, 1993). Linaceae is placed in the order Linales by some taxonomists (Cronquist, 1981), but most recently the family has been placed in the order Malpighiales (APG II, 2003). Important genera in the family includes: *Linum* (230 species), *Hugonia* (40 species), *Reinwardtia* (two–four species), *Anisadenia* (two species), *Roucheria* (eight species) and *Radiola* (Heywood, 1993).

The genus *Linum* is traditionally divided into five sections, *Linum*, *Linastrum*, *Cathartolinum*, *Dasylinum*, and *Syllinum* (Winkler, 1931) with an additional section, *Cliococca*, added by Ockendon and Walters (1968). Cultivated flax, *Linum usitatissimum*, is placed in the section *Linum*. The taxonomy and classification of *Linum* has changed with increased knowledge. Many researchers classified *Linum* species either on the basis of morphological characters or center of origin (Linnaeus, 1857; De Candolle, 1904; Tammes, 1925; Vavilov, 1926; Winkler, 1931; Dillman, 1933; Dillman, 1953; Richharia, 1962). Alternatively, other researchers grouped *Linum* species based on chromosome number (Kikuchi, 1929; Nagao, 1941; Ray, 1944; Osborne and Lewis, 1962; Gill, 1966; Ockendon, 1971; Chennaveeraiah and Joshi, 1983; Gill, 1987). However, there is no single prevailing classification scheme for this genus. The grouping of 41 *Linum* species proposed by Gill (1987), based on morphological, cytological, and interspecific compatibility evidence, will be followed in this paper.

Phylogenetic studies based on molecular markers are limited. An amplified fragment length polymorphism (AFLP) based phylogeny of 17 species of *Linum* is not compatible with traditional sections of the species (e.g., Winkler, 1931; Ockendon and Walters, 1968; Diederichsen and Richards, 2003), although there is evidence of five species clusters (Vromans, 2006). McDill and Simpson (2005) conducted a more comprehensive phylogenetic study of *Linum* based on DNA sequence variation from multiple chloroplast markers and the nuclear encoded internal transcribed spacer region (ITS). Their analysis of approximately 70 species indicates that blue-flowered

*Linum* species were sister to a predominantly yellow-flowered lineage. These lineages initially diversified in Eurasia and members of both the blue and yellow-flowered lineages appear to have independently colonized North America. The subsequent diversification of the yellow-flowered *Linum* species in North America includes members previously classified as separate genera: *Hesperolinon*, *Sclerolinon digynum*, and *Cliococca selaginoides*.

Karyotype number is not reflective of phylogenetic relationships among *Linum* species. For example, an analysis based on RAPD data indicate that *L. decumbens* ( $2n = 30$ ) is clustered with *L. grandiflorum* ( $2n = 16$ ), not with other species that share the same chromosome number (e.g., *L. angustifolium* and *L. usitatissimum*; Fu et al., 2002). *Linum perenne* group can be easily distinguishable from other *Linum* species morphologically (Ockendon, 1968), but the molecular study of Vromans (2006) indicate that classification among the *L. perenne* group is still complicated. Neither *L. perenne* nor *L. austriacum* form a specific group, even though *L. austriacum* is considered a member of *L. perenne* group (Diederichsen, 2007) and they have the same haploid karyotype number of nine (Nagao, 1941; Gill, 1987).

Additional molecular studies have focused on within-species variation of *L. usitatissimum*. Mansby et al. (2000) used isozyme markers to study the genetic diversity in flax and defined five groups, but with low variation within the groups. An unexpectedly high genetic diversity within accessions led to the conclusion that the large heterozygosity found in *L. usitatissimum* may be the result of more outbreeding than earlier believed (Mansby et al., 2000). This finding was unexpected as flax is reported to be an obligate inbreeding species (Durrant, 1986). In a study on geographic patterns of flax variability, Fu (2005) pointed out that accessions from the East Asian and European regions were most diverse, whereas accessions from the Indian subcontinent and Africa were the most distinct. Overall, comparatively more variation existed in landraces than cultivars. Considerable difference within and among the four groups of cultivated flax cultivars was observed in quantitative traits; however, RAPD and two qualitative characters did not show marked differences (Diederichsen and Fu, 2006). A molecular study comparing fiber and oil flax indicated that fiber cultivars have a narrower and more homogenous genetic base than oil cultivars (Fu et al., 2002). Vromans' (2006) AFLP study supports this finding and he further speculated that linseed cultivars and a wild relative *L. bienne* could be important sources for the introduction of favorable traits to fiber flax.

## VARIABILITY IN CHROMOSOME NUMBERS

Karyotypic analysis of *Linum* species began more than a half century ago, which has allowed several species to be recognized and differentiated (Tutin et al., 1968). The genus

*Linum* has a large number of diploid species that exhibit a remarkable diversity in chromosome number including  $n = 8, 9, 10, 12, 14, 15, 16, 18, 30$ , and  $> 30$  (Darlington and Wylie, 1955; Gill, 1987). Diversity in chromosome numbers may be due to polyploidy and aneuploidy (Chennaveeraiah and Joshi, 1983). Initial studies of the chromosome number of cultivated flax estimated the chromosome number to be  $2n = 32$  (Martzenitzin, 1927; Lutkov, 1939). However, later cytogenetic and interspecific hybridization studies confirm the chromosome number to be  $2n = 30$  (Kikuchi, 1929; Dillman, 1938; Nagao, 1941; Ray, 1944; Richharia, 1962; Gill, 1966; Chennaveeraiah and Joshi, 1983). The reasons for the conflicting results were the small size of the chromosomes in *Linum*, the tendency of the observed fragments to retain some stain (Ray, 1944; Gill, 1966), and an accidental segmentation in the somatic mitosis (Martzenitzin, 1927).

There were some disagreements among various researchers regarding the chromosome numbers of other *Linum* species (Table 1). For example, Kikuchi (1929) classified *L. alpinum* as a member of group III with chromosome number  $n = 18$ , whereas, Ray (1944) and Nagao (1941) have grouped this species as  $n = 9$  (Table 1). Gill (1966) indicated uncertainty in the chromosome number of this species (Table 1). The *Linum alpinum* specimen from which Kikuchi (1929) counted chromosomes may be a Japanese tetraploid (Simonet and Chopinet, 1939), which could account for the variability in the results. *Linum narbonneuse* was grouped as  $n = 14$  (Ray, 1944), but Kikuchi (1929) and Nagao (1941) observed  $n = 9$ , and  $2n = 18$  and/or  $36$  (Gill, 1966). *Linum monogynum* has been reported, with qualification, as  $n = 43$  and  $2n = 86$  (Kikuchi, 1929). *Linum hirsutum* has a variable reported chromosome count of  $n = 8$  (Ray, 1944),  $n = 9$  (Nagao, 1941),  $n = 15$  (Seetaram, 1972), and  $n = 16$  or  $18$  (Gill, 1966; Table 1).

## CENTER OF ORIGIN AND EVOLUTION OF *L. USITATISSIMUM*

The center of origin of cultivated flax is uncertain (Lay and Dybing, 1989) with many existing theories. Among the eight independent centers of origin of the world's most important cultivated plants (Vavilov, 1926), *Linum* species were reported to have originated in four, the Central Asiatic, the Near Eastern, the Mediterranean, and the Abyssinian Center. Gill (1987) and Richharia (1962) have also discussed these four probable centers of flax origin. Alternatively, other researchers believe that Egypt could be a center of dissemination (De Candolle, 1904). Finally, an area east of the Mediterranean toward India has been suggested as another center of origin because a diverse form of flax is found in the area (De Candolle, 1904; Zeven, 1982).

