

## Research Insight

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# Metabolic Network Reprogramming During Leaf Senescence in *Arabidopsis*

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**Abstract** This study collates the changes in primary metabolism of *Arabidopsis thaliana* during leaf senescence, from being dominated by “accumulation” to being dominated by “decomposition and reuse”, and introduces the degradation of starch, the decomposition of proteins and membrane lipids, as well as the significant roles of amino acid and polyamine metabolism in nutrient mobilization and energy supply. The functions of the phenylpropane pathway, flavonoids and other secondary metabolites in antioxidation and stress response were summarized. How transcription factors such as NAC, WRKY and bZIP, as well as hormone signals such as ethylene, ABA, JA and SA, together form a multi-level regulatory network was discussed. Moreover, through multi-omics and systems biology methods, the metabolic network and gene regulatory network related to aging were integrated and reconstructed. This study aims to construct a more systematic framework for regulating leaf senescence metabolism, providing a reference for a deeper understanding of senescence mechanisms and genetic improvement of crops.

**Keywords** *Arabidopsis thaliana*; Leaf senescence; Metabolic network reprogramming; Transcriptional regulatory networks; Hormonal and stress signaling

## 1 Introduction

Leaf senescence is the final developmental stage in a plant's life. The main feature of this stage is that the leaves shift from “producing nutrients” to “redistributing nutrients to other tissues”. This process is very important for the growth, adaptation and survival of plants (Guo et al., 2021). Leaf senescence is not only regulated by genes, but also affected by internal factors such as hormones and age, and is disturbed by external conditions such as light and stress (Tian et al., 2020). In model plants such as *Arabidopsis thaliana*, genetic and molecular biological studies have identified many genes and signaling pathways related to aging. These results indicate that leaf senescence is a process jointly controlled by multiple regulatory mechanisms, involving multiple aspects such as transcriptional regulation, epigenetic regulation, post-transcriptional regulation and protein modification (Liu et al., 2019). During this process, the most obvious phenomena are the reduction of chlorophyll and the yellowing of leaves. Meanwhile, macromolecules such as proteins, lipids and nucleic acids will also be gradually decomposed, and their metabolic products will be recycled and transported to the newly grown tissues or seeds (Kim et al., 2018).

During the senescence process of leaves, the metabolic network of cells undergoes significant changes, shifting from “nutrient accumulation” to “nutrient decomposition and reuse” (Kamranfar et al., 2018). During this period, dynamic changes will occur in the transcriptome, proteome and metabolome. Transcription factors such as NAC and WRKY play a key role in the regulatory network. They coordinate multiple pathways, such as degradation pathways, energy metabolism pathways and signal transduction pathways (Meng et al., 2019). For instance, these transcription factors can regulate a large number of aging-related genes (SAGs), promoting the decomposition of proteins, lipids, starches, etc., thereby enabling plants to redistribute elements such as carbon and nitrogen (Kim et al., 2018). At the same time, the structure and function of organelles such as chloroplasts and mitochondria will also be adjusted during aging to ensure energy supply and metabolite transport. At the entire plant level, these metabolic changes not only affect aging itself, but are also closely related to stress resistance, yield and quality (Guo et al., 2021).

This study analyzed the changes in metabolic networks during the senescence process of *Arabidopsis thaliana*

leaves, including transcriptional regulation, metabolic pathway alterations, and their integration with external signals. By integrating multi-omics data and network analysis, multiple key regulatory factors and their regulatory modules were identified, and it was explained how the dynamic reconstruction of metabolic networks affects the senescence process and nutrient redistribution. This study aims to provide theoretical references for improving the yield, quality and stress resistance of *Arabidopsis thaliana* and other crops.

