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Biodiversity in Ethiopian linseed (*Linum usitatissimum* L.): molecular characterization of landraces and some wild species

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Abstract

Molecular characterization of germplasm is important for sustainable exploitation of crops. DNA diversity was measured using inter-retrotransposon-amplified-polymorphism (IRAP) and inter-simple-sequence-repeat (ISSR) markers in 203 Ethiopian landraces and reference varieties of linseed (flax, *Linum usitatissimum*) and wild *Linum* species. Molecular diversity was high (PIC, 0.16; GD, 0.19) compared to other reports from the species. Genotyping separated reference from landrace accessions, and clustered landrace accessions from different altitudes and geographical regions. Collections showed evidence for recent introduction of varieties in some regions. The phylogeny supported *L. bienne* Mill. as the progenitor of domesticated *L. usitatissimum*. Markers developed here will be useful for genetic mapping and selection of breeding lines. The results show the range of characters that can be exploited in breeding lines appropriate for smallholder and commercial farmers in Ethiopia, producing a sustainable, secure, high-value crop meeting agricultural, economic and cultural needs.

Key words/phrases: Biodiversity, Ethiopia, linseed, *Linum usitatissimum,* landraces, *L. bienne*, altitude, region, diversity

Introduction

Knowledge about the extent and distribution of genetic diversity in crop plants is important for any breeding program (Rahman et al. 2016; Khan et al. 2007). *Linum usitatissimum* L. is a multipurpose crop grown in many environments for fibre, food, industrial, feed and potentially pharmaceutical uses. The bast or phloem fibres have outstanding mechanical properties (Fernández 2016) with strength and flexibility. Fibre orientation and ratio of fibre length-to-diameter are important characters (Sliseris et al. 2016). Linseed oil has high α-linolenic acid (an omega-3 fatty acid) content (Schardt 2005) and seeds are used whole or crushed in foods. The hardening oil is used for varnishes, linoleum and putty and for leather preparation. It is in local medicines as demulcent, emollient and laxative, and is taken orally in bronchial infection and diarrhoea (Gul et al. 2016). Cellulosic and lignified material remaining after extraction of fibre or oil is used for manufacture of paper and straw board, and for animal feed or bedding. As a food, linseed is eaten with *wot* (a stew) particularly in the north of Ethiopia, and used to make a beverage of fasting days. Linseed is one of the oldest cultivated plants and was an important commercial crop before the invention of petroleum and extensive use of cotton (McHughen, 1990). Because of the diverse uses and sustainability, there is renewed interest in its cultivation. Additional information on the genetic basis of variation is required to enable modern breeding exploiting the biodiversity in the species and its wild relatives (McKenzie et al. 2008; Kurt and Evans 1998). In Ethiopia, a Vavilovian centre of crop diversity (Zohary and Hopf 2000) and a major part of the Horn of Africa endemism hot-spot (Harrison and Noss 2017), linseed is valued for food and is also exported. We have assessed the morphological variation in the highly diverse Ethiopian germplasm (Worku et al.

2015), which is grown over topical and sub-tropical environments from 3 to 15°N and from 1200 m.a.s.l. to 3500 m.a.s.l. Ethiopia has several agro-ecological zones (Hurni 1998) and other crops such as durum wheat (Mengistu 2016); enset (*Ensete ventricosum* Welw*)* (Olango et al. 2015); tef (*Eragrostis tef* (Zucc.)) Tortter (Bedane et al. 2015; Ayalew et al. 2011); coffee (Tadele et al. 2014); sesame (Gebremichael and Parzies 2011); and barley (Muhe and Assefa 2011) have many landraces shown to have high genetic diversity in Ethiopia.

DNA-based molecular markers have advanced genetic studies in the last three decades. Reports from different authors (Uysal et al. 2010; Cloutier et al. 2009; Fu et al. 2002; Wiesnerova and Wiesner 2004) showed that RAPDs, AFLPs, SSRs and ISSRs have low diversity in linseed germplasm, although Pali et al. (2015) reported higher variation within 48 Indian accessions. Oil seed cultivars have been considered to have more genetic diversity than fibre cultivars (Fu et al. 2002). As might be expected from genetic bottlenecks during selection, diversity is higher among wild species, and within cultivated germplasm it decreases from landraces to breeding lines and then to cultivars (Smykal et al. 2011; Habibollahi 2015). The wide variation in chromosome number found in the genus *Linum* (2n = 16 up to 84) indicates that hybridization and polyploidy have played a role in its evolution (Bolsheva et al. 2015). To contribute to a better understanding of the genetic diversity and species relationships in this genus, Bolsheva et al. (2015) recommended comparative studies of karyotypes, and the development of suitable molecular markers will complement this approach.

A high proportion of the linseed genome is composed of repetitive DNA sequences with motifs present in tens to millions of copies (Cullis 2005). Primers amplifying DNA between SSRs (inter simple sequence repeat, ISSR), retroelements (inter retroelement amplified polymorphisms, IRAP) or SSRs and retroelements (retrotransposon-microsatellite amplification polymorphism, REMAP) have proved to be valuable as informative and polymorphic markers (Kalendar and Schulman 2006; Teo et al. 2005; Alsayied et al. 2015). Both SSR (evolving by slippage replication or recombination) and retroelements (amplifying through an RNA intermediate and reinserting in the genome) can evolve rapidly and hence show polymorphisms in germplasm with little diversity. Different markers reveal different classes of variation (Powell et al. 1996), and evolve at different rates, so a primer useful in distinguishing varieties of a crop may be too polymorphic to be of use in wild germplasm of the same species. Saeidi et al. (2006 and 2008) analysed a collection of of wild *Aegilops tauschii* Coss. germplasm from Iran, finding that SSR markers developed for use in the D genome (derived from *Ae. tauschii*) of wheat cultivars were very highly polymorphic. Thus, while useful in the cultivated hexaploid, the SSR markers gave little information about relationships in the wild species because of their fast evolutionary rate compared to the time of diversification of the accessions. In contrast, within the *A. tauschii* sub-species, another marker class, IRAP markers, were able to show phylogeographic evolutionary patterns over the northern Iran region.

Here, we aimed to exploit molecular (ISSR and IRAP) markers to determine the levels and patterns of genetic diversity and polymorphism of Ethiopian linseed accessions comparing with other international lines and wild relatives. This work towards developing a functional genomic map underpins work to identify candidate genes for agronomic or morphological characters; provides data on linseed crop diversity; can help choose parents for crosses in breeding program; and suggests appropriate sites for increased activity in germplasm conservation or collection.

Materials and methods

Plant materials

Linseed germplasm from cultivated and some wild *Linum* species included 203 lines of which 192 were linseed accessions (of these 17 genotypes were segregating landraces which were divided during the study) and 11 (one accession represented by two genotypes) were accessions from seven wild *Linum* relatives (Table S1). The germplasm was acquired from international and in country gene banks, Research Centres, and local farmers' on-farm holdings (Worku et al. 2015). One hundred and four (92 original + 12 segregating) accessions came from the Ethiopian Biodiversity Institute (EBI); 22 (17+5 segregating) lines from Ethiopian Agricultural Research Centres; 57 collections from local farmers; six varieties (AC Carnduff, AC Emerson, AC McDuff, AC Watson, CDC Bethune and Macbeth: AC McDuff represented by two genotypes) were from Canadian Crop Development Centres; one Irish origin linseed cultivar; 10 (PI 253971 with two genotypes) accessions of six wild *Linum* species from USDA (PI 650336, PI 650322, PI 650318, PI 650315, PI 522290, PI 253971, PI 253971, PI 231886, PI 650297, PI 440472); and *L. volkensii* Engl. was a new collection from the Ethiopia. Germplasm from Agricultural Research Centres is coded ARCxx and from local farmers is coded WLxxxx. Selection of accessions acquired from EBI took into consideration their distribution to represent the different parts of the country and agro-ecosystems.

For spatial diversity analysis altitude information grouped into eight classes using Agarwal (1996) formula: $I = \left(\frac{L-S}{K}\right)$; where *I* is class width; *L* is the largest (3449 m.a.s.l.) and *S* is the smallest (1410 m.a.s.l.) values from altitude records, respectively; *K* is number of classes obtained from $K = 1 + 3.322 log_{10}^{n}$; and *n* is total number of observations, which is 130. Former administrative regional divisions of Ethiopia were used to study the regional diversity in the germplasm.

