Multiple Layers of Regulation on Leaf Senescence: New Advances and Perspectives

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Leaf senescence is the last stage of leaf development and is an orderly biological process accompanied by degradation of macromolecules and nutrient recycling, which contributes to plant fitness. Forward genetic mutant screening and reverse genetic studies of senescence-associated genes (SAGs) have revealed that leaf senescence is a genetically regulated process, and the initiation and progression of leaf senescence are influenced by an array of internal and external factors. Recently, multi-omics techniques have revealed that leaf senescence is subjected to multiple layers of regulation, including chromatin, transcriptional and post-transcriptional, as well as translational and post-translational levels. Although impressive progress has been made in plant senescence research, especially the identification and functional analysis of a large number of SAGs in crop plants, we still have not unraveled the mystery of plant senescence, and there are some urgent scientific questions in this field, such as when plant senescence is initiated and how senescence signals are transmitted. This paper reviews recent advances in the multiple layers of regulation on leaf senescence, especially in post-transcriptional regulation such as alternative splicing.

Keywords: leaf senescence, senescence-associated genes, multi-omics, gene regulatory network, alternative splicing

INTRODUCTION

Plant leaves are the main organ for photosynthesis, converting light energy into chemical energy stored in carbohydrate molecules, which is the main source of energy for all organisms on earth. Senescence is the final stage of leaf development process, which is a slow and complex biological process including the initiation, progression, and terminal phases (Guo and Gan, 2005; Lim et al., 2007). The degradation of chlorophyll and chloroplasts occurs in the later phase of leaf senescence, accompanied by the degradation of macromolecules such as proteins, lipids, and nucleic acids. In annual plants, the nutrients released from senescent leaves are transferred to actively growing young leaves and seeds to increased reproductive success. In perennial plants such as deciduous trees, the nitrogen from leaf proteins is relocated to form bark storage proteins (BSP) in phloem tissues, and then remobilized and reutilized for spring shoot growth. Therefore, the timing of leaf senescence plays an important role in ensuring nutrient recycling, adaptation to the environment, and reproduction in plants. A number of studies in crops such as wheat and rice revealed that alteration of leaf senescence process could significantly affect the yield and quality of crops.
Extended lifespan of leaves in apple trees greatly improved fruit quality in apple trees (Han et al., 2020; Hu et al., 2020), and increased fruit yield and sugar content in tomato (Solanum lycopersicon) (Lira et al., 2017; Ma et al., 2018). Moreover, delayed leaf senescence conferred enhanced drought resistance in tobacco or cassava (Zhang et al., 2010). Therefore, an in-depth understanding of the regulatory mechanisms of leaf senescence is of great importance.

Leaf senescence is not a passive and disorderly process, but a highly programmed degenerative process (Guo and Gan, 2005). The initiation and progression of leaf senescence are influenced by numerous endogenous developmental signals and external environmental factors. Leaf age is the most important endogenous cue that determines the initiation of leaf senescence. However, the nature of age and how age information is perceived remains a mystery (Jing et al., 2002). Plant hormones such as ethylene, jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), brassinosteroid (BR), and strigolactone (SL) promote leaf senescence and are extensively involved in response to various abiotic and biotic stresses, whereas auxin, cytokinins (CKs), and gibberellins (GAs) delay leaf senescence (Lim et al., 2007; Miao and Zentgraf, 2007; Li et al., 2013; Zhang et al., 2013; Yamada and Umehara, 2015; Kim et al., 2020). Hormone signaling pathways often mediate or influence development and environmental responses to regulate leaf senescence (Lim et al., 2007). Interestingly, changes in the circadian rhythm of plants also impact leaf senescence, but the causal relationship between them needs to be further explored (Song et al., 2018).

In addition to being regulated by plant age or phytohormones, leaf senescence can also be caused by numerous environmental stresses such as darkness, nutrient deficiency, drought stress, and pathogen infection (Lim et al., 2007; Chen et al., 2013; Yamada and Umehara, 2015; Kim et al., 2020). There is much information about age- or abiotic stress-induced leaf senescence, whereas little is known about the molecular basis of biotic stress-triggered senescence. Recently, it was found that the secretory effector protein PevD1 (Protein elicitor from Verticillium dahliae) plays an important role in the V. dahliae-induced senescence process. PevD1 interacts with ORESARA1 (ORE1), one core transcription factor regulating plant senescence (Lim et al., 2007), and attenuates the NLA-mediated degradation of ORE1, thereby enhancing ethylene biosynthesis by directly binding the promoter of 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6 (ACS6) (Zhang Y. et al., 2021). This research provides a mechanism for previous observations that ethylene contributes to V. dahliae-induced premature leaf senescence.

Here, we reviewed the recent advances in the molecular regulation of leaf senescence, including chromatin level, transcription level, as well as post-transcriptional, translational, and post-translational level (Figure 1). We also summarized the key players involved in the multilevel regulation of leaf senescence (Table 1).

### Chromatin Level

In eukaryotic cells, DNA is packaged into chromatin and its functional units are nucleosomes. The basic unit of chromatin is the nucleosome core particle, a structure in which ~146 bp of DNA is wrapped around a protein octamer consisting of two subunits each of core histones H2A, H2B, H3, and H4 (Luger et al., 1997; Davey et al., 2002; Marino-Ramirez et al., 2005). The globular region of the histone forms the core of the nucleosome, while the N-terminal tail protrudes from the nucleosomes and is enriched for various post-translational modifications (PTMs), including acetylation, methylation, phosphorylation, and ubiquitination (Bannister and Kouzarides, 2011). These modifications have important regulatory roles, including gene repression, gene activation, and replication (Kouzarides, 2007; Morgan and Shilatifard, 2020). Histone modifications and the enzymes that implement them can facilitate chromatin compaction, nucleosome dynamics, and transcription. These modifications can respond to intrinsic and external stimuli (Kouzarides, 2007). Dysregulation of these processes can alter the balance of gene expression and thus is often observed in many human diseases or plant development, either by gain or loss of function, overexpression, repression through promoter hypermethylation, chromosomal translocation, or mutation of histone-modifying enzymes/complexes, or even histone modification sites (Zhao and Shilatifard, 2019).

