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RESEARCH ARTICLE

Microsatellite markers-based genotyping, population structure analysis and field screening of chickpea (*Cicer arietinum* L.) genotypes against Fusarium wilt

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Abstract

As an important source of protein, vitamins and minerals, chickpeas (*Cicer arietinum* L.) are the most significant self-pollinated pulse crop. The main cause of its low production is Fusarium wilt, which is brought on by the fungal disease *Fusarium oxysporum* f. sp. *ciceris*. Thus, the present investigation aimed to conduct field-level screening of 71 chickpea genotypes against Fusarium wilt disease as well as microsatellite markers-based analysis in the laboratory. In the field investigation, one genotype was found to be resistant, 13 genotypes were moderately resistant, 34 genotypes moderately susceptible, 14 genotypes susceptible, while 9 genotypes were highly susceptible at the reproductive stage under wilt sick plot. Out of 22 markers, 13 markers were found to be polymorphic and the highest PIC value was shown by the marker TA200 followed by H3A12, TA110, GA137, GA20, TR2, TS79, TA37, TR19 and H1B06. Based on the dendrogram, all 71 genotypes were grouped into 6 clusters. In this investigation, a structured population in tested chickpea genotypes was demonstrated. All genotypes were stratified into 2 populations (P1, P2), representing 50.70% and 49.29% of genotypes used in structure analysis respectively. Based on both sick plot and molecular screening result analysis, it can be concluded that the genotypes viz., JG315, RVSSG84, JAKI 9218, ICC 4958, SAGL-152339, RVSSG 52 and RVSSG 74 are resistant against Fusarium wilt and therefore, may be effectively used by the breeders in Fusarium resistant chickpea breeding development programmes.

Keywords

chickpea; Fusarium wilt; molecular markers; resistant; SSRs; wilt sick plot

Introduction

Chickpea (*Cicer arietinum* L.), sometimes referred to as the garbanzo bean, Egyptian pea, Bengal gram or gram, is a high-quality plant protein provider that increases soil fertility through biological nitrogen fixation. The Fabaceae family comprises the chickpea, an annual legume species that is self-pollinated and diploid (having 2n = 2x = 16) with a genome size of 738 Mb (1). It is mostly cultivated on residual soil moisture during the winter season in approximately 50 nations across the globe on diverse soil types and agro-climatic conditions (2). India has been the top producer of chickpeas, with a global annual production of 11.91 million tons from an area of 10.94 million ha, with an average yield of 1.09 tons ha^{-1} (3).

Unpredictable variations in the duration and severity of some extreme weather conditions and climate change are the major issues that have a negative impact on chickpea production. These abiotic stresses can alter plant-pathogen interactions by making the host plant more vulnerable to pathogen infection and insect attack (4). At present, numerous abiotic stresses including drought, salinity, heat, cold and biotic stresses *viz*., Ascochyta blight, Fusarium wilt and pod borer, etc. contribute to the reduced productivity of chickpeas (5, 6). *Fusarium oxysporum* f. sp. *ciceris* (foc) is the primary biotic stressor that restricts chickpea productivity, causing Fusarium wilt, a serious soil-borne disease. It is mostly found in arid, warm areas and can result in yield losses of up to 10 - 15% annually; during epidemics, crop losses can reach up to 100% (1, 5). About 77 - 94% of yield loss is attributed to early wilting, which manifests as dull green discoloration within 25 days of planting. In the occurrence of "late wilt", dropping petioles and leaf yellowing symptoms appear during the podding stage, resulting in 24 - 65% yield reductions (7).

In chickpeas, simple field screening and selection in wilt sick plots (WSPs) resulted in the identification and release of several FW-resistant donors and varieties. The wilt-sick plot (WSP) approach is the most frequent and widely employed method for detecting Fusarium wilt-resistant genotypes (8). The basic benefit of the WSP approach is that it enables field screening of a huge number of genetic materials (9). Furthermore, the development of resistant cultivars is hampered by genetic heterogeneity in the pathogen population as well as the creation and upkeep of uniform wilt sick plots for the purpose of selecting resistance lines. Molecular breeding techniques have been used recently to solve these issues.

Marker-assisted breeding combines conventional plant breeding, especially using newly developed markers, which may prove helpful in knowing the genetic constitution of plants. Whereas classical breeding cannot notice the genetic constitution of a plant, it assesses only the basis of physical appearance (1). Markers have also exhibited improvement in the effectiveness of the selection and development of new cultivars. Marker-aided breeding has been widely employed in breeding programs aimed at improving disease resistance in chickpeas (10). Microsatellite markers or simple sequence repeats (SSRs) have shown effective in the breeding of chickpeas and other legume crops for the evaluation of genetic diversity and subsequent selection, including MAS (11, 12).

In early plant growth, resistant genotypes can be screened using simple sequence repeats that are tightly linked to Fusarium wilt resistance genes, all without exposing the plants to pathogens. By using marker-assisted selection and the gene pyramiding strategy, these markers, which are simple, stable, effective and easy to use, can be used to accelerate the production of cultivars that are high-yielding and resistant to FW (13, 14). Because SSR markers are uniformly distributed short tandem repeats with high polymorphic information content (PIC) and repeatability over the entire genome, they are highly valuable for genotype discrimination, pedigree analysis, determining genetic distances among genotypes and variety identification (15, 16). So, the present investigation was carried out to screen Fusarium wilt-resistant chickpea genotypes at the field level and in the laboratory to identify superior genotypes for future improvement of this important legume.

Materials and Methods

Plant Material

In this study, 71 genotypes of chickpeas (Table 1) were obtained from the All India Coordinated Research Project (AICRP) on Chickpea, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, India and the RAK College of Agriculture, Sehore, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV), Gwalior, India. Two checks *viz*., JG315 (highly resistant) and JG62 (extremely susceptible) were included in this set of 71 genotypes. JG315 is resistant to *Foc*, however, JG62 is an early wilting genotype that is particularly vulnerable to *Foc* races 1-5 (17).

