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**Original Research Article**

# Molecular Characterization of Vegetable-Type Pigeonpea Genotypes Using SCoT Markers

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**A B S T R A C T**

Fifteen Start Codon Targeted (SCoT) markers were used to test the molecular diversity among the sixteen pigeonpea germplasm lines for the vegetable purpose. The clear-cut bands were shown by only eight markers, out of which six marker showed 100% polymorphism and 2 marker *i.e.* SCoT 12 showed 35.7%, and SCoT 15 showed 60% polymorphism. The PIC value of the markers ranged from 0.4145 (SCoT-2) to 0.844 (SCoT-13). The average PIC value of the marker was 0.7345. The similarity matrix was constructed based on Jaccard's similarity coefficient, 16 genotypes were classified into two super clusters. Super cluster I had the maximum number of genotypes *i.e.* 15, and super cluster II had only one genotype. Within the super cluster I two sub-clusters was observed *i.e.* sub-cluster A and sub cluster B. Again within sub cluster A two sub sub-clusters were observed. The cluster analysis revealed a high diversity was observed in PKV-TARA and AKTM-1644. But in a broad sense a very low level of DNA polymorphism was observed among the selected genotypes of pigeonpea.

**Keywords**

Pigeonpea, SCoT markers, genotypes, dendrogram

## Introduction

Legumes are of prime importance in human diet and animal feed contributing the major source of vegetable protein. They are an economic source of not only protein but of carbohydrate, minerals and B-complex vitamins particularly in a vegetarian diet (Salunkhe *et al.*, 1985) and hence correctly called as poor man's meat (Heiser 1990). Besides being a source of protein, pulses are also important for sustainable agriculture as they improve Physical, Chemical, and Biological properties of the soil and function as mini- nitrogen factories. Pulses also have an inherent quality to trap moisture from the lower strata of the soil, therefore, they are considerably known to be drought tolerant and fit well in a rainfed environment. Pigeonpea is one of the major pulse crops of dry land agriculture because of its deep tap root system and inherent drought resistance. Its ability to produce a high amount of biomass per unit area makes it useful as fodder and for thatching for the rural masses. Large quantities of foliage drop add

to the organic matter of the soil. The deep and well-spread root system helps in soil aeration after decomposing pigeonpea. Hence, it is highly valued by the farmers. It is a sturdy crop and can be grown on a wide range of soils and under diverse agro-ecological environment. The legume can be utilized in several diverse ways while the high genetic variability exists within the cultivated and wild relatives remains to be explored for further uses.

The importance of plant genetic diversity is now being recognized as a specific area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in the developing countries. Breeding crops with higher yield and better nutritional traits are the need of the hour and the success of any breeding programme depends on the diversity in the germplasm. Molecular markers are the key tools to assess the variability. Molecular characterization using markers help us in analyzing the diversity at the genetic level. Gupta *et al.,* (1994) and Williams *et al.,* (1990) worked on DNA markers produced by polymerase chain reaction (PCR) using single primers that are designed from the short conserved region flanking the ATG start codon that is conserved for all genes.

Therefore, in principle, this technique is similar to RAPD or ISSR or single primer amplification reaction because a single primer is used as the forward and the reverse primer. Davis *et al.,* (1995) used SCoT technique based on the single primer amplified region principle since it uses a single primer as a forward and reverses primers, like the RAPD or ISSR technique. However, PCR amplification using SCoT primers targets gene regions surrounding the ATG initiation codon on both DNA strands. It is possible that some SCoT markers would be co-dominant due to insertion-deletion mutations; these would be the minority like co-dominant RAPDs.

## Materials and Methods

### Plant material

The germplasm lines of pigeon pea for the vegetable purpose were acquired from the germplasm maintained at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and different sources (Table 1) *viz.* Badanapur, Bihar, TNAU, Tamil Nadu, ICRISAT, Hyderabad NAU, Navsari, CSAUAT, Kanpur, Nagpur for the present investigation. A field experiment was conducted at the experimental farm of Agricultural Botany, Dr. P.D.K.V, Akola, Maharashtra, India. Field experiments were laid out in a randomized complete block design (RCBD) with three replications.

### Genomic DNA Isolation

Young and tender leaves were plucked from different genotypes of the pigeon pea seedling in control and stressed condition both and DNA isolation was done using CTAB method as suggested by Saghai Maroof *et al.,* (1984).