Previous investigations revealed that epigenetic modification participates in the plant leaf senescence process. Chromatin immunoprecipitation sequencing (ChiP-seq) analysis using the trimethylation of histone H3 at lysine 4 (H3K4me3) and the trimethylation of histone H3 at lysine 27 (H3K27me3) antibodies reveals the relationship between histone modifications and leaf senescence in Arabidopsis (Brusslan et al., 2012, 2015). Mutation of histone deacetylase AtHD1, a histone modification-related gene, altered leaf senescence process in Arabidopsis (Pandey et al., 2002). The histone acetylation status of specific parts of chromatin is determined by histone acetylases (HATs) and histone deacetylases (HDACs) and their relative activities. Histone acetyltransferase 1 (HAT1) promotes leaf senescence by targeting Ethylene-responsive transcription factor expression with the transcription factor FUS3027. A positive regulator of leaf senescence (Hinckley et al., 2019). HISTONE DEACETYLASE 9 (HDA9), HDA15, HDA19, HISTONE DEACETYLASE 2C (HDC2), and SIRTUIN 1 (SRT1) play a potential role in promoting leaf senescence (Buszewicz et al., 2016; Zheng et al., 2016; Liu et al., 2017; Ueda et al., 2018; Hu et al., 2019; Shen et al., 2019). ChiP-seq and Fluorescence in situ hybridization (FISH) reveal that the chromatin structure changes as the leaf ages in Arabidopsis. Overexpression of SUVH2, a SU(VAR)3-9 (KMTase1) histone methyltransferase gene, delayed leaf senescence by increasing...
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FIGURE 1 | Multiple Layers of Regulation on Plant Leaf Senescence. Plant leaf senescence is finely regulated by endogenous developmental signals, and external environmental cues in an age-dependent manner. The multiple layers of regulation on leaf senescence includes epigenetic regulation at the chromatin level involved in histone proteases, DNA methylation modifying enzymes and chromatin remodeling factors, the master transcription factors involved in transcription regulation such as WRKYs and NACs, also includes miRNA and alternative splicing involved in the post-transcriptional level, and translation initiation and elongation factors involved in the regulation of translation level, as well as post-translational ubiquitination and phosphorylation. SAP, senescence-associated protein; Ub, ubiquitin; TF, transcription factors.

the dimethylation of histone H3 at lysine 27 (H3K27me2) and H3K27me3 levels in the promoter region of WRKY53, a master positive regulator of leaf senescence, and then suppressing its transcripts (Ay et al., 2009). JUMONJI DOMAIN-CONTAINING PROTEIN 16 (JM16), a specific H3K4 demethylase containing JmjC-domain, regulates negatively leaf senescence through repressing the expression of WRKY53 and SENESCENCE-ASSOCIATED GENE 201 (SAG201), two positive regulators of leaf senescence in Arabidopsis. Moreover, genome-wide analysis reveals widespread hypermethylation of H3K4me3 at JM16 binding genes, including WRKY53 and SAG201, and coordinated upregulation of their expression in the jm16 mutant compared with the wild type (Liu et al., 2019). To screen the upstream regulator of NONYELLOWING1 (NYE1) that regulates chlorophyll degradation during leaf senescence by Yeast one-Hybrid (Y1H) approach, the histone H3K27me3 demethylase RELATIVE OF EARLY FLOWERING6 (REF6) was found to directly interact with the NYE1 promoter through its zinc finger domain and up-regulates gene expression of positive regulators of leaf senescence such as ETHYLENE INSENSITIVE 2 (EIN2) and ORE1 (Wang et al., 2019). In addition, overexpression of SUVH2 also inhibits the gene expressions of senescence-associated WRKY (Sen-WRKY) and Sen-NAC (NAM/ATAF/CUC) transcription factors, central components of the leaf senescence process, in Arabidopsis leaves upon treatment with bleomycin (BLM), a genotoxic chemical that induces double-strand breaks (DSBs) (Li et al., 2020a).

ATP-dependent chromatin remodeling enzyme involved in chromatin remodeling is also associated with leaf senescence. Mutations of DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRDI) and DECREASED DNA METHYLATION 1 (DDM1), two SWI2/SNF2 chromatin remodeling proteins, delay leaf senescence (Cho et al., 2016). In contrast, loss-of-function of BRAHMA (BRM), another SWI/SNF2 chromatin remodeling ATPase (Archacki et al., 2017), accelerates leaf senescence (Efroni et al., 2013; Li C. et al., 2016). Further studies are needed in the future to reveal how various epigenetic modifications coordinately regulate leaf senescence.

Transcription Level

Large-scale reprogramming of the transcriptome is a core step in plant leaf senescence. Approximately a dozen percent of genes are up-regulated or down-regulated during leaf senescence in Arabidopsis. Furthermore, master TFs-mediated transcriptional regulation plays a crucial role in the regulation of leaf senescence (Zentgraf et al., 2010; Breeze et al., 2011). A WRKY transcription factor is one of the plant-specific TF families controlling the leaf senescence process. Members of WRKY TFs, including WRKY6, WRKY22, WRKY42, WRKY45, WRKY46, WRKY53, WRKY54, WRKY55, WRKY57, WRKY70, and WRKY75, coordinate with endogenous hormones to finely regulate the leaf senescence...
### TABLE 1 | List of the key genes involved in multiple layers of regulation on leaf senescence.