The progenitor of cultivated flax is also uncertain (Gill, 1987). Many authors reported cultivated flax is derived from two or more ancestral forms (De Candolle,

**Table 1. Comparison of various groupings of *Linum* species based on chromosome numbers, including only those species for which cytological information is available.**

Kikuchi (1929)	Ray (1944)	Nagao (1941)	Gill (1987)
Group I ( <i>n</i> = 9) <i>L. altaicum</i> Fisch. <i>L. austriacum</i> L. <i>L. extraaxillare</i> Kit. <i>L. hologynum</i> Reichb. <i>L. lewisii</i> Pursh <i>L. muelleri</i> Moris. <i>L. narbonense</i> L. <i>L. perenne</i> L. <i>L. sibiricum</i> DC	Group I ( <i>n</i> = 8) <i>L. grandiflorum</i> Desf. <i>L. hirsutum</i> L.	Group I ( <i>n</i> = 8) <i>L. grandiflorum</i> Desf.	Group I (2 <i>n</i> = 18) <i>L. alpinum</i> Jacq. (2 <i>n</i> = 18,36) <i>L. altaicum</i> Ldb. <i>L. anglicum</i> Mill. <i>L. austriacum</i> L. <i>L. grandiflorum</i> Desf. (2 <i>n</i> = 16,18) <i>L. tenuifolium</i> L. <i>L. hologynum</i> Reichb. <i>L. julicum</i> Hayek. <i>L. lewisii</i> Pursh. <i>L. narbonense</i> L. (2 <i>n</i> = 18,28) <i>L. perenne</i> L. <i>L. strictum</i> L.
Group II. ( <i>n</i> = 15) <i>L. americanum</i> L. <i>L. angustifolium</i> Huds. <i>L. corymbiferum</i> Desf. <i>L. flavum</i> L. <i>L. usitatissimum</i> L.	Group II ( <i>n</i> = 9) <i>L. alpinum</i> Jacq. <i>L. altaicum</i> Fisch. <i>L. austriacum</i> L. <i>L. collinum</i> Guss. <i>L. hologynum</i> Reichenb. <i>L. lewisii</i> Pursh. <i>L. loreyi</i> Jord. <i>L. perenne</i> L. <i>L. strictum</i> L. <i>L. tommasinii</i> Nym.	Group II ( <i>n</i> = 15,16) <i>L. angustifolium</i> Huds. <i>L. crepitans</i> Dum. <i>L. usitatissimum</i> L.	Group II (2 <i>n</i> = 30) <i>L. africanum</i> L. (2 <i>n</i> = 30,32) <i>L. album</i> Kotschy <i>L. angustifolium</i> Huds. (2 <i>n</i> = 30,32) <i>L. bienne</i> Mill. (2 <i>n</i> = 30,32) <i>L. corymbiferum</i> Desf. (2 <i>n</i> = 18,30) <i>L. decumbens</i> Desf. <i>L. flavum</i> L. (2 <i>n</i> = 28, 30) <i>L. hispanicum</i> Mill. <i>L. humile</i> Mill <i>L. medium</i> Planch. (2 <i>n</i> = 30,36) var. <i>texanum</i> <i>L. nervosum</i> Waldst. <i>L. pallescens</i> Ldb. <i>L. rigidum</i> Pursh. var. <i>filifolium</i> Rog. <i>L. rigidum</i> Pursh. var. <i>rigidum</i> Rog. <i>L. sulcatum</i> Riddell. <i>L. tenue</i> Desf. <i>L. usitatissimum</i> L.
Group III ( <i>n</i> = 18) <i>L. alpinum</i> Jacq.	Group III ( <i>n</i> = 10) <i>L. gallicum</i> L.	Group III ( <i>n</i> = 14) <i>L. campanulatum</i> L. <i>L. flavum</i> L.	Group III (2 <i>n</i> = 28) <i>L. campanulatum</i> L. <i>L. capitatum</i> L. (2 <i>n</i> = 24,28) <i>L. dolomiticum</i> Borb.
Group IV ( <i>n</i> = 43) <i>L. monogynum</i> Forst.	Group IV ( <i>n</i> = 14) <i>L. capitatum</i> Kit. <i>L. narbonense</i> L.	Group IV ( <i>n</i> = 9) <i>L. hirsutum</i> L. <i>L. maritimum</i> L.	Group IV (2 <i>n</i> = 16) <i>L. catharticum</i> L. (2 <i>n</i> = 16,57) <i>L. hirsutum</i> L. (2 <i>n</i> = 16,18) <i>L. viscorum</i> L.
	Group V ( <i>n</i> = 15) <i>L. angustifolium</i> Huds. <i>L. flavum</i> L. <i>L. medium</i> Britton. <i>L. usitatissimum</i> L.	Group V ( <i>n</i> = 9) <i>L. alpinum</i> Jacq. ( <i>n</i> = 18) <i>L. altaicum</i> Ldb. <i>L. austriacum</i> L. <i>L. extraaxillare</i> Kit. <i>L. hologynum</i> Reichb. <i>L. lewisii</i> Pursh. <i>L. montanum</i> Schleich <i>L. muelleri</i> Moris. <i>L. narbonense</i> L. <i>L. perenne</i> L. <i>L. sibiricum</i> DC. <i>L. tenuifolium</i> L.	Group V Others <i>L. gallicum</i> L. (2 <i>n</i> = 20) <i>L. marginale</i> A. Cunn. (2 <i>n</i> = 80) <i>L. maritimum</i> L. (2 <i>n</i> = 20) <i>L. monogynum</i> Forst. (2 <i>n</i> = 86) <i>L. rupestra</i> Engelm. (2 <i>n</i> = 36) <i>L. schiedeanum</i> S. & C. (2 <i>n</i> = 36)

1904; Vavilov, 1926; Richharia, 1962). The species cultivated by ancient Egyptians were believed to be different from those indigenous to Russia and Siberia. Alternatively, it was suggested that cultivated flax originated from a single wild species *L. angustifolium* (Heer, 1872). This hypothesis is supported by morphological (Dillman, 1936; Diederichsen and Fu, 2006) and cytological studies (Kikuchi, 1929; Ray, 1944; Gill and Yermanos, 1967a; Gill and Yermanos, 1967b). A RAPD analysis of seven *Linum* species revealed that *L. angustifolium* and *L. usitatissimum* have a high RAPD similarity and these two species consistently clustered in the same group (Fu et al., 2002). A different AFLP study indicates that *L. bienne* is the sister species to *L. usitatissimum* (Vromans, 2006), although some consider *L. angustifolium* and *L. bienne* to be the same species (Tutin et al., 1968; Zohary and Hopf, 2000). However, genome comparisons with molecular markers of these three species (*L. angustifolium*, *L. bienne*, and *L. usitatissimum*) confirm that they are very closely related genetically and *L. bienne* can be considered as a subspecies of *L. usitatissimum*, rather than a separate species (Muravenko et al., 2003).

