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ORIGINAL ARTICLE

# Inter Simple Sequence Repeat Analysis of Genetic Diversity and Relationship in Four Egyptian Flaxseed Genotypes

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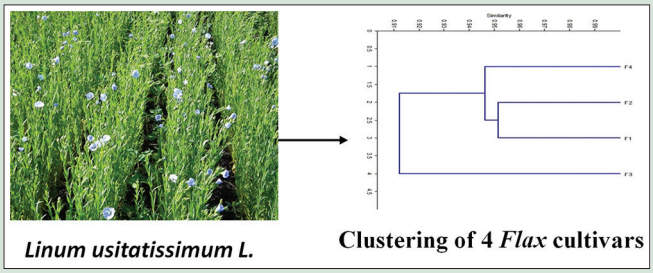
## ABSTRACT

**Background**: Flaxseed is a highly important industrial and medicinal plant worldwide. **Objective**: To use inter simple sequence repeat (ISSR) technique for making unique fingerprint for the four newly produced genotypes of flax in Egypt. **Materials and Methods**: The genetic diversity among four promising Egyptian flax (*Linum usitatissimum* L.) genotypes was premeditated by means of polymerase chain reaction-based ISSR markers. The phenotypic variation among the four flax genotypes, namely, promising strains 533/39/5/3 (F1), S.402/3/3/7 (F2), S.421/3/6/4 (F3), and S.11 (F4) was studied during the two successive winter seasons of 2014/2015 and 2015/2016 in randomized complete block design through four replications. **Results**: The promising strain (F3) surpassed the other flax genotypes regarding seed yield/feddan, oil yield/feddan, and oil percentage. Twelve ISSR primers were used for the genetic examination yielding 139 loci, of which 31 were polymorphic. The middling number of amplified loci and the middling number of polymorphic loci per primer were 11.6 and 2.6, correspondingly, while the percent of loci polymorphism ranged from 0.0% to 58.0% with a middling of 21.4% crosswise all the flax genotypes. The more informative primers were GAC $(GATA)_4$ and $(GATA)_4$ GC, while the less informative were $(AC)_8T$ and $(GT)_8G$. Unweighted pair group method with arithmetic mean derived dendrogram clearly discriminated the flax genotypes in three clusters. The Jaccard's similarity coefficient along with the genotypes ranged from 0.91 to 0.95. **Conclusion**: This study identified S. 421/3/6/4 (F3) strain to be the mainly assorted genotype and recommended its use in propagation programs and for upward mapping populations.

**Key words:** Cluster analysis, genotypes, linseed, molecular marker, polymorphism

## SUMMARY

Four promising Egyptian flax (*Linum usitatissimum* L.) genotypes was studied using ISSR. The more informative primers were GAC$(GATA)_4$ and $(GATA)_4$ GC. The strain S. 421/3/6/4 (F3) was identified as the most diverse genotype.




**Abbreviations Used:** CTAB: N-cetyl-N,N,N-trimethylammonium bromide; EDTA: ethylenediaminetetraacetic acid; ISSR: Inter Simple Sequence Repeat; PCR: polymerase chain reaction; RAPDs: random amplified polymorphic DNAs; RCBD: Randomized Complete Block Design; UPGMA: Unweighted Pair Group Method with Arithmetic Mean; SSR: simple sequence repeat.

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## INTRODUCTION

Family Linaceae encompass 22 genera, of which genus *Linum* is the majority well known. This genus includes the refined species *Linum usitatissimum* and the ornamentals *Linum grandiflorum* and *Linum perenne*. However, the concluding two species are of little economic importance.[1]

Flax (*L. usitatissimum* subsp. *usitatissimum*) is one of the beginning crops in Egypt cultivation is a diploid, annual plant variety, which is mainly measured to be inbreeding. Ever since the domestication of flax, there has been a partiality for mounting it either for its fiber or oil. The twofold idea of flax was already known in ancient Egypt, linen (consequent from the fiber) was used for covering the regal mummies, and as well linseed oil was old to embalm the bodies of late Pharaohs.[2]

Over the last two decades, there has been rehabilitated attention in the use of flaxseed for its fiber, oil, and functional food production.[3] Being a highly industrial and medicinal plant, the worldwide demand of flaxseed species is increasing day by day; therefore, more researches have been conducted in the development of linseed genotypes. Miscellany assessment of flax was former attempted by means of morphological parameters[4] and isozyme markers.[5]

Molecular categorization of flax germplasm has been finished through various molecular techniques to assess genetic diversity of the cultured flax and to inspect evolutionary dealings of undomesticated flax species.[6] The use of DNA-based markers to learn flax diversity was early reported by Oh *et al.* (2000).[4] Among the molecular markers, microsatellites called simple sequence repeat (SSR) markers were the most suitable for several applications because of the effortlessness in



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handling, reproducibility, co-dominant inheritance, and genome-wide coverage.[7] Inter SSR (ISSR) region can be amplified using different protocols. Protocol of ISSR-polymerase chain reaction (PCR) for agarose gel electrophoresis is a fast and efficient technique for standardizing primers and quick polymorphism testing including mapping. Microsatellites are highly useful markers in cultivars identification as they have been shown to be highly polymorphic and genotype specific. Thus, ISSR technique is highly robust and reproducible; hence, it was rapidly replacing random amplified polymorphic DNAs (RAPDs) as a tool for genotype segregation.[8]

