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Research Article

DEVELOPMENT OF BACKCROSS MAPPING POPULATION FOR SEED ZINC CONTENT IN PIGEONPEA

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Abstract: The study was taken up with an objective to develop a backcross mapping population for seed zinc content in pigeonpea as a prerequisite for any molecular breeding programme. An elite variety BRG-1 was used as a recurrent parent in development of backcross population with a high zinc containing germplasm line. After crossing, the true F1 were identified using SSR marker system. Four true F1s obtained were backcrossed to BRG-1 and selfed in next season to develop the BC1F2 population. This mapping population serves a useful genetic resource for molecular breeding studies for zinc content in pigeonpea.

Keywords: Zinc deficiency, mapping population, pigeonpea and backcross

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Introduction

Zinc deficiency is one of the risk factors included in the global burden of disease study and inturn has put about 49% of the world's population at risk of zinc deficiency [1]. The probable solutions to correct zinc deficiency in humans may be supplementation of food, diversification of diet and fortification of food or biofortification. The former two programmes require purchasing power, market accessibility and centres for health care and constant funding, which are confined in their own way [2]. Moreover, such programmes favour the urban population with purchasing power, especially in the developing countries. On the other hand, biofortification is the best option for alleviating zinc deficiency. Biofortification is the generating of micronutrient-dense staple crops using the best traditional breeding methods and modern biotechnology [3]. It will be accessible to both the rural and urban populations. It could be achieved either through genetic biofortification or agronomic biofortification. There is an increasing field of research on biofortification of food crops with zinc. This involves the breeding of new varieties of crops with the genetic potential to accumulate a high density of zinc in edible parts or genetic engineering approaches enabling the development of transgenics for accumulation of more zinc or reduction of antinutritional factors coming in the way of nutrient absorption (genetic biofortification) and the use of zinc fertilizers to increase zinc density (agronomic biofortification). Although the use of fertilizers is the fastest method to improve the zinc density in diets, plant breeding method is likely to be the most cost-effective approach in the long run [2]. Prior to plant breeding programme it is necessary to screen the existing germplasm for trait of interest. Make crosses between selected lines and develop the mapping population in order to map the genomic region responsible for 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 in this present study to develop backcross population (BC1F2) specific for seed zinc content in pigeonpea, so that this plant recourse could be used in future studies to improve the nutritive quality of the pigeonpea crop.

Material and Methods

Plant material

Seeds of ICP6443 containing greater Zn content and BRG-1 an elite variety were used in this study. BRG-1 was used and recurrent parent. Seeds of both the lines were sown in Kharif season to carry out crossing.

Crossing technique

Emasculation is required for artificial hybridization in pigeonpea. The buds most likely to shed pollen the next day are selected for emasculation [4]. The buds which were approximately 65-75 % the size of a mature bud and which were tightly closed and if the corolla of such a bud were greenish yellow in color were selected for emasculation. For best results only two buds on one inflorescence were emasculated and total of two to ten buds were emasculated on a branch, while all other buds were removed [5]. The pigeonpea stigma is receptive before anthesis; therefore, pollination was done immediately after emasculation. Before emasculation, the flowers in which the anthers had burst and pollens grains were available were kept in Petri-dishes on moist filter papers neatly labeled. The pollination was carried out in the cool hours of the morning between 6am to 8am. The corolla of the pollen-source bud was removed so that the staminal columns and anthers were uncovered. To transfer pollen, the flower was held in one hand and touched the anthers to the stigma of an emasculated bud. Care was take to avoid cross pollination and undesirable pollination by growing the crop under netted structures.

Pod development

A week after pollination the pods became visible and completed their development in 15—20 days. The seed attained physiological maturity within 30-35 days and were ready for harvest in and around 40 days.

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 DNA extraction as per modified CTAB (Cetyl Trimethyl Ammonium Bromide) method of [6].

Genotyping with SSR markers

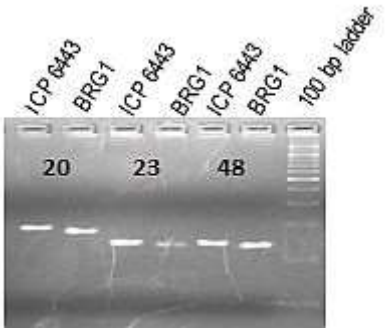
In the present study, 71 genic SSR markers [7] were chosen for parental polymorphism survey between the parental genotypes which were used as parents for development of BC1F2mapping population. A, 96 well PCR systems (Bio-Rad and Eppendorf) were used for amplification of the desired SSR sequence.

Polymerase chain reaction

The template DNA from the parental lines was amplified using each genic microsatellite marker. The volume of reaction mixture per one reaction is as follows:PCRs were carried out in 15 µl volume containing1.5 µl of 10X reaction buffer,0.20µl of 10 mM dNTPs (133 I M),1.5 µl each of forward and reverse primers (10 pmol),I µl (62.5 ng) of template genomic DNA and0.15 µl (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. The bands which didn't get resolved in agarose gel, for such products 3% Metaphor agarose gels containing 0.1 µg/ml ethidium bromide was being used. The gels were visualized and photographed in gel documentation system (BioRad).



