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Identification of microsatellite markers for fingerprinting popular Indian flax (*Linum usitatissimum* L.) cultivars and their utilization in seed genetic purity assessments

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Abstract

Fingerprinting with molecular markers allows precise and rapid variety identification. The present investigation was carried out with the following objectives: (i) to identify informative SSR marker allele(s) for fingerprinting popular flax cultivars cultivated in Chhattisgarh state of India, and (ii) to validate the utility of the genotype specific SSR markers in seed genetic purity assessments. A set of 38 SSR markers located across the 30 chromosomes of flax were used for fingerprinting the selected flax cultivars, out of which 28 SSR markers were observed to be informative enough to distinguish the cultivars considered in the study. A set of two SSR markers (LU 1 and LU 7) exhibited unique alleles for four flax cultivars (Kartika, Deepika, Indira Alsi 32 and RLC 92) and could serve as molecular IDs (Identities) for these cultivars. To validate the utility of the SSR markers in genetic purity assessment, certified seed lots of Kartika, Deepika, Indira Alsi 32 and RLC 92 were assessed for their genetic purity using both SSR marker analysis and 18 morphological characters in a grow-out test (GOT). The impurities detected in the SSR marker analysis were 2-3% higher as compared to those detected based on morphological characters in GOT. The results indicated practical utility of the SSR markers in assessing the genetic purity of the flax cultivars.

Key words: DNA Fingerprinting; DUS; Flax; Genetic purity; Grow-out-test; Microsatellite marker; Multiplex-PCR.
Abbreviations: CTAB_Cetyl Trimethyl Ammonium Bromide, DUS_Distinctness Uniformity Stability, GOT_Grow-Out-Test, PAGE_Poly Acryl-amide Gel Electrophoresis, PCR_Polymerase Chain Reaction, PIC_Polymorphic Information Content, SPS_ Single Plant Selection, SSR_Simple Sequence Repeats, UPOV_Union for Protection of New Plant Varieties.

Introduction

Flax (*Linum usitatissimum* L.), also called common flax or linseed, is an annual herb, which is the third largest natural fibre crop and one of the five major oilseed crops in the world. Flax is a small size and self-pollinated herb that has been thought to be the model plant for the best fibre plants (Millam et al., 2005). High-quality seeds and elite cultivars play a crucial role in its production. However, since new cultivars normally arise from hybridizations between members of an elite group of genetically similar parents, the amount of genetic variability among newly developed cultivars is likely to become even smaller (Rahman et al., 2009), which makes it more difficult to unambiguously distinguish cultivars from the others with morphological characteristics and isozyme electrophoresis patterns because of influences by environmental factors. Fingerprinting with molecular markers allows precise, objective and rapid cultivar identification, which has been proved to be an efficient tool for crop germplasm characterization, collection and management. SSR markers have been widely used for genetic analysis and cultivar identification because of their abundance, co-dominance inheritance, high polymorphism, reproducibility, and ease of assay by PCR (Kuleung et al., 2004; Xie et al., 2011).

The genetic purity of varieties is conventionally determined by the grow-out test (GOT), which is based on assessment of morphological and floral characters called "descriptors" in plants grown to maturity. However, it is a time and resource consuming exercise, influenced by environmental factors and the results are often subjective. Sometimes, a new cultivar may also arise from hybridization between members of popular, but genetically close varietal groups. In this case, the amount of morphologically detectable genetic variability among the newly developed cultivars is likely to become even smaller. This will further complicate the task of unambiguously identifying new cultivars by the use of conventional characters alone. Biochemical methods such as, isozyme analysis and seed protein electrophoresis cannot discriminate closely related genotypes due to limited polymorphism and environmental influence and also they do not provide accurate estimates of genetic distances among cultivars (Ainsworth and Sharp, 1989). There are thus compelling reasons to develop more rapid and cost effective procedures, which can directly assess the seed genetic purity with greater accuracy. The above limitations can be managed effectively by employing molecular markers, particularly DNA based markers which can be assessed through the technique of PCR.