Other studies were carried out on genetic assortment of flax varieties through RAPD technique. DNA (RAPD) markers were used to cram multiplicity in 61 flax varieties including Canadian cultivars and landraces, where low genetic unpredictability was reported.[9] RAPD markers were also used to analyze the genetic difference, genetic attrition, and connection in 54 North American flax cultivars.[10] Furthermore, phenotypic and RAPD disparity inside four infraspecific groups of flax (dehiscent flax, fiber flax, large-seeded flax, and intermediate flax) was studied to recognize phenotypic and genotypic demarcation within the cultivated gene pool.[11]

The ISSR practice for flax fingerprinting was optimized using re-amplification technique and through arithmetical correlation of free energy of dissociation of ISSR primers.[12,13] An ISSR primer assay was reported in the cram of flax germplasm.[14,15] The molecular genetic analysis realized by the ISSR method made it possible to obtain the objective data on genetic relations between flax genotypes.[15] Fingerprinting the marketable flax genotypes based on molecular markers is a crucial measure for unambiguous and quick identification of similar or closely related genotypes.

Flaxseed oil is becoming increasingly popular as a nutritional and functional food in the Western world due to its high content of therapeutic health-promoting substances such as omega-3 fatty acid, soluble and insoluble fiber, and lignans, and its suitability for use in bread, breakfast cereals, muesli bars, and other food products.[16] Flaxseed straw contains bast fibers which can be used for the production of paper, coarse textiles, rope, fiber board, molded panels, and as insulation material.[17,18] Hence, there has been increased interest in 1 breeding and growing dual-purpose linseed cultivars which can be harvested for both seed and fiber.[19]

In the current study, we planned to investigate the difference between the commercial genotype Sakha 1 and the four promising flax genotypes concerning fiber and oil yields. In addition, the ISSR technique was chosen for making unique fingerprint for the four newly produced genotypes of flax to evaluate the genetic diversity among them, with the aim of providing facts and tools to increase the assortment for future flax propagation and assisting in developing and planning breeding strategies for crop improvement programs in Egypt.

# MATERIALS AND METHODS

## Plant germplasm and phenotyping

Two field experiments were conducted at Giza Agriculture Research Station, Giza Governorate, Egypt, during 2014/2015 and 2015/2016 winter seasons to study the performance of five flax genotypes concerning straw, fiber seed, and oil yields as well as their related characters. The genotypes included the local cultivar Sakha 1 as well as the promising strains 533/39/5/3 (F1), S.402/3/3/7 (F2), S.421/3/6/4 (F3), and S. 11 (F4) [Table 1]. The experiments trails were arranged in a randomized complete block design with four replications. Sowing date was in the first week of November in both seasons; the plot size was 10.5 $m^2$. Plant density of 2500 seeds/$m^2$ was used and seeds were broadcasted regularly within each plot. Normal cultural practices for flax production as recommended were followed.[20] At maturity, straw, seed, fiber, and oil yields/fad were calculated from the hole plot area basis. The seeds of the four promising flax genotypes are held in reserve in the herbarium of the Pharmacognosy Department, College of Pharmacy, Cairo University as Voucher specimen no. 2092016. Samples of the leaves, obtained from seedlings of the germinated seeds, were stored at −70°C, freeze-dried, and ground to a well grind using a coffee chopper before DNA isolation. Studied characters were straw yield/feddan (t.); seed yield/feddan (kg); fiber yield/feddan (kg); oil yield feddan; fiber percentage (%); and oil percentage (%).

**Table 1:** Pedigree, classification, and characteristic of four Egyptian flax genotypes

| Genotype | Type | Pedigree |
|---|---|---|
| Sakha 1 | Dual | Bombay X I.1485 |
| 533/39/5/3 (F1) | Fiber | S.420/140/5/10 X Bombay |
| S.402/3/3/7 (F2) | Dual | 235 X Giza 5 |
| S.421/3/6/4 (F3) | Dual | S.162/12 X S. 6/2 |
| S.11 (F4) | Fiber | H. (420/140/5/10 X S, 401/2) X I, 1563 |

## Materials for DNA mapping

Buffers: The following buffers were used: Extraction buffer: 0.7 MNaCl, 100 mM Tris (pH 7.5), 0.01 M ethylenediaminetetraacetic acid (EDTA), 1% (w/v) N-cetyl-N, N, N-trimethylammonium bromide (CTAB), and 1% (v/v) β-mercaptoethanol (added immediately before use); washing buffer: 1:76% ethanol, 0.2 M Na-acetate; 2:76% ethanol as washing buffer, 10 mM $NH_4$ O-acetate, TE-buffer; 10 mM tris (pH 8.0), 1 mM EDTA, ×10; reaction buffer: 100 mM tris (pH 8.3), 500 mM KCl, 0.01% (w/v) gelatin, chloroform/isoamyl alcohol 24:1 (v/v), isopropanol, d NTP, Taq DNA polymerase.

Primers: Twelve primers, ISSR (Applied Biosciences), were used in the detection of polymorphism and used in the present analysis [Table S1]. ISSR practice was carried out in triplicates using genomic DNA with 12 decamer primers for reproducibility of the consequences.

