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Research Article

TESTING DNA VARIATION IN KABULI CHICKPEA SEEDS THROUGH ISSR-PCR

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ABSTRACT

Objective: Chickpea is an annual legume known for its high seed protein thus beneficial for one's health and well-being. Of the two chickpea seed types, Kabuli seeds have reasonably high-protein and -carbohydrate contents, as a result, set up more commercial demand.

Methods: Inter simple sequence repeat (ISSR) markers are useful in the areas of genetic diversity. The present study attempted ISSR-PCR experimentation in testing DNA variation predominantly in Kabuli chickpea seed accessions employing 10 different set of ISSR primers.

Results: *In vitro* amplification by PCR revealed an average number of bands per primer per accession which accounted to 8.7. Maximum number of bands (25) scored by the primer UBC-836 while least number of bands (12) were displayed by UBC-811.

Conclusion: The accession JGK-1 and IPCK-12-288 presented divergent DNA polymorphic among all chickpea accessions tested and therefore found distinct. The genetic divergence at DNA level existed between these chickpea accessions can be used proficiently for future plant breeding program.

Keywords: Kabuli chickpea, Inter simple sequence repeat-PCR, Plant breeding, MIPS gene, Chickpea apoptosis.

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INTRODUCTION

Chickpea is a well-accepted food legume plant belonging to the family *Leguminosae*. [1]. The plant species *Cicer arietinum* L. is the only cultivated species represented by various cultivars that are grown commonly. Chickpea seeds are the most preferred source of protein and afford good source of carbohydrates, minerals, and protein [2]. In addition, seeds of chickpea have certain medicinal properties [3,4]. It also constitutes a better source of livestock feed.

Seeds of chickpea are broadly categorized in two types that are Kabuli and Desi seed accessions representing two assorted set of gene pools [5]. The chickpea seeds have dissimilar protein and antinutritional contents [6-8]. Chickpea seeds of both Kabuli and Desi do contain various antinutritional elements. Nutritional levels, however, prominently get enhanced on germination and antinutritional contents become degenerated [9,10]. Earlier studies stated that desi chickpea seed types are more sensitive to various physical methods removing antinutritional contents than Kabuli when subjected to normal and treated environments [11]. Further, gamma-rays effectively enhance the antioxidant level in chickpea seeds [12].

Moreover, seeds encompass flavonoids and many health promoting components and an important protein called lectins [13]. Lectins are carbohydrate binding proteins [14,15]. Chickpea seed contents and chickpea lectins are being characterized substantially [16,17]. This includes screening of lectins/phytohemagglutinin in more than 100 genotypes [18], purification and characterization [16,17,19], X-ray crystallography [20], microbial resistance [16], antifungal activity [17], MIPS gene [21], anticancer activities [3], apoptosis inducing activity [22], and its various pharmacological significance [4].

DNA markers have an advantage of not being influenced by environment. PCR is an *in vitro* technique of DNA amplification that utilizes various markers of known sequences. Various PCR-based markers that are most frequently employed by research community includes RAPD and inter simple sequence repeat (ISSR) markers. Both of these markers are rapidly being used in various fields of plant analysis/improvement. These markers are useful in the areas of genetic diversity [23-26], phylogenetic studies [27], and in a wide range of other legume crop species [28].

In ISSR-PCR technique, SSRs are used as primers to amplify mainly the inter-SSR regions. ISSRs segregate mostly as codominant markers following simple Mendelian inheritance. ISSR-PCR technique overcomes most of the limitations of *in vitro* PCR amplification and allied marker systems [29].

Knowledge of genetic diversity and relatedness in the germplasm of cultivated species is a prerequisite for better utilization in crop improvement program [30]. Genetic markers have been widely used to detect genetic variation at individual gene *loci* among several *loci*, their combinations, both between individual plants in a population, or between populations [31]. Interspecific hybridization, seed storage protein profiles, isozymes, karyotype, and molecular markers have been used as different criteria to investigate species relationships in the chickpea seed accessions [13,23-26,32-39]. Same experimental approach was, moreover, experimented in other legumes such as mung bean and moth bean [40-42]. Being a best source of food proteins, the present study makes an effort employing ISSR markers to screen out chickpea seed accessions to study genetic polymorphisms at DNA level, if any.

METHODS

Plant material

A typical group of seven Kabuli chickpea seed accessions were analyzed employing polymerase chain reaction. Agronomic particulars of all these accessions are given in Table 1. All accessions were obtained from Indian Institute of Pulses Research, Kanpur (U.P.), following MTA understanding.