DNA isolation, ISSR and IRAP-PCR analysis

Genomic DNA was isolated from freshly harvested leaves of a young, single plant per accession. One hundred and fifty mg lyophilized dry leaves from 30 to 45-day old seedlings were ground to fine powder using a Silamat plus Vivadent machine in 1.5mL micro centrifuge tube with 8-10 glass beads. To the finely ground and chilled powdered leaves, a pinch of PVP (polyvinylpyrrolidine) and 1mL preheated 2% CTAB with β-mercaptoethanol solution was added. Then the homogenized content was transferred to 2mL tube and incubated for 30 min at 65^{o}C in a water bath and mixed occasionally 2-3 times by inverting the tubes. Tubes were cooled to ambient temperature and 500µL chloroform:isoamyl alcohol (24:1) was added to each tube. The content in the tubes was rocked at 180^o with 36 turns/min for 30 min using an orbital shaker, then centrifuged for 10 min at 13,000 rpm and the supernatant transferred to a new 2mL tube and the chloroform extraction steps repeated for the second time. To the final supernatant 600µL ice-cold isopropanol was added, rocked gently and high molecular weight DNA was spooled out. The DNA extracts were washed using 1mL wash buffer and dried then the dried pellets were again dissolved in 150 to 250 mL TE pH 8.0 buffer. RNAas treatment was conducted and DNA quality and concentration was checked using 0.8% agarose gel electrophoresis and a Nanodrop (Thermo Scientific NanoDrop-1000 Spectrophotometer, USA) for A260/A280 and A260/A230 ratios. Finally, the DNA extracts were stored at -20^{o}C until needed for PCR and other experiments.

Two ISSR primers (3PCT1 and 3PCT2) from Wiesner et al. (2001), and a set of six IRAP primers: Sukula, Nikita, 3’LTR, 5’LTR, LTR6149 and LTR6150 (Kalendar et al. 2006; Teo et al. 2005; Saeidi et al. 2008) were used (Table S2). After tests with some genotypes and optimization of annealing temperature, two ISSR and six IRAP primers giving clear and polymorphic patterns were used for further analysis of DNA from single plants of the accessions.

PCR amplification reactions were conducted in a reaction volume of 15µL using appropriate single or pairs of primers, 8.15 – 9.15µL sigma water, 1.5µL 10x buffer, 1µL of 250mM $MgCl_2$, 1.2µl of 10mM dNTP mix, 1µL forward primer, 1µL reverse primer where needed, 1µL of 100ng template

DNA and 0.15µL Taq polymerase (KAPA Biosystems). The annealing temperature was optimized using gradient PCR and the annealing temperatures for 3PCT2, Sukula, Nikita and 3’LTR were 60°C, 48°C, 44°C, and 48°C, respectively. The PCR programme for ISSR (3PCT2) primer was set at 94°C for 4 min for initial denaturation and then for 30 cycles each cycle comprised 1 min denaturation at 94°C, 45 s annealing at 60°C temperature, 2 min extension at 72°C with 7 min final extension at the end of the 30th cycle and pause at 4°C. The PCR programme for IRAP primers was 95°C for 2 min for initial denaturation and then for 30 cycles each cycle comprised 1 min denaturation at 95°C, 45 s ramping +0.5^{o}Cs^{-1} to annealing temperature for each primer, 2 min + 3 s extension at 72°C with 10 min final extension at the end of the 30th cycle, and pause at 4°C. Fragments were size-separated by electrophoresis in 2% agarose gels, for IRAP primers, using one part “high resolution” agarose and three parts normal agarose.

Analysis

Each marker band was scored for presence (1) or absence (0), and the data were analysed using the genetic analysis package PowerMarker ver. 3.23. Genetic distances were calculated from the data table using Nei et al. (1983) frequencies between each pair of accessions (Lapitan et al. 2007; Yoon et al. 2012). ISSR and IRAP data was analysed using Neighbour-joining with Nei (1983) genetic distances. In the cluster analyses for the associations between each of two individuals or groups of assays were replicated. Based on the number of individuals or groups of individuals, 100 and 1000 replications were made for cluster analyses or for grouping assays into more genetically related groups. Bootstrap percentages from 50% - 70% were used to determine members of a cluster and phylogeny trees were displayed using Geneious (www.Geneious.com).

Results

ISSR and IRAP primers amplified multiple, polymorphic products from genomic DNA of linseed (*L. usitatissimum*) and seven wild species. The total numbers of bands, degree of polymorphism, number of unique alleles, range of band size, gene diversity (GD) and polymorphism information content (PIC) exhibited by ISSR and IRAP primers from linseed and wild genotypes were calculated (Table 1a-1d). In total, 203 genotypes studied using four optimized PCR primer amplifications, 435 independent marker bands were scored in 41 gels; 88,305 band positions were scored for presence or absence of marker bands. From the 203 defined accessions, 11,008 marker bands (with only 4.80% missing data where amplification or gel failed or was not tested) were analysed. Genotypes were split into cultivated and wild species groups and the results from the parameters for each group are indicated in Table 1a-d. Wild species were polymorphic with all primers and had multiple (up to 36 out of 59) unique alleles (Table 1c). Table 1d shows analysis of the Ethiopian flax and linseed accessions excluding the Canadian reference lines; three of the 22 unique alleles were present in the Canadian germplasm only.

The gene diversity (GD) value at individual primer level varied from 0.004 to 0.500 in the assayed genotypes (for the related metric of polymorphism information content (PIC), the maximum value was 0.375). The mean GD value from regional groups of genotypes ranged between 0.209±0.152 from Gondar region and 0.385±0.139 from Kefa region (mean PIC 0.176±0.113 and 0.301±0.106 respectively). The Canadian lines scored 0.210±0.188 mean GD (0.171±0.148 PIC), similar to the least diverse Gondar/Shewa accessions. It was also notable that the lines from the ARC Ethiopian research centres had relatively little diversity (GD 0.242±0.150 and PIC 0.202±0.113).

Germplasm was grouped into its origin in 13 former administrative regions of Ethiopia where these data were available (see Figure 2, Table S1 and Table 2a). Individual regional groups of accessions (Table 2a) had showed polymorphism levels from 61.90% to 99.65%: the lowest was in Canadian lines and the highest from Gondar region. Ethiopian accessions with collection information were grouped into eight altitude classes (Table 2b). Like regional groups, all altitude classes showed less than 100% polymorphism (91.11% to 96.82%) with the least from altitude 7 (high, around 3000m) and the most from altitude 2 (low, below 2000m), almost from the extremes of altitude ranges.

Phylogenetic relationships

The genetic-marker distance data were used for phylogenetic analysis. The overall 203 genotypes were split into different populations based on their species, region and altitude classifications and used to create trees.

The 11 wild *Linum* accessions (Table S1) were chosen to represent four sections of the genus and various chromosome numbers (2n=16, 18, 20, 28, 30). *L. hirsutum* L. was used as an outgroup: it is in section Dasylinum, and groups with *L. volkensii* (an old-world yellow-flowered flax) and *L. trigynum* (both section Linopsis; see McDill et al. 2009). The two *L. austriacum* L. (sect Linum), from German and Russia place in different groups: from Russia forms independent cluster and from German groups with *L. narbonense* L. (sect Linum) and *L. flavum* L. (sect Syllinum). The four *L. bienne* accessions (sect Linum) form a well-supported cluster.

When grouped by the 13 former regions (Figure 2) used for collections, there were five reasonably well-supported clusters with greater than 65% bootstrap (Figure 1b). Accessions from North Ethiopia (Tigray), grouped into cluster I; accessions from North and northern central parts of Ethiopia (Gondar and Shewa regions) grouped into cluster II; accessions from northwest, southwest and South Ethiopia (Gojam, Wellega, Illubabour, Kefa, Gomugofa and Sidamo) grouped into cluster III; accessions from southeast Ethiopia (Bale and Arsi) grouped into cluster VI; and accessions from eastern Ethiopia (Wollo and Hararghe) grouped into cluster V.

The eight altitude classes from a range of 1410-3449m (Table 2b) were grouped into four clusters by the relationship tree: the high altitude class 8 (3195-3449m); altitude classes 1 (1410-1665m) and 3 (1920-2174); altitude classes 4, 5, 6 and 7 (2175-3194 m); and altitude class 2 (1665-1919).

Discussion

Genetic diversity and polymorphism

IRAP and ISSR markers were appropriate for assay of genetic diversity in the Ethiopian linseed accessions and the *Linum* genus. Levels of polymorphism and shared bands enabled inferences about genetic structure, diversity and biogeography.