## 2 Physiological and Molecular Features of Leaf Senescence

### 2.1 Visible traits and biochemical signatures: chlorophyll loss, nutrient remobilization, ROS accumulation

The most obvious feature of leaf senescence is the yellowing of leaves, mainly due to the continuous decomposition of chlorophyll (Zhou et al., 2022). Chlorophyll degradation is essentially a process in which photosynthetic pigments and photosystem complexes are disassembled. This process requires the participation of multiple enzymes, such as NYE1/SGR1, PAO, etc. Meanwhile, in the aging leaves, large molecules such as proteins, nucleic acids and lipids will also be decomposed. The nutrients such as nitrogen and phosphorus released by them will be retransported to new tissues or seeds with the help of transport proteins (Guo et al., 2021). During aging, reactive oxygen species (ROS) also increase significantly. ROS, on the one hand, promotes aging as a signal; on the other hand, it can cause cell damage and even trigger programmed cell death (Doan et al., 2022). The accumulation of ROS is often associated with weakening of the antioxidant system (Zheng and Yu, 2020).

### 2.2 Senescence-associated genes (SAGs) and hallmark molecular events

Leaf senescence is a process strictly controlled by programs. Hundreds of aging-related genes (SAGs) are involved in regulation, and their expression levels increase significantly during aging (Guo et al., 2021; Zhou et al., 2022). The proteins encoded by these genes are involved in multiple functions, such as macromolecular degradation, ROS clearance and signal transduction (Doan et al., 2022). Common aging marker genes include *SAG12*, *SAG13* and *SAG29* (Peng et al., 2024). The expression of these genes is influenced by multiple levels of regulation, such as transcription, post-transcriptional regulation, translation control, and epigenetic regulation, among which epigenetic mechanisms include H3K27me3 demethylation, etc. (Guo et al., 2021). Transcription factors such as NAC and WRKY play a key role in regulating SAGs (Doan et al., 2022; Zhou et al., 2022). Furthermore, m<sup>6</sup>A RNA methylation also affects the stability of some aging-related transcripts (Sheikh et al., 2023).

### 2.3 Hormonal and environmental signals governing senescence initiation

Leaf senescence is jointly regulated by multiple hormones and the external environment. Ethylene, abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) promote aging, while cytokinin, gibberellin, and auxin generally delay aging (Tian et al., 2020; Zheng and Yu, 2020). In the ethylene pathway, EIN2 and ORE1 are important nodes for controlling aging (Peng et al., 2024). External factors such as light intensity, nitrogen supply, darkness, drought and ozone stress can all affect the rate of aging by regulating hormone levels and signaling pathways (Buelbuel et al., 2023). For example, low red/far-red light ratio or prolonged darkness will accelerate aging, while strong red light can slow down aging (Tian et al., 2020). Furthermore, ROS also plays an integrating role in senescence, which can link environmental stress signals with the responses within plants (Doan et al., 2022).

## 3 Primary Metabolic Network Reprogramming

### 3.1 Carbon metabolism remodeling: starch degradation, sugar signaling, carbon flux changes

During the senescence of leaves, carbon metabolism shifts from being dominated by “synthesis” to being dominated by “decomposition”. The degradation of starch is significantly enhanced, providing energy and a carbon skeleton for cells. The NAC transcription factor RD26 can directly activate some genes related to starch degradation and sugar transport, such as amylase *AMY1* and sugar transporter *SWEET15*, thereby promoting starch degradation and increasing the content of monosaccharides and disaccharides. These sugars will be redistributed to the growing tissues (Kamranfar et al., 2018; Huang et al., 2020). The nuclear pore complex component Nup98 is also involved in regulating starch degradation. Its mutants will show starch accumulation and premature aging, suggesting that abnormal energy metabolism may be a major cause of the initiation of aging

(Xiao et al., 2020). In addition, sugar signaling pathways, such as SnRK1 kinase, also regulate the expression of carbon metabolism-related genes, thereby influencing the rate of aging. The synthesis, degradation and transport of sugar act together to help effectively transfer carbon resources in senescent leaves to “sink” tissues (Huang et al., 2020).

### **3.2 Nitrogen metabolism transitions: protein degradation, amino acid interconversion, nutrient mobilization**

During the senescence of leaves, proteins, especially those in chloroplasts, are decomposed in large quantities, and the amino acids produced are reused as nitrogen sources. Transcription factors such as RD26 can activate protein-degrading genes, such as *CV* and *LKR/SDH*, thereby enhancing the decomposition and transformation of proteins and amino acids. The changes in the content of different amino acids, such as the reduction of glutamic acid and aspartic acid, while the increase of GABA and leucine, indicate that nitrogen is being redistributed within plants. The mobilization efficiency of nitrogen is often related to the degree of aging. Premature aging materials generally exhibit a faster rate of nitrogen redistribution. Proteases and autophagy pathways jointly participate in protein degradation and nitrogen recovery, thereby ensuring that nitrogen can be smoothly transported to new organs or seeds (James et al., 2018; Kamranfar et al., 2018).