| Gene       | Species                  | Effects       | Regulation        | References               |
|------------|--------------------------|---------------|-------------------|--------------------------|
| AtHD1      | Arabidopsis thaliana     | Delay         | Chromatin level   | Pandey et al., 2002      |
| AtSRT1     | Arabidopsis thaliana     | Delay         | Chromatin level   | Liu et al., 2017         |
| Brahma     | Arabidopsis thaliana     | Delay         | Chromatin level   | Efroni et al., 2013      |
| DRD1/DDM1  | Arabidopsis thaliana     | Promote       | Chromatin level   | Cho et al., 2016         |
| HAC1       | Arabidopsis thaliana     | Promote       | Chromatin level   | Hinckley et al., 2019    |
| HDA9       | Arabidopsis thaliana     | Delay         | Chromatin level   | Zheng et al., 2016       |
| HDA15      | Arabidopsis thaliana     | Delay         | Chromatin level   | Shen et al., 2019        |
| HDA19      | Arabidopsis thaliana     | Delay         | Chromatin level   | Ueda et al., 2018        |
| HD2C       | Arabidopsis thaliana     | Delay         | Chromatin level   | Buszewicz et al., 2016   |
| JMU16      | Arabidopsis thaliana     | Promote       | Chromatin level   | Liu et al., 2019         |
| SLVH2      | Arabidopsis thaliana     | Delay         | Chromatin level   | Ay et al., 2009          |
| REF8       | Arabidopsis thaliana     | Promote       | Chromatin level   | Wang et al., 2019        |
| WRKY6      | Arabidopsis thaliana     | Promote       | Transcription level| Robatzek and Somssich, 2001|
| WRKY22     | Arabidopsis thaliana     | Promote       | Transcription level| Zhou et al., 2011        |
| WRKY42     | Arabidopsis thaliana     | Promote       | Transcription level| Ni et al., 2020          |
| WRKY45     | Arabidopsis thaliana     | Promote       | Transcription level| Chen et al., 2017        |
| WRKY46     | Arabidopsis thaliana     | Promote       | Transcription level| Zhang D. et al., 2021    |
| WRKY53     | Arabidopsis thaliana     | Promote       | Transcription level| Zentgraf et al., 2010    |
| WRKY54/WRKY70 | Arabidopsis thaliana | Delay         | Transcription level| Besseau et al., 2012    |
| WRKY55     | Arabidopsis thaliana     | Promote       | Transcription level| Wang et al., 2020        |
| WRKY57     | Arabidopsis thaliana     | Delay         | Transcription level| Jiang et al., 2014       |
| WRKY75     | Arabidopsis thaliana     | Promote       | Transcription level| Guo P. et al., 2017      |
| GhWRKY42   | Gossypium hirsutum       | Promote       | Transcription level| Gu et al., 2018          |
| GhWRKY91   | Gossypium hirsutum       | Delay         | Transcription level| Gu et al., 2019          |
| CpWRKY71   | Chimonanthus praecox     | Promote       | Transcription level| Huang et al., 2019       |
| OsWRKY42   | Oryza sativa             | Promote       | Transcription level| Han et al., 2014         |
| OsWRKY93   | Oryza sativa             | Promote       | Transcription level| Li Y. et al., 2021       |
| BnaWR1     | Brassica napus           | Promote       | Transcription level| Cui et al., 2020         |
| BnaWGR1    | Brassica napus           | Promote       | Transcription level| Yang et al., 2018        |
| BrWRKY6    | Brassica rapa var. parachinensis | Promote | Transcription level| Fan et al., 2018 |
| AtNAP      | Arabidopsis thaliana     | Promote       | Transcription level| Guo and Gan, 2006        |
| AtNAC3     | Arabidopsis thaliana     | Promote       | Transcription level| Hickman et al., 2013     |
| AtADF1     | Arabidopsis thaliana     | Promote       | Transcription level| Garapati et al., 2015    |
| ANAC016    | Arabidopsis thaliana     | Promote       | Transcription level| Kim et al., 2013         |
| ANAC017, VNH1, and ANAC090 | Arabidopsis thaliana | Delay         | Transcription level| Woo et al., 2016         |
| ANAC019    | Arabidopsis thaliana     | Promote       | Transcription level| Lee et al., 2015         |
| ANAC032    | Arabidopsis thaliana     | Promote       | Transcription level| Mahmood et al., 2016     |
| ANAC046    | Arabidopsis thaliana     | Promote       | Transcription level| Oda-Yamamizo et al., 2016|
| ANAC072    | Arabidopsis thaliana     | Promote       | Transcription level| Li S. et al., 2016       |
| NAC075     | Arabidopsis thaliana     | Delay         | Transcription level| Kan et al., 2021         |
| ANAC102    | Arabidopsis thaliana     | Promote       | Transcription level| Nakashima et al., 2012   |
| JUB1       | Arabidopsis thaliana     | Delay         | Transcription level| Wu et al., 2012          |
| NLT9       | Arabidopsis thaliana     | Promote       | Transcription level| Yoon et al., 2008        |
| ORF1       | Arabidopsis thaliana     | Promote       | Transcription level| Kim et al., 2009         |
| PIF4 and PIF5 | Arabidopsis thaliana | Promote       | Transcription level| Balazadeh et al., 2011   |
| VNII       | Arabidopsis thaliana     | Delay         | Transcription level| Sakuraba et al., 2014; Song et al., 2014|
| BnaNAC87   | Brassica napus           | Promote       | Transcription level| Yan et al., 2018         |
| lbnNAc1    | Ipomoea batatas          | Promote       | Transcription level| Chen et al., 2016        |
| MpsNAC67   | Musa x paradisiaca       | Promote       | Transcription level| Tak et al., 2018         |
| MINAC5     | Miscanthus lutarioriparius| Promote      | Transcription level| Yang et al., 2015        |
| NNAC080    | Nicotiana tabacum        | Promote       | Transcription level| Li et al., 2018          |
| NAM-B1     | Triticum turidum         | Promote       | Transcription level| Uauy et al., 2006        |