### Purification and Confirmation of genomic DNA

Purification of DNA is essential to remove RNA, proteins and polysaccharides which are considered to be the major contaminants in the DNA precipitates. The inclusion of CTAB in DNA extraction buffer helps elimination of polysaccharides. RNA was removed by RNAase treatment and proteins were removed by phenol-chloroform extraction. The DNA obtained after extraction was confirmed by running it on 0.8% agarose gel (containing ethidium

bromide @ 0.5 mg/ml) in a horizontal gel electrophoresis system.

## SCoT-PCR Analysis

For SCoT-PCR analysis, 15 primer sequences were employed for diversity analysis of pigeonpea genotypes. A reaction mixture to a final volume of 20.0µl Containing 1.0µl sample DNA, 2 µl primer, 0.5µl dNTPs, 2.5µl $MgCl_2$ and 0.3µl Taq DNA polymerase, 2.0µl 10X PCR buffer and 11.7 µl Sterile double distilled water. The list of SCoT primers used in the present study is given in Table 2. PCR products were separated on 1.4% agarose gels, stained with ethidium bromide. Amplification products in the gel images were scored for presence (1) or absence (0) missing and doubtful cases were scored. Homology of bands based on the distance of migration of amplified DNA fragments according to their molecular weight in the gel was determined. Molecular weights of the amplicons were estimated using 1kb DNA Ladder (MBT formats) as standard. The polymorphic percentages of the obtained amplicons were calculated by using the formula:

Polymorphism% = (No. of polymorphic amplicons /Total amplicons) x 100.

Jaccard similarity coefficient was calculated and the similarity matrix was subjected to UPGMA (Unweighted pair group method for arithmetic mean) for cluster analysis. The dendrogram was generated based on the similarity matrix and the clustering of genotypes was done based on the dendrogram.

## Results and Discussion

Molecular markers are the key tools to assess the variability. Molecular characterization using markers help us in analyzing the diversity at the genetic level. Sixteen genotypes of pigeonpea were examined for DNA polymorphism using 15 primers out of which eight primers showed amplification shown in Fig. 1. Out of the eight primers, six primers showed 100% polymorphism and SCoT 12 showed 35.7%, and SCoT 15 showed 60% polymorphism. In the present study, the Polymorphism Information Content (PIC) value ranged from 0.4145 to 0.844. High PIC value indicates a high degree of polymorphism among the genotypes which in turn helps to estimate genetic distance with more precision. The highest PIC value was found in the primer SCoT-13 (0.844) followed by SCoT- 19 (0.8397) and SCoT-18(0.8102). The wide range of PIC values indicated their utility for assessment of genetic diversity. The similar range has been reported by Pakseresht *et al.,* (2013) using SCoT markers in chickpea and Petchiammal *et al.,* (2015) using SSR markers in pigeonpea.

The similarity matrix was constructed based on Jaccard's similarity coefficient that revealed, similarity values ranging from 0.056 to 0.762. The highest similarity was found between AMAR and AKTM-11-06. The lowest similarity coefficient was observed between SKN-0632 and AKTM-11-06. A lower similarity coefficient value indicates high diversity among the genotypes. UPGMA dendrogram was constructed using Jaccard's similarity coefficient which is mentioned in Fig 2. The dendrogram showed clear-cut classification of crops into different clusters. The genotypes were clustered into two super clusters viz. super cluster-I and super cluster-II at 40 % cut-off level. Super cluster-I included maximum no. of pigeonpea genotypes *i.e.*15 genotypes. Super cluster- II included only one pigeonpea genotype *i.e.* AKTM-1644.