The relationship between the seven *Linum* wild species (Figure 1a) were broadly consistent with the results based on a combined ITS and chloroplast (ndhF+trnK intron +trnL-F) topology by McDill et al. (2009). Based on EST-SSR primer data Fu (2011), reported *L. bienne* Mill. and *L. decumbens* (both sect Linum) collected from different countries grouped into different clusters. Linseed and six of the seven *Linum* species did not have common alleles for ISSR and IRAP primers used for the study. Similarity between linseed and all four *L. bienne* Mill. genotypes (Fig 1a, Table 1) was expected since *L. bienne* Mill. is the progenitor of *L. usitatissimum* (Soto-Cerda 2011); one allele was common to all linseed and *L. bienne* Mill. genotypes but not to other *Linum* species. Melnikova et al. (2014) also reported *L. usitatissimum* and *L. bienne* were grouped under Sect. Linum. *L. strictum* L., *L. keniense* Fries., *L. holstii* Engl. ex R Wilczek (likely to be a synonym of *L. volkensii* Engl.) and *L. trigynum* L. var *sieberi* (Planch.) Cuf. are the four wild *Linum* species in Ethiopia (Edwards 1991). Even these more distant germplasm pools may be useful to increase variation by crossing with linseed, since several *Linum* species can produce viable and fertile hybrids (Seetharam 1972; Friedt 1989). Diederichsen and Richards (2003) and Cullis (2011) reported the *L.*

bienne Mill. itself is more diverse in morphology than *L. usitatissimum*: in the accessions in the present study, the three *L. bienne* Mill. showed variation in their major morphological and maturity characters, with heterogeneity in maturity, plant height, boll size and seed characters (Worku et al. 2015), supported by the high genotypic diversity revealed here (Figure 1a; Tables 1c and S1). Additional collections and detailed studies on wild relatives may increase the germplasm pool available to breeders for introgression of novel alleles.

The GD and PIC from the present study showed the presence of high GD and PIC in Ethiopian linseed accessions as compared to the results obtained for samples collected from different countries (Soto-Cerda et al. 2012: Žiarovská et al. 2012). Soto-Cerda et al. (2012) used a core set of linseed germplasm developed from 60 accessions as representative of 16 countries from three continents and obtained 0.34 mean value of GD from 150 microsatellite (85 gSSR and 65 EST-SSR). Žiarovská et al. (2012) reported PIC values from four ISSR and two IRAP primers ranged from 0.12 to 0.37 and from 0.22 to 0.28, respectively from 18 accessions collected from 11 countries. These values were similar to the range of results from the present study. In *Piper betle,* Grewal et al. (2016) reported ISSR polymorphism was between 43% and 100% (PIC 0.17 to 0.45, mean 0.32). IRAPs were also informative with *Crocus*, where the lack of diversity within the saffron crocus and high diversity in other members of the genus were evident (Alsayied 2015). Diederichsen (2001) compared the genetic variation from Canadian linseed with world collections and reported Canada linseed cultivars showed higher rate of loss in genetic variation because of the breeding programs which largely use selected varieties (and reflected in the GD and PIC values of Canadian lines here). The selected accessions from Ethiopian Agricultural Research Centres also showed relatively low GD and PIC values.

Despite the substantial morphological variation in all the flax accessions here (Worku et al. 2015), the molecular genotype markers were able to group accessions by both altitude and region (Figure 1) indicating lack of gene flow across the country and/or selection for particular genotypes in each environment. Ethiopia is a mountainous country including dry areas (Engels and Hawkes 1991; Friis et al. 2010) and diverse topography (particularly altitude), important for agro-ecological zonation (Hurni 1998). Linseed grows in tropical highlands where mean temperatures during the growing period correlate with altitude. Selection may be related to agronomic performance and also regional use differences (Worku et al. 2005, 2012). Previously, Ali et al. (2015) showed clusters of variation between the Ethiopian linseed landraces and non-native cultivars for majority of morphological traits. Noormohammadi et al. (2015) reported wild *L. austriacum* L. populations at intermediate altitudes have greater diversity than populations at lower and higher altitudes. The existing diversity of linseed in Ethiopia is the contribution of diversities in agroecological systems, cultural history of the people and farmers knowledge (Engels and Hawkes 1991; Kiros-Meles et al. 2008). In addition to agroecological (Hurni 1998), morphological (Worku et al. 2015) and molecular similarity, and geographical proximity, linseed in the Arsi and Bali regions is cultivated for cash and most growers used selected linseed varieties to match their demand for higher price with the demand of oil pressing factories for higher oil content from the crop (Worku et al. 2012). Some of the research centres and sites, like Sinana, Bekoji, Kulumsa, Arsi-Robe, for linseed and other crops breeding are located in the two regions, so knowledge awareness and seed acquisition are easily accessible to farmers in the regions.

Gondar and Gojam are major linseed producing regions while Kefa and Gamugofa are not. The attention given to linseed in major producing regions (including by the government and local administrators to improve their linseed production) means new and uniform varieties may be more prevalent in these regions: Victory, Concurrant, CI-1525 and CI-1652 are examples of the four selected varieties introduced to Ethiopia and released in 1978 and 1984 (Belayneh 1991), where oil pressing machines are used and linseed is a cash crop. Accessions were collected in different decades by EBI from different regions in Ethiopia. In the cluster analysis (see Figure S2 and Table S3a & b), cultivars collected after 2000 by EBI grouped. This suggested that newly introduced lines are currently substituting the native linseed cultivars in Ethiopia on farm. The introduction of uniform lines would be cause for genetic erosion in Ethiopian linseed cultivars (Belayneh, 1991). However, during the expansion years, the Tigray Region was not accessible. Worku et al. (2012) reported only a single variety name, Yehagerie or Yehabesha, meaning cultivar from own country, whereas from other regions at least two different additional names of introduced ‘improved’ varieties: Yeferenj (cultivar introduced from abroad) and Yemengist (cultivar given from the state).

For breeding and improvement programs, unimproved germplasm (with high diversity and genetic distances) must be included in crosses (as has been successful in wheat; eg Ali et al. (2014), particularly with requirement to meet new climatic stress factors. Landrace linseeds and other crops, once identified and integrated into a program, can maintain diversity; while enabling adoption of varieties with improved performance, it is important to mitigate loss of potentially valuable and adapted genotypes.

Conclusions

In Ethiopia linseed is traditionally grown on marginal lands and farmers accept low crop yields since they do not contribute any input for its growth (Worku et al. 2012) and it is drought tolerant (Durant 1976; Seegeler 1983). Nevertheless, there are strong agro-ecological associations between linseed and other grain crops, and a cultural value in the multi-use crop within Ethiopian communities (Mulatu et al. 2002). The presence of different socio-cultural conditions and the diverse tropical and sub-tropical, mountainous topography in Ethiopia support high genetic diversity in linseed both among regional and altitude groups. In contrast, temperate linseed varieties (Canadian here) have lower genetic diversity and polymorphism, expected because of strong selection made by breeders, farmers and researchers.

Exploitation of diversity within linseed, hybridization with *L. bienne*, and backcrossing with other wild *Linum* species, can introgress novel and useful characters to linseed for quality (oil and fibre), yield, biotic and abiotic stress resistances. The regional and altitude diversity revealed here suggests genes are available for improvement, particularly with respect to abiotic stress resistances. Both genetic mapping and perhaps association genetics using a combination of DNA markers and performance or morphological data may be valuable for in country breeding centres, and have the potential to expand linseed producing regions and productivity under developing agroecological conditions.

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Ethical Statements

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Conflict of interest: The authors declare that they have no conflict of interest.

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LEGENDS TO TABLES

Table 1. Polymorphism and gene diversity exhibited by ISSR and IRAP primers: a) in 193 accessions from cultivars + 11 accessions from seven wild *Linum* spp; b) in 193 linseed accessions (185 from Ethiopia + seven [one doubled] from Canada + one from Ireland); c) in 11 accessions [one doubled] from seven wild spp. [one from Ethiopia]; and d) in 185 Ethiopian linseed accessions.

Table 2. Genetic diversity, degree of polymorphism and number of unique alleles: a) within regional grouping; and b) within altitude classes.

Supplementary Table 1. The accessions of cultivated *Linum usitatissium* and wild *Linum* species analysed in this work showing taxon, accession number and origin/source. PI: USDA – North Central Regional Plant Introduction Station.

Supplementary Table 2. IRAP and ISSR primer used for the study of polymorphism, with their sequences and T_a.

Supplementary Table 3. a) Distribution of 192 cultivated + 11 wild *Linum* accessions among the 13 clusters from Supplementary Figure 1. The total number of the samples used for Table S3a result was 201. (Accession PGRC/E243797 was represented by two seedlings was omitted). b) Distribution of 190 linseed accessions among the 10 clusters from Supplementary Figure S2.

LEGENDS TO FIGURES

Figure 1. Neighbour joining trees based on ISSR and IRAP data showing the genetic relationships among linseed and other *Linum* accessions. Numbers represent bootstrap re-sampling values for support at nodes (%). a) 11 accessions of seven wild *Linum* species; b) 13 former Ethiopian Administrative Regions for their linseed germplasm genetic diversity based on 163 linseed germplasm; c) Eight altitude classes for their linseed germplasm genetic diversity based on 107 linseed germplasm Altitude class 8 is used as an outgroup.