The *sad* gene is responsible for converting stearyl-ACP to oleoyl-ACP and, thus, has been used for manipulation of unsaturated fatty acids (Ohlrogge and Jaworski, 1997). In a molecular study it was estimated that the genetic diversity of the stearyl-ACP desaturase II (*sad2*) locus in cultivated flax is low compared to pale flax (*L. angustifolium*) suggesting flax was first domesticated for oil, not for fiber (Allaby et al., 2005).

### Interspecific Hybridization in *Linum*

Hybridization between crop species and wild relatives has played a role in the evolution of many crop plants (Arnold, 1997) and is also responsible for the expression of new characters not found in either parent (Briggs and Knowles, 1967). Hybridization of several closely related species of *Linum* might have played a role in the evolution of *L. usitatissimum* in the Mediterranean and Southeast Asia where a diverse form of flax has been found (De Candolle, 1904; Richharia, 1962; Gill, 1966; Zeven, 1982). The studies of interspecific hybridization of *L. usitatissimum* with its wild relatives enable estimates of crossability and provide information to predict potential gene flow between *Linum* species (Kikuchi, 1929; Gill, 1966; Gill and Yermanos, 1967a).

Heterostyly must be taken into consideration when selecting *Linum* species for interspecific hybridization (Rogach, 1941). Heterostylous species have two (distyly) or three (tristyly) contrasting flower types. The plants that have flowers with long styles and short stamens are known as “pin” and the reverse is called “thrum”. Several species of yellow flowered *Linum* were found to be heterostylous (Ockendon, 1968). Hand pollinations in *L. grandiflorum* of

pin × thrum or thrum × pin were highly fertile (85–97%), but self pollination of pin or thrum flowers were only 3.0% successful (Kostopoulos, 1970).

### *Linum* Hybrids among Taxa with $n = 15$

The first interspecific hybridization in *Linum* was reported by Kolreuter between *L. usitatissimum* and *L. narbonense*, but later *L. narbonense* was considered to be synonymous with *L. angustifolium* (Tammes, 1928). There have been many reports of successful hybridization between *L. usitatissimum* and *L. africanum*, *L. angustifolium*, *L. corymbiferum*, *L. floccosum*, *L. pallescens*, and *L. tenue* (Tammes, 1928; Kikuchi, 1929; Ray, 1944; Gill, 1966; Gill and Yermanos, 1967a; Bari and Godward, 1970; Seetaram, 1972). All these crosses produced fertile  $F_1$  hybrids in at least one direction, presumably due to their similarity in ploidy levels and size of chromosomes (Bari and Godward, 1969; Seetaram, 1972). Crosses among five taxa, *L. africanum*, *L. angustifolium*, *L. corymbiferum*, *L. decumbens*, and *L. usitatissimum* were highly successful in at least one direction with  $F_1$  progeny exhibiting 80 to 90% germination (Gill, 1966). In a cytogenetic study Gill (1966) reported that *L. usitatissimum* differs by one translocation from three closely related species, *L. africanum*, *L. angustifolium*, and *L. decumbens*, but did not differ from *L. corymbiferum* in this respect. *Linum angustifolium* differs from the other three wild species (*L. africanum*, *L. corymbiferum*, and *L. decumbens*) by two translocations, each involving two nonhomologous chromosomes. Hybridization of *L. usitatissimum* with *L. decumbens*, *L. hirsutum*, and *L. nervosum* were reported but with low  $F_1$  fertility (Sharma and Khanna, 1964; Bari and Godward, 1970; Seetaram, 1972; Fig. 1).

Hybridization events among species other than cultivated flax are also successful. When *L. strictum* was used as a male parent, it successfully hybridized with *L. africanum*, *L. angustifolium*, and *L. floccosum* (Seetaram, 1972). *Linum crepetans* and *L. humile* pollen have produced fertile plants when crossed with *L. hirsutum* and *L. hispanicum*, respectively (Gill and Yermanos, 1967a; Seetaram, 1972; Fig. 1).

In summary, interspecific hybridization studies indicate that cultivated flax has the potential to hybridize with at least nine wild relatives with karyotype  $n = 15$  (Fig. 1). *Linum africanum*, *L. angustifolium*, and *L. pallescens* were crossed with *L. usitatissimum* and all reciprocal crosses produced fertile  $F_1$  plants (Fig. 1). Therefore, further studies should be conducted to determine if hybrids between these three species occur and retain transgenes from novel flax in the natural ecosystem, not only through backcrossing, but also by hybridization and introgression with other wild relatives (Fig. 1). All three species have produced fertile  $F_1$  seeds in crosses with at least two of the following species: *L. decumbens*, *L. floccosum*, *L. hirsutum*, *L. strictum*, and *L. tenue* (Sharma and Khanna, 1964; Gill, 1966; Seetaram, 1972; Fig. 1).

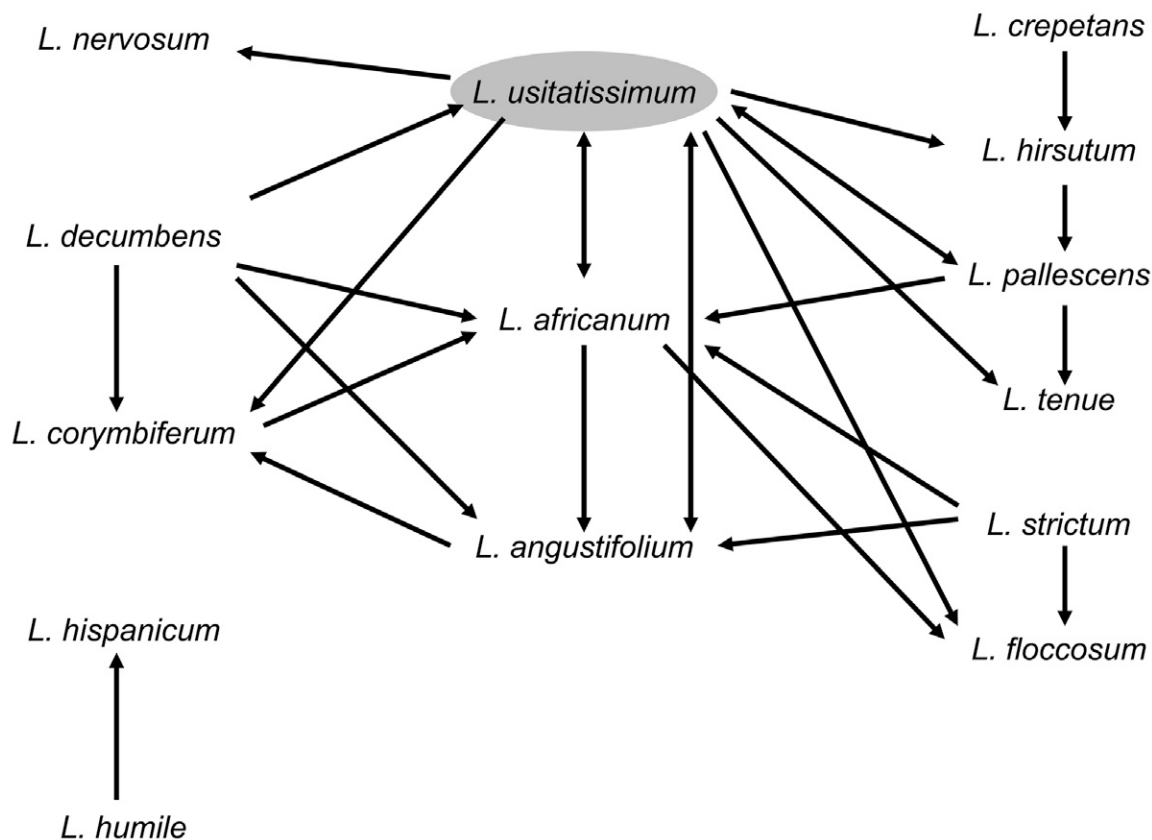


Figure 1. Artificial interspecific crosses among *Linum* species ( $n = 15$ ) that resulted in fertile progeny. Arrows indicate the direction of the cross (male to female). These are the related species with the greatest potential to hybridize with flax.