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| Gene                     | Species                  | Effects | Regulation       | References                  |
|--------------------------|--------------------------|---------|------------------|-----------------------------|
| TaNAC-S                  | Triticum turgidum        | Delay   | Transcription    | Zhao et al., 2015           |
| ONAC011                  | Oryza sativa             | Promote | Transcription    | El Mannai et al., 2017      |
| ONAC096                  | Oryza sativa             | Delay   | Transcription    | Kang et al., 2019           |
| ONAC106                  | Oryza sativa             | Delay   | Transcription    | Sakuraba et al., 2015       |
| OsNAC2                   | Oryza sativa             | Promote | Transcription    | Mao et al., 2017            |
| OsNAP                    | Oryza sativa             | Promote | Transcription    | Liang et al., 2014          |
| OsDOS                    | Oryza sativa             | Delay   | Transcription    | Kong et al., 2006           |
| SiNAP1 and SiNAP2         | Solanum lycopersicon     | Promote | Transcription    | Ma et al., 2018             |
| SiORE1S06, SiORE1S03, and SiORE1S02 | Solanum lycopersicon | Promote | Transcription    | Lira et al., 2017           |
| SiNAC1                   | Setaria italica          | Promote | Transcription    | Ren et al., 2018            |
| GmNAC065 and GmNAC085    | Glycine max              | Promote | Transcription    | Melo et al., 2018           |
| GmNAC81                  | Glycine max              | Promote | Transcription    | Pimenta et al., 2016        |
| GhNAC12                  | Gossypium hirsutum       | Promote | Transcription    | Zhao et al., 2016           |
| AIF2                     | Arabidopsis thaliana     | Delay   | Transcription    | Kim et al., 2020            |
| AP2                      | Arabidopsis thaliana     | Delay   | Transcription    | Qi et al., 2015             |
| bHLH03, bHLH13, bHLH14, and bHLH17 | Arabidopsis thaliana | Delay   | Transcription    | Balanza et al., 2018        |
| CRF1, CRF2, CRF3, CRF5, and CRF6 | Arabidopsis thaliana | Promote | Transcription    | Raines et al., 2016         |
| CBF2                     | Arabidopsis thaliana     | Delay   | Transcription    | Sharabi-Schwager et al., 2010|
| DEAR1                    | Arabidopsis thaliana     | Promote | Transcription    | Tsutsui et al., 2009        |
| FYF                      | Arabidopsis thaliana     | Delay   | Transcription    | Chen et al., 2011           |
| IAA17                    | Arabidopsis thaliana     | Promote | Transcription    | Shi et al., 2015            |
| KH21 and KH22            | Arabidopsis thaliana     | Promote | Transcription    | Yan et al., 2017            |
| MYC2, MYC3, and MYC4     | Arabidopsis thaliana     | Promote | Transcription    | Qi et al., 2015             |
| MYCS                     | Arabidopsis thaliana     | Promote | Transcription    | Song et al., 2017           |
| JAZ7                     | Arabidopsis thaliana     | Promote | Transcription    | Yu et al., 2016             |
| MdBHLH3                  | Malus domestica          | Promote | Transcription    | Hu et al., 2020             |
| AtMYBL                   | Arabidopsis thaliana     | Promote | Transcription    | Zhang et al., 2011          |
| MYB2                     | Arabidopsis thaliana     | Promote | Transcription    | Buchanan-Wollaston et al., 2005|
| MYBR1                    | Arabidopsis thaliana     | Delay   | Transcription    | Jaradat et al., 2013        |
| MYBH                     | Arabidopsis thaliana     | Promote | Transcription    | Huang et al., 2015          |
| ORE15                    | Arabidopsis thaliana     | Delay   | Transcription    | Kim J. H. et al., 2018      |
| OsMYC2                   | Oryza sativa             | Promote | Transcription    | Uji et al., 2017            |
| OsMYB102                 | Oryza sativa             | Delay   | Transcription    | Piao et al., 2019           |
| Rap2.4f                  | Arabidopsis thaliana     | Promote | Transcription    | Xu et al., 2010             |
| REVOLUTA                 | Arabidopsis thaliana     | Promote | Transcription    | Xie Y. et al., 2014         |
| RAV1                     | Arabidopsis thaliana     | Promote | Transcription    | Woo et al., 2010            |
| ScMYB25S1                | Sugarcane                | Promote | Transcription    | Guo X. et al., 2017         |
| SIERF36                  | Solanum lycopersicon     | Promote | Transcription    | Upadhyay et al., 2013       |
| SUB1A                    | Oryza sativa             | Delay   | Transcription    | Fukao et al., 2012          |
| SIFYFL                   | Solanum lycopersicon     | Delay   | Transcription    | Xie Q. et al., 2014         |
| SIMBP11                  | Solanum lycopersicon     | Delay   | Transcription    | Guo X. et al., 2017         |
| TCP2, TCP4, and TOP10    | Arabidopsis thaliana     | Promote | Transcription    | Schommer et al., 2008       |
| CFM4                     | Arabidopsis thaliana     | Promote | Post-transcription | Lee et al., 2014 |
| ERF4                     | Arabidopsis thaliana     | Promote | Post-transcription | Koyama et al., 2013; Riester et al., 2019 |
| miR156                   | Arabidopsis thaliana     | Delay   | Post-transcription | Wang, 2014                  |
| miR164                   | Arabidopsis thaliana     | Delay   | Post-transcription | Kim et al., 2009            |
| miR172                   | Zea mays                 | Delay   | Post-transcription | Wu et al., 2016             |
| miR319                   | Arabidopsis thaliana     | Promote | Post-transcription | Schommer et al., 2008       |
| miR840                   | Arabidopsis thaliana     | Delay   | Post-transcription | Yujun et al., 2019          |
| ONAC054                  | Oryza sativa             | Promote | Post-transcription | Sakuraba et al., 2020       |
| PRR26                    | Populus                  | Delay   | Post-transcription | Wang et al., 2021           |
| SlymiR208                | Solanum lycopersicon     | Promote | Post-transcription | Zhang Y. et al., 2020       |
| ScMYB2                   | Saccharum officinarum    | Promote | Post-transcription | Guo X. et al., 2017         |

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TABLE 1 | (Continued)

| Gene       | Species                  | Effects               | Regulation                  | References                               |
|------------|--------------------------|-----------------------|-----------------------------|------------------------------------------|
| u11-48k    | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Xu et al., 2016                          |
| eIF5A      | Picrocriza kurrooa       | Delay                 | Translation level           | Parkash et al., 2014                     |
| LrEF1A4    | Petunia hybrida          | Delay                 | Translation level           | Sun et al., 2020                         |
| SPL33      | Oryza sativa             | Delay                 | Translation level           | Wang et al., 2017                        |
| AtSAFRK    | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Xu et al., 2011                          |
| AtWAGL10   | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Li L. et al., 2021                       |
| ATG4a/4b   | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Yoshimoto et al., 2004                   |
| ATG9       | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Hanaka et al., 2002                      |
| ATG10      | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Phillips et al., 2008                    |
| ATG11a     | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Xiong et al., 2005                       |
| EDR1       | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Frye et al., 2001                        |
| MKK4/5,MKPK1/2 | Arabidopsis thaliana | Promote               | Post-transcriptional level  | Zhang Y. et al., 2020                    |
| MAPK18     | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Matsuzaka et al., 2015                   |
| MPK6/MKP9  | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Zhou et al., 2009                        |
| GmSAFRK    | Glycine max              | Promote               | Post-transcriptional level  | Li et al., 2006                          |
| OsMAPK18K1 | Oryza sativa             | Promote               | Post-transcriptional level  | Wang et al., 2015                        |
| PUB12/PUB13| Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Zhou et al., 2015                        |
| RPN10      | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Lin et al., 2011                         |
| RPN5a      | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Book et al., 2009                        |
| SAUR49     | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Wen et al., 2020                         |
| SERK4      | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Li et al., 2019                          |
| ULP5       | Arabidopsis thaliana     | Delay                 | Post-transcriptional level  | Zentgraf et al., 2010                    |
| UBP12/UBP13| Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Park et al., 2019                        |
| UBA2       | Arabidopsis thaliana     | Promote               | Post-transcriptional level  | Kim et al., 2008                         |

process (Robatzek and Somssich, 2001; Miao et al., 2004; Zentgraf et al., 2010; Zhou et al., 2011; Besseau et al., 2012; Jiang et al., 2014; Chen et al., 2017; Guo P. et al., 2017; Niu et al., 2026; Wang et al., 2020). Recently, WRKY46 was found to interact with Non-expressor of Pathogenesis-Related gene 1 (NPR1) and combined with the WRKY6 promoter to induce its expression in response to SA signals, thereby establishing an NPR1-WRKY46-WRKY6 signaling cascade to regulate leaf senescence (Zhang D. et al., 2021). Several WRKY TFs coordinate leaf growth and senescence in plants, including GhWRKY42 and GhWRKY91 in Gossypium hirsutum, BrWRKY6 in cabbage (Brassica rapa), CpsWRKY71 in Chimonanthus praecox, OsWRKY93 in Oryza sativa, BnaWSR1 (WRKY regulating SA and ROS 1) and BnaWGR1 (WRKY generating ROS 1) in Brassica napus (Fan et al., 2018; Gu et al., 2018, 2019; Yang et al., 2018; Huang et al., 2019; Cui et al., 2020; Li Y. et al., 2021).

