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Research Article

# DETECTION OF SSR MARKER BASED POLYMORPHISM IN PIGEONPEA LINES CONTRASTING FOR SEED ZINC

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**Abstract:** Pigeonpea germplasm lines were extensively screened for seed zinc content and four each of high and low seed zinc types were selected. The objective of the study was to detect the molecular polymorphism between the pigeonpea lines contrasting for seed zinc content. Attempt was made to cross the contrasting lines and effective crosses were obtained. SSR based marker system was employed to screen the contrasting lines for parental polymorphism. SSR polymorphism revealed higher polymorphism between ICP6992 & ICP8513 and least polymorphism between ICP6974 & ICP8602. Based on the parental polymorphism results, the cross ICP6992 × ICP8513 was taken further to develop a mapping population for further studies.

**Keywords:** Zinc, parental polymorphism, SSR, Pigeonpea

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## Introduction

Micronutrient deficiencies in plants are becoming ever more important globally. Intensive cultivation of high yielding cultivars with heavy applications of N, P and K fertilizers lead to the occurrence of micronutrient deficiencies in many countries [1]. In plants, Zn deficiency affects not only the grain yield [2], but also the nutritional quality of grain [3] and eventually nutritional quality of human diet [4]. Zn and Fe deficiencies are of greater importance afflicting more than 3 billion and one-third of the population worldwide [1]. These resulted in serious health and productivity problems for various population groups, especially among resource-poor women, infants and children [5]. Since the primary sources of all nutrients for people come from agricultural products, if agricultural system fails to provide products containing adequate quantities of Zinc, results in dysfunctional systems that cannot support healthy lives; unfortunately this is the core for many agricultural systems in many developing nations in global foundation [5]. It is unfortunate that the use of zinc fertigation does not completely alleviate zinc deficiency due to factors such as subsoil constraints, topsoil drying or disease interactions [6]. Because of the wide spread problems of zinc deficiency and difficulties in alleviating it via fertigation, a promising alternative may be the identification of zinc efficient genotypes. Tolerance of plant genotypes to soils with low plant-available Zn is usually called Zn efficiency and defined as the ability of a plant to grow and yield well in soils deficient in Zn [4]. Although the use of fertilizers is the quickest method to elevate the zinc density in diets, molecular plant breeding method is likely to be the most cost-effective approach in the long run [7]. Breeding programme requires the screening of the existing germplasm for trait of interest. Make crosses between selected lines and develop the mapping population to map the genomic region determining the trait of interest. This information can be further used in marker assisted selection (MAS) breeding to develop elite variety with desirable traits. Hence it is very important to develop suitable plant material for a quality research. In this direction, attempt has been made to make crosses between zinc contrasting lines of pigeonpea and screen for parental polymorphism to select the best cross for mapping population development.

## Material and Methods

### Plant material

Seeds of ICP7118, ICP6974, ICP14954, ICP6992 the high zinc types and ICP10654, ICP8602, ICP8863, ICP8513 the low zinc types were sown in *Kharif* season to carry out crossing.

### Crossing technique

Artificial hybridization in pigeonpea is done by emasculation method. The buds which are about to shed pollens are selected for emasculation [8]. The tightly closed buds, which were approximately 3/4th, the size of a mature bud and greenish yellow in colour were chosen carefully for emasculation. Two to ten buds per branch were retained and all other buds were removed for best crossing results [9]. Pollination was done immediately after emasculation as stigma remains receptive before anthesis in pigeonpea. Prior to emasculation, pollens were collected from the flowers in which anthers had dehiscenced and kept in petri-dishes on moist filter papers neatly labelled. The buds were pollinated the cooler hours of the morning between 6am to 8am. To transfer the desired pollens, the emasculated bud was held in one hand and dusted with the anthers on to the stigma of the emasculated bud. Care was taken to avoid cross pollination and undesirable pollination by growing the crop under netted structures.

### Pod development

A week after pollination the pods completed their development by 15—20 days of pollination. By 30 to 35 days after pollination, the seeds in the pod attained physiological maturity and were ready for threshing at around 40 days after pollination.

### Sample collection and DNA isolation

Young healthy leaves were collected and frozen in liquid nitrogen from individual parental lines in the field. Frozen leaves were used to grind. The fine powder obtained from each sample was used for extraction of DNA as per modified cTAB (Cetyl Trimethyl Ammonium Bromide) method of [10].