Table 1. Description of microsatellite markers used for identification of flax cultivars

S.No	Primers	Sequence 5'--> 3'	K	T_a (^{0}C)	Allele size (bp)	PIC value
SSR Primers						
1	LU 1	F:TCATTCATCTCCTTCCACTAAAA R:TTGAAAGCCCTAGTAGACACCA	4	58	146-179	0.750
2	LU 2	F:TCCGGACCCTTTCAATATCA R:AACTACCGCCGGTGATGA	2	58	125-150	0.375
3	LU 3	F: GCTCGTGATCTCCTTCATCC R: AAAACCACGTCCAGATGCTC	2	58	153-173	0.375
4	LU 4	F: TTATTTCCGGACCCTTTCAA R: AAACTACCGCCGGTGATGAT	2	58	150-155	0.375
5	LU 5	F:GTCACTGGGTGTGTGTTTGC R:AGCAGAAGAAGATGGCGAAA	2	58	134-150	0.375
6	LU 6	F:CCCCATTTCTACCATCTCCTT R:CAACAGCGGAACTGATGAAA	2	58	125-140	0.375
7	LU 7	F:CATCCAACAAAGGGTGGTG R:GGAACAAAGGGTAGCCATGA	4	58	134-154	0.750
8	LU 8	F:TCCCGTAATATTCTATGTTCTTCC R:TGAGTTGGACCTTACAAGACTCA	2	58	181-221	0.375
9	LU 9	F:TTGCGTGATTATCTGCTTCG R:ATGGCAGGTTCTGCTGTTTC	2	58	123-175	0.375
10	LU 10	F:GCCTAAAGCTGATGCGTTTC R:TGTCAGGCTCCTTCTTTTGC	2	58	141-148	0.375
11	LU 11	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	2	58	100-150	0.375
12	LU 13	F: AAGATGACGTCGGTGGTGAT R: CGGAACCTTCCATTTTCCTC	2	58	95-100	0.375
13	LU 14	F: GCTTGCGAGAAGAAGGAGAA R: TCACCAAAGGCATTCACAAA	2	58	141-150	0.375
14	LU 15	F:TGGACGACGATGAAGATGAA R:CCGCCGGGTACACTACTACT	2	58	114-120	0.375
15	LU 16	F: TTATTCTTGCCTGCCAATCG R: TCCAGCTCTTGCTCGTTCTT	2	58	153-167	0.375
16	LU 18	F:AGAGGCGGAGGGCATTAC R:TTGGAGAGTTGGAATCGAGA	2	58	140-150	0.375
17	LU 20	F:TTCAACCAGGCAAATTTCAA R:CAAGAAGAGGCCCAGAATTG	2	58	122-125	0.375
18	LU 21	F:AAGGGTGGTGGTGGGAAC R:GTTGGGGTGAAGAGGAACAA	3	58	135-150	0.625
19	LU 22	F:GATGGGGTTGAAGCCAGTAG R:CCCACCCCATCTATCATTTG	2	58	132-148	0.375
20	LU 24	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	2	58	95-138	0.375
21	LU 25	F:TCTACAGAGTTCAATTCCCGTAA R:GTTGGACCTTACAAGACTCACTG	3	58	200-250	0.625
22	LU 27	F:GTTTGAGAAGAGGGCATCCA R:GTTGGGGTGAAGAGGAACAA	2	58	152-173	0.375
23	LU 29	F:GGGCAGTGATTGATTGGTTT R:GGCGGCAATTGCTACATT	2	58	102-115	0.375
24	LU 31	F:TCTTTGTTTGGTGCCAAAGTT R:TTCATGATCTCACCTAACCTGA	2	58	102-117	0.375
25	LU 32	F:ACGCGTAAACTTTCCGTTTC R: ATAATGTCGGCTGCTTCTGC	2	58	120-125	0.375
26	LU 33	F:TTCTCCATCATCTCACATCCA R: CCAAATCAGAATGTGCGTGT	2	58	149-155	0.375
27	LU 34	F:GGAAGAATTGGAAGAGGAAGG R:CCTTCTCCCATGATCAAACAA	2	58	110-130	0.375
28	LU 35	F: CCAACGGATCATCCTCTAGC R: GGACAGAAAGGGGAAAGGAA	2	58	143-149	0.375

K: number of allele; T_a: annealing temperature; PIC: polymorphic information content.