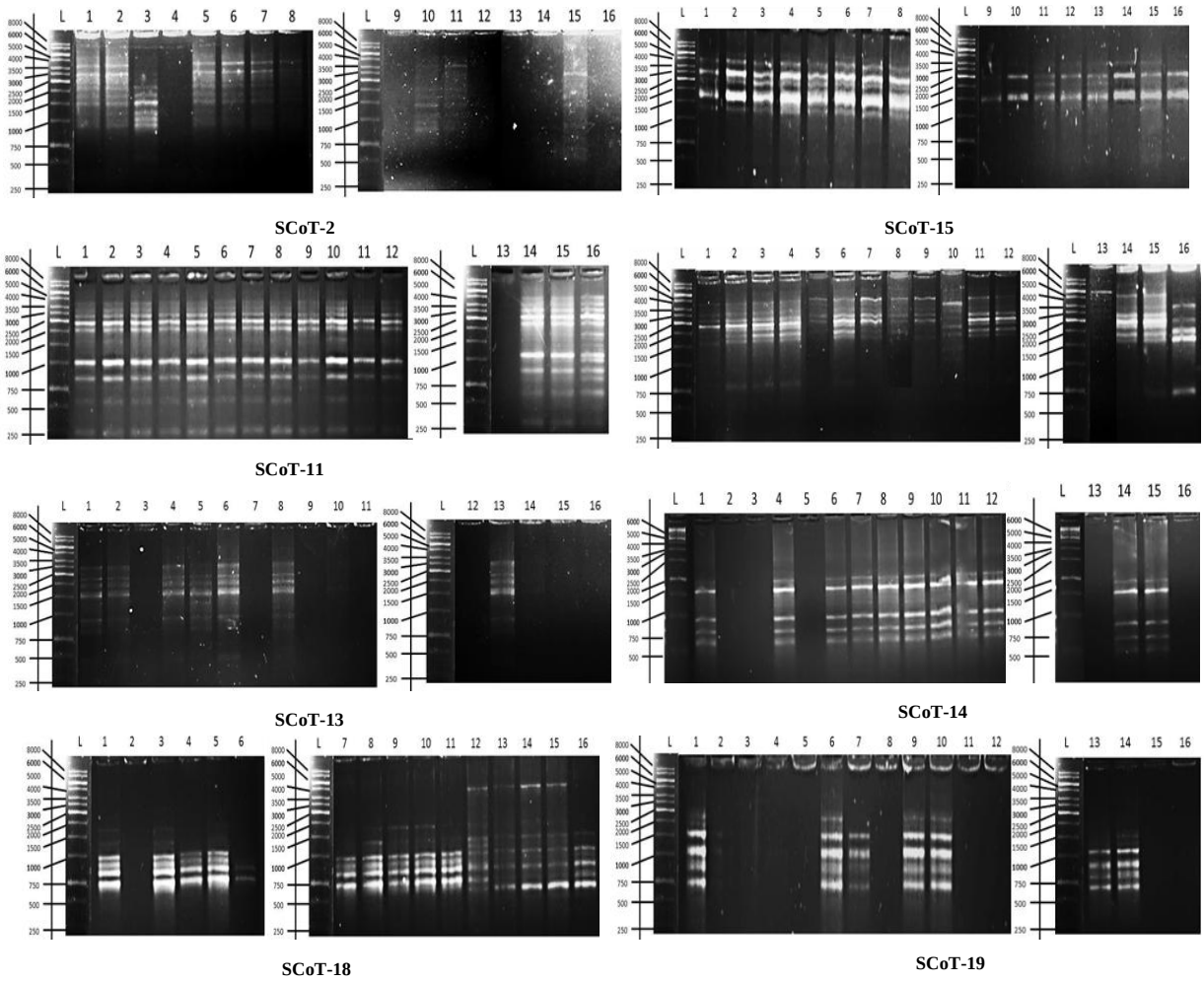
**Table.1** List of 16 genotypes of vegetable pigeonpea and their sources

| No. | Genotypes | Source |
|---|---|---|
| 1 | AKTM-11-06 | Dr. PDKV, Akola |
| 2 | AKTM-11-07 | Dr. PDKV, Akola |
| 3 | AKTM-11-10 | Dr. PDKV, Akola |
| 4 | AKTM-11-11 | Dr. PDKV, Akola |
| 5 | AKTM-11-12 | Dr. PDKV, Akola |
| 6 | AMAR | CSAUAT, Kanpur, U.P. |
| 7 | AZAD | CSAUAT, Kanpur, U.P. |
| 8 | BAHAR | Bihar |
| 9 | BDN-2004-2 | Badnapur,M.S. |
| 10 | BDN-716 | Badnapur, M.S. |
| 11 | CORG-9701 | TNAU, T.N. |
| 12 | ICPL-151 | ICRISAT, Hyderabad |
| 13 | GT-100(check) | Navsari Agri. University, Gujurat |
| 14 | PKV-TARA(check) | Dr. PDKV, Akola |
| 15 | SKN-0632 | Badnapur |
| 16 | AKTM-1644 | Dr. PDKV, Akola |

