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Genetic divergence, path analysis and molecular diversity analysis in cluster bean (*Cyamopsis tetragonoloba* L. Taub.)

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ABSTRACT

Cluster bean, an important export commodity, is a drought tolerant crop of arid and semi-arid regions. This legume is not only grown for food, feed and fodder purpose but also having a vast range of diverse and unique industrial applications. Presence of galactomannan gum makes it an important industrial crop with commercial outlook. Owing to its high industrial qualities, there is a requirement for well documentation to increase cluster bean production. In this study the genetic/diversity was assessed among 31 cluster bean genotypes using morpho-physiological, yield and SSR markers. A total of 17 morphological traits were studied for the analysis of variance, correlation and path coefficient. The genotypes showed considerable amount of variability for most of the traits with high heritability (>51%). All the genotypes were classified into five groups on the basis of standardized morphological data. SSR amplification with 17 primers detected 33 alleles with a mean of 1.83 alleles/SSR. The clustering revealed five distinct groups at 0.68 cut-off value. The cluster analysis showed low genetic variation among the genotypes studied. The phenotype-based cluster did not correspond with the molecular-based cluster as the correlation coefficient for the two clustering matrices was negative ($r = -0.72$). The results of the present study indicated that molecular markers as a complementary tool should be used in conjunction with morphological characterization for better description of the level and pattern of genetic diversity, and for crop improvement.

1. Introduction

Cluster bean or guar (*Cyamopsis tetragonoloba*) is an important self-pollinated crop with chromosome number 2n = 14 (Hymowitz and Upadhya, 1963). Guar is a well-adapted and deep rooted hardy leguminous herb of arid and semi-arid parts of the world (Pathak et al., 2011). It is believed to have originated from Africa but it's been grown throughout southern Asia, especially India which accounts for 80% of the total guar produced in the world. It is also cultivated in Pakistan as a cash crop and, to a limited extent in other parts of the world such as Australia, Bangladesh, Myanmar, South Africa, and Srilanka (Kuravadi et al., 2014). It is an ancient crop of rained eco-system and is an important source of nutrition to both human being and animals. Cluster bean is used for human consumption and cattle feed (30–40%), industrial purpose (50–55%), medicinal (5%) as well as for soil improvement and other miscellaneous purposes.

Galactomannan gum, a polysaccharide derived from guar seeds, is a natural hydrocolloid soluble in cold water and forms a thick solution at low concentrations and extensively used in paper, mining, food, cosmetic, textile, oil and pharmaceutical industries around the world (Brahmi et al., 2004). As a result, because of its gum content, cluster bean has emerged as a new industrial crop. Cluster bean endosperm is a rich source of good galactomannan (78–82%; Das and Arora, 1978). In addition to its industrial applications, it has medicinal value in the treatment of diabetics and high cholesterol cases (Kumar et al., 2013). Therefore, demand for cluster bean has increased globally in recent years, leading to crop introductions in several countries (Undersander et al., 1991) including U.S.A., South Africa, Australia, Brazil, Zaire and Sudan. But, in spite of the importance of this crop in agricultural production, productivity of guar is very low.

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The studies on phenotypic, genetic and molecular diversity are essential to determine the genetic distance among genotypes and to identify groups with similar genetic backgrounds for conserving, evaluating and utilizing germplasm for hybridization (Shabanimofrad et al., 2013). Thus, assessment of genetic variability present in the germplasm and the extent to which it is heritable is critical factor in managing and exploiting the diversity.

Traditionally, genetic diversity is determined through the analyses of morphological parameters. Despite high variability at morphological level, variation in SSR in guar germplasm has not been studied in detail which can offer a clear picture of molecular diversity. Previously many researchers have reported the nature and extent of genetic diversity among the different genotypes of guar using morphological characters (Pathak et al., 2011; Girish et al., 2012; Kumar et al., 2013; Jukanti et al., 2015). But, morphological markers could be misleading particularly for quantitative traits, which are controlled by multigenes. Moreover, phenotype based genetic analysis are influenced by environmental factors; hence does not reliably reflect true genetic variation (Pathak et al., 2009). Therefore, attempts have been made to analyse untapped genetic diversity of guar for breeding and crop improvement (Punia et al., 2009; Kumar et al., 2013; Sharma et al., 2014).

Information regarding the extent and pattern of genetic variation in cluster bean is limited (Sharma et al., 2014). Biochemical marker studies using allozyme (Brahmi et al., 2004) have also been carried out in guar. Though, with the advent of recent methods in molecular biology, different molecular markers have been applied to the study of molecular diversity in cluster bean like RAPD (Punia et al., 2009; Pathak et al., 2010), rDNA (Pathak et al., 2011) and ISSR (Sharma et al., 2014) but there is paucity of sequence based markers especially simple sequence repeats. Thus due to availability of limited molecular and genomic resources like molecular markers, the pace of cluster bean breeding has been hindered and slow (Kuravadi et al., 2014).

