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From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants

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The dissection of gene-trait associations and its translation into practice through plant breeding is a central aspect of modern plant biology. The identification of genes underlying simply inherited traits has been very successful. However, the identification of gene-trait associations for complex (multi-genic) traits in crop plants with large, often polyploid genomes has been limited by the absence of appropriate genetic resources that allow quantitative trait loci (QTL) and causal genes to be identified and localised. There has also been a tendency for genetic resources to be developed in germplasm not directly relevant to the breeding community limiting effective implementation. In this review, we discuss approaches to mapping genes and the development of Multi-parent Advanced Generation Inter-cross (MAGIC) populations derived from breeder-relevant germplasm as a platform for a new generation of gene-trait analysis in crop species.

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Introduction

Most traits of biological and economic interest in crop plants are of a quantitative nature, displaying continuous variation within or between species and are under polygenic control [1]. The term quantitative trait loci (QTL) was introduced by Geldermann [2] to describe those regions of the genome underlying a continuous trait. The majority of QTL in plants have been identified by two approaches, either bi-parental crosses exploiting recent recombinations or association analysis which exploits historical recombination. Both methods have limitations in facilitating candidate gene identification, whilst evaluating numerous alternative alleles and investigating epistatic interaction in breeder-relevant genetic backgrounds.

To detect QTL four elements are required: a population of plants that is genetically variable for the target phenotype; marker systems allowing genotyping of the population; reproducible quantitative phenotyping methodologies; and finally, appropriate experimental and statistical methods for detecting and locating QTL.

This paper provides a review of experimental systems available for QTL and candidate gene identification in crops and is divided into two broad categories: first, those based on selection or natural populations and second, experimental populations. We have also included the development of second-generation mapping resources (Multi-parent Advanced Generation Inter-cross, MAGIC) in which we are involved.

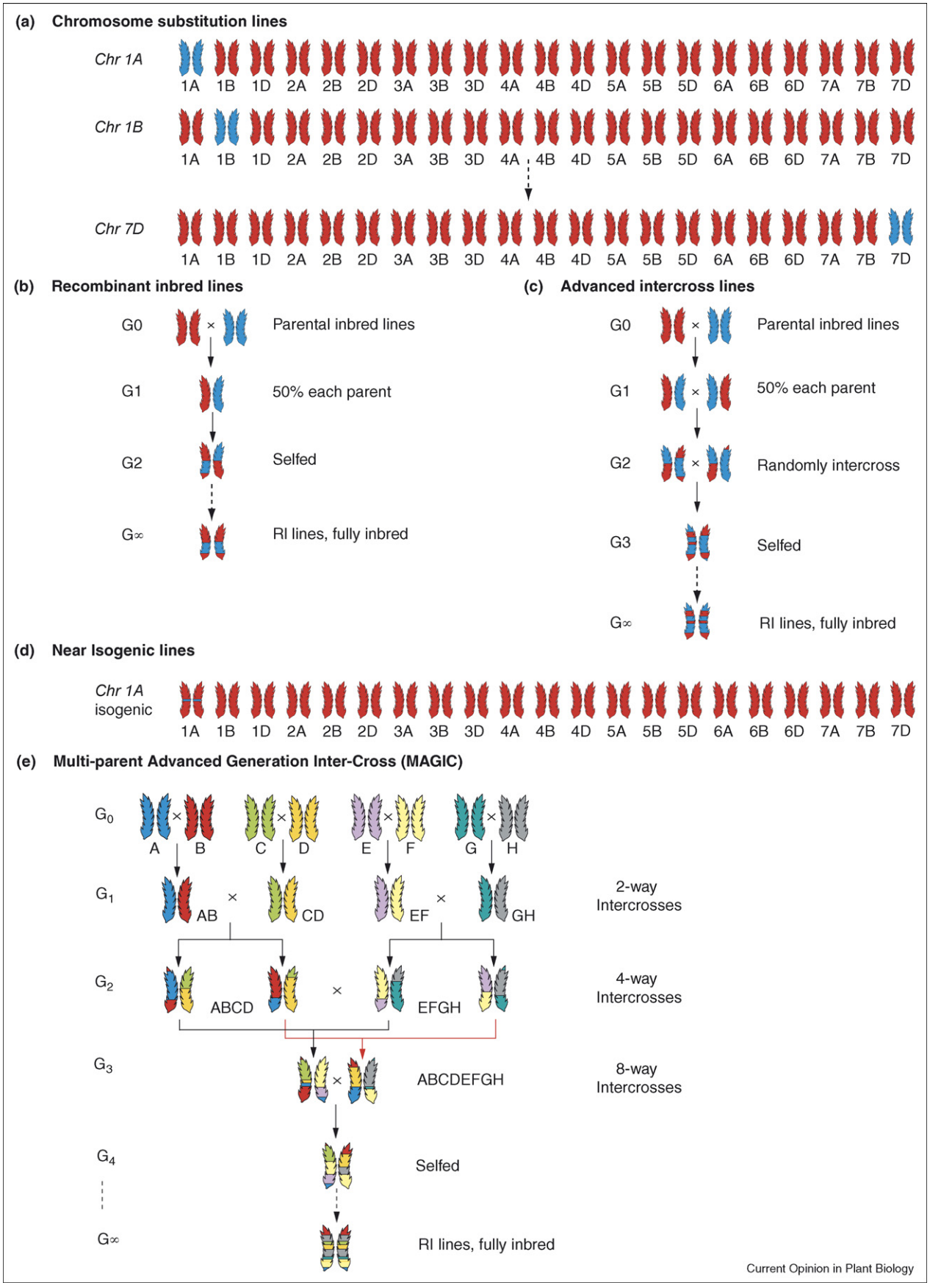
Approaches to gene-trait dissection using selection and natural populations

