International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 8(4): 805-817 December 2015
DOI Number: 10.5958/2230-732X.2015.00089.3

Genetics and Plant Breeding

An Overview on Flax (*Linum usitatissimum* L.) and its Genetic Diversity

Sumita Nag[1*], Jiban Mitra[2] and P.G. Karmakar[2]

[1] Ramakrishna Mission Vivekananda University, Narendrapur, Kolkata – 700103, West Bengal, India
[2] Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata-700120, West Bengal, India

*Corresponding author: sumi11nag@gmail.com

Paper No.: 373 Received: 04 Jan 2015 Accepted: 08 Dec. 2015

Abstract

Flax (*Linum usitatissimum* L.) is an annual, self-pollinated species with a genome size of ~ 370 Mb. Flax provides raw materials for food, medicine and textiles and hence it has been of great importance to human culture and development. Linseed oil is well-known for its health benefits mainly attributed to its high content of omega-3 alpha linolenic acid (55-57%). Consumption of grounded seeds adds nutritional benefits because flax seeds are also a rich source of lignans, having anticancer properties. India contributes to almost 20% of world's linseed production and governs linseed production among the Asian countries whereas, the scenario is just reverse in case of fibre flax. In the last decade, the fibre industry has devoted some effort to develop high-value products from linseed stems with applications in the pulp, technical fibre and biofuel industries. Therefore, understanding its genetic diversity is important for the continued improvement of this crop and as well as for its utilization as a truly dual purpose crop. Diversity analysis based on morphology alone has a significant limitation in the fact that it is highly influenced by the environment, to overcome this problem; molecular characterization can play an important role. However, systematic studies regarding the genetic diversity of flax in India are meagre. Hence, in-depth studies based on both morphological and molecular markers will help in better conceiving the genetic diversity of flax germplasm.

Highlights

- Flax (*Linum usitatissimum*) is an ancient crop having diverse utility and importance.
- Germplasm characterization is an important link between the conservation and utilization of plant genetic resources.
- In-depth studies based on morphological and molecular markers will help in understanding the genetic diversity of germplasm.

Keywords: Flax, genetic diversity, morphological marker, molecular marker

Flax belongs to the genus *Linum*, one of the ten genera in the family Linaceae. The genus encompasses more than hundred annual and perennial species. Cultivated flax pertains to the species, *Linum usitatissimum*, having two types: one is grown for oil (linseed) and the other for fibre (fibre flax). Textile properties of flax fibre are superlative to cotton. Flax is the third largest natural fibre crop and one of the five top oilseed crops in the World. It is a small size, self-pollinated herb that has been thought to be the best model fibre plant (Millam *et al.* 2005). Flax is commonly also known as, tisi, kshuma, lin, llion, liner, linum, line, linen, lein and lan. The plant is an annual which grows to a height of 110-120 cm and about 1.4-1.6 mm in diameter. In India flax is grown predominantly for linseed oil for human consumption and commercially it is utilized for paint, varnish, finished leather and printing ink. The history of flax dates back to 7000 BC when it was used by the Mesopotamians. Later on Egyptians, Babylonians, Greeks, Romans and other civilizations cultivated flax for its fibre. During the middle ages, linen was the most indispensable textile product. Hence flax is one of the antique and most interesting plants cultivated. The art of weaving flax was so advanced that wearing of 'linen

cloth' was considered to be a sign of aristocracy and gleaming whiteness of linen as a symbol of purity. In fact, the word 'candidate' used for office seekers has its origin from the latin word 'candidus' which means white linen. The Egyptian art of weaving flax was gradually introduced in India, where linen was worn by many cases before the use of cotton. Presently as reported flax fibres producing countries are Belgium, Russia, Switzerland, Brazil, England, France and Argentina. The chief producer of flax fibre is the erstwhile Soviet Union, but the world's best thread derives from Belgium and adjoining countries. In India the manufacturer of line fabrics, import the flax fibres from European countries and does not utilize the flax produced in India. The reasons for this are, Indian flax does not match with the quality standards of imported flax. But now several dual purpose varieties released are competent for both oil and fibre purpose. Among the oilseed crops, flax is next to rapeseed and mustard. India occupies 25% of world acreage and ranks first in the area (4.368 lac ha), fourth in production (1.725 lac tonnes) and eighth in productivity (395.0 Kg/ha) of the flax crop. The yield of fibre flax is about 10-15 quintal/ha. In India, small farmers grow linseed mainly for local consumption. This crop is not only commercially very important, but for the rural poor, it is a necessary means of survival. In spite of flax value as a food source, research directed toward the improvement of cultivated flax has been limited.

Therefore, germplasm characterization is an important link between the conservation and utilization of plant genetic resources. The diversity among varieties may be assessed based on morphological, biochemical and molecular markers. However, diversity analysis based on morphology alone has a significant limitation in the fact that the environment highly influences it. To overcome this problem, molecular characterization can play an important role. The development of molecular and biochemical techniques help researchers not only to identify genotypes but also in assessing and exploiting the genetic variability (Whitkus *et al.* 1994). However, systematic studies regarding the genetic diversity of flax through DUS descriptors as well as molecular markers in India are inadequate. Hence, in-depth studies based on morphological and molecular markers will help in understanding the genetic diversity of germplasm for the analysis of population structure, identification, conservation and utilization of authentic and superior crop materials.

Crop History

Flax or linseed is among the oldest crop plants cultivated for the purpose of oil and fibre for more than 6000 years, and it is among the first plants to be domesticated. The botanical name, *Linum usitatissimum* was given by Linnaeus in his book "Species Plantarum" (Linnaeus, C. 1857). It was already cultivated in ancient Egypt and Samaria 10,000 years ago (Zohary and Hopf, 2000) to provide both fiber and oil. Recently, 30,000-year old processed and colored flax fiber was found, indicating that early humans made fabric or threads from the flax (Kvavadze *et al.* 2009). In ancient Egypt, linen was used for wrapping the royal mummies and additionally linseed oil was used to embalm the bodies of deceased Pharaohs (Dewilde, 1983).

In China and India domesticated flax was cultivated by at least 5,000 years ago (3,000 BCE). Portraits on tombs and temple walls at Thebes illustrate flowering flax plants. The use of flax fibre in the manufacturing of cloth in northern Europe dates back to Neolithic times. In Northern America, flax was first pioneered by the Puritans. For a long time flax has been cultivated as a dual-purpose crop, but now fibre flax and linseed represent different gene pools. Fibre flax has been cultivated in the Netherlands and most likely in Belgium and Northern France since ancient times. The quality and fineness of the linen have been proven ever since.

