Evangelista D, Hotton S, Dumais J. 2011. The mechanics of explosive dispersal and self-burial in the seeds of the filaree, Erodium cicutarium (Geraniaceae). Journal of Experimental Biology 214, 521–529.

Guo Z, Chen D, Schnurbusch T. 2015. Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. Journal of Experimental Botany 66, 3099–3111.

Guo Z, Schnurbusch T. 2015. Variation of floret fertility in hexaploid wheat revealed by tiller removal. Journal of Experimental Botany 66, 5945–5958.

Janson CH. 1983. Adaptation of fruit morphology to dispersal agents in a neotropical forest. Science 219, 187–189.

Mach J. 2015. Domesticated versus wild rice? Bring it awn! The Plant Cell 27, 1818.

Maydup ML, Antonietta M, Guiamet JJ, Graciano C, Lopez JR, Tambussi EA. 2010. The contribution of ear photosynthesis to grain filling in bread wheat (Triticum aestivum L.). Field Crops Research 119, 49–58.

Motzo R, Giunta F. 2002. Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat. Australian Journal of Agricultural Research 53, 1285–1293.

Rebetzke GJ, Bonnett DG, Reynolds MP. 2016. Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. Journal of Experimental Botany 67, 2573–2586.

Rebetzke GJ, Richards RA. 2000. Gibberellin acid-sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Australian Journal of Agricultural Research 51, 235–245.

Slafer GA, Elia M, Savin R, García GA, Terrile II, Ferrante A, Miralles DJ, González FG. 2015. Fruiting efficiency: an alternative trait to further rise wheat yield. Food and Energy Security 4, 92–109.

Wehrich RA, Carver BF, Martin BC. 1995. Photosynthesis and water-use efficiency of awned and awnletted near-isogenic lines of hard red winter-wheat. Crop Science 35, 172–176.

Xie Q, Mayes S, Sparkes DL. 2015. Carpel size, grain filling, and morphology determine individual grain weight in wheat. Journal of Experimental Botany 66, 6715–6730.

Insight

Harnessing epigenome modifications for better crops

James Giovannoni

US Department of Agriculture Robert W. Holley Center and Boyce Thompson Institute, Tower Road, Cornell University campus, Ithaca, NY 14853, USA

james.giovannoni@ars.usda.gov; or jjg33@cornell.edu

Chemical DNA modifications such as methylation influence translation of the DNA code to specific genetic outcomes. While such modifications can be heritable, others are transient, and their overall contribution to plant genetic diversity remains intriguing but uncertain. In this issue, Gouil and colleagues (pages 2655–2664) characterize the epigenome phenomenon termed ‘paramutation’ underlying a tomato photosynthesis-related gene defect, and in doing so expand our understanding of how epigenome modifications are conferred with exciting implications for crop improvement.

It has long been recognized that DNA harbors information of richer texture and fidelity than the DNA sequence alone. Just as recorded texts contain information while volume, emphasis and accent contribute to the ultimate meaning conveyed by language, DNA sequence presents the genetic code while epigenome modifications influence specific conversion of this code into phenotypic traits. Epigenome contributions to genetic diversity and outcomes are gaining in appreciation as genomes and their modifications become ever more readily characterized and cataloged.

As examples, whole genome sequencing and genomic DNA–protein interaction studies have revealed that many genomes, including those of important crops, include large repertoires of DNA- and RNA-derived mobile genetic elements rendered silent via chemical modifications, including cytosine methylation and methylation or acetylation of DNA-associated histone proteins (Cui and Cao, 2014). Also heritable epigenome reprogramming is critical in plant embryo development (Schmitz and Ecker, 2012), while developmental epigenome changes contribute to fruit maturation and ripening (Zhong et al., 2013), with additional modifications acquired in response to stress conditions. Recent evidence suggests the existence of molecular mechanisms in place to erase these induced modifications prior to meiotic transfer (Iwasaki and Paszkowski, 2014). A number of epialleles (stable genetic variants in DNA methylation patterns, but not DNA sequence) have been reported (Weigel and Colot, 2012), confirming that some heritable genetic diversity is anchored in the epigenome.

