



Linseed (*Linum usitatissimum* L.) genetic resources for climate change intervention and its future breeding

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Received: September 12, 2016; Revised received: February 3, 2017; Accepted: May 15, 2017

Abstract: Linseed or flax (*Linum usitatissimum* L.), a multiple purpose crop valued for its seed oil, fibre, probiotic and nutraceutical properties, is adapted to different environments and agro-ecologies. Modern breeding techniques using only limited number of selected varieties have resulted in a loss of specific alleles and thus, reduction in total genetic diversity relevant to climate-smart agriculture. However, well-curated collections of landraces, wild linseed accessions and other *Linum* species exist in the gene banks and are important sources of new alleles. This review is primarily focused on the studies of genetic diversity of linseed species and evaluation related to tolerance to abiotic and biotic stress factors that could be useful for improving linseed through future promising breeding programs in addition to briefly discussing different morphotypes and nutraceutical importance. Wide diversity in linseed germplasm indicates a considerable potential for improving this crop for both agronomic and quality traits required for developing climate-resilience tailored to specific environments. Recent release of the flax genome sequence coupled with wide range of genomic and analytical tools in public domain has furthered understanding of molecular mechanisms for detailed study of the genes underlying flax adaptation to stress and diversity in commercially important accessions. Important climate related traits and their constituent genes are presented and key developments for the future highlighted emphasizing the urgent need to increase the use of genetically diverse germplasm to meet the emerging challenges in agricultural production and to conserve valuable genetic resources for the future.

Keywords: Climate change, Genebank, Genetic resources, Germplasm characterization, Linseed

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is an important crop produced for natural textile fibre (linen) or oil for industrial application as well as culinary purpose. Recently the market has evolved around linseed as a functional food laden with health promoting and nutraceutical properties further highlighting its importance and increased demand. The total world production of linseed reached approximately 2.56 million tons in the year 2014, with Canada (34 %), the Russian Federation (15 %), and China (13 %) being the main producers (FAOSTAT, 2016). India is the sixth largest producer in the world with contribution to global linseed area and production 13 % and 5.5 %, respectively. However, the average national productivity (403 kg/ha) is far below the world average (851 kg/ha) because of its cultivation in marginal and sub-marginal areas, rain-fed soils, sensitivity to fungal diseases, lack of seed of improved varieties, meager or no application of fertilizers and it's sowing in *utera* cultivation by resource poor farmers.

In near future, climate change may put strong pressure on breeding community to develop cultivars which can adapt to changing eco-climatic conditions. Access to

genetic diversity is essential and prerequisite for improvement as well as adaptation of any crop to changing environment or market needs and the crucial role of germplasm in this regard has been well recognized. Gene bank collections are sources of diverse germplasm collections such as landraces, wild relatives, commercial, obsolete and elite varieties, pure and breeding lines, mutants, polyploids, and hybrids. In world germplasm collections, there are 46,513 linseed/flax accessions reported (with perhaps 10,000–15,000 unique accessions), of which *L. bienne* (the wild progenitor of cultivated flax) is rarely represented (279 accessions only) in genebanks (Diederichsen, 2007). The National Bureau of Plant Genetic Resources (NBPGR), India is involved in collection, evaluation, conservation, documentation and scientific studies to identify donor germplasm for different traits, has ≈2900 linseed accessions under its long term storage facility. Three main germplasm resources available to plant breeders are commercial varieties, landraces (primitive cultivars), and a range of wild relatives and weedy races. Linseed germplasm is also represented by cultivars, landraces, wild relatives and other wild ancestral species which breeders can exploit to improve cultivars for future climatic adaptations (Heslop-

Harrison and Schwarzacher, 2012; Diederichsen and Fu, 2008). Early linseed breeding and comprehensive agro-botanic analyses of variation (Vavilov, 1951; Dillman, 1953) revealed the importance of germplasm collections in 19th century. More information on germplasm diversity has been generated over time by studies of Vavilovian collections (Kutuzova, 1998). Further, the use of landraces for fibre flax breeding was described by Zhuchenko and Rozhmina (2000). Such studies have proven to be useful tools for efficiently preserving and using flax germplasm collections (Diederichsen, 2007; Diederichsen and Fu, 2008; Diederichsen *et al.*, 2013). These primary evaluations of flax germplasm collections were followed by numerous secondary evaluations for different characters related to tolerance to biotic and abiotic stress factors (Brutch, 2002; Zhuchenko and Rozhmina, 2000) with recent focus of germplasm screening on monogenic traits, such as disease resistances (Rashid, 2003). Thus, wide diversity available in linseed germplasm collections coupled with characterization and evaluation data is a potential source worth utilization in breeding for improved traits.