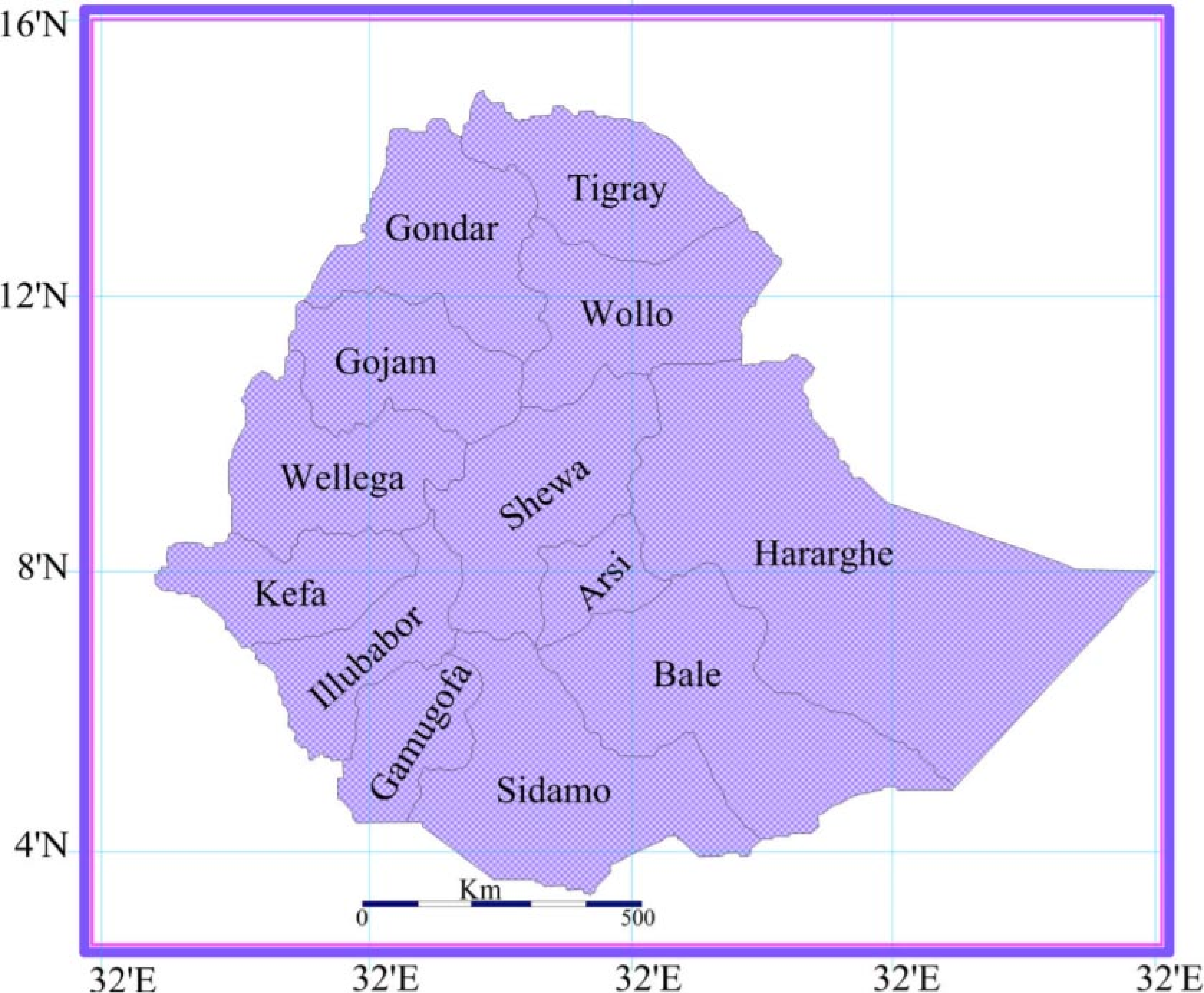
Figure 2. Map of Ethiopia: with Latitude-Longitude position information (Wikimedia Commons); and its former administrative regions.

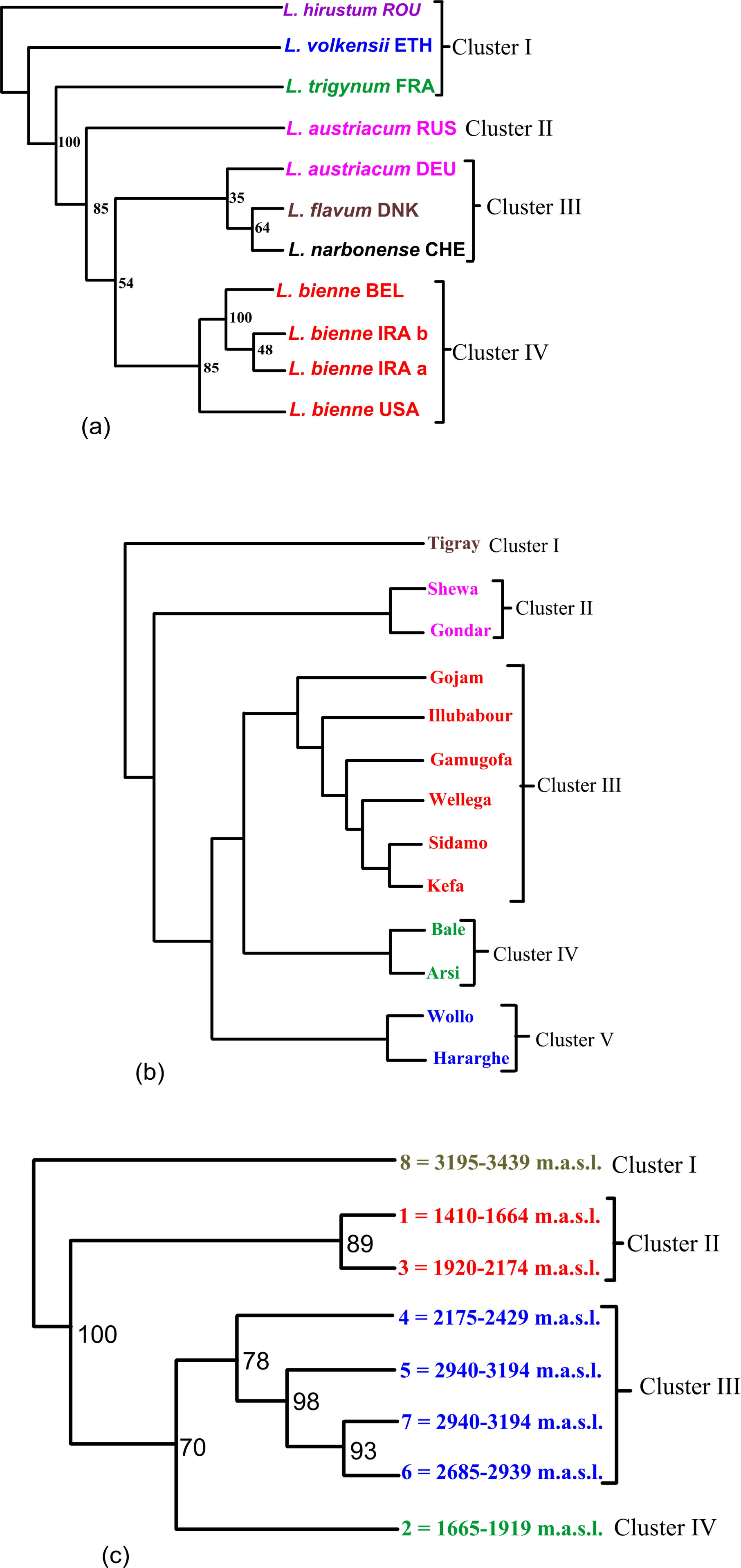
Supplementary Figure S1. Dendrogram showing genetic relationships among 192 linseed and 11 wild *Linum* accessions.

🟦 Canadian lines
🟧 Genotypes from local farmers
🟦 Genotypes from ARC
🟥 Wild genotype
🟪 EBI collections after 2000
🟩 EBI collections before 1977
🟪 EBI collections between 1978-2000

Supplementary Figure S2. Dendrogram showing genetic relationships among 190 linseed accessions based on ISSR and IRAP data using Neighbour-joining algorithm and Nei, 1983 genetic distance.