### **3.3 Lipid metabolism and membrane dismantling: phospholipid breakdown, fatty acid turnover**

When leaves age, the cell membranes and chloroplast membranes will gradually disintegrate. The enhanced activity of lipases such as phospholipase D (PLD) enables the decomposition of phospholipids (such as PC) into phosphatidic acid (PA), accelerating the breakdown of membrane lipids. The cycle transformation of fatty acids is fast, and some of them will enter the  $\beta$ -oxidation pathway to provide substrates for energy metabolism or the synthesis of signaling molecules (Kamranfar et al., 2018). Some lipid degradation products, such as PA, lysophospholipids and liposomes, will continuously accumulate, further damaging the membrane structure and amplifying aging signals. Lipid-binding proteins (such as ACBP3) can also regulate membrane lipid metabolism and the stability of autophagy-related proteins, thereby influencing membrane disintegration and the autophagy process of cells.

## **4 Secondary Metabolism and Defense-Related Reprogramming**

### **4.1 Activation of phenylpropanoid pathway and antioxidant network**

During the senescence of leaves, the phenylpropane pathway is significantly activated. It is a core pathway in secondary metabolism and can promote the synthesis of lignin, flavonoids and other antioxidants. The expression of key enzymes such as C4H will increase, causing the continuous accumulation of flavonoids, thereby enhancing antioxidant capacity, reducing ROS content and minimizing oxidative damage (Hou et al., 2023). Meanwhile, transcription factors such as NAC and WRKY also regulate antioxidant enzyme genes, such as *CAT*, *APX* and *SOD*, to help maintain the REDOX balance within cells and thereby delay aging (Doll et al., 2020; Kan et al., 2021). The synthesis of flavonoids and anthocyanins in some autophagy-deficient mutants is affected, which also indicates that the phenylpropane pathway and its downstream products play an important role in antioxidant defense.

### **4.2 Flavonoid, terpenoid, and alkaloid metabolism under senescence progression**

Flavonoids accumulate in both leaf senescence and stress response. The content is higher in young leaves, which can relieve the oxidative stress of photosystem II and reduce lipid peroxidation (Sperdouli et al., 2021). Transcription factors such as MYB4 finely regulate flavonoid metabolism by regulating genes related to phenylpropane and flavonoid synthesis. Relevant transcriptome and metabolome studies have shown that the synthesis of flavonoids such as anthocyanins and quercetin is closely related to the aging process. In addition, some terpenoids, alkaloids and other secondary metabolites also change during the aging process and participate in cellular signal regulation and defense responses (Guo et al., 2021).

### **4.3 Interaction between secondary metabolism and stress response pathways**

Leaf senescence often occurs simultaneously with multiple stresses such as drought, salt stress, and pathogen infection. At this point, secondary metabolites will play a “bridge” role in the defense response. Phenylpropane

and flavonoids can not only directly eliminate ROS, but also regulate hormone signals such as ABA, ethylene, JA and SA, thereby jointly regulating aging and stress responses (Asad et al., 2019; Wang et al., 2021). For example, in the early stage of aging, JA and ROS signals are simultaneously activated, promoting the expression of some defense-related genes (Figure 1) (Doll et al., 2020; Chen et al., 2024). In addition, sugar signaling also works in conjunction with hormones and secondary metabolic pathways to regulate the aging process and enhance resilience. Through these complex network regulations, plants can better cope with stress during the aging stage, while also completing nutrient redistribution and survival adaptation.