Evaluation of FW-resistant genotypes in Fusarium wilt diseased sick plot

In Rabi 2022-2023, a collection of 71 chickpea genotypes, including checks, was subjected to epiphytotic conditions at the Fusarium wilt diseased sick plot, College of Agriculture, RVSKVV, Indore, India, to determine their level of resistance against Fusarium wilt. Two rows of 2 m each were planted in each line, with a row-to-row and plant-to-plant spacing of 30 and 10 cm. To compute PDI (% disease incidence), data on disease incidence was collected at the seedling and reproductive stages. To determine the Fusarium wilt incidence a method suggested by Irulappan et al. (18) was followed. The percentage of Fusarium wilt incidence was computed using the initial plant count and the total number of wilted plants. Over the course of the crop season, the pathogen burden in the sick plot was enhanced by the addition of additional pathogen culture.

$$\text{Percent Disease Incidence (\%)} = \frac{\text{Number of plants exhibiting with symptoms}}{\text{Tootal number of plants observed}} \times 100$$

DNA extraction and quantification

Cetyl tri-methyl ammonium bromide (CTAB) technique was used for genomic DNA extraction from young leaves of chickpea genotypes (19). Using a sterile pestle and mortar, 2.0 g of leaf samples were ground into a fine powder using liquid nitrogen. 2 mL microcentrifuge tubes containing 1 mL of extraction buffer were filled with 100 mg of leaf powder. For protein separation, sample tubes were heated at 65 °C for an hour and then centrifuged at 10000 rpm for 15 min using a phenol-chloroform-isoamyl alcohol (25:24:1) mixture. After transferring the supernatant to a new tube, cooled isopropanol was used to precipitate the DNA. Precipitated DNA was then dried and dissolved in 50 µL of nuclease-free water. The purity of the DNA was examined on a 0.8% agarose gel and the quantification was evaluated with a nano-drop spectrophotometer. DNA was diluted to a

Table 1. Details of experimental material with their parentage

Sl. No.	Name of genotypes	Pedigree/Parentage	Sl. No.	Name of genotypes	Pedigree/ Parentage
1	ICCV 201211	JNKVV, Jabalpur	37	SAGL- 162387	ICC 4958 x BG 1003
2	ICCV 201210	JNKVV, Jabalpur	38	SAGL- 152227	JSC 52 x ICC 4812
3	ICCV 201109	JNKVV, Jabalpur	39	SAGL- 162381	JSC 52 x RSG 888
4	ICCV 20116	JNKVV, Jabalpur	40	SAGL- 162364	SC 36 x JSC 37
5	ICCV 201115	JNKVV, Jabalpur	41	SAGL- 152356	RAK, Sehore
6	ICCV 201214	JNKVV, Jabalpur	42	SAGL- 152337	ICC 4958 x KAK 2
7	ICCV 201112	JNKVV, Jabalpur	43	SAGL- 153226	RAK, Sehore
8	ICCV 201205	JNKVV, Jabalpur	44	SAGL- 152336	KAK2 x JG130
9	ICCV 201104	JNKVV, Jabalpur	45	SAGL- 152222	JSC 19 x ICCV 96029
10	ICCV 201206	JNKVV, Jabalpur	46	SAGL- 152318	JSC 19 x JG 16
11	ICCV 201117	JNKVV, Jabalpur	47	SAGL- 152258	JG 135 x FG 711
12	ICCV 201207	JNKVV, Jabalpur	48	SAGL- 152231	ICC 4958 x BG 362
13	Pant Gram-5	PG035 X HC5	49	SAGL- 152223	RAK, Sehore
14	H12-55	HC 1 X H 00-216	50	SAGL- 152234	JSC 19 x ICC 4958
15	RVG 202	(JAKI 9226 X DCP 20) X JG 412	51	SAGL- 152329	PG9425-9 x BG2064
16	SAGL 22-110	IPCK-1010 X JG 11	52	SAGL- 162376	JSC 52 x RSG 888
17	SAGL 22-116	RVG 201 X JSC 11	53	SAGL- 162377	JSC 36 x JSC 52
18	SAGL 22-117	RVG 201 X JSC 37	54	RVSSG 84	RAK, Sehore
19	SAGL 22-118	RVG 204 x RVSSG 74	55	JG 315	Selection form WR 315
20	SAGL 22-119	RVG 202 x RVSSG 74	56	RVSSG 74	RAK, Sehore
21	SAGL 22-120	JG 6 x ICPK 1010	57	JG 130	([PhuleG5 X Narshinghpur bold] X JG 74)
22	SAGL 22-121	IPCK 9294 x KAK 2	58	RVSSG 83	RAK, Sehore
23	SAGL 22-122	RVG 204 xNBeG 47	59	JAKI 9218	(ICCC 37 x GW5/7) x ICCV 107
24	SAGL 22-123	RVG 205 x ICC 4958	60	RVG 204	ICCV10 X ICCL87322
25	SAGL 22-124	JG 315 x JAKI 9218	61	JG 6	(ICCV10 x K850) x (H208x RS11)
26	SAGL-152327	KAK 2 x JSC 19	62	RVSSG 92	RAK, Sehore
27	SAGL- 152324	IPC 4958 X IPC 9494	63	ICC 4958	Germplasm collection
28	SAGL- 152237	BG 2064 x KAK -2	64	RVSSG 71	RAK, Sehore
29	SAGL- 152278	JSC 37 x JSC 36	65	RVSSG 52	RAK, Sehore
30	SAGL- 152250	KAK 2 x BG 2064	66	RVSSG 68	RAK, Sehore
31	SAGL- 152330	ICC 4958 x PHULE G 5	67	SAGL- 161024	JAKI 9218 x BGD 112
32	SAGL- 152238	PG -9425-9 x IPC 9494	68	SAGL- 163006	ICC 4812 x ICC 506
33	SAGL- 152405	RAK, Sehore	69	SAGL- 161025	JSC 52 x BGD 112
34	SAGL- 152339	JG16 x KAK 2	70	SAGL- 163007	ICC 4812 x ICC 506
35	SAGL- 152344	IPC9494 x JG16	71	JG 62	Local bulk selection
36	SAGL- 162299	JSC 52x JSC 36			

final concentration of 20-25 ng/µL for further analysis.