DNA isolation

For DNA extraction, single seed was used following the CTAB extraction method as described by Talebi *et al.* [43] with slight alteration. Seed

material (100 mg) was crushed in liquid nitrogen and homogenized with freshly prepared extraction buffer (1 ml). To this, 20% SDS was added and incubated at 60°C for 30 min. Then after, 92 µl of 5 M NaCl was added, and subsequently, 75 µl of CTAB solution was mixed and reincubated at 65°C for 15 min. To this cocktail, 300 µl of chloroform: isoamyl alcohol mix (24:1) was added and subjected centrifugation at 12,000 g for 15 min in cold using Remi C-24 centrifuge. Chloroform: isoamyl alcohol mixture was added for a 2nd time to the supernatant in 1:1 volume and recentrifuged at 12,000 g for 15 min. Subsequently, DNA precipitation was done by adding ice-cold isopropanol 40% v/v as a final concentration. The precipitated DNA was centrifuged. The ethanol washed DNA was air dried overnight and dissolved in 100 µl of Tris-EDTA buffer (19 mM Tris-HCl pH 8.0; 1 mM EDTA pH 8.0). Isolated DNA was stored at −20°C.

DNA electrophoresis and quantification

The electrophoresis of the soluble DNA was performed on 1% agarose gel in Tris acetate-EDTA buffer comprised ethidium bromide (0.5 $\mu g.ml^{-1}$) as stain. The electrophoretic separation was carried out at 100 V for 45 min and DNA bands were visualized under a UV transilluminator. The quantification has been done by comparisons with lambda DNA mass marker of *MBI, Fermentas, Richlands B. C*. The quality of isolated DNA was checked by calculating absorbance ratio at 260 and 280 nm wavelength in Systronics UV–Visible spectrophotometer.

ISSR analysis

The information of ISSR primers is provided in Table 2. The polymerase chain reaction was carried out using a cocktail of the following in a final volume of 25 µl containing 10 mM Tris-HCl pH 9.0; 50 mM KCl; 0.1% Triton X-100; 1.5 mM $MgCl_2$; 0.1 mM dNTP; 2 mM primer; 0.5 unit of *Taq* DNA polymerase (*MBI, Fermentas, Richlands B. C.*); and 25 ng template DNA. Amplifications were carried out in a Bio-Rad 3.03 version thermocycler program was set for 35 cycles with an initial melting at 94°C, for 4 min, this was followed by denaturation at 94°C for 1 min. The annealing was performed at 37°C for 1 min, which then was followed by polymerization at 72°C for 2 min and a final extension step at 72°C for 7 min.

RESULTS AND DISCUSSION

ISSR amplification

PCR analysis shown DNA variation amongst the Kabuli chickpea seed accessions. Each primer yielded obvious bands of intensities and so used for counting. Out of 10 ISSR primers tested, five could amplify. While other five did not, such kind of non-amplifying primers were previously also been reported in chickpea [30]. Rest of the five amplifying ISSR primers yielded a total of 87 bands in a molecular weight range of 500–2000 bps. The average number of bands per primer per accession accounted to 8.7. Maximum number of bands scored by the primer UBC-836 was 25 while least number of bands displayed by UBC-811 was 12. The representative ISSR-PCR amplification patterns as generated by primer UBC-836 and UBC-835 are shown in Fig. 1.

Table 1: Agronomic particulars of Kabuli chickpea seed accessions used in the study

S. No.	Accession	Agronomic features
1.	IPCK-12-286	Kabuli, white and bold seeded, wilt resistant
2.	IPCK-12-287	Kabuli, white and normal seeded, wilt resistant
3.	IPCK-12-291	Kabuli, white and medium seeded, dwarf
4.	IPCK-12-277	Kabuli, white and small seeded, wilt resistant
5.	JGK-1	Kabuli, white and bold seeded, released variety
6.	IPCK-12-288	Kabuli, white and small seeded, wilt resistant
7.	KAK-2	Kabuli, white and normal seeded, released variety