Selection-based approaches

Selection experiments have had a long and successful history in quantitative genetics [1,3]. Marker allele frequency changes, following selection, can reveal the existence of linked QTL which have responded to that selection [4]. Such experiments require many generations of breeding, but the advantage is that the difference in phenotype between the selected and initial population (or between high and low selections) may be greater than available in alternative mapping populations, indicating a potential increase in the number of QTL that can be detected. The detection of QTL relies on linkage disequilibrium (LD, the non-random association of alleles at separate loci located on the same chromosome), between QTL and closely linked markers persisting over generations. By crossing extreme individuals from generation 70 of the Illinois long-term corn selection experiment, followed by 10 generations of intermating to reduce LD between loosely linked loci, Laurie *et al.* [5••] were able to detect 50 QTL accounting for about 50% of the genetic variance in oil content with a resolution of the order of a few centimorgans.

Selection itself will generate LD around a QTL, but this decays with crossing at a rate dependent on the recombination fraction between the QTL and marker. As a result, the power and precision to locate QTL is complex, dependant on QTL effect, allele frequency, intensity of

Figure 1


(a) Chromosome substitution lines
Chr 1A
Chr 1B
Chr 7D
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D
(b) Recombinant inbred lines
G0 Parental inbred lines
G1 50% each parent
G2 Selfed
G∞ RI lines, fully inbred
(c) Advanced intercross lines
G0 Parental inbred lines
G1 50% each parent
G2 Randomly intercross
G3 Selfed
G∞ RI lines, fully inbred
(d) Near Isogenic lines
Chr 1A isogenic
(e) Multi-parent Advanced Generation Inter-Cross (MAGIC)
G0 A B C D E F G H
G1 AB CD EF GH
2-way Intercrosses
G2 ABCD EFGH
4-way Intercrosses
G3 ABCDEFGH
8-way Intercrosses
G4 Selfed
G∞ RI lines, fully inbred
Current Opinion in Plant Biology

selection, recombination fraction, and number of generations selected.

The process of crop domestication itself can be viewed as a long selection experiment (typically thousands of years). Comparisons of allele frequencies in domesticated crops with wild ancestors can detect genes and genomic regions which have been the subject of selection during domestication in the absence of any phenotype information. This approach, termed hitchhiking mapping [6], has been used to detect loci subject to selection during the domestication of maize [7].