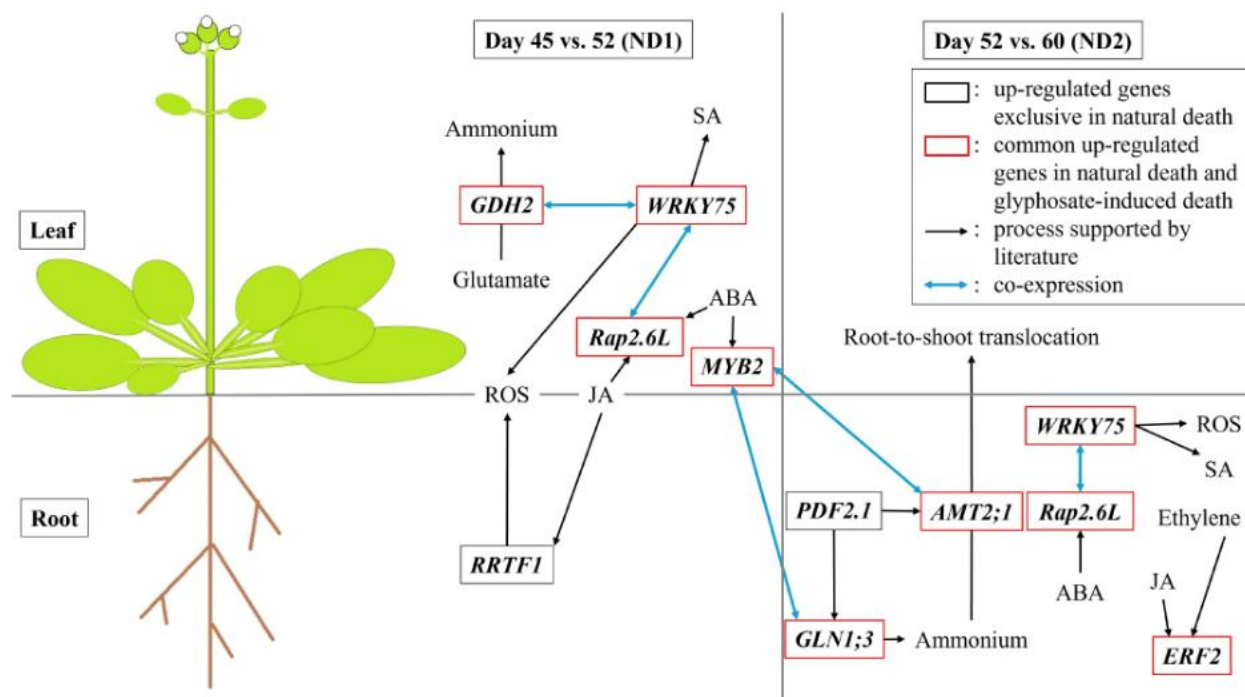


Figure 1 Regulation model of transcriptional gene-gene and gene-phytohormone interactions in leaves and roots during the whole-plant senescence in *Arabidopsis* (Adopted from Chen et al., 2024)

Image caption: The phytohormone JA triggers early signaling of senescence. In leaves, JA, along with another phytohormone ABA, directly or indirectly induces the transcription factors *Rap2.6L* and *WRKY75*, subsequently increasing *GDH2* expression to respond to local C/N imbalances caused by chloroplast degradation. On the other hand, *RRTF1* and *PDF2.1* transmit JA signals to initiate root senescence. In the later stages of senescence, *PDF2.1* co-expressed with *AMT2;1* and *GLN1;3*, which are involved in assimilation of ammonium and the translocation of ammonium from roots to shoots. These genes scavenge the ammonium released during root death and contribute to nutrient recycling in senescing roots (Adopted from Chen et al., 2024)

