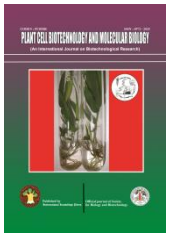

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Screening of *Alternaria* Blight Resistant Linseed (*Linum usitatissimum*) Genotypes Based on Disease Indexing and Gene Specific SSR Markers

Shruti Paliwal [a*], Manoj Kumar Tripathi [a,b], Sushma Tiwari [b], Niraj Tripathi [c], P. N. Tiwari [b] and R. S. Sikarwar [a]

[a] Department of Genetics and Plant Breeding, Rajmata Vijayaraje Scindia Krishi, Vishwavidyalaya, Gwalior, Madhya Pradesh, 474002, India.
[b] Department of Plant Molecular Biology & Biotechnology, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior, Madhya Pradesh, 474002, India.
[c] Directorate of Research Services, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur-482004, Madhya Pradesh, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: shruti1paliwal@gmail.com;*

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ABSTRACT

In present investigation, disease scoring and SSR-based molecular analysis of 92 linseed genotypes was performed against *Alternaria* blight resistant line (s) identification. Total 20 markers were used for the study out of which only seven markers showed polymorphism between the resistant and non-resistant lines. A total of 24 alleles were identified with an average of 3.4 alleles per locus for polymorphic SSR markers. The Polymorphic Information Content (PIC) values varied between 0.530-0.662 (Lu_899 to LuC_8166563_1) with an average of 0.60. The primer which showed highest PIC values was LuC_8166563_1 while the lowest was observed for the primer Lu_899. The major allele frequency varied between 0.32 (LUSc_270_01, LU_934, LuC_8166563_1) to 0.54 (Lu_899) with a mean value of 0.40. Population structure analysis revealed that 29 genotypes were assigned to group1 (G_1), 33 genotypes to G_2 and 30 genotypes were assigned to admixture group G_3. The relationship between groups derived from STRUCTURE explained that G_1 and G_2 comprised of distinct types. The present investigation has helped in differentiating the genotype based on their resistance for the disease. Six genotypes *viz.,* LMS-2015-42, LMS-2014-20, IC0498675, IC0498724, IC0498761 and IC0424878 were identified as highly resistant. While genotypes including IC0525920, IC0498538, IC0498768, IC0526514, IC0305053, IC0385383, and IC0499156 were considered as resistance. Twenty-one were found to be moderately resistant to disease while forty-two moderately susceptible and sixteen susceptible.

Keywords: Alternaria blight; disease scoring; molecular-marker; PIC values; SSR.

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.) is an annual plant with self-pollinating nature having chromosome number thirty with a small genome size. It is one of the earliest and most widely grown crops [1]. The Latin name of this crop means "very beneficial," perhaps because it contains three major bioactive ingredients *viz*., lignans, dietary fibre, and α-linolenic acid [2-4]. Due to climate changes and alteration in agro-ecological conditions, diseases which were previously categorised as minor is now becoming a major prevalent problem for the crops [4]. An array of diseases affects linseed plants. A significant disease that reduces the yield and oil content of linseed (*Linum usitatissimum* L) is *Alternaria* blight. The symptoms initially emerge as a black spot on the lower leaves, which progressively grow in size to become round, oval, or irregular in shape. Spots clump together and cover a significant portion of the leaves when infections are severe. The other foliar portion of the plant is likewise attacked by the pathogen. Two fungi that are known to produce *Alternaria* leaf blight in linseed are *Alternaria linicola* Groves & Skolko and *Alternaia lini*. In contrast to *Alternaria linicola A. lini,* dominated infected tissue, according to studies on the relative dominance of pathogens linked to the sick tissue. Dey [5] initially documented the disease from flower buds in Kanpur, Uttar Pradesh. Subsequently, *Alternaria* blight was found on linseed cultures at IARI in New Delhi and various locations across the nation [6]. After the disease was originally reported in 1933 [5], the fungus was given the name *Alternaria lini* [2].