NAC family is one of the largest gene families in plants and plays a central role in regulating leaf senescence. NAC TFs function as positive regulators of leaf senescence, including ORE1/ANAC092, AtNAP/ANAC029, ORE1 SISTER1 (ORS1/ANAC059), ATAF2/ANAC081, ATAF1/ANAC002, ANAC019, AtNAC3/ANAC055, RESPONSIVE TO DESICCATION 26 (ATRD26/ANAC072), ANAC102, ANAC032, ANAC046, ANAC016, and NAC TRANSCRIPTION FACTOR-LIKE 9 (NTL9) or negative regulators such as JUNGBRUNNEN1 (JUB1/ANAC042), ANAC017, VND-INTERACTING1 (VNI1/ANAC082), VND-INTERACTING2 (VNI2/ANAC083), ANAC090, and ANAC075 (Guo and Gan, 2006; Yoon et al., 2008; Kim et al., 2009, 2013; Balazadeh et al., 2011; Nakashima et al., 2012; Wu et al., 2012; Hickman et al., 2013; Garapati et al., 2015; Lee et al., 2015; Takasaki et al., 2015; Kim H. J. et al., 2016, 2018; Li S. et al., 2016; Mahmood et al., 2016; Oda-Yamamizo et al., 2016; Woo et al., 2016; Nagahage et al., 2020; Kan et al., 2021). Future studies need to investigate whether there is communication between these positive and negative regulatory NAC-TFs, which will help to gain insight into the fine regulatory mechanisms of leaf senescence. On the one hand, to investigate whether there are direct interactions between these factors and whether they are synergistic or antagonistic to each other. On the other hand, ChIP-Seq data should be used to analyze whether their target genes overlap to develop a gene regulatory network of leaf senescence. Indeed, some studies have already started to address this aspect. For example, members of NAC-TFs and WRKY-TFs have been found to interact with each other to change the expression of downstream target genes, which in turn triggers leaf senescence (Kim et al., 2009; Zentgraf et al., 2010; Balazadeh et al., 2011; Besseau et al., 2012; Kim H. J. et al., 2016, 2018). Since the process of leaf senescence is accompanied by nutrient return, genes that regulate senescence are likely to regulate crop yield. In supporting this hypothesis, several NAC-TFs regulate crop yield by fine-tuning the initiation and progression of leaf senescence, such as NAM-B1 and TaNAC-S in wheat (Uauy et al., 2006; Zhao et al., 2015; Kang et al., 2019, 2018; Li S. et al., 2016; Mahmood et al., 2016; Oda-Yamamizo et al., 2016; Woo et al., 2016; Nagahage et al., 2020; Kan et al., 2021), which provides a molecular strategy to improve crop yield or quality by finely regulating the leaf senescence process.
The basic helix-loop-helix (bHLH) family TFs also regulate leaf senescence. Members of bHLH subgroup IIe factors, including myelocytomatosis protein 2 (MYC2), MYC3 and MYC4, antagonistically interact with the bHLH subgroup IIId factors bHLH03 (JAM3), bHLH13 (JAM2), bHLH14, and bHLH17 (JAM1), and mediate JA-induced leaf senescence by directly binding the promoter of SAG29 in Arabidopsis (Qi et al., 2015; Goossens et al., 2017). In addition, MYC5 also positively regulates JA-induced leaf senescence (Song et al., 2017). Darkness induces the protein accumulation of JASMONATE ZIM-domain 7 (JAZ7), which in turn inhibits dark-induced leaf senescence by suppressing MYC2 (Yu et al., 2016). In rice, OsMYC2 acts as a positive regulator of leaf senescence by regulating the transcript levels of SAGs (Uji et al., 2017). These findings suggest that MYC2 regulates leaf senescence via multiple signaling pathways. ACTIVATION-TAGGED BRI1 (BRASSINOSTEROID-INSENSITIVE1)-SUPPRESSOR1 (ATBS1)-INTERACTING FACTOR2 (AIF2) is a non-DNA-binding bHLH TF and delays dark or BR-induced leaf senescence (Kim et al., 2020). Phytochrome-interacting bHLH transcription factors (PIFs) such as PIF4 and PIF5 promote leaf senescence under natural or dark conditions in Arabidopsis (Sakuraba et al., 2014; Song et al., 2014; Li N. et al., 2021). MdbHLH3 regulates leaf senescence by promoting the expression of dehydratase-eluase-phosphatase complex 1 (MdDEP1) in Malus Domestica (Hu et al., 2020). These results imply that bHLH TFs are involved in the regulation of leaf senescence in both annuals and perennial woody plants.

There is growing evidence that multiple TF families of genes are involved in the regulation of leaf senescence, including MYB-TFs such as MYB2 (Buchanan-Wollaston et al., 2005), MYB DOMAIN PROTEIN R1 (MYBRI1) (Jaradat et al., 2013), MYB HYPOCOTYL ELONGATION-RELATED (MYBH) (Huang et al., 2015), AtMYBL (Zhang et al., 2011), OsMYB102 (Piao et al., 2019), ScMYB251 (Guo J. et al., 2017); PLANT A/T-RICH SEQUENCE- AND ZINC-BINDING PROTEIN (PLATZ) family transcription factor (ORE15) (Kim J. H. et al., 2018); AP2/ERF transcription factors such as CRF1/2/3/5/6 in Arabidopsis (Raines et al., 2016), SIERF36 in Tomato (Upadhyay et al., 2013), and SUBMERGENCEA1A (SUB1A) in Rice (Fukao et al., 2012); AP2/DREB transcription factors (DEAR1 and Rap2.4f) (Tsutsui et al., 2009; Xu et al., 2010); CCCH zinc-finger family [K-homolog (KH) proteins, KHZ1 and KHZ2] (Yan et al., 2017); AUXIN RESISTANT 3 (AXR3)/INDOLE-3-ACETIC ACID INDUCIBLE 17 (IAA17), one member of Auxin response factors (ARF) family, is a positive regulator of natural leaf senescence (Shi et al., 2015); TEOSINTE BRANCHED1/CYCOIDEA/PCF (TCP) family transcription factor (TCP2/4/10) (Schoenmer et al., 2008); Homeodomain-leucine zipper family (REVOLUTA) (Xie Y. et al., 2014); MADS box transcription factors such as FOREVER YOUNG FLOWER (FYF) in Arabidopsis and SIFYFL in Tomato (Chen et al., 2011; Xie Q. et al., 2014; Guo X. et al., 2017), as well as RAV family transcription factor (RAV1) (Woo et al., 2010).

A large number of studies have shown that TF plays a key regulatory role in leaf senescence, however, most of the studies have mainly focused on a few families, including the NAC or WRKY families, and more studies are needed in the future to analyze whether other family TFs are also involved in leaf senescence. A recent transcriptomic study revealed that 115 Sen-TFs from 31 families are involved in autumn leaf senescence in poplar (Wang et al., 2021), further supporting this suggestion.