### ***Linum* Hybrids among Taxa Other than ( $n = 15$ )**

There have been studies of successful hybridization among taxa other than  $n = 15$  (Fig. 2). The taxa with  $n = 9$ , constitute the largest group in the genus *Linum* (Gill, 1966). Some crosses between species of taxa  $n = 9$ , *L. alpinum*, *L. altaicum*, *L. austriacum*, *L. julicum*, *L. narbonense*, and *L. perenne*, produced fertile  $F_1$  plants (Gill, 1966; Gill and Yermanos, 1967b). The pairing of chromosomes of these  $n = 9$  species revealed that *L. altaicum* differs by one reciprocal translocation from *L. alpinum*, *L. austriacum*, *L. julicum*, *L. narbonense*, and *L. perenne* (Gill and Yermanos, 1967b). They further speculated that *L. austriacum* and *L. narbonense*, and *L. julicum* and *L. narbonense* also apparently differ by one translocation, whereas *L. narbonense* and *L. perenne* differ by two translocations.

The chromosomes not involved in translocations formed normal bivalents, indicating that the genomes of the six species were sufficiently homologous for normal pairing to occur. However, a difference of two translocations was discovered between *L. alpinum* and *L. perenne* involving three nonhomologous chromosomes (Gill, 1966). When *L. perenne* was crossed with *L. austriacum*, hybrids were produced but only by embryo culture (Laibach, 1929; Fig. 2). The diploid *L. perenne* was successfully hybridized with autotetraploid *L. alpinum* (Kikuchi, 1929). Meiosis in this cross was studied and trivalents, bivalents, and univalents were observed at metaphase I (Nagao, 1941).

Interspecific hybridization between *Linum* species with different chromosome numbers was also studied. Crosses between *L. alpinum* ( $n = 9,18$ ), *L. austriacum* ( $n = 9$ ), *L. vulgare* ( $n = 9$ ), and *L. usitatissimum*; as well as crosses between other species with  $n = 15$  (i.e., *L. crepetans*, *L. hirsutum*, *L. strictum*, *L. usitatissimum*) and *L. grandiflorum* ( $n = 8$ ), either did not produce any seeds, or failed to produce fertile  $F_1$  plants (Kikuchi, 1929; Ray, 1944; Sharma and Khanna, 1964; Gill, 1966; Bari and Godward, 1970; Seetaram, 1972; Fig. 2). These results suggest karyotype plays an important role in interspecific hybridization in *Linum* species (Gill, 1966; Bari and Godward, 1970; Fig. 2).

Thus, only hybridization between species with equal chromosome numbers was successful in producing fertile  $F_1$  plants (Rogach, 1941; Richharia, 1962; Gill, 1966; Bari and Godward, 1970; Seetaram, 1972; Fig. 2). When cultivated flax is crossed with species having a different chromosome number, not a single cross has produced fertile plants. These greenhouse studies suggest that species with different chromosome numbers have no or minimal risk of gene flow to them.

### **GEOGRAPHIC DISTRIBUTION**

*Linum* species' distribution records are grouped into regions in accordance with the standard publication of Hollis and Brummitt (1992), which divides the terrestrial world into nine areas: Africa, Antarctic, Asia-Temperate, Asia-

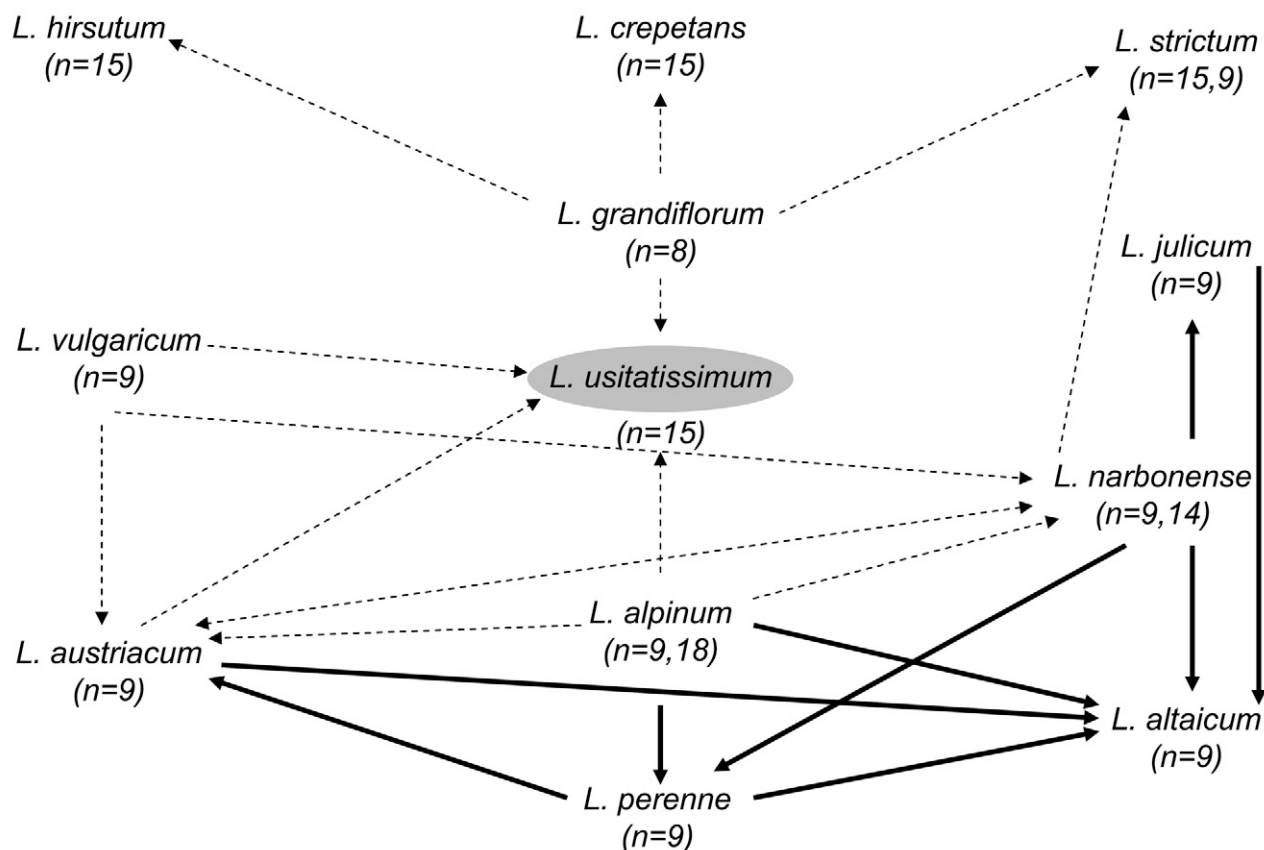


Figure 2. Interspecific hybridization in *Linum* (species with different chromosome numbers). Arrows indicate the direction of the cross (male to female). Solid lines indicate fertile  $F_1$  hybrids were obtained with viable seed production. Dotted lines indicate hybridization occurred, but  $F_1$  hybrids were not obtained with embryo rescue and/or treatments with colchicine.

