A late spring in Europe and the adaptability of plants

Epigenetic regulation seems to play a major role in plants' ability to adapt to and remember unusual climate patterns

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After an unusually long winter in Europe and a very cold and wet spring that lasted until late May, many perennial plants have come late into leaf and flower. As such, 2013 will probably see a late harvest and possibly reduced yields, despite the hot weather that came later in July.

This response by plants to a long cold period is a normal adaptation to adverse weather conditions, but this unusual period of weather has given biologists a chance to understand how plants determine the optimal time for essential processes such as flowering or fruit ripening in response to current and even past climate conditions. Such knowledge could be applied to agriculture both to deal with adverse weather and to breed plant varieties better adapted to harsher climates, especially in colder regions.

Plants use various environmental cues to determine their flowering time in the spring. Some species respond directly to temperature changes whereas others use day length, which results in less variation in the timing of their leaf and flower development between successive springs. Some observers, however, have noticed that some plants seem to respond not just to the immediate season but also to the previous one: during a cold spring they will still come into leaf and bud at the normal time if the previous spring was warmer than usual. This suggests that plants are not just subject to the whims of the weather, but that they are capable of adapting to long-term environmental conditions by taking two or more years of weather patterns into account. Such observations are informing the search for the molecular mechanisms and genes involved in these adaptations, with the hope of developing

plant varieties that have a high tolerance for a range of stress factors, including drought, parasites and cold. There is also the potential to alter the time at which plants come into flower and fruits ripen, which would have great commercial advantage for farmers.

One study that provides particularly good insight into the varying response of plants to the recent cold European spring compares the transcriptomes of the ripening berries of a single grapevine clone in three successive growing seasons [1]. The study, conducted at the University of Verona in Italy between 2006 and 2008, investigated phenotypic plasticity in the grapevine species *Vitis vinifera* cultivar *Corvina* at 11 vineyards over a period of two years. It analysed how the local environmental conditions and vinicultural practices of the vineyards modified the transcriptome of the grapevines and found that the most coordinated responses were attributable to weather patterns during the early spring. It so happened that, of the three years, 2007 was an unusually warm spring in the area, while both 2006 and 2008 were normal and quite similar. Vines, similarly to many other plants, are highly sensitive to the temperatures they experience during the early growing season. As such, the authors suspected that the vines' transcriptomes might have been significantly reprogrammed by the unusually warm weather of early 2007, resulting in the expression of a range phenotypes.

When the analysis was complete, it turned out that 2007 was characterized by the specific upregulation of genes related to disease resistance, abiotic stress adaptation

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and oxidative burst, reflecting the higher spring temperatures. However, the authors also found a significant upregulation of genes involved in DNA/RNA metabolic processes and transcription in plants grown in 2008, but not in those grown in 2006, which was unexpected given that the weather was similar in those two seasons.

The Italian scientists concluded that the transcriptome state in the 2008 plants had been affected by the unusual weather of the previous year and began to speculate about the mechanisms involved. One possibility was that it was a form of epigenetic memory of the past year, presumably through DNA methylation. However, the study's lead author, Silvia dal Santo at the University of Verona's Department of Biotechnology, was careful to emphasize that epigenetic memory was not the only possible explanation. "The influence of the previous season or seasons on the 2008 transcriptome may be explained in different ways," she said. "Firstly, of course it can be epigenetic memory, but this would need specific analysis to be demonstrated as this issue was never studied in perennial plants and future work will address this attractive hypothesis. Secondly, it could be related to the specific accumulation, in terms of amount and tissue location, of storage compounds from the previous year that may have been influenced by the particular environmental

conditions. Thirdly, it could be determined by the inter-annual phenology relationships including bud dormancy induction during the late summer and fall, and the flowering induction and differentiation which take place one year before inflorescence appearance." In fact, all three hypotheses might be correct. After all, many plants do store compounds from the previous year, partly to get off to a good start in the new season. Similarly, many plants form their flower buds in the previous year and then rely on a process known as vernalization to prevent them from opening until the winter is over.

Vernalization is a good starting point to investigate epigenetic factors, as it is subject to epigenetic regulation. Vernalization suppresses critical genes until after a sufficiently long period of cold weather, often measured in 'chill hours', which presumably equates to the extent of a typical winter (see illustration). These processes might all be part of the overall epigenetic regulatory apparatus for regulating response to environmental cues. In that case, it might be splitting hairs to try to distinguish between alternative hypotheses

as contributors to a 'memory' of previous seasons, as the ability of plants to modify their transcriptome in response to their environmental history and current conditions probably relies on a range of factors.

This ability has obvious potential for plant breeders. For decades, breeders have sought to create varieties of cereal crops, notably wheat, that are better adapted to cold climates in order to produce higher yields in countries such as Canada and Russia, which have vast open spaces suitable for arable agriculture but long, cold winters. One study that made significant progress in understanding the transcriptional adaptation of wheat to cold was conducted at Bristol University in the UK [2]. The study was set up initially to identify genes involved in vernalization, as wheat varieties grown over the winter require a period of cold to initiate flowering, while spring-planted varieties do not. But it turned out that many of the same genes were also responsible for tolerance to cold and other environmental stresses, and that epigenetic mechanisms are involved in the reprogramming. "The flowers that require vernalization must by necessity be cold tolerant," explained Mike Winfield, lead author

of the Bristol study. "So there tends to be a relationship between those genes involved in cold tolerance and vernalization."