### Post-transcriptional Level

Post-transcriptional regulation, including RNA editing, polyadenylation, mRNA stability, and alternative splicing, is related to leaf senescence. Multiple organellar RNA editing factors 9 (MORF9), one of the core proteins of plant editosomes, are involved in the RNA editing in chloroplasts, and its mRNA level declined in senescent leaves (Tian et al., 2019). MicroRNA (miRNA) is involved in leaf senescence by regulating the expression of SAG genes. For example, miR156, miR164, miR172, and miR840 regulate leaf senescence by suppressing their target genes (Kim et al., 2009; Wu et al., 2016, 2020; Tian et al., 2019; Yujun et al., 2019; Roussin-Leveillee et al., 2020). In addition to miRNAs, circular RNA (circRNA), and long non-coding RNA (lncRNAs) participate in leaf senescence of rice by a competitive endogenous RNA (CeRNA) network (Huang et al., 2021a,b). Interestingly, EIN3 and clock-associated PSEUDO-RESPONSE REGULATOR 9 (PRR9) up-regulate the transcription level of ORE1 by inhibiting the transcription of miR164 (Li et al., 2013; Kim H. et al., 2018). miR398 participates in regulating leaf senescence by post-transcriptional regulation of ASCORBATE PEROXIDASE 6 (APX6) (Chen et al., 2021). SlymiR208 regulates leaf senescence by controlling the expression of isopentenyl transferases SIPT2 and SIPT4 (Zhang Y. et al., 2020).

Alternative splicing (AS) is widely used in RNA splicing and processing after gene transcription in higher eukaryotes, which can increase the diversity of transcriptome and proteome. AS events can be mainly classified into five categories: IR, skipping exon and mutually exclusive exons, as well as alternative 5'-splice sites and alternative 3'-splice sites. In animals, splicing factors control cellular senescence by regulating the splicing process of RNA precursors (Fregoso et al., 2013). In plants, AS acts as a regulatory mechanism of plant development or adaptation to environmental stress factors. RNA splicing factor RNA-BINDING PROTEIN 25 (RBM25) responds to ABA stress in Arabidopsis (Zhan et al., 2015). Loss-of-function of CRM FAMILY MEMBER SUBFAMILY 4 (CFM4) leads to abnormal rRNA processing during chloroplast RNA splicing, and exhibited plant growth retardation and delayed senescence (Lee et al., 2014). An interesting discovery shows that the differential expression of sugarcane MYB TF ScMYB2 alternative splicing transcripts may be an important post-transcriptional regulatory mechanism for controlling drought stress and leaf senescence (Guo J. et al., 2017).

The splicing mechanism occurs in the spliceosome, which is composed of five small nuclear RNAs (snRNAs) and a series of related protein factors. The spliceosome can recognize the splice site of the precursor RNA and catalyze the splicing reaction. There are major spliceosomes (U2) and minor spliceosomes (U12) that support splicing functions (Sharp, 2005). Although the splicing efficiency of the U12-type spliceosome is relatively lower,
splicing errors will affect the normal growth and development of plants. The U12-type intron-specific small spliceosome mainly removes the small U12 intron from the precursor mRNA. Mutation of u11-48k causes defects in growth and development, such as short plant size, increased lotus-like leaves, and delayed senescence (Xu et al., 2016), indicating that the regulation of the RNA splicing process has a potentially important effect on plant leaf senescence. ETHYLENE RESPONSE FACTOR4 (ERF4) has two different isoforms, ERF4-R and ERF4-A, produced by alternative polyadenylation of its pre-mRNA. ERF4-R contains an ERF-associated amphiphilic repression (EAR) motif and acts as a repressor, whereas the other form, ERF4-A, is lacking this motif and acts as an activator. ERF4-R and ERF4-A can directly bind to the promoter of catalase3 (CAT3) but have antagonistic effects on gene expression. The ratio of ERF4-A to ERF4-R mRNA changed as the plant ages and caused a complex age-dependent regulation of CAT3 activity. Interestingly, overexpression of ERF4-R but not of ERF4-A led to accelerated senescence (Koyama et al., 2013; Riester et al., 2019). ONAC054 was shown to participate in ABA-induced leaf senescence by directly activating OsaA15 in rice (Sakuraba et al., 2020). Interestingly, the ONAC054 transcript (ONAC054a) has an alternatively spliced form, ONAC054b, encoding a small truncated protein. Overexpression of ONAC054a or ONAC054b promotes leaf senescence (Sakuraba et al., 2020). A recent study reported that an alternative splicing event retaining the first intron of the PtRD26 pre-mRNA occurred in a senescence-associated manner in poplar. The intron retention (IR) event in PtRD26 led to an alternative splicing variant, PtRD26IR, which encodes a truncated protein. PtRD26IR forms heterodimers with multiple hub Sen-NAC TFs, including PtNAC039, PtNAC055, PtNAC076, PtNAC086, PtNAC099, and PtNAC109, represses their DNA binding activity to target genes, and delays age-, dark-, and PtRD26-induced leaf senescence in poplar, tobacco, and Arabidopsis. PtRD26 regulates Sen-NAC TFs by directly binding their promoters or indirectly through protein-protein interactions using its splicing variant, PtRD26IR, thereby forming a multiply-interlocked feed-forward loop to finely tune the leaf senescence process. Functional analysis of senescence-associated splicing factors (SF) revealed that PtU2A2A, PtU2A2B-1, or PtU2A2B-2 (U2 auxiliary factor large subunit A or B) are involved in AS of PtRD26IR. Silencing separately or simultaneously of these SFs significantly decreased the transcript levels of PtRD26IR and accelerated leaf senescence. Based on these findings, it is found that the products of AS have different functions and regulate plant development such as plant senescence through different mechanisms. With the application of multi-omics technology, more AS events will be found to be involved in the regulation of leaf senescence, which will further deepen the mechanistic understanding of plant aging.

**Translation Level**

Senescence is a long-term state of cell cycle arrest arising from cells that have suffered sublethal damage. Although senescent cells no longer replicate, they remain metabolically active and further develop a distinct and stable phenotype not seen in proliferating cells (Guo and Gan, 2005; Lim et al., 2007). On the one hand, along with leaf senescence, a large number of proteins are degraded and translation efficiency decreases; on the other hand, senescence-specific regulatory factors are synthesized to inhibit or retard the leaf senescence process (Guo and Gan, 2005; Lim et al., 2007). Thus, translation in senescent cells paradoxically includes a general inhibition of translation triggered by numerous stresses and a selective increase in translation of specific proteins, including SAG protein.