“The pathogen was identical to *Alternaria brassicae* (Berk) Sacc. var. macrospora (Broun) in appearance, pathogenicity, and physiology, according to Arya and Prasad [7], who described a significant epidemic of the disease in Delhi in 1949. Every component of the plant that is aerial is affected by the disease. According to Vloutoglou” [8], the three most common species on flax are *Alternaria infectoria* Simmons, *A. linicola*, and *A. alternata*. Additional species include *A. linicola* Skolko and Groves, which is endemic to India and loves temperatures ranging from 26 to 33 degrees Celsius, as well as humidity [9]. *A. linicola* persists as thick-walled chlamydospores in flax waste and in the soil [8]. Early season conidia infect flax seedlings. “The fungus that causes seedling blight can be propagated by infected seeds” [9]. The first indication of *A. linicola* is the brown rot or seedling blight brought on by the seed-borne inoculum. In one to two weeks, stunted seedlings with dark red lesions on their cotyledons and hypocotyls entirely collapse. The sick and dying seedlings produce a rich reservoir of inoculum, which spreads to infect healthy plants. Dark brown lesions appear on infected leaves and

usually spread to cover the entire leaf before it turns chlorotic and eventually dies [10]. This information makes it necessary to identify sources of resistance against *Alternaria* blight in linseed.

Although there are not many genuinely resistant lines of linseed in the gene pool, a small number of moderately resistant variations have been documented [11]. Critical phenotypic observations and arduous field trials are part of the traditional breeding procedures for developing disease-resistant varieties [12]. But this procedure take time for development of resistant variety and possess a significant lag due to the environmental effect which can hinder the result [13]. However, the use of new molecular tools such as simple sequence repeats (SSR) markers can easily differentiate between the resistant and the non-resistant line [14-26]. "Moreover, DNA-based genetic markers are being used more frequently lately for quick cultivar identification, cultivar fingerprinting, and cultivar diversity analysis [14-26]. Due to their high abundance, reproducibility, simplicity, co-dominant inheritance, higher rate of polymorphism, and wider genome coverage, SSRs have emerged as the top choice among different types of DNA-based markers for plant breeders and biotechnologists to assess genetic diversity and cultivar selection" [14-44].

In this context, linseed genotype(s) resistant to *Alternaria* blight needs to be introduced so that major yield loss due to this disease could be minimized and productivity may be improved without affecting yield and oil quality. Therefore, the present investigation was undertaken to screen resistant linseed genotype (s) against *Alternaria* blight disease based on disease scoring and disease-linked microsatellite markers.

2. MATERIALS AND METHODS

2.1 Plant Materials

A total of 92 genotypes of linseed of which 90 was acquired from All India Coordinated Research Project on linseed, Crop Research Farm Mauranipur, BUA & T, Banda U.P., India including two checks resistance against *Alternaria* blight *viz*., LMS-2015-42 and LMS-2015-81 and two (SLS-135, SLS-140) from AICRP on Linseed, Sagar, M.P., India were used in present investigation (Table1). The field trial was conducted at Research Farm, Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior, M.P., India during *Rabi* 2022-23.

Table 1. List of genotypes used in present investigation

S.No	Genotype	S. No	Genotype	S. No	Genotype	S. No	Genotype
1.	IC0096672	24.	IC0199753	47.	IC0426926	70.	IC0346107
2.	IC0525976	25.	IC0413173	48.	IC0498517	71.	IC0523801
3.	IC0498660	26.	IC0053273	49.	IC0342805	72.	IC0305053
4.	IC0499165	27.	IC0096540	50.	IC0342799	73.	IC0498392
5.	IC0526058	28.	IC0526118	51.	IC0498482	74.	IC0498938
6.	IC0448872	29.	IC0054981	52.	IC0585324	75.	IC0498795
7.	IC0526063	30.	IC0585316	53.	IC0967423	76.	IC0385383
8.	IC0526118	31.	IC0342801	54.	EC0718843	77.	IC0498427
9.	IC0498486	32.	IC0449113	55.	IC0498489	78.	IC0259404
10.	IC305141	33.	IC0499201	56.	IC0498561	79.	IC0526105
11.	IC0525920	34.	IC0498517	57.	IC0499155	80.	IC0498675
12.	IC0118855	35.	IC0498689	58.	IC0499128	81.	IC0498724
13.	IC0526166	36.	IC0564592	59.	IC0572912	82.	IC0498761
14.	IC0526087	37.	IC0498768	60.	IC0499013	83.	IC0424878
15.	IC0498866	38.	IC0096638	61.	IC0599415	84.	IC0499156
16.	IC0356352	39.	IC0498880	62.	IC0510935	85.	IC0356165
17.	IC0385396	40.	IC0498786	63.	IC0096678	86.	IC0118861
18.	IC0394130	41.	IC0394118	64.	IC0998770	87.	LMS-2014-20
19.	IC0424547	42.	IC0498989	65.	IC0296039	88.	LMS-2018-22
20.	IC0498538	43.	IC0498843	66.	IC0498605	89.	LMS-2015-42(AB)
21.	IC0621689	44.	IC0526514	67.	EC0041621	90.	LMS-2015-81(AB)
22.	IC0620658	45.	IC0521450	68.	IC0526162	91.	SLS-135 (D)
23.	IC0448921	46.	IC0521455	69.	IC0564677	92.	SLS-140 (D)

