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Research Article

RAPD ANALYSIS IN KABULI CHICKPEA SEED ACCESSIONS

NEHA GUPTA, SAMEER SURESH BHAGYAWANT*

School of Studies in Biotechnology, Jiwaji University, Gwalior, Madhya Pradesh, India.
Email: sameerbhagyawant@gmail.com

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ABSTRACT

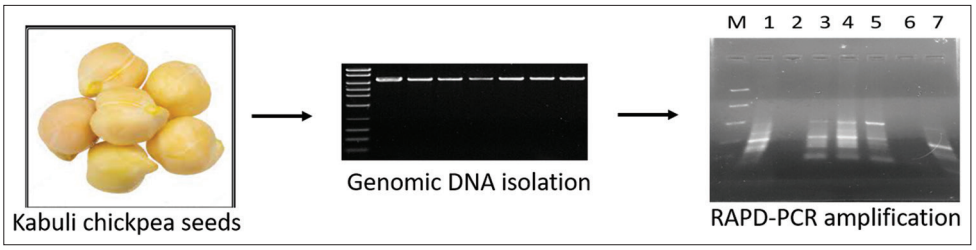
Objective: Random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction (RAPD-PCR) methodology promoted the development of molecular assay system to detect DNA polymorphisms. Analysis of genetic diversity among exclusive Kabuli accessions collected from IIPR, Kanpur, was demanding.

Methods: The present study apprised application of RAPD in assessing genetic diversity predominantly in Kabuli chickpea seed accessions.

Result: Of the 10 RAPD primers tested, the average number of bands per primer per accession accounted to 9.5. Maximum number of bands scored by the primer OPA-17 was 28 while least number of bands displayed by OPG-04 was 14.

Conclusion: The accession KAK-2 exhibited polymorphic among all and therefore diverse. Thus, it can be utilized as one of the parents in future chickpea breeding program.

Graphical Abstract



Keywords: Random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction, Kabuli chickpea, Genetic diversity.

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INTRODUCTION

The second most important edible food legume in the world is "chickpea" popularly called as "channa" in the Indian continent. The seeds of chickpea supplement exceptional source of proteins and nutrition that is required for human growth [1]. As such, there exit two leading categories of chickpea seed cultivars that are grown generally. They are Kabuli and desi type seed accessions demonstrating two diverse set of gene pools [2]. These seeds have different protein and antinutritional composition [3]. In addition, seeds enclose flavonoids and various health promoting elements [4] and an important protein called lectins [5]. Lectins are carbohydrate-binding proteins [6,7]. Chickpea seed contents and chickpea lectins are being characterized substantially at our research laboratory [2]. This includes screening of lectins/phytohemagglutinin in more than 100 genotypes [8], purification and characterization [9-11], X-ray characterization [12], antimicrobial activity [9], fungal resistance [10], MIPS gene [13], anticancer activities [14], apoptosis inducing activity [15], and its various pharmacological attributes [16].

Seeds of pulse when consumed in raw state can cause negative side effects in humans mentioned as anti-nutritional [17]. Chickpea seeds of both Kabuli and desi do contain such anti-nutritional elements. Published report describes that nutritional levels, however, get elevated on germination and anti-nutritional contents gets deteriorated [18,19]. Earlier studies stated that desi chickpea seed types are more sensitive to these physical methods removing anti-nutritional elements than Kabuli when subjected to normal and treated environments [20]. Furthermore, gamma-rays have been observed effective in enhancing antioxidant levels in chickpea seeds [21]. Consequently, chickpea seeds can be made available as a fortified food products in coming days.

Genetic diversity analysis enhances the chances of selecting better genotypes for breeding based on polymerase chain reaction (PCR) [22]. The differences at the deoxyribonucleic acid (DNA) level make available information about genetic relationships. PCR is an *in vitro* DNA amplification method, using arbitrary primers, has been widely employed in plant genotyping [23]. The random amplified polymorphic DNA (RAPD) amplifications based on the PCR are one of the most commonly used molecular markers. RAPD markers are the amplification products of anonymous DNA sequence using single, short, and arbitrary oligonucleotide primers thus they do not require prior knowledge of DNA sequence [24]. RAPD identification techniques can be used at any stage of plant development and they are not affected by environment factors [25]. It is rapidly being used by the research community in various fields of crop improvement. The technique was successfully employed in studying chickpea genetic diversity [26,27], phylogenetic studies [28], genome mapping [29], and evolutionary biology in a wide

range of other crop species [22]. The aim of this study was to appraise Kabuli chickpea seed accessions employing RAPD markers to provide information on DNA polymorphism.