Fig 1. Molecular profiles of 4 flax cultivars obtained with microsatellite marker LU 1. The numbers of lanes 1 to 20 correspond to the flax cultivars: Lane 1-5 — Kartika, Lane 6-10 — Deepika, Lane 11-15 — Indira Alsi 32, Lane 16-20 — RLC 92, M—50 bp DNA ladder.

Among the molecular markers, microsatellites also called Simple Sequence Repeat (SSR) markers are most suitable for many applications because of the ease in handling, reproducibility, co-dominant inheritance and genome-wide coverage (McCouch et al., 1997). The use of SSR markers for assessing genetic purity of parental lines and hybrids has been reported in agricultural crops like rice (Yashitola et al., 2002; Nandakumar et al., 2004; Sundaram et al., 2008), maize (Mingsheng et al., 2006) and sunflower (Antonova et al., 2006). Fingerprinting the commercial flax cultivars based on molecular markers is a crucial measure for unambiguous and quick identification of similar or closely-related cultivars. However, no study on fingerprinting of the main commercial flax cultivars under cultivation at present in Chhattisgarh state of India was reported up to now. The present study therefore aimed to identify informative microsatellite markers of flax which can serve as distinct molecular fingerprints/IDs for popular Indian flax cultivars and to validate their utility in monitoring seed genetic purity.

Results

DNA fingerprinting database and cultivar identification based on SSR analysis

An elementary DNA fingerprinting database of the main four commercial flax cultivars was successfully constructed based on the twenty-eight polymorphic SSR primer pairs. A set of 38 SSR markers located across the 30 chromosomes were employed for fingerprinting the chosen cultivars. Based on the polymorphic status, 28 SSR markers were found to be informative for discriminating the genotypes and showed a total of 61 alleles. The number of alleles amplified by each primer pair ranged from 2-4 with an average value of 2.17. The polymorphic information content (PIC) for these 28 SSR markers ranged from 0.37 to 0.75 with an average of 0.41. The number of alleles amplified by each of the polymorphic SSR markers along with their PIC values is given in Table 1. Based on the results of the present study, SSR markers selected could be considered as 'highly informative' as evident from their PIC values. Out of the 38 SSR markers employed for molecular fingerprinting, a set of 28 SSR markers were observed to be informative enough to distinguish cultivars considered in the study. The SSR markers LU 1 and LU 7 generated a maximum number of four alleles (Fig. 1 and Fig. 2) while, LU 21 and LU 25 exhibited three polymorphic alleles and the rest of markers generated only two alleles. Out of 28 SSR markers, a set of two markers (LU 1 and LU 7) exhibited unique alleles for four cultivars. These can serve as molecular IDs for these cultivars. These two markers could distinguish one cultivar from the other by amplification of a specific allele for that cultivar. Molecular IDs developed using the two SSR markers for the four cultivars and their specific allele for a cultivar are shown in Table 1. The graphical demonstration of most informative polymorphic markers is given in Fig. 3.

Genetic purity assessement through utilization of SSR markers and Grow-out-test

For genetic purity assessment using GOT, four flax cultivars (Kartika, Deepika, Indira Alsi 32 and RLC 92), for which unique 'molecular fingerprints' or 'molecular IDs' were available, were selected. Four hundred plants of each of the selected cultivars were studied to determine if they were true-to-type for eighteen morphological characters in GOT. Of the 18 morphological characters analyzed, seven characters (time of flowering, flower size, petal venation colour, anther colour, plant height, seed colour and seed weight) differentiated the impurities in certified seed lot. Among these, time of flowering and seed weight exhibited maximum variation (Table 2). The characters of few individuals showing deviation from the standard characters were identified as off-type. Genetic purity detected by morphological markers ranged from 85.4 to 100%. In the certified seed-lot of Deepika, a maximum of 14 impure plants were observed based on morphological characters (85.4% genetic purity), followed by Indira Alsi 32 with 7 impure plants (92.7% genetic purity), RLC 92 with 6 impure plants (93.7% genetic purity), whereas Kartika showed 94.8% genetic purity with a total of 5 impure plants with respect to the morphological characters. Genetic purity detected by molecular markers ranged from 83.3 to 100%. In the analysis using the genotype specific SSR markers LU 1 and LU 7, cultivar Deepika showed 16 impure plants (83% genetic purity) followed by Indira Alsi 32 with 10 impure plants (89.5% genetic purity). The cultivars Kartika and RLC 92, displayed 92.7% genetic purity (7 impure plants) each with respect to the markers LU 1and LU 7. The variants plants identified on the basis of morphological characters also showed variation on molecular basis. Similar observations were made in rice by (Kalaichelvan, 2009). Fig. 4 displays the genetic purity analysis using the marker LU 1 in a set of seeds belonging to the certified seed-lot of Deepika. The plants which were identified as impure based on GOT analysis were also identified as impure throgh SSR analysis. The results of the field grow-out test (GOT) and SSR marker test were comparable. The percentage of contaminants detected based on SSR marker analysis was higher than those detected by conventional GOT assay (Keshavulu, 2006). Molecular markers detected some additional impurities, which were not detected through morphological markers. For example in certified seed lots, some additional impure plants were detected based on SSR analysis, which was not detected by GOT analysis. This demonstrated the better discriminatory power and efficiency of SSR markers in genetic purity