Taxonomy and Nomenclature

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Malpighiales
Family	:	Linaceae
Genus	:	Linum
Species	:	*L. usitatissimum*

More than 200 species present in the genus *Linum* are distributed worldwide divided into five subsections (Tutin *et al.* 1968), of which subsection *Linum* contains the cultivated species *Linum usitatissimum* L. and the two ornamentals *L. grandiflorum* and *L. perenne*. *Linum* is the largest genus within the family and is found both in the Mediterranean region and the Americas. The two major user types are connected to morphotypes, broadly designed as oil (linseed; convar. mediterraneum), fiber (flax; convar. elongatum), and intermediate (convar. usitatissimum) varieties, although this infra-specific grouping is not unified (Diederichsen and Fu, 2006). The chromosome number of *Linum* species show a wide

range extending from 2n = 16 to 2n = 72 (Fedorov, 1974). *L. usitatissimum* and its wild relatives comprising 2n = 30 chromosomes (Muravenko *et al.* 2003). The genome size (1C) of cultivated flax is 686 Mbp (Bennett and Leitch, 2004).

Origin Distribution and Domestication

Centre of origin

The centre of origin of flax (*Linum usitatissimum* L.) is uncertain. It is considered that *L. bienne* as the progenitor of small seeded flax, originating from Kurdistan and Iran, whereas it is also sometimes considered that *L. angustifolium* containing high oil content and seed weight, as progenitor, originating from the Mediterranean region (Murre, 1955; Zeven and de Wet, 1975). Others suggest that *L. bienne* and *L. angustifolium* are the same species, and are widely distributed over Western Europe, the Mediterranean basin, North Africa, the Near East, Iran and Caucasus (Tutin *et al.* 1968; Zohary and Hopf, 1993). Contemporarily, a study with molecular markers advocated that the three species originated from one common ancestor, *L. angustifolium* being most primitive (Muravenko *et al.* 2003). *L. usitatissimum* is an annual crop species whereas, the wild forms can also be biennial or perennial. All species are predominantly self-pollinated (Zohary and Hopf, 1993). Cross pollination may occur (Williams, 1988) by artificial means through insects.

Domestication

Flax is indigenous to the region expanding from the eastern Mediterranean to India and was presumably first domesticated in the Fertile Crescent. It is cultivated throughout the world including Canada, India, China, United States, Ethiopia and all over Europe (FAOSTAT, 2013). Since the domestication of flax, there has been an inclination for growing flax either for its fibre or oil. In the Western region of Eurasia, flax is mostly grown for its fibre, whereas in the Eastern area of Eurasia flax is cultivated for its oil (Gill, 1987).

Crop Botany

Flax cultivars are homogenous, and individual plants are considered homozygous (Anonymous, 1996). Flax is an annual plant growing 120 cm tall, with slender stems. The leaves are green, 20-40 mm long and 3 mm broad. The flowers are majorly pure pale blue and of various other colors, 15-25 mm diameter, with five petals. The fruit is a round, dry capsule 5-9 mm diameter, which may contain up to ten seeds when filled (Freeman, 1995). Seeds are glossy brown and 4-7 mm long. The kernels have a crisp and chewy texture and a pleasant, nutty taste (Carter, 1993). It is a herbaceous plant with shallow taproot system that may extend to a depth of 92-122 cm in the coarse textured soil.

Growth Habit

The cultivars grown with intention to make fibre are tall growing with straight culms and have fewer secondary branches, and are conventionally grown in cool temperate regions of the world, The cultivars grown primarily for seed/oil purpose are relatively short in height and possess more secondary branches and seed bolls (seed capsule), and generally prefer the warmer climates such as those in the Mediterranean area, India, Canada and USA. Flax is mainly self-pollinated, but natural crossing is possible through insect. The frequency of cross-pollination seems to be related with varietal differences and environmental factors. In flax, individual flowers open in the first few hours after sunrise on clear, warm days, and the petals usually fall before noon. Most of the commercial varieties have blue petals. Petals may also be white or different shades of purple, blue or pink. The seeds are of various shades of yellow, brown, greenish-yellow, greenish-brown, or nearly black. Seed colour of most commercial varieties is light brown. Flax is an excellent companion crop to help establish small-seeded grasses and legumes. Plant characteristics that advocate its use as a companion crop are: (1) limited leaf area and short stature that allow enough light to reach the forage seedlings, (2) early maturity and (3) less extensive root system than many crops which reduces competition for soil moisture. Flax is an annual spring crop with 90 to the 110-day growing season. The typical life cycle consists of 45 to 60-day vegetative period, followed by a 15 to 25-day flowering period, and 30 to 40 day maturation period. Proper harvest time is critical in flax production. Early harvest diminishes yield while late harvest can change the chemical make-up of the oil and thus its quality and value.

Agronomical Aspects

Fibre flax and linseed perform best in different regions. Fibre flax is mainly grown in climates with a relatively low temperature and high air humidity, which is characteristic for northern temperate regions. The subtropical regions and highlands are ideal locations for linseed cultivation, and, therefore, linseed should be more tolerant to prolonged periods of drought (Bunting, 1951). Although the soil type is not the most important

factor in flax cultivation, the sandy clay soils are very suitable for fibre flax cultivation. Flax requires a wide crop rotation of about seven years. Also, the preceding crop is important for growing flax to prevent the occurrence of diseases and lodging. As a rule of thumb, flax is sown at day 100 of the year and harvested on day 200, which is a growing period of 100 days. However, this depends somewhat on the cultivar and environmental conditions. The high sowing density of fibre flax of 110-130 kg/ha results in plant elongation due to the competition for light. It is important to obtain long, high-quality unbranched fibres. Linseed is sown with a lower density, 25-55 kg/ha (Rowland, 1998) to stimulate branching to obtain greater numbers of flowers and an increased seed yield. Flax starts to bloom approximately 11 to 14 weeks after sowing. The flowers are open for only a couple of hours in the morning, after which the petals fall off, and sepals close. Ten to 14 days after flowering the fruit reaches its final size, after which the weight remains stable until it decreases as a consequence of the ripening process. At the end of the development the flax plant hardens, turns to yellow (senescence) and loses its leaves. At a certain point, the plants are ready for the retting process, although the seeds might not be fully ripened. The retting process is the most crucial phase of flax cultivation because it determines the yield and quality of the fibre.

