

MAGIC populations improved traits and provided different accomplishments

ABSTRACT

Plant breeding is a crucial aspect while ensuring food security by applying updated techniques and utilizing available resources to the fullest extent possible. For instance, the development of the Multi-parent Advanced Generation Intercrosses (MAGIC) population is a prominent example of this. This population uses a wide range of parents from different regions of the world and produces a population with a broad genetic base and significant phenotypic differentiation. Using quantitative trait loci (QTL), certain traits can be identified that can be used for the development of breeding lines for use in a breeding program for the development of advance varieties. As part of this review, we provided some examples of improved traits and different accomplishments achieved through the use of MAGIC populations.

Key words: MAGIC, QTL mapping, experimental design, diversity and genetic base

INTRODUCTION

With limited food sources, increasing food supply is a very difficult task, but plant breeding allows us to enhance the genetics of crops, ultimately improving crop growth and development. For the increasing population their demand must be reached with the help of plant breeding and by enhancing the genetic base and genetic gain of the crops (Ray *et al.* 2013; Zaw *et al.* 2019). In the green revolution of the 1960s which is by the use of a traditional approach of combining two or more genotypes/genomes of phenotypically dissimilar parents

to get multiple resistance. But some consequences were there like a narrow genetic base, using multiple lines for cultivation. To avoid these consequences an intermediate complex experimental design is developed which is MAGIC. MAGIC initiated by Mackay and Powell (Mackay *et al.* 2007) is mating between the large sets of inbred lines collected from different areas whose genomes are from different varieties worldwide. In the traditional approach occurrence of alleles is at a very high frequency due to the limited number of parents but here in this MAGIC population, the frequency occurs at a low rate due to the greater diversity because of polymorphism, but the initial investment of time and capability in the MAGIC population is more than a normal biparental cross. By carefully choosing the genetic material for the construction of a MAGIC population, it is possible to increase its applicability to a broader range of breeding populations, which can result in long-term benefits by providing a valuable genetic resource panel. Previous theoretical and experimental research on MAGIC populations has shown that they have the potential to significantly enhance the identification of QTLs (Quantitative Trait Loci). With the help of QTL mapping analysis, genes for the agronomic trait of interest can be identified, and this identification significantly contributes to improving traits related to quality and yield. For the identification of QTLs in crops, four key elements are necessary: (1) a diverse segregating population with parents that exhibit contrasting traits for the phenotype of interest, (2) marker systems that can genotype the population, (3) reliable quantitative phenotyping techniques, and (4) a suitable experimental design that accounts for environmental factors and statistical approaches for identifying and locating QTLs.

The first multi-parental intermating population was the collaborative cross done in the mice where a variety of species have been taken. In this collaborative cross, eight species were selected, this multiparent population crossed together,

and then inbred lines were created by sibling mating. Concurrently, some eight progenitors were used to create a diverse outbred population for related heterogeneous stock (HS). These populations identified and mapped genes for coat colour traits and for serum cholesterol. In plants, the primary MAGIC population was created in *Arabidopsis thaliana* using different species in their known history.

METHODOLOGY

Design for population development is a consideration that should be carefully addressed, this will not only define the novelty of the population but also helps with answering the practical questions related to research. For the production of MAGIC, the design is an important factor because of the time needed for the creation and different factors which affect diversity, adaptability, and superiority. Hence, the purpose should be cleared for the population before undertaking population development. Both factors will be included which are concerned with the pedigree and how these lines which we have found will be intermated, and also look at some considerations that will improve the efficiency of the design.

Founder selection is an initiation step in population development, so first there will be a selection of founder lines and which may be based on phenotypic or genotypic diversity between the lines the more diverse lines are the more we are getting the benefit, it can be either in the form of a constrained set of materials which can be some widely adapted genotypes, high yielding cultivars or the cultivars based on their diverse origin (geographically from distant places). Selecting the genotypes and hoping for greater diverse lines is not an easy task, the greater can be achieved by selecting landraces but ultimately generalizability to the current populations will be reduced, and there may be an incompatibility in some specific crosses. Differences in chromosome structure, such as rearrangements or the introduction of foreign genetic material from wild

or non-native sources, that are specific to a particular plant variety, can impact the development of the resulting population and its usefulness for genetic mapping purposes. A narrow genetic base is another criterion that should be kept in mind for estimating the founder probabilities and preventing researchers so they cannot fully exploit the potential of these populations.