Table 2. Comparison of seed genetic purity assessment on the basis of molecular and morphological markers.

S.No	Cultivars	Class of seed	Morphological Purity %				Molecular Purity %		
			Number of contaminants	Purity %	Impure plant numbers	Variation in morphological characters	Number of contaminants	Purity %	Impure plant numbers
1	Kartika	NSP-SPS Nucleus seed	0	100	-	----------	0	100	-
		CSSCA-Certified seed	5	94.8	9,20,37,55,76	Time of flowering and seed weight	7	92.7	9,20,37,55,76, 81,93
2	Deepika	NSP-SPS Nucleus seed	0	100	-	----------	0	100	-
		CSSCA-Certified seed	14	85.4	26,28,29,30,58, 59,60,61,62,63, 64,83,92,94	Time of flowering, flower size, petal venation colour, anther colour, plant height, seed colour and seed weight	16	83.3	26,27,28,29,30, 40,58,59,60,61, 62,63,64,83,92, 94
3	Indira Alsi 32	NSP-SPS Nucleus seed	0	100	-	----------	0	100	-
		CSSCA-Certified seed	7	92.7	7,13,47,54,79, 83,95	Time of flowering, petal venation colour, seed colour, and seed weight	10	89.5	7,13,30,41,47, 54,61,79,83,95
4	RLC 92	NSP-SPS Nucleus seed	0	100	-	----------	0	100	-
		CSSCA-Certified seed	6	93.7	5,21,33,49,61, 90	Time of flowering, plant height, anther colour and seed weight	7	92.7	5,21,33,49,61, 72,90

NSP- National Seed Project, CSSCA- Chhattisgarh State Seed Certification Agency

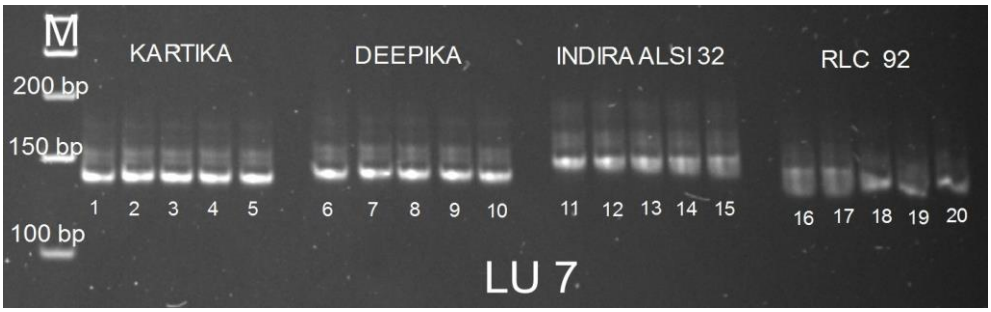

Fig 2. Molecular profiles of 4 flax cultivars obtained with microsatellite marker LU 7. The numbers of lanes 1 to 20 correspond to the flax cultivars: Lane 1-5 — Kartika, Lane 6-10 — Deepika, Lane 11-15 — Indira Alsi 32, Lane 16-20 — RLC 92, M—50 bp DNA ladder.

assessments and these markers could even accurately detect residual heterozygosity in the seed. Similar results have been reported by (Nandakumar et al., 2004; Yun et al., 2005; Sundaram et al., 2008). Based on the results obtained from this study, it is clear that there is a need to critically assess the genetic purity of popular cultivars of premium quality at each and every stage of seed multiplication and processing with the help of molecular markers so that the seeds cultivated by farmers are true-to-type and fetches premium price.