Importance

The flax is considered as one of the ancient and most utilitarian crops having separate utility. Cultivar development of flax for consumption is currently focused on augmenting the oil content and nutritional value to meet the requirement of nutraceutical market supply, as a substitute of fish oil, a rich source of eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). Flaxseed is also rich in soluble and insoluble fibres and lignans, which makes it useful as a dietary supplement. Consumption of flaxseed in daily diet simplifies the risk of cardiovascular diseases such as coronary heart disease and stroke. There is also evidence that flax has anticancer effects in the breast, prostate and colon cancers. Flax fibre is used in the textile industry for making linen cloth and also in paper industry. The fibres of flax have great tensile strength, staple length, durability and fineness. They are used in the manufacture of linen cloth and thread, canvas, duck, strong twine, carpets, fish and seine lines, cigarette paper, writing paper and insulating materials. Fibres from the stalks of flax grown for seed are too harsh and brittle for spinning but may be used for other purposes. Some of the benefits of linen are that it is allergy-free, absorbs humidity and allows the skin to breathe, antistatic, antibacterial and low elasticity (fabrics don't deform). Linen can be washed many times without alteration. It can absorb moisture up to 20 times its weight before it feels damp. The residues remaining after the oil extraction from linseed contains about 35-40% protein and 3-4% oil, a rich source of feed to livestock like cattle. Flax is naturally high in polyunsaturated fatty acids (PUFA), more specifically in ω-3 fatty acids; and hence flax seed as a component of poultry meal that can provide ω-3 enriched eggs. Rapid "drying" property of linseed oil is used for several purposes in industry, including paint and flooring (linoleum) industries. Due to its novel oil profile, flax may also be a suitable platform crop for the synthesis of exceptional industrial and nutraceutical products.

Area and Production

Linseed is an old world crop that was probably cultivated first in Southern Asia and Mediterranean region. There has been a general downward trend in area planted to linseed that began shortly after World War–II. During

Table 1: Top ten fibre flax and linseed producing countries in the World (FAOSTAT 2013)

	Fibre Flax and Tow			Linseed	
Sl. No.	**Country**	**Production (tonnes)**	**SL No.**	**Country**	**Production (tonnes)**
1	France	83100.00	1	Canada	712000.00
2	Belgium	67300.00	2	China Mainland	398809.00
3	Belarus	44925.00	3	Russian Federation	325756.00
4	Russian Federation	39039.00	4	Kazakhstan	295020.00
5	China	24356.00	5	India	147000.00
6	China Mainland	24056.00	6	Sweden	22900.00
7	United Kingdom	14000.00	7	Argentina	17070.00
8	Netherland	11237.00	8	France	16147.40
9	Egypt	8500.00	9	Brazil	9734.00
10	Italy and Chile	3000.00	10	Spain	8500.00
	World + Total	303113.00		World + Total	2305369.07

2006, the total global linseed area was 3.02 million hectares with a production of 2.57 million tonnes and productivity of 852 kg/ha. According to FAOSTAT 2013, the total global production of fibre flax and linseed is given in Table 1.

In India, linseed is cultivated in about 4.68 lakh ha and the total linseed production is 1.63 lakh tonnes with 349 kg/ha productivity (2007-08). Madhya Pradesh, Uttar Pradesh, Chhattisgarh, Maharastra, Bihar and Orissa are major linseed producing states in India. It is grown to a small extent in Jharkhand, Karnataka, Assam, Rajasthan, West Bengal, Himachal Pradesh, etc. There has been a continuous decline in linseed area in the country during last four decades. Linseed recorded annual compound growth rates of -2.11, -1.19 and 0.93% in area, production and productivity during 1950-51 and 2006-07. Presently in India linseed area harvested is about 4.31 lakh ha and production is 1.52 lakh tonnes. These data are not available in case of fibre flax and tows for India. The total world area harvested for linseed is approx 2.34 million ha and for fibre flax and tows it is 2.18 lakh ha. (FAOSTAT 2012).

India imports fibre flax worth ₹ 150 crore approximately. Belgium is the largest supplier of fibre flax accounting for imports worth ₹ 104 crore followed by France and Netherlands, which export fibre flax worth ₹ 40 crore and ₹ 6 crores respectively. Kolkata Sea accounted for 78.9% of imports followed by Nhava Sheva Sea and Tuticorin Sea, which account for 9.7% and 8.4% of imports respectively (www.zauba.com).

Genetic Diversity and its Importance

Biodiversity can be defined at genetic, species and community levels of biological organization. Even though genetic diversity is in the lowest in the hierarchy, without genetic diversity, a population cannot

Table 2. Details of different studies about the genetic diversity in flax using morphological characters

Parameters /Characters studied	Material(s) used for study	References
Dimensional Parameters such as Length, Width, Thickness, Geometric mean diameter, Surface area and Aspect ratio.	Kernels of Twelve linseed varieties.	S. Sharma and K. Prasad , 2013
Plant height (PH); Days to flowering (DF); Days to maturity (DM); Primary branches per plant (PB/P); Secondary branches per plant (SB/P); Number of bolls per plant (B/P); Number of seeds per boll (S/B); Seed yield per plant (SY/PT); Seed yield per plot (SY/PT); Thousand seed weight (TSW) Oil content (Oil%) Oil yield per plot (Oil yield).	Sixty accessions of linseed, mainly from Ethiopia.	W. Adugna, M.T. Labuschagne and C.D. Viljoen, 2006
Eighteen morphological characters in a grow-out test (GOT).	Four flax cultivars (Kartika, Deepika, Indira Alsi 32 and RLC 92).	Palli *et al*. 2014
Straw yield/plant (g); Biological yield/plant (g); Seed yield/plant (g); No. capsules/plant; technical length (cm) and plant height (cm) and between biological yield/plant (g) with each of No. capsules/ plant; length of the fruiting zone (cm); technical length (cm) and plant height (cm) and between seed yield/plant (g) with length of the fruiting zone (cm) and plant height (cm) and between No. capsules/plant with technical length (cm) and plant height (cm) and finally between both plant height (cm) and technical length (cm).	Six flax cultivars of diverse origins were grown during three successive seasons with three sowing dates in each growing season.	Ottai M.E.S *et al*. 2011
Seven quantitative traits measured on whole seeds and three quantitative traits measured on longitudinal seed section.	Six linseed genotypes from two harvested years – 2010 (5 genotypes), 2012 (5 genotypes).	Janka Nôžková *et al*. 2014
Sixteen quantitative charismas.	Twenty one parent flax genotypes and twenty F_2 hybrids.	IAA Kandil *et al*. 2012
Developed and used a set of morphological descriptors to determine levels and patterns of diversity in Ethiopian germplasm.	One hundred ninety eight Ethiopian traditional varieties.	Worku *et al*. 2014
Morphological and seed-oil characters were used to describe the phenotypic diversity.	2331 flax accessions.	Diederichsen, 2001
Investigated variation and relationships among seed colour, seed weight and seed oil content in cultivated flax (*Linum usitatissimum* L. ssp. *usitatissimum*).	2934 flax accessions from 72 countries for describe the variation of the seed characters.	Diederichsen and Raney, 2006

metamorphose and adapt to environmental changes. The genetic diversity has an impact on the higher levels of biodiversity (Templeton, 1991, 1993). Genetic biodiversity discovers its natural resources in wild species for which it is necessary to reveal out the amount of genetic variability by the way of morphological, biochemical and molecular markers. Characterization of diversity has long been based on morphological traits mainly. However, morphological variability is often restricted, characters may not be visible at all stages of the plant development, and appearance may be affected by environment (Neilsen, 1985). Nowadays, a variety of different genetic markers has been proposed to assess genetic variability as a complementary strategy to more traditional approaches in genetic resources management (Sharopova *et al.* 2002; Hirata *et al.* 2006). Understanding the molecular basis of the essential biological phenomena in plants is crucial for the effective conservation, management, and efficient utilization of plant genetic resources (PGR). In particular, sufficient knowledge of existing genetic diversity, wherein a plant population it is found and how to best utilize it, is of paramount interest for basic science and applied aspects like the efficient management of crop genetic resources. The improvement of crop genetic resources is dependent on continuous infusions of wild relatives, traditional varieties and the use of modern breeding technologies. These processes require an assessment of diversity at some level, to select resistant, highly productive varieties.