Phenotypic diversity is also an aspect of genetic diversity; we can produce a resource that will be practically utilized based on phenotypic diversity. Taking into account traits like the time of maturity in the founder lines can prevent the inheritance of undesirable values in the offspring. This can have a practical impact on the process of making crosses and can also affect subsequent phenotypic evaluations. More diverse selected lines may result in a wide variety of traits biologically but the selection of these founder lines based on breeding program and for specific traits will surely result more quickly in superior breeding lines in a MAGIC population. The population should fit each market segment and should not only focus on allelic diversity.

Mixing the selected lines from various parts of the world and getting intercrossing them will result in a wider genetic base. The heterogeneous stock was first done by McClearn *et al.* (1970), which created an outbred population by using multiple parents. In this stage, these inbred lines are paired getting intercrossed with a systemic order for each line, known as a *funnel*. The mixing stage will require n generations as the recombinant inbred line (RIL) is the product of the 2^n way cross. The result after making these crosses will be a set of genotypes that have genomes from every founder. Broman (2005) showed that the contribution of these lines depends on the structure of the funnel; hence, these funnels are selected and which design is used in the cross will affect the performance of the population.

Funnel's great variety generates stronger checking for factors, but the time and cost investment will be more. Simulation helps to provide more information for

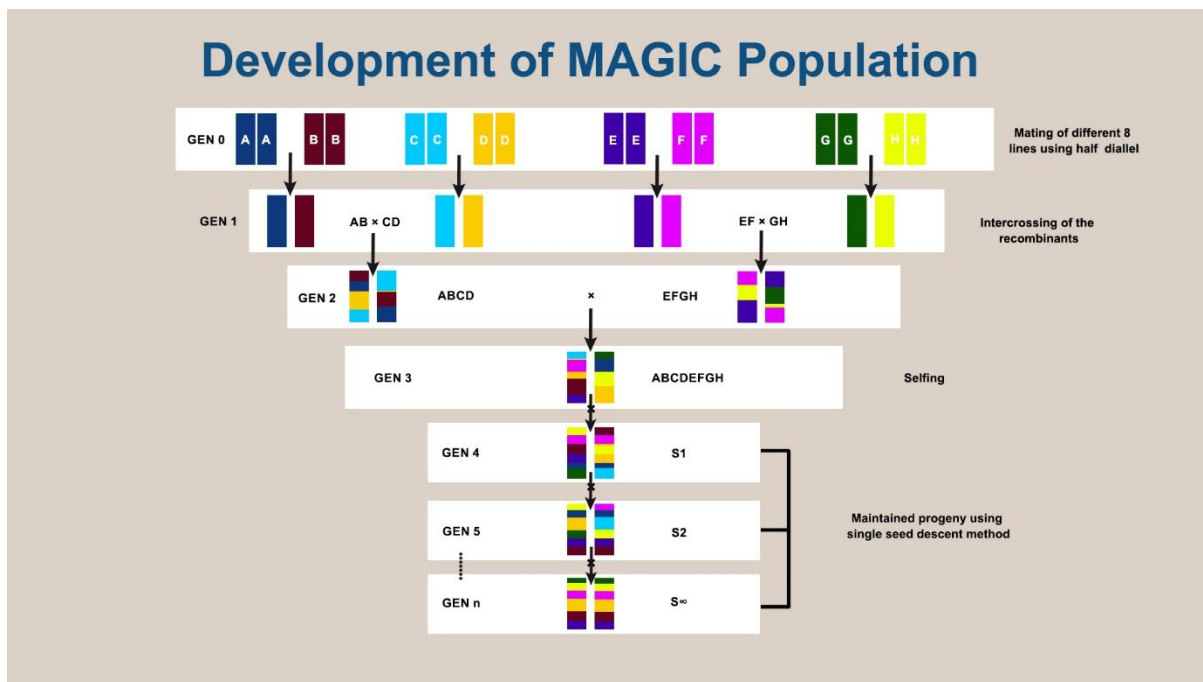
balancing these factors [Simulation crucial tool for estimating the potential of different designs and different simulation packages are there as R/qtl (Broman *et al.* 2003) and R/mpMap (Huang and George 2011) provide genotypes information for selection as founders].

The mixed lines obtained from these intercrossed are now getting serially arranged in the second stage as advanced intercross (Darvasi and Soller 1995). Yamamoto *et al.* (2014) achieved intercrossing in different genome structures and stated that for a major improvement in QTL mapping, six cycles of intercrossing should be done.