Molecular weight markers: 100 bp ladder (Promega Corporation, USA).

## Equipment

ADNA thermocycler (Hybaid PCR Express, USA) was used for the amplification of DNA, and agarose gel electrophoresis implement (Biorad Wide Mini Sub Cell, USA) was used for the separation of ISSR fragments according to size and ultraviolet (UV) Polaroid camera used for the apparition of ISSR wreckage.

## Methods for molecular investigations

### *DNA extraction*

DNA analysis was conducted at Food Technology Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Giza, Egypt, in 2016. DNA was extracted using CTAB method.[21] Fifty milligrams of frozen leaf were pulverized in liquid nitrogen, extracted with 0.8 ml CTAB, and precipitated with isopropanol.

### *Assessment of DNA deliberation*

DNA concentration was determined by diluting the DNA 1:5 in distilled $H_2O$. The DNA samples were electrophoresed in 1% agarose gel against 10 μg of a DNA size marker. This marker covers a range of concentration between 95 ng and 11 ng. Thus, valuation of the DNA concentration in a prearranged sample was achieved by comparing the intensity of fluorescence of the unknown DNA band with the dissimilar bands in the DNA size marker.

### *Magnification of inter simple sequence repeat markers*

The PCRs were conceded out using 100 ng of genomic DNA template subsequent a thermal cyclic program.[22]

### *Thermocyling profile*

Magnification of PCR was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems, USA) automatic to accomplish 35 cycles later than an early denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 45°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer expansion segment was wholesale to 7 min at 72°C in the closing cycle. The augmentation products were determined by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1 × TBE buffer at 95 volts. A 1 kb DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (Bio-Rad 2000, Germany).

### *Inter simple sequence repeat – polymerase chain reaction analysis*

DNA was extracted from fresh leaves using the QiagenDNeasy kit. PCR magnification was performed in a Perkin-Elmer/GeneAmp® PCR system 9700 (PE Applied Biosystems, USA). Intensification products were determined by electrophoresis, visually examined and scored for the occurrence (1) or absence (0) of DNA bands. The resemblance matrix was obtained through the cluster analysis of data using unweighted pair-group method with arithmetic average (UPGMA). For estimating genetic distance among the tested samples, each of DNA bands was treated as a unit character.[23] A dendrogram was constructed using the UPGMA with the SAHN module of NTSYS-pc to show a phenetic representation of genetic relationships as exposed by the similarity coefficient.[24]

## Data analysis

Every data were statistically analyzed by the study of variance method according to Snedecor and Cochran.[25] Differences between means were tested by least significant difference at the level of 0.05. Bartlett test of homogeneity was adapted indicating no statistical evidence for heterogeneity, thus combined analysis of variance (ANOVA) for genotypes over seasons was worked out according to Le Clerg *et al.*[26]

The banding patterns generated by ISSR-PCR marker analyses were scored as present (1) or absent (0), each of which was treated as a sovereign character in spite of its intensity. Only major and reproducible bands obtained for each ISSR primer were measured. By comparing the banding patterns of species for a primer, species-specific bands were identified. Faint or indistinct bands were not measured.

# RESULTS

## Phenotypic variations, correlations, and analysis of variance for the four genotypes

Statistical analysis of all phenotypic data was conducted to account for the phenotypic variation characters of the four promising Egyptian flax genotypes [Table 1]. Large phenotypic variations were observed concerning the yields (mean values) of straw, seed, fiber, and oil/feddan (fed.) as well as fiber and oil percentages. Results obtained from the combined analysis over the two winter seasons 2014–2015 and 2015–2016 are presented in [Table 2]. Data offered in Table 2 revealed significant differences among the four flax genotypes regarding all the six characters (straw yield/fed., seed yield/fed., fiber yield/fed., oil yield/fed., and fiber and oil percentage). ANOVA indicated that the promising strain S. 533/39/5/3 (F1) ranked first in the yields of straw, fiber, and fiber percentage with values of 4.70 T/fed., 814.35 kg/fed., and 18.30%, respectively. The superiority ratios of S. 533/39/5/3 (F1) over Sakha 1 were 28.77%, 36.40%, and 11.86% for the previously mentioned characters, respectively.

On the other hand, the promising strain 421/3/6/4 (F3) gave the maximum estimates for seed yield (743.00 kg/fed.), oil (304.85 kg/fed.), and oil percentage (41.03%); the superiority ratios of S. 421/3/6/4 (F3) over the variety Sakha 1 were 17.94%, 21.89%, and 3.35% for seed kg/fed., oil kg/fed., and oil percentage, respectively. The other flax genotypes laid intermediate position between highest estimates and the lowest one.

