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SHORT RESEARCH ARTICLE

Genetic diversity and population structure analysis in popular cowpea (*Vigna unguiculata* (L.) Walp) cultivars

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Abstract

Genetic diversity and population structure of 21 cowpea (*Vigna unguiculata* (L.)Walp) popular genotypes were evaluated using simple sequence repeat (SSR) molecular markers. A set of 144 primer pairs were used; out of which 71 gave polymorphic amplicons. A total of 184 amplicons with an average of 2.562 alleles per polymorphic marker were observed. The average effective number of alleles (Ne) was 1.928 alleles per genotype. The presence of a higher Shannon's information index (I) of 0.651 indicates higher diversity in the given set of cowpea lines. The total accessions were stratified into 2 groups (K=2) in population structure analysis. In phylogenetic analysis, 3 major clades were formed. The results give insight into existing diversity in the popular cowpea cultivars and a framework for future studies aimed at the selection of parental lines for cowpea genetic improvement programmes.

Keywords: Cowpea, Diversity, Popular cultivars, SSR markers.

Introduction

The genome of cowpea (*Vigna unguiculata* (L.)Walp) is diploid with 2n = 22, and has a genome size of about 620 million base pairs. The productivity of cowpea is limited by numerous abiotic and biotic constraints and the farmers have limited access to quality seeds of improved varieties. The shrinking genetic resources of crop varieties, including cowpea pose a great threat to agricultural production. Proper selection of materials containing the desired gene(s) is a prerequisite to achieve the breeding objectives and shorten the breeding period. Modern molecular genetic tools could be used efficiently for cowpea improvement. Therefore, genetic diversity study is important for breeding varieties with target traits and utilizing germplasm resources to improve cultivars (Tan et al. 2012). In the past decades, chloroplast DNA polymorphisms (Vaillancourt and Weeden 1992), RAPD (Diouf and Hilu 2005), RFLP (Fatokun et al. 1993), DAF (Simon et al. 2007), AFLP (Coulibaly et al. 2002), ISSR (Ghalmi et al. 2010), and SSR (Desalegne et al. 2016) have been used to analyze the genetic variation among cowpea varieties. More recently, with the next generation sequencing (NGS), single nucleotide polymorphism (SNP) markers have been increasingly used in these types of studies and have been shown to be effective (Desalegne et al. 2017). As far as SSRs markers are concerned, they are extremely effective tools in diversity studies because, in addition to being abundant and randomly distributed in the genome, they are highly polymorphic, heritable, co-dominant, easily reproducible and traceable with simple screening (Li Wang et al. 2008). This study was undertaken to assess the genetic diversity and population structure of a given set of 21 cowpea cultivars, landraces and germplasm.

A set of 21 popular cowpea genotypes (VBN1, PL1, IT3895-1, KBC2, DC15, RC19, PL3, PL4, C152, RC101, PL2, GC3, DC16, GC4, VBN3, PCP 03061, KBC9, TVX944, KM5, GC6 and GC5) released through the Varietal Release committee of India were used in the current study and have been named as CP1 - CP 21, respectively in the current study. Total genomic DNA 50 mg was extracted from the well-developed

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trifoliate leaves with the DNeasy plant mini kit (Qiagen), as per the manufacturer's procedures. DNA integrity and concentration were checked for further studies. The cowpea (Honglin et al. 2017) and adzuki bean SSR markers (Wang et al. 2004) reported in previous studies and few cowpea specific primers designed through MISA using the reference cowpea genome available at NCBI were used in the study. Primers were synthesized by Eurofin Scientific.

PCR, gel-electrophoresis, diversity and population structure analysis

PCR was performed in a total reaction volume of 15 µL containing 1X PCR buffer, 2 mM of $MgCl_2$, 0.2 mM of dNTPs, 1.25 U of Taq DNA polymerase (Genei laboratories Pvt Ltd) and 0.33 pM each of forward and reverse primers with the following cycling conditions: 96°C for 3 minutes, 30 cycles each of 96°C for 30 seconds, 55 to 65°C for 30 seconds, 72°C for 30 seconds and 72°C for 7 minutes. The amplicons were separated on 4% agarose gel and primer pairs yielding clear unambiguous bands were used for assessing polymorphism taking 100 bp ladder as a reference through the GelOne (Cleaver Scientific, Cambridge, UK) gel documentation system.

A set of 144 SSR primer pairs were used for assessing the genetic diversity. GenAlEx version 6.5 was used to calculate the genetic diversity indices (Peakall and Smouse 2012) and cluster analysis for polymorphic SSR loci was performed using DARwin version 6.0.15 (Perrier and Collet 2006) to assess the phylogenetic relationship. The Bayesian clustering method was used for inferring the population structure of the cowpea accessions using the STRUCTURE version 2.3 software. The Burn-in period and Monte Carlo Markov Chain (MCMC) iterations both were set to 10,000 with an admixture model to deduce the number of clusters using K values between 1 and 10. Five independent runs were performed for each K value. The best K- value for estimating a suitable population size was identified by the *Evanno* method in the online based Structure Harvester program (Earl et al. 2012).

