Development of MAGIC population in pigeon pea: a powerful genetic resource for mapping, genetic analysis and identification of potential breeding lines

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Multi-parent advanced generation intercross (MAGIC) population, is a powerful resource for genetic studies, molecular mapping and development of high-yielding pigeon pea genotypes. In this study, eight diverse pigeon pea genotypes were used as founder parents and crossed in two-, four- and eight-way approach to develop a MAGIC population, which could be useful for delineating the genomic regions/quantitative trait loci for various traits of interest. The population can also prove useful for selection of high-yielding pigeon pea breeding lines adapted to various agro-ecological regions of India.

Keywords: Breeding lines, genomic regions, mapping population, pigeon pea, quantitative trait loci.

THE knowledge of genetic mechanisms underlying a trait is the primary requirement towards its genetic manipulation for further improvement. Most of the traits of economic importance in plants puzzle the breeders by their complex inheritance coupled with environmental influences. For gaining useful information about such traits, it is essential to have suitable genetic resources and powerful analytical approaches, which are often not met with. The bi-parental mapping populations such as F_2, backcross, recombinant inbred lines (RILs), near-isogenic lines (NILs), etc. are routinely used to map quantitative trait loci (QTLs) for various key traits in different crops[1,2]. However, these populations have inherent shortcomings. Being restricted to the genetic variation between two parental genotypes only, these populations capture just a small snapshot of the genetic variations for the traits resulting in poor resolution. Such limitations can be overcome by deploying a robust molecular marker system and appropriate mapping population. Therefore, a new generation of mapping approaches, i.e. association mapping (AM)[3] and next-generation mapping populations such as nested association mapping (NAM)[4] and multi-parent advanced generation intercross (MAGIC)[5] have been developed. Unlike the bi-parental mapping populations, the next-generation mapping approaches use multiple parental genotypes and efficiently capture far greater diversities. Further, these approaches map the genetic factors with higher precision and often deliver recombinants with desirable traits, which can be advanced through appropriate breeding approaches. However, development of MAGIC population takes a longer time and involves crossing of many founder parental lines and hybrids in several generations, which is often difficult in many leguminous crops, including pigeon pea. Nevertheless, MAGIC populations have been developed in many crops, including maize[6], rice[7], soybean[8] and pigeon pea[9]. Compared to other crops, efforts to generate MAGIC population in pigeon pea are limited. Therefore, it is important to develop a MAGIC population in pigeon pea involving genotypes which differ for morpho-physiological traits, including yield and stress tolerance. In the present study, we develop an effective MAGIC population through two-way, four-way and eight-way hybridization approaches. The population would serve as a powerful resource for genetic studies, molecular mapping and development of high-yielding pigeon pea genotypes adapted to various agro-ecological regions of India.

The selection of founder genotypes is a critical step in the development of a MAGIC population. In this study, eight diverse pigeon pea genotypes were selected as founder parents, which hugely vary for several traits such as plant architecture (compact to loose), plant stature (tall to dwarf), branching pattern (spreading to erect), growth habit (determinate to indeterminate), number of branches/plant (uniculm type to multiple branching), flower structure (wrapped to normal unwrapped flowers), seed coat colour (black to white) (Figure 1), seed size (small to large), seed weight (ranging from 6.00 to 13.30 g), waterlogging tolerance and cold tolerance. Table 1 depicts the characteristic features of the founder genotypes.

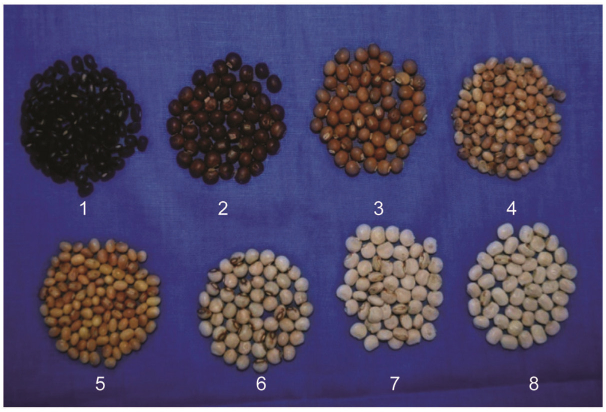

Figure 1. Variation in the seed-coat colour of founder genotypes used in the MAGIC population: 1, ICP 5028; 2, Sel. Richa 2000; 3, MAL-13; 4, ICP 7574; 5, Sel. ICP 10509; 6, ICP 14276; 7, Sel. ICP 11182; 8, ICP 12107.