History of domestication and morphotypes: Linseed is an annual, self-pollinated and only cultivated species under the genus *Linum* which is believed to be originated in either the Middle East or Indian regions and spread throughout Asia and Europe, and later into the New World (Green *et al.*, 2008; Soto-Cerda *et al.*, 2013). The domestication of flax was also observed on the Indian subcontinent near the Mediterranean Sea and this region is known to have high biological diversity of genus *Linum* (Allaby *et al.*, 2005; Fu, 2011). Cultivated species (*Linum usitatissimum*) arose from a single domestication event from *L. bienne*, involving selection for annual habit, non-shattering of capsules and more efficient self-fertilization (Fu, 2011). However, there are also other species in the genus *Linum* which are of vital and emerging interest to linseed researchers, although they belong to the secondary, or even the tertiary, genepool (Kutuzova, 1998). Some of these species are ornamental and their utilization may increase in future *e.g.* blue and occasional white flowers are frequent in the different species of the *L. perenne* and *L. narbonense*; yellow flowers characterizing the *L. flavum* group and red flowered *L. grandiflorum* are often used as ornamental plants. Divergent selection applied over thousands of years has resulted in fiber (taller and less branched) and linseed types (shorter, more branched, larger seeded) which are the same species but differ considerably in morphology, anatomy, physiology and agronomic performance (Diederichsen and Ulrich, 2009). Fiber flax is currently grown in the cool-temperate regions of China, the Russian Federation and Western Europe while linseed cultivars are grown in Canada, India, China, the United States and Argentina (Green *et al.*, 2008).

Currently the oil-type is fifth largest oil crop and the fibre type is the third largest textile fibre crop in the world (Ottai *et al.*, 2011), although with a markable lower production than the major oil crops (Brassica, soybean and palm) or other fibres (cotton and jute).

A multipotential crop: Linseed provides raw materials for seed oil, stem fibre, paper, wax, nutraceutical and food processing industries. The flax fibre is a valuable raw material for linen clothing, the straw and short fibre for thread, rope and other packaging materials; cigarette paper, currency notes (Cullis, 2011), while the seed oil has been used in production of paints, inks, varnish and quality linoleum flooring because of its unique drying properties (Kurt and Bozkurt, 2006). In addition, linseed varieties with oils suitable for culinary use are also available (Hosseinian *et al.*, 2004). Now a days, these two basic types of uses are supplemented by using of common flax as a functional food (Bassett *et al.*, 2009). Consumption of ground seeds adds nutritional benefits because flax seeds are also a rich source of lignans, compounds that have anticancer properties (Westcott and Muir, 2003). Its oil, characterized by a high concentration of omega \square 3 alpha linolenic acid (55-57 %) is used in anti-hypercholesterolemic drugs for cardiovascular diseases (Simopoulos, 2000). As a rich source of soluble fibre, linseed mucilage constitutes an excellent alternative to inulin (polysaccharide with prebiotic effects isolated from chicory root) exclusively required for the food processing industry (Diederichsen *et al.*, 2006). Recently, there has been a growing interest in the probiotic properties of linseed and its beneficial effects on coronary heart disease, neurological and hormonal disorders (Huang and Ziboh, 2001; Simopoulos, 2002), colon tumor (Dwivedi *et al.*, 2005), breast cancer (Chen *et al.*, 2006) and atherosclerosis (Wang *et al.*, 2005). Keeping in view of increasing demand of linseed due to numerous health benefits, there is consistent need to increase genetic seed yield potential as well as its development into a truly multi-purpose crop.