🟪 EBI collections after 2000
🟨 EBI collections before 1977
🟧 EBI collections between 1978-2000
🟦 Genotypes from local farmers
🟩 Canadian lines
🟥 Genotypes from ARC


16'N
12'N
8'N
4'N
Tigray
Gondar
Wollo
Gojam
Wellega
Shewa
Hararghe
Arsi
Kefa
Illubabor
Bale
Gamugofa
Sidamo
Km
0
500
32'E
32'E
32'E
32'E
32'E


L. hirustum ROU
L. volkensii ETH
L. trigynum FRA
Cluster I
L. austriacum RUS
Cluster II
L. austriacum DEU
L. flavum DNK
L. narbonense CHE
Cluster III
L. bienne BEL
L. bienne IRA b
L. bienne IRA a
L. bienne USA
Cluster IV
100
85
54
35
64
100
48
85
(a)
Tigray
Cluster I
Shewa
Gondar
Cluster II
Gojam
Illubabour
Gamugofa
Wellega
Sidamo
Kefa
Cluster III
Bale
Arsi
Cluster IV
Wollo
Hararghe
Cluster V
(b)
8 = 3195-3439 m.a.s.l.
Cluster I
1 = 1410-1664 m.a.s.l.
3 = 1920-2174 m.a.s.l.
Cluster II
4 = 2175-2429 m.a.s.l.
5 = 2940-3194 m.a.s.l.
7 = 2940-3194 m.a.s.l.
6 = 2685-2939 m.a.s.l.
Cluster III
2 = 1665-1919 m.a.s.l.
Cluster IV
89
100
78
98
93
70
(c)

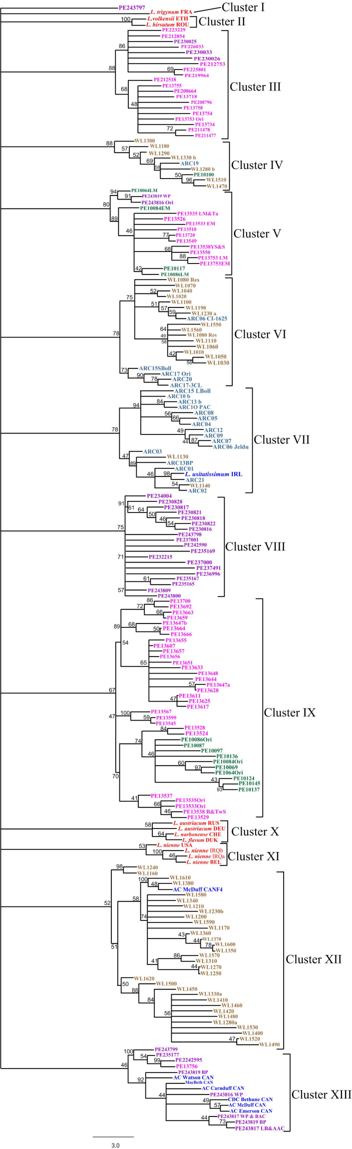

PE243797
L. trigynum FRA
Cluster I
L.volkensii ETH
L. hirsutum ROU
Cluster II
PE223229
PE212854
PE230025
PE226033
PE230033
PE230026
PE212753
PE225801
PE219964
PE212518
PE13755
PE208664
PE13718
PE208796
PE13758
PE13754
PE13753 Ori
PE13734
PE211478
PE211477
Cluster III
WL1300
WL1180
WL1290
WL1330 b
ARC19
WL1280 b
PE10100
WL1510
WL1470
Cluster IV
PE10064LM
PE243819 WP
PE243816 Ori
PE10084EM
PE13535 LM&Ta
PE13526
PE13533 EM
PE13510
PE13720
PE13549
PE13538YS&S
PE13550
PE13753 LM
PE13753EM
PE10117
PE10086LM
Cluster V
WL1080 Res
WL1070
WL1040
WL1020
WL1100
WL1190
WL1230 a
ARC06 CI-1625
WL1550
WL1560
WL1080 Res
WL1110
WL1060
WL1010
WL1050
WL1030
ARC15SBoll
ARC17 Ori
ARC20
ARC17-3CL
Cluster VI
ARC15 LBoll
ARC10 b
ARC13 b
ARC1O PAC
ARC08
ARC05
ARC04
ARC12
ARC09
ARC07
ARC06 Jeldu
ARC03
WL1130
ARC13BP
ARC01
L. usitatissimum IRL
ARC21
WL1140
ARC02
Cluster VII
PE234004
PE230828
PE230817
PE230821
PE230818
PE230822
PE230816
PE243798
PE237001
PE242590
PE235169
PE232215
PE237000
PE237491
PE236996
PE235167
PE235165
PE243809
PE243800
Cluster VIII
PE13700
PE13692
PE13663
PE13659
PE13647b
PE13664
PE13666
PE13655
PE13607
PE13657
PE13656
PE13651
PE13633
PE13648
PE13644
PE13647a
PE13628
PE13611
PE13625
PE13617
PE13567
PE13599
PE13545
PE13528
PE13524
PE10086Ori
PE10087
PE10097
PE10136
PE10084Ori
PE10069
PE1064Ori
PE10124
PE10145
PE10137
PE13537
PE13535Ori
PE13533Ori
PE13538 B&TwS
PE13529
Cluster IX
L. austriacum RUS
L. austriacum DEU
L. narbonense CHE
L. flavum DUK
Cluster X
L. nienne USA
L. nienne IRQb
L. nienne IRQa
L. nienne BEL
Cluster XI
WL1240
WL1160
WL1610
WL1380
AC McDuff CANF4
WL1580
WL1340
WL1210
WL1230b
WL1200
WL1590
WL1170
WL1360
WL1370
WL1600
WL1350
WL1570
WL1310
WL1270
WL1250
WL1620
WL1500
WL1450
WL1330a
WL1410
WL1460
WL1420
WL1480
WL1280a
WL1530
WL1400
WL1520
WL1490
Cluster XII
PE243799
PE235177
PE2242595
PE13756
PE243819 BP
AC Watson CAN
MacBeth CAN
AC Carnduff CAN
PE243816 WP
CDC Bethune CAN
AC McDuff CAN
AC Emerson CAN
PE243817 WP & BAC
PE243819 BP
PE243817 LB&AAC
Cluster XIII
3.0

Table 1.

Primer		*Ta*	*Nº of observation*	*Total bands*	*Polymorphic bands*	*Degree of polymorphism (%)*	*Nº of unique allele*	*Band size*		*Gene diversity*	*PIC*
								min	*max*		
Table 1a											
ISSR	3PCT2	60	199	102	102	100	25	190	3000	0.149±0.157	0.125±0.122
IRAP	Sukula	48	184	114	114	100	25	228	3169	0.161±0.154	0.136±0.121
	Nikita	44	199	117	117	100	28	165	3043	0.143±0145	0.122±0.115
	3’LTR	48	191	155	155	100	21	264	2858	0.140±0109	0.125±0.089
									Mean	**0.148±0.140**	**0.127±0.110**
Table 1b											
ISSR	3PCT2	60	188	71	70	98.59	2	190	3000	0.208±0.160	0.174±0.121
IRAP	Sukula	48	173	84	84	100	1	360	3169	0.213±0.152	0.179±0.117
	Nikita	44	188	98	98	100	13	165	3043	0.168±0.147	0.143±0.115
	3’LTR	48	180	133	133	100	6	264	2858	0.165±0.108	0.145±0.087
									Mean	**0.184±0.140**	**0.157±0.108**
Table 1c											
ISSR	3PCT2	60	11	59	59	100	36	236	3000	0.252±0.119	0.213±0.083
IRAP	Sukula	48	11	63	63	100	32	228	2754	0.269±0.120	0.226±0.083
	Nikita	44	11	48	48	100	25	343	3043	0.279±0.132	0.232±0.090
	3’LTR	48	11	44	44	100	23	335	2500	0.279±0.134	0.232±0.092
									Mean	**0.269±0.125**	**0.225±0.086**
Table 1d											
ISSR	3PCT2	60	180	70	69	98.57	1	190	3000	0.208±0.163	0.174±0.123
IRAP	Sukula	48	165	84	84	100	3	360	3169	0.214±0.154	0.179±0.118
	Nikita	44	180	95	95	100	13	165	3043	0.170±0.146	0.145±0.114
	3’LTR	48	172	133	133	100	6	264	2858	0.165±0.110	0.145±0.088
									Mean	**0.185±0.141**	**0.158±0.109**

Table 2.

