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Evaluation of genetic purity among chickpea (*Cicer arietinum*) seeds with different colours through SSR markers

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Introduction

Maintaining high genetic purity in crop cultivars is mandatory for achieving certification presenting reasonable agronomic performance in the field, and attracting farmers and investors. DNA-based technologies were developed to provide better discrimination of varieties for breeders (Smith et al., 2008).

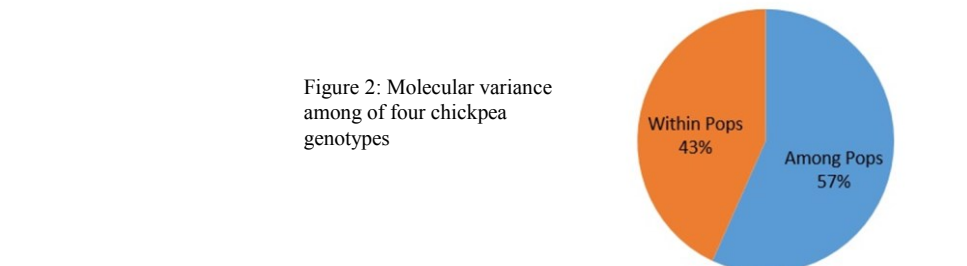
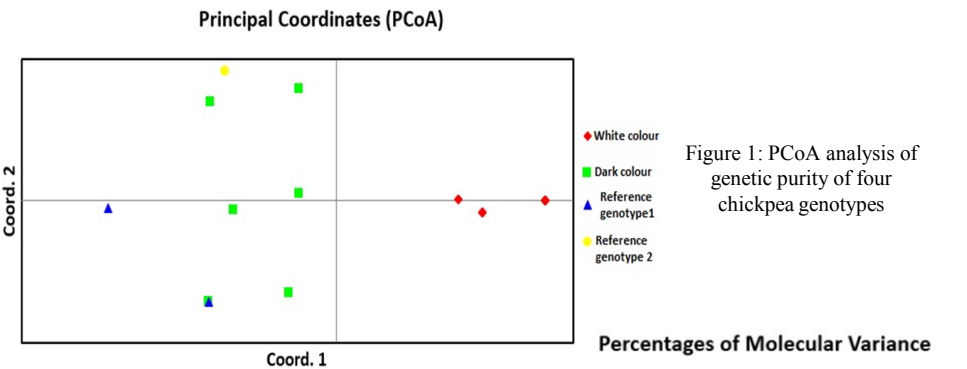
Materials and methods

Chickpea genotypes with two different colours along with two reference seeds, were investigated to discriminate molecular characterization at molecular markers Laboratory. Genomic DNA extraction was isolated from leaves as described by Doyle (Doyle, 1991). DNA quantification and qualification were measured using NanoDrop (ND-1000, Thermo Fisher Scientific) at 260 nm. Two SSR primer pairs were used to identify genetic purity of the studied chickpea genotypes. PCR amplification and poly acrylamide gel electrophoresis were run through the highly polymorphic primer sets. Both SSR primers exhibited polymorphisms, as shown in Table 1. Amplified DNA fragments were scored based presence and absence of the alleles. The total number of alleles per locus, allele frequency, PCOA (Principal Coordinate Analysis), and molecular variance were calculated using the statistical analysis software GenAEx version 6.5. Polymorphic Information Content (PIC), heterozygosity index (HI), marker Index (MI) and discriminating power (D) were calculated using the software POWER MARKER, version 3.23 (Table 1).

Table 1: polymorphism indices of two SSR markers among 4 chickpea populations

SSR Primers		Sequence(5-3)	Tm	Genetic purity test				
				Alle le/n	PIC	HI	MI	D
SSR-H1A06	Forward	TGGATAATTGTAGGGTAAGAAATGC	60	5	0.66	0.71	0.71	0.71
	Reverse	TGTGTAATTAAAGTGTGGGGGTATT						
SSR- H3C11	Forward	GCCCATATTCAATTCTTACCATTATTA	58	4	0.61	0.66	0.66	0.44
	Reverse	ACCTTTAACGCTAATAGAGTGAGTTTA						

Tm, annealing temperature; PIC, polymorphism information content; heterozygosity index (HI); marker Index (MI) and discriminating power (D)



Results and Discussion

In this study, two sets of SSR markers (SSR-H1A06, H3C11) were employed to determine the genetic purity of two chickpea genotypes introduced by a breeder along, along with two reference genotypes from our seed bank. Both markers demonstrated polymorphism in the chickpea genotypes during the purity test, whereas marker SSR-H1A06 showed the greater efficiency based on polymorphism indices (Table 1).). SSR markers are widespread molecular markers due to their highly abundance and polymorphic status (Zane et al, 2002; Xu et al, 2013). SSR polymorphism produced satisfactory results for plants genetic research and molecular breeding due to its multi-allelic nature and variation in DNA segment length (Shu et al, 2021). The highest values of the genetic variation parameters were observed in the dark-coloured chickpea genotype (Figure 1). The PCOA analysis of SSR data grouped the four chickpea populations into three main clusters: white- coloured chickpea was clustered separately, while the dark-coloured chickpea grouped with the two reference genotypes (Figure 1). Further, the white and dark -coloured chickpea seeds showed no complete conformity with our seed bank genotypes. Our results revealed 43% molecular variance within populations and 57% among populations (Figure 2). Thus, this study demonstrates the potential of two SSR markers to distinguish different colours of a chickpea genotypes and assess their genetic purity.

Conclusion

The SSR markers would be the trustworthy genetic means to evaluate genetic purity of chickpea genotypes. Besides, these two SSR markers showed proper potential to determine genetic purity of a chickpea genotype based on different colours.

Keywords: Chickpea; Genetic purity; SSR markers; Polymorphism; Genetic variation

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