METHODS

Plant material

An authenticated representative set of seven Kabuli chickpea seed accessions was analyzed employing PCR. 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 cetyltrimethylammonium bromide (CTAB) extraction method as described Talebi *et al*. [30], 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 6°C for 30 min. Then after, 92 µl of 5M 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.

RAPD primers

A set of selected 10 RAPD primers that were synthesized indigenously (courtesy: Operon Tech. Inc., Almeda, USA) and available in the laboratory was used in the study. Particulars of primers are signified in Table 2.

RAPD-PCR

The PCR procedure for DNA amplification was followed with some minor modifications [30]. Reaction carried out in 25 µl volume contained 10 mM Tris-HCl pH 9.0; 50 mM KCl; 0.1% Triton-x-100; 1.5 mM MgCl2; 0.1 mM dNTP; 2 mM primer; 0.5 unit of Taq DNA polymerase (MBI, Fermentas, Richlands B. C., Qld) and 25 ng template DNA. Amplification reactions was processed in a Bio-Rad 3.03 version thermocycler. The reactions were programmed for 35 cycles with an initial melting at 94°C for 4 min followed by denaturation at 94°C for 1 min. The annealing was performed at 37°C for 1 min, then followed by polymerization at 72°C for 2 min. Final extension was done at 72°C for 5 min.

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

Accession	Agronomic features
IPCK-12-286	Kabuli, white and bold seeded, wilt resistant
IPCK-12-287	Kabuli, white and normal seeded, wilt resistant
IPCK-12-291	Kabuli, white and medium seeded, dwarf
IPCK-12-277	Kabuli, white and small seeded, wilt resistant
JGK-1	Kabuli, white and bold seeded, released variety
IPCK-12-288	Kabuli, white and small seeded, wilt resistant
KAK-2	Kabuli, white and normal seeded, released variety

Table 2: RAPD primers deployed in the study

Primer	Sequence motif	Tm	GC content (%)
OPA-18	5'-AGGTGACCGT-3'	32°C	60
OPG-11	5'-TGCCCGTCGT-3'	34°C	70
OPG-04	5'-AGCGTGTCTG-3'	32°C	60
OPA-12	5'-TCGGCGATAG-3'	32°C	60
OPA-17	5'-GACCGCTTGT-3'	32°C	60
OPA-11	5'-CAATCGCCGT-3'	32°C	56
OPA-08	5'-GTGACGTAGG-3'	32°C	80
OPAC-06	5'-GGCTTCGCAA-3'	32°C	60
OPZ-06	5'-GTGCCGTTCA-3'	32°C	60
OPZ-10	5'-CCGACAACC-3'	30°C	62.2

Agarose gel electrophoresis and dendrogram construction

The PCR products were separated on 1.5% agarose gel prepared in 1× TAE; electrophoresis at 100 V for 3 h and bands were detected by ethidium bromide staining. Three kb standard molecular weight of MBI, Fermentas, Richlands B.C, was used as a marker. Clearly resolved bands were scored visually for their presence or absence. The DNA fragment profiles representing a consensus of two independent replicates were scored in the form of a matrix with "1" and "0," indicating the presence and absence of bands in each accession. Jaccard's similarity coefficient [31] was estimated from these binary data using Past software [32].

RESULTS AND DISCUSSION

RAPD amplification

RAPD analysis done by PCR revealed DNA polymorphism at a small scale among the Kabuli chickpea accessions that are experimented. Each primer yielded detectable bands of variable intensities and therefore used for scoring. Out of 10 RAPD primers tested, five could amplify while other five did not. Such kind of non-amplifying primers were previously also been reported in chickpea [33]. Rest of the five amplifying RAPD primers yielded a total of 95 bands in a molecular weight range of 500–2000 bps. The average number of bands per primer per accession accounted to 9.5. Maximum number of bands scored by the primer OPA-17 was 28 while least number of bands displayed by OPG-04 was 14. The representative RAPD-PCR amplification patterns as generated by primer OPA-17 and OPA-18 are shown in Fig. 1.