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Table 1. Detailed characteristic features of parents utilized in the development of MAGIC population

Genotype	Characteristic features
ICP 5028	Black colour of seeds, waterlogging-tolerant.
Sel. Richa 2000	Uniculm-type stem, bold seed with red mottled seed coat.
MAL-13	Spreading plant type, high-yielding, round seed with hilum ring, national check/NEPZ, highest recovery of *dhal*, higher consumer preference due to better cooking quality and taste.
ICP 7574	High number of secondary and tertiary branches, very small seed, heavy flowering, short stature.
Sel. ICP 10509	Angle < 20°, highly erect, tall, frost-tolerant, higher number of primary branches.
ICP 14276	Wrapped flower, coloured ring on seed and anthocyanin-led purple colour stem.
Sel. ICP 11182	Curly pod, droopy stem, red flower, highly sensitive to frost.
ICP 12107	White seeds, green pod, determinate, sensitive to salt stress.

Figure 2. Stem colour variation in the genotypes ICP 14276 (purple) and Mal-13 (green).

While selecting the founder parents, attention was given to include genotypes with specific features that may help developing recombinant genotypes fit for the changing climatic conditions. The anthocyanin content improves the adaptability of a genotype to low temperature. Further, it protects the plant from UV rays[10]. ICP 14276 and Mal-13, two founder parental genotypes, have purple and green stem respectively (Figure 2). Further, ICP 14276 has a unique flower structure with elongated standards, which prevents out-crossing and thus helps in maintaining the genetic purity of the seeds.

The anthocyanin content in ICP 14276 and Mal-13 was estimated using the HPLC system. The HPLC chromatogram of the anthocyanins of ICP 14276 exhibited four peaks at retention time of 11.28, 11.79, 12.02 and 13.09 min interval, while only two peaks were observed in case of Mal-13 at retention time of 12.06 and 12.98 min interval (Figure 3). Considering all the constitutive peak areas of anthocyanins, total anthocyanin content of ICP 14276 and Mal-13 was recorded to be 37.21 and 2.11 mg/g of the sample respectively. Recombinants developed from it would help in mapping this trait effectively.

Similarly, a frost-tolerant line ICP 10509 was used as one of the founder parents. Potential of this genotype as a donor of frost tolerance has already been established[11] (Figure 4). On the other hand, ICP 11182 is a frost-sensitive genotype with determinate growth, wavy primary branch, curly pod and red flowers. The MAGIC-RIL developed here will not only facilitate mapping of frost tolerance but also in the development of frost-tolerant, high-yielding genotypes of pigeon pea.

ICP 5028, a waterlogging-tolerant line[12] and ICP 7574, a short statured genotype with high number of secondary and tertiary branches and tiny seeds, were included as diverse founder parents. Similarly, Mal-13, a high-yielding national check variety with spreading plant type, round seed with hilum ring and having highest recovery of *dhal* besides better cooking quality and palatability was also included in the crossing programme. The richness of genetic diversity among the founder parental genotypes will ensure effective mapping and understanding the genetic control of the traits. Visible display of the traits in various combinations has indicated possible segregation of the alleles in the selfing generations, which would facilitate the discovery and effective deployment of alleles with desirable effects.

During *kharif* 2016–17, the founder parents were grown and crossed in pairs (four pairs). The seeds of the four putative hybrids (F_1s) were grown during *kharif* 2017–18, and true hybrid plants were identified using dominant morphological markers such as seed coat colour, branching pattern, plant stature, growth habit, anthocyanin pigmentation, flower morphology, etc. The F_1s (two-parent hybrid) were then crossed in pairs (two pairs) to obtain the seeds of four-parent hybrids, which were then grown during *kharif* 2018–19 and crossed pairwise (only one pair) to produce the eight-parent hybrid. Figure 5 depicts the crossing programme adopted to develop the hybrids.

