Reprogramming rice leaves: another layer of senescence regulation

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This article comments on:
Zhang Y, Li Y, Zhang Y, Zhang Z, Zhang D, Wang X, Lai B, Huang D, Gu L, Xie Y, Miao Y. 2022. Genome-wide H3K9 acetylation level increases with age-dependent senescence of flag leaf in rice (Oryza sativa). Journal of Experimental Botany 73, 4696–4715.

How does a leaf switch its gene expression from active photosynthesis to senescence, ending in death of the organ? Making sure this process is tightly regulated is especially important in a major crop such as rice where the remobilization occurring during senescence is critical to the development of the seed and ultimately crop yield. Zhang et al. (2022) now show that epigenetic reprogramming through genome-wide histone H3 acetylation (H3K9ac) has an important role to play in rice leaf senescence.

The most obvious visible sign of leaf senescence is yellowing, caused by the ordered disassembly of the photosynthetic machinery and the breakdown of chlorophyll (Kuai et al., 2018). Leaf senescence is therefore sometimes neglected as an end-of-life process in which metabolism shuts down, and all cells ultimately die. However, we know from a wealth of studies in model plants such as Arabidopsis thaliana (Breeze et al., 2011; Guo et al., 2021) that it is in fact a carefully regulated active developmental process in which transcript levels of suites of genes increase as well as decrease. The key function of senescence is in the remobilization of nutrients to other parts of the plant, crucially in monocarpic crops such as rice, to the developing seed (Lee and Masclaux-Daubresse, 2021). Although leaf senescence is a developmental process, it is also affected by environmental factors such as drought, cold, and other abiotic factors as well as pathogen attack. These signals are mediated by phytohormones: of particular importance are positive regulators: abscisic acid (ABA), ethylene, jasmonic acid (JA), salicylic acid (SA), and brassinosteroids, while cytokinin and gibberellin act as negative regulators of senescence, and the role of auxin is less clear (Ahmad and Guo, 2019). It is assumed that environmental signals are perceived at the cell surface and transduced through receptor-like kinases (RLKs) and mitogen-activated protein kinases (MAPks), and an increasing number of RLKs and MAPks are being associated with leaf senescence signalling (Ahmad and Guo, 2019). These in turn modulate the expression of transcription factors important for orchestrating the transcriptional shift during senescence (Fig. 1). Two important families of transcription factors have been associated with regulating transcription during senescence: the NAC and WRKY families, although MYB family transcription factors, also associated with stress responses, and transcription factors from other families, such as bHLH, C2H2, AP2/ERF, bZIP, and HB, are also involved in rice (Lee and Masclaux-Daubresse, 2021) and Arabidopsis (Balazadeh et al., 2008). Importantly, these transcription factors act as networks with hubs responding to overlapping sets of signals (Hickman et al., 2013).

An increasing number of developmental shifts and environmental responses in plants are being revealed to be under epigenetic control, and a key process is histone modification (Zhao et al., 2019). Modifications can repress transcription such as the dimethylation of histone H3 at lysine 9 (H3K9me2) or can activate it such as acetylation of histone H3 at lysine 9 (H3K9ac) (Zhou et al., 2010; Fig. 1). By applying ChIP-seq to map where modified histones bind across the genome, and comparing the pattern with changes in the transcriptome, it is now possible to obtain a dynamic profile of the effects of histone modification on gene expression.

Several histone marks have recently been associated with leaf senescence. These include the repressive mark H3K27me3...
(Wang et al., 2019), as well as H3K9ac and H3K4me3, both linked to active gene expression (Brusslan et al., 2015). In Arabidopsis, H3K9ac was shown to be associated with genes whose expression rises during leaf senescence, indicating it may play a role in senescence regulation (Brusslan et al., 2015). In this earlier study, a number of genes were found associated with the H3K9ac mark, but surprisingly not the widely accepted senescence marker SAG12 which was instead associated with the H3K4me3 mark. H3K9ac has also been implicated in several other plant responses to their environment including de-etiolation (Charron et al., 2009), and response to drought (Kim et al., 2012), as well as in developmental reprogramming such as that occurring during flowering (Adrian et al., 2010).

Is binding to H3K9ac important for senescence progression?