## Polymorphism revealed by inter simple sequence repeat analysis

The banding profile of the four promising genotypes of flax produced by the 12random primers is illustrated in Tables 3-5 and Figure 1a-e. ISSR bands were treated as there or absent, exclusive of considering their percentage. The middling number of augmented loci and the middling number of polymorphic loci per primer were 11.6 and 2.6, respectively, as the percent of loci polymorphism ranged from 0.0% to 58.0% with an average of 21.4% crossways all the flax genotypes. An entirety of 139 dissimilar fragments have been recorded and formed mainly by six of the 12 used primers, showing 14 bands by primer ISSR-2 ranging from 1.15 to 0.15 Kbp, 12 bands by primer ISSR-5 ranging from 0.85 to 0.17 Kbp, and 11 bands by primer ISSR-3 and ISSR-10 ranging from 1.20 to 0.21 and 1.45, 0.70 Kbp, respectively. On the other hand, primers ISSR-1, ISSR-4, and ISSR-8 produced 10 bands. Moreover, primers R-5, R-6, and ISSR-6 produced only 7 and 6 bands, respectively. The analysis of ISSR-PCR data can thus select the use of primers ISSR-2, ISSR-5, ISSR-3, and ISSR-10 for the selective discrimination of flax genotypes from other commercial varieties. These primers may be used as an indicator for obtaining genetic markers. Out of 139 loci detected, the polymorphic, monomorphic, and unique loci were 31, 108, and 13, respectively. All flax cultivars were discriminated by the presence or absence of unique piece in ISSR outline.

**Table 2:** Mean values of straw, seed, fiber, and oil yields, as well as fiber and oil percentages for five flax genotypes from the combined analysis over the two seasons (2014/2015) and (2015/2016)

| Genotype | Straw (ton/feddan) | Seed (kg/feddan) | Fiber (kg/feddan) | Oil (kg/feddan) | Fiber (%) | Oil (%) |
|---|---|---|---|---|---|---|
| Sakha 1 | 3.65 | 630.00 | 597.14 | 250.11 | 16.36 | 39.70 |
| S.533/39/5/3 (F1) | 4.70 | 470.00 | 814.35 | 174.37 | 18.30 | 37.10 |
| S.402/3/3/7 (F2) | 3.78 | 725.00 | 650.09 | 294.35 | 17.22 | 40.60 |
| S.421/3/6/4 (F3) | 3.95 | 743.00 | 687.30 | 304.85 | 17.40 | 41.03 |
| S.11 (F4) | 4.06 | 430.00 | 730.80 | 157.81 | 18.00 | 36.70 |

These results are means of four replications

## Cluster analysis

A dendrogram was generated based on the resemblance matrix by the UPGMA, in which the flax genotypes were grouped in three clusters. The Jaccard's similarity coefficient among the genotypes ranged from 0.91 to 0.95 [Figure 2].

# DISCUSSION

To go for a proper conservation, management, and selection of parental lines for large-scale cultivation, a prior knowledge on the existing genetic diversity is most important. For improvement of crop genetic resource, it is necessary to have continuous mixing of wild relatives and use of effective breeding methods. Considering the need of molecular characterization of the crop, studies were undertaken on DNA fingerprinting using ISSR and RAPD markers.[27]

Flax is a highly important crop valued for its fixed oil and fibers which are used in different industries, in addition to the medicinal importance of its lignans. To enrich the genetic pool of the species, screening and selection of genotypes are essential, and this can be only achieved through proper genetic diversity study. Several earlier works were done on different flax cultivars, but this is the first attempt to fingerprint the four promising flax genotypes derived from the main commercial variety Sakha 1 which is cultivated in Egypt. The DNAs from the fresh leaves of the four flax genotypes have been compared. Study of the degree and allotment of genetic diversity in harvest vegetation was performed for optimizing variety and breeding strategies.

ISSRs are arbitrary multilocus markers produced by PCR amplification with a single anchored microsatellite primer. They are beneficial because no genomic in order is necessary for their employ. This provides a suitable and rapid appraisal of the differences in the genetic composition of closely related individuals at the DNA level and has been working in a large number of plant species for categorization and estimation of genetic assortment because of their pace and simplicity in handling.[28] ISSR markers were successfully applied to evaluate genetic diversity and relations among 22 Canadian cultivars, 29 selected world cultivars and 10 landraces of flax.[9,29]

In the present research, ISSR analysis made it possible to detect the changeability in the majority of loci of different parts of the genome and obtain the objective evidence about the genetic relations between flax genotypes. The four promising genotypes of flax were subjected to realized analysis with the help of 12 effective random primers, ISSR-1, ISSR-2, ISSR-3, ISSR-4, ISSR-5, ISSR-6, ISSR-7, ISSR-8, ISSR-9, ISSR-10, R-5, and R-6. The number of ISSR-PCR fragments indicated that the 12 primers were reproduced. Each DNA band was treated as a unit character. There were 139 loci obtained randomly and distributed over genome and 108 common for all cultivars fragments of amplification, as well as 31 polymorphic fragments. The three primers 5`GAC $(GATA)_4$, 5`$(GATA)_4$GC, and 5`$(AGAC)_4$GC exhibited 58%, 50%, and 44% polymorphism.