Association mapping

Association mapping exploits LD to localise QTL in diverse populations. In humans, this approach has recently proven remarkably successful, with 24 genetic risk factors identified in genome scans for 7 common human diseases [8]. These studies were, however, large collaborative efforts among over 50 research groups in which 500 000 markers were genotyped across 17 000 individuals. The scale and precision of these studies are currently beyond the reach of crop research where a number of factors limit its effective implementation (reviewed in Mackay and Powell [9•]). However, in maize in particular, association mapping has proven to be successful [10,11], aided by large diversity and lower levels of population structure and a high-density marker map.

One of the major impediments to association mapping is that population structure can lead to spurious associations if not dealt with an appropriate manner [12••]. Secondly, most crop species lack a reliable high-density consensus map, making it difficult to estimate accurately the relationship between genetic distance and decline in LD. Recently it was estimated that 250 000 single nucleotide polymorphisms (SNPs) were adequate for genome association studies in *Arabidopsis* [13•]. Clearly it will be some time before such marker density will be available for routine screening in many crops species. Finally, some plant studies have been underpowered with sample sizes around 200: association mapping generally provides greater precision than linkage mapping but has lower power to detect QTL. Studies utilising larger sample sizes have reported a common architecture for QTL effects, whereby many small effect QTL contribute to the genetic architecture [5••]. Power and precision to detect QTL are two important factors when designing any genetic study. Power refers to the probability of detecting a QTL that is segregating within a population whilst precision refers to the location error associated with the predicted QTL and the actual QTL.

Mapping in pedigrees

Contemporary varieties of many crops are members of an extended pedigree. This pedigree structure can be exploited for linkage analysis in the same manner as in animal and human genetics [14]. In humans, this approach has led to the positional cloning of causative genes for many Mendelian-inherited diseases, though it has been much less successful for more complex traits [15]. For some crops, for example coconut and other perennial species, the development of experimental populations may be difficult and this approach offers a practical approach to mapping QTL in germplasm of direct relevance to breeders. Even if pedigree structure is not known, relationships between varieties can be estimated using molecular markers and used in its place [16]. Pedigree structure also allows the implementation of association testing. The best-known method is the transmission disequilibrium test (TDT) [17] in which the transmission frequency of an allele from heterozygous parents to offspring selected on phenotype is compared to its expected value of 0.5. The TDT is a test for association in the presence of linkage. A TDT-like method has been developed for crops [18] and because it does not detect marker-trait associations between unlinked loci is robust to population structure.

Approaches to gene-trait dissection using experimental populations

Genetic libraries

Mutant populations

The analysis of spontaneous mutations is a well-established method for gene discovery and elucidation of gene function [19,20]. Mutagenesis is a way of increasing the mutation frequency across the genome affecting many traits of interest. There are numerous alternative methods such as ethylmethane sulphonate, fast neutron radiation and heavy ion irradiation utilised in crop species. Creating mutagenised populations is relatively straight forward, but large resources are required for genotypic and phenotypic screens. Polyploidy complicates the ability to identify mutants via phenotypic screens because of the duplication or triplication of genetic information.

A more sophisticated method for detecting mutations at known loci is the Targeting Local Lesions IN Genomes (TILLING) approach, a PCR-based reverse genetics approach that searches the genome for induced or natu-

Experimental designs commonly used in crops to identify QTL using the wheat genome as a model: **(a)** chromosome substitution lines are created by generating a set of lines each of which contains a single introgressed chromosome from a donor and the remainder from a recurrent parent; **(b)** bi-parental recombinant inbred lines (RILs) are generated by inbreeding F2-derived progeny to create a population homozygous at all loci for genetic analysis; **(c)** advanced inter-cross lines are created by inter-crossing successive F2-derived generations. RILs may then be derived by selfing; **(d)** near isogenic lines (NILs) are created by backcrossing with a recurrent parent until the entire genome except the gene/region of interest, in this example chromosome 1A has the introgressed region; **(e)** MAGIC populations are created by inter-crossing *n* lines for *n*/2 generations until all founders are combined with equal proportions in the inter-crosses, RILS may be derived immediately or additional rounds of intermating.

rally occurring causative SNPs [21]. The method does not require prior phenotypic screening, however, prior knowledge of the gene controlling the target trait is required. TILLING has been implemented in a number of species including wheat [22], barley [23], maize [24] and legumes [25].

