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Mechanism of Generating MAGIC Population and Its Utility for Crop Improvement

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Abstract

The identification of gene-trait associations for complex (multi-genic) traits needs a mapping population. Mainly two methods viz., biparental crosses and association mapping or linkage disequilibrium mapping, have been used for a long time in the identification of gene-trait associations. The major disadvantage of a biparental population are narrow genetic base, reduction of genetic heterogeneity and only two allelic variations can be analysed in these populations. Association mapping requires large samples and is predominantly influenced by unknown population structure. Recently, the QTL mapping using multi-parent populations has become very popular. The use of MAGIC populations for QTL analysis can fill the gap between biparental mapping and association mapping using natural or breeding populations. MAGIC provides more abundant genetic diversity than a biparental population and higher allele balanced frequency than the panels consisting of diverse accessions in AM and increased mapping resolution by taking the advantages of both historical and synthetic recombination.

Introduction

MAGIC stands for “Multi-parent Advanced Generation Inter-Cross”. It is a simple extension of advanced intercross. Multiple inbred founder lines are intermated for several generations prior to creating inbred lines, resulting in a diverse population whose genomes are fine scales mosaics of contribution from all founders. First proposed and applied in mice taking 8 inbred strains by Mott and co-workers in 2000. The term ‘MAGIC’ was coined by Mackay and Powell in 2007. In plants, it was first developed and described in *Arabidopsis thaliana* by Kover *et al.* (2009) taking 19 founders. MAGIC can be described as ‘heterogeneous stock’.

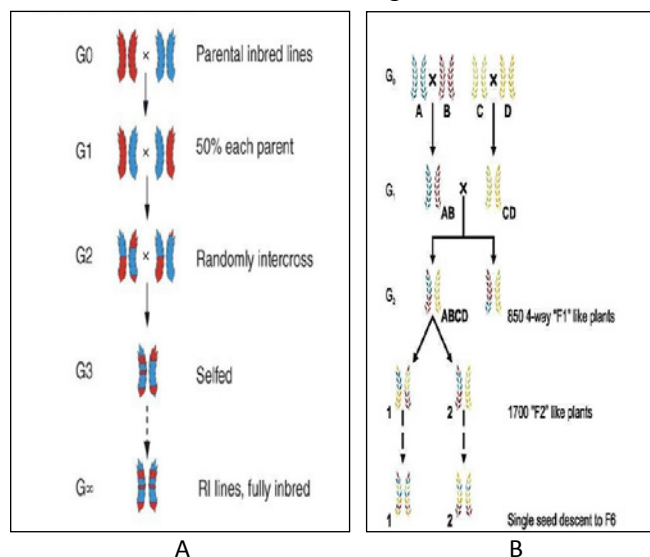


Figure 1: Showing comparison between (A) Advanced Intercrossed Lines, and (B) Four way MAGIC Population

Why MAGIC?

The identification of gene-trait associations for complex (multi-genic) traits needs a mapping population. Mainly two methods viz., biparental crosses and association mapping or linkage disequilibrium mapping, have been used for a long time in the identification of gene-trait associations.

Biparental Crosses

Traditional experimental populations combine the genomes of two parents with contrasting phenotypes to identify regions of the genome affecting the trait.

Limitations

- Only two alleles are analysed and that genetic recombination in these populations.
- Biparental crosses have narrow genetic base which limits the resolution for QTL detection and genetic mapping.
- It is only possible to detect those genomic regions which differ between two founders.

Association Mapping/ Linkage Disequilibrium Mapping

A method of mapping quantitative trait loci (QTLs) that take advantage of historic linkage disequilibrium to link phenotypes to genotypes by sampling distantly related individuals.

Limitations

- It is predominantly influenced by unknown population structure, leading to spurious (fake) association.
- Requires very large samples to have sufficient power to detect genomic regions of interest, and may have difficulty detecting rare alleles of importance.

Hence, the weakness of existing designs have led to this new type of complex experimental design *i.e.* MAGIC population, which is intermediate to biparental and association mapping designs in terms of power, diversity and resolution.