The numeric numbers on the gel picture are the ASSR series of genic SSR markers

Plate-1 Representative gel showing PCR amplified product of genic SSR markers in the genomic DNA of parental lines

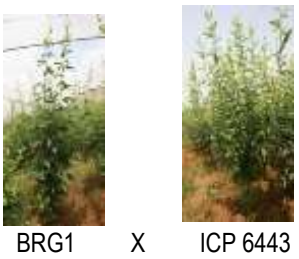
Results and Discussion

Genotying of parents

Crossing by emasculation between BRG1 and ICP6443 resulted in generation of 32 putative F_1 seeds. These F_1 seeds were sown in the subsequent Kharif season and F_1 plants were grown. [Plate-1] shows the architecture of the parental lines and the F_1 population, while [Plate-2] represents the bud and corolla pigmentation in parents and F_1 plants. Among the 32 seeds sown, 18 putative F_1 plants survived. Once the crop was established, fresh and young leaf samples were collected from the parental lines as well as the 18 F_1 plants. A good quality DNA was extracted from the leaf samples. The DNA sample was used for molecular screening using the 71 genic SSR markers. First the parental DNA was screened using 71 genic SSRs for polymorphism detection. Out of 71 SSRs, 15 SSR markers turned to be polymorphic between the parents. A polymorphism of 21 percent was detected between the parents BRG1 and ICP6443 using 71 genic SSR markers. [Plate-3] represents the gel picture of parental polymorphism detection in 3 % agarose gel stained with ethidium bromide.

Genotying of progenies

Once 15 of the SSR markers were found to be polymorphic between the parents, all these 15 markers were amplified in the 18 putative F_1 plants. [Plate-4] is the representative ethidium bromide gel showing expected PCR amplified product for the polymorphic markers from genomic DNA of F_1 plants of BRG1 X ICP6443 cross. In the gel, the true F_1s are the ones where both the parental alleles have been amplified and thus the lane showing double bands are the true F_1. The plants that showed the presence of both the parental alleles *i.e.*, double band amplification for maximum number of polymorphic markers were chosen as true F_1s. Thus, screening of 18 putative F_1s with 15 polymorphic SSR markers resulted in the selection of four true F_1 lines for further research studies.



F_1 (32 seeds)

Sowed and 18 plants survived

F_1 plants

Plate-2 Plant architecture in ICP6443, BRG1 and F_1 plants

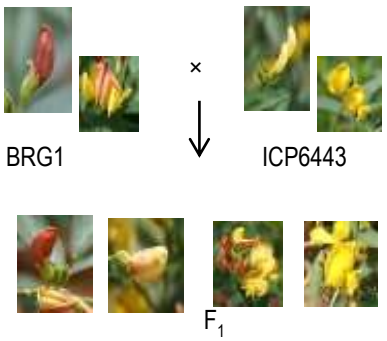
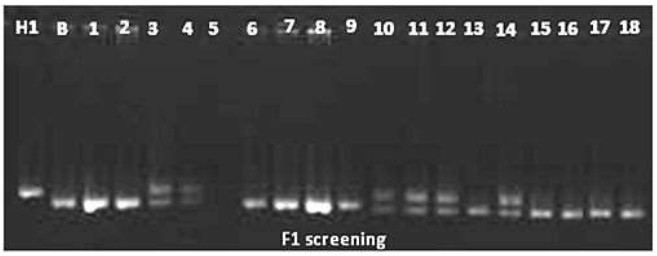


Plate-3 Bud & corolla pigmentation in parental lines and F_1 plants

Generation of backcross population

During the sowing of F_1 seeds, staggered sowing of the recurrent parent BRG-1was also carried out, so that backcrossing could be carried out in the true F_1 plants. At the flowering stage, the four selected true F_1s were being backcrossed by the BRG-1 pollens. The manually crossed flowers were tagged. The seeds were collected from the pods developed from the manually crossed flowers. The collected seeds were the BC1F1 seeds. In the following Kharif season the BC1F1 seeds were sown under isolated conditions under netted structure to promote selfing, in order to develop BC1F2 seeds. A backcross population (BRG1 X ICP6443) BC1F2was developed. Developing mapping population and construction of genetic linkage maps are prerequisite for identifying genomic region linked to trait of interest for molecular breeding programme in pigeonpea. DNA markers technology is a prominent genetic tool with reliability and accuracy for the assessment of hybrid purity in crop breeding.



BRG1 and H1 (ICP6443) – parents, 1 to 18 – F1 plants of BRG1 X H1 cross

Plate-4 Representative Ethydium bromide gel showing expected PCR amplified product for the polymorphic markers from genomic DNA of F1 plants of BRG1 X H1 cross

The fact that the SSR assay is sensitive to single base changes, contributes to a higher efficiency in screening the entire genomes for polymorphisms. Also SSR are capable of detecting both the alleles of the locus facilitates the precise mapping [8]. Thus, microsatellite markers have been widely and successfully applied for parentage identification, hybrid identification, and purity status testing in some crop plant species, for example, cotton and maize [9,10]. Our study also illustrates the successful application of the SSR markers in parental polymorphism identification and true F_1 screening in pigeonpea. Various populations may be utilized for mapping within a given species of plant, with each population type holding advantages and disadvantages [11,12]. The simplest types of mapping populations than can be developed are F_2 populations, derived from F_1 hybrids, and backcross (BC) populations, derived by crossing the F_1 hybrid to one of the parents [13]. The present study was focused on developing backcross population BC1F2 using BRG-1 as a recurrent parent.

Conclusion

Zn enrichment traits are present within the genomes of crops that could allow considerable increase in the Zn concentration of edible parts with no negative impact on yield. Enhancing the amount of Zn in food crops can improve the status of Zn in people. Furthermore, the use of Zn-dense seeds results in enhanced seedling vigour and increased crop yields when the seeds are sown in Zn-poor soils. Hence, the mapping population developed in this study forms the basis for future research of mapping the QTLs responsible for the Zn enrichment trait and using them in the MAS breeding programme to develop a better variety.

Application of research: BC1F2 can be further backcrossed with the recurrent parent to develop trait introgressed lines. Screening of the mapping population across regions for seed Zn content must be carried out to check the consistency of the lines.

Abbreviations:

QTL: Quantitative trait loci
MAS: Marker assisted selection

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