2a. Within a region

S.Nº	Region	Nº of assay	Total band Nº	Polymorphic band	Degree of polymorphism (%)	Number & % of unique band	GD	PIC
1	ARC	22	167	157	94.01	48(28.74)	0.242±0.150	0.201±0.113
2	Arsi	18	206	199	96.60	37(17.96)	0.295±0.142	0.241±0.103
3	Bale	20	195	187	95.90	26(13.33)	0.296±0.145	0.242±0.105
4	Gamugofa	7	149	137	91.95	66(44.30)	0.330±0.138	0.2660±.100
5	Gojam	6	176	164	93.18	108(61.36)	0.329±0.108	0.269±0.076
6	Gondar	42	289	288	99.65	64(22.15)	0.209±0.152	0.176±0.113
7	Hararghe	8	112	97	86.61	46(41.07)	0.249±0.130	0.210±0.100
8	Illubabour	4	158	147	93.04	102(64.56)	0.353±0.116	0.284±0.087
9	Kefa	4	124	112	90.32	75(60.48)	0.385±0.139	0.301±0.106
10	Shewa	12	214	209	97.66	95(44.39)	0.300±0.141	0.245±0.098
11	Sidamo	1	71	---	---	---	---	---
12	Tigray	16	212	203	95.75	29(13.68)	0.302±0.127	0.249±0.091
13	Wellega	10	145	130	89.66	26(17.93)	0.303±0.141	0.247±0.106
14	Wollo	14	195	187	95.90	57(29.23)	0.289±0.146	0.236±0.105
15	Canada	6	84	52	61,90	17(20.24)	0.210±0.188	0.171±0.148
	Mean	**12.67**	**173.40**	**162.07**	**91.48**	**57.87(34.29%)**	0.292±0.140	0.238±0.104

2b. within an altitude class

S.Nº	Altitude class	Nº of assay	Total band Nº	Polymorphic band	Degree of polymorphism (%)	Number & % of unique band	GD	PIC
1	1410-1664	11	169	158	93.49	47(27.81)	0.320±0.153	0.257±0.110
2	1665-1919	21	220	213	96.82	45(20.45)	0.267±0.135	0.222±0.099
3	1920-2174	22	218	211	96.79	31(14.22)	0.284±0.138	0.234±0.102
4	2175-2429	13	235	227	96.60	100(42.55)	0.263±0.139	0.218±0.100
5	2430-2684	17	179	170	94.97	26(14.53)	0.302±0.144	0.246±0.105
6	2685-2939	7	146	134	91.78	74(50.68)	0.298±0.126	0.246±0.093
7	2940-3194	10	135	123	91.11	44(32.59)	0.315±0.167	0.252±0.119
8	3195-3449	6	144	135	93.75	54(37.50)	0.360±0.130	0.287±0.093
	Mean	**13.375**	180.75	171.38	**94.81**	**52.63(29.11%)**	0.288±0.129	0.235±0.094

Table S1.

Item №	Taxon	Accession number	Origin/source	Item №	Taxon	Accession number	Origin/source
1	*L. trigynum*	PI 650336	France	52	*L. usitatissimum*	13644	Ethiopia- Welega
2	*L. norbonense*	PI 650322	Switzerland	53	*L. usitatissimum*	13647	Ethiopia- Gondar
3	*L. hirsutum*	PI 650318	Romania	54	*L. usitatissimum*	13648	Ethiopia- Gondar
4	*L. flavum*	PI 650315	Denmark	55	*L. usitatissimum*	13651	Ethiopia- Arsi
5	*L. bienne*	PI 522290	United States, Iowa	56	*L. usitatissimum*	13655	Ethiopia- Arsi
6	*L. bienne*	PI 253971	Iraq	57	*L. usitatissimum*	13656	Ethiopia- Gamo Gofa
7	*L. bienne*	PI 253971	Iraq	58	*L. usitatissimum*	13657	Ethiopia- Gamo Gofa
8	*L. bienne*	PI 231886	Belgium	59	*L. usitatissimum*	13659	Ethiopia- Welega
9	*L. austriacum*	PI 650297	Germany	60	*L. usitatissimum*	13663	Ethiopia- Welega
10	*L. austriacum*	PI 440472	Russian Federation	61	*L. usitatissimum*	13664	Ethiopia- Welega
11	*L. volkensii*	NEW Collec	Ethiopia	62	*L. usitatissimum*	13666	Ethiopia- Welega
12	*L. usitatissimum*	PI 522756	Ireland	63	*L. usitatissimum*	13674	Ethiopia- Gondar
13	*L. usitatissimum*	AC Carnduff	Canada	64	*L. usitatissimum*	13692	Ethiopia- Wollo
14	*L. usitatissimum*	AC Emerson	Canada	65	*L. usitatissimum*	13700	Ethiopia- Wollo
15	*L. usitatissimum*	AC McDuff	Canada	66	*L. usitatissimum*	13718	Ethiopia- Wollo
16	*L. usitatissimum*	AC McDuff	Canada	67	*L. usitatissimum*	13720	Ethiopia- Wollo
17	*L. usitatissimum*	AC Watson	Canada	68	*L. usitatissimum*	13734	Ethiopia- Wollo
18	*L. usitatissimum*	CDCBethune	Canada	69	*L. usitatissimum*	13753	Ethiopia- Welega
19	*L. usitatissimum*	MacBeth	Canada	70	*L. usitatissimum*	13754	Ethiopia- Illubabur
20	*L. usitatissimum*	10064	Ethiopia- Gamo Gofa	71	*L. usitatissimum*	13755	Ethiopia- Illubabur
21	*L. usitatissimum*	10069	Ethiopia- Gojjam	72	*L. usitatissimum*	13756	Ethiopia- Illubabur
22	*L. usitatissimum*	10084	Ethiopia- Illubabur	73	*L. usitatissimum*	13758	Ethiopia- Kefa
23	*L. usitatissimum*	10086	Ethiopia- Kefa	74	*L. usitatissimum*	208664	Ethiopia- Hararghe
24	*L. usitatissimum*	10087	Ethiopia- Kefa	75	*L. usitatissimum*	208796	Ethiopia- Arsi
25	*L. usitatissimum*	10097	Ethiopia- Shewa	76	*L. usitatissimum*	211477	Ethiopia- Gamo Gofa
26	*L. usitatissimum*	10100	Ethiopia- Shewa	77	*L. usitatissimum*	211478	Ethiopia- Shewa
27	*L. usitatissimum*	10117	Ethiopia- Shewa	78	*L. usitatissimum*	212518	Ethiopia- Shewa
28	*L. usitatissimum*	10124	Ethiopia- Sidamo	79	*L. usitatissimum*	212753	Ethiopia- Gondar
29	*L. usitatissimum*	10136	Ethiopia- Welega	80	*L. usitatissimum*	212854	Ethiopia- Bale
30	*L. usitatissimum*	10137	Ethiopia- Welega	81	*L. usitatissimum*	219964	Ethiopia- Tigray
31	*L. usitatissimum*	10145	Ethiopia- Wollo	82	*L. usitatissimum*	225801	Ethiopia- Gamo Gofa
32	*L. usitatissimum*	13510	Ethiopia- Arsi	83	*L. usitatissimum*	226033	Ethiopia- Gojjam
33	*L. usitatissimum*	13524	Ethiopia- Gondar	84	*L. usitatissimum*	230025	Ethiopia- Bale
34	*L. usitatissimum*	13526	Ethiopia- Bale	85	*L. usitatissimum*	230026	Ethiopia- Bale
35	*L. usitatissimum*	13528	Ethiopia- Bale	86	*L. usitatissimum*	230033	Ethiopia- Bale
36	*L. usitatissimum*	13529	Ethiopia- Shewa	87	*L. usitatissimum*	230034	Ethiopia- Bale
37	*L. usitatissimum*	13533	Ethiopia- Arsi	88	*L. usitatissimum*	230816	Ethiopia- Hararghe

38	*L. usitatissimum*	13535	Ethiopia- Bale	89	*L. usitatissimum*	230817	Ethiopia- Hararghe
39	*L. usitatissimum*	13537	Ethiopia- Bale	90	*L. usitatissimum*	230818	Ethiopia- Hararghe
40	*L. usitatissimum*	13538	Ethiopia- Bale	91	*L. usitatissimum*	230821	Ethiopia- Hararghe
41	*L. usitatissimum*	13545	Ethiopia- Arsi	92	*L. usitatissimum*	230822	Ethiopia- Hararghe
42	*L. usitatissimum*	13549	Ethiopia- Arsi	93	*L. usitatissimum*	230828	Ethiopia- Hararghe
43	*L. usitatissimum*	13550	Ethiopia- Arsi	94	*L. usitatissimum*	232215	Ethiopia- Arsi
44	*L. usitatissimum*	13567	Ethiopia- Gojjam	95	*L. usitatissimum*	234004	Ethiopia- Tigray
45	*L. usitatissimum*	13599	Ethiopia- Shewa	96	*L. usitatissimum*	235165	Ethiopia- Tigray
46	*L. usitatissimum*	13607	Ethiopia- Gojjam	97	*L. usitatissimum*	235167	Ethiopia- Tigray
47	*L. usitatissimum*	13611	Ethiopia- Gondar	98	*L. usitatissimum*	235169	Ethiopia- Tigray
48	*L. usitatissimum*	13617	Ethiopia- Gondar	99	*L. usitatissimum*	235177	Ethiopia- Tigray
49	*L. usitatissimum*	13625	Ethiopia- Gondar	100	*L. usitatissimum*	236996	Ethiopia- Arsi
50	*L. usitatissimum*	13628	Ethiopia- Bale	101	*L. usitatissimum*	237000	Ethiopia- Arsi
51	*L. usitatissimum*	13633	Ethiopia- Wollo	102	*L. usitatissimum*	237001	Ethiopia- Arsi