In the third stage, the advanced intercrossed lines which have been created now have to be homozygous, and these lines *i.e.* RILs can be formed either by single seed descent (Goulden 1939) or doubled haploid production (Blakeslee *et al.* 1922; Maluszynski *et al.* 2003). The production of double haploid lines is faster than the formation of RILs, the selfing will add more recombination to the lines.

The only progeny that will be fully inbred is double haploid at intermediate generations lines, and the remaining heterozygosity can give benefits or create problems for us. In genotyping, polyploidy mapping of heterozygotes may be an issue for some markers (Cavanagh *et al.* 2013) and genotyping-by-sequencing (GBS) approaches (Elshire *et al.* 2011). (Borman 2012) defines the probabilities of genotype at intervening generations with considerable heterozygosity. So, average plants need to be selfed for more than five generations to attain less than 3% heterozygosity in the genome. Generally, 8 crop seasons are the minimum requirement for the MAGIC population to reach the S5 stage.

Fig 1. Development of MAGIC population in various crop



- First 8 different founder lines are selected from around the world on the basis of phenotypic or genotypic diversity between them and mated in half diallel pattern.
- Intercrossing will be done of the recombinants produced by these crosses
- After intercrossing, to stabilize the generation we will do selfing and maintain this generation using single seed descent method, selfing of the plants should be done 5 times.

1. WHY MAGIC?

By the detection of QTL, we can ensure the information of the molecular marker and genotyping output (Chen *et al.* 2014; Mammadov *et al.* 2012) with the help of advancement in the technology of next-generation sequencing (NGS) and cost-effective sequencing for the manipulation of genetic material (Flint-Garcia *et al.* 2003; Stadlmeier *et al.* 2018). Association mapping and linkage analysis are used as a conventional approach for QTL mapping and it generally has genomes of two parents, the populations included in the mapping are F₂, backcross, recombinant inbred lines, double haploids, and near-isogenic lines (Rakshit *et al.* 2012; Collard *et al.* 2005). The MAGIC population is a special group that has a diverse set of original genetic variants. It falls between

a group of plants that have two parents and a group that is created through association mapping. The MAGIC population focuses on smaller marker density, but higher diversity of alleles, leading to better mapping resolution and statistical power in studying population substructure. This makes it a more efficient and effective approach compared to bi-parental populations (Pascual *et al.* 2015).

Table 1. Utilization of Multiparent advance generation intercross (MAGIC) population in various crop

Utilization of Multiparent Advance Generation Intercross (MAGIC) population in various crop			
Economically Important Traits	Genetic Analyses	Genetic Resource Development	Multi-trait Analyses
Abiotic Stress	Coarse mapping	Development of NILs	Detection of pleiotropic QTL
Biotic Stress	Fine mapping	Development of elite breeding lines	Detection of closely linked QTL
Yield related traits	Gene functional analysis	Development of Recombinant lines	

The utilization of the MAGIC population ensures the better production of crop varieties in different ways which can be estimated economically or genetically. Economically the protection from different types of stresses like biotic and abiotic and using of high-yielding diverse varieties as parents in the MAGIC population will also help in the production of high-yielding cultivars for the beneficiary of the farmers. Genetically with the help of QTL mapping, the progeny of diverse parents can be mapped and analysed for trait selection, genetic resources will also be enhanced by using diverse genotypes from different parts of the world.

CROP IMPROVEMENT THROUGH MAGIC

MAGIC population requires lots of resources and complexity in development and despite these challenges, the increased number of MAGIC population among all the crops is tremendous, and this number can be observed in any crop like legumes, cereals, and vegetables. Agronomic traits improved using the MAGIC population (Diaz *et al.* 2020) as these improved traits help solve the crops' biotic and abiotic stress constraints. In maize, corn borers are among the important pests, and the detection of multiple single nucleotide polymorphisms (SNPs) of low effect which is related to resistance to stalk tunnelling by corn borers, and in groundnut for the resistance of late leaf spot disease total 10 MAGIC lines with incubation of more than 11.0 days and two lines with a long latent period (>27 days) were identified than the resistant parent by field screening at two different locations ICRISAT and ARS, Kasbe Digraj (Wankhade *et al.* 2021). For abiotic stress, in common bean (*Phaseolus vulgaris* L.) the MAGIC population is produced from the eight Mesoamerican breeding lines, and the drought resistance lines were developed using Genotyping by Sequencing (GBS) and Whole Genome Sequencing (WGS) (Diaz *et al.* 2020).