The high level of polymorphism observed in this study indicated a elevated level of genetic variation among the 4 genotypes analyzed, these results were in accordance with Rakoczy-Trojanowska and Bolibok,[30] who reported highly polymorphic patterns when retort primers based on microsatellite sequences in plants were working. Our work was also in accordance with that reported by Blair *et al.*,[31] where primers with poly-GA motifs produce on average, a larger number of amplified loci. In the nearby effort, primers with 5' anchoring showed more monomorphic loci (seven from 12 primers) [Table S1]. These outcomes could be doable because primers anchoring in 5` include in the amplified creation the whole microsatellite sequence, and thus, the variability in the number of nucleotides inside a microsatellite repeat would consequence in length polymorphism when using a 5` anchored primer.[32]

The dendrogram obtained by the UPGMA method allowed the identification of two major clusters. The first one comprised F1 and F2 which had 95% similarity, whereas the subsequent comprised $F_1$, $F_2$, and $F_4$ which had 94% resemblance to each other. On the extra hand

**Table 3:** The sequence and names of inter simple sequence repeat primers used in the fingerprinting of the five Egyptian cultivars

| Name | Sequence | Primer | T° (°C) |
|---|---|---|---|
| ISSR-1 | 5'-AGAGAGAGAGAGAGAGC-3' | $(AG)_8C$ | 50 |
| ISSR-2 | 5'-AGAGAGAGAGAGAGAGG-3' | $(AG)_8G$ | 50 |
| ISSR-3 | 5'-ACACACACACACACACT-3' | $(AC)_8T$ | 51 |
| ISSR-4 | 5'-ACACACACACACACACG-3' | $(AC)_8G$ | 53 |
| ISSR-5 | 5'-GTGTGTGTGTGTGTGTG-3' | $(GT)_8G$ | 51 |
| ISSR-6 | 5'-CGCGATAGATAGATAGATA-3' | CGC $(GATA)_4$ | 49 |
| ISSR-7 | 5'-GACGATAGATAGATAGATA-3' | GAC $(GATA)_4$ | 58 |
| ISSR-8 | 5'-AGACAGACAGACAGACGC-3' | $(AGAC)_4GC$ | 55 |
| ISSR-9 | 5'-GATAGATAGATAGATAGC-3' | $(GATA)_4GC$ | 50 |
| ISSR-10 | 5'-GACAGACAGACAGACAAT-3' | $(GACA)_4AT$ | 49 |
| R-5 | 5′-ACACACACACACACACA-3' | $(AC)_8A$ | |
| R-6 | 5'-ACACACACACACACACC-3' | $(AC)_8C$ | |

A: Adenine; T: Thymine; G: Guanine; C: Cytosine; T°: Annealing temperature; °C: Centigrade degree; ISSR: Inter simple sequence repeat

**Table 4:** Degree of polymorphism and polymorphic information content for interspecies genetic relationship in 4 flax cultivars*

| Primers | Gel polymorphism | | | | | | |
|---|---|---|---|---|---|---|---|
| | TL | ML | PL polymorphic (without unique) | Unique bands | Polymorphic (with unique) | Polymorphism (%) | Mean of band frequency |
| $(AG)_8C$ | 12 | 10 | 0 | 2 | 2 | 17 | 0.9 |
| $(AG)_8G$ | 15 | 14 | 1 | 0 | 1 | 7 | 1.0 |
| $(AC)_8T$ | 11 | 11 | 0 | 0 | 0 | 0 | 1.0 |
| $(AC)_8G$ | 11 | 10 | 0 | 1 | 1 | 9 | 0.9 |
| $(GT)_8G$ | 12 | 12 | 0 | 0 | 0 | 0 | 1.0 |
| CGC $(GATA)_4$ | 8 | 6 | 0 | 2 | 2 | 25 | 0.8 |
| GAC $(GATA)_4$ | 12 | 5 | 6 | 1 | 7 | 58 | 0.7 |
| $(AGAC)_4GC$ | 18 | 10 | 5 | 3 | 8 | 44 | 0.8 |
| $(GATA)_4GC$ | 10 | 5 | 3 | 2 | 5 | 50 | 0.8 |
| $(GACA)_4AT$ | 14 | 11 | 2 | 1 | 3 | 21 | 0.9 |
| $(AC)_8A$ | 8 | 7 | 0 | 1 | 1 | 13 | 0.9 |
| $(AC)_8C$ | 8 | 7 | 1 | 0 | 1 | 13 | 0.9 |
| Total | 139 | 108 | 18 | 13 | 31 | | |
| Mean | 11.58 | 9 | 1.5 | 1.08 | 2.58 | 21.41 | |

*Flax genotypes: Namely, promising strains (F1) 533/39/5/3, (F2) S.402/3/3/7, (F3) S.421/3/6/4, and (F4) S.11. TL: Total loci; ML: Monomorphic loci; PL: Polymorphic loci; PIC: Polymorphic information content