Chromosome engineering

Substitution lines

Diversity is central to the genetic advancement of any crop species, and exotic breeding material such as landraces and wild relatives are used to increase diversity and productivity in crops [26]. With the molecular and analytical tools available, a more detailed and directed approach to wild introgressions will be possible (reducing linkage drag) and facilitate increased productivity and quality improvements for many crops. The potential of wild relatives as sources of genetic variation has long been recognised [27] and more recently harnessed to develop some of today's modern varieties [28]. Recombinant chromosome substitution lines are common in wheat where they have had a long history [29]. Typically chromosome substitution sets involve all chromosomes (except one) being derived from a recurrent parent and the remaining chromosome from a donor parent (Figure 1a). To define the position of genes on substitution chromosomes, recombinant inbred chromosome substitution lines (RICSLs) can be developed [30,31] and have been successful in the cloning of genes underlying traits in agriculture [32–35].

The tomato has led the way in regard to the introgression of exotic chromosomes, with the successful identification of a gene increasing the provitamin A (β-carotene) by 15-fold [36]. Gur and Zamir [37] have demonstrated the benefits of harnessing natural diversity from wild relatives of tomatoes to increase yields using introgression lines. Using an introgression from *Triticum dicoccoides* (wild emmer wheat) Uauy *et al.* [35] have identified a gene improving senescence, grain protein, zinc and iron content in bread wheat.

Deletion lines

In wheat, the large genome size, polyploidy and limited polymorphism has led to the creation of around 400 deletion lines for *cv* Chinese spring [38]. These deletion bins are widely used for the assignment of markers to physical chromosomes. The usefulness of these deletion lines has been extended with the mapping of 725 microsatellites [39] across 21 chromosomes providing a link between genetic and physical maps. In addition, a large number of ESTs have been mapped utilising the deletion lines (http://wheat.pw.usda.gov/GG2). The use of these stocks in fine mapping genes affecting polygenic traits, though powerful, is constrained because of the large number of genes that are deleted. To this extent the use of genetic modification (such as RNAi) is important for providing a tool to test candidate genes underlying QTL [35].

Bi-parental populations

Bi-parental populations have been widely employed by selecting lines displaying large phenotypic differences for one or more traits, usually with unrelated parents selected to maximise marker polymorphism (for a review see Ref. [40]). These populations in crops involve crossing lines differing in the performance for the trait/s of interest and creating a single population such as recombinant inbred lines (RILs) or doubled haploids (DHs). All progenies are homozygous at each locus with varying contributions from either parent (Figure 1b).

These methods generally locate QTL to within 10–30 cM regions and are attractive as seed can be retained indefinitely and grown at multiple sites to test environmental effects. An extension of RILs, the advanced inter-crosses (AICs) proposed by Darvasi and Soller [41], consists of a repeatedly intermated F2 population, followed by selfing after which RILs are derived for QTL analysis (Figure 1c). The additional rounds of intermating reduce the level of LD and increase the precision of QTL location (for a review see [42]).

Subsequent to these approaches has been the 'mendelisation' of populations by creating near isogenic lines (Figure 1d), designed to eliminate background effects and localise the gene of interest [43]. This approach has been successful in a number of species [44–51].

Genetic analysis in bi-parental populations only allows mapping of pairs of alleles for which the parents differ. There are a number of ways of incorporating a larger portion of the genetic variation available in the gene pool into such studies. One way is to include a number of studies in a meta-analysis to evaluate statistically identified QTL [52] or combining experiments [53], however, these do not offer the possibility to examine epistasis.