Genetic Diversity in Flax

The availability and knowledge about the extent of genetic diversity of genetic resource material play a major role in identifying parental lines and developing new varieties with desirable traits. Morphological trait-based diversity assessment has been widely used in crop plants including linseed (Diederichsen, 2001; Diederichsen and Raney, 2006; Saeidi, 2008) (Table 2); however the morphological characters are not only sensitive to environmental factors but they also require labour intensive field evaluation over extended periods of time.

Table 3. List of Molecular markers developed in flax for the genetic diversity analysis

Markers generated	Motif identified	Primer designed	Material used for study	Application of marker	References
Expressed sequence tags (ESTs)	Eighty three SSR motifs were identified.	662 primer pairs	23 flax accessions	Development of genetic and physical maps, quantitative trait loci mapping, genetic diversity studies, association mapping and fingerprinting cultivars.	Sylvie Cloutier *et al.* 2009
Putative simple sequence repeats (SSRs)	Out of 1,506 putative SSRs, 1,164 were derived from BAC-end sequences (BESs) and 342 from expressed sequence tags (ESTs). Trinucleotide = most abundant and Dinucleotide = most polymorphic.	673 (58 %) and 145 (42 %) primer pairs being polymorphic in the BESs and ESTs, respectively.	Panel of 16 flax accessions	Useful in genetic, quantitative trait loci (QTL) and association mapping as well as for anchoring the physical/genetic map with the whole genome shotgun reference sequence of flax.	Sylvie Cloutier *et al.* 2012
SSR markers	Contigs and the singlets contained 1,842 microsatellite motifs, with dinucleotide motifs as the most abundant repeat type (54%) followed by trinucleotide motifs (44%).	290 SSR markers were designed	Panel of 27 diverse linseed genotypes	Utility of next-generation sequencing technology for efficiently discovering a large number of microsatellite markers in non-model plants.	Sandip M. Kale *et al.* 2012
Simple sequence repeats (SSRs)	SSR sequences were isolated from the flax genome using a modified fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) procedure.	92 SSRs	12 flax varieties and seven related *Linum* species	Markers add to the resources available for genetic mapping and variety identification in flax.	Cory L Bickel *et al.* 2011
Microsatellite markers	38 SSR markers located across the 30 chromosomes of flax were used for fingerprinting the selected flax cultivars.	28 SSR markers	four flax cultivars (Kartika, Deepika, Indira Alsi 32 and RLC 92)	Practical utility of the SSR markers in assessing the genetic purity of the flax cultivars.	Palli *et al.* 2014

Table 4. Details of some major marker systems used for the genetic diversity analysis of flax reflecting the material studies

Marker system	Primer/Isozyme details	Material(s) used for study	References
AFLP	Sixteen primer combinations: E-ACA/M-CAC; E-ACA/M-CAG; E-ACA/M-CAT; E-ACA/M-CTA; E-AAC/M-CAA; E-AAC/M-CAG; E-AAC/M-CAT; E-AAC/M-CTT; E-AGC/M-CAC; E-AGC/M-CAG; E-AGC/M-CAT; E-AGC/M-CTA; E-AGC/M-CTC E-AGC/M-CTT; E-AAG/M-CAA; E-AGG/M-CAA	Characterized and evaluated the level of genetic diversity among some of the prominent (45) Indian genotypes of linseed.	Chandrawati *et al.* 2014
RAPD	Nine RAPD primers	Evaluated the genetic relationship among three ecotypes of flax.	*T.H.S. Abou El-Nasr and Heba A. Mahfouze*, 2013
RAPD	120 (RAPD) markers	Molecular characterization of 40 linseed varieties/genotypes.	*Ambreen Ijaz et al. 2013*
AFLP	Seven pairs of AFLP primers: M2, M3, M4, M5, M6, M7, M8	Genetic diversity of Sixty accessions of linseed.	Adugna Wakjira *et al.* 2005
Inter-Retrotransposon Amplified Polymorphism (IRAP)	10 IRAP primers: 1826, 1833, 1838, 1845, 1846, 1854, 1868, 1881, 1886, 1899	Evaluated genetic diversity among 708 accessions of cultivated flax comprising 143 landraces, 387 varieties, and 178 breeding lines.	P. Smykal *et al.* 2011
ISSR and RAPD	One ISSR primer (UBC 889) and two RAPD primers (UBC 556 and 561)	The microspore origin of anther-culture-derived 16 Flax plants.	Y. Chen *et al.* 1998
RAPD	Twenty-nine primers (UBC primers 220, 250, 301, 310, 335, 336, 337, 338, 365, 373, 388, 391, 396, 502, 526, 540, 542, 548, 556, 569, 574, 586, 711, 731, 737, 743, 775, 790, and 795)	Genetic diversity and relationships in 22 Canadian cultivars, 29 selected world cultivars and 10 landraces of flax	Yong-Bi Fu *et al.* 2002
RAPD	29 informative RAPD primers	Genetic diversity in12 flax accessions representing seven flax species in the genus *Linum*.	Yong-Bi Fu *et al.* 2002
RAPD, ISSR, REMAP, SLP (simple length polymorphism) and IRAP markers.	A set of 10 RAPD and 15 ISSR primers	Genetic diversityin 18 flax accessions.	Jana Žiarovská *et al.* 2012
ISSR	Twelve ISSR primers	Seventy Indian flax genotype.	Rajwade *et al.* 2010
RAPD		54 North American flax cultivars.	Yong *et al.* 2004
RAPD		3101 accessions of cultivated flax (*Linum usitatissimum* L. subsp. *usitatissimum*).	Diederichsen and Yong, 2006
AFLP	Six AFLP primer combinations		Everaert *et al.* 2001
ISSR	Twenty-four ISSR primer	Characterize a set of flax germplasm, along with one Turkish cultivar, one Russian cultivar, five winter and four dehiscent type accessions of cultivated flax.	Hüseyin *et al.* 2010
(EST-SSR) primer pairs	49 informative expressed sequence tag-derived simple sequence repeat (EST-SSR) primer pairs.	Assess genetic relationships of 63 *Linum* accessions representing seven typical groups of cultivated flax and its wild progenitor, pale flax (*Linum bienne* Mill.).	Yong, 2011

On the other hand DNA-based molecular markers have several advantages like abundance, environment independent early and rapid assessment and non-tissue specific characteristics. Oh *et al.* first reported the use of DNA-based markers to study flax diversity, (2000) who compared RAPD and RFLP techniques and generated a preliminary genomic map based on these marker data. Molecular characterization of flax germplasm has been made using various molecular techniques to assess genetic diversity of cultivated flax and to examine evolutionary relationships of wild flax species. High-quality seeds and elite cultivars play a crucial role in flax production.