Mutation of ORE4, which encodes the plastid ribosomal small subunit protein 17 that is a component of the plastid ribosome, reduces the translation rate in the chloroplast and thus extends leaf longevity in Arabidopsis (Woo et al., 2002), suggesting a possible link between decreased metabolism and extended longevity of the leaves. Translation initiation, the first step in the protein synthesis process, is the main regulatory step controlling translation and involves a large number of translation initiation factors. Studies in plants have revealed that these translation initiation factors affect various aspects of plant growth and development, in addition to their role in protein synthesis (Wang et al., 2001). Mutation of EUKARYOTIC ELONGATION FACTOR 5a (eIF5a) significantly inhibits plant nutrition and reproductive growth and delays leaf senescence in Arabidopsis and Picrorhiza (Picrorhiza kurrooa Royle ex Benth.) (Reviron et al., 1992; Parkash et al., 2014). Translation initiation factor eIF3h is involved in the signal activation and restart of rapamycin (TOR) and affects the growth and development of plants (Schepetilnikov et al., 2013). In addition, euukaryotic translation elongation factors (eEF) also involved leaf senescence. For instance, mutation of Spotted Leaf 33 (spl33), encoding a eEF1 alpha (eEF1A)-like protein, induces early leaf senescence (Wang et al., 2017). Ectopic expression of Lilium regales EUKARYOTIC elongation translation elongation factor 1 alpha 4 (LrefE1A4), encoding the α subunit of elongation factor 1 from a Lilium regale cucumber mosaic virus (CMV), delayed leaf and flower senescence in petunia (Petunia hybridra) (Sun et al., 2020). Interestingly, two subunits of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the key enzyme that determines the rate of carbon assimilation in photosynthesis, is controllable at the translation level, and affect plant growth and development, including the leaf senescence process (Suzuki and Makino, 2013; Woo et al., 2013).

**Post-translational Level**

Post-translational modifications (PTM), including methylation, acetylation, phosphorylation, ubiquitination, and deubiquitination affect the structure and function of proteins. Previous studies found that the PTM of a large number of SAG proteins changed with leaf senescence (Wang and Schippers, 2019). This implies a close relationship between leaf senescence and PTM, but the causal relationship is not clear.

Transcriptomics analysis reveals that a large number of SAGs are involved in PTM, such as receptor-like kinase (RLK) and mitogen-activated protein kinase (MAPK) (Ahmad and Guo, 2019). RLK is an ideal candidate for senescence-inducing signal receptors, which often have an N-terminal extracellular binding domain for ligand binding, a transmembrane domain spanning the plasma membrane, and a cytoplasmic kinase domain (Shiu and Bleecker, 2001; Gish and Clark, 2011). The
largest subfamily of RLK is the leucine-rich repeat-like protein kinase (LRR-RLK), containing more than 200 members, and lots of them are involved in the regulation of leaf senescence (Shiu et al., 2004). GmSARK (Glycine max Senescence-Associated Receptor-like Kinase), a senescence-associated LRR-RLK isolated from soybean (Glycine max) and its homolog AtSARK in Arabidopsis are positive regulators of leaf senescence (Li et al., 2006; Xu et al., 2011). SARK-mediated signaling pathway positively regulates leaf senescence through suppressing SMALL AUXIN-UP RNA 49 (SAUR49), a negative regulator of leaf senescence, and activating SENESCENCE-SUPPRESSED PROTEIN PHOSPHATASE (SSPP), an accelerator of leaf senescence (Xiao et al., 2015; Wen et al., 2020). In contrast, the somatic embryogenesis receptor-like kinase 4 (SERK4) and the cell wall-associated kinase 10 (AtWAK10) act as the negative regulators of leaf senescence (Li et al., 2019; Li L. et al., 2021). Interestingly, a common receptor can work with multiple receptors in different signaling pathways. AtSARK and SERK4 may be part of the receptor complex that regulates plant aging by acting with other LRR-RLKs (Brandt and Hothorn, 2016; Cui et al., 2018).

The mitogen-activated protein kinase cascade MAPKKK- MAPKK-MAPK is one of the most important signal transduction pathways in plants and animals. Recently, MAP KINASE 4/5 (MKK4/5)-MITOGEN-ACTIVATED PROTEIN KINASE 1/2 (MPK1/2), MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 18 (MAPKKK18), and OsMAPKKK1 have been found to be the positive regulators of leaf senescence (Matsuoka et al., 2015; Wang et al., 2015; Zhang J. et al., 2020). By contrast, Enhanced Disease Resistance 1 (EDR1), a MAPKK, functions as a negative regulator by coordinating biotic stress response and ethylene-induced senescence (Frye et al., 2001; Tang and Innes, 2002). MKK9 phosphorylates the target MPK6, which stabilizes the leaf senescence transcription factor EIN3 by promoting the cleavage and nuclear translocation of ORE3/EIN2 (Zhou et al., 2009; Zhang Y. et al., 2016). These findings suggest that RLKs and MAPKs regulate leaf senescence by affecting the phosphorylation status of target proteins.

The leaf senescence process is accompanied by protein degradation. The main protein degradation pathways are autophagy and the ubiquitin-proteasome system (UPS), which precisely regulate the turnover of organelles and the degradation of abnormal proteins and maintain protein homeostasis. Autophagy and protein ubiquitination are synergistic in the cell. Ubiquitination acts as a signal to induce organelles to target autophagy. Mitophagy and chloroplast protein degradation is the result of the synergistic effect of ubiquitination and autophagy (Geisler et al., 2010; Kikuchi et al., 2020). Interestingly, autophagy seems to prevent aging, whereas the proteasome acts as a positive regulator of aging (Wang and Schippers, 2019). Chaperone-mediated autophagy is one of the main types of autophagy in cells, with high selectivity. Autophagy-related genes (ATG) involved in autophagy are up-regulated with the occurrence of plant senescence (Masclaux-Daubresse et al., 2014). Mutation of several ATG genes, including ATG4a/4b, ATG9, ATG19, and ATG18a, promotes leaf senescence under nitrogen-starvation conditions (Hanaoka et al., 2002; Yoshimoto et al., 2004; Xiong et al., 2005; Phillips et al., 2008; Wang and Schippers, 2019). Although most studies support the role of autophagy in delaying aging, ATG8 promotes senescence by interacting with the ABNORMAL SHOOT3 (AB3S). This non-autophagic ATG8-AB3 pathway interacts with the classic autophagy pathway to balance aging and survival (Jia et al., 2019). Therefore, the components of autophagy may have a dual role in the initiation and progression of senescence. 26S proteasome is mainly responsible for degrading ubiquitinated proteins. The recognition of ubiquitinated substrates in the process of ubiquitin/proteasome-mediated proteolysis (UPP) is directly mediated by the proteasome subunits RPN10 (REGULATORY PARTICLE NON-ATPase 10) and RPN13. The loss of the potential UPP ubiquitin receptor RPN10 significantly delays senescence (Lin et al., 2011), and overexpression of RPN5a leads to premature senescence (Book et al., 2009). In contrast to the overall up-regulation of ATG genes, transcript levels of only a small part of the proteasome subunit genes were increased during leaf senescence (Guo and Gan, 2012). In the senescent leaf of rape and barley (Hordeum vulgare L.), the proteasome is very active (Poret et al., 2016; Velasco-Arroyo et al., 2016). Interestingly, an application of protease inhibitor delays the onset of senescence symptoms (Pak and van Doorn, 2005). Taken together, these observations imply that autophagy and proteasome seem to have different effects on the onset of senescence, and they coordinately regulate the progression of leaf senescence.