2.2 Disease Assessment

Ten days old pure culture of *Alternaria spp.* multiplied on Potato Dextrose Agar (PDA) was used for inoculation. The spore suspension was prepared in sterilized distilled water having a spore load of 50-75 per microscopic field (10x). This suspension was sprayed at 30 DAS by using hand atomizer. Inoculation was sprayed after noon. After inoculation, the entries were regularly watched for recording the observations of disease severity. The first observations were made after ten days of inoculation on ten randomly selected plants. The disease score of each selected plants were recorded using 0-5 scale as earlier suggested by Kumar and Tripathi [27] based on per cent blighted area of leaf. Finally, maximum disease score for each genotype was recorded as List 1.

2.3 Morphological Description of *Alternaria linicola* isolate

The isolate of the *Alternaria lini* culture exhibited black substratum colour and a grey colony with white borders. It took 10–14 days for the isolates of *A.linicola* to sporulate. Previous accounts indicates that, including the beak, the typical size of Conidia of *A. linicola* is around 50 × 14 µm. In contrast, *A. lini* generates airborne conidia that resemble flasks and measure 24 × 7 µm, including the beak. Simple conidiophores that appear individually or in bundles are the defining feature of the seed-borne pathogen *A. linicola* [28]. Fig. 1 represent the grey mycelial fungal colony grown on PDA media and conidia shape.

2.4 Genomic DNA Isolation and PCR Amplification

DNA isolation was performed 20-days-old leaves of seedling grown in field from all the linseed genotypes. Leaf samples were treated with ethanol and wiped to remove infections of diseases. The genomic DNA was extracted using CTAB method [29] with minor modification as suggested by Tiwari et al. [30]. The quality of the DNA was checked on 1% agarose gel and the DNA concentration was estimated with the nanodrop spectrophotometer (Thermo-fisher). The DNA concentration for use in polymerase chain reaction (PCR) was diluted to 20-30 ng/µl based on spectrophotometer reading. A set of 20 gene-specific simple sequence repeats (SSR) molecular markers were selected for screening of linseed genotypes for *Alternaria* blight diseases (Table 2) as suggested by Singh et al. [31].

List 1. 0-5 scale adapted to indicate degree of resistance against *alternaria* blight of linseed

Scale	Disease Intensity	Disease Reaction
0	Free from disease	Free (F)
1	1-10% infection	Resistant (R)
2	10.1-25% infection	Moderately resistant (MR)
3	25.1-50% infection	Moderately susceptible (MS)
4	50.1-75% infection	Susceptible (S)
5	75.1-100% infection	Highly Susceptible (HS)

(a) **(b)**

Fig. 1 (a) *Alternaria lini* isolate grown on PDA media, grey mycelial fungal colony (b) Multiseptate conidia of *Alternaria lini*

Table 2. List of gene based SSR molecular marker used for screening of linseed genotypes

