MAGIC: A new genetic resource for multiple trait improvement and QTL identification in Cotton (*G. hirsutum L*)

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Introduction:

- Breeders and Molecular Geneticists have routinely used populations derived from bi-parental crosses for
 - i) Varietal development

- ii) Mapping QTLs for traits of interest
- Breeders have attempted multi-parent crosses such as three –way or double crosses to increase the genetic variation in breeding population

Limitations of bi-parental crosses

- 1. The recombination takes place only in F1 generation and not enough time is available to shuffle the genome in small fragment.
- 2. Narrow genetic base
- 3. It allows mapping of only the allelic pairs present in two parents and thus whole genome cannot be exploited in such studies.

Multiparental

Advanced

Generation

nter

Cross

- It is a simple extension of the advance intercross (Darvasi and Soller, 1995)
- The method was proposed and applied in mice (Mott et al. 2000)
- The word MAGIC was coined by Mackay and Powell (2008) and advocated by them for crops.
- MAGIC population was first developed and described in Arabidopsis (Kover et al., 2009)
- Funnel Breeding

Limitations of Multi-parental cross

- Intensive labour for crossing
- Extensive segregation
- Large population size is required for recovery of recombinants with all the desirable traits
- More time is required to develop the resource population
- Large scale phenotyping is required for a particular trait.
- Requires more inputs







Advantages of using Multi-parental populations

- More targeted traits from each parents can be analysed based on the selection of the parents used to make multi-parental cross.
- Increased precision and resolution with which the QTLs can be detected due to increased level of recombination.
- MAGIC population helps in shuffling of genes across different parents enabling novel rearrangements of alleles.
- To interrogate multiple allele
- Greater genetic variability
- Chances to get best combination of desirable genes.
- Phenotypic selection in advance generation reduce the frequency of deleterious/undesirable alleles from donors.
- MAGIC population will be a permanent mapping population for precise QTL mapping.

These populations are now attractive for researches due to

- The development of high throughput SNP (Single nucleotide polymorphism) genotyping platform which has reduced the cost of genotyping
- speed of analysis
- Advances in statistical methods to analyse the data for such populations

Requirements for Mapping population

- A population of plants that is genetically variable for target phenotypes
- Marker system that allows genotyping of the populations
- Reproducible quantitative genotyping

Comparison between biparental linkage analysis, association mapping and MAGIC

Properties	Biparental	Association	MAGIC
Founder Parents	2	≥ 100	≥8
Crossing requirement	Yes	No	Yes
Time to establish	Moderate	Low	Long
Population size	~200	~100	~1000
Suitability for coarse mapping	Yes	No	Yes
Suitability for fine mapping	No	Yes	Yes
Amount of genotyping required	Low	High	High
Amount of phenotyping required	Low	High	High
Statistically complexity	Low	High	High
Use of germplasm variation	Low	High	High
Practical Utility	Low	High	High
Relevance over time	Low	High	High

Use Of MAGIC Lines In Breeding Programs

- MAGIC population can directly be used as source material for the extraction and development of breeding lines and varieties.
- Development of varieties with several agronomically beneficial traits.
- Variety which can adopt to several diverse regions of the country and suitable for diverse climatic conditions.
- An assessment and understanding the potential of enhanced recombination in generating novel diversity.

Contd.

- MAGIC populations have the potential for identifying useful QTLs from a diverse set of parents
- The MAGIC population can be explored to study gene x gene interactions.
- The MAGIC population is well suited as a platform for Fine mapping QTLs and gene isolation

Wheat

Cockram (2014) utilised MAGIC strategy for fine mapping quantitative loci, gene isolation and pattern of linkage dis-equilibrium

- The genetic diversity in comparison to bi-parental cross and an association mapping population was found to be 142% and 94 % respectively.
- Identified resistance to major foliar disease *Puccinia striiformis f. sp. trilici* (wheat yellow stripe rust) controlled by three QTLs on the long arm of Chr 2D, 2A and 1A.
- Identified a strong diagnostic marker for awn presence /absence on chr 5BL which is also associated with Fusarium wilt resistance.

Cockram et al. (2015) adopted eight founder MAGIC population to fine map the wheat Snn1 locus which confers sensitivity to the Parastagonospora nodorum necrotrophic effector SnTox1

- The lines showed a range of sensitivity to SnTox1 sensitivity, with transgressive segregation evident in the progenies.
- SnTox1 showed high heritability with QTL analysis.
- Snn1 Locus was fine mapped on the short arm of chromosome 1B which was previously identified on the long arm of the chromosome 5A.
- The peak SNP for Snn1 locus was converted into KASP genotyping platform (Kompetitive Allelic Specific PCR).