Tropical (in this manuscript, we have considered Asia as a single region), Australia, Europe, North America, Pacific, and South America. However, a distributional report for a taxon in a geographical or political region does not necessarily imply widespread occurrence in that region, but indicates that a literature citation or other evidence (i.e., herbarium specimen) records the presence of the species (USDA-NRCS, 2006). State or provincial distributions were not itemized for taxa widespread within countries, except in North America. Here we discuss the geographic distribution of 41 *Linum* species (Table 2).

Flax is cultivated in almost all continents with temperate climates (Gill, 1987). In Europe, it is grown primarily for fiber, except in Germany, Hungary, Poland, and Romania where it is grown as an oilseed. *Linum angustifolium* Huds, a putative wild progenitor of flax, is a perennial species of the Mediterranean and sub Mediterranean area, Ireland, and the southern UK (Tammes, 1928). There are many other perennial species found in the Mediterranean extending up to Asia including *L. alpinum*, *L. campanulatum*, *L. capitatum*, *L. dolomiticum*, *L. hologynum*, *L. julicum*, and *L. viscorum* (Tutin et al., 1980; Table 2). Many species such as *L. austriacum*, *L. flavum*, *L. grandiflorum*, *L. hirsutum*, *L. narbonense*, and *L. perenne* have attractive flowers and so these species are frequently cultivated in European

and Canadian botanical gardens and available in nurseries as ornamental plants.

There are many *Linum* species native to Asia (Zeven, 1982). Two species, *L. mysorensis* and *L. usitatissimum* were recorded in many states of India (Cooke, 1903). Hooker (1875) reported two additional species, *L. perenne* and *L. strictum*. *Linum angustifolium* and *L. grandiflorum* were introduced in India as ornamental plants (Richharia, 1962). *Linum perenne* was reported in escaped clusters and this species might have been in cultivation in India during the Dravidian period (around 2000–1500 BC; Richharia, 1962). Many species including *L. altaicum*, *L. angustifolium*, *L. flavum*, *L. nervosum*, *L. pallescens*, *L. perenne*, and *L. tenuifolium* are native to the Russian Federation and distributed extensively within that region (Greuter et al., 1984; Table 2). *Linum marginale* and *L. monogynum* are distributed in Australia and New Zealand (Willis, 1972; Hnatiuk, 1990; Table 3). Detailed information on geographical ranges of individual species is given in Table 2.

### **Linum Species in the New World**

There are more than 63 *Linum* species distributed in the New World throughout the U.S., Canada, and Mexico (Small, 1907; Budd, 1987; Diederichsen, 2007; Table 3). Flax is grown primarily for seed in the Canadian prairies,

Table 2. Geographical distribution of *Linum* species classified by Gill, 1987.

Taxon	Location (endemic and/or naturalized)	Reference
Group I (2n = 18)		
<i>L. alpinum</i> Jacq. ( <i>L. perenne</i> subsp. <i>alpinum</i> (Jacq.) Stoj.& Stef.) (2n = 18, 36)	Europe: Austria, Bulgaria, France, Greece, Italy, Spain, Switzerland, Yugoslavia	Tutin et al. (1980); Greuter et al. (1984); Huxley (1992)
<i>L. altaicum</i> Ldb.	Asia: Kazakhstan, Mongolia, Russian Federation	Czerepanov (1995)
<i>L. anglicum</i> Mill. ( <i>L. perenne</i> subsp. <i>anglicum</i> (Mill.) Ockendon)	Europe: England, Scotland	Ockendon (1968)
<i>L. austriacum</i> L.	Africa: Algeria, Morocco Asia: Albania, Armenia, Azerbaijan, Bulgaria, Greece, Iran, Italy, Russian Federation, Turkey, Western Siberia Europe: Austria, Czechoslovakia, France, Germany, Hungary, Poland, Romania, Spain Switzerland, Ukraine, Yugoslavia	Tutin et al. (1980); Greuter et al. (1984)
<i>L. grandiflorum</i> Desf.	Africa: Algeria	Greuter et al. (1984); Huxley (1992)
<i>L. hologynum</i> Reichb.	Europe: Albania, Bulgaria, Greece, Romania, Yugoslavia	Tutin et al. (1980); Greuter et al. (1984)
<i>L. julicum</i> Hayek ( <i>L. perenne</i> subsp. <i>alpinum</i> (Jacq.) Stoj & Stef.)	Europe: Austria, Bulgaria, France, Greece, Italy, Spain, Switzerland, Yugoslavia	Tutin et al. (1980); Greuter et al. (1984)
<i>L. lewisii</i> Pursh	North America: Canada: AB, BC, MB, ON, QC, SK, YT U.S.: CA, CO, ID, MT, NV, OR, ND, SD, UT, WA, WV, WY	Hitchcock et al. (1969); Cody, (1996)
<i>L. narbonense</i> L. (2n = 18, 28)	Africa: Algeria, Morocco Europe: Albania, France, Italy, Portugal, Spain, Yugoslavia	Tutin et al. (1980); Greuter et al. (1984)
<i>L. perenne</i> L.	Asia: India, Russian Federation Europe: Albania, Austria, Belarus, Bulgaria, Czechoslovakia, France, Germany, Greece, Hungary, Italy, Moldova, Poland, Romania, Russian Federation, Spain, Switzerland, Ukraine, United Kingdom, Yugoslavia North America: Canada: MB, ON, SK, Victoria Island, YT Mexico U.S.: AK	Richharia (1962); Komarov (1969); Tutin et al. (1980)
<i>L. strictum</i> L.	Africa: Algeria, Egypt, Ethiopia, Libya, Morocco, Tunisia Asia: Iran, Iraq, Israel, Jordan, Pakistan, Syria, Turkey Europe: Albania, Bulgaria, France, Greece, Italy, Portugal, Spain, Yugoslavia	Rechinger (1963); Tutin et al. (1980); Meikle (1985); Hooker, (1875)
<i>L. tenuifolium</i> L.	Asia: Armenia, Azerbaijan, Georgia, Iran, Russian Federation, Syria, Turkey	Komarov (1969); Tutin et al. (1980)
Group II (2n = 30)		
<i>L. africanum</i> L. (2n = 30, 32)	Africa: Cape Province	Bond and Goldblatt (1984); Arnold and DeWet (1993)
<i>L. album</i> Kotschy	Asia: Iran, Syria	Guest et al. (1966)
<i>L. angustifolium</i> Huds. ( <i>L. bienne</i> Mill) (2n = 30, 32)	Asia: Armenia, Azerbaijan, Cyprus, Georgia, India, Iran, Iraq, Israel, Lebanon, Russian Federation, Syria, Turkey Africa: Algeria, Libya, Morocco, Portugal, Tunisia Europe: Albania, Bulgaria, France, Greece, Ireland, Italy, Portugal, Spain, UK, Ukraine, Yugoslavia	Guest et al. (1966); Tutin et al. (1980); Meikle (1985)
<i>L. corymbiferum</i> Desf.	Africa: Algeria, Tunisia North America: U.S.: IA	Greuter et al. (1984); USDA, NCRPIS, personal communication (2006)
<i>L. decumbens</i> Desf.	Europe: Germany	PGRC, personal communication (2006)
<i>L. flavum</i> L. (2n = 28, 30)	Asia: Russian Federation, Turkey Europe: Austria, Czechoslovakia, Germany, Hungary, Poland, Belarus, Moldova, Ukraine, Albania, Bulgaria, Italy, Romania, Yugoslavia	Davis et al. (1988); Huxley (1992)
<i>L. hispanicum</i> Mill.	Europe: Albania, Bulgaria, France, Greece, Ireland, Italy, Portugal, Spain, Ukraine, United Kingdom, Yugoslavia	Komarov (1969); Davis et al. (1988)
<i>L. humile</i> Mill ( <i>L. usitatissimum</i> var. <i>humile</i> Mill Pers.)	Africa: Mediterranean	Komarov (1969); Tutin et al. (1980)



Table 2. Continued.