Polymerase chain reaction (PCR) and gel electrophoresis

Based on factors including heterozygosity, allelic richness and polymorphic information content (PIC), a total of 22 Fusarium wilt resistance-associated simple sequence repeat (SSR) markers were chosen based on previous literature. Standardized PCR parameters were used to validate the markers. The annealing temperature of the markers was determined on the basis of the literature from which the markers were selected. Amplification was carried out in 10 µL reaction volume using Thermal Cycler (BioRad, USA) according to the method already established (11). The amplified PCR products were subsequently resolved on 3% agarose gel using 0.5X TBE running buffer through electrophoresis. The separated bands were visualized under UV light and photographed employing a Gel Documentation System (Syngene, USA).

Molecular and population structure analysis

The genetic profile of 71 chickpea genotypes (JG62 as a positive control and JG315 as a negative control) was scored based on the differences in allele size using SSR markers. GenAlEx v.6.51b2 (20) and Power Marker v.3.25 (21) software was employed for calculating summary statistics of scored alleles. Allele frequency and other parameters were analysed with the help of Power Marker v.3.25. MEGA V11.0.9 software (22) was used to visualize the dendrogram tree. STRUCTURE v.2.3.4 software (23) was used for constructing the Bayesian model-based population structure of studied genotypes and Structure Harvester Web V0.6.94 was used for visualizing the output of STRUCTURE software. To summarize the significant variance by population, an analysis of molecular variance

(AMOVA) within and among populations was also performed using GenAlEx v.6.51b2.

Results and Discussion

Identification of resistant chickpea genotype (s) against Fusarium wilt under wilt sick plot

The signs of wilting on chickpea plants that were biologically infected are leaf yellowing prior to drying out, which was followed by a slow and steady yellowing, drooping and withering of the entire plant or its branches. The afflicted plant roots displayed brown to black discoloration in the xylem vessels on the split aperture. It was reported similar findings earlier (24). *F. oxysporum* f. sp. *ciceris* causes FW by clogging the xylem vessels within the host plant (25). This obstruction disrupts the normal water transport inside the plant, ultimately leading to wilting and the complete collapse of the plant onto the ground.

At the seedling and reproductive phases, the illness incidence of 71 genotypes was noted (Table 2). Under field screening, the assessment of various chickpea genotypes against Fusarium wilt produced encouraging findings. Many genotypes displayed resistance reactions at the seedling stage, while some genotypes demonstrated resistance during the reproductive stage. The age and growth stage of the plants, which are essential sources of parental materials to determine the slow wilting type, were linked to the resistance. Field screening at the reproductive stage seems to be a more reliable method to guarantee the dependability of the breeding programme because the resistant lines at that stage also turned susceptible (26). According to this study, 9 genotypes were found highly susceptible during the reproductive stage under the wilt sick plot, whereas one genotype was found to be resistant, 13 genotypes to be moderately resistant, 34 genotypes to be moderately susceptible, while 14 genotypes to be susceptible (Table 3).

Genotype JG315 showed resistance to FW throughout the examinations and exhibited susceptibility to 3.70% at the seedling stage and 6.58% at the reproductive stage. However, genotype JG62 has demonstrated a highly susceptible response to FW, showing 100% susceptibility during the reproductive stage and 95.49% susceptibility during the seedling stage (Table 2). After inoculating the chickpea variety JG62 after 25 days, saw wilting symptoms (27). They deduced that the earliest symptoms were light yellowing and drooping of the leaves, followed by wilting of the host. Genotype JAKI9218, RVSSG 74, RVSSG 84, ICC4958, RVSSG 52, ICCV201207, RVG 202, SAGL 22-110, SAGL 22-118, SAGL-152339, SAGL-152227, SAGL-162381 and SAGL-152234 exhibited a moderate resistance reaction to FW at reproductive stage during crop season (Table 3). Comparable investigations were also carried out (28), who noted that 50 chickpea entries had different responses to *Foc*: 6 were extremely resistant, 31 were resistant, 8 were somewhat resistant, 2 were moderately susceptible and 3 were highly susceptible. In a field study, it was examined 31 genotypes against Fusarium wilt and observed 10 resistant, 18 moderately resistant and 28 susceptible genotypes (29). It was also investigated the genotype-specific incidences of Fusarium wilt in chickpeas (30).

SSR markers-based analysis

Marker-assisted selection (MAS) in crop development programmes needs molecular markers as a prerequisite. According to a study, the development of improved cultivars of chickpeas involves the identification of highly diverse germplasm, highly polymorphic trait-specific molecular markers and effective molecular breeding strategies (31). For this reason, characterizing the chickpea germplasm has the potential to be extremely important for upcoming international breeding initiatives (5, 31), particularly in India, the country that produces the most chickpeas. SSRs have been extensively used in many crops, including chickpeas, for genetic diversity study, germplasm grouping and population structure analysis (32). Using 22 SSR genetic markers, the current study screened 71 genotypes of chickpeas and identified the resistance genotype(s) against Fusarium wilt.