Value of germplasm for important genes and underlying traits under climate change: Evaluations of flax germplasm collections for disease resistance (Kutuzova, 1998; Zhuchenko and Rozhmina, 2000; Rashid, 2003; Singh, 2004) or adaptation to dry and warm regions (Diederichsen *et al.*, 2006b) deliver information facilitating the use of germplasm collections. Limited success was reported in initial attempts to grow flax in saline-alkaline soil to avoid competition for land with other food crops. Using different strategies, flax breeders have made great efforts to develop a salt tolerant flax cultivar (McHughen, 1987; El-Beltagi, 2008). One of the main factors limiting yield of flax is physiological drought in many regions of the world (Gupta, 2007). This situation, predicted to be the result of global climatic changes, significantly

Table 1. Recent advances detailing important genes and underlying traits in linseed.

Trait	Description of upregulated/down regulated genes	References
Drought tolerance	LEA genes coding for late embryogenesis abundant protein LEA5, dehydrin, brassinosteroid-regulated protein BRU1 precursor, calmodulin-binding heat-shock protein genes, genes encoding cytochrome P450 family proteins, AP2/ERF domain containing transcription factor, brassinosteroid insensitive 1-associated receptor kinase 1, and histone h2b	Dash <i>et al.</i> , 2014
Fibre quality	Cell wall related genes (cellulose synthase catalytic subunit c1532) involved in cellulose biosynthesis, genes involved in secondary cell wall hemicellulose synthesis, phenylpropanoid/lignin biosynthesis, gene (xyloglucan endotransglycosylase c5939) involved in cell wall expansion, sucrose partitioning and gene (fasciclin-like AGP 2 - c51183) that has previously been associated with both flax fibre formation and G-fibre formation in tree reaction wood	Roach and Deyholos, 2007; 2008; Fenart <i>et al.</i> , 2010; Gorshkova <i>et al.</i> , 2010
Saline and alkaline stress	miRNA targeted genes (Lus-miRNAs) Myb domain protein, Mitochondrial transcription termination factor family protein, Transcription regulators, Auxin signaling F-box,	Yu <i>et al.</i> , 2016; Yu <i>et al.</i> , 2014; Barvkar <i>et al.</i> , 2013; Younas and Barozai, 2012
Nutrient use efficiency	miRNA targeted genes ubiquitin-conjugating enzyme E2 (<i>UBE2</i>) gene	Melnikova <i>et al.</i> , 2014, 2015; Neutelings <i>et al.</i> , 2012
Pathogen resistance	Genes encoding cysteine proteases known to play an important role in programmed cell death (PCD) associated with the hypersensitive response (HR), genes (c7149 and c48400) encoding germin-like proteins potentially associated with ROS production and cell-wall cross-linking in defense, gene (Nbi-D c37826) involved in flax resistance to rust (<i>Melampsora lini</i>)	Fenart <i>et al.</i> , 2010; Lorenc-Kukula <i>et al.</i> , 2007
Transgenic flax for agronomic and quality traits	Genes encoding chalcone synthase, chalcone isomerase and dihydroflavonol reductase; SsGT1 gene, higher resistance to <i>Fusarium</i> infection and significant increase of the flavonoid glycoside content; high oleic flax through RNAi-mediated multiple FAD2 gene silencing	Lorenc-Kukula <i>et al.</i> , 2005; 2009; Chen <i>et al.</i> , 2015; Ludviková <i>et al.</i> , 2015; Soto-Cerda <i>et al.</i> , 2014