The seeds of the first selfing generation (F_2) of the eight-parent hybrid were grown during *kharif* 2019–20. A wide range of variations was observed in the morphological traits, including seed coat colour due to segregation of the alleles at various loci (Figure 6). The selfing generations with about 1500 plants are being advanced each year to attain homozygosity at all the segregating loci by F_6–F_7. The MAGIC-RIL population (~1500 RILs) thus

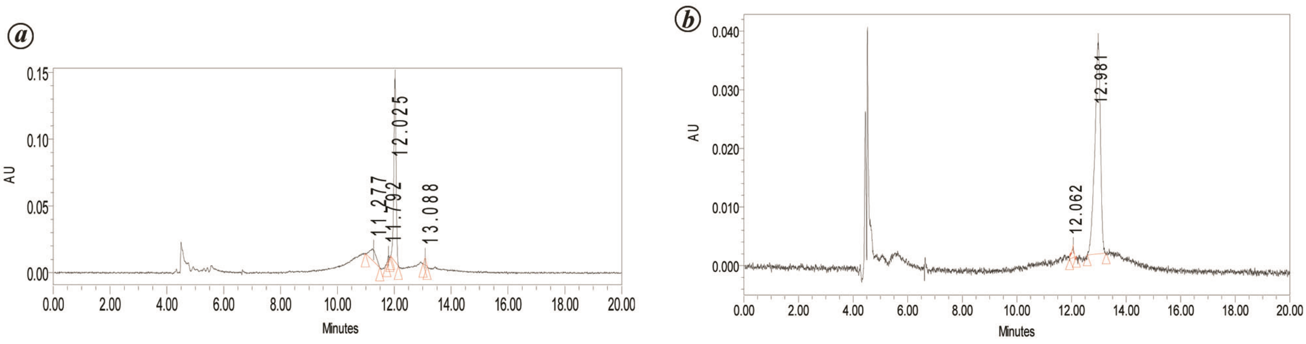

Figure 3. HPLC chromatogram of the anthocyanins in (***a***) ICP 14276 and (***b***) Mal-13.

Figure 4. The genotype Sel. ICP 10509 (left) is tolerant to frost, whereas Sel. ICP 11182 (right) is highly sensitive to frost. F_1 (middle) developed by crossing these two genotypes is also tolerant to frost.

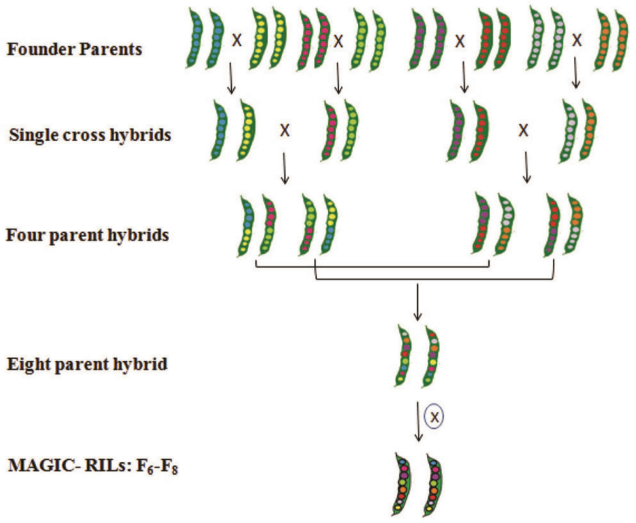

Figure 5. Schematic crossing programme for development of the MAGIC population.

developed would be a powerful resource in pigeon pea for understanding the genetic control of various traits including yield. In addition, lines with desirable traits would be fit for testing in large plots to be released as a variety.