Marker efficiency and allele frequency

Out of 22 SSR gene-based markers employed, 13 markers (59.09%) were found reproducible and polymorphic with an average of 3.23 alleles per SSR marker (Table 4). The number of alleles ranged from 2-4, whereas the maximum alleles were observed for TA200, H3A12, GA20 and GA137. The major allele frequency of 13 SSR markers ranged between 0.2816 (28.16%) for the marker TA200 and 0.5774 (57.74%) for the marker TA59. A marker with a PIC value = 0.5 or more indicates the presence of high informativeness (33). Here, PIC values ranged from 0.3689 (GA16) to 0.7011 (TA200) with an average value of 0.5521. PIC values were higher for primers containing more alleles, such as TA200, H3A12, GA20 and GA137 (Table 4). According to a study, microsatellites that are better suited for diversity analysis have higher PIC values (32). In the investigation of chickpea RILs, it was discovered 12 markers associated with wilt resistance in chickpea crops (34). Among these markers, TA96, CS27, TA110 and TA59 were found to be more consistently associated. These markers can be utilized for marker-assisted selection (MAS) because the results demonstrated their high prediction accuracy for desirable genotypes. The percentage of polymorphism in the current study was average (59.09%). Earlier reports are also documented very low levels of polymorphism such as 9.5% and 11.57% (35), 13.45% (36) and 16.7% (37).

SSR-based diversity analysis

In order to address breeding programs and validate the value of varied germplasm lines in crop breeding programmes, it is helpful to evaluate genetic diversity and comprehend the genetic linkages within the germplasm collection (38). SSR markers were employed in the current work to analyze the genetic diversity among 71 genotypes of chickpeas in order to determine whether or not these marker systems could be useful in breeding programmes. Following research, the genetic diversity of 71 genotypes of chickpeas was evaluated.

Table 2. Disease reactions under Fusarium wilt sick plot conditions

Sl. No.	Genotypes	Mean SS (%)	Reaction (SS)	Mean RS (%)	Reaction (RS)
1	ICCV 201211	21.20	MS	29.58	MS
2	ICCV 201210	25.52	MS	33.59	S
3	ICCV 201109	10.29	MR	24.78	MS
4	ICCV 20116	15.09	MR	30.48	S
5	ICCV 201115	16.99	MR	35.83	S
6	ICCV 201214	32.78	S	47.60	S
7	ICCV 201112	11.20	MR	26.45	MS
8	ICCV 201205	16.20	MR	54.39	HS
9	ICCV 201104	19.15	MR	23.33	MS
10	ICCV 201206	18.70	MR	31.75	S
11	ICCV 20117	18.34	MR	32.48	S
12	ICCV 201207	6.46	R	15.34	MR
13	Pant Gram 5	8.89	R	20.89	MS
14	H12-55	13.70	MR	27.16	MS
15	RVG 202	6.20	R	14.61	MR
16	SAGL 22-110	9.09	R	15.74	MR
17	SAGL 22-116	16.99	MR	38.95	S
18	SAGL 22-117	9.53	R	28.72	MS
19	SAGL 22-118	6.46	R	18.12	MR
20	SAGL 22-119	11.46	MR	21.45	MS
21	SAGL 22-120	14.02	MR	22.04	MS
22	SAGL 22-121	11.46	MR	24.31	MS
23	SAGL 22-122	16.20	MR	27.92	MS
24	SAGL 22-123	9.53	R	28.72	MS
25	SAGL 22-124	14.02	MR	54.39	HS
26	SAGL- 152327	13.70	MR	35.74	S
27	SAGL- 152324	8.70	R	25.77	M.S
28	SAGL- 152237	10.29	MR	54.78	HS
29	SAGL- 152278	15.09	MR	27.26	MS
30	SAGL- 152250	6.46	R	23.67	MS
31	SAGL- 152330	4.23	R	23.33	MS
32	SAGL- 152238	11.20	MR	26.45	MS
33	SAGL- 152405	16.20	MR	33.80	S
34	SAGL- 152339	8.89	R	15.34	MR
35	SAGL- 152344	18.70	MR	28.72	MS
36	SAGL- 162299	13.70	MR	40.74	S
37	SAGL- 162387	16.99	MR	29.58	MS
38	SAGL- 152227	23.70	MS	17.58	MR
39	SAGL- 162381	14.71	MR	17.08	MR
40	SAGL- 162364	23.70	MS	62.74	HS
41	SAGL- 152356	13.70	MR	32.88	S
42	SAGL- 152337	8.89	R	20.89	MS
43	SAGL- 153226	6.61	R	55.74	HS
44	SAGL- 152336	15.49	MR	44.78	S
45	SAGL- 152222	6.46	R	62.56	HS
46	SAGL- 152318	17.42	MR	62.74	HS
47	SAGL- 152258	13.70	MR	24.31	MS
48	SAGL- 152231	8.70	R	23.07	MS
49	SAGL- 152223	19.62	MR	24.03	MS
50	SAGL- 152234	6.46	R	18.12	MR
51	SAGL- 152329	3.98	R	27.16	MS
52	SAGL- 162376	3.83	R	20.37	MS
53	SAGL- 162377	11.46	MR	24.31	MS
54	RVSSG 84	8.89	R	15.34	MR
55	RVSSG 74	6.76	R	13.21	MR
56	JG 130	8.70	R	25.77	MS
57	RVSSG 83	9.53	R	22.66	MS
58	JAKI 9218	8.89	R	12.56	MR
59	RVG 204	11.20	MR	26.45	MS
60	JG 6	8.70	R	25.77	MS
61	RVSSG 92	9.77	R	35.83	S
62	ICC 4958	9.53	R	16.60	MR
63	RVSSG 71	9.09	R	27.16	MS
64	RVSSG 52	7.26	R	17.58	MR
65	RVSSG 68	6.61	R	27.16	MS
66	SAGL- 161024	16.99	MR	29.58	MS
67	SAGL- 163006	6.33	R	36.59	S
68	SAGL- 161025	14.71	MR	29.58	MS
69	SAGL- 163007	21.20	MS	55.02	HS
70	JG 315 (Check)	3.70	R	6.58	R
71	JG 62 (Check)	95.49	HS	100.00	HS

where, **SS**= Seedling stage; **RS**=Reproductive stage; **R**=Resistance; **MR**= Moderate resistance; **MS**=Moderate susceptible; **S**=Susceptible; **HS**= Highly susceptible