An Eight-Parent Multiparent Advanced Generation Inter-Cross Population for Winter-Sown Wheat: Creation, Properties, and Validation

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- 90,000 SNPs were used for the analysis and found the population was well suited as a platform for fine mapping of QTLs and gene isolation.
- Genetic marker diversity among the founder lines was 74 %.
- Pattern of linkage dis-equilibrium showed that the population was highly recombined.
- They identified highly diagnostic marker for the morphological trait awn presence /absence.

Arabidopsis thaliana

Huang et al. (2011) followed MAGIC in Arabidopsis thaliana and created six subpopulations (MPRILS).

- Flowering time and leaf morphology trait QTLs was determined
- Fine mapped a number of QTLs in the selfed progeny of lines
- Among the QTLs detected in MPRIL population several are likely to correspond to the QTLs identified before in bi-parental populations for which the candidate genes are known.

RICE

Bandillo et al. (2013)

- They have developed MAGIC population each for *indica* and *japonica* rice. They have also inter-crossed the two to form 16 parent Globle MAGIC population.
- The population was phenotyped for multiple traits including blast and bacterial blight resistance, salinity and submergence tolerance and grain quality.
- GWAS mapping identified several known major genes and QTLs including *Sub1* association with submergence tolerance and Xa4 and xa5 associated with resistance to bacterial blight.
- Also identified potential novel loci associated with essential traits for rice improvement.

Yamamoto et al. 2014 in rice used this population to detect the linked QTLs

- Populations that includes more recombination sites is expected to be an effective way to resolve the problems that results from linkage QTLs.
- They have tried to investigate: whether the linked QTLs are the function of the no. of cycles of recurrent crossing.
- They have come to the conclusion that when population is derived from eight parental lines, the use of fewer than two cycles does not improve the power to detect linked QTLS. However, increasing to six cycles dramatically improved the detection power, suggesting that advanced intercrossing can help to resolves the problems derived from linkage among QTLs.

Characterization of Three *Indica* Rice Multiparent Advanced Generation Intercross (MAGIC) Populations for Quantitative Trait Loci Identification

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- 8-way population had a higher gene diversity than the 4-way populations
- 8-way population was more powerful than the other two population for QTL identification.
- The association analysis identified 2 and 3 QTL for plant height and heading date respectively
- A novel QTL for plant height was identified on chr 12 using 8way population



MAGIC lines segregating for resistance and susceptibility

Maize

Acqua et al. 2015 have studied the genetic properties of MAGIC maize population

- A flowering time QTL was reported on chr 10 having ZmCCT as casual gene which might have contributed to early flowering of Founder 7.
- Identified three QTLs for grain yield. They searched genes in QTLs and could identify 45 such genes for grain yield QTL.
- The major QTL for grain yield was a locus on the short arm of chr 6 pleiotropic to plant height and ear height.
- GRMZM2G054651 is one of the four differentially expressed genes within a smaller QTL for grain yield on chr 4.
- GRMZM2G101875 is the other gene associated with grain yield QTL on chr 10.
- Identified several QTL loci for Pollen shed. Identified 101 genes within these QTL.
- Found a pleiotropic QTL on chr 8 explaining 19 % of flowering time variation and having effect on plant height and ear height.

Cow pea

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A multi-parent advanced generation inter-cross population for genetic analysis of multiple traits in cowpea (Vigna unguiculata L. Walp.)

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- Using F8 lines, lines diverse for agronomic traits including flowering traits, growth habit, maturity, yield potential and seed characteristics across environment were identified.
- Markers with major effects for early flowering time were located on Chr 9 while markers with smaller effects were located on chr 5 and 11.
- Markers significantly associated with plant growth habit were identified on chr 1 and 9.
- For grain yield, significant peak was detected which was located on chr 8 under restricted irrigated while no significant markers were identified for grain yield under normal irrigation.
- Markers with major effects were identified for seed size on chr 8 based on data from both water restricted and full-irrigation condition.