S. No.	Marker name (SSR)	Forward sequence	Reverse sequence
1	Lu 2472	TAAATGTTTCCCGCCAAAAC	TTTGGAAATGGGAAGTGAGG
2	LuC 8166563_1	CCGACCCATCTGGAATTATG	TCAATATTGTGCATGGGATTT
3	LUSc 898_3_12	GGAAGAGAGGAAATGGGGAG	GGAAGAGAGGAAATGGGGAG
4	Lu 3078	AGGGGGAAGACACAGATGAG	CTTAAGGCTGTAAGAAGGTCAAT
5	Lu_3043	TTCCGAGAGAGGAGAGTTGG	CAGAGGCGGGCAGATATAAA
6	Lu_207198	TCAGCAAAGGAATCGGATCT	GTTTCCAGGGTTCCAGAGC
7	Lu 899	CTGCATGCATGTCCAGTACG	CAGGCTTGGAGGATTATTGG
8	LU_934	GCTTCAACACCAGTCACCAA	CCGGTCCAAAGCTTGAAGA
9	LUSc_270_01	TAAAACTGGTGCTGGAAGCC	TATACGACGACGGTGGGAAG
10	LUSc_257_01	CACTATAACTTCGCTGCACCTG	ATGAGTACCAGAATGCTCCCAC
11	Lu1171	CAAATTTTAAATGAATACAAAACATGA	GTTGGCCAGAGAGAGATTCG
12	Lu897	CTAACGACCGGTTCATGGAT	AATTTACATACAAGCTACACAAAAA
13	Lu3209	CATCCAAGCCAATGGTCTCT	ATAGGGCTGGAGCAGTGAGA
14	Lu3211	AATAGAAGCAGACAGGCTGAGG	AACAACTGAATCACCCATTCG
15	Lu3212	GGTTTAATTTGCAGTCCAGTACG	GAAAAAGCCAGGAGTCATGC
16	Lu2373	TTTTGGACCAACTCCACCTC	GATACTTAGCCCGCATCAGC
17	Lu2377	GCGGAGCTAGGAATGTGAAG	AATTGTCAGGAGGCATCCAC
18	Lu870	TGAAACGAAGAACTGGTCCAA	ACGTCTTCGAAAGTGCCTGT
19	Lu3217	AAAAGGGAGAGTTCCCCAAG	AGCAGCCAGCAATCTGTACC
20	LU_934	GCTTCAACACCAGTCACCAA	CCGGTCCAAAGCTTGAAGA

Polymerase chain reaction was performed in 10 µl reaction mixture comprising of 1X PCR buffer, 0.1 U Taq DNA polymerase, 1 µl dNTP (1 mM), 0.5 µl of forward and reverse primers each (10 pM) and 2 µl (20 ng/µl) of genomic DNA in a thermocycler (Bio-Rad, USA). The PCR protocol comprised of initial denaturation step of 94ºC for 3 min followed by 35 cycles of 94ºC for 1 min, annealing at 55ºC for 30 sec, elongation at 72ºC for 1 min with final extension at 72ºC for 10 min. The PCR products were resolved on 3% agarose gel at 120V for 2-3 hrs and documented using Gel Documentation System (Syngene, USA). Gel scoring was done by base pair analysis using ladder based on banding pattern. Data sheet was prepared to run in population structure and allele pattern A/A was used if band was in the upper side and pattern B/B was used if the band was in the lower side, in heterozygous condition banding pattern was A/B and in case of no amplification -/- was employed.

2.5 Population Structure Analysis

"Based on the allele size the genetic profile of 92 linseed genotypes was scored using SSR markers. The observations were recorded for major allele frequency, polymorphism information content (PIC) and genetic distance by using Unweighted Pair Group Method for Arithmetic average (UPGMA) tree using Power Marker v3.25 software" [32] and the dendrogram was constructed using MEGA 6.0 software [33]. SSR data was again subjected to cluster analysis followed by bootstrap analysis with 1000 permutations for all the genotypes using Mega 6.0 software. The population structure for 92 linseed genotypes comprising both germplasm lines and check varieties was inferred using Structure 2.3.4 software [34]. "The structure outputs were visualized using Structure Harvester from which Evanno plots were constructed" [35]. Principle Co-ordinate Analysis (PCoA) based on origin was recorded using GenAlex software.

3. RESULTS AND DISCUSSION

3.1 Field Screening

Out of the 92 genotypes that were included in the study none were investigated either extremely vulnerable to the disease or free from it. Six genotypes *viz.,* LMS-2015-42, LMS-2014-20, IC0498675, IC0498724, IC0498761 and IC0424878 were identified as highly resistant (Table 3; Fig. 2). Genotypes including IC0525920, IC0498538, IC0498768, IC0526514, IC0305053, IC0385383, and IC0499156 were considered as resistance. Twenty-one were found to be moderately resistant to disease while forty-two moderately susceptible and sixteen