Table 3. Reaction of chickpea genotypes against Fusarium wilt under epiphytotic field conditions

Disease reaction	Number of genotypes		Name of genotypes	
	Seedling stage	Reproductive stage	Seedling stage	Reproductive stage
Resistant	31	1	ICCV 201207, Pant Gram 5, RVG202, SAGL 22-110, SAGL 22-117, SAGL 22-118, SAGL 22-123, SAGL- 152324, SAGL- 152250, SAGL- 152330, SAGL- 152339, SAGL- 152337, SAGL- 153226, SAGL- 152222, SAGL- 152231, SAGL- 152234, SAGL- 152329, SAGL- 162376, RVSSG 84, RVSSG 74, JG 130, RVSSG 83, JAKI 9218, JG 6, RVSSG 92, ICC 4958, RVSSG 71, RVSSG 52, RVSSG 68, SAGL- 163006, JG 315	JG 315
Moderately Resistant	33	13	ICCV 201109, ICCV 20116, ICCV 201115, ICCV 201112, ICCV 201205, ICCV 201104, ICCV 201206, ICCV 20117, H12-55, SAGL 22-116, SAGL 22-119, SAGL 22-120, SAGL 22-121, SAGL 22-122, SAGL 22-124, SAGL- 152327, SAGL- 152237, SAGL- 152278, SAGL- 152238, SAGL- 152405, SAGL- 152344, SAGL- 162299, SAGL- 162387, SAGL- 162381, SAGL- 152356, SAGL- 152336, SAGL- 152318, SAGL- 152258, SAGL- 152223, SAGL- 162377, RVG 204, SAGL- 161024, SAGL- 161025	ICCV 201207, RVG 202, SAGL 22-110, SAGL 22-118, SAGL- 152339, SAGL- 152227, SAGL- 162381, SAGL- 152234, RVSSG 84, RVSSG 74, JAKI 9218, ICC 4958, RVSSG 52
Moderately Susceptible	5	34	ICCV 201211, ICCV 201210, SAGL- 152227, SAGL- 162364, SAGL- 163007	ICCV 201211, ICCV 201109, ICCV 201112, ICCV 201104, Pant Gram 5, H12-55, SAGL 22-117, SAGL 22-119, SAGL 22-120, SAGL 22-121, SAGL 22-122, SAGL 22-123, SAGL- 152324, SAGL- 152278, SAGL- 152250, SAGL- 152330, SAGL- 152238, SAGL- 152344, SAGL- 162387, SAGL- 152337, SAGL- 152258, SAGL- 152231, SAGL- 152223, SAGL- 152329, SAGL- 162376, SAGL- 162377, JG 130, RVSSG 83, RVG 204, JG 6, RVSSG 71, RVSSG 68, SAGL- 161024, SAGL- 161025
Susceptible	1	14	ICCV 201214	ICCV 201210, ICCV 20116, ICCV 201115, ICCV 201214, ICCV 201206, ICCV 2011,7 SAGL 22-116, SAGL- 152327, SAGL- 152405, SAGL- 162299, SAGL- 152356, SAGL- 152336, RVSSG 92, SAGL- 163006
Highly susceptible	1	9	JG 62	ICCV 201205, SAGL 22-124, SAGL- 152237, SAGL- 162364, SAGL- 153226, SAGL- 152222, SAGL- 152318, SAGL- 163007, JG 62

Table 4. Molecular analysis among chickpea genotypes using SSR markers

Sl. No.	Marker	Major Allele Frequency	Number of Allele	Gene Diversity	PIC value
1	TA110	0.3521	4	0.7018	0.6434
2	TA59	0.5774	3	0.5578	0.4828
3	TA200	0.2816	4	0.7482	0.7011
4	TA37	0.5070	3	0.6165	0.5447
5	TS74	0.5492	2	0.4951	0.3725
6	TS79	0.4788	3	0.6181	0.5410
7	H3A12	0.4225	4	0.7054	0.6557
8	H1B06	0.5352	3	0.5768	0.4954
9	TR19	0.5070	3	0.6094	0.5347
10	TR2	0.4225	3	0.6427	0.5669
11	GA16	0.5774	2	0.4879	0.3689
12	GA20	0.4084	4	0.6915	0.6338
13	GA137	0.3661	4	0.6958	0.6370
Mean		**0.4604**	**3.23**	**0.6267**	**0.5521**

Fig. 1. Chickpea genotypes affected by Fusarium wilt (*foc*) under wilt sick plot.

Gene diversity among the markers

Table 4 shows that the average genetic dissimilarity of the SSR markers was 0.6267, with a range of 0.4879 to 0.7482. The markers TA200 (0.7482), H3A12 (0.7054), TA110 (0.7018), GA137 (0.6958), GA20 (0.6915), TR2 (0.6427), TS79 (0.6181), TA37 (0.6165), TR19 (0.6094), H1B06 (0.5768) and TA59 (0.5578) had the highest gene diversity. High molecular variation among genotypes is indicated by a high amount of genetic diversity; this conclusion is consistent with the earlier findings (32, 34). It was mapped *Foc2* and *Foc3* with the flanking markers H3A12 and H1B06 respectively (36). The marker TA37 was found to be polymorphic (PIC=0.5447) and generated alleles corresponding to resistance and susceptibility in different genotypes. It was reported a linkage of this marker with the *Foc-1* locus for resistance against race 1 of the Fusarium wilt (39). A number of markers including CS27A, TA27, TA59, TA96, TA103, TA110, TA194, Tr19, H1B06, H1F05, H1F22, H1P09/2 and H6D11, H3A12 were mapped on LG2 of the chickpea reference map in earlier studies (40-43).