However, since novel cultivars in general arise from hybridizations between members of an elite group of genetically alike parents, the amount of genetic instability among newly developed cultivars is likely to become even insignificant (Rahman *et al.* 2009), which makes it more tiresome to decipher ably distinguish cultivars from the others with morphological characteristics and biochemical markers because of influences by environmental factors (Table 2). Fingerprinting with molecular markers allows precise, objective and rapid cultivar identification, which has been proved to be an efficient tool for crop germplasm characterization, collection and management. Multipurpose uses with whole plant utilisation for several purposes including industrial food, animal feed, fibre, nutraceutical, pharmaceutical and bioproduct markets. Genetic diversity analysis of linseed germplasm can reveal the extent of genetic relatedness among accessions by estimating their genetic distance and is useful in the conservation of genetic resources. It is also necessary for cultivar identification and seed certification programmes.

Under the International Union for the Protection of New Varieties of Plants (UPOV, 1991), plant breeders' rights (PBR) are based on criteria of distinctiveness, uniformity and stability (DUS) of genotypes. To identify potentially novel genotypes among the flax accessions, and to assess genetic diversity for both germplasm management and core collection (Frankel and Brown, 1984) assembly, molecular markers are highly useful. A variety of marker systems, including Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP) and simple sequence repeat (SSR) been used to analyze flax germplasm (Cloutier *et al.* 2009; Diederichsen and Fu, 2006; Everaert *et al.* 2001; Fu, 2002, 2005; Fu *et al.* 2002a, b, 2003; van Treuren *et al.* 2001; Wiesnerova and Wiesner, 2004) (Table 3 and Table 4). Taken together, these studies show that cultivated flax has low genetic diversity compare to wild relatives or some other crops (Smykal *et al.* 2008a), possibly resulting from a domestication bottleneck.

The details of molecular marker developed in flax have been given in Table 3. In apparent contradiction to the lack of diversity indicated by marker studies, the flax genome shows environmentally induced yet heritable genomic changes, a phenomenon of interest for many

Table 5. List of Association mapping studies in flax

Marker system utilized	Population studied	Software used	Result	Reference
150 microsatellite loci	To assess the population structure, genetic diversity and LD of a set of 60 flax cultivars/ accessions capturing the breadth of SM (seed mucilage) variation in flax germplasm.	Structure	Collection could be useful in AM studies aimed at the discovery of genes/alleles involved in SM.	Soto-Cerda *et al.* 2013
448 microsatellite markers	407 globally distributed flax accessions	Structure	Core collection is suitable for AM studies targeting multiple agronomic and quality traits aiming at the improvement of flax as a true dual purpose crop.	Braulio J. Soto-Cerda *et al*. 2012
460 microsatellite markers	390 accessions	Structure	The candidate QTL identified herein will establish the foundation for future marker-assisted breeding in linseed.	Braulio J. Soto-Cerda *et al*. 2014
Agronomic traits	35 linseed genotypes	Structure	The combined application of the stability, AM and QQE analyses could accelerate the development of marketable linseed cultivars adapted to Southern Chile.	Braulio J. Soto-Cerda *et al*. 2014

years (Evans *et al.* 1966). Conventional breeding methods for this crop, which produce pure lines through successive Generations of inbreeding, are time-consuming. Breeding flax using haploid techniques has the potential advantages of the rapid development of completely homozygous lines within one generation and the development of efficient means of genotypic selection. Currently, anther culture is the most successful method for producing doubled haploid lines in flax (Friedt *et al.* 1995). Given such recent development of linseed cultivars and the historical significance of flax cultivation, it is surprising to learn that few studies have been made to assess the genetic diversity of flax germplasm with molecular techniques. Analyses of the extent and distribution of genetic variation within and among various flax germplasms are essential for understanding genetic relationships among accessions, and for sampling genetic resources for breeding and conservation purposes (Ayad *et al.* 1997). The details about the molecular diversity of flax have been given in Table 4.

Association Mapping Studies in Flax

Association mapping (AM) takes advantage of an extensive range of germplasm including natural populations and collections of varieties and breeding lines to map traits by linkage disequilibrium (LD), which is the non-random association of alleles at different loci (Myles *et al.* 2009). The brief description of Association mapping and QTL mapping in flax from early reviews is given in Table 5 and Table 6. The main advantage of AM is its high resolution in predicting the correlation between polymorphisms and QTL based on thousands of meiotic events accumulated during the shared history of the individuals in a population. Since *L. usitatissimum* has a long and complex domestication and different breeding history and considering its limited gene flow, it is expected that flaxseed populations exhibit complex population structures. Information on population structure has important implications because population structure is the primary source of spurious associations (Chao *et al.* 2010; Flint-Garcia *et al.* 2003). Therefore, population structure must be investigated to determine the potential for association analyses (Song *et al.* 2009). Moreover, assessing the genetic relatedness among the accessions of the targeted population is an essential prerequisite for the identification of nonredundant core collections suitable for optimizing LD estimation and association studies (Maccaferri *et al.* 2005).