Taxon	Location (endemic and/or naturalized)	Reference
<i>L. medium</i> Planch. (2n = 30, 36)	North America: Canada: ON  U.S.: AL, AR, FL, GA, IA, KS, LA, OK, MO, NC, SC, TX, VA	Rogers (1963); Scoggan (1993); Magee and Ahles (1999)
<i>L. nervosum</i> Waldst.	Asia: Armenia, Azerbaijan, Georgia, Iran, Russian Federation, Turkey Europe: Bulgaria, Ukraine, Romania, Yugoslavia,	Tutin et al. (1980); Davis et al. (1988)
<i>L. pallescens</i> Ldb.	Asia: Kazakhstan, Kyrgyzstan, Russian Federation, Tajikistan,	Komarov (1969)
<i>L. rigidum</i> Pursh.	North America: Canada: AB, MB, ON, SK	Rogers (1963); Rogers (1968); Scoggan (1993)
var. <i>filifolium</i> Rog.	Mexico	
var. <i>rigidum</i> Rog.	U.S.: FL, TX	
<i>L. sulcatum</i> Riddel.	North America: Canada: MB, ON, QC  U.S.: AL, AR, GA, IL, IA, KS, KY, LA, MD, MS, MN, MO, NE, NC, ND, OK, PA, TN, TX, VA, WI	Rogers (1963); Scoggan (1993)
<i>L. tenue</i> Desf.	Africa: Algeria, Morocco Europe: Portugal, Spain	Tutin et al. (1980)
<i>L. usitatissimum</i> L. Group III (2n = 28)	Cultivated species of <i>Linum</i> , grown in almost all continents	Richharia (1962); Gill (1966)
<i>L. campanulatum</i>	Europe: Italy, France, Spain	Tutin et al. (1980)
<i>L. capitatum</i> L. (2n = 24, 28)	Europe: Albania, Bulgaria, Greece, Italy, Yugoslavia	Tutin et al. (1980)
<i>L. dolomiticum</i> Borb. Group IV (2n = 16)	Europe: Hungary	Tutin et al. (1980)
<i>L. catharticum</i> L. (2n = 16, 57)	Africa: Morocco Asia: Azerbaijan, Georgia, Iran, Russian Federation, Turkey Europe: Albania, Austria, Belarus, Belgium, Bulgaria, Czechoslovakia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Norway, Sweden, United Kingdom, Netherlands, Poland, Switzerland, Russian Federation, Ukraine, Portugal, Romania, Spain, Yugoslavia North America: Canada: NF, NS, ON, QC U.S.: MI, PA	Rogers (1963); Scoggan (1993)
<i>L. hirsutum</i> (2n = 16, 18)	Europe: Bulgaria, Czechoslovakia, Hungary, Yugoslavia	Greuter et al. (1984); Tutin et al. (1980)
<i>L. viscorum</i> L. Group V Others	Europe: Austria, France, Germany, Italy, Spain, Yugoslavia	Tutin et al. (1980)
<i>L. gallicum</i> L. ( <i>L. trigynum</i> L.)	Cyprus, Lebanon, Syria, Turkey	Davis et al. (1988); Komarov (1969)
<i>L. marginale</i> A. Cunn. (2n = 80)	Australia: New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia	Willis (1972); Hnatiuk (1990)
<i>L. maritimum</i> L.	Africa: Algeria, Morocco	Tutin et al. (1980); Greuter et al. (1984)
<i>L. monogynum</i> Forst. (2n = 86)	Australia: Australia and New Zealand	Allan (1961); Hnatiuk (1990)
<i>L. rupestre</i> (Gray) Engelm. Ex Gray	North America: U.S.: NM, TX	USDA-NRCS (2006)
<i>L. schiedeanum</i> S. & C.	Mexico  U.S.: FL, TX	Rogers (1969)

Kansas, Minnesota, Montana, Nebraska, North Dakota, and Wisconsin (Scoggan, 1993; USDA, 2007). Rogers (1963; 1968), who developed an extensive classification and distribution of *Linum* species in North America, reported that *L. rigidum* and closely related species are believed to be the most primitive in North America. These species are distributed in southern Florida and also have a vast range in the Great Plains extending from northern Mexico to western Canada (Mosquin and Hayley, 1967). There are eight species of *Linum* distributed in Canada (Scoggan, 1993). Plant Gene

Resources of Canada (PGRC) has a germplasm collection of 5296 accessions of *L. usitatissimum* and 76 identified flax wild relatives (Diederichsen, 2007). *Linum sulcatum* ( $n = 15$ ) is extensively distributed in several states of the United States and provinces of Canada (Table 3).

*Linum* species in North America can be divided into three groups: blue, white, and yellow flowered species (see Rogers, 1969 for relationships among these three groups). There are three basic karyotypes in the North American species, each representing an invasion from the Old

**Table 3. Distribution of *Linum* species ( $n = 15$ ) in North America.**

Species	Distribution	Reference
<i>L. alatum</i> (Small) Winkler	U.S.: TX	Rogers and Harris (1966)
<i>L. aristatum</i> Engelm.	U.S.: AZ, CO, NM, UT, TX	Rogers and Harris (1966)
<i>L. australe</i> Heller	U.S.: AZ, NM	Rogers (1963)
<i>L. corymbiferum</i> Desf.	U.S.: IA	USDA, NCRPIS, personal communication (2006)
<i>L. hudsonioides</i> Planch.	U.S.: KS, NM, OK, TX	Harris (1968); Correl and Johnston (1970)
<i>L. imbricatum</i> (Raf.) Shinnars	U.S.: TX	Osborne and Lewis (1962)
<i>L. puberulum</i> (Engelm.) Heller	U.S.: FL, OK, TX	Mosquin and Hayley (1967)
<i>L. rigidum</i> Pursh		
var. <i>rigidum</i>	Canada: AB, MB, ON, SK	Mosquin and Hayley (1967); Rogers (1968); Scoggan (1993)
var. <i>berlandieri</i>	U.S.: FL, NM, TX	
var. <i>compactum</i>		
var. <i>filifolium</i>		
var. <i>carteri</i>		
<i>L. sulcatum</i> Riddell	Canada: MB, ON, QC, SK. U.S.: AL, AR, CT, GA, IL, IN, IA, KS, KY, LA, MD, MI, MS, MN, MO, NE, NJ, NY, NC, ND, OH, PA, PA, TN, TX, VT, WV, WI	Rogers (1963); Rogers and Harris (1966); Scoggan (1993)

World, (i)  $n = 8$  (*Linum catharticum*), (ii)  $n = 9$  (blue flowered species), and (iii)  $n = 18$  (yellow flowered species) (Harris, 1968) as well as many species with  $n = 15$  (Fig. 3). A large number of interspecific crosses of two Florida tetraploids ( $n = 30$ ) *Linum rigidum* var. *rigidum* and *L. rigidum* var. *carteri*, were attempted with two diploid species ( $n = 15$ ) of the Great Plains, *L. alatum* and *L. aristatum*, but all of the crosses either failed to produce seed or the seeds failed to develop into mature plants (Mosquin and Hayley, 1967). However, crosses of both the Florida tetraploids have resulted in successful hybridization with the diploids of the Great Plains, *L. rigidum* var. *berlandieri*, *L. rigidum* var. *rigidum*, and *L. elongatum*.