RICE

It belongs to the *Poaceae* family and has two major diploid ($2n=24$) varieties cultivated worldwide: *Oryza sativa indica* and *Oryza sativa japonica*. It is a staple food that provides instant energy in form of carbohydrates (starch). Rice has relatively small if compared to other cereals which is 430 Mb. Mainly the improvement of rice has been done using biparental population and MAGIC approach in rice improving traits is an attractive approach. Introducing MAGIC in rice genetic recombination improves trait dissection and synthesis. Genotypes with distinct characters have been introduced into the pedigrees, and how much genotypic diversity is created is not sure for the achievement of genetic gain in different traits.

Abiotic stress

A study by Zhang *et al.* identified the QTLs for salt-tolerant related traits, MAGIC population created by intercrossing of four elite *indica* varieties was used. In that study, researchers found 7 different locations on chromosomes 1, 2, 5, and 9 that are responsible for certain traits. One of these locations, called qRRL2, is located on chromosome 2 and is linked to relative root length. Another location, known as qSLST1/qRDSW1/qRB1, is a multi-trait QTL located on chromosome 1 that is associated with root biomass, root dry weight, and shoot length specifically under conditions of salt treatment. (Zhang *et al.* 2020).

Yield

In a study, eight *japonica* and eight *indica* elite parents were selected and made crosses with each other, either *indica* x *indica* or *japonica* x *japonica*. Total 60 QTLs were identified using Bayesian networking and with the help of GWAS 38 and 34 QTLs were detected with 22 marker-marker associations, 32 trait-trait associations and 65 marker-trait associations (Zaw *et al.* 2019). At headquarter of the International Rice Research Institute (IRRI) GWAS is performed for the identification of QTL for 14 different traits which include yield, and other related traits to yield using elite *indica* lines (DC1, DC2, 8-way) and a total of 26 QTL on all chromosomes were identified except 7, 9, and 11 for a total of 10 traits in dry and wet seasons (Meng *et al.* 2016).

WHEAT

Wheat has 6 major varieties: *Triticum aestivum*, *T. vulgare*, *T. spharococcum*, *T. dicoccum*, *T. durum*, *T. monococcum* with different ploidy levels, among these *Triticum aestivum* is the most cultivated covered almost 87% of the area having hexaploid level and chromosome number ($2n=6x=42$) having genome size approximate 17Gb. To obtain greater diversity and better-performed traits novel approaches should be used to develop new lines so that the need of the

population can be met, and the genome of wheat is complex and it requires new approaches to study its genetic dissection, then the researcher's uptake MAGIC. In Australia, the first MAGIC population was produced (Huang *et al.* 2012) using four spring-type Australian cultivars. An eight-parent winter-sown wheat population has also been created consisting of 1091 F7 lines (Mackay *et al.* 2014). The development design for the eight-parent population is to mix all founder lines using various funnels without intercrossing to develop RILs similar to the *indica* rice population.

Plant height

A MAGIC population WM-800 derived from 8 European winter wheat cultivars, with the help of GWAS 14 QTLs identified which plant height including known genes *Rht-B1* and *Rht-D1* and a new QTL found on chromosome 5A, also find out that epistatic effects-controlled plant height (Sannemann *et al.* 2018)

Biotic stress

In wheat, there is fungal pathogen *Zymoseptoria tritici* of the septoria tritici blotch (STB) disease. The eight-founder "NIAB Elite MAGIC" population helped to identify QTLs for STB resistance and a total of 25 QTLs were found, these genomic regions serve as beneficial resources for the rapid selection, resistance, breeding of favourable genes for developing new STB-resistant lines (Riaz *et al.* 2020).

TOMATO

Cultivation of tomato (*Solanum lycopersicum* L.) $2n=2x=24$ comes under the most important vegetable crops in the world. It belongs to Solanaceae, which almost contain 3000 species and among them, *Solanum lycopersicum* is the most cultivated specie. Tomato has attractive features like compound leaves, juicy fruits and sympodial shoot branching (Townsend, Manning, Schmitz). It

also has a short generation time, basic ploidy level, and low maintenance. MAGIC was introduced in tomato to utilize its attractive features in a variety for the better performance of complex traits. A previously sequenced eight founder lines have been used to develop the MAGIC population (Pascual *et al.* 2015).