Conclusion

Assessment of genetic variability is the first step in any crop improvement programme. Diversity analysis is an essential process for precise and accurate identification of the genetic relatedness of the available genetic resources. It is also required for effective choice of parents for next crossing and selection of the progenies. Flax (*Linum usitatissimum subsp. usitatissimum*) is one of the founding crops with diverse importance. Since flax, and, in particular, fibre flax, has been such an important cultivated crop, it is of great significance to conserve as

Table 6. Brief description about the QLT mapping and Linkage mapping studies in flax

Marker used	Population	Linkage group	QTL identified	Relevance	Reference
143 SSR markers	F2 population of 300 individuals generated from a cross between the susceptible cultivar NorMan and a resistant cultivar Linda	The 15 linkage group map covered 1241 cM	Three PM resistance QTL located on LG1, 7, and 9 were identified	Understanding the genetics of PM resistance in flax and map-based cloning of candidate genes underlying the QTL.	Parvaneh Asgarinia *et al.* 2013
Consensus Genetic and Physical Maps of Flax					
Marker used	**Population**	**Linkage map**	**feature**	**Relevance**	**Reference**
SSR primers	CDC Bethune/ Macbeth, E1747/Viking and SP2047/UGG5-5 containing between 385 and 469 mapped markers each.	Three linkage maps of flax (Linum usitatissimum L.)	The total length of the consensus genetic map is 1,551 cM with a mean marker density of 2.0 cM. A total of 670 markers were anchored to 204 of the 416 finger printed contigs of the physical map corresponding to *274 Mb or 74 % of the estimated flax genome size of 370 Mb	Resource for comparative genomics, genome organization, evolution studies and anchoring of the whole genome shotgun sequence.	Sylvie Cloutier *et al.* 2012

widely genetic material of flax as possible for future utilisation in breeding. To maintain and exploit these genetic resources efficiently, an understanding of the amount and distribution of genetic variation within and among accessions in a collection is required. Thus, study of the extent and distribution of genetic variation within and among various flax germplasms are essential for understanding genetic relationships among accessions, and for sampling genetic resource for breeding and conservation purposes. Further study research in this area shortly will facilitate germplasm management and enhance the utilization of germplasm in designing specific flax breeding programme.

References

Abou, El-Nasr, T.H.S. and Mahfouze, H.A. 2013. Genetic Variability of Golden Flax (*Linum usitatissimum* L.) using RAPD markers. *World Applied Sciences Journal* **26**(7)**:** 851-856.

Adugna, W., Labuschagne, M.T. and Viljoen, C.D. 2006. The use of morphological and AFLP markers in diversity analysis of linseed. *Biodiversity and Conserv.* **15:** 3193–3205.

Anonymous, 1996. Growing flax: production, management and diagnostic guide, 3rd edn. Flax Council of Canada, Winnipeg.

Anonymous, 1997. General Recommendation. *In*: Proceedings of the first workshop on Jute DUS Testing, Organized by Strengthening Seed Certification Agency Project, Seed Certification Agency, Gazipur held in Bangladesh Jute Research Institute, Dhaka, 7 September, 1997.

Asgarinia, P., Cloutier, S., Duguid, S., Rashid, K., Mirlohi, A.F., Banik, M. and Saeidi G. Mapping Quantitative Trait Loci for Powdery Mildew Resistance in Flax. (*Linum usitatissimum* L.). *Crop Science* **53**(6)**:** 2462-2472.

Ayad, W.G., Hodgkin, T., Jaradat, A. and Rao, V.R. 1997. Molecular genetics techniques for plant genetic resources. Rep. IPGRI Workshop, 9-11 October 1995, *Int. Plant Genet. Res. Inst.,* Rome, Italy.

Bennett, M.D., Leitch, I.J. 2004. Plant DNA C-values database (release 3.0).

Bickel, C.L., Gadani, S., Lukacs, M. and Cullis, C.A. 2011. SSR markers developed for genetic mapping in flax (*Linum usitatissimum* L.). *Research and Reports in Biology* **2:** 23-29.

Braulio, J. Soto-Cerda, Diederichsen, A., Ragupathy, R. and Cloutier, S. 2013. Genetic characterization of a core collection of flax (*Linumusitatissimum* L.) suitable for association mapping studies and evidence of divergent selection between fibre and linseed types. *BMC Plant Biology* **13:** 78.

Brown, A.H.D. 1989a. A case for core collections. *In*: The use of plant genetic resources (Brown AHD, Frankel OH, Marshall DR, and Williams JT, eds). Cambridge: Cambridge University press. 136-156.

Brown, A.H.D. 1989b. Core collections: a practical approach to genetic resources management. *Genome* **31:** 818-824. Clayton D., 2001.Choosing a set of haplotype tagging SNPs from a larger set of diallelic loci. ftp://ftp-gen.cimr.cam.ac.uk/software.

Bunting, A.H. 1951. Linseed. *Paint Manufacturing* **21:** 444-447.

Carter, J. F. 1993. Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. *Cereal Foods World*. **38**(10):753-759.

Chandrawati, Maurya, R., Singh, P.K., Ranade, S.A. and Yadav, H.K. 2014. Diversity Analysis in Indian Genotypes of Linseed (*Linum usitatissimum* L.) using AFLP Markers. *Gene* **549:** 171-178.

Chao, S., Dubcovsky, J., Dvorak. J., Luo, M.C., Baenziger, S.P., Mat-nyazov, R., Clark, D.R., Talbert, L.E., Anderson, J.A., Dreisi-gacker, S., Glover, K., Chen, J., Campbell, K., Bruckner, P., Rudd, J.C., Haley, S., Carver, B.F., Perry, S., Sorrells, M.E., Akhunov, E.D. 2010. Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). *BMC Genomics* **11:** 727.

Chen, Y., Kenaschuk, E. and Dribnenki, P. 1998. High frequency of plant regeneration from anther culture in flax, *Linum usitatissimum* L. *Plant Breeding* **117:** 463–467.

Cloutier, S., Miranda, E., Ward, K., Radovanovic, N., Reimer, E., Walichnowski, A., Datla, R., Rowland, G., Duguid, S., Ragupathy, R. 2012. Simple sequence repeat marker development from bacterial artificial chromosome end sequences and expressed sequence tags of flax (*Linum usitatissimum* L.). *TheorAppl Genet* **125**(4)**:** 685–694.

Cloutier, S., Niu, Z., Datla, R., Duguid, S. 2009. Development and analysis of EST-SSRs for flax (*Linum usitatissimum* L.). *TheorAppl Genet* **119:** 53–63.

Dewilde, B., 1983. 20 eeuwen vlas in Vlaanderen: 439 pp.

Diederichsen, A. 2001. Comparison of genetic diversity of flax (*Linum usitatissimum* L.) between Canadian cultivars and a world collection. *Plant Breeding* **120**(4): 360-362.

Diederichsen, A. and Raney, J. P. 2006. Seed colour, seed weight and seed oil content in *Linum usitatissimum* accessions held by Plant Gene

Resources of Canada. *Plant Breeding* **125** (4)**:** 372-377.

Diederichsen, A. and Fu, Y.B. 2006. Phenotypic and molecular (RAPD) differentiation of four infraspecific groups of cultivated flax (*Linum usitatissimum* L. subp. *usitatissimum*). *Genet Resour Crop Evol* **53**(1)**:** 77–90.

Evans, G.M., Durrant, A. and Rees, H. 1966. Associated nuclear changes in induction of flax genotrophs. *Nature* **212:** 697–699.