Table 1: Comparison between Biparental Linkage Analysis, Association Mapping and MAGIC Population

Sl. No.	Features/ Properties	Biparental Linkage Analysis	Association Mapping	MAGIC Population
1	Founder parents	2	>100	>8
2	Crossing requirement	Yes	No	Yes
3	Time to establish	Moderate	Low	Long
4	Population size	~200	~100	~1000
5	Suitability for course mapping	Yes	No	Yes
6	Suitability for fine mapping	No	Yes	Yes
7	Amount of genotyping required	Low	High	High
8	Amount of phenotyping required	Low	High	High
9	Statistically complexity	Low	High	High
10	Use of germplasm variation	Low	High	High
11	Practical utility	Low	High	High
12	Relevance over time	Low	High	High

MAGIC populations are now attractive for researchers due to-

- Presence of marker systems that allow genotyping of the populations by providing a high-throughput SNP (Single nucleotide) polymorphism genotyping platform.
- Advances in statistical methods *i.e.*, Requirements for a better mapping population, genetic variability for target phenotype, reproducible quantitative genotyping.

Procedure of Generating MAGIC Population

There are four steps involved in development of MAGIC population:

1. Founder selection

2. Mixing of parents

3. Advanced intercrossing

4. Inbreeding

1. Founder Selection

Founders are selected based on genetic and/or phenotypic diversity. It may be elite cultivars with geographical adaptation or materials of more diverse origins *i.e.* worldwide germplasm collections, distant relatives.

2. Mixing of Parents

Multiple founder lines are intercrossed to form a broad genetic base. The inbred founders are paired off and inter-mated in a prescribed order for each line, known as funnel. In this stage we get such set of lines

whose genome is contributed by each of the founders.

3. Advanced Intercrossing

Now mixed lines from different funnels are randomly and sequentially intercrossed as in the advanced intercrossed. The main goal advanced intercrossing is to increase the number of recombination. At least six cycles of intercross are required for constructing a good QTL map.

4. Inbreeding

The advanced intercrossed lines (AILs) are used to Development of homozygous individuals. Recombinant inbred lines (RILs) are produced through single seed descent (SSD) or double haploid production. Double haploid production is often faster than SSD. Multiple generations of selfing introduces additional recombination.

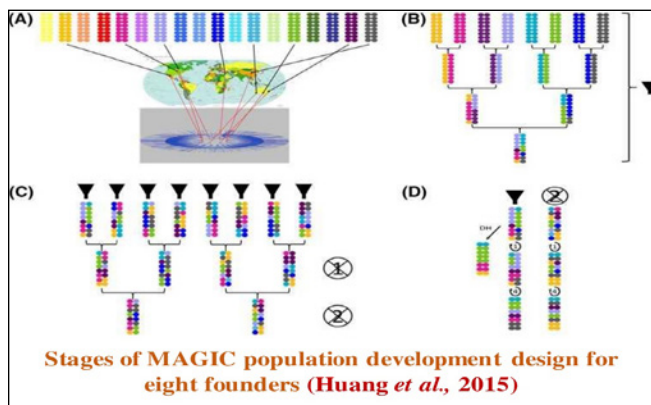


Figure 2: Stages of MAGIC population development design for 8 founder lines

Genetic Analysis of MAGIC Populations

There are two main approaches for genetic analysis of MAGIC populations. These are linkage map construction and QTL mapping approaches.

1. Linkage Map Construction

- The large number of polymorphic markers across all founders and accumulation of recombination events through many generations of the MAGIC pedigree can be used to achieve dense and high resolution mapping of the genome.
- The first linkage map from a MAGIC population was constructed in wheat by Huang and co-workers in 2012.
- The higher levels of recombination in the MAGIC population can be seen most clearly in the region around centromeres.

2. QTL Mapping Approaches

- The use of heterogeneous stock improves the power to detect and localize QTLs.
- The large number of parental accessions increases the allelic and phenotypic diversity.
- The larger number of accumulated recombination events

increase the mapping accuracy of the detected QTL compared to a biparental F₂ cross.

“Thus, MAGIC lines occupy an intermediate position between naturally occurring accessions and synthetic populations.”

Role of MAGIC Lines in Crop Improvement

- MAGIC populations can be used in the development of variety with several agronomically beneficial traits.
- MAGIC can help in the development of variety which can adopt several diverse regions of the world and suitable for diverse climatic conditions.
- MAGIC populations can be used directly as a source material for the extraction and development of breeding lines and varieties.
- MAGIC can provide solution to a range of production constrains (biotic and abiotic).
- MAGIC has the potential to increase the speed and efficiency of breeding.
- MAGIC will direct impact on the production of farms as well as the ability to change the way of scientists to identify the genes that control the quality and disease resistance.
- It can help to create a novel diversity.