## 5 Regulatory Networks Controlling Metabolic Reprogramming

### 5.1 Transcriptional regulation: NAC, WRKY, bZIP, MYB families in metabolic network control

NAC and WRKY are the earliest and most significant families of age-regulating transcription factors to be studied. Genes such as *ORE1* (*ANAC092*), *NAP* (*ANAC029*), and *JUB1* (*ANAC042*) in the NAC family have been proven to be core factors regulating leaf senescence in *Arabidopsis thaliana*. They accelerate or slow down the aging process by regulating the expression of aging-related genes (SAGs) (Kan et al., 2021; Cao et al., 2023; Ma et al., 2025). There are also mutual regulatory relationships among these NAC members, which act in a relay manner in chronological order to help plants initiate and advance senescence (Kim et al., 2018). The role of the WRKY family is equally important. For example, *WRKY53*, *WRKY75* and *WRKY42* can promote aging, while *WRKY57*, *WRKY54* and *WRKY70* can inhibit aging (Tian et al., 2020). *WRKY71* can also directly activate ethylene signaling genes *EIN2*, *ORE1* and ethylene synthesis gene *ACS2*, thus accelerating leaf senescence. In addition, the bZIP and MYB families are also involved in the regulation. bZIP mainly regulates SAGs through ABA signaling, while MYB is involved in ABA metabolism and response. However, its specific function in *Arabidopsis* senescence still requires further study (Asad et al., 2019; Cao et al., 2023).



## 5.2 Hormonal signaling regulatory hubs (ethylene, JA, SA, ABA) and their interactions

Ethylene, JA, SA and ABA are the main hormonal signals regulating leaf senescence. Ethylene and SA can jointly promote aging. The synergistic effect of EIN3 and NPR1 can activate ORE1 and SAG29, thereby promoting aging (Wang et al., 2021). There is also a positive feedback between ABA and ethylene. NAP is activated by these two hormones, and NAP, in turn, can promote their continued synthesis, forming a signal loop that accelerates aging (Asad et al., 2019; Ueda et al., 2020; Ma et al., 2025). JA and SA show antagonism when regulating systemic ROS signaling: JA suppresses ROS waves, while SA enhances them, thereby affecting the systemic aging response (Myers et al., 2022). Furthermore, ABA can also regulate transcription factors such as bZIP, NAC, and WRKY, enabling plants to integrate environmental stress signals with their own hormone signals to regulate SAGs and metabolic changes (Asad et al., 2019).

## 5.3 Integration of signaling pathways, transcriptional cascades, and metabolic flux regulation

The metabolic reprogramming of leaf senescence requires the coordination of multiple layers including signaling pathways, transcriptional regulation and metabolic fluxes. Transcription factors such as NAC and WRKY are key nodes for signal integration. They can simultaneously respond to hormone, environmental and developmental signals, and regulate downstream SAGs and metabolism-related genes (Kim et al., 2018; Cao et al., 2023). For instance, *NAP* and *ORE1* not only respond to hormones but also directly regulate the synthesis genes of ethylene and ABA, gradually shifting the metabolic flow towards the aging pathway (Ma et al., 2025). The WRKY network interacts with signals such as ROS, SA, and JA to connect environmental stress with endogenous signals, thereby achieving dynamic regulation of metabolism (Doll et al., 2020; Tian et al., 2020). bZIP regulates energy metabolism and stress resistance through ABA signaling, while MYB affects ABA metabolism and regulates carbon-nitrogen metabolism (Asad et al., 2019; Cao et al., 2023). These multi-layer regulatory networks jointly ensure the orderly progress of metabolic reprogramming during the leaf senescence process.

## 6 Multi-Omics Insights into Network Reprogramming

### 6.1 Transcriptome dynamics during senescence and gene network reconfiguration

When leaves senescence occurs, large-scale changes occur in the transcriptome, and many senescing-related genes (SAGs) are up-regulated or down-regulated (Guo et al., 2021). Mononuclear and temporal transcriptome analyses indicated that the senescence processes of different cell types were synchronous, but also had their own characteristics (Guo et al., 2025). Among these regulatory networks, NAC and WRKY are the most core families of transcription factors. Some NAC members (such as ANAC017, ANAC082, ANAC090) form an “inhibitory combination” in the early senescence stage. They prevent leaves from entering senescence too early by regulating the signals of salicylic acid (SA) and reactive oxygen species (ROS) (Kim et al., 2018; Doan et al., 2022). In addition, the transcriptome and small RNAs (such as miRNA and circRNA) also interact with each other, forming a multi-level regulatory system.

