MAGIC: A Genetic Strategy for Discovering and Harnessing Natural Allele Variants for Trait Manipulation

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Natural variation - also referred to as genetic diversity or biodiversity - is pervasive in all living organisms. It is important for their survival, providing the raw material for evolution, speciation and adaptation. From the viewpoint of agriculture, all crop plants have been domesticated from genetic diversity present in the wild. Breeders are well aware of the significance of natural variation, because it is the force that propels their enterprise. Unfortunately, however, as breeding advances, it also results in an erosion of genetic diversity, leading to the narrowing of the germplasm (Tanksley and McCouch, 1997).

Scientists such as Vavilov and Burbank recognized many years ago that agricultural breeding tended to reduce genetic diversity, and their efforts led to the creation of germplasm collection centers or gene banks to complement the diversity that remains present at the centers of origin (or "centers of diversity") for each crop species (reviewed in McCouch, 2004). One key premise for establishing the gene banks was that they would act as a source of genes and genetic diversity for future plant breeding endeavors. However, breeders have not tapped into this resource as expected over the years, in part for lack of techniques to discover and then utilize useful variation from them (Tanksley and McCouch, 1997). Much of the genetic variation relevant to agriculture is complex and it remains hidden, surfacing only under certain environments or genetic backgrounds (Hoisington, 1999).

As a consequence, breeding for many crops is mostly done by reshuffling the same set of genes/alleles already available to breeders in their modern germplasm. Although it still manages to generate 1-2% gain annually, many wonder about the longer term sustainability of this endeavor because reshuffling the same gene pool can boost plant performance only so much. Indeed, many breeding programs are beginning to show fatigue already, as gains are getting smaller and smaller over time. A new influx of genes will need to take place or the agricultural enterprise will be unable to meet future demands.

Fortunately, there is a renewed interest in the understanding and utilization of natural variation for crop improvement, thanks largely to advances in quantitative genetics and genomic technologies. A part of the reason for this 'back-to-nature' trend for identifying agronomic genes comes from the somber realization that much-anticipated promises of transgenic technology to boost plant yields sustainability may not be fulfilled as quickly as had been hoped. More importantly, however, much of this excitement in exploring and exploiting biodiversity derives from a growing appreciation of natural variation as a ready resource of genes for all sorts of agricultural intervention (Tanksley and McCouch, 1997; Zamir, 2001). This variation, which has been created and selected by nature over millions of years of evolution, represents a huge and largely untapped source of genetic variants of both basic and practical value. If mined and

harnessed effectively, this resource can be utilized to improve production and sustainability of our crops significantly. In fact, it has already been demonstrated in a few cases that genes/alleles can be extracted from wild germplasm that have the ability to break yield barriers of agricultural plants (reviewed in Tanksley and McCouch 1997; Gur and Zamir, 2004).

The traditional way of judging the potential agronomic value of any line is by phenotypic screens. However, phenotype is not always a good predictor of a line's worth, and, in fact, it can be misleading. For instance, a given line may harbor a defective allele for a step in a pathway that leads to a trait of interest. Because of the negative impact of this defect on the trait phenotype, this line will be thrown away even though it may contain highly desirable alleles of genes that control other steps in the pathway (Johal et al., 2008). Such considerations and observations have prompted scientists to stress that we need to stop using phenotypic screens alone for exploring diversity and should also resort to other genetic and genomic methods for discovering useful genes in diverse germplasm (Tanksley and McCouch, 1997). These methods include quantitative trait loci (QTL) analysis, association mapping, fingerprinting diversity selections with high density molecular markers, and ecotilling etc. (Doerge, 2002; Flint-Garcia et al., 2005; Hake and Rocheford, 2004). However, all of these approaches take substantial amounts of time, expense and effort. In addition, many of them hinge inevitably, at some stage in any protocol, on having extensive phenotypic data for hundreds of plants or populations. Thus, it remains a formidable challenge to sift through the enormous diversity available in our gene banks and in the wild.

MAGIC

To alleviate some of the problems discussed above, we have devised a simple yet effective method to discover and characterize useful alleles. This method, which we have called MAGIC (for **M**utant-**A**ssisted **G**ene **I**dentification and **C**haracterization), makes use of the phenotype of a mutant (for a gene affecting the trait of interest) as a reporter to discover and analyze trait-enhancing or trait-suppressing alleles present naturally in the germplasm (Johal et al., 2008). It involves crossing a mutant to diverse germplasm and then evaluating the *mutant progeny* for transgressive changes (both suppressed and severe) in the mutant phenotype(s).