**Genotyping with SSR markers**

In the present study, 71 genic SSR markers [11] were chosen for parental polymorphism study between the parental genotypes which were used for crossing. A 96 well PCR systems (Bio-Rad and Eppendorf) were used for amplification of the desired SSR sequence.

**Polymerase chain reaction**

Genic SSR markers were used to amplify the SSR regions in the template DNA of parental lines. The volume of reaction mixture per one reaction was as follows: PCRs were carried out in 10 µl volume containing1 µl of 10X reaction buffer,0.10µl of 10 mM dNTPs,1µl each of forward and reverse primers (5pmol), 1µl (60 ng/µl) of template genomic DNA and0.04µl (0.75 U) of Taq DNA polymerase (Kappa Taq).

**Fragment analysis**

PCR products were resolved by electrophoresis in 3% Agarose gel containing 0.1 µg/ml ethidium bromide in 1X TBE buffer at 130 V for 4 h,. For greater resolution 3% metaphor agarose gels with 0.1µg/ml of ethidium bromide was being used. Gel documentation system (Biorad)was used to visualize and photograph the amplified bands present in the gel.

**Results and Discussion**

**Crossing of pigeonpea lines contrasting for zinc content**

Crossing of high zinc type lines with the low zinc type lines generated putative $F_1$ seeds. The cross ICP7118 × ICP10654 generated 113 putative $F_1$ seeds, similarly 20 seeds from ICP6974 ×ICP8602, 5 seeds from ICP14954 ×ICP8863 and 11 seeds from the cross ICP6992 ×ICP8513 were obtained. [Table-1] shows the list of putative $F_1$ seeds obtained from each cross. [Plate-1] depicts the pod and seed colour differences in all the pigeonpea lines used for crossing programme. To select the true $F_1$s, one of the methods would be to screen the parental lines at molecular level using molecular marker system. In order to find the true $F_1$s the parental lines were screened using SSR marker system.

Table-1 Number of effective crosses between contrasts pigeonpea genotypes

| SN | Crosses | No of seeds |
|---|---|---|
| 1 | ICP 7118 X ICP 10654 | 113 |
| 2 | ICP 6974 X ICP 8602 | 20 |
| 3 | ICP 14954 X ICP 8863 | 5 |
| 4 | ICP 6992 X ICP 8513 | 11 |

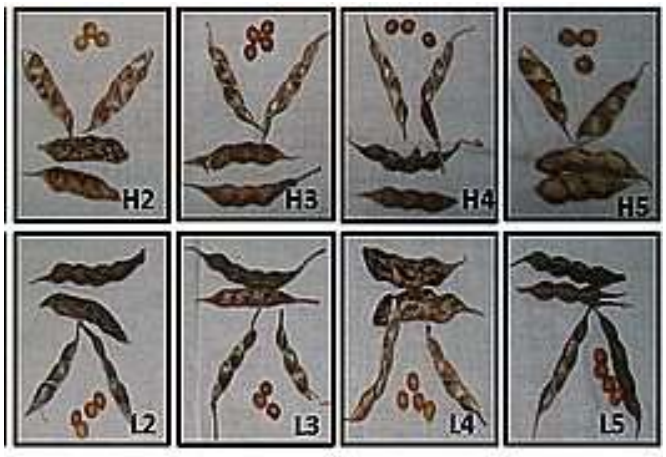


Plate-1 seed color in contrasting parents [Seed pod characteristics

*H series are the high Zn types. H2: ICP7118; H3: ICP6974; H4:ICP14954; H5: ICP6992. L series are the low Zn types. L2: ICP10654; L3: ICP8602; L4: ICP8863; L5: ICP8513*

**SSR screening to detect parental polymorphism**

Once the crop was established, fresh and young leaf samples were collected from the parental lines. A good quality DNA was extracted from the leaf samples. The quantity and quality of DNA was checked and diluted accordingly, to maintain concentration of 60 ng/µl to carryout PCR reactions. Firstly, the annealing temperature for a set of 71 genic SSR markers was standardized using single DNA sample. Once the annealing temperature was standardized, the PCR was carried out in the parental DNA samples in combination with all the 71 genic SSR markers. A few of the genic SSRs were found to be polymorphic between the parental lines. [Plate-2] shows the representative gel image of PCR amplified product of genic SSR marker ASSR59 in the genomic DNA of parental lines. The parental polymorphism between the parental lines was found to be 7 % for ICP7118 × ICP10654, 4.2 % for ICP6974 × ICP8602, 5.6 % for ICP14954 ×ICP8863 and 8.4 % for ICP6992 × ICP8513.

