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Genetic Variability for Yield and Yield Contributing Traits of Chickpea (*Cicer arietinum* L.) through SSR Markers

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Abstract: ***The molecular genetic diversity analysis with six chickpea mutants of genotype-V2 (IC265298) with one control (untreated) to determine their transferability in chickpea, 24 SSR markers taken from adzukibean, common bean, cowpea, mungbean, chickpea, and pigeonpea were employed". The effectiveness of transferable SSRs was also examined in several genotypes of this species". The results revealed among the twenty-four SSR markers utilized, a total of 71 alleles were amplified, with the number of alleles ranging from 2 to 4 and a mean of 2.63 alleles per locus. Particularly, highest (4) number of alleles were amplified by markers SSR 1,4. The amplified products with 24 SSRs were ranged from 116 to 277 bp. The highest PCR fragment (277 bp) was generated by primer 22 and the lowest size fragment (116 bp) by SSR 16 & 18. Total of 71 alleles generated by using 24 SSR markers and the obtained PIC values. These findings indicate a high level of polymorphism among 6 chickpea mutants with one control genotype. Generally, genotypes have lower PIC value described that lower mutants and higher PIC values indicates higher mutants with higher genetic diversity.***

Keywords: Chickpea, Induced EMS, SSR markers, Yield attributes, Genetic variability

1. Introduction

Chickpea (*Cicer arietinum* L.) is an important agricultural crop and 4th most important grain-legume crop after soybean, bean, and pea (Upadhyaya et al. 2008). Chickpea is an important grain legume as a cheap protein source in most developing countries in Asia and Africa. (Saeed et al. 2011; Jannatabadi et al. 2014). Assessment of the genetic diversity within crop germplasm is fundamental for breeding and conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids (Talebi et al. 2008). The conservation and use of diverse collections of plant genetic resources is the backbone in plant breeding programs, so this genetic variability is the raw material for crop breeding industry on which selection acts to evolve superior genotypes (Saeed et al. 2011; Upadhyaya et al. 2008). Molecular genetic markers have been widely used in the last decades for both assessment of original material and search for valuable plant phenotypes. Genetic diversity in the chickpea has been explored using a range of molecular markers such as SSR (Saeed et al. 2011; Ghaffari et al. 2014), RAPD and AFLP (Talebi et al. 2008). In recent years, molecular markers have been reported to be used for accurate genetic diversity estimation, identification of pure lines or cultivars for germplasm maintenance, selection of diverse parental combinations to produce segregating offspring with the greatest genetic variability, and introgression of desirable traits into existing cultivars to broaden the genetic base (Sari D et al, 2023, Aggarwal et et al., 2015*)*. "The creation of genomic libraries for SSR marker development is extremely expensive and time-consuming". However, identification of transferable markers from the related species has proved to be an important way to develop SSR markers and it was successfully achieved in the other food legume crops (Millan, et al, 2010). "To determine genetic variability in chickpea, 24 SSR markers taken from adzuki bean, common bean, cowpea, mungbean, chickpea, and pigeonpea were employed.