One of the well-characterized PTMs involved in the regulation of leaf senescence is ubiquitination/deubiquitination modification. Protein ubiquitination requires the synergy of ubiquitin activation (E1), ubiquitin-binding (E2), and ubiquitin ligase (E3). Members of E2 and E3 have been found to be involved in the regulation of leaf senescence (Shu and Yang, 2017; Park et al., 2018). Among them, RING-type E3 and U-box-type E3 ligases have been shown to act as regulators of leaf senescence by mediating ABA signaling. For example, PLANT U-box (PUB) E3 ubiquitin ligase PUB12 and PUB13 ubiquitinated FLS2 (FLAGELLIN-SENSITIVE 2) for protein degradation, thereby down-regulating flagellin signaling and negatively regulating stress-induced leaf senescence (Zhou et al., 2015). In addition, HECT-type ubiquitin E3 ligase (UPL1-UPL7) plays a critical role in cell death and leaf senescence (Lan and Miao, 2019). Mutation of UBQUITIN PROTEIN LIGASE 5 (UPL5) leads to the accumulation of WRKY53 and induces early leaf senescence (Zentgraf et al., 2010). Ubiquitin-specific protease (UBP1)-associated protein 2a (UBA2a), UBA2b, and UBA2c positive regulators of leaf senescence (Kim et al., 2008). Likewise, the potato (Solanum tuberosum) RNA-binding protein StUBA2a/b is homologous to Arabidopsis UBA2s. Constitutive overexpression of StUBA2a/b increases the expression of the SAG13 gene, pathogen-related genes (PR), and autophagy-related genes, and promotes leaf senescence in Arabidopsis (Na et al., 2015). The process of protein ubiquitination is reversible, and deubiquitinating enzymes (DUBs) can remove mono-ubiquitin molecules or polyubiquitin chains on proteins. UBP is the largest DUB subfamily, and members of the UBP family are involved in a variety of physiological processes, including leaf senescence (Zhou et al., 2017). Out of them,
UBIQUITIN-SPECIFIC PROTEASE 12 (UBP12) and UBP3 are involved in the regulation of circadian clock and flowering (Cui et al., 2013), and accelerate nitrogen starvation-induced leaf senescence by counteracting the effect of E3 ligase NLA (Nitrogen Ubiquitin-Protein Ligases DNA) to maintain the homeostasis of ORE1 (Park et al., 2019).

CONCLUSIONS AND PERSPECTIVES

Leaf senescence is a highly complex process of orderly degradation of cell structure and is controlled by multiple layers regulatory network (Figure 1), in which different regulatory factors at different levels may interact to fine-tune the initiation and progression of leaf senescence (Table 1). Although regulation is artificially divided into multiple levels (Woo et al., 2013), leaf senescence is a highly dynamic regulatory process (Woo et al., 2019), and there is no single way to regulate it. For example, changes in chromatin structure affect gene expression, protein translation, and thus the function of transcription factors, which in turn cause changes in the senescence process of plant leaves. Moreover, the regulation of leaf senescence involves not only the interactions between proteins, proteins, and DNA, but also the exchange of information between cells and organelles, thus synergistically regulating the initiation of leaf senescence, which guarantees the return of nutrients and the survival of plants. Therefore, we should combine genome, transcriptome, proteome, metabolome, and the latest translation comics data to discuss the general mechanism of regulate senescence and understand how senescence and death are systemically integrated within the entire plant (Kim J. et al., 2016).

With the aid of forwarding or reversing genetics strategies and the development of multi-functional CRISPR genome editing technology, a large number of senescence-related mutants will be generated. For example, quintuple mutants of oss40s-cr generated using CRISPR technology displays stay-green phenotype (Habiba et al., 2021), which will further deepen our understanding of leaf senescence. The model plant Arabidopsis has played an important role in revealing the molecular or genetic regulation mechanisms of plant senescence, but we still know little about leaf senescence and do not fully understand the biological significance of senescence (Kim J. et al., 2007). The relatively short life cycle of Arabidopsis has limitations for our understanding of plant aging. Along with the genomic information revealed for a variety of plants, it provides the possibility to systematically study plant senescence by comparative genomics.

It's unclear how these transcription factors regulate, such as the WRKY family and NAC family, and epigenetic factors co-regulate the senescence process of plants. The function of hormone signaling on leaf senescence has been widely recognized (Hu et al., 2017). It is necessary to further explore how plant signals and environmental signals are integrated into the hormone signaling pathway, and how post-translational modifications such as phosphorylation and ubiquitination are passed through transcription factors, kinases, and protease, finely control these signals to regulate gene expression and protein turnover during leaf senescence. The senescence symptoms of leaf senescence have always been detected at the organ level. However, in senescent leaves, leaf cells are usually at different developmental ages or senescence stages, which makes it impossible to better understand the biological process of leaf senescence. Fortunately, the application of single-cell sequencing technology may offer the possibility to resolve the cytological basis of leaf senescence.

In addition to the loss- or gain-of-function of mutants, ecotypes of various species will greatly contribute to the understanding of the molecular mechanisms underlying leaf senescence. Through analysis of naturally occurring DNA methylation variation regions (NMRs) between Col-0 and C24 accessions of Arabidopsis thaliana, a retrotransposon named NMR19-4 (naturally occurring DNA methylation variation region 19) was identified to be involved in the regulation of leaf senescence (He et al., 2018). NMR19-4 is an environmentally associated epiallele that controls leaf senescence by regulating the expression of PHEOPHYTIN PHEOPHORBIDE HYDROLASE (PHH), which is involved in chlorophyll breakdown (Schelbert et al., 2009; He et al., 2018). By mapping the quantitative trait locus (QTL) of leaf senescence between the Col-0 and Ct-1 accessions of Arabidopsis thaliana, ACCELERATED CELL DEATH 6 (ACD6) was identified as the causal gene (Jasinski et al., 2020). Using two rice subspecies indica and japonica, variations were found in the promoter regions of the Stay-Green (OsSGR) gene encoding a chlorophyll-degrading enzyme. This promoter variations trigger higher and earlier induction of OsSGR, which in turn accelerates leaf senescence in indica (Shin et al., 2020).

AUTHOR CONTRIBUTIONS

ZL conceived the project and designed the manuscript. HG and XX designed part of the manuscript. Y-MZ collected the data and organized figure. PG organized table. All authors have read and agreed to the published version of the manuscript.

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