The realization that a number of genetic diseases are traceable to the epigenome, including some cancers (Timp and Feinberg, 2013), has accelerated interest and inquiry into mechanisms of epigenome modification and resulting manifestations that can contribute to illness. Similarly, research in plants is spurred on by the knowledge that a clearer mechanistic picture of epigenome mechanisms will facilitate our understanding of their relative contributions to crop genetic diversity and open doors to their use for crop improvement.

Paramutation facilitates genetic diversity via epigenome modification

Paramutation has been studied extensively in maize, though examples have been reported in additional species, including...
tomato, at the sulfurea (*sulf*) locus. The phenomenon was first described as an apparent exception to Mendel’s First Law of allelic segregation (Brink, 1956) where an inactive allele of the maize gene *r1* was observed to convert, or paramutate, an active (paramutable) *R1* allele to the inactive form. The resulting paramutated allele was both heritable and capable of conferring the same effect on other active alleles when transferred via sexual hybridization. While the underlying DNA has been shown to encode tandemly repeated copies of an HLH transcription factor regulating anthocyanin biosynthesis (Dooner et al., 1991), better characterized examples of maize paramutation at the *b1* and *pl1* loci (Hollick, 2012) indicate that small interfering RNAs (siRNAs) are associated with DNA methylation changes responsible for these paramutagenic phenomena (Box 1).

**Tomato as a model for epigenome analysis**

While maize is among the most important staple crops and a model for plant genetics, the cultivated tomato (*Solanum lycopersicum*) offers a number of advantages for experimentation. These include a smaller and less repetitive sequenced genome; the ability to create stable inbred and true-breeding lines; efficient means of stable and transient DNA transformation; and a large collection of characterized genetic mutations, variants and inter-fertile wild species facilitating genetic analyses (The Tomato Genome Consortium, 2012 and references therein).

Gouil et al. (2016) took advantage of these features in characterizing the paramutagenic tomato *sulf* locus, which manifests as sectors of chlorosis (i.e. yellowing, thus the *sulfurea* designation) in *sulf/+* leaves where paramutation has rendered the corresponding somatic genotype *sulf/sulf*. Genetic segregation in a population derived from a cross with the wild progenitor (*Solanum pimpinellifolium*) of the cultivated tomato confirmed prior localization of *sulf* to a region of chromosome 2. The tomato genome sequence allowed identification of a collection of linked gene candidates and further narrowed this group to a subset with differential gene expression via whole genome transcriptome analysis of normal and mutant genotypes.

The most downregulated gene was *SLTAB2*, whose Arabidopsis ortholog was previously linked to translation of mRNAs encoding photosystem proteins (Barneche et al., 2006). In the absence of proper photosystem assembly, chloroplast membrane structure and photosynthetic capacity is dramatically compromised leading to the yellowing phenotype characteristic of both *sulf* and a corresponding Arabidopsis T-DNA mutant. Promoter methylation profiles revealed regions of differential methylation in *sulf* versus wild-type *SLTAB2*. Especially noteworthy was a hypermethylated

---

**Box 1. Endogenous paramutation and targeted gene silencing**

Endogenous paramutation and targeted gene silencing – e.g. virus-induced gene silencing (VIGS) – confer siRNA-mediated DNA methylation changes on susceptible loci. In this example the hypomethylated and transcriptionally active (*) *P* allele is converted to the hypomethylated and inactive *p* allele by either mechanism. A range of genetic outcomes could be achieved through differential methylation highlighting the potential for targeted epigenome and trait engineering.
region that encompassed the transcription start site, likely contributing to downregulation of SLTAB2 in sulf genotypes and termed DMR1 (Differentially Methylated Region 1). This region was also associated with elevated levels of 23–24-nucleotide siRNAs associated with RNA-mediated DNA methylation. DNA sequence variation between the cultivated and wild species alleles of the sulf locus further allowed monitoring of paramutation in the segregating F1 population where the wild species allele also became hyper-methylated at DMR1 in the presence of the sulf allele.