Polymorphism of SSRs

Out of 144, 71 markers were polymorphic that were used for further analysis. Markers with only 1 allele but a corresponding null allele in one or few genotypes were also considered polymorphic. Genotypic data reveals the presence of 1 to 7 alleles (Na) of different markers. The markers like CEDG 300, CEDG 141, CEDG 21, CEDG 50, CEDG 67 etc. had only one amplicon or null allele; likewise, other markers gave 6 and 7 amplicons, like Vu 19 and Vu 17 markers.

Allelic frequency determined was in the range of 0.048–0.952, revealing the presence of rare alleles in some genotypes along with the major alleles. A total of 184 amplicons were documented from 71 markers with an average of 2.562 amplicons, most of the markers had 2 to 3 amplicons.

The effective number of alleles (Ne) ranged from 1.00 (all markers with only 1 amplicon) to 4.523 (Vu 17, marker with 7 alleles) with an average of 1.928 alleles per genotype. Similarly, Shannon's information index (I) ranged from 0.00 (all markers with only 1 amplicon) to 1.67 (Vu 17, marker with 7 alleles) with an average of 0.651 that suggests presence of higher diversity in the current set of genotypes. The average observed heterozygosity (Ho) and expected heterozygosity (He) was found to be 0.115 and 0.387, respectively.

Phylogenetic analysis

The genotypes being studied were stratified into two main clusters with few admixtures (Fig. 2), as revealed by STRUCTURE analysis. Cluster1 includes 13 genotypes (CP 1, CP 2, CP 3, CP 6, CP 7, CP 8, CP 10, CP 11, CP 12, CP 14, CP 16, CP 20 and CP 21, depicted in red bars) while Cluster 2 includes only 8 genotypes (CP 4, CP 5, CP 9, CP 13, CP 15, CP 17, CP 18, CP 19, depicted in green bars). The analysis revealed admixture in genotypes 7, 9, 15 and 18.

A similar clustering was observed in the phylogenetic analysis done using DARwin; wherein 3 clades were observed. Clade 1 includes all lines present in Cluster 2 and Clade 2 and 3 includes all genotypes stratified in Cluster 1 (Fig. 2).

Genotyping using crop-specific and heterologous SSR markers revealed amplification of 1-7 alleles in the population indicating allelic richness in the variety panel studied. Our results are in congruence with previous studies in other legumes, e.g., variation in number of alleles

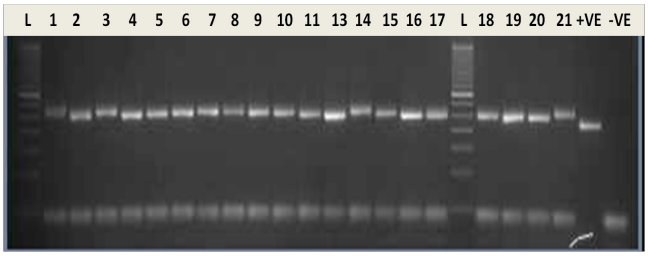

Fig. 1. A representation gel electrophoresis among 21 genotypes using CEDG 003 primers along with 50 bp ladder

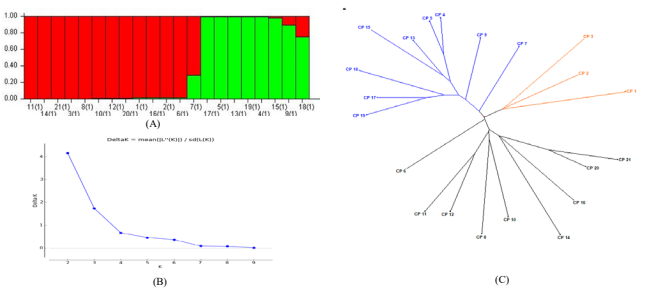

Fig. 2. (A) Estimated population structure of 21 cowpea accessions at *K* = 2 reveals presence of 2 subgroups. (B) Delta K (1K) for different numbers of subpopulations (K). (C) Phylogenetic analysis of 21 cowpea lines depicts the presence of 3 major clades

amplified in chickpea from 1 to 8 (Winter et al. 1999), alfalfa from 9 to 14 (Mengoni et al. 2000) and soybean from 11 to 26 (Rongwen et al. 1995). Presence of high Shannon information index (I) reveals the presence of higher diversity in the selected set of lines. Variations in SSRs is reflective of the extent of genetic variation present in the cowpea gene pool. This study thus documents good genetic diversity in the variety panel studied.

We also observed the presence of three major clusters in both phylogenetic as well as population structure analysis which is in accordance with Xiong et al. (2016). Although there are attempts to study genetic diversity present in cowpea germplasm, no study has been conducted to study the diversity present in the released varieties. This kind of study is imperative to know the origin of different varieties, whether any parent is used repeatedly or if different parental lines have been used.

Authors Contribution

Conceptualization of research (MR); Designing of the experiments (MR, AP, SK, NPS); Execution of field/lab experiments and data collection (PS, JP, SK, MR); Execution of field/lab experiments and data collection (MR, AP, SK, NPS); Analysis of data and interpretation (KK, MR), Preparation and editing of manuscript (SK, KK, MR, AP, NPS).

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