Phylogenetic analysis

A phylogenetic tree (Fig. 3) based on UPGMA analysis of 71 chickpea genotypes was constructed to analyze genetic resemblance among and between genotypes studied. According to a study, UPGMA is the most popular and dependable sort of hierarchical clustering technique (44). The chickpea genotypes studied were sorted into 2 primary groups and 6 clusters by the UPGMA-based dendrogram (Table 5 and Fig. 3). As shown in Table 5, Cluster I had 14 genotypes, Cluster II had 10 genotypes, Cluster III had 5 genotypes, Cluster IV had 12 genotypes, Cluster V had 14 genotypes, whereas Cluster VI had 16 genotypes. The results suggested that only limited genotypes showed polymorphic alleles with resistant check JG315 using these SSR markers (Fig. 3). Cluster

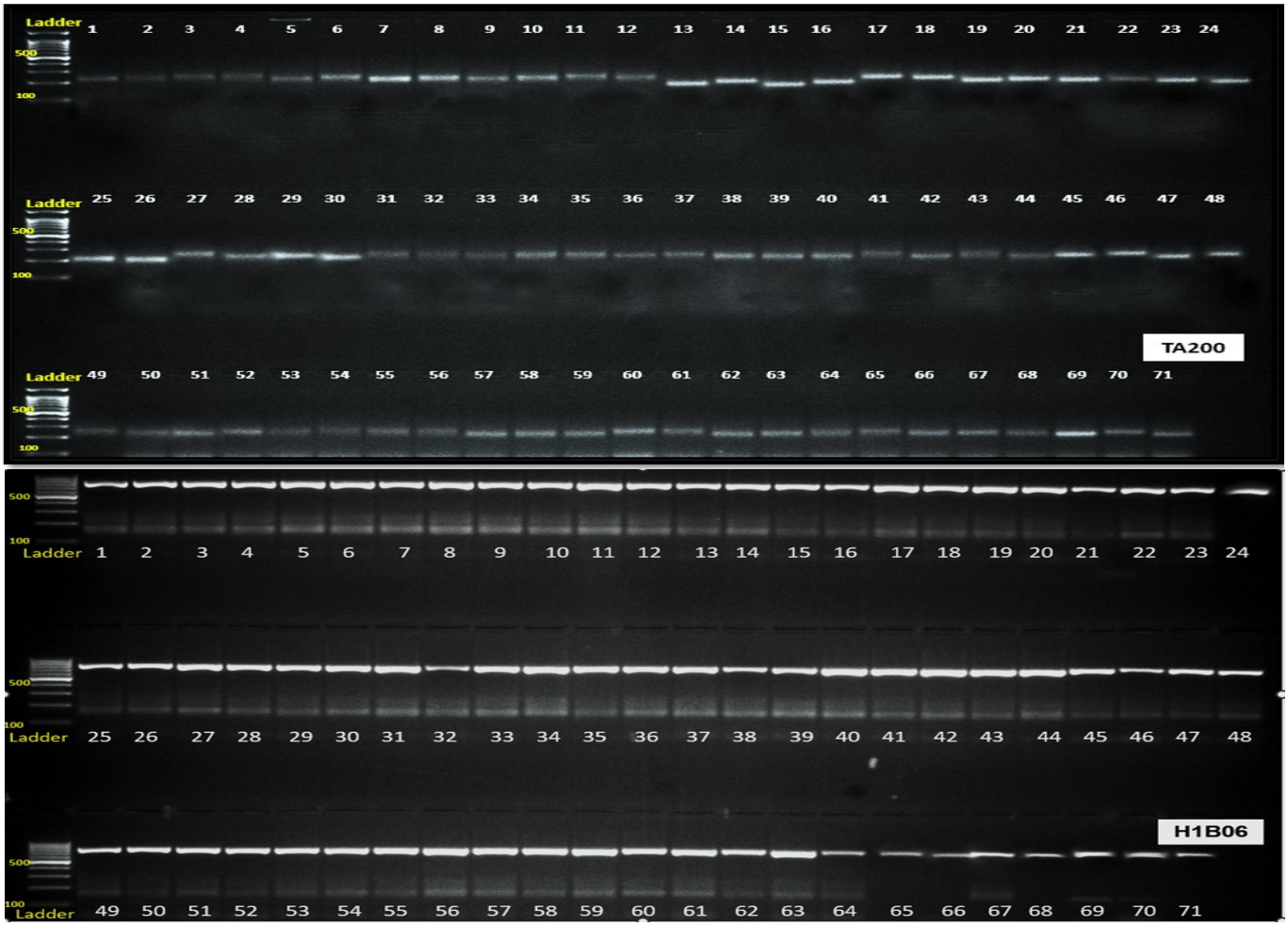

Fig. 2. Agarose Gel electrophoresis image representing PCR product of TA200 and H1B06 SSR molecular markers.

Table 5. Grouping of chickpea genotypes into clusters based on the similarity index of SSR markers

Cluster	Name of genotypes
Cluster I	SAGL-161025, SAGL-163006, SAGL-163007, SAGL-152237, SAGL-152405, SAGL-161024, ICC 4958, JAKI 9218, SAGL-152278, SAGL-152238, SAGL-152250, SAGL-152344, SAGL-162299 and SAGL-162387
Cluster II	SAGL-15230, SAGL-152339, SAGL-152223, JG 6, RVSSG 52, SAGL-162377, SAGL-152324, RVSSG 84, JG 315 and RVSSG 74
Cluster III	RVSSG 68, RVG 204, RVSSG 71, RVSSG 83 and RVSSG 92
Cluster IV	JG 62, SAGL-152318, SAGL-152337, SAGL-152356, SAGL-162364, SAGL-153226, SAGL-152222, SAGL-152231, SAGL-152258, SAGL-152336, SAGL-152227 and SAGL-162381
Cluster V	ICCV 201104, ICCV 201112, ICCV 201214, SAGL-152234, ICCV 201205, ICCV 201206, ICCV 201207, JG 130, SAGL-152329, SAGL22-117, H12-55, ICCV 201115, RVG 202 and SAGL 22-110
Cluster VI	ICCV 201109, SAGL 22-124, ICCV 201210, ICCV 201210, SAGL 22-116, SAGL-152327, Pant Gram-5, SAGL 22-118, SAGL 22-122, SAGL 22-119, SAGL 22-120, ICCV 20116, SAGL 22-121, SAGL 22-123, ICCV 201117 and SAGL-162376