Item №	Taxon	Accession number	Origin/source	Item №	Taxon	Accession number	Origin/source
103	*L. usitatissimum*	237491	Ethiopia- Wollo	154	*L. usitatissimum*	WL1080	Ethiopia- Bale
104	*L. usitatissimum*	242590	Ethiopia- Tigray	155	*L. usitatissimum*	WL1090	Ethiopia- Bale
105	*L. usitatissimum*	242595	Ethiopia- Tigray	156	*L. usitatissimum*	WL1100	Ethiopia- Bale
106	*L. usitatissimum*	243797	Ethiopia- Wollo	157	*L. usitatissimum*	WL1110	Ethiopia- Bale
107	*L. usitatissimum*	243798	Ethiopia- Wollo	158	*L. usitatissimum*	WL1550	Ethiopia- Bale
108	*L. usitatissimum*	243799	Ethiopia- Wollo	159	*L. usitatissimum*	WL1560	Ethiopia- Bale
109	*L. usitatissimum*	243800	Ethiopia- Wollo	160	*L. usitatissimum*	WL1120	Ethiopia- Gondar
110	*L. usitatissimum*	243809	Ethiopia- Wollo	161	*L. usitatissimum*	WL1130	Ethiopia- Gondar
111	*L. usitatissimum*	243816	Ethiopia- Gondar	162	*L. usitatissimum*	WL1140	Ethiopia- Gondar
112	*L. usitatissimum*	243819	Ethiopia- Gojjam	163	*L. usitatissimum*	WL1150	Ethiopia- Gondar
113	*L. usitatissimum*	10064 Late	Ethiopia- Gamo Gofa	164	*L. usitatissimum*	WL1160	Ethiopia- Gondar
114	*L. usitatissimum*	10084-Early	Ethiopia- Illubabur	165	*L. usitatissimum*	WL1170	Ethiopia- Gondar
115	*L. usitatissimum*	10086 Late	Ethiopia- Kefa	166	*L. usitatissimum*	WL1180	Ethiopia- Gondar
116	*L. usitatissimum*	13533-Early	Ethiopia- Arsi	167	*L. usitatissimum*	WL1190	Ethiopia- Gondar
117	*L. usitatissimum*	13535 Tallest	Ethiopia- Bale	168	*L. usitatissimum*	WL1200	Ethiopia- Gondar
118	*L. usitatissimum*	13538 -Yellow	Ethiopia- Bale	169	*L. usitatissimum*	WL1210	Ethiopia- Gondar
119	*L. usitatissimum*	13753-Early	Ethiopia- Welega	170	*L. usitatissimum*	WL1220	Ethiopia- Gondar
120	*L. usitatissimum*	13753-Late	Ethiopia- Welega	171	*L. usitatissimum*	WL1230	Ethiopia- Gondar
121	*L. usitatissimum*	243797 Late	Ethiopia- Wollo	172	*L. usitatissimum*	WL1240	Ethiopia- Gondar
122	*L. usitatissimum*	243816-Bp	Ethiopia- Gondar	173	*L. usitatissimum*	WL1250	Ethiopia- Gondar
123	*L. usitatissimum*	243816-wp	Ethiopia- Gondar	174	*L. usitatissimum*	WL1260	Ethiopia- Gondar
124	*L. usitatissimum*	243817-wp	Ethiopia- Gondar	175	*L. usitatissimum*	WL1270	Ethiopia- Gondar
125	*L. usitatissimum*	Belay-96	Ethiopia- ARC01	176	*L. usitatissimum*	WL1280	Ethiopia- Gondar
126	*L. usitatissimum*	Berene	Ethiopia- ARC02	177	*L. usitatissimum*	WL1290	Ethiopia- Gondar
127	*L. usitatissimum*	CDC-1747	Ethiopia- ARC03	178	*L. usitatissimum*	WL1300	Ethiopia- Gondar
128	*L. usitatissimum*	Chilalo	Ethiopia- ARC04	179	*L. usitatissimum*	WL1320	Ethiopia- Gondar
129	*L. usitatissimum*	CI-525	Ethiopia- ARC05	180	*L. usitatissimum*	WL1330	Ethiopia- Gondar
130	*L. usitatissimum*	Jeldu	Ethiopia- ARC06	181	*L. usitatissimum*	WL1340	Ethiopia- Gondar
131	*L. usitatissimum*	Kasa1	Ethiopia- ARC07	182	*L. usitatissimum*	WL1350	Ethiopia- Gondar
132	*L. usitatissimum*	Kasa2	Ethiopia- ARC08	183	*L. usitatissimum*	WL1360	Ethiopia- Gondar
133	*L. usitatissimum*	Kulumsa-1	Ethiopia- ARC09	184	*L. usitatissimum*	WL1570	Ethiopia- Gondar
134	*L. usitatissimum*	LLA S’PS’ 21	Ethiopia- ARC10	185	*L. usitatissimum*	WL1590	Ethiopia- Gondar
135	*L. usitatissimum*	LLA S’PS’ 21	Ethiopia- ARC10	186	*L. usitatissimum*	WL1580	Ethiopia- Gondar
136	*L. usitatissimum*	Omega	Ethiopia- ARC12	187	*L. usitatissimum*	WL1590	Ethiopia- Gondar
137	*L. usitatissimum*	PGRC/E10306	Ethiopia- ARC13	188	*L. usitatissimum*	WL1600	Ethiopia- Gondar
138	*L. usitatissimum*	PGRC/E10306	Ethiopia- ARC13	189	*L. usitatissimum*	WL1410	Ethiopia- Shewa
139	*L. usitatissimum*	PI-523353	Ethiopia- ARC15	190	*L. usitatissimum*	WL1420	Ethiopia- Shewa
140	*L. usitatissimum*	PI-523353	Ethiopia- ARC15	191	*L. usitatissimum*	WL1380	Ethiopia- Shewa

Item №	Taxon	Accession number	Origin/source	Item №	Taxon	Accession number	Origin/source
141	*L. usitatissimum*	PI-523353	Ethiopia- ARC15	192	*L. usitatissimum*	WL1390	Ethiopia- Shewa
142	*L. usitatissimum*	R12-M20G’	Ethiopia- ARC17	193	*L. usitatissimum*	WL1400	Ethiopia- Shewa
143	*L. usitatissimum*	R12-M20G’	Ethiopia- ARC17	194	*L. usitatissimum*	WL1430	Ethiopia- Shewa
144	*L. usitatissimum*	R12-N27G’	Ethiopia- ARC19	195	*L. usitatissimum*	WL1440	Ethiopia- Shewa
145	*L. usitatissimum*	Tole	Ethiopia- ARC20	196	*L. usitatissimum*	WL1450	Ethiopia- Tigray
146	*L. usitatissimum*	Geregera -Adet	Ethiopia- ARC21	197	*L. usitatissimum*	WL1460	Ethiopia- Tigray
147	*L. usitatissimum*	WL1010	Ethiopia- Arsi	198	*L. usitatissimum*	WL1470	Ethiopia- Tigray
148	*L. usitatissimum*	WL1020	Ethiopia- Arsi	199	*L. usitatissimum*	WL1480	Ethiopia- Tigray
149	*L. usitatissimum*	WL1030	Ethiopia- Arsi	200	*L. usitatissimum*	WL1500	Ethiopia- Tigray
150	*L. usitatissimum*	WL1040	Ethiopia- Arsi	201	*L. usitatissimum*	WL1510	Ethiopia- Tigray
151	*L. usitatissimum*	WL1050	Ethiopia- Arsi	202	*L. usitatissimum*	WL1520	Ethiopia- Tigray
152	*L. usitatissimum*	WL1060	Ethiopia- Bale	203	*L. usitatissimum*	WL1530	Ethiopia- Tigray
153	*L. usitatissimum*	WL1070	Ethiopia- Bale				

Note: Cultivated *Linum* accession numbered from 10064- 243819 have PGRC/ET prefix or tag

Table TS2

Marker Category	Name	Repeat motif	Primer sequence (5'-3')	Ta (^{o}C)	Sources (references)
IRAP	Sukula ➡		GATAGGGTCGCATCTTGGGCGTGAC	48	Teo *et al.* (36); Saeidi *et al.* (32); Alavi-Kia *et al.* (2008)
IRAP	Nikita ➡		CGCATTTGTTCAAGCCTAAACC	44	Teo *et al.* (36); Saeidi *et al.* (32); Alavi-Kia *et al.* (2008)
IRAP	3'LTR ➡		TGTTTCCCATGCGACGTTCCCCAACA	48	Teo *et al.* (36); Saeidi *et al.* (32); Alavi-Kia *et al.* (2008)
ISSR	3PCT2	(CT)15	5' VRV (CT)15	60	Wiesner *et al.* (37); Wiesnerova and Wiesner (23)