Biotechnological approaches have not been fully utilized for the improvement of this important commercial crop (Randhawa and Verma, 2014). The cloning of β- mannan synthase gene from developing guar endosperm (Dhugga et al., 2004) and analysis of ESTs for studying galactomannan synthesis (Naoumkina et al., 2007) has dragged the attention of molecular biologist on this crop. Recently, Kuravadi et al. (2014) has mined SSR sequences from 16,476 expressed sequence tags (ESTs) of guar followed by identification and characterization of 187 SSR markers in guar. Despite great industrial value, so far no attempt has been made in guar to study the genetic variation using SSR markers and morphometric data simultaneously.

Therefore, in present study, attempts have been made to study phenotypic, genotypic and genetic divergence in guar and detection of genetic relationships among genotypes using a collective approach of morphological traits and molecular markers to accelerate the future crop improvement program of guar.

2. Materials and methods

2.1. Plant materials and field evaluation

A total of 31 released guar varieties were raised at Agronomy farm, Anand Agricultural University, Anand in three replications. The average rainfall of the zone is around 750 mm. The soil of the experiment farm is sandy loam in texture and poor in organic carbon. The experiment was laid out in a randomized complete block design with ten plants per variety in each replication. Rows were spaced 0.45 m apart with 0.15 m plant to plant distance. The standard package of agronomical practices, were followed to raise a healthy experimental crop.

During the course of this study, data on the 17 characters were recorded from randomly selected five competitive plants in each variety in each replication except for days to 50% flowering and days to maturity which were recorded on the plot basis. The crude oil content of seed was chemically determined using the Soxhlet extraction method, while the semimicro-Kjeldahl method was used to assess crude protein content. For estimation of gum content, the procedure given by Das and Arora (1978) was used.

2.2. DNA isolation and SSR analysis

DNA was extracted from 2 g young leaves collected from 10-day-old seedlings (bulked samples consisted of randomly selected 5–10 plants from each accession) using the protocol described by Doyle and Doyle (1990).Integrity of DNA was determined by 0.8% agarose gel electrophoresis and quantified by Nanodrop ND-1000 (Thermo, USA). The DNA was diluted to a working concentration of 20 ng/μL.PCR was performed in a 10 μL reaction mixture containing 1.0 × PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 10 pmol of each primer, 1 unit of *Taq* polymerase, and 20 ng of genomic DNA. A touch down PCR amplification profile with 94 °C for 3 min of initial denaturation, followed by first 5 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, with 10 °C decrease in annealing temperature per cycle, then 30 cycles of 94 °C for 30 s with constant annealing temperature 60 °C and 72 °C for 1 min followed by a final extension for 5 min at 72 °C. PCR amplicons were resolved on 6% non-denaturing PAGE.

2.3. Morphological characterization and statistical analysis

During the study, data were recorded from the five competitive plants of each genotype in each replication. For 17 morphological traits, data were analysed for analysis of variance (ANOVA) and LSD test was performed to identify genotypes that were significantly different from each other. SAS (SAS Institute, Inc., 1996) procedures and programmes were used for these calculations. Genetic parameters were estimated to identify genetic variability among accessions and determine genetic and environmental effects on various characters. These genotypic and phenotypic components of variance, coefficients of variability, broad sense heritability and genetic advance were estimated by adapting the formulae suggested by Allard (1960) and Singh and Chaudhary (1977).

i. Genotypic variance ($\hat{\sigma}^2 g$) = (MSG − MSE)/r, where MSG = mean square of accession, MSE = mean square of error, and r = number of replications

ii. Environmental variance ($\hat{\sigma}^2 e$) = MS_e

iii. Phenotypic variance ($\hat{\sigma}^2 p$) = $\hat{\sigma}^2 g + \hat{\sigma}^2 e$

iv. Genotypic coefficient of variation (GCV%) = $\frac{\sqrt{\hat{\sigma}^2 g}}{\bar{X}} \times 100$

v. Phenotypic coefficient of variation (PCV%) = $\frac{\sqrt{\hat{\sigma}^2 p}}{\bar{X}} \times 100$

vi. Heritability in broad sense (h_2%) = $\frac{\hat{\sigma}^2 g}{\hat{\sigma}^2 p} \times 100$

Where, h_2 = Heritability (broad sense), $\hat{\sigma}^2 g$ = Genotypic variance, $\hat{\sigma}^2 p$ = Phenotypic variance.

vii. Genetic advance as per cent of mean

Expected genetic advance (GA) = $K \times \frac{\hat{\sigma}^2 g}{\hat{\sigma}^2 p} \times \sigma_p$

Where, K = Standardized selection differential (K = 2.06 at 5% selection intensity),

$\frac{\hat{\sigma}^2 g}{\hat{\sigma}^2 p}$ = Heritability in broad sense, σ_p = Phenotypic standard deviation, GA (as% of the mean) = $\frac{GA}{\bar{X}} \times 100$ X refers to mean of the trait being evaluated.

To evaluate the relationship among the different variables in the experiment, correlation coefficients were used by SAS 9.2. As correlation alone cannot explain relationships among the characters,