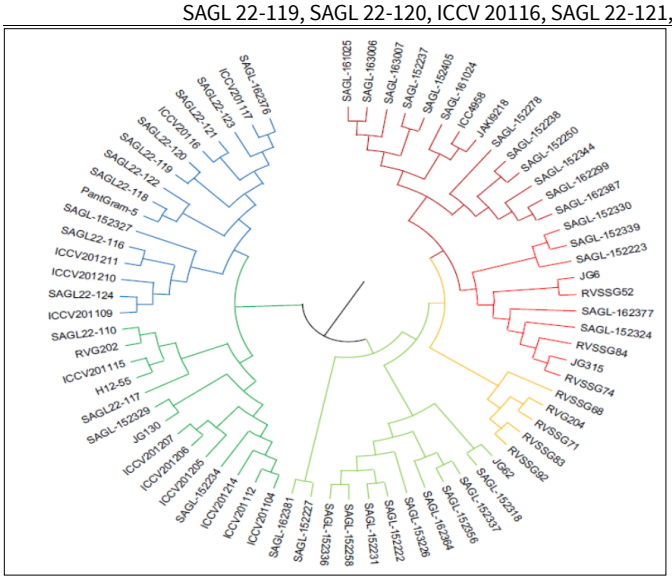

Fig. 3. Phylogenetic tree of 71 chickpea genotypes using the UPGMA method.

analysis was described by earlier researchers (45, 46).

Principal coordinate analysis (PCoA)

In addition to the phylogenetic analysis, PCoA was utilized to analyze multi-dimensional connections that indicate the percentage of genetic variance in the dataset (47). Every genotype was dispersed throughout the plot, as demonstrated by the PCoA. Based on similarity indices, the scatter plot produced by PCoA divided the 71 genotypes into 2 groups (Fig. 4).

Population structure analysis

Population structure was utilized to classify individuals into populations to identify admixed and migratory individuals and distinguish unique genetic groups. The data's log probability, LnP (D), grew steadily as K (the number of groups or populations) increased. Using all 71 genotypes and 13 polymorphic markers with varied K from 1 to 14, a

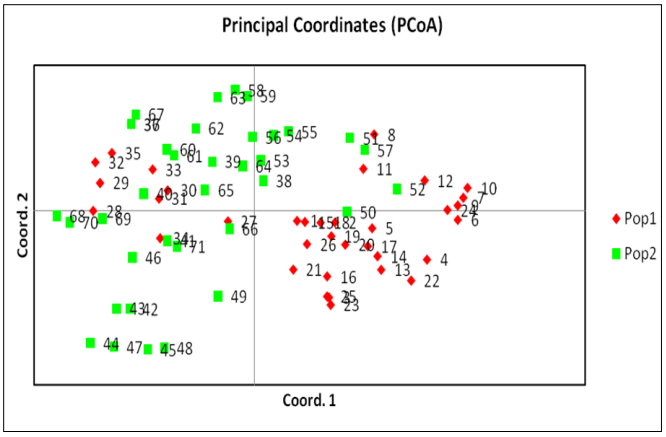

Fig. 4. Principal coordinates analysis (PCoA) of 71 chickpea genotypes using 13 SSR markers.

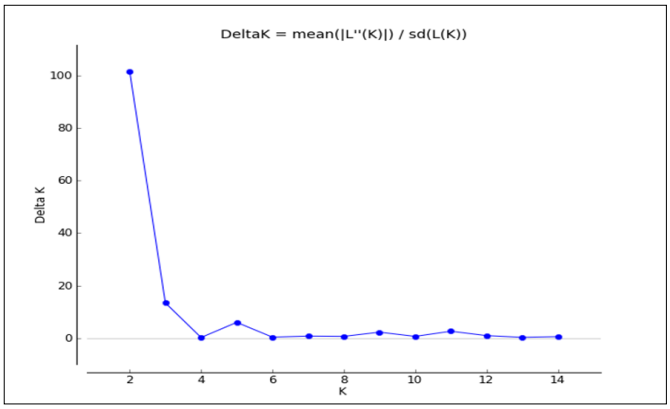

Fig. 5. Estimation of the population using LnP(D) derived ΔK with K ranged from 1 to 10.

population structure model was examined (Fig. 5). The presence of 2 populations in the tested genotypes was indicated by the largest peak, which was detected at K = 2, with a ΔK = 104 value in the ΔK analysis (Fig. 5). Two groups were created in the population structure, which likewise showed this pattern (Fig. 6). There were also 2 minor peaks at K = 3 (ΔK = 14.50) and K = 5 (ΔK = 6). Two populations, *viz*., P1 and P2, representing 50.70% and 49.29% of the genotypes used in the structural study respectively, were formed at K=2 from all genotypes. The accessions with membership proportion (Q) of 80% or higher were categorized as pure, while the rest were categorized as admixtures. 57 pure and 14 admixture lines were investigated at an 80% threshold (Fig. 7). Fig. 7 shows that of the 57 pure lines, 28 were found in Group-1 (G1 = red) and 29 in Group-2 (G2 = green). Based on membership fractions, genotypes in these 2 populations were categorized as either pure or admixture populations: P1 showed 77.77% pure and 22.22% admixed individuals, while P2 showed 82.85% pure and 17.14% admixed individuals. Other researchers who conducted similar analyses include (46, 48, 49).