Multiplex-PCR

An important aspect of the present study is deployment of the strategy of PCR multiplexing for detecting genetic impurities in seed-lots of cultivars. PCR multiplexing is a cost saving strategy, wherein analysis can be carried out simultaneously using two or more markers in a single PCR with negligible addition to the total cost of assay and with enhanced accuracy (Fig. 5). Sometimes analysis using single markers (Yashitola et al., 2002; Nandakumar et al., 2004) may not help in accurate estimation of seed impurities in certain cases, and wherever possible, it is better to deploy more than one marker through multiplex PCR to get authentic results (Sundaram et al., 2008).

Discussion

This is the first attempt to fingerprints the four main commercial flax cultivars under cultivation at present in Chhattisgarh state of India using SSR markers, and the fingerprinting data obtained can be used for cultivar identification in practice. Distinctness, uniformity and stability (DUS) testing based on DNA markers can effectively augment the process of characterization of the cultivars (Singh et al., 2004). The increasing deployment of molecular markers in varietal profiling, characterization and plant variety identification indicates their utility in seed purity analysis. Molecular markers like SSRs, which are present in the functional regions of the genome, as demonstrated in the present study, can be effectively utilized as supplementary information for carrying out DUS tests in order to distinguish essentially derived varieties (EDVs) from the original varieties. Based on the results of the present study, it is suggested that the 28 hyper-variable SSR markers identified can be deployed as supplementary data in DUS testing of these flax cultivars. A set of two SSR markers (LU 1 and LU 7) clearly distinguished four flax varieties (Kartika, Deepika, Indira Alsi 32 and RLC 92). A set of molecular descriptors (i.e. molecular IDs) were constructed based on the 2 SSR markers for identifying all four cultivars analyzed in the present study. The four SSR markers can be considered highly informative due to their high PIC values and also due to their capability to distinguish closely related cultivars, which have a common pedigree. All the plants that were identified to be true-to-type in the marker-based assay were also identified to be true-to-type in a grow-out test, indicating that marker-based assays could be gainfully deployed for seed purity assessments. Results from the present study also demonstrate that carefully selected hyper-variable SSR markers are indeed highly useful for genetic purity assessment of flax cultivars as a supplement to the time consuming GOT. The procedure for seed genetic purity analysis with SSR markers is relatively simple and rapid and can be completed within a short time. A set of two or more highly-informative SSR markers (when analyzed through multiplex PCR) may be sufficient enough to distinguish closely related cultivars and assays based on such markers can be deployed for reliable estimation of genetic purity of the crop varieties, as the SSR markers represent functional regions of the crop genome (Sundaram et al., 2008). Interestingly, the percentage impurity detected through molecular marker analysis was 2-3% higher as compared to that detected in GOT. Similar observations have been reported by several workers (Nandakumar et al., 2004; Yun et al., 2005; Jaikishen et al., 2007; Sundaram et al., 2008) indicating that the markers selected in the study are indeed highly informative and can be highly useful in marker-based seed purity assessments (Yashitola et al., 2002; Sundaram et al., 2008).

Materials and Methods

Plant materials

The present study was carried out at Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Indira Gandhi Agricultural University (IGAU), Raipur, India during *Rabi* 2011-2012. The plant material for this study comprised of SPS nucleus seeds of four popular cultivars of flax (Table 3) collected from National Seed Project (NSP), IGAU, Raipur and certified seeds were collected from Chhattisgarh State Seed Certification Agency (CGSSCA), Raipur, India.

Genomic DNA extraction

Genomic DNA was extracted from young leaves of the flax plants according to the CTAB method as described by (Kang et al., 1998) with some modifications. The concentrations and quality of the genomic DNA samples were estimated on spectrophotometer ND-2000 (Nanodrop, USA). Finally, all the genomic DNA samples were diluted to a final concentration

Table 3. Parentage, special features of four flax cultivars.