On the basis of the results of interspecific hybridization experiments, we conclude that chromosome number is an important factor in hybridization and introgression between *Linum* species (Fig. 1 and 2). With the one exception of *L. corymbiferum*, none of the native North American species presented in Fig. 3 have been included in reports of hybridization studies with cultivated flax. Because all these species have the same chromosome number ( $n = 15$ ), there may be the potential for transgene introgression from transgenic flax to them (Fig. 3). However, hybridization of crop-weed complexes can be influenced by environmental, temporal, and spatial variables (Ellstrand et al., 1999; Hall et al., 2006). Hybridization is also influenced by many other factors including sympatry of crop and weedy species, availability of pollinators, duration of pollen viability, synchronicity of flowering, floral morphology, genetic relatedness, direction of hybridization, heterostyly, and sexual compatibility (Kostopoulos, 1970; Govindaraju, 1988; Ellstrand and Hoffman, 1990; Rieseberg and Wendel, 1993). The geographic distribution of North American wild relatives with  $n = 15$  is given in Table 3.

## BIOLOGY AND ECOLOGY

Limited information is available on the biology and ecology of *Linum* species. Almost all *Linum* species are noted for their value in mixes for erosion control and in beautification. A long period of flowering makes the plant more aesthetically appealing (USDA, 2007) but also increases the potential flowering synchronicity with cultivated flax. Most of the species are fire resistant because leaves and stems stay green with relatively high moisture content during most of the fire season (USDA, 2007). The following is information on specific *Linum* species.

### *Linum usitatissimum* L. ( $2n = 30$ )

*Linum usitatissimum* (cultivated flax) is grown for seed oil and fiber. Linseed type flax is a relatively short plant which produces many more secondary branches compared to the fiber type (Gill, 1987). The flowers are hermaphroditic, hypogynous, and slightly protandrous (Eyre and Smith, 1916) with five sepals, five petals, five stamens, and a compound pistil of five carpels in a radially symmetrical arrangement (Dillman, 1938). The fruit is a capsule, containing 8 to 10 seeds. Flax is predominantly a self pollinated species but cross pollination rates have been reported in the range of 1 to 5% (Eyre and Smith, 1916; Robinson, 1937; Dillman, 1938; Gill, 1987), with important pollinators being honeybees, bumble bees, and butterflies (Dillman, 1938; Gubin, 1945). The life cycle of a flax plant consists of a 45 to 60 d vegetative period, 15 to 25 d flowering period, and a fruit maturation period of 30 to 40 d (Anonymous, 2006). In addition to being cultivated, *L. usitatissimum* is found as an escape in waste places, along roadsides (Richharia, 1962), in disturbed land habitats, and in unmanaged ecosystems (CFIA, 1994; Thomas

et al., 1997). The establishment and spread of flax in disturbed habitats warrants further study.

Flax grows best on soils with high water holding capacity and good inherent fertility. It does not thrive on sandy soils unless a large supply of moisture is available (Anonymous, 2006). Although flax is considered to be a cool season crop, air temperature below 10°C in the spring may inhibit growth and development, which can delay flowering (Gusta et al., 1997). In a recent study on seed color, seed weight, and seed oil content in several flax accessions, Diederichsen and Raney (2006) revealed that yellow seeded flax had a higher seed weight and oil concentration than brown seeded flax. In vigour tests, yellow seed had lower seed vigour than brown seed (Saeidi and Rowland, 1999).

Poor management practices may result in large numbers of seeds being returned to the soil during harvest. This can result in an increase in the flax seed bank and resulting in volunteer weed problems in succeeding crops (Leeson et al., 2003). Flax is a poor competitor (Friesen, 1988; Wall, 1994), and volunteer flax does not usually result in yield losses in crops like cereals and canola (*Brassica napus* L.). However, it can cause considerable difficulty at harvest time (Anonymous, 2006). Thomas et al. (1997) reported that volunteer flax was present in twice as many fields under zero tillage, but at lower densities when compared to conventional tillage systems. A recent survey on volunteer flax emergence indicated that it varied

throughout the growing season from 0 to 189 plants m<sup>-2</sup> in the direct seeded plots to 1 to 1510 plants m<sup>-2</sup> in the conventional seeded plots (Dexter et al., 2006). These data infer that volunteer flax can also contribute to substantial gene flow if not controlled. The distribution of small clumps of volunteer flax seedlings in a field indicates that many seeds germinate within seed bolls, rather than as single dispersed seeds (unpublished data, 2006).

### *Linum perenne* (2n = 18)

*Linum perenne* is a perennial that grows 20 to 80 cm in height with stems arising from the cotyledonary node, and linear to lanceolate-linear leaves. Inflorescences are loose cymes containing white to blue flowers. The flowers are heterostylous, as in many of its species group (Gill, 1966). It is known as blue flax (USDA, 2007) or perennial flax (Scoggan, 1993). It is commonly found in hills and eroded banks over the northern plains, prairies, and in open fields in moist, well-drained, calcareous soils (Scoggan, 1993).

Blue flax is noted to have forage value for livestock and wildlife because plants stay green throughout the growing season. Birds use seeds and capsules in the fall and winter. It is also considered desirable for deer (*Odocoileus hemionus*, *O. virginianus*), antelope (*Antilocapra americana*), and birds, either as herbage or seed (USDA, 2007). Blue flax is a native to Eurasia and has been distributed not only in the U.S. (USDA, 2007), but also in some provinces of Canada (Scoggan, 1993).