affects India where flax is extensively cultivated in rain-fed conditions in central India. Despite recent advances in research on drought stress in a number of important crops such as wheat, rice and mustard, related progress in linseed is very limited, except few studies such as genome-wide analysis of drought induced gene expression (Dash *et al.*, 2014) addressing the response of flax to drought at the molecular level. A major effect of climate change will be to alter the intensity as well as incidence of linseed diseases. There are germplasm sources with tolerance/resistance to biotic factors that could be incorporated into the cultivars through developmental breeding or germplasm enhancement. For the same purpose the genetics of several disease resistance traits have been investigated in linseed, and associated genes have been assigned (Keijzer and Metz, 1992; Kutuzova, 1998). Wild germplasm-pathogen systems have generally been ignored by plant pathologists; only a limited number of wild germplasm-pathogen systems (wild populations of *Linum marginale* and flax rust (*Melampsora lini*) have been extensively studied (Burdon and Leather, 1990). Considerable variability for resistance has been recognized in coevolved systems of wild germplasm and their pathogens. Knowledge of diseases and pathogens of wild germplasm allows construction of maps of geographic patterns of disease resistance that can facilitate more effective collection of resistant germplasm. Areas with a rich diversity of pathogens and a corresponding diversity of resistant plants ("pathogen parks") are excellent sites for in situ studies

of diseases and pathogens of wild crop relatives. Analysis of agro-morphological diversity and oil content in Indian linseed germplasm resulted in identification of promising donors for oil content, early flowering and early maturity (Dikshit and Sivaraj, 2015). Genetic diversity study in indigenous and exotic linseed germplasm (Abdul Nizar and Mulani, 2015) led to identification of exotic as well as indigenous trait specific promising accessions for taller stalk, high seed yield, high oil content, more capsules per plant and earliness. However, despite great potential for the unknown future threats and wide recognition, the use of germplasm in breeding programs is still limited due to breeder's reluctance to use germplasm largely due to non-availability of reliable information economically important traits and fear of linkage drag (Upadhyaya *et al.*, 2011). Therefore, to enhance utilization of germplasm, core collection and pre-breeding concepts were introduced recently. The main purpose of the core fraction a subset of accessions (5–10% of the base collection) is to provide efficient access to the whole collection with minimum repetitiveness and widest range of variability useful for breeders or other users. Pre-breeding is the early phase of breeding utilizing germplasm. The end product of pre-breeding is although deficient in certain desirable characters but useful to plant breeders due to greater potential for direct utilization in a breeding programme than the original unadapted exotic sources. Prior to the conception of the core collection concept, a "genetic collection" of flax was established during the 1970s at the N.I. Vavi-

lov Institute in St. Petersburg, which includes about 250 lines with genetically defined morphological, agronomic or disease-resistance characters (Brutch, 2002). A catalogue listing 50 accessions as donors for specific genes controlling traits, such as resistance to rust, wilt, anthracnose, and pasmo, fibre content and quality, lodging resistance, earliness, seed oil content, seed size and capsule indehiscence, was published by the Vavilov Institute (Kutuzova, 2000). The Centre for Genetic Resources at Wageningen in The Netherlands has established a flax core collection of 84 accessions from 506 fibre flax accessions (van Soest and Bas, 2002). This core subset was used in evaluations for fibre characters (Booth *et al.*, 2004). A core collection comprising of 381 accessions from the flax world collection of 3378 accessions maintained by Plant Gene Resources of Canada (PGRC) was also assembled recently (Diederichsen *et al.*, 2013).

Linseed tailored to specific environments through the development of genomic tools: Anthropogenic climate changes *viz.* rising global temperature, scarce water availability, increased salinity, as well as changes in disease and pest prevalence pose a serious threat to food security due to direct impacts on crop production. Ever rising global population, fast diminishing natural resources coupled with declining soil fertility further complicate these already complex issues. Western Canada and parts of Russia are important linseed or fibre flax production regions, and, during the past century, average temperatures have increased, annual precipitation became less evenly distributed with a trend to more heavy precipitation events in these areas (Gitay *et al.*, 2002). As germplasm (landraces and wild material in the wider *Linum* gene pool) is reservoir of beneficial alleles required for crop improvement, efficient quantification and utilization of these resources are underway through the continued development and implementation of strategies, for instance more powerful genetic tools and genomic information. In recent years, different molecular markers have been used to measure genetic variation and relationships in cultivars and landraces of linseed. The molecular characterization of *Linum* by Fu (2005) employed random amplified polymorphic DNA (RAPD) to examine a flax world collection of 2,727 germplasm accessions, while Cloutier and co-workers (2009, 2011, and 2012) employed simple sequence repeats (SSR) for mapping of QTLs underlying fatty acid composition traits. Fortunately, detailed study of diversity and further understanding of transcriptional level molecular mechanisms of flax adaptation to stress (Table 1) has been greatly accelerated by recent release of the flax genome sequence (Wang *et al.*, 2012). Genomic regions underlying agronomic traits in linseed (*Linum usitatissimum* L.) were revealed very recently in 407 globally distributed flax accessions by association mapping using 448 microsatellite markers (Soto-Cerda *et al.*,