It is well known that genetic background influences the penetrance (whether or not a given phenotype is detected) and expressivity (the strength of a given phenotype) of mutations. As a result, when one seeks to identify a gene underlying a trait or phenotype of interest, the mutation is introgressed into an inbred background to help clarify the inheritance pattern of the mutation. Indeed, it is generally regarded as useful to cross a mutation into several inbred backgrounds and look for the background that gives the most consistent and pronounced phenotype, then discard the rest (Freeling and Fowler, 1993). We reasoned that rather than being a confounding problem, the variation observed for a mutation among different inbred backgrounds (so-called "background effects") is instead a valuable asset for discovering interacting genes and QTL.

The principle underlying MAGIC is similar to the mutagenesis approach that geneticists use to isolate extragenic suppressors or enhancers of individual mutations in genetic screens (Page and Grossniklaus, 2002). However, the genes/alleles identified with MAGIC have existed in natural populations for millions of years and have been defined and refined by natural

selection. Alleles in natural populations may also have more complex changes than single base mutations (or other consequences of conventional mutagenesis).

MAGIC was conceived during a study designed to investigate the genetic background factors impacting the phenotype of the *lesion mimic23* (*les23),* a recessive lesion mimic mutant characterized by formation of yellowish-brown cell death patches on leaves. Disease lesion mimic mutants like *les23,* which belong to more than 50 different loci in maize, show spontaneous cell death resembling what is caused by pathogen attack, but do so in the absence of the pathogen (Johal, 2007). Some maize *les* mutants have phenotypes so extreme that they are lethal in one genetic background, yet have a nearly undetectable, benign phenotype in another background. This easily discerned, visible phenotype manifests in a developmentally specific, cell-autonomous manner, and the sensitivity of *les* mutations to background effects can be exploited to determine genetically the nature of these background factors.

To accomplish this for *les23,* an F2 population was generated between this mutant (in the Va35 background) and the inbred Mo20W, which is known to suppress a number of *les* mutations (Johal, 2007). Approximately 3,000 F_2 plants were grown in the field, and ~900 were *les23/les23* homozygotes. We phenotyped these plants and grouped them into ten discrete classes ranging from very severe to highly suppressed. In addition, we noted the timing of lesion onset for all 900 *les23* segregants. Genotypes of these *les23* segregants were determined using 103 SSR markers and QTL mapping established associations between the genotype and phenotype. We identified a major suppressor of cell death (*Slm1*) that accounted for ~70% and ~90% of the phenotypic variation in overall symptom severity and the timing of lesion initiation, respectively (Penning et al., 2004). The use of *les23* as a reporter thus facilitated the identification of a novel and QTL, which our recent results suggest protects plants from a number of abiotic stresses, including excess light and heat. It is likely that without the assistance of the mutant phenotype of *les23, Slm1* would have stayed cryptic and probably never revealed.

While MAGIC was conceptualized using a recessive mutant, dominant or partially dominant mutations are even more well-suited because they can provide information right in the F_1 populations. F_2 populations (F_1 wild type X mutant sib mating) can also be generated to uncover recessive suppressors or enhancers, if any. MAGIC screens can also be used on cytoplasmic traits, identifying modifiers of these traits that are nuclear genes. In fact, restorers of fertility (Rf) in cytoplasmic male sterile maize and other plants were identified using essentially this approach (Duvick, 1956). Similarly, transgenic variants, made either by overexpression or RNAi knockdown of a gene, could also be used to reveal QTL in diverse germplasm for the trait of interest in a single generation.

The power of MAGIC resides in the fact that it provides a straightforward method to quickly survey hundreds and thousands of lines for useful genes and alleles. However, it also facilitates the recovery of transgressive segregants, thereby allowing the identification and buildup of potentially ueful gene combinations that could help increase plant performance. Thus, MAGIC can facilitate breeding efforts in two ways: first, by revealing where best alleles underlying a given trait could be found, and second, by facilitating their enrichment into a single genotype that can be easily recognized.

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