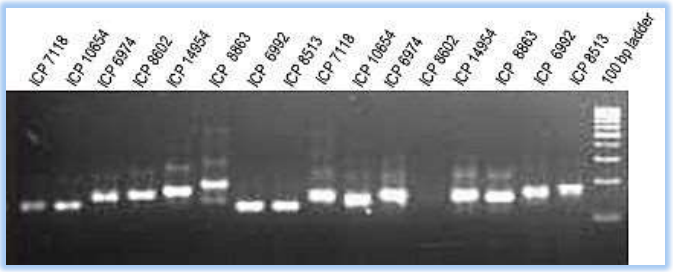


Plate-2 Representative gel showing PCR amplified product of genic SSR marker ASSR 59 in the genomic DNA of parental lines

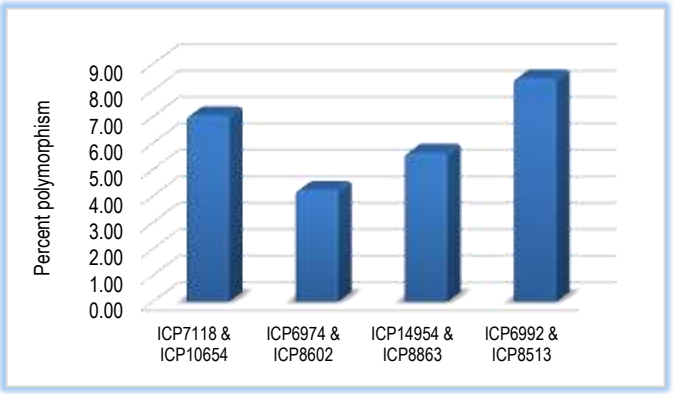


Fig-1 Number of polymorphic genic SSR markers between the parental lines, expressed in percent polymorphism

[Fig-1] shows the graph of parental polymorphism that was observed between the parental lines. Based on the parental polymorphism results, the cross ICP6992 × ICP8513 having relatively maximum polymorphism was selected for development of mapping population. Pigeonpea is a chief grain legume crop grown in tropical and subtropical regions of the world. Though pigeonpea is believed to have narrow genetic base, huge genetic resources are available for its genetic improvement. For genetic improvement, it's necessary to screen the existing germplasm for trait of interest. Screening results in selection of contrasting genotypes for trait of interest. These lines can further be used in molecular breeding programmes to develop a high yielding variety with desirable traits. Molecular breeding involves selection of contrast, crossing them to develop mapping population and molecular characterization using marker system to develop linkage map, discover QTL and carry out marker assisted selection breeding. The most significant use for linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest; such maps may then be referred to as 'QTL' (or 'genetic') maps [12]. It is critical that adequate polymorphism exists between parents in order to construct a linkage map [13]. In many cases, parents that provide enough polymorphism are particularly selected on the basis of the level of genetic diversity between parents [14]. In our study, we screened between four parental combinations to observe for maximum polymorphism. Additionally the choice of DNA markers used for mapping may depend on the accessibility of characterised markers or the suitability of particular markers for a particular species. The fact that the SSR assay is sensitive to single base changes, adds to a greater efficiency in screening the whole genomes for polymorphisms. Also SSR are capable of detecting both the alleles of the locus assist for the precise mapping [15]. Thus microsatellite markers have been widely and effectively applied for parentage identification, hybrid identification, and purity status testing in some crop plant species, for example, cotton and maize [16,17].

Our study also illustrates the successful application of the SSR markers in parental polymorphism identification in pigeonpea.

**Conclusion**

Molecular plant breeding develops useful genetic diversity for crop improvement. In this direction attempt was made to cross the pigeonpea lines contrasting for seed zinc content and discover the extent of molecular polymorphism that existed between the crossed parental lines. SSR marker based polymorphism was found to be considerably less in pigeonpea compared to other crops found in literature. Among the four crosses made, the parental polymorphism was found to be realtively higher between the cross ICP6992 × ICP8513. This cross will form a useful genetic recourse to develop various mapping population, which can be used in future research to develop linkage maps, QTLs and used in marker assisted selection breeding programmes.

**Application of research**: The crosses obtained can be used to develop genetic resources such as mapping populations, which will find its application development of linkage map and discovery of QTLs for zinc related traits.

**Research Category:** Plant genetics

**Abbreviations**:
SSR : Simple sequence repeat
QTL: Quantitative trait loci

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**Sample Collection:**

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

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