2013; 2014). Digital gene expression for the identification of several differentially expressed genes and saline-alkaline tolerant miRNAs in flax (Lus-miRNAs) using over 2.7 million unique sRNAs mapped to the flax genome were reported for the first time (Yu *et al.*, 2014; 2016). Dash *et al.* (2014) conducted microarray analysis to study transcriptome associated with induced drought in flax and identified 183 differentially expressed genes (DeGs). Using a combination of molecular techniques such as high-throughput genome-wide molecular markers and next-generation sequencing technology instrumentation, mobilizing and tracking putatively advantageous target alleles, and mutant stocks to facilitate proof of gene function or phenotypic response are powerful tools (Ragupathy *et al.*, 2011). For the identification of mutant alleles, approaches such as targeting induced local lesions in genomes (TILLING; Colbert *et al.*, 2001) can be highly informative and exceptionally promising emerging alternatives. Sequence and epigenetic variation across the majority of the gene complement of linseed can thereby be revealed, catalogued and made accessible to the breeding community.

Looking to the future: Plant breeders are continuously challenged to develop flax cultivars adapted to changing environments and market needs which is further stimulated due to importance of the health-related properties of flax in human and animal nutrition. To ensure long term sustainability of flax production, the diverse germplasm stored in genebanks is essential for search of new traits relevant to changing environmental and socio-economic needs, as on-farm diversity has already mostly disappeared. Concerted breeding efforts involving distant and exotic germplasm, collected from main centers of diversity are required in view of narrow genetic base of the modern varieties. On the other hand, the enormous merits of the landraces coupled with the threat of genetic erosion indicate the importance of landraces and to plan future work of major priority. The potential contributions of *L. bienne* as wild progenitor of cultivated flax, presently rare in genebanks deserves more attention as a genetic resource for flax breeding. The International Treaty on Plant Genetic Resources for Food and Agriculture did not cover the genus *Linum* (Fowler, 2004), thus, it is important to ensure that countries conserving *Linum* diversity be made aware of the present status of conserved germplasm and coordination of future conservation activities. Intense pre-breeding and priority in evaluation to major genes essentially unavailable in the cultivated gene pool for development of short duration and heat tolerant high yielding varieties of linseed particularly for paddy fallows, and for *utera* cultivation carry immense importance. Effective trait mining should be focused using statistical approaches like focused identification of germplasm strategy (FIGS) combining environmental

and geographical data with trait information. Furthermore, the international initiative DIVSEEK (2016) established to unlock the potential of crop diversity stored in genebanks worldwide and make it available to all to promote effective utilization of genetic variation in plant improvement, enhanced agricultural productivity, and sustainability ensures intelligent use of genetic diversity across the globe.

Conclusion

Linseed is predominantly grown under rain-fed (63 %), *utera* (25 %) and input starved conditions in India, due to which the increase in yields of this crop has not been so considerable. Till date the major research work carried out by linseed breeders lies in the area of varietal development mainly for yield and disease resistance for different agro-ecological regions of the country. Since there is no major study on trait specific evaluation and utilization of linseed germplasm in Indian context, present review is intended to emphasize the importance of unlocking the potential of these genetic resources through evaluation of genebank material. Measurements of agro-morphological variation will be helpful in the selection of suitable parents for breeding programs, while knowing the population structure of crop genotypes from morphological and DNA markers will be helpful for association studies through linkage disequilibrium in populations for identifying particular alleles associated with a given phenotype. Genetic enhancement for seed yield, early duration for *utera* cultivation to avoid moisture constraint, quality traits assume importance for rain-fed linseed agriculture from the consideration of growth, equity and sustainability.

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