### 6.2 Proteomics and metabolomics mapping of pathway activation and metabolic shifts

Proteomic and metabolomic data indicate that in senescent leaves, protein degradation, lipid metabolism, and pathways such as amino acids and organic acids are significantly enhanced. Proteins in chloroplasts (such as Rubisco) and pigments are gradually broken down, providing materials for the recovery of nitrogen and carbon. Some intermediate products of the TCA cycle also show significant changes in the pre-aging stage (Zhu et al., 2021). Lipid droplet proteins (such as AtSRP1 and CALEOSIN3) are involved in regulating lipid droplet formation and lipid redistribution, which is different from seed lipid droplets. Metabolomics analysis also revealed that some aromatic and branched-chain amino acids, as well as some stress-related secondary metabolites (such as glucosides and galactol), accumulate in senescent leaves. These changes all indicate that metabolism is shifting from “accumulation” to “mobilization” (Zhu et al., 2021).

### 6.3 Network modeling and systems biology approaches to reconstruct metabolic regulatory networks

By means of systems biology methods (such as dynamic Bayesian networks, co-expression networks and deep learning models), researchers integrated multi-omics data to reconstruct the regulatory networks during the aging process. These models reveal key regulatory factors, such as transcription factors like NAC, WRKY, bHLH, etc.,

which interact spatiotemporal with their respective target genes and miRNAs (Kim et al., 2018; Mishra et al., 2018). Through network perturbation and simulation, researchers also predicted some new regulatory factors, such as MAF5, and speculated on its possible role in aging (Guo et al., 2024). Furthermore, single-cell/monomic omics combined with weighted co-expression analysis identified hundreds of possible hub genes, which provided a new perspective for understanding carbon and nitrogen allocation and the source-reservoir relationship (Guo et al., 2021; Guo et al., 2025).

## 7 Case Study: Network Reprogramming in a Senescence-Delayed Arabidopsis Mutant

### 7.1 Background: selection of stay-green or delayed-senescence mutant lines

In *Arabidopsis thaliana*, leaf senescence is a strictly regulated process. It involves many aging-related genes (SAGs) and complex signaling networks. Researchers have identified many mutants that can “retain green” or delay aging through forward genetic screening, such as *ore1*, *nac016*, *pif5*, *pao4*, *sast1*, etc. Under conditions of natural aging or induced aging, the leaves of these mutants remain green for a longer time, photosynthesis declines more slowly, and the expression of SAGs also decreases (Figure 2) (Liebsch et al., 2022; Cheng et al., 2024). Therefore, they are often used as good materials for studying the regulation of aging and metabolic reprogramming.