Everaert, I., De Riek, J., De Loose, M., Van Waes, J. and Van Bockstaele, E. 2001. Most similar variety grouping for distinctness evaluation of flax and linseed (*Linum usitatissimum* L.) varieties by means of AFLP and morphological data. *Plant Var Seed* **14:** 69–87.

FAOSTAT: http://www.faostat.org.

Federov, A.A. 1974. Chromosome numbers of flowering plants. George Allen and Unwin ltd. London. pp.519.

Flint-Garcia, S., Thornsberry, J.M., Bukler, E.S., 2003. Structure of linkage disequilibrium in plants. *Annu Rev Plant Biol* **54:** 357–374.

Frankel, O.H., 1984. Genetic perspectives of germplasm conservation. *In*: Genetic manipulation: impact on man and society (Arber W, Llimensee K, Peacock WJ, and Starlinger P, eds) Cambridge: Cambridge University Press. 161-170.

Frankel, O.H., and Brown, A.H.D., 1984. Plant genetic resources today: a critical appraisal. *In*: Crop genetic resources: conservation and evaluation (Holden JHW and Williams JT, eds). London: George Allen and Unwin. 249-257.

Freeman, T. P. 1995. *Structure of flaxseed, In*: Flaxseed in human nutrition, Champaign, AOCS Press, pp. 11-21.

Friedt, W., Bickert, C., Schaub, H. 1995. In vitro breeding of high-linolenic, doubled-haploid lines of linseed (Linumusitatissimum L.) via androgenesis. *Plant Breed* **44:** 322%326.

Fu, Y.B. 2005. Geographic patterns of RAPD variation in cultivated flax. *Crop Science* **45:** 1084–1091.

Fu, Y.B. 2005. Geographic patterns of RAPD variation in cultivated flax. *Crop Science* **45**(3)**:** 1084–1091.

Fu, Y.B. 2011. Genetic evidence for early flax domestication with capsular dehiscence. *Genet Resour Crop Evol* **58**(8):1119–1128.

Fu, Y.B., Diederichsen, A., Richards, K.W. and Peterson, G. 2002. Genetic diversity within a range of cultivars and landraces of flax (*Linumusitatissimum* L) as revealed by RAPDs. *Genet Resour Crop Evol* **49**(2)**:** 167–174.

Fu, Y.B., Rowland, G.G., Duguid, S.D., Richards, K.W. 2003. RAPD analysis of 54 North American flax cultivars. *Crop Sci* **43**(4)**:** 1510–1515.

Gill, K.S., 1987. Linseed. Indian Council of Agricultural Research, New Delhi, India. pp. 386.

Hirata, M., Cai, H., Inoue, M., Yuyama, N., Miura, Y., Komtasu, T., Takamizo T., Fujimori M. 2006. Development of simple sequence repeat (SSR) markers and construction of an SSR-based linkage map in Italian ryegrass (*Loliummultiforum* Lam.). *Theoretical and Applied Genetics* **113**: 270–279.

https://www.**zauba**.com/import-yu-hs-code.html

Ijaz, A., Shahbaz, A., Ullah, I., Ali, S., Shaheen, T., Rehman, M., Ijaz, U. and Samiullah 2013. Molecular Characterization of Linseed Germplasm using RAPD DNA Fingerprinting Markers. *American- Eurasian J. Agric. and Environ. Sci.* **13**(9)**:** 1266-1274.

Jhala, A.J. and Hall, L.M. 2010. Flax (*Linum usitatissimum* L.) : Current Uses and Future Applications. *Australian Journal of Basic and Applied sciences* **4**(9)**:** 4304-4312.

Kale, S.M., Pardeshi, V.C., Kadoo, N.Y., Ghorpade, P.B., Jana, M.M., and Gupta, V.S. 2012. Development of Genomic Simple Sequence Repeat Markers for Linseed using Next Generation Sequencing Technology. *Molecular Breeding* **30:** 597-606.

Kandil, A.A., Shareif, A.E., Abo-Zaied, T.A. and Moussa, A.G.T 2012. Multivariate Analysis of some Economic Characters in Flax. *Pakistan Journal of Biological Sciences* **15**(2)**:** 85-91.

Kvavadze, Dordrecht, E. 2009. *Science.* **325:** 1359.

Linnaeus, C., 1857. Species Plantarum. The Royal Society of London, London, UK. p. 300.

Maccaferri, M., Sanguineti, M.C., Noli, E., Tuberosa, R. 2005. Population structure and long-range linkage disequilibriumin durum wheat elite collection. *Mol Breed* **15:** 271–289.

Mardi, M. 2007. *Ann. Appl. Biol.* **152:** 81-91.

Millam, S., Bohus, O. and Anna, P. 2005. Plant cell and biotechnology studies in *Linum usitatissimum*-A review. *Plant Cell Tissue Organ Cult.* **82:** 93-103.

Muravenko, O.V., Amosova, A.V., Samatadze, T.E., Popov, K.V., Poletaev, A.I. and Zelenin, A.V. 2003. 9-aminoacridin- an efficient reagent to improve human and plant chromosome banding patterns and to standardize chromosome image analysis. *Cytometry A.* **51:** 52–57.

Murre, M.V. 1955. Meppel: Uitgeverij Ceres. 112.

Myles, S., Peiffer, J., Brown, P.J., Ersoz, E.S., Zhang, Z., Costich, D.E., Bukler, E.S. 2009. Association

mapping: critical 48 Euphytica. 2014. **196:** 35–49. 123 Author's personal copy considerations shift from genotyping to experimental design. *Plant Cell* **21:** 2194–2202.

Nielsen, G. 1985. The use of isozymes as probes to identify and label plant varieties and cultivars. *In*: M.C. Rattazzi, Scandalios, J.G. and Whitt, G.S. (Eds), Isozymes: Current Topics In Biological and Medical Research. **12:** 1-32. Alan R. Liss, New York.

Nô ková, J., Remeselníková, K., and Bjelková, M. 2014. Characterization and evaluation of flax seeds (*Linum usitatissimum* L.) on selected genotypes. *Journal of Central European Agriculture.* **15**(1)**:** 193-207.

Oh, T.J., Gorman, M. and Cullis, C.A. 2000. RFLP and RAPD mapping in flax (L. *usitatissimum*). *TheorAppl Genet* **101:** 590–593.

Ottai, M.E.S., Al-Kordy, M.A.A. and Afiah, S.A. 2011. Evaluation, Correlation and Path Coefficient Analysis among Seed Yield and Its Attributes of Oil Flax (*Linum usitatissimum* L.) Genotypes. *Australian Journal of Basic and Applied Sciences*. **5**(11)**:** 252-258.

Palli, V., Verma, S.K., Xalxo,M.S., Saxena, R.R., Mehta, N. and Verulkar, S.B. 2014. Identification of microsatellite markers for fingerprinting popular Indian flax (*Linum usitatissimum* L.) cultivars and their utilization in seed genetic purity assessments. *AJCS* **8**(1)**:** 119-126.