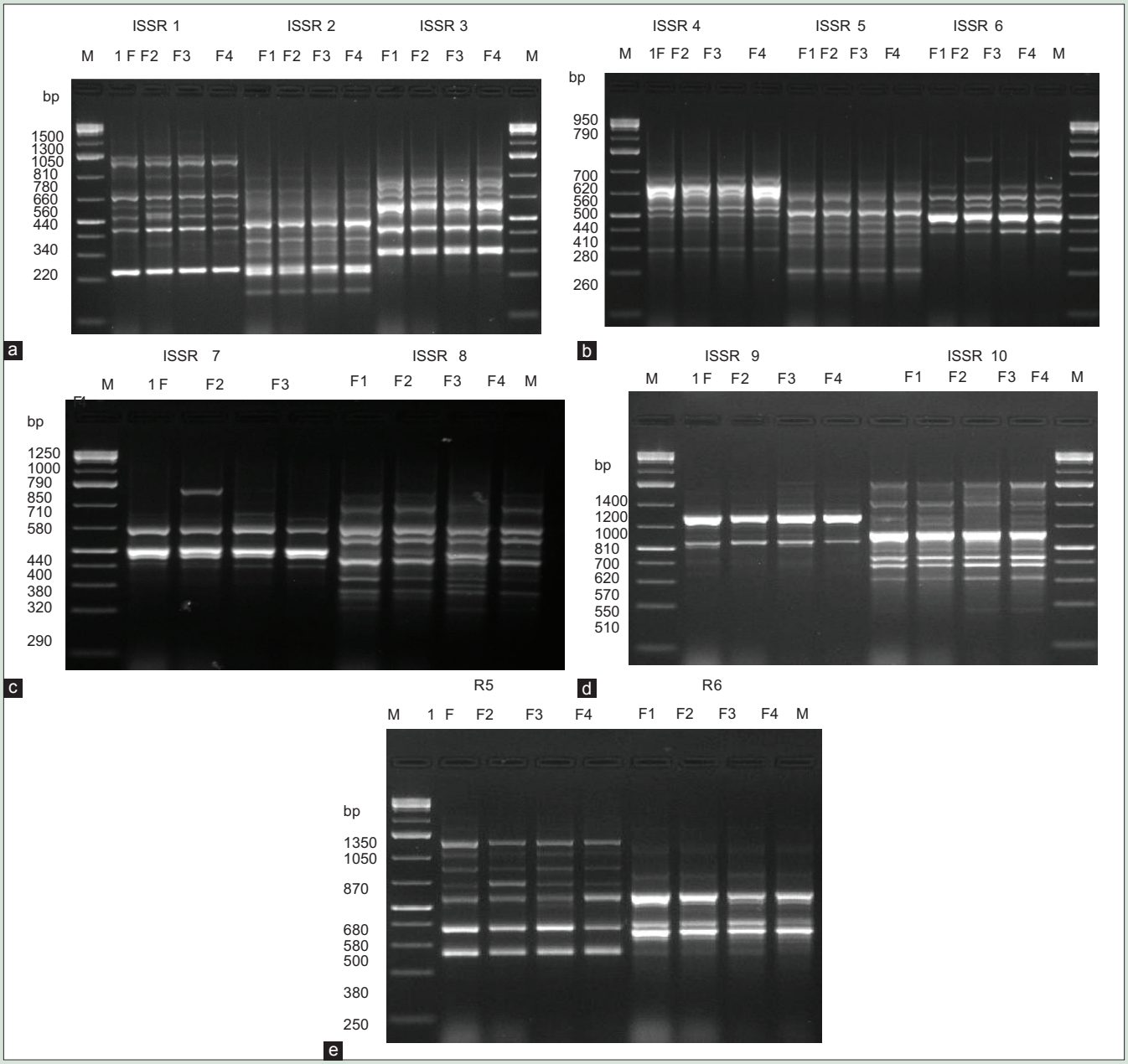


**Figure 1:** (a) Inter simple sequence repeat – polymerase chain reaction profiles were obtained using a primers inter simple sequence repeat 1, inter simple sequence repeat 2, and inter simple sequence repeat 3 in different flax cultivars F1=, F2=, F3=, F4 = respectively; (lanes: 1-4)=, Lane M = DNA ladder. Arrows show cultivers specific band. (b) inter simple sequence repeat – polymerase chain reaction profiles were obtained using a primers inter simple sequence repeat 4, inter simple sequence repeat 4, and inter simple sequence repeat 5 F1=, F2=, F3=, F4 = respectively; (lanes: 1-4) =, Lane M = DNA ladder. (c) inter simple sequence repeat – polymerase chain reaction profiles were obtained using a primers inter simple sequence repeat 7 and inter simple sequence repeat 8 F1=, F2=, F3=, F4 = respectively; (lanes: 1-4)=, Lane M = DNA ladder. (d) inter simple sequence repeat – polymerase chain reaction profiles were obtained using a primers inter simple sequence repeat 9 and inter simple sequence repeat 10 F1=, F2=, F3=, F4 = respectively; (lanes: 1-4)=, Lane M = DNA ladder. (e) Inter simple sequence repeat analysis carried out with primers R5 and R6 F1=, F2=, F3=, F4 = respectively; (lanes: 1-4)=, Lane M = DNA ladder

over, the third group comprised F3, which had 91% similarity to the second group.

The phenotypic variations of the four flax genotypes on the basis of the straw, seed, fiber, and oil yield have also supported the segregation among them. Where, F3 genotype exhibited the highest seed yield (743 Kg/fed.) as well as oil yield (304.85 Kg/fed.) and oil percent (41.03%). Industrially, flaxseed oil is an important ingredient in the manufacture of paint, varnish, and linoleum.[17] Flaxseed oil also contains α-linolenic acid, a polyunsaturated fatty acid that has nutritional and health benefits (Wood, 1997).[18] Our study therefore identified F3 to be the major assorted genotypes and recommended their use in breeding programs and for upward mapping populations.