**Epigenome modification as a tool for crop improvement**

Paramutation and its consequences were not only directly monitored in the segregating population via promoter methylation, but were elegantly reconstituted through transient expression of DMR1 sequences in wild-type tomato plants. Expression of non-transcribed sequences has been linked to RNA-mediated DNA methylation of the corresponding genomic sequences (Box 1) with consequences for expression of linked genes (Bond and Baulcombe, 2015). Indeed expression of DMR1 sequences in normal tomato plants resulted in elevated levels of both DMR1 promoter methylation and corresponding 23–24-nucleotide siRNAs, in addition to leaf chlorosis analogous to the sulf mutation.

These results not only provide confirmation of the DNA sequence target and nature of epigenome changes conferring the sulf phenotype, but also demonstrate the potential for creating novel genetic variants through targeted epigenome modification. Such modifications would be expected to yield a range of phenotypes corresponding to the degree of methylation modification and with applications to specific and desired phenotypic outcomes. As an example, one might imagine a collection of alleles that confer increased fruit ripening delays tailored to localized post-harvest storage needs. Alternatively, a range of expression levels from a locus conferring salt tolerance, but at a cost to yield, could be tailored to localized soil conditions so as to facilitate acceptable crop performance while minimizing adverse side effects.

**Contribution of the epigenome to natural genetic diversity**

The dramatic effect of epigenome state on phenotype highlighted by the tomato sulf allele begs the question of the role of epigenome variation in available germplasm that is deployable for crop improvement. Many domesticated species, including tomato, are derived from a relatively small slice of the pie of available genetic diversity, yet retain considerable phenotypic variability. In inbred species, where tomato is again an example, DNA sequence diversity may be limited and epigenome diversity may play a larger role in trait variability. Indeed tomato breeders have long noted that while phenotypic diversity abounds in germplasm collections, DNA sequence-based molecular markers facilitating selection of desired traits are usually difficult to identify. Further investigations into epigenomes of model crop systems such as tomato will reveal the contributions of the epigenome to genetic diversity, and should highlight methods to select for and possibly manipulate phenotypic range and eventually culminate in the development of targeted methods for tailoring epigenomes toward enhancement of desired crop traits. The detailed characterization of the sulf gene by Gouil et al. (2016) suggests these aims are achievable.

Key words: Auxin, DNA methylation, epigenome, genetic diversity, paramutation, sulfurea, tomato.

Journal of Experimental Botany, Vol. 67 No. 9 pp. 2535–2537, 2016 doi:10.1093/jxb/erw143

**References**

Barneche F, Winter V, Crèvecoeur M, Rochaix J-D. 2006. ATAB2 is a novel factor in the signalling pathway of light-controlled synthesis of photosystem proteins. The EMBO Journal 25, 5907–5918.

Bond D, Baulcombe DC. 2015. Epigenetic transitions leading to heritable, RNA mediated de novo silencing in Arabidopsis thaliana. Proceedings of the National Academy of Sciences, USA 112, 917–922.

Brink R. 1956. A genetic change associated with the R locus in maize which is directed and potentially reversible. Genetics 41, 872–889.

Cui X, Cao X. 2014. Epigenetic regulation and functional expatiation of transposable elements in higher plants. Current Opinion in Plant Biology 21, 83–88.

Dooner H, Robbins T, Jorgensen R. 1991. Genetic and developmental control of anthocyanin biosynthesis. Annual Review of Genetics 25, 173–199.

Gouil Q, Novák O, Baulcombe D. 2016. SLTAB2 is the paramutated SULFUREA locus in tomato. Journal of Experimental Botany 67, 2655–2664.

Hollick JB. 2012. Paramutation: a trans-homolog interaction affecting heritable gene regulation. Current Opinion in Plant Biology 15, 536–543.

Iwasaki M, Paszkowski J. 2014. Identification of genes preventing transgenerational transmission of stress-induced epigenetic states. Proceedings of the National Academy of Sciences, USA 111, 8547–8552.

Schmitz R, Ecker J. 2012. Epigenetic and epigenomic variation in Arabidopsis thaliana. Trends in Plant Science 17, 149–154.

The Tomato Genome Consortium. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485, 635–641.

Timp W, Feinberg A. 2013. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nature Reviews Cancer 13, 497–510.

Weigel D, Colot V. 2012. Epialleles in plant evolution. Genome Biology 13, 249.

Zhong S, Fei Z, Chen Y, et al. 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nature Biotechnology 31, 154–159.