Rahman, M.S., Molla, M.R., Alam, M.S. and Rahman, L. 2009. DNA fingerprinting of rice (*Oryzasativa* L.) cultivars using microsatellite markers. *Aust J Crop Sci* **3**(3)**:** 122-128.

Rajwade, A.V., Arora, R.S., Kadoo, N.Y., Harsulkar, A.M., Ghorpade, P.B., Gupta, V.S. 2010. Relatedness of Indian flax genotypes (*Linumusitatissimum* L.): an inter-simple sequence repeat (ISSR) primer assay. *MolBiotechnol.* **45**(2)**:** 161–170.

Rowland, G.G., 1991. An EMS-induced low-linolenic acid mutant in McGregor flax (*Linum usitatissimum* L.). *Can J. Plant Sci.* **71:** 393-396.

Rowland, G.G., 1998. Growing Flax : Production , Management and Diagnostic Guide. Flax Council of Canada and Saskatchewan Flax Development commission 56.

Rowland, G.G., McHughen, A., Bhatty, R.S., Mackenzie, S.L. and Taylor, D.C. 1995. The application of chemical mutagenesis and biotechnology to the modification of linseed (*Linum usitatissimum* L.). *Euphytica* **85**(1-3)**:** 317-321.

Saeidi, G. 2008. Genetic variation and heritability for germination, seed vigour and field emergence in brown and yellow-seeded genotypes of flax. *Int. J. Plant Prod.* **2:** 15–22.

Sharma, S. and Prasad, K. 2013. Comparative Physical Characteristics of Linseed (*Linum usitatissimum* L.) Kernels. *International Journal of Agriculture and Food Science Technology* **4**(7)**:** 671-678.

Sharopova, N., McMullen, M.D., Schultz, L., Schroeder, S., Sanchez-Villeda, H., Gardiner, J., Bergstrom, D., Houchins, K., Melia-Hancock, S., Musket, T., Duru, N., Polacco, M., Edwards, K., Ruff, T., Register, J.C., Brouwer, C., Thompson, R., Velasco, R., Chin, E., Lee,M., Woodman-Clikemen, W., Jane Long, M., Liscum, E., Cone, K., Davis, G., Coe, E.H. Jr. 2002. Development and mapping of SSR markers for maize. *Plant Molecular Biology* **48:** 463–481.

Smy´kal, P., Bac¡ova´-Kerteszova,´ N., Kalendar, R., Corander, J., Schulman, A.H., Pavelek, M. 2011. Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotrasnsposon-based markers. *TheorAppl Genet* **122:** 1385–1397.

Song, B.H., Winsor, A.J., Schmid, K.J., Ramos-Onsins, S., Schranz, M.E., Heidel, A.J. and Mitchell-Olds, T. 2009. Multilocus patterns of nucleotide diversity, population structure and linkage disequilibrium in Boecherastricta, a wild relative of Arabidopsis. *Genetics* **181:**1021–1033.

Soto-Cerda, B.J., Maureira-Butler, I., Muñoz, G, Rupayan, A., Cloutier, S. 2012. SSR-based population structure, molecular diversity and linkage disequilibrium analysis of a collection of flax (Linumusitatissimum L.) varying for mucilage seed-coat content. *Mol Breed* **30**(2)**:** 875–888.

Templeton, A.R., 1991. Genetics and conservation biology. *In*: Seitz, A. and Loeschche V. (Ed.), Species Conservation a Population biological Approach. Basel, Birkhauser Verlag. 15 29.

Templeton, A.R., 1993. Translocation as conservation tool. *In*: Szaro R. (Ed.), Biodiversity in mangrove landscapes, theory and practice. Oxford University Press.

Tutin, T.G., Heywood, V.H., Burges, N.A., Murre, D.M., Valentine, D.H., Walters, S.M. and Webb, D.M. (ed.). 1968. Flora Europaea: Rosaceae to Umbelliferae. Cambridge Univ. Press, Cambridge. UK.

Uysal, H., Fu, Y.B., Kurt, O., Peterson, G.W., Diederichsen, A. and Kusters, P. 2010. Genetic diversity of cultivated flax (*Linum usitatissimum* L.) and its wild progenitor pale flax (*Linum bienne* Mill.) as revealed by ISSR markers. *Genet Resour Crop Evol.* **57:** 1109-1119.

Van Treuren, R., van Soest, L.J.M. and van Hintum, T.J.L. 2001. Marker assisted rationalisation of genetic

resource collections: a case study in flax using AFLPs. *TheorAppl Genet.* **103:** 144-152.

Wakjira, A., Viljoen, C.D. and Labuschagne, M.T. 2005. Analysis of genetic diversity in linseed using AFLP markers. *Ethiop. J. Sci.* **28**(1)**:** 41–50.

Whitkus, R., Doebley, J. and Wendel, J. F. 1994. Nuclear DNA reassessment. *Am. Nat*. **140:** 725–742. Markers in systematics and evolution. pp. 116–141 *In:* DNA-Based Ott, J., 1991 Analysis of Human Genetic Linkage. The John Hopkins Markers in Plants, edited by Phillips, R.L. and Vasil, I.K. Kluwer University Press, Baltimore. Academic Publishers.

Wiesnerová, D. and Wiesner, I. 2004. ISSR-based clustering of cultivated flax germplasm is statistically correlated to thousand seed mass. *MolBiotechnol* **26**(3)**:** 207–213.

Williams, I.H. 1988. The pollination of linseed and flax. *Bee World* **69:** 145–152.

Worku, N., Heslop-Harrison, J.S. and Adugna, W. 2014. Diversity in 198 Ethiopian linseed (*Linum usitatissimum*) accessions based on morphological characterization and seed oil characteristics *Genet Resour Crop Evol.*

Yong-Bi Fu. 2011. Genetic evidence for early flax domestication with capsular dehiscence. *Genet Resour Crop Evol*. **58:** 1119–1128.

Zeven, A.C. and De Wet, J.M.J. 1975. Dictionary of cultivated plant and their regions of diversity. Wageningen: Pudoc, Centre for agricultural publishing and documentation. Pp 263.

Ziarovska, J., Razna, K., Senkova, S., Stefunova, V. and Bezo, M. 2012 Variabilty of *Linum usitatissimum* L. based on Molecular Markers. *ARPN Journal of Agricultural and Biological Sciences* **7**(1)**:** 50-58.

Zohary, D. and Hopf, M. 1993. Domestication of Plants in the Old World. The Origin and Spread of Cultivated plants in West Asia, Europe, and the Nile Valley. 2nd Edition. Oxford Science Publications, Clarendon Press, Oxford. ISBN.

Zohary, D. and Hopf, M. 2000. Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Oxford University Press, Oxford.