Simultaneously mapping multiple populations in one study will also increase the sampled genetic variation. This strategy is part of an ongoing project in maize where 5000 RILs have been created by crossing 25 diverse maize inbreds to a single common inbred line to create 200 RILs for each cross. Each parent is being sequenced (http://www.panzea.org/info/RIL_phenotyping_press_rel.html) and all RILs typed with markers. Including a common parent across populations facilitates the incorporation of genetic heterogeneity into the model and genetic interactions between the common parent and different backgrounds. These populations are, however, restricted in assessing background-specific interactions because of the common parent in all populations. The strength of this method is the utilisation of historical LD

information from the parents to allow more precise positioning of QTL and the ability to incorporate a large number of alleles from the gene pool.

Multi-parent populations

The AIC has been extended in mice by including multiple parents (called heterogeneous stock) and has proven successful in fine mapping many QTL controlling complex traits in mice to small confidence intervals [54]. Yalcin *et al.* [54] used the strain distribution pattern (SDP) at a locus to prioritise functional candidate genes and map a QTL explaining 10% of the phenotypic variation in anxiety to a 4.8 Mb region and identify 14 SNPs from 15 000 variants as the possible functional polymorphism. This type of resolution in crop breeding may seem fanciful now, but it highlights the need to develop biological resources to take advantage of molecular advances probable in crops. A further development in mice is the creation of a large multi-parent RIL population [55] which in crops has been termed MAGIC. These MAGIC populations (Figure 1e) allow the use of both linkage and association methodologies without the difficulties of highly structured populations. Sampling a greater proportion of the genetic variation will also occur and seed from any generation can be saved and utilised to develop RILs suitable for both coarse and fine mapping. The incorporation of multiple parents ensures the population is segregating for multiple QTL for multiple traits and cytoplasm effects can be modelled.

Modelling precision and power to detect QTL, Valdar *et al.* [56•] show that an 8-parent RIL population with 1000 progenies is capable of mapping resolution in the sub-centimorgan range.

The underlying theory for mapping these populations in mice is well advanced [57••]. There is now the opportunity to extend and exploit this theoretical framework for fine mapping in crops. One compelling advantage of the multi-parent RIL is that a large proportion of the genetic variation that exists in modern crops may be included within the parental lines selected. For selfing species especially, large numbers of lines can be cheaply generated to allow adequately powered experiments. Table 1 compares the relative strengths and weaknesses of current approaches for localising genes affecting complex traits.

This approach has many additional advantages for the crop science community. With many sequencing projects in their infancy, MAGIC populations will be an ideal resource for creating high-density maps using germplasm relevant to breeders.

These populations have other distinct applications. If a large set (>1k) of RILs are produced, two-way and three-way epistatic interactions can be assessed to shed light on the complex architecture of many traits associated with crop performance and product quality. The large sample size will also allow the screening of specific subsets of the population for traits (such as yield under drought), where specific phenology (flowering time) can be exploited whilst still incorporating genetic diversity within a defined population structure.

Table 1

Relative strengths and weaknesses of three methods for the identification of QTL in crops, bi-parental linkage analysis (linkage), association mapping (association) and Multi-parent Advanced Generation Inter-crosses (MAGICs)

Application	Linkage	Association	MAGIC
Suitability for coarse mapping	+	−	+
Suitability for fine mapping	−	+	+
Low genotyping requirement	+	−	−
Low phenotype requirement	+	−	−
Resistant to population substructure	+	−	+
Relevance to breeders	−	+	+
Relevance over time	−	+	+
Time to establish	−	+	−

Conclusions

Technological advances in the mammalian genomics arena are already impacting crop biology. To capture and benefit from this opportunity more emphasis needs to be placed on the creation of populations that will allow both the discovery and deployment of new gene function for traits that exhibit complex inheritance patterns. The MAGIC design exemplifies a population that provides a platform for a community-based approach to the discovery, characterisation and deployment of genes responsible for complex traits. Most importantly it provides a common route to the establishment of a system-based scheme directed at gene isolation for crop scientists.

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