**Table.2** List of SCoT primers along with their sequences used for diversity analysis

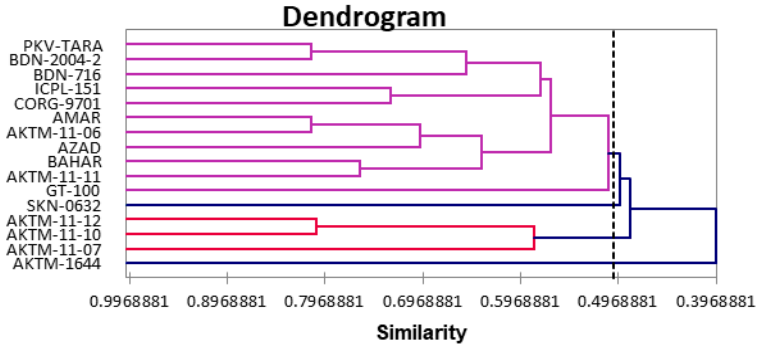
| SCoT primer | Sequence (5′-3′) | %GC |
|---|---|---|
| **SCoT 01** | CAACAATGGCTACCACCA | 50 |
| **SCoT 02** | CAACAATGGCTACCACCC | 56 |
| **SCoT 04** | CAACAATGGCTACCACCT | 50 |
| **SCoT 05** | CAACAATGGCTACCACGA | 50 |
| **SCoT 08** | CAACAATGGCTACCACGT | 50 |
| **SCoT 09** | CAACAATGGCTACCAGCA | 50 |
| **SCoT 11** | AAGCAATGGCTACCACCA | 50 |
| **SCoT 12** | ACGACATGGCGACCAACG | 61 |
| **SCoT 13** | ACGACATGGCGACCATCG | 61 |
| **SCoT 14** | ACGACATGGCGACCACGC | 67 |
| **SCoT 15** | ACGACATGGCGACCGCGA | 67 |
| **SCoT 16** | ACCATGGCTACCACCGAC | 56 |
| **SCoT 17** | ACCATGGCTACCACCGAG | 61 |
| **SCoT 18** | ACCATGGCTACCACCGCC | 67 |
| **SCoT 19** | ACCATGGCTACCACCGGC | 67 |

**Fig.1** Amplification profile of 16 pigeonpea genotype obtained using SCoT-2, SCoT-15, SCoT-11, SCoT-12, SCoT-13, SCoT-14, SCoT-18 and SCoT-19 marker




L: 1kb ladder, 1: AKTM-11-06, 2: AKTM-11-07, 3: AKTM-11-10, 4: AKTM-11-11, 5: AKTM-11-12, 6: AMAR, 7: AZAD, 8: BAHAR, 9: BDN-2004-2, 10: BDN-716, 11: CORG-9701, 12: ICPL-151, 13: GT-100, 14: PKV-TARA, 15: SKN-0632, 16: AKTM-1644

**Fig.2** UPGMA dendrogram of vegetable pigeonpea based on the Jaccard's similarity co-efficient



Cluster-I was further classified at 46 % cut off level into cluster A and B. Sub-cluster A was further divided into $a_1$ and $a_2$ at 50 % cut off level. $a_1$ included eleven no. of genotype like AKTM-11-06, AKTM-11-11, AZAD, BAHAR, AMAR, GT-100, BDN-2004-2, BDN-716, PKV-TARA, CORG-9701, ICPL-151 while $a_2$ had only one genotype *i.e.* SKN-0632. Three genotypes *i.e.* AKTM-11-07, AKTM-11-10, AKTM-11-12 were included in Sub-cluster B.

The present study revealed a high diversity was observed in PKV-TARA and AKTM-1644. But in broad sense a very low level of DNA polymorphism was observed among the selected genotypes of pigeonpea, similar kind of results was reported by Yang *et al.*, (2006) in pigeonpea, Yadav *et al.*, (2012), Mishra *et al.*, (2013) and Walunjkar *et al.*, (2014).

Low level of genetic diversity within the genotypes is likely to represent a significant mishap in any genetic improvement program for this crop with these selected genotypes.

The adaption of genetically homogeneous cultivars has led to depletion of plant genetic diversity.

Narrow genetic variability is highly prone to diseases and pests epidemics as evidenced in many other crops. It is often said that pigeonpea has reached its performance plateau (Saxena 2008). Although ample morphological diversity is exhibited by pigeonpea, the same is not true at the molecular level. (Yang *et al.*, 2006).

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