Limitations

- It needs intensive labour for crossing.
- Extensive segregation.
- Large population size is required for recovery of recombinants with all the desirable traits.
- It requires more time to develop the resource population.
- Large scale phenotyping is required for a particular trait.
- It requires more inputs.
- Incompatibility between the parents can cause a large reduction in number of progeny.
- Better marker system is necessary to identify QTLs.

Table 2: Institutes involved in the development of MAGIC populations

Sl. No.	Crop	Institute
1	Rice	IRRI, Manila Philippines
2	Bread wheat	NIAB, Hyderabad
3	Durum wheat	University of Bologna, Italy
4	Barley	SAC,
5	Oat	IBERS, Aberystwyth
6	Cowpea	IITA, Nigeria
7	Sorghum	ICRISAT, Hyderabad

Case Studies

1. Bandillo *et al.* (2013) selected eight *indica* and eight *japonica* rice founder lines. These lines were known to exhibit good grain quality, high yield potential and tolerance to a range of biotic and abiotic stresses. At the S_4 stage of single seed descent (SSD), they genotyped a subset of MAGIC *indica* using single nucleotide polymorphism (SNP) markers. They phenotyped these populations for multiple traits *viz.*, grain quality, blast and bacterial blight resistance, salinity and submergence tolerance.

Outputs of Their Study

- Developed *indica* MAGIC (8 *indica* parents), *japonica* MAGIC (8 *japonica* parents), MAGIC plus (8 *indica* parents with two additional rounds of 8-way F_1 intercrossing) and MAGIC global (16 parents- 8 *indica* and 8 *japonica*) populations.
- Developed thousands of lines and now checking for wider adaptability.

Several major genes and QTLs were identified that includes:

- Blast: Chromosome 2(26 Mb), 3(3.5 Mb), 7(27 Mb), 10(13 Mb).
- Bacterial blight: Chromosome 11(27.3-27.9 Mb), 5(0.4 Mb).
- Salt tolerance: Chromosome 1(11.8 Mb).
- Submergence tolerance: Chromosome 9(6.2-6.3 Mb).

2. Kover *et al.* (2009) using heterogeneous stock of 19 inter-mated accessions of the plant *Arabidopsis thaliana*, developed a set of 527 recombinant inbred lines (RILs). They genotyped these lines along with 19 founders with 1,260 SNP and phenotyped for development related traits. After all, attempted these lines to a fine QTL map.

Outputs of Their Study

- QTL affecting natural variation in flowering time, identified on Chromosome 5 (~3.5 Mb).
- Detected two QTLs on Chromosome 3 and 4 for the trait number of days to germination.
- QTL on Chromosome 3 (~15.9 Mb) for nitrilase gene transfer.

3. Meng *et al.* (2016) using eight elite *indica* rice varieties from different breeding programs, developed three new rice (*Oryza sativa* L.) multi-parent advanced generation intercross (MAGIC) populations. These three populations were two recombinant inbred line (RIL) populations derived from two 4-way crosses, DC1 and DC2, and one RIL population derived from an 8-way cross. They genotyped populations using an

Illumina Infinium rice 6K SNP chip for plant height (PH) and heading date (HD). A population of 248 IRRI breeding lines and a population of 323 Chinese breeding lines were also included to compare genetic diversity and linkage disequilibrium (LD) pattern.

Outputs of Their Study

- Three rice MAGIC populations were developed to better integrate QTL identification and breeding.
- The association analysis identified two and three QTL for PH and HD.
- A novel QTL for PH was identified on Chromosome 12 using the 8-way population.

Besides rice and *Arabidopsis thaliana*, MAGIC population have also been developed in several crops *viz.*, soybean, tomato, brassicas, chickpea, wheat *etc.*, in last decade for various biotic and abiotic constrains. MAGIC development in some crops like pigeonpea, peanut, sorghum, oat *etc.* is in progress.

Conclusion

The MAGIC lines are a new panel of genetically diverse and highly recombinant inbred lines. It is a powerful method to increase the precision of genetic markers linked to the QTLs. They represent a significant improvement over standard RILs. Multi-parental populations of all types are still in their infancy. MAGIC populations are likely to bring model shift towards QTL analysis, gene mapping, variety development *etc.* in plant species.

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