Abiotic stress

In tomato heat stress main factor in defining yield quantity if the temperature above 30°C diminishes the reproduction which ultimately affects the fruit yield quantity, Bineau *et al.* experimented on eight parent MAGIC population and evaluated 11 traits for the effect of heat at QTL and transcriptome levels and by linkage and genome-wide association analysis 98 QTLs were identified (Bineau *et al.* 2021). A total of 250 MAGIC lines covered a great diversity in tomato crop and were tested for salinity resistance as compared to controlled conditions for contributing traits of quality and yield of fruit (number of fruits, fruit weight, soluble solid content, firmness), a vegetative trait, leaf length and phenology traits (time to flower ripe). Among these, 54 QTLs were found using 1,345 SNP markers which further revealed there were 15 of them showed the interaction between genotype X environment and 65% (35 QTL) were treatment specific (Diouf *et al.* 2018).

FUTURE PROSPECTS

MAGIC population helps the crop to demonstrate the fine QTL mapping of their genetic and phenotypic diversity, yet there is a large range left to be exploited.

Multivariate (combination of many lines)

The production of MAGIC lines has been demonstrated as less loss in the genetic diversity of the crop though it was reported as an increase in the overall diversity of the crop as it was produced by the crossing of diverse (from each other) lines and by introducing wild varieties in these intra-specific crosses and broaden the genetic base of these cultivated species which ultimately helps in controlling the different type of stresses and loss in variability will be less but in the other methods of plant breeding the loss of genetic diversity gradually noticed (Smykal *et al.* 2018; Purugganam *et al.* 2019).

The MAGIC population can be used as having multiple genomic fragments or introgression of undomesticated variety into a cultivated species. A MAGIC population of chickpeas produced using 8 elite cultivars (ICC 4958, ICCV 10, JAKI 9218, JG 11, JG 130, JG 16, ICCV 97105 and ICCV 00108) from Ethiopia, Tanzania, Kenya, and India, all are diverse in their contrasting characters, that helps in producing cultivars with more variation. This allows the study of QTL in a mixed population which will help in the production of genetically characterized elite cultivars and these cultivars can be integrated into different breeding programs. In the current scenario due to climate change, there is an expected rise in temperature between 1.5 and 2 ° C by the end of this decade, so in this study, these high-yielding lines ICCML10088, 11136, 11055, 10363, 10649 and 10070 were produced which showed much less fluctuation in producing yield under heat stress condition than the normal cultivars.

MAGIC Populations in Breeding Pipelines

The MAGIC population can also be utilised as elite material for the breeders apart from using it as a mapping population in the identification of genes and QTLs. In the MAGIC population as parents are selected are more diverse, then a new type of phenotypic lines will be produced and these lines may have improved characters in them which can be further released directly as a cultivar by the selection within MAGIC populations and these elite cultivars have been

released in chickpea and rice (Samineni *et al.* 2021; Li *et al.* 2013) and many more, and in this way, the MAGIC population is homozygous and the frequency of individual lines can be determined by multiplying the frequency of the desired alleles for each specific locus found among the original genetic resources. Due to the large size of the MAGIC population, it is possible to identify various QTLs or genes that have been combined and can be directly used by breeders in their breeding programs. To make this process easier, Scott *et al.* suggested the use of "packages" that include the MAGIC population material along with detailed information about its genetic makeup and phenotypic characteristics. These packages would make it much simpler for breeders to incorporate the MAGIC materials into their breeding pipelines.

MAGIC lines may be particularly useful for hybrid parents. By crossing these lines with testers and evaluating their specific and general combining abilities (Labroo *et al.* 2021), it is possible to identify MAGIC lines that produce high-performing hybrids with already established elite lines or even establish new heterotic groups within the MAGIC population. The genotyping data available for MAGIC populations can also aid in the prediction of hybrid performance by using genetic distances or other genetic parameters among the lines (Alves *et al.* 2019).

CONCLUSION

The MAGIC populations are produced using diverse parent intercrossing and then made homozygous either with the help of double haploid or single decent method. Despite the considerable effort required to develop these populations, both theoretical and real-world studies have shown that they are powerful tools for detecting QTLs present in the parent set. In fact, they offer significant benefits over bi-parental and genetic resources for QTL identification. After the identification of these QTLs, these lines can be released as varieties, and to analyse these varieties new tools are getting produced for estimating the

efficacy of the MAGIC population. As the result we have seen so far by producing a MAGIC population, these lines will help breeders to address different challenges like genetic erosion, climate change and the demand for food. The MAGIC population holds the potential for producing high-yield, diverse with capabilities to resistance against various stresses.

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