Furthermore, many of those genes are also involved in adapting to other principal stress factors such as drought, heat and pathogens that suck out nutrients and damage tissues. "There are certain modules of proteins that can recognize families of genes, and that family is modulated in response to a lot of environmental insults," Winfield said. He added that this makes sense from both a mechanistic and an evolutionary standpoint, as the impact of a wide variety of these insults on plants, and the ways in which the plants respond to them, are quite similar. "Even cold can be seen as like a kind of drought for the plant, because if water is frozen it is not available."

Winfield pointed out there are subtle variations between the responses of plants to different stress factors. Yet, even though some specific transcription factors modulate the expression of particular genes, there are several common genetic pathways that could be exploited by plant breeders. For instance,

breeders could focus on genes known to be associated with vernalization in order to improve cold tolerance. As Winfield pointed out, some studies have discovered close connections between the gene networks involved in vernalization and those involved in acclimation to frost in both wheat and barley [3]. It might therefore be possible to create new phenotypes by manipulating the epigenetic regulation of genes that confer tolerance to various stresses, rather than by manipulating those genes directly. In the short term at least, the role of epigenetics research might be more directed towards identifying the genes involved in these networks rather than modifying the transcriptome around existing genes.

Work on plant transcriptomes might also provide new ways to generate desirable traits without any genetic or epigenetic engineering. Winfield has studied the interplay of light and cold during October, when days are naturally getting shorter, and how this leads to the epigenetic reprogramming of the transcriptome in wheat [4]. He found that artificially sustaining high light intensity and duration during the expected drop in average temperature caused stress-related patterns of gene expression. He concluded that light and temperature work together in reprogramming the transcriptome, both for adapting to winter and for vernalization, and has developed the idea that inducing vernalization in crops such as wheat might be possible by the use of light alone. Such techniques could have a commercial application, as vernalization and the related process of cold hardening to resist frost are required for hardy winter wheat varieties [5]. "If you could mimic exposure to cold by altering the light quality, you might find it economically advantageous," Winfield said.

While cold tolerance and vernalization are important traits to control in crop plants grown mainly at higher latitudes, the control of ripening is vital for many crops grown all over the world. Owing to the need to transport produce over large distances, or in order to meet market demands, it is often necessary to induce ripening at a specific time. So far, this has been done by administering the natural hormone ethylene, which is most commonly used to accelerate the ripening of tomatoes. However, ethylene's effect is quite limited: it does not work on young plants and it is most effective close to the time the ripening process would begin anyway.

Conventional genetic research had been unable to discover why ethylene only works within a limited and narrow time window, until a joint US/Chinese study began to look for epigenetic triggers for the whole ripening process, including recruitment of ethylene. To assess whether the cue for ripening and the effect of ethylene involved epigenetic reprogramming, the researchers injected unripe tomatoes with the methyltransferase inhibitor 5-azacytidine, which inhibits the enzymes that methylate DNA. The injected tomatoes ripened prematurely, strongly indicating that DNA methylation regulates ripening [6]. Altogether, 52,095 differentially methylated regions, representing 1% of the genome, were covered by the analysis. The study also found that the binding sites for Ripening Initiation Factor, already known to be one of the main transcription factors involved in ripening, were frequently localized in the promoters of numerous ripening genes, with binding taking place in concert with demethylation. This suggests that the control of ripening is distributed across the whole fruit, which could help explain why Dal Santo's group, working on vines and other plants, found that ripening time can even vary within individual berries on the same plant.

Generally, it seems that plants make use of epigenetic changes to control crucial processes such as fruit ripening or vernalization because these changes are reversible. According to Jerzy Paszkowski, a plant epigeneticist from the University of Geneva in Switzerland, there is a stochastic probabilistic component to epigenetic changes, which means that even though some are highly stable and persist through generations of cell division, and occasionally even survive into successive generations, there is at each stage a probability that they will be reversed. This distinguishes epigenetics from genetics: unlike alleles, which persist indefinitely, subject to mutation and natural selection, epi-alleles are always reversible. According to Paszkowski, there are few examples of epi-alleles that have been demonstrated to be heritable, but those that have been found tend to control fundamental functions, such as sex determination and flowering time.

One of the best-known examples concerns the *Arabidopsis* flowering wageningen (FWA) gene, which determines flowering time and is epigenetically regulated to take account of environmental conditions [7]. FWA is only expressed from maternal genes

and is likely to be regulated by genomic imprinting, according to Paszkowski. FWA suppresses flowering, which is then initiated by the silencing of the FWA promoter. By silencing the maternal FWA gene, plants can flower earlier without the need for environmental conditioning.

It seems that epigenetic control of the transcriptome enables plants to better fine-tune many processes that are dependent on environmental conditions. As a consequence, a better understanding of plant epigenetics and transcriptome reprogramming will help plant scientists to identify new ways of engineering desirable traits of commercial value or nutritional importance, especially as the critical processes of stress adaptation, flowering and fruit ripening seem to be controlled by related genetic networks that are under the control of epigenetic factors.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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