Based on this RAPD-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 an assistance. A similarity matrix was constructed employing the Jaccard coefficient using binary data. 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 two accessions that are IPCK-12-287 and IPCK-12-288. Cluster II included IPCK-12-286, IPCK-12-277, IPCK-12-291, and JGK-1 while KAK-2 came as an outgroup.

Analysis of genetic diversity among only Kabuli accessions collected from IIPR, Kanpur, was demanding. Earlier, for a long time, genetic diversity exploration has been done based on the morphological characteristics. Morphological traits display a continuous change in phenotypic behavior and as a quantitative trait, their expression is mostly controlled by multigenes. Qualitative traits are equally effective in diversity assessment in plant species with molecular markers [29].

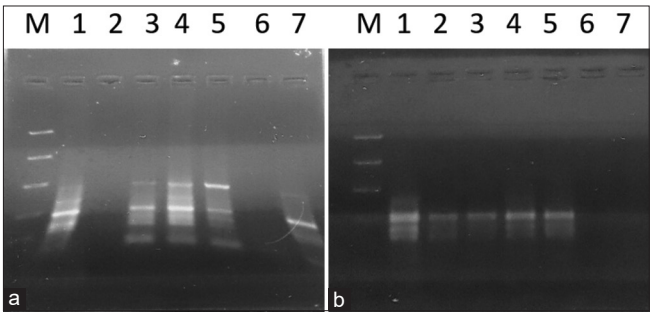

Fig. 1: Amplification of Kabuli accessions with primer (a) OPA-18 and (b) OPA-17 M: markers Lane 1 -07: IPCK-12-286 IPCK-12-287 IPCK-12-291 IPCK-12-277, JGK-1, IPCK-12-288, and KAK-2

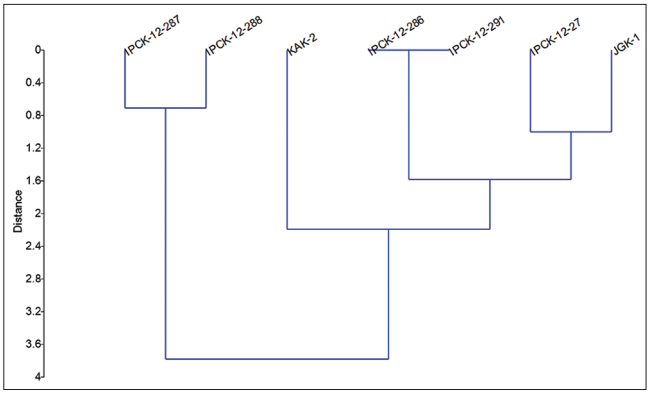

Fig. 2: Dendrogram of chickpea accessions based on random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction amplification

Then after, another era occurred, in which biochemical markers, namely, isoenzyme and protein-based markers were explored as a means of tapping genetic diversity. Total storage proteins were able to address extent of genetic diversity using SDS-PAGE [34,35]. In addition, seed storage proteins were investigated employing 2G electrophoresis coupled with mass spectroscopy [36]. Same experimental approach was moreover experimented in other legumes such as mung bean and moth bean [37-39]. Being a best source of food proteins, chickpea seed proteins are reported to provide not only a food source but as a good source of medicine when experimented *in vitro* and *in vivo* [9,11,40-44].

With the advent of time, PCR-based molecular markers are being routinely exercised for tapping genetic differentiation and/or understanding species relationships, if any. Genetic diversity normally described as an average sequence variation between any of the two individual species for a given loci. The degree of polymorphism though at a small scale in this study compared to other reports appears belonged to a different Kabuli chickpea seed accessions. In this study, RAPD produced a higher number of bands because RAPDs are random in nature and can anneal anywhere in the genome. In parallel to RAPD, other maker like ISSR is proven equally applied in assessing genetic diversity [45-47]. The technique of ISSR-PCR was equally worked well in other legumes crops like moth bean too [48].

CONCLUSION

RAPD-PCR methodology promoted the development of molecular assay system to detect DNA polymorphisms. The various molecular markers, however, have technical differences in terms of cost and reproducibility. Looking at the massive chickpea germplasm available in the gene bank and being a self-pollinated crop, genotype screening using DNA-based markers must be performed continuously unless whole germplasm is totally evaluated, like we have attempted herein. Such published reports can be documented by chickpea research organizations and gene banks to provide guidelines in executing future breeding programs. The present research provides small-scale polymorphisms at DNA level in Kabuli chickpea seed accessions by RAPD markers.

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