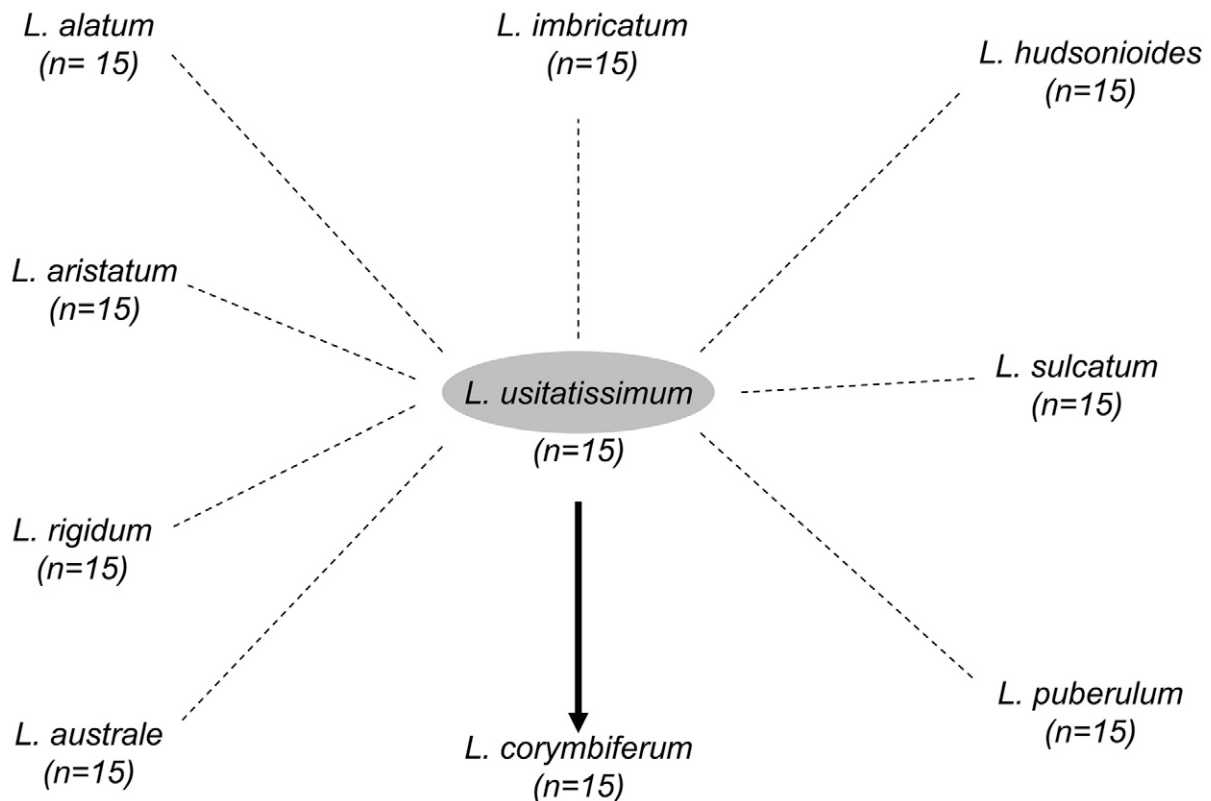


Figure 3. Potential hybridization of flax with related species ( $n = 15$ ) in the New World. A dotted line indicates species that may hybridize with flax *L. usitatissimum*, but no evidence of hybridization has been reported, while a solid arrow indicates a successful hybridization from male to female.

*Linum perenne* is cultivated for horticultural or revegetation purposes in Iowa, Oregon, and North Dakota. Seed yields of 600 to 700 pounds per acre of blue flax can be expected under irrigated conditions and 200 to 300 pounds per acre under dry land conditions (USDA, 2007). Flowering is indeterminate and there is the possibility of some flowers present at harvest. Some seeds will shatter once capsules open. Seeds retain viability for several years under 15% moisture conditions (USDA-NRCS, 2006).

### ***Linum sulcatum* Riddell (2n = 30)**

*Linum sulcatum*, known as grooved flax, is generally considered an annual plant species, but has also been recorded as a biennial in North Carolina (Radford et al., 1968). Leaves are alternate, sessile, about 2 cm long and 2 mm wide, with a single midrib. Plants vary in height from 20 to 70 cm and inflorescences are axillary loose panicles or racemes (Gill, 1966). Flowers have five yellow petals that are rounded at the apex. Grooved flax is insect-pollinated and probably self-compatible (Zaremba, 2003). Rogers (1963) has divided *L. sulcatum* in two cultivars, *L. sulcatum* var. *harperi* (Small), distributed in Southern America; and *L. sulcatum* var. *sulcatum*, widely distributed from Manitoba to Texas and east to Georgia and New Hampshire. Like cultivated flax, *L. sulcatum* is  $n = 15$  (Dillman, 1933), so it may have potential to hybridize with flax. Rogers (1963) has classified *L. sulcatum* as an intermediate form between two complexes in the genus *Linum*, which was supported by Giannasi and Rogers (1970) and by a cytogenetic study of Harris (1968). Attempts to hybridize *L. sulcatum* with its near relatives to assess chromosome similarities were unsuccessful (Harris, 1968).

Grooved flax can be considered a weed (Stevens, 1932). The seeds are known to persist in the soil seed bank (Blake, 1935). Zaremba (2003) observed that plants are first evident in early June and begin to flower by late June continuing through the early fall, and that even small plants (4 cm) can produce flowers and seeds. It can also colonize new sites easily. There is no detailed information available on pollination mechanisms, but it is believed to be insect pollinated (Zaremba, 2003). Robertson (1971) reported that many species of genus *Linum* are homostylous, so *L. sulcatum* may be self-compatible.

## **CONCLUSIONS**

Most of the cultivated crop plants diverged from their wild relatives less than a few thousand generations ago, and it is unlikely that complete isolation—halting the flow of domesticated alleles from crop species to progenitors—has occurred (Ellstrand et al., 1999). Whereas flax has been cultivated for >5000 years (De Candolle, 1904; Dillman, 1938; Richharia, 1962; van Zeist and Bakker, 1975; Gill, 1987; Fu et al., 2002) it can be artificially crossed with several wild relatives and produce fertile progeny (Gill

and Yermanos, 1967a; Yermanos and Gill, 1967; Bari and Godward, 1970; Seetaram, 1972; Fig. 1 and 2). If fertile hybrids can be produced from crosses between *L. usitatissimum* and closely related species, a transgene may be able to transfer to these wild species. This is particularly noteworthy since cultivated flax and wild relatives may grow in sympatry in several locations.

Although artificial hybridization of *Linum* species under controlled conditions does not predict the success of hybridization in the natural ecosystem, it can establish potential cross compatibility between those species (Ellstrand et al., 1999). In nature, the hybridization rate is predicted to be lower than that of greenhouse studies. However, the number of plants in the environment increases the chance that a successful cross will eventually occur. A limited distribution of wild populations and weed control practices would also reduce the population size of wild plants and volunteer flax and thus minimize gene flow.

Western Canada is the largest flax growing region in the world and only three wild species are distributed in this area. Two of them, *L. rigidum* and *L. sulcatum*, have the same karyotype as cultivated flax. While interspecific outcrossing has not been documented for these species (Fig. 3), hybridization of flax with other  $n = 15$  species suggests outcrossing may occur (Fig. 1). *Linum lewisii*,  $n = 9$ , seems less likely to outcross with cultivated flax (Gill, 1987). Among the *Linum* species in the United States, *L. corymbiferum* is the only species where successful hybridization with cultivated flax has been documented but there are several other species for which outcrossing with flax has not been reported (see Fig. 3). Little is known about the distribution, flowering time, preferred habitat, or population size of these species. Further research on species, including a greenhouse study to quantify outcrossing potential with cultivated flax is warranted to determine whether introgression of a transgene can occur.

The assessment of the potential for gene flow to wild relatives is one of many components of an environmental risk assessment before the release of a new transgenic crop. Other concerns are the movement of transgenes via pollen to conventional crops, seed and volunteer mediated gene flow, influences on non-target species, and the potential harm to biodiversity. Details on the specific trait and the consequence of its expression are required before determining whether the transgene could have an impact on wild species, should introgression occur.

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