Cultivars	Year of release	Parentage	Special features
Kartika	2005	Kiran × LCK 88062	Dwarf in height, light brown coloured seed, moderately resistant to wilt, powdery mildew and bud fly, oil content- 42.93%.
Deepika	2006	Kiran × Ayogi	Medium in height and early maturity, blue flower, brown seeded, resistant to powdery mildew, oil content- 41.39%.
Indira Alsi 32	2005	Kiran × RLC 29	Dwarf statured, blue flower, dark brown seeded, resistant to powdery mildew, oil content- 39.18%.
RLC 92	2008	Jeevan × LCK 9209	Tall in height, tinge blue flower, brown seeded, tolerant to bud fly and resistant to wilt, powdery mildew, oil content- 39%.

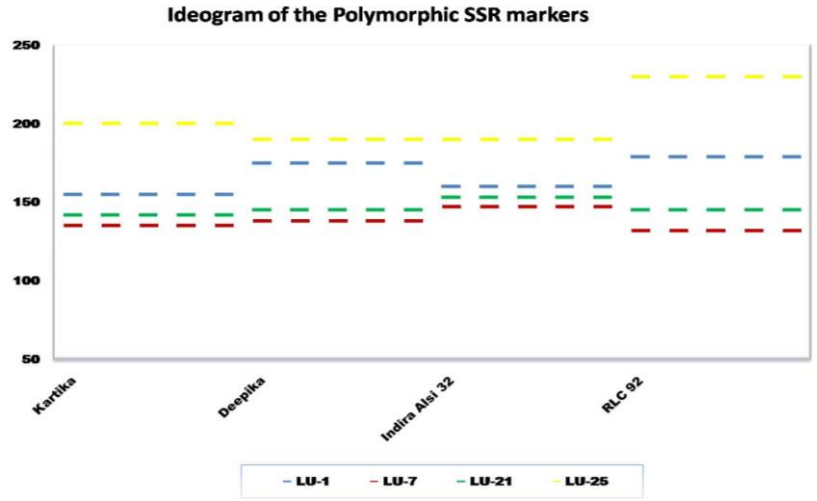

Fig 3. Graphical demonstration of SSR polymorphic markers. SSR markers LU 1 and LU 7 distinguished all four cultivars from each other while LU 21 and LU 25 markers distinguished three cultivars from each other.

Table 4. PCR components using SSR markers.

Components	Microsatellite (SSR) (in µl)
Nanopure water	13.5
10 X PCR Buffer	2
dNTPS 10mM	1
Primer (F) 5 pmol	0.5
Primer (R) 5 pmol	0.5
Taq 1U/ µl	0.5
Template DNA (40 ηg/µl)	2
Total volume	20

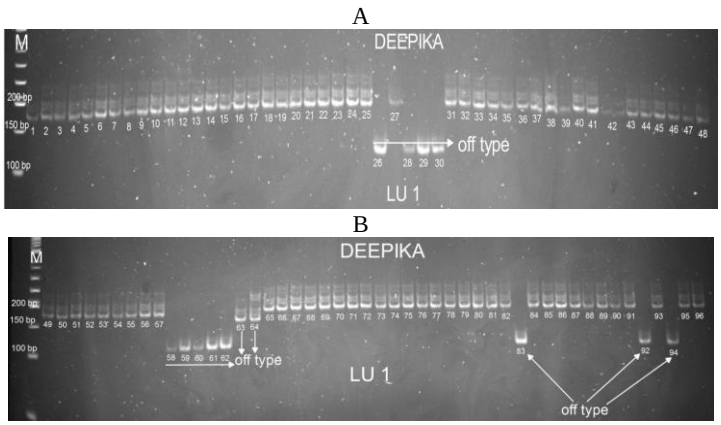

Fig 4. (a and b) Detection of impurities in the Indian flax cultivar-Deepika using the microsatellite marker LU 1. The numbers of lanes 1 to 96 correspond to the certified seed lot of Deepika cultivar. White arrow indicates impurities in the test samples as detected by the marker. M— 50 bp DNA ladder.

Table 5. Temperature profile used for PCR amplification of SSR markers.