➡Arrows indicate primer direction; V = A+C+G, R = A+G nucleotides

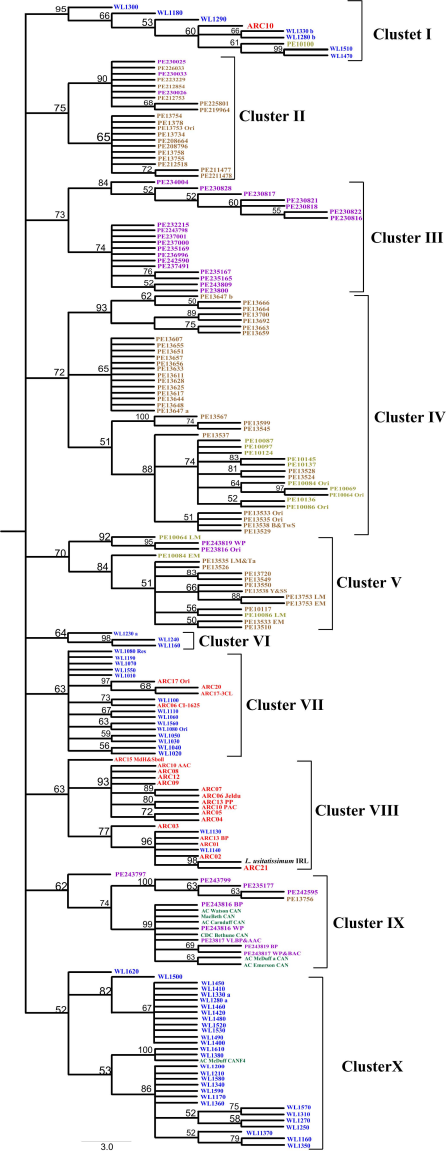
Table S3

S3a. Distributions among 13 clusters

Cluster Nº	Accessions in the cluster and their number	Approximate group name	Cluster Nº	Accessions in the cluster and their number	Approximate group name
I	1 accession: *L. trigynum*	Wild *Linum* from Sec. Linopsis	**VIII**	19 accessions: 15 PGRC/E23xxxx and 4 PGRC/E24xxxx	Late or third phase IBC/ETH collections
II	2 accessios: *L. volkensii* and *L. hirsutum*	Wild *Linum* from Secs. Linopsis and Dasylinum	**IX**	40 accessions: 30 PGRC/E13xxx; 6 PGRC/E100xx and 4 PGRC/E10xxx	Early or first phase IBC/ETH collections
III	20 accession: 6 PGRC/E21xxxx; 6 PGRC/E137xx; 4 PGRC/E2300xx; 2 PGRC/E208xxx; 1 PGRC/E223229 and 1 PGRC/E226033	Middle period or second phase IBC/ETH collections	**X**	4 accessions: 2 *L. austriacum*; 1 *L.narbonense* and 1 *L. flavum*	Wild *Linum* from Secs. Linum and Syllinum
IV	9 accessions: 7 WLxxxx; ARC19 and PGRC/E10100	On-farm holdings from Gondar and Tigray local farmers	**XI**	4 accessions: all *L. bienne* from three countries	Wild *Linum* from Sec. Linum
V	16 accession: 10 PGRC/E13xxx; 3 PGRC/E100xx; 2 PGRC/E24xxxx and PGRC/E10117	Middle period or second phase IBC/ETH collections	**XII**	33 accessions: 32 WLxxxx and 1 Canadian (duplication from McDuff line)	On-farm holdings from Gondar, Shewa and Tigray local farmers
VI	20 accessions: 15 WLxxxx and 5 ARCxx	On-farm holdings from Aris and Bale local farmers	**XIII**	15 accessions: all the 6 Canadian lines; 7 PGRC/E24xxxx; PGRC/E235177; and PGRC/E13756	Canadian lines and last EBI/ETH collections
VII	19 accessions: 16 ARCxx linses; 2WLxxxx collections; and 1 Irish accession	Agricultural Research Centre lines			

S3b. Distributions among the 10 clusters

Cluster Nº	Accessions in the cluster and their number	Approximate group name	Cluster Nº	Accessions in the cluster and their number	Approximate group name
I	9 accession: 5 WLxxxx from Gondar and 2 from Tigray; ARC19; and PRGC/E10100	On-farm holdings from Gondar and Tigray local farmers	**VI**	3 accession: WL1230a, WL1240, and WL1160	On-farm holdings from Gondar local farmers
II	20 accessions: 6 PGRC/E21xxxx; 6 PGRC/E13xxx; 3 PGRC/E22xxxx; 3 PGRC/E2300xx and 2 PGRC/E20xxxx	Middle period or second phase IBC/ETH collections	**VII**	18 accession: all 9 WLxxxx from bale; all 5 WLxxxx from Arsi; 3 ARCxx; and 1 WLxxxx from Gondar	On-farm holdings from Bale and Arsi local farmers
III	19 accessions: 15 PRGC/E23xxxx; and 4 PRGC/E243xxx	Late or third phase IBC/ETH collections	**VIII**	19 accession: 16 ARCxx; 2 WLxxxx from Gondar regions; and the Irish linseed	Agricultural Research Centre lines
IV	40 accessions: 30 PGRC/E13xxx and 10 PGRC/E10xxx	Early or first phase IBC/ETH collections	**IX**	16 accessions: 6 Canadian; 8 PGRC/E24xxxx; PGRC/E235177; and PGRC/E13756	Canadian lines and last IBC/ETH collections
V	16 accession: 10 PGRC/E13xxx; 4 PGRC/E10xxx; and 2 PGRC/E24xxxx	Early or first phase IBC/ETH collections	**X**	30 accessions: 19 WLxxxx from Gondar; 5 from Tigray; 5 from Shewa; and 1 Canadian [duplicated]	On-farm holdings from Gondar, Shewa and Tigray local farmers


Clustet I
WL1300
WL1180
WL1290
ARC10
WL1330 b
WL1280 b
PE10100
WL1510
WL1470
Cluster II
PE230025
PE226033
PE230033
PE223229
PE212854
PE230026
PE212753
PE225801
PE219964
PE13754
PE1378
PE13753 Ori
PE13734
PE208664
PE208796
PE13758
PE13755
PE212518
PE211477
PE2211478
Cluster III
PE234004
PE230828
PE230817
PE230821
PE230818
PE230822
PE230816
PE232215
PE2243798
PE237001
PE237000
PE235169
PE236996
PE242590
PE237491
PE235167
PE235165
PE243809
PE23800
Cluster IV
PE13647 b
PE13666
PE13664
PE13700
PE13692
PE13663
PE13659
PE13607
PE13655
PE13651
PE13657
PE13656
PE13633
PE13611
PE13628
PE13625
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PE13644
PE13648
PE13647 a
PE13567
PE13599
PE13545
PE13537
PE10087
PE10097
PE10124
PE10145
PE10137
PE13528
PE13524
PE10084 Ori
PE10069
PE10064 Ori
PE10136
PE10086 Ori
PE13533 Ori
PE13535 Ori
PE13538 B&TwS
PE13529
Cluster V
PE10064 LM
PE243819 WP
PE23816 Ori
PE10084 EM
PE13535 LM&Ta
PE13526
PE13720
PE13549
PE13550
PE13538 Y&SS
PE13753 LM
PE13753 EM
PE10117
PE10086 LM
PE13533 EM
PE13510
Cluster VI
WL1230 a
WL1240
WL1160
Cluster VII
WL1080 Res
WL1190
WL1070
WL1550
WL1010
ARC17 Ori
ARC20
ARC17-3CL
WL1100
ARC06 CI-1625
WL1110
WL1060
WL1560
WL1080 Ori
WL1050
WL1030
WL1040
WL1020
Cluster VIII
ARC15 MdH&Sboll
ARC10 AAC
ARC08
ARC12
ARC09
ARC07
ARC06 Jeldu
ARC13 PP
ARC10 PAC
ARC05
ARC04
ARC03
WL1130
ARC13 BP
ARC01
WL1140
ARC02
L. usitatissimum IRL
ARC21
Cluster IX
PE243797
PE243799
PE235177
PE242595
PE13756
PE243816 BP
AC Watson CAN
MacBeth CAN
AC Carnduff CAN
PE243816 WP
CDC Bethune CAN
PE23817 VLBP&AAC
PE243819 BP
PE243817 WP&BAC
AC McDuff a CAN
AC Emerson CAN
ClusterX
WL1620
WL1500
WL1450
WL1410
WL1330 a
WL1280 a
WL1460
WL1420
WL1480
WL1520
WL1530
WL1490
WL1400
WL1610
WL1380
AC McDuff CANF4
WL1200
WL1210
WL1580
WL1340
WL1590
WL1170
WL1360
WL1570
WL1310
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WL1250
WL11370
WL1160
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