RESEARCH ARTICLE

Open Access



A MAGIC population-based genome-wide association study reveals functional association of *GhRBB1_A07* gene with superior fiber quality in cotton

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- By employing GWAS (Genome wide association study) they able to identify markers significantly associated with fibre quantitative trait loci.
- Identified 86 fibre QTLs distributed on 24 chromosomes. More of the fibre QTL on D genome when compared to A sub genome
- 16 QTL clusters were identified, with two to five QTL in a cluster.
- Identified and validated one QTL cluster associated with four fibre quality traits (SFC, Strength, UHML and UI) on chr A 07.
- Identified GhRBB1_A07 gene as candidate gene for superior fibre quality in upland cotton.

Li et al. (2016) carried out polymorphism analysis of MAGIC population of upland cotton

- Gene diversity indices and polymorphism information content were calculated using polymorphism analysis.
- Identified 17 pairs of specific SSR primers on A chromosome sub group and 24 pairs of specific SSR primers on B chromosome sub group of upland cotton.

Tomato

Plant Biotechnology Journal



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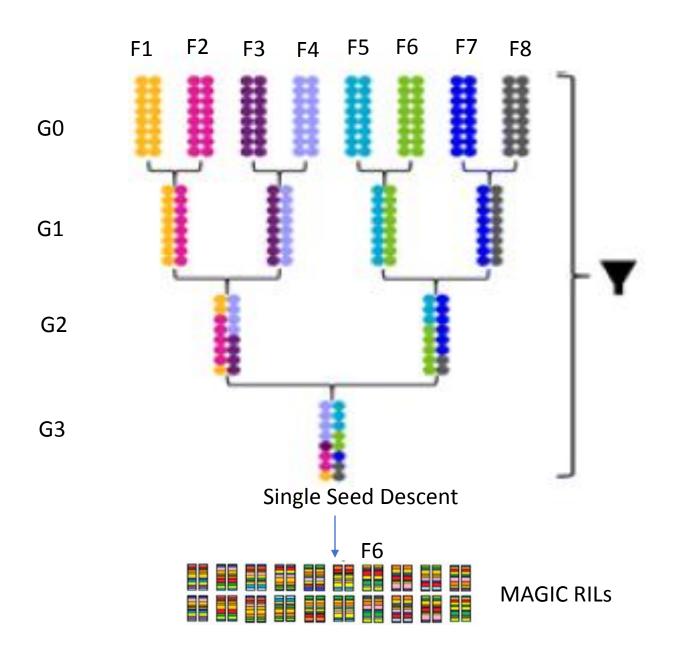
doi: 10.1111/pbi.1228

Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era

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- The linkage map showed an increase of 87 % in recombination frequency compared to bi-parental population
- Three stable QTLs and six specific QTLs of a location was detected.
- The number of candidate polymorphisms was reduced from 12284 (on 800 genes) to 96 (in 54 genes).

Crossing Scheme for Development of MAGIC Population



F: Founder lines

LRA 5166

N170

NH 615

28 I

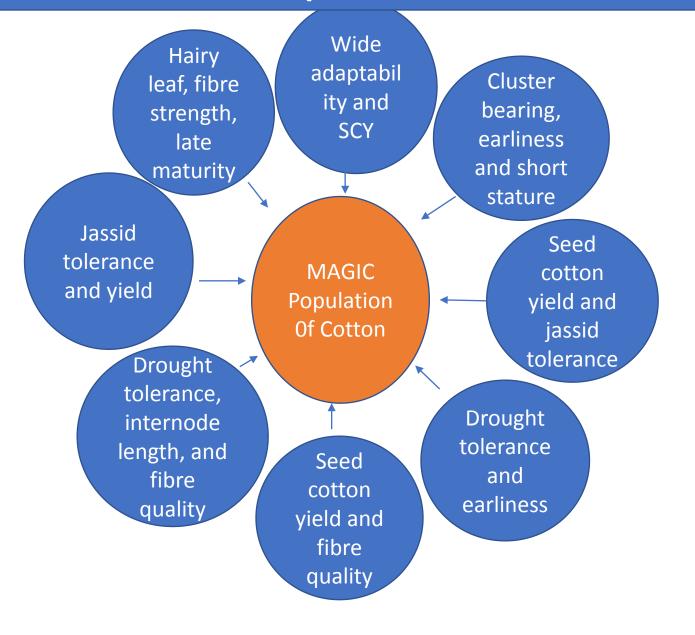
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Characteristic features of parents involved in development of MAGIC



Variation in morphological characters in parents