The incidence of environmentally induced transmissible changes in definite flax varieties has been shown to be accompanied by changes in the genomic DNA.[33] Introduction of modern cultivars and using them in breeding is the only way to ensure the decrease in genetic variation.[34,35] Shrinking of the genetic diversity consequently reduces options to ensure diverse nutrition, to enhance food production and to face climate

**Table 5:** Genetic similarity matrix based on inter simple sequence repeat – polymerase chain reaction data among* four flax genotypes (F1–F4) estimated according to Jaccard's method

| | Similarity matrix | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| F1 | 100 | | | |
| F2 | 95 | 100 | | |
| F3 | 91 | 91 | 100 | |
| F4 | 94 | 95 | 91 | 100 |

*Flax genotypes: Namely, promising strains (F1) 533/39/5/3, (F2) S.402/3/3/7, (F3) S.421/3/6/4 and (F4) S.11

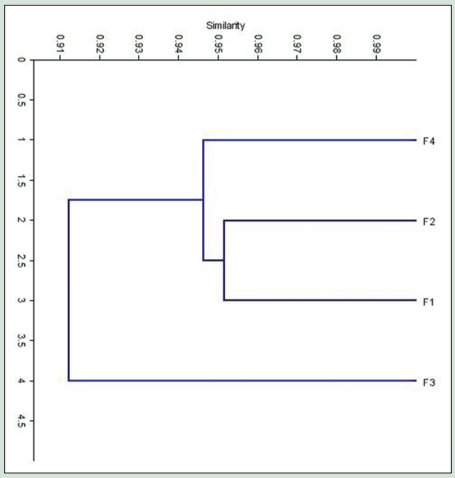


**Figure 2:** Unweighted pair-group method with arithmetic average dendrogram showing clustering of 4 flax cultivars based on inter simple sequence repeat – polymerase chain reaction data

change. Crop wild relatives hosted in gene bank collections have received some attention because they harbor untapped genetic variation available for domesticated crops.[36,37] Despite the few marker-trait associations identified, herein, we provided a proof of thought for brown flax functional variants as potentially useful for cultivated flax improvement.

In the present study, we demonstrated that the ISSR primers can be effectively working to evaluate the level of polymorphism and diversity in flax genotypes. The results obtained in this study legalize once more that ISSRs are useful markers in genetic variety studies, due to the very high polymorphism level detected by the primers. The possibility of classification of every individual examined offers a promising perception as a molecular tool for varietal recognition and breeding program applications.

## CONCLUSION

To the best of our awareness, this is the first exertion to judge genetic relationships among the Egyptian flax genotypes and to use molecular markers for categorization of the influential flax genotypes actively concerned in Egyptian flax breeding programs. These findings will be certainly helpful for the breeders in the selection of genotypes for the future breeding and improvement programs.

## Supporting information

Data of the unique and common bands for the four flax cultivars obtained with 12 primers in PCR amplification is available as supporting information.

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## Conflicts of interest

There are no conflicts of interest.

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**Table S1:** The unique and common bands for flax genotypes (F1–F4) obtained with 12 decamer primers in polymerase chain reaction amplification

| Primers | Cultivars (specific unique bands [bp]) | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| ISSR-1 [Figure 2a] | 1500 | 1500 | 1500 | 1500 |
| | 1300 | 1300 | 1300 | 1300 |
| | 1050 | 1050 | 1050 | 1050 |
| | 810 | 810 | 810 | 810 |
| | 780 | 780 | 780 | 780 |
| | 660 | 660 | 660 | 660 |
| | - | 590 | - | - |
| | 540 | 540 | 540 | 540 |
| | - | 510 | - | - |
| | 440 | 440 | 440 | 440 |
| | 340 | 340 | 340 | 340 |
| | 220 | 220 | 220 | 220 |
| ISSR-2 [Figure 2a] | 1150 | 1150 | 1150 | 1150 |
| | 1000 | 1000 | 1000 | 1000 |
| | 790 | 790 | 790 | 790 |
| | 690 | - | - | 690 |
| | 590 | 590 | 590 | 590 |
| | 510 | 510 | 510 | 510 |
| | 480 | 480 | 480 | 480 |
| | 420 | 420 | 420 | 420 |
| | 360 | 360 | 360 | 360 |
| | 320 | 320 | 320 | 320 |
| | 300 | 300 | 300 | 300 |
| | 280 | 280 | 280 | 280 |
| | 230 | 230 | 230 | 230 |
| | 210 | 210 | 210 | 210 |
| | 150 | 150 | 150 | 150 |
| ISSR-3 [Figure 2a] | 1200 | 1200 | 1200 | 1200 |
| | 940 | 940 | 940 | 940 |
| | 880 | 880 | 880 | 880 |
| | 730 | 730 | 730 | 730 |
| | 620 | 620 | 620 | 620 |
| | 580 | 580 | 580 | 580 |
| | 520 | 520 | 520 | 520 |
| | 420 | 420 | 420 | 420 |
| | 290 | 290 | 290 | 290 |
| | 270 | 270 | 270 | 270 |
| | 210 | 210 | 210 | 210 |
| ISSR-4 [Figure 2b] | 950 | 950 | 950 | 950 |
| | 790 | 790 | 790 | 790 |
| | 700 | 700 | 700 | 700 |
| | 620 | 620 | 620 | 620 |
| | 560 | 560 | 560 | 560 |
| | 500 | 500 | 500 | 500 |
| | 440 | 440 | 440 | 440 |
| | 410 | 410 | 410 | 410 |
| | - | - | 370 | - |
| | 280 | 280 | 280 | 280 |
| | 260 | 260 | 260 | 260 |
| ISSR-5 [Figure 2b] | 850 | 850 | 850 | 850 |
| | 730 | 730 | 730 | 730 |
| | 680 | 680 | 680 | 680 |
| | 510 | 510 | 510 | 510 |
| | 480 | 480 | 480 | 480 |
| | 430 | 430 | 430 | 430 |
| | 370 | 370 | 370 | 370 |
| | 330 | 330 | 330 | 330 |
| | 300 | 300 | 300 | 300 |
| | 270 | 270 | 270 | 270 |
| | 170 | 170 | 170 | 170 |