susceptible. The effectiveness of promising lines or cultivars of linseed against the *Alternaria* blight disease was also investigated by Das et al. [36], three enhanced check varieties including Neelam, T397, and Kiran and 440 linseed germplasm line were tested to determine the extent of promising genotypes at the yield level against *Alternaria* blight. Eight genotypes *viz*., A-225B, A-75, A-226, A-364, A-232, A-66, A-202, and A-184, had the lowest percentages of disease intensity, 12.67, 21.50, 22.34, 22.54, 23.25, 24.25, 24.77, and 25.00 percent, respectively. In a different investigation conducted by Prasad and Manapure [37], *Alternaria* blight incidence ranged between 9.00 and 16.30 among the parents. Padmini (9.00) was the parent with the least amount of *Alternaria* blight infestation, followed by JRF-5 (9.30), GS234 (10.60), and NL-97 (10.65). GS-234 x Padmini (7.85), EC-1424 x Padmini (8.00), JRF-4 x PKVNL-260 (9.05), JRF4 x Padmini (9.30), and IC-15888 x Padmini (9.70) were the crosses that displayed the least intensity of *Alternaria* blight infection. In the experiment of Kumar and Tripathi [27], seven genotypes were found to be resistant (R), 66 to be moderately resistant (MR), 102 to be moderately susceptible (MS), and 25 to be susceptible (S) to linseed blight disease out of 200 genotypes; disease free (F) and highly susceptible (HS) germplasm were not detected.

3.2 Screening of Genotypes Based on Gene-Specific SSR Molecular Markers

In present investigation, 92 genotypes were considered to validate a total of 20 reported gene- specific SSR markers against *Alternaria* blight disease. Out of these 20 markers, 7 were found to have variation between tolerant and sensitive germplasm lines. A set of 7 allele specific primers were amplified across 92 linseed genotypes along with two checks. A total of 24 alleles were identified with an average of 3.4 alleles per locus for polymorphic SSR markers (Table 4). The gene diversity and Polymorphic Information Content (PIC) values varied between 0.530274-0.662316 (Lu_899 to LuC_8166563_1) with an average of 0.60 respectively (Table 4). The primer which showed highest gene diversity and PIC values was LuC_8166563_1 while the lowest gene diversity and PIC values was observed for the primer Lu_899. The major allele frequency ranged from 0.32 (LUSc_270_01, LU_934, LuC_8166563_1) to 0.54 (Lu_899) with a mean worth of 0.40

Table 3. Categorization of linseed genotypes based on disease score

S. No.	Disease reaction	Number of genotypes	Name of genotypes
1.	Highly Resistant	6	LMS-2015-42, LMS-2014-20, IC0498675, IC0498724, IC0498761, IC0424878
2.	Resistant	7	IC0525920, IC0498538, IC0498768, IC0526514, IC0305053, IC0385383, IC0499156
3.	Moderate resistant	21	IC0526087, IC0394130, IC0199753, IC0499201 IC0564592, IC0498786, IC0521455, IC0342799 EC0718843, IC0498489, IC0499013, EC0041621 IC0296039, IC0498795, IC0526105, LMS-2018-22 SLS-135, SLS-140, IC0498427, LMS-2015-81 IC0259404
4.	Moderate susceptible	42	IC0096672, IC0526063, IC0526118, IC0498486 IC305141, IC0498392, IC0498938, IC0096638 IC0498880, IC0118855, IC0526162, IC0564677 IC0346107, IC0523801, IC0526166, IC0413173 IC0526105, IC0599415, IC0510935, IC0096678 IC0356165, IC0118861, IC0053273, IC0096540 IC0526118, IC0054981, IC0585316, IC0342801 IC0498561, IC0499155, IC0499128, IC0572912 IC0449113, IC0967423, IC0498517, IC0498689 IC0424547, IC0394118, IC0498989, IC0498843 IC0426926, IC0521450
5.	Susceptible	16	IC0525976, IC0498660, IC0621689, IC0998770 IC0620658, IC0448921, IC0499165, IC0526058 IC0448872, IC0498866, IC0356352, IC0498605 IC0385396, IC0498482, IC0585324, IC0498517

Table 4. Allele specific SSR marker presenting major allele frequency, numbers of alleles, gene diversity and PIC value

Marker	Major Allele Frequency	Genotype No.	Gene Diversity	PIC value
LuC_8166563_1	0.326	4	0.716	0.662
Lu_3043	0.413	3	0.656	0.582
Lu_207198	0.500	3	0.616	0.542
Lu_899	0.543	3	0.598	0.530
LU_934	0.326	4	0.707	0.650
LUSc_270_01	0.326	4	0.703	0.644
Lu_897	0.369	3	0.664	0.590
Mean	**0.400**	**3.420**	**0.660**	**0.600**

Fig. 2. Showing different plant parts affected with the blight (a) Leaf spot of blight, (b) Infected capsule (c) Infected immature capsule died down to disease (d) Tip of infected capsule