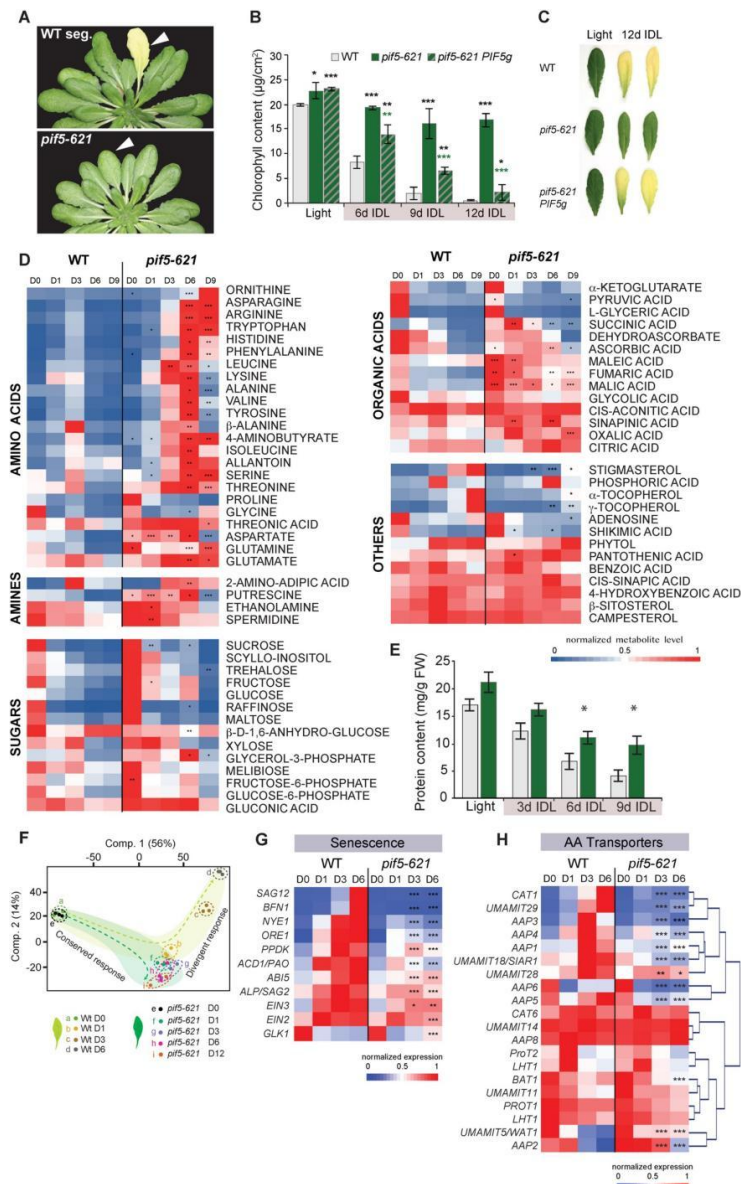


Figure 2 Phenotypic, metabolic, and transcriptomic characterization of *pif5-621* during dark-induced senescence (Adopted from Liebsch et al., 2022)

## 7.2 Multi-omics analysis: transcriptomics, proteomics, metabolomics, and regulatory network reconstruction

The addition of multi-omics technology has enabled researchers to have a deeper understanding of anti-aging mutants. Transcriptome analysis revealed that the expression of many SAGs decreased in these mutants, including key transcription factor families such as NAC and WRKY (Kim et al., 2018). Proteomics and metabolomics also showed that certain amino acids (such as arginine, ornithine), polyamines (such as spermidine, spermidine), sugars, and some antioxidant substances were significantly accumulated in the mutants. These substances can maintain energy balance and enhance antioxidant capacity (Cheng et al., 2024). Network analysis further indicates that the activity of some regulatory core nodes, such as ORE1, NAC016, ANAC046, WRKY42/71, etc., is weakened in these mutants. This will affect their downstream metabolic modules, including amino acid metabolism, glucose metabolism, ethylene signaling and ROS-related pathways, thereby delaying aging as a whole (Kim et al., 2018; Liebsch et al., 2022).

## 7.3 Key insights: altered regulatory hubs, metabolic modules, and mechanisms contributing to delayed senescence

Among these mutants, the expression of transcription factors such as NAC and WRKY is generally low. For example, NAC016 and ORE1 would have directly initiated the expression of a large number of SAGs, but their signals were weakened in mutants (Kim et al., 2018). Meanwhile, arginine, ornithine and polyamine metabolic pathways are more active, and there are more carbohydrates and antioxidant substances. These changes help cells maintain energy stability and avoid the aging signal of “energy deficiency” (Liebsch et al., 2022; Cheng et al., 2024). Polyamines (such as spermidine) can also affect ethylene signaling. They stabilize EBF1/2, thereby inhibiting aging. Sugar signals also affect the rate of aging through the TOR and SnRK1 pathways. Amino acid and polyamine metabolism can also interact with signals such as ROS and ethylene, forming a feedback loop that inhibits aging and helps regulate cell death and nutrient redistribution.

## 8 Implications for Crop Improvement and Stress Adaptation

### 8.1 Enhancing nutrient use efficiency through senescence network manipulation

Leaf senescence is a crucial stage for plants to shift from “producing nutrients themselves” to “redistributing nutrients”, and this process can affect the yield and quality of crops. Studies on model plants such as *Arabidopsis thaliana* have found that some important genes, such as NAC transcription factor, amino acid transporter, and purple acid phosphatase *AtPAP26*, all affect the recovery efficiency of nutrients such as nitrogen and phosphorus (Jiang et al., 2020; Guo et al., 2021; Chen et al., 2024). When aging slows down, leaves can maintain photosynthesis for a longer time, but the rate of nitrogen redistribution will decline. When aging accelerates, the protein content in seeds may increase, but the total yield may be affected. Therefore, how to make the aging process “just right”, ensuring both the continuous photosynthesis and the efficient transport of nutrients, is the key to increasing crop yields and fertilizer utilization efficiency.