Table 2: ISSR primers deployed in the study

S. No.	Primer	Anchor sequence	Tm (°C)
1.	UBC-808	5'AGAGAGAGAGAGAGAGC3'	50°C
2.	UBC-811	5'-GAGAGAGAGAGAGAGAC-3'	52°C
3.	UBC-835	5'AGAGAGAGAGAGAGAGAC3'	54°C
4.	UBC-836	5'AGAGAGAGAGAGAGAGCA3'	54°C
5.	UBC-866	5'CTCCTCCTCCTCCTCCTC3'	60°C
6.	UBC-864	5'-ATGATGATGATGATGATG-3'	48°C
7.	UBC-869	5'-GTTGTTGTTGTTGTTGTT-3'	41°C
8.	UBC-871	5'-TATTATTATTATTATTAT-3'	28°C
9.	UBC-879	5'-CTTCACTTCACTTCA-3'	36°C
10.	UBC-880	5'-GGAGAGGAGAGGAGA-3'	45°C

Based on this ISSR-PCR amplification data, a consensus was scored using a binary matrix indicating absence (0) and presence (1). For this observation, software PAST was taken for assistance. A similarity matrix was constructed employing the Jaccard coefficient using binary data [44]. For dendrogram construction, these data were further subjected to UPGMA cluster analysis, as shown in Fig. 2. Based on the cluster analysis, Kabuli accessions grouped into two clusters. Cluster I contained three accessions that are IPCK-12-277, KAK-2, and JGK-1 came as an out group. Cluster II included IPCK-12-291, IPCK-12-286, and IPCK-12-287 while IPCK-12-288 represented an out-group.

Previously, Bhagyawant and Srivastava [30] performed the genetic fingerprinting of chickpea cultivars using ISSR primers using grown cultivars only. Twelve *Cicer* cultivars were screened using 10 ISSR primers for PCR studies and revealed 5.85 bands per primer per genotype. A molecular evaluation of five chickpea varieties was conducted by Tahir and Karim [45] to access the genetic diversity and relationship of chickpea cultivars using RAPD and ISSR markers. All five primers used were polymorphic and generated 6.6 bands per primer in a total of 33 bands. Present results are in agreement with previous one.

The extent of polymorphism detected in the present study corroborate previously reported ISSR markers [46,47], though at a small scale. This may be due to obligatory self-pollination and an extensively

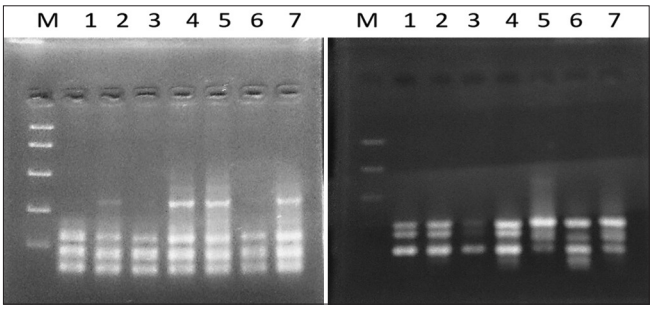

Fig. 1: Amplification of Kabuli accessions with primer (a) UBC-836 and (b) UBC-835 M: Markers Lanes 1–7: IPCK-12-286, IPCK-12-287, IPCK-12-291, IPCK-12-277, JGK-1, IPCK-12-288, and KAK-2

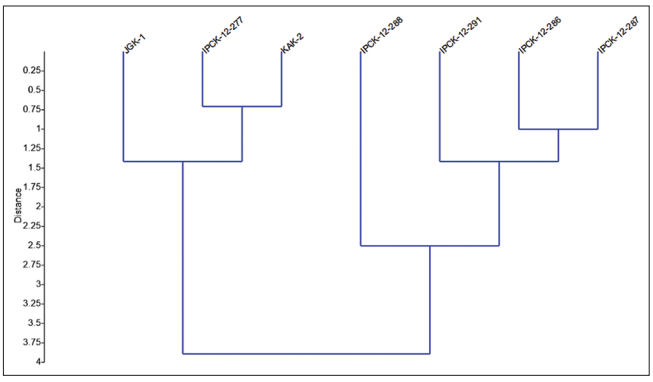

Fig. 2: Dendrogram depicting grouping in chickpea accessions due to ISSR-PCR

monotonous genome of chickpea. In the present scenario, ISSR markers are the efficient marker systems because of their ability to expose various informative *loci* from a single amplification. The idea behind such study was to provide better knowledge about genetic variability so that this information can prove be informative in the management of genetic resources of chickpea. Genetic information obtained from ISSR data can be used in categorizing chickpea cultivars and can also harmonize the genetic studies generated from morphological traits. Further, the genetic divergence that mounted among Kabuli chickpea accessions can be resourcefully used in plant breeding schedule.

CONCLUSION

ISSR-PCR analysis detected DNA polymorphismsin kabuli chickpea seed accessions. This information can assist breeders in forecasting future breeding program of chickpea.

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