*Contd...*

**Table S1:** Contd...

| Primers | Cultivars (specific unique bands [bp]) | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| ISSR-6 [Figure 2b] | - | 1250 | - | - |
| | 790 | 790 | 790 | 790 |
| | 710 | 710 | 710 | 710 |
| | 600 | 600 | 600 | 600 |
| | 490 | 490 | 490 | 490 |
| | 460 | 460 | 460 | 460 |
| | 410 | - | - | - |
| | 390 | 390 | 390 | 390 |
| ISSR-7 [Figure 2c] | - | 1250 | 1250 | - |
| | - | - | 1000 | - |
| | 850 | - | 850 | - |
| | 790 | 790 | 790 | 790 |
| | 710 | 710 | 710 | 710 |
| | 580 | 580 | 580 | 580 |
| | 480 | 480 | 480 | 480 |
| | 440 | 440 | 440 | 440 |
| | 400 | 400 | - | - |
| | - | 380 | 380 | 380 |
| | 320 | 320 | - | - |
| | - | 290 | 290 | 290 |
| ISSR-8 [Figure 2d] | 1150 | 1150 | - | 1150 |
| | - | - | 960 | - |
| | 900 | 900 | - | 900 |
| | 860 | 860 | 860 | 860 |
| | 660 | 660 | 660 | 660 |
| | 640 | 640 | 640 | 640 |
| | 560 | 560 | 560 | 560 |
| | 460 | 460 | - | 460 |
| | 450 | 450 | 450 | 450 |
| | - | - | 440 | - |
| | 410 | 410 | 410 | 410 |
| | 380 | 380 | 380 | 380 |
| | 350 | 350 | 350 | 350 |
| | 310 | 310 | 310 | 310 |
| | - | - | 280 | - |
| | 260 | 260 | 260 | 260 |
| | 230 | 230 | - | - |
| | 200 | - | 200 | - |
| ISSR-9 [Figure 2d] | - | - | 1400 | - |
| | 1200 | 1200 | 1200 | 1200 |
| | 1000 | 1000 | 1000 | 1000 |
| | 810 | 810 | 810 | 810 |
| | 700 | 700 | 700 | 700 |
| | 620 | 620 | 620 | - |
| | - | - | - | 570 |
| | 550 | 550 | 550 | 550 |
| | 510 | - | 510 | - |
| | 380 | 380 | 380 | - |
| ISSR-10 [Figure 2d] | 1450 | 1450 | 1450 | 1450 |
| | - | - | 1150 | - |
| | 1050 | 1050 | 1050 | 1050 |
| | 950 | 950 | 950 | 950 |
| | 810 | 810 | - | 810 |
| | - | 750 | - | 750 |
| | 700 | 700 | 700 | 700 |
| | 590 | 590 | 590 | 590 |
| | 550 | 550 | 550 | 550 |
| | 470 | 470 | 470 | 470 |
| | 420 | 420 | 420 | 420 |
| | 370 | 370 | 370 | 370 |
| | 290 | 290 | 290 | 290 |
| | 170 | 170 | 170 | 170 |

*Contd...*

**Table S1:** Contd...

| Primers | Cultivars (specific unique bands [bp]) | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | F4 |
| R5 [Figure 2e] | 1350 | 1350 | 1350 | 1350 |
| | 1050 | 1050 | 1050 | 1050 |
| | 870 | 870 | 870 | 870 |
| | 680 | 565 | 565 | 565 |
| | 580 | 580 | 580 | 580 |
| | - | - | 500 | - |
| | 380 | 433 | 433 | 433 |
| | 250 | 369 | 369 | 369 |
| R6 [Figure 2e] | 820 | 820 | 820 | 820 |
| | 600 | 600 | 600 | 600 |
| | 550 | 550 | 550 | 550 |
| | 380 | 380 | 380 | 380 |
| | 340 | 340 | 340 | 340 |
| | - | - | 320 | 320 |
| | 300 | 300 | 300 | 300 |
| | 250 | 250 | 250 | 250 |

Flax genotypes: Namely, promising strains (F1) 533/39/5/3, (F2) S.402/3/3/7, (F3) S.421/3/6/4 and (F4) S.11. ISSR: Inter simple sequence repeat