Zhang et al. (2022) have revisited the role of H3K9ac in an important crop—rice. Six time points were sampled from green mature leaves through to the beginning of visible senescence at the leaf tip to leaves that were obviously yellowing across the whole blade. The distribution of H3K9ac binding across each gene showed a similar pattern to that in Arabidopsis (Brusslan et al., 2015), with most binding in intragenic and promoter regions. This conservation across taxonomically divergent species might suggest an early evolution of this epigenetic regulation and indeed H3K9ac is associated with actively transcribed genes across animals as well (Zhou et al., 2010). Association

![Diagram of Senescence Regulation](image-url)
with H3K9ac rose during senescence, indicating an increasing importance for this regulator as senescence progressed. The types of genes associated with a loss of H3K9ac binding are consistent with our understanding of senescence in which photosynthesis and biosynthesis shut down (Lee and Masclaux-Daubresse, 2021). Likewise, pathways most associated with an increase in H3K9ac binding included defence and metabolic processes, again associated with up-regulation during leaf senescence (Breeze et al., 2011; Guo et al., 2021). These findings confirm that, indeed, H3K9ac binding is consistent with a regulation of known senescence processes.

**How H3K9ac may affect transcription**

Histone acetylation on lysine residues increases transcription by relaxing the chromatin structure. In Arabidopsis, this can occur in response to growth regulators, mediated via transcription factors. For example, under high auxin conditions, Arabidopsis bZIP11-related transcription factors bind to the promoters of auxin-responsive genes and, via an adaptor protein, they recruit histone acetyltransferase complexes, resulting in upstream histone acetylation (Weiste and Dröge-Laser, 2014). Growth regulator signalling pathways were targeted by H3K9ac during rice leaf senescence (Zhang et al., 2022), including the major stress- and senescence-related regulators: ABA, ethylene, and JA, as well as auxin and SA. In addition, transcription factors from >30 different families were also associated with changes in expression during leaf senescence and the H3K9ac mark. It will be interesting to see if further work can elucidate how the acetylation of histone H3 is modulated and whether similar or different mechanisms from that seen above for auxin regulation in Arabidopsis apply.

Zhang et al. (2022) also provide insights into a quantitative effect of the H3K9ac binding: in most highly expressed genes, binding was spread across a wider area; indeed, binding extended into the gene body as was also previously found in Arabidopsis (Brusslan et al., 2015). Again, a clear mechanism for this is missing but may be important in understanding the fine-tuning of the effects on transcription.

**Dynamic layers of regulation**

The sequence of different control points during senescence is critical to the ordered disassembly of chloroplasts and efficient remobilization of nutrients. In Arabidopsis microarray analysis identified the precise sequence of gene expression changes, showing that up-regulation of autophagy and reactive oxygen species were early events followed by an increase in the expression of genes related to ABA and JA signalling, all of which preceded the down-regulation of genes related to photosynthesis (Breeze et al., 2011). In rice, down-regulation of photosynthesis also coincides with the first signs of leaf yellowing (Zhang et al., 2022), and activation of OsPME1 involved in JA biosynthesis (Fang et al., 2016) also starts early. This suggests conservation of at least some of the chronology of events between rice and Arabidopsis leaf senescence.

MiRNAs represent another layer of gene expression regulation that controls both developmental and environmental responses, and miRNA control of leaf senescence in Arabidopsis is now well established (Qin et al., 2016). Many of the miRNA families involved in Arabidopsis leaf senescence are conserved in rice. Zhang et al. (2022) provide new evidence that miRNA synthesis may be under control of H3K9 acetylation. AGO1 is required for miRNA biosynthesis (Vaucheret, 2008), and both OsAGO1d expression and its association with H3K9ac increased during leaf senescence. An interaction between H3K9ac marks and miRNA regulation has been found in response to pathogens in tomato (Crespo-Salvador et al., 2020), where expression of a WRKY transcription factor is associated with H3K9ac marks but is also miRNA regulated, and the miRNA structural gene is itself linked to H3K9ac marks in response to the pathogen. This interaction between epigenetic layers is worthy of further exploration to better understand the role of other histone marks, interactions with DNA methylation, and the possibility of coordinated control in the important regulatory pathways in leaf senescence.

**Conclusions and perspectives**

Understanding how the different layers of epigenetic control mesh with each other and with growth regulator control and other signalling networks in regulating the initiation and progression of leaf senescence will open up new avenues for its potential manipulation to optimize crop productivity. Importantly, we also need to understand the operation of these networks during stress-induced senescence to mitigate the effects of a changing climate and pathogen landscape. Current approaches to manipulating leaf senescence have largely focused on growth regulators and transcription factors (Guo et al., 2021), and encouragingly have been most successful in improving grain yield under stress (e.g. Peleg et al., 2011). However, new tools are needed to improve food security and, by applying an improved understanding of epigenetic control, it may be possible to fine-tune senescence towards optimal grain production for the future.

**Keywords:** ChiP seq, histone modifications, H3K9ac, leaf senescence, rice.

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