Analysis of molecular variance (AMOVA)

The results of the analysis of molecular variance among the 71 genotypes showed that genetic differences within populations accounted for 96% of the variance, whereas genetic differentiation within populations explained 4% of the variance.

Conclusion

In order to boost productivity and lower yield losses, it is imperative to create high-yielding, disease-resistant cultivars of chickpeas, a significant legume crop in many regions of the world. In chickpeas, Fusarium wilt is still a very harmful vascular disease. In our investigation, we used sick plots and SSR molecular markers to screen 71 different genotypes of chickpeas against Fusarium wilt. This study revealed that one genotype *viz*., JG315 was found to be resistant, 13 genotypes *namely*, JAKI 9218, RVSSG74, RVSSG84, ICC 4958, RVSSG52, ICCV201207, RVG

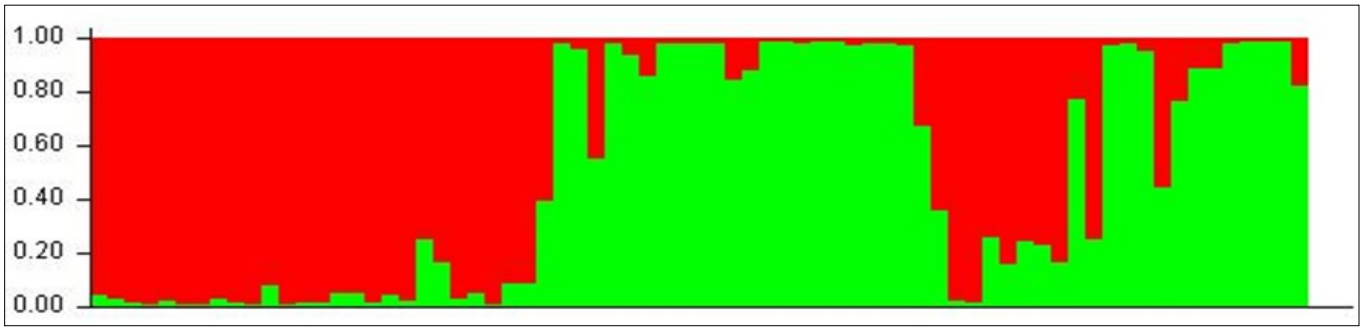

Fig. 6. Population structure (K = 2) inferred from STRUCTURE analysis for the 71 chickpea genotypes.

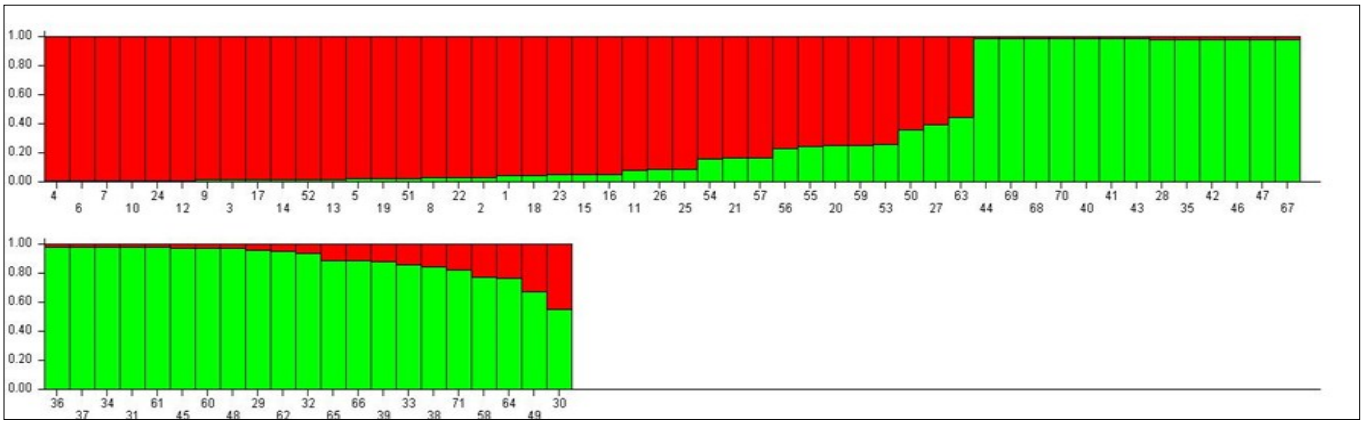

Fig. 7. Graphical representation of 71 chickpea genotypes using 13 markers for K = 2.

202, SAGL 22-110, SAGL 22-118, SAGL-152339, SAGL-152227, SAGL-162381 and SAGL-152234 were moderately resistant at reproductive stage under wilt sick plot. Future initiatives aimed at improving chickpeas may find great value in the resistant genotypes that have been found. Because molecular markers provide advantages over conventional approaches, they have become an essential component of breeding programs. SSR marker-based genetic diversity study shed light on the genetic connections between different chickpea cultivars. The SSR genotyping results suggested that only limited genotypes including SAGL-152330, SAGL-152339, SAGL-152223, JG6, RVSSG 52, SAGL-162377, SAGL-152324, RVSSG 84 and RVSSG 74 showed polymorphic alleles with resistant check JG315 using SSR makers. Genotypes *viz*., JG315, RVSSG84, JAKI9218, ICC4958, SAGL-152339, RVSSG 52 and RVSSG 74 responded to wilt resistance under both sick plot and SSR genotyping. Thus, these genotypes may be considered wilt resistant. The breeder may include these genotypes as a donor for disease-resistance sources in MAS breeding programmes to develop wilt-resistant cultivars.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process :

During the preparation of this work, the author(s) used (Grammarly) in order to (improve language of the manuscript). After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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