### 8.2 Improving stress resilience by engineering metabolic network robustness

Leaf senescence is also strongly associated with various stresses, such as drought, flooding and nutrient deficiency. Studies on *Arabidopsis thaliana* have shown that by regulating amino acid metabolism, polyamine content, ABA signaling, and ROS balance, aging can be delayed and plants can thrive better in adverse conditions (Ostrowska-Mazurek et al., 2020; Liebsch et al., 2022). For instance, altering the synthesis of polyamines or the transport of amino acids can enhance the drought or flood resistance of plants. Regulatory factors such as NAC transcription factors and RD26 will readjust the metabolic network under adverse conditions to ensure energy supply and nutrient recovery. In addition, some epigenetic regulatory methods (such as DNA methylation and histone modification) can also affect the rate of aging, providing a new direction for improving the stress resistance of crops.

### 8.3 Translating Arabidopsis findings to crop species for yield and longevity improvement

The aging regulatory network of *Arabidopsis thaliana* is very similar to that of many crops, such as rice, wheat, corn and barley, etc. The NAC gene family, amino acid and polyamine metabolism regulation, ABA signaling, etc.,

also play similar roles in these crops. Applying the key genes and regulatory modules already identified in *Arabidopsis thaliana* to crops through gene editing or molecular breeding can help achieve the goals of delaying aging, increasing yield or enhancing stress resistance. For instance, genes such as *OsNAP* in rice, *NAMI* in wheat, and greenness retention in corn have all been proven to be related to yield, quality, and stress tolerance (Sekhon et al., 2019; Brar et al., 2025; Roeder et al., 2025).

## 9 Challenges and Future Directions

### 9.1 Complexity and redundancy of metabolic regulatory networks

The regulation of leaf senescence is very complex, involving many levels of metabolic changes and gene expression regulation. Many transcription factors are involved, such as NAC, WRKY, bHLH, ERF, MYB, etc. These factors, along with their downstream target genes, form a regulatory network that is large in structure and changes rapidly. Research has found that if one of the transcription factors is knocked out, it is often replaced by other family members, so the phenotypic change is not obvious. This situation indicates that there is obvious redundancy in the network and that the regulation method is very complex. In addition to transcriptional regulation, epigenetic modifications (such as H3K27me3 demethylation) and post-translational modifications of proteins (such as ubiquitination and phosphorylation) also jointly affect the aging process at different levels. At present, it remains a significant challenge to fully understand these mutually compensating and mutually influencing relationships and identify the truly crucial regulatory points.

### 9.2 Linking metabolic network reprogramming to whole-plant development and ecological fitness

Leaf senescence is not merely a change in the leaves themselves; it is closely related to the development of the entire plant, nutrient redistribution, and ecological adaptability. When aging, nutrients such as nitrogen and carbon are decomposed and reused. Many of them will be transferred to new organs such as seeds, which is very important for improving the reproductive success rate and long-term survival of plants. However, there is still a lack of systematic explanations on how the metabolic reprogramming that occurs in leaves during senescence is integrated with the overall development of the plant, the source-reservoir relationship, and stress signals. Current research is more focused on the leaves themselves. In the future, more research will be needed on the signal exchanges and metabolic product flows between different organs to understand the aging process from a more holistic perspective.

### 9.3 Future perspectives: single-cell multi-omics, spatial metabolomics, AI-based network modeling

With the development of new technologies such as single-cell multi-omics and spatial metabolomics, researchers can observe the metabolic and gene regulation characteristics of different cell types during aging in greater detail. Spatial metabolomics can also help us observe the distribution changes of metabolites in different regions of leaves and better understand the transformation process between the source and the reservoir. In addition, artificial intelligence and deep learning methods (such as the DEGRN model) have also been used to analyze large-scale data and construct regulatory networks. They can predict new regulatory factors and help us understand more complex network structures. In the future, integrating single-cell technology, spatial metabolomics and AI models will help to more systematically reveal the regulatory methods of leaf senescence and better explain the relationship between senescence and the overall development and ecological adaptability of the plant.

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## Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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