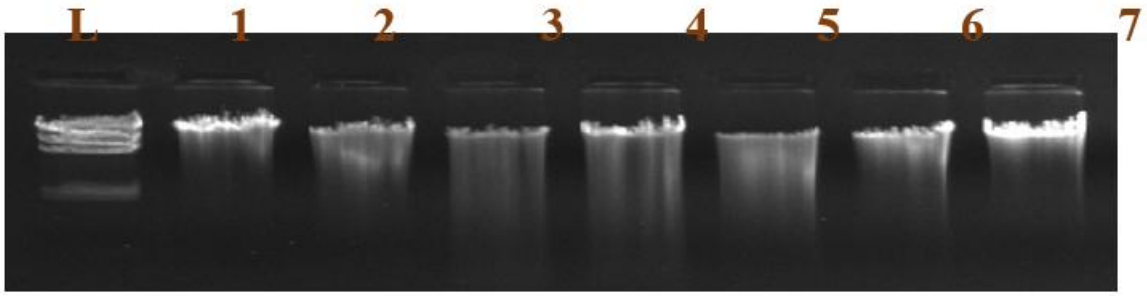
2. Materials and Methods

The present study was done with 6 chickpea mutants with one control genotype to determine genetic diversity among mutants and the genotypes using SSR (Simple Sequence Repeats) markers. In the present study 24 yield related SSR markers recorded polymorphism during their respective amplifications and results in different banding patterns among them. The results indicate that the majority of SSR markers displayed polymorphism. These polymorphic SSRs were then employed to analyse the genetic variation among the chosen mutants and control genotype. "The SSR markers amplified by polymerase chain reaction (PCR) in a volume of 20 *µl*, containing 15ng genomic DNA', 1 U of *Taq* DNA polymerase (Cinagene), 2 µM of each primer, 100 µM of each dNTP (Cinagene), 2 µl (10×) PCR buffer, 2.5 mM $MgCl_2$ (Cinagene) and ddH_2O, using a Eppendorf PCR System (Master Cycler Gradiant, Eppendorf). Amplification was carried for 38 cycles, each consisting of a denaturation step at 95 °C for 1 min, annealing at 50-60°C for 1 min and an extension step at 72°C for 2 min. PCR products were analyzed using 3% 'Metaphor' agarose electrophoresis gels stained with ethidium bromide. Frequencies of incidence of all polymorphic alleles for each SSR markers were calculated and used for determining statistical parameters. Total genomic DNA was extracted from Chickpea leaves by CTAB method given by Murray & Thompson, (1980) with some modifications. Approximately 1gm of Rice leaves was transferred to mortor and masticated to fine suspension with CTAB buffer (100 mM Tris, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, pH 8.0). This fine suspension was taken into 2 ml eppendorf tubes. These tubes were incubated in a water bath at 65^0C for 40 minutes with intermittent mixing for every 15

minutes. The incubated samples were allowed to attain room temperature and an equal volume of Chloroform : Isoamyl Alcohol (C:IAA= 24:1) was added, gently mixed for 15 minutes and centrifuged at 10,000rpm for 5 minutes. The aqueous layer obtained is transferred to 1.5 ml eppendorf tubes. To these tubes containing supernatant, an equal volume of chilled Isopropanol was added and gently mixed by inverting the tubes. These tubes were incubated in refrigerator for 15 minutes and centrifuge at 10,000 rpm for 5 minutes. The supernatant obtained is discarded and the pellet is washed with 100μl of 70% Ethanol, followed by centrifugation at 10,000 rpm for 5 minutes. The pellet is air dried and dissolved in 50-100μl sterile distilled water/ $T_{10}E_{0.1}$ buffer, where $T_{10}E_{0.1}$ buffer facilitates for longer storage whereas sterile distilled water for shorter storage. The samples were stored in -20^0 C refrigerator for further use.