Comparative analysis of molecular sequence data enables the determination of proximity or distance between genotypes and displays clusters of genotypes by constructing a phylogenetic tree. For this purpose, cluster analysis was performed among linseed by the neighbour-joining method of the maximum composite likelihood substitution model, identifying three clustered groups. Considering the higher cophenetic correlation coefficient, the dendrogram was assumed to represent the similarity matrix very well. The genetic relationships among linseed genotypes are presented in SSR based UPGMA tree (Fig. 3). All the genotypes are grouped into 3 clusters. The genotypes in cluster 1 (red colour) include twenty-nine genotypes. Cluster 2 (green colour) had thirty-one genotypes while cluster 3 (blue colour) includes thirty-two genotypes. Cluster I comprise the check variety resistant against disease along with other genotypes. Possibility exist that these genotypes may also be resistant to the *Alternaria* blight disease. Cluster II include group of genotypes which are moderately resistant to the disease and include some moderately susceptible individuals too. Whereas cluster III consist of moderately susceptible and susceptible genotypes. The clustering of genotypes in the UPGMA tree is in complete correspondence to STRUCTURE analysis (Fig. 5).

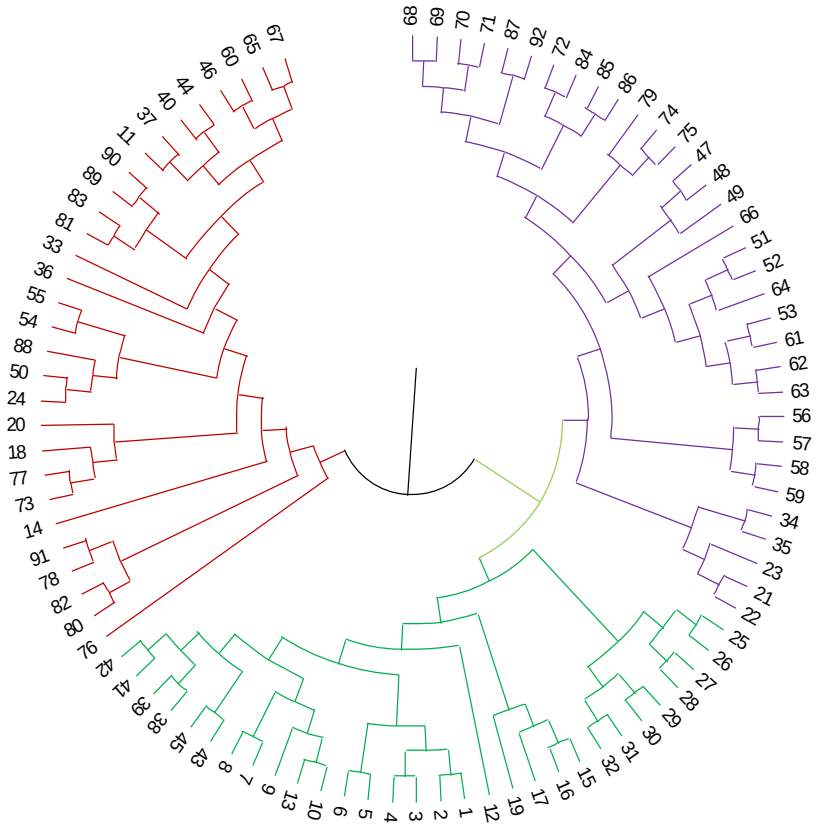

Fig. 3. Dendrogram of 92 linseed genotypes based on banding pattern analysis of gene based SSR markers using MEGA 6.0 software

3.3 Principal Co-ordinate Analysis for SSRs

Principal coordinate analysis (PCoA) is a multivariate dataset that provides the ability to find and archive key patterns in multiple loci and multiple samples. With this technique, the distances between the groups, which are based on the two-dimensional diagram formed by the similarity or distance matrix between the individuals, reflect actual distances [38]. PCoA is used to provide a spatial representation of the relative genetic distances between populations [39]. Principle Co-ordinate analysis (PCA) based on origin formed 3 major population groups. Population group 1 included accessions which showed moderate susceptibility and susceptible genotypes. While group 2 include moderate resistant to moderate susceptible genotypes and group 3 contained genotypes that are resistant to highly resistant including checks. The distribution of genotypes in PCoA (Fig. 4) showed complete correspondence with STRUCTURE analysis and UPGMA (Fig. 3) phylogenetic tree.