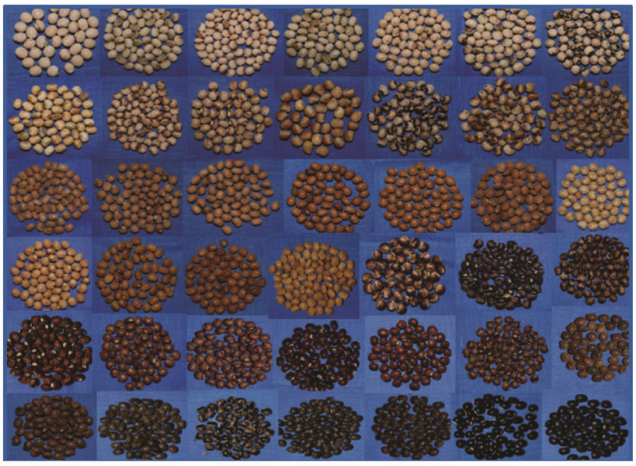

Figure 6. Segregation of seed coat colour in F_2 seeds of the MAGIC population.

The size of the mapping population is an important factor that determines the precision of mapping of a trait. A larger population is highly desired for mapping traits of complex inheritance. In this study, the targeted mapping population was 1500 RILs, which is sufficiently large for mapping traits pertaining to plant architecture and abiotic stress tolerance. The development of a MAGIC-RIL (~1300 RILs) in pigeon pea has been reported in 2020 (ref. 9), where the founder parents differed for early maturity, high seed protein content, high yield, disease resistance, etc. Using model-based analysis, Valdar *et al.*[13] reported that an eight-founder parent MAGIC-RIL population with 1000 progenies is good enough to map QTLs with high resolution. Thus, the current MAGIC population is not only of optimum size (1500 RILs), but also of diverse composition for effective mapping and possible discovery of genes for the target traits.

Compared to other crops, the progress of molecular mapping of genes/QTLs in pigeon pea has been rated as sub-optimal. Limited availability of a robust molecular marker system has been identified as the primary cause for its limited success. However, after the whole genome was sequenced and made publicly available[14], the dearth of molecular markers in pigeon pea has become a matter of the past. In addition, a 62K genic-SNP chip was used to map and analyse the genes/QTLs for reasonable reliability and higher precision[15].

In conclusion, pigeon pea breeders will be able to map complex traits, identify the gene/genomic region(s) controlling these traits, characterize them and use them for genetic improvement. The MAGIC population will also deliver desirable genotypes for future testing and possible release for cultivation under various climates.

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Camera traps reveal coat colour variation in an isolated population of golden jackals

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Long-term monitoring of golden jackals along the coastline of the southern Western Ghats, India, provided interesting results. We observed various coat colour patterns such as leucism, melanism, semi-melanism and rusty coats among jackals resembling dogs. The melanistic variation and rust colour expression may be due to the acquired genetic properties during cross-breeding with the free-ranging dogs, as the associated mutations have been previously documented in dogs and not in jackals. Genetic studies can assist us in understanding the causes and consequences of coat colour variation.

Keywords: Coat colour variation, golden jackal, hybridization, isolated populations.

GOLDEN jackals (*Canis aureus*) are a species of least concern according to the IUCN Red List. Jackals were common in reserve and non-reserve areas as well as the countryside around 30–40 years ago. The species is presently restricted to individual isolated pockets owing to changing land patterns and human population expansion. Ecological studies on jackals are less prioritized, and a comprehensive record of the meta-populations is not available. Group size of the jackals can be up to 20 and have a home range of 21.2 km^2 in their natural habitats and 6.6 km^2 in human-dominated habitats[1]. When plenty of food is available in a region, the usual social organization is lacking[2]. The population density of individual species is declining worldwide, owing to indiscriminate land-use patterns. The pace of urbanization is leading to a reduction in environmental heterogeneity and a collapse of ecological niches[3]. Thus, the dwindled and isolated jackal meta-populations may undergo hybridization with associated feral species. Hybridization in the wild is not always encouraging, as it can lead to endangerment and extinction through genetic swamping[4].

The southern Western Ghats coastline extends from Mangaluru in Karnataka to Kanyakumari in Tamil Nadu, encompassing three states in India with a large stretch in Kerala. According to geologists, inward redress of the sea

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