Steps	Temperature (°C)	Duration (min.)	Cycles		Activity
1	95	4	1		Initial Denaturation
2	95	1			Denaturation
3	58	1			Annealing
4	72	1		32	Extension
5	72	7	1		Final Extension
6	4	24 hrs.			Storage

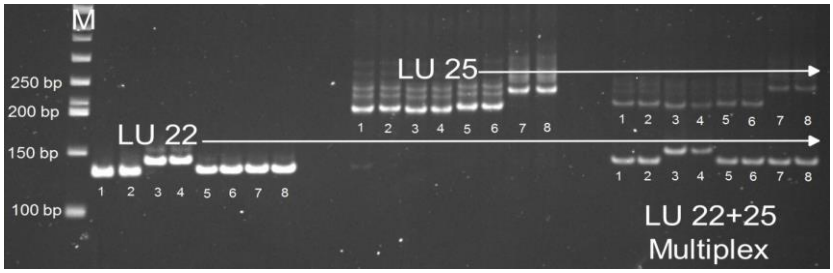

Fig 5. Multiplex PCR assay for distinguishing flax cultivars using the SSR markers LU 22 and LU 25. Lane 1-2 —Kartika, Lane 3-4 — Deepika, Lane 5-6 — Indira Alsi 32, Lane 7-8 — RLC 92. White arrow indicates the combination of both markers LU 22 and LU 25 in the same PCR assay. M—50 bp DNA ladder.

of 40 ng/µl with TE buffer (10 mM Tris-HC1, pH 8.0; 1 mM EDTA) and stored at -20^{0}C for further use.

SSR-PCR amplification

A total of thirty-eight flax SSR primers covering all the chromosomes of flax were used in this study. Polymerase chain reaction for amplifications of DNA preparations were carried out in a 20 µl volume for SSR (Table 4 and Table 5). All PCR reactions were carried out in a Veriti Thermal Cycler (Applied Biosystems, USA). PCR products were separated using 5% PAGE, stained with ethidium bromide and photographed under UV light using Image Gel Doc Lab$_{TM}$ software Version 2.0.1 (Bio-Rad, USA).

Data analysis

The band profiles were scored only for distinct, reproducible bands as present (1) or absent (0) for each SSR primer pair. The polymorphism information content (PIC) value of SSR markers was calculated using the following formula (Anderson et al., 1993).

$$PIC = 1 - \sum_{i=1}^{k} p_i^2$$

Where k is the total number of alleles (bands) detected for one SSR locus and P_i is the frequency of the i^{th} allele (band) in all the samples analyzed.

Genetic purity assessment using genotype specific molecular markers and through grow-out test (GOT)

For the purpose of validation of genotype specific SSRs in seed genetic purity assessments, four cultivars, *viz.*, Kartika, Deepika, Indira Alsi 32 and RLC 92 were used for molecular fingerprinting. Certified seed lots of these four cultivars, collected from the Chhattisgarh State Seed Certification Agency (CSSCA), Raipur, India were assessed for genetic purity using the genotype specific SSR markers and through GOT during the *Rabi* season 2011-12 at Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Agricultural University, Raipur, India using the 18 morphological characters as per the DUS guidelines on linseed (UPOV, 1995). For testing genetic purity through GOT, the field experiment was laid out, keeping the plot size of 4 m × 3 m with a spacing of 30 cm between rows and 10 cm between plants. Each sample was sown in three replicate plots. The authentic (breeder) seed samples of four selected cultivars were also raised in adjacent plots to identify impure plants in the certified seed lot by visual comparison. In each replication, 400 plants of each cultivar were studied for their genetic purity by comparing 18 morphological characters with the authentic seed plot throughout the growing season up to maturity. Plants which deviated from the original character of a cultivar were tagged and recorded as impure at maturity.

Conclusion

In this study, an elementary DNA fingerprinting database of the four main commercial flax cultivars under cultivation in Chhattisgarh state of India was built using twenty-eight SSR primer pairs, which could be expended as the number of additional cultivars and molecular markers (systems) increasing. The present study clearly demonstrated the ability of SSR markers to generate locus specific allelic information for the elite Indian flax cultivars and the utility of highly informative SSR markers in seed genetic purity assessments has been validated and compared with GOT. SSR markers are quick, effective and results are generally consistent with morphological analysis in the field study. Primers identified in the study could be utilized for routine genetic purity testing of cultivars. The SSR markers information developed through this study will be of immense help for seed industry to select appropriate marker combinations and assess genetic purity of the crop.

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