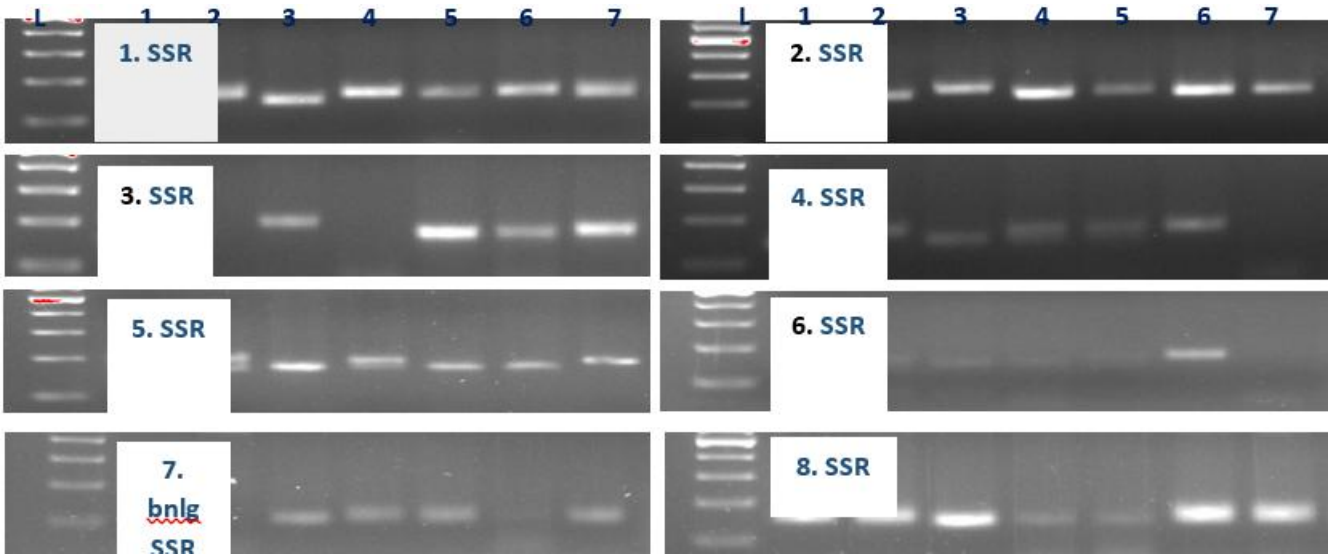
3. Results and Discussions

Molecular genetic markers have been widely used in the last decades for both assessment of original material and search for valuable plant phenotypes. Genetic diversity in the chickpea has been explored using a range of molecular markers such as SSR, RAPD and AFLP etc. More recent studies have demonstrated that highly polymorphic and Co-dominant SSR markers can be used for gene mapping and diversity fingerprinting in chickpea. Thus, SSR markers can be widely used for constructing chickpea related genetic linkage maps.



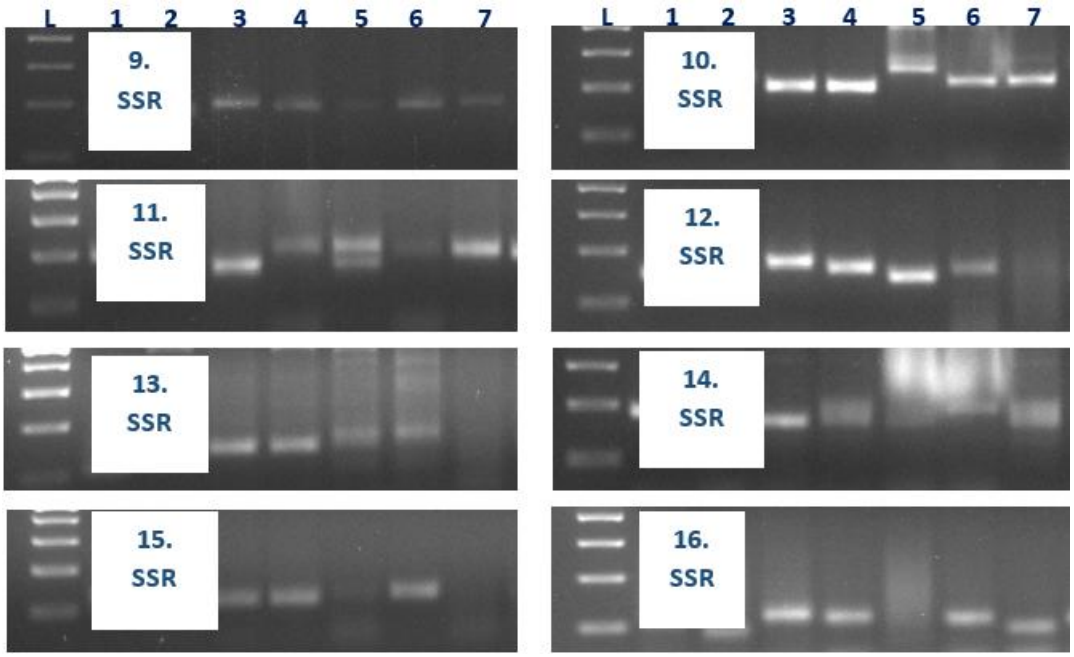
Elution of DNA, L- DNA Ladder (100bp), 1 – 6 mutants and 1 control

Figure 1: Genomic DNA extraction in 6 mutants and 1 control chickpea



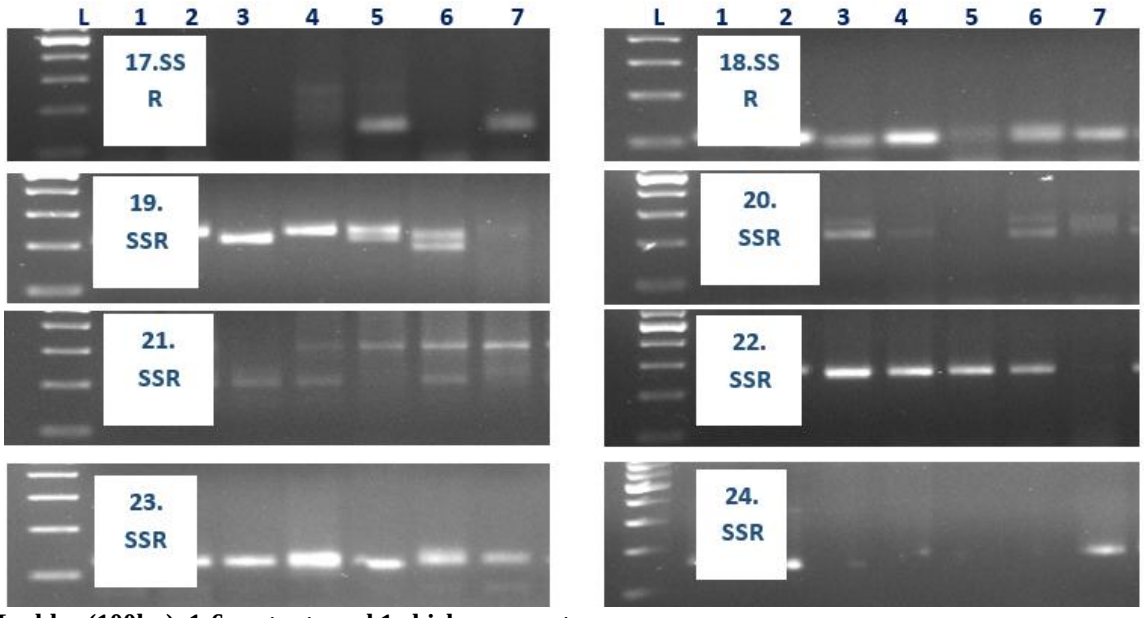
L- DNA Ladder (100bp), 1-6 mutants and 1 chickpea genotype