3.4 Population Structure Analysis

The population structure of the 92 linseed genotypes was estimated using STRUCTURE v2.3.3 software based on SSR markers. The optimum K value was determined by using Structure Harvester, where the highest peak was observed at delta K = 3 which indicated that the entire population can be grouped into three sub groups. The number of sub populations (K) was identified based on maximum likelihood and delta K (dK) values, with two core and pure groups and an admixture group (Fig. 5 and Fig. 6). Using a membership probability threshold of ≥ 80 %, 29 genotypes were assigned to group1 (G_1), 33 genotypes to G_2 and 30 genotypes were assigned to admixture group G_3. The relationship between groups derived from STRUCTURE explained that G_1 and G_2 comprised of distinct types. This indicated that the population structure was in accordance with clustering of linseed genotypes formed using UPGMA tree based on SSR data [40].

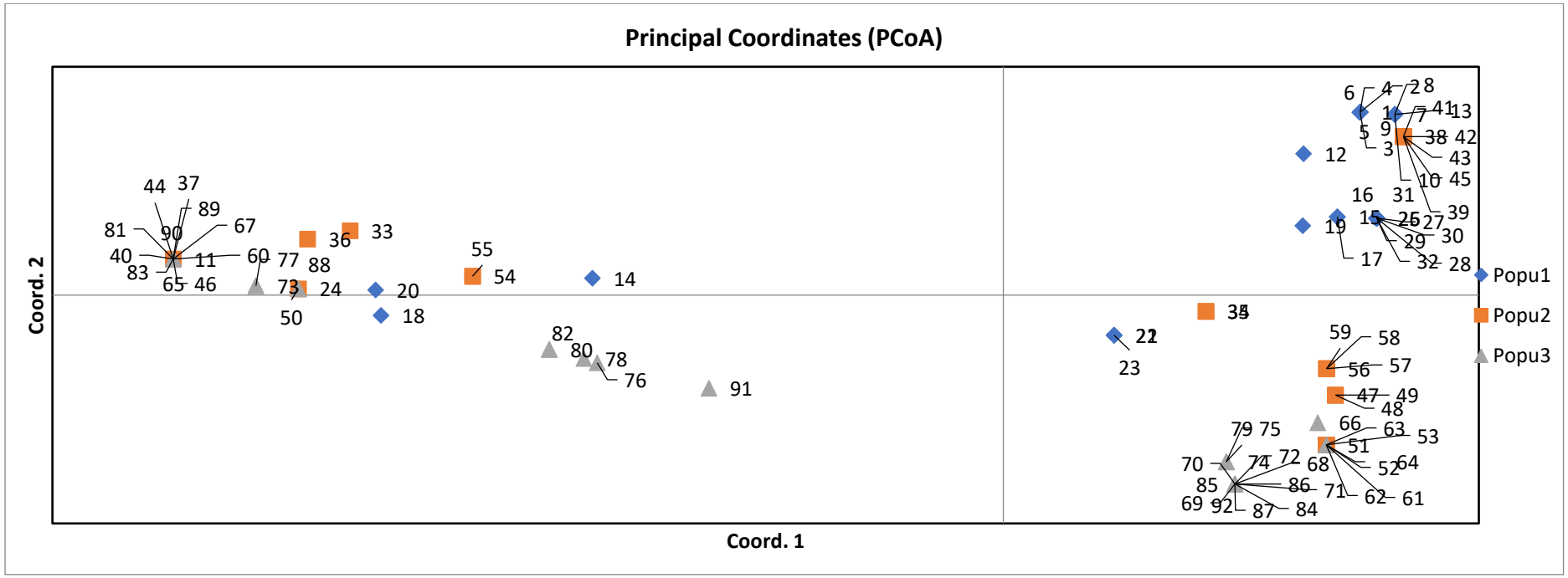

Fig. 4. Principal Coordinates Analysis (PCoA) based on allelic variation using SSR markers representing origin of linseed genotypes using GenAlex software