Figure 2: PCR amplification profile of 6 mutants and 1 control chickpea generated by SSR primers



L- DNA Ladder (100bp), 1-6 mutants and 1 Chickpea genotype

Figure 3: PCR amplification profile of 6 mutants and 1 control chickpea generated by SSR primers



L- DNA Ladder (100bp), 1-6 mutants and 1 chickpea genotype

Figure 4: PCR amplification profile of 6 mutants and 1 control chickpea generated by SSR primers

The results indicate that the majority of SSR markers displayed polymorphism. These polymorphic SSRs were then employed to analyse the genetic variation among the chosen mutants and control genotype.

Table 1: Characteristics 24 SSR Number of allele, product size, PIC value and heterozygosity

S. No.	Marker	No. of alleles	Product size	PIC	Heterozygosity
1	TA64	4	141-182	0.83	0.85
2	TA46	3	216-229	0.85	0.88
3	TR7	2	157, 162	0.79	0.81
4	TA28	3	185-198	0.82	0.86
5	TA27	4	158-202	0.72	0.77
6	TAASH	2	161, 165	0.75	0.82
7	TA117	2	131, 142	0.73	0.77
8	TA113	2	139, 151	0.71	0.74
9	GA26	2	178, 182	0.83	0.85
10	TA179	3	187-193	0.74	0.77
11	TAA58	2	177, 182	0.78	0.81
12	TR43	4	138-159	0.72	0.77
13	TA18	3	130-153	0.74	0.78
14	TA22	4	157-172	0.72	0.77
15	TA135	3	161-169	0.85	0.87
16	CaSTM2	3	116-128	0.78	0.82
17	GA26	2	1,18,126	0.77	0.8
18	TA72	2	116, 123	0.71	0.74
19	TA180	3	196-218	0.85	0.83
20	H3E04	2	225, 233	0.75	0.77
21	TR2	2	214, 221	0.7	0.77
22	CaSTMS15	2	265, 277	0.75	0.81
23	TA130	2	140, 142	0.72	0.76
24	TR29	2	146, 151	0.81	0.83
Mean		2.63		0.77	0.8

Among the twenty-four SSR markers utilized, a total of 71 alleles were amplified, with the number of alleles ranging from 2 to 4 and a mean of 2.63 alleles per locus. Particularly, highest (4) number of alleles were amplified by markers SSR 1 and 4. The amplified products with 24 SSRs were ranged from 116 to 277 bp. The highest PCR fragment (277 bp) was generated by primer 22 and the lowest size fragment (116 bp) by SSR 16 & 18. Total of 71 alleles generated by using 24 SSR markers and the obtained PIC values (Table 1). These findings indicate a high level of polymorphism among 6 chickpea mutants with one control genotype. Generally, genotypes have lower PIC value described that lower mutants and higher PIC values indicates higher mutants with higher genetic diversity. Similar genetic diversity were found by Sefera et al., 2011; Choudhary et al., 2012; Aggarwal et al., 2015 in chickpea genotyeps.

4. Conclusion

The results showed that particularly, highest (4) number of alleles were amplified by markers SSR 1 and 4. The highest PCR fragment (277 bp) was generated by primer 22 and the lowest size fragment (116 bp) by SSR 16 & 18. Total of 71 alleles generated by using 24 SSR markers and the obtained PIC values. These findings indicate a high level of polymorphism among six chickpea mutants with one control genotype.

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