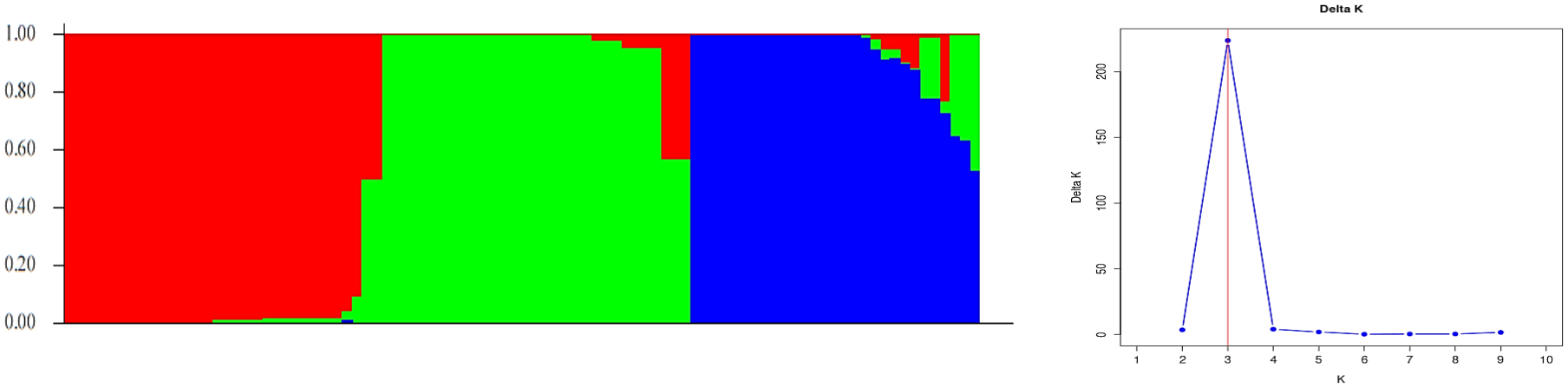

Fig. 5. Population structure of 92 accessions (*K* = 3) and graph of estimated membership fraction for *K* = 3. The maximum of adhoc measure ΔK determined by structure harvester was found to be *K* = 3, which indicated that the entire population can be grouped into three subgroups (SG1, SG2 and SG3)

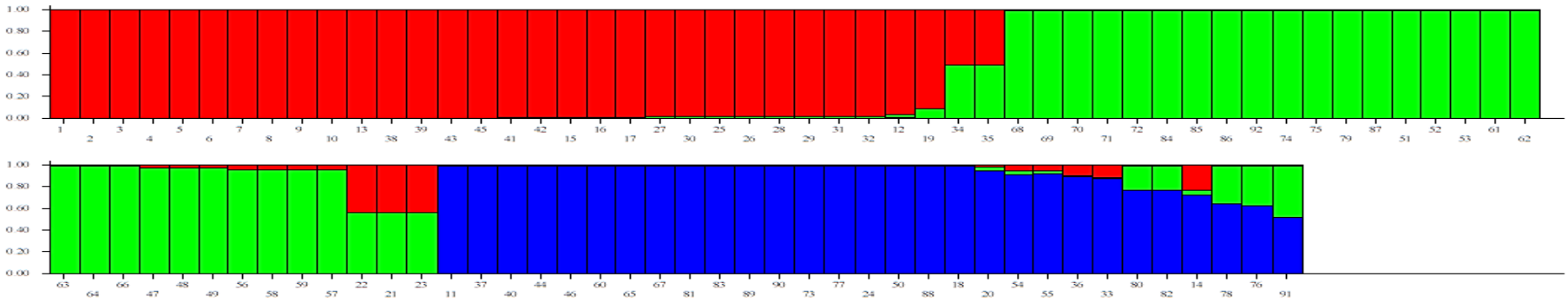

Fig. 6. Population structure of 92 accessions arranged based on inferred ancestry. Based on the membership fractions, the accessions with the probability of ≥ 80 % were assigned to corresponding subgroups with others categorized as admixture

Molecular analysis of linseed based on *Alternaria* blight through gene-specific SSR markers was previously investigated by Singh et al. [41]. Out of 1720 SSRs, 216 were determined to be polymorphic between the parents. The linkage map was created using a total of 191 SSRs, which were divided into 15 linkage groups and covered a genomic length of 1802.4 cM. Two QTLs were identified for *Alternaria* blight. Another study conducted by Singh et al. [31] and they reported that out of hundred only ten markers showed polymorphism and only three markers *viz*. LUSc 898_3_12, Lu2472 and Lu 3078 were able to differentiate between resistant and non-resistant genotypes. Similar results were received in present investigation.

4. CONCLUSION

Identifying the resistant line (s) to be included in the diverse breeding programme to develop *Alternaria* blight resistant cultivars through gene-specific SSR molecular markers identified six highly resistant and seven resistant genotypes among the ninety-two genotypes included in the study. These results were backed with field screening too. From the economic point of view use of these resistant line (s) is the cheapest method for disease control in any breeding programme. Hence these results can be further incorporated in integrated disease management programme to help increase crop productivity further and breed new *Alternaria* blight resistant cultivar (s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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