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Review Article

Research Progress of Leaf Senescence Related Genes in Tobacco

Xiaoming Gao, Yongfeng Guo*

Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao, China

Email address:

gaoxiaoming@caas.cn (Xiaoming Gao), guoyongfeng@caas.cn (Yongfeng Guo)

*Corresponding author

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Abstract: Tobacco (*Nicotiana tabacum* L.) is an important model plant that has been widely used in plant biological research. Tobacco is also an economic crop in which mature yellow leaves are harvested. Leaf maturity and the yellowing process have a decisive influence on the quality of tobacco products. The mechanism, influencing factors and regulating genes of plant leaf senescence was briefly summarized in this review.The research progress in genes related to tobacco leaf senescence and the effects of exogenous genes on the senescence of tobacco was also summarized. It is of great importance to study tobacco leaf senescence process and related genes in order to improve the quality of tobacco.

Keywords: Tobacco, Leaf Senescence, Regulatory Genes

1. Introduction

Senescence is the terminal phase of plant growth and development. Plant senescence is the process of decline of growth in cell, tissue, organ, or whole plant and is regulated by genes and influenced by internal and external factors. Senescence eventually leads to the death of the whole plant [1]. Leaf senescence is the main feature of plant senescence. Normally, natural leaf senescence mainly depends on leaf age and development stage in the whole life cycle of the plant [2]. In addition, many environmental cues, such as drought stress, extreme temperatures, darkness, pathogen infection, and internal factors, such as hormones and reproductive growth, can induce and regulate leaf senescence [1, 3]. Chloroplasts are the first degraded organelles during leaf senescence. Leaf yellowing caused by chlorophyll degradation is the main feature of leaf senescence. During leaf senescence macromolecules such as proteins, lipids, and nucleic acids are degraded, and nutrients released from the catabolism of these macromolecules as well as other nutrients are recycled to actively growing new buds, young leaves, and developing fruits and seeds [4].

Tobacco (*Nicotiana tabacum*) is an economic crop widely grown in the world. In addition, as a model plant, tobacco plays an important role in the field of plant genetics and transgenic research. Tobacco leaves are processed to make cigarettes and other tobacco products to meet the needs of people. Mature leaves are used for these tobacco products. The process of tobacco leaf maturation is actually the process of senescence and has great influence on the quality of tobacco leaves and tobacco products. In the process of tobacco cultivation, “topping” block the transfer of nutrients to the reproductive organs and young stem, so that photosynthetic products can accumulate more in the leaves, which is beneficial to the accumulation of dry matter in tobacco leaves. But at the same time this practice changes the normal aging pattern of tobacco leaves. Therefore, tobacco leaf senescence can be regarded as a special aging process, which has important value in the study of the molecular mechanism of plant senescence. In recent years, the molecular mechanisms of leaf senescence have been well studied in model plants as well as major food crops. In the past two years, metabolome data and transcriptome data of tobacco leaf senescence were obtained [5, 6]. But the research on tobacco leaf senescence is still limited.

2. Plant Leaf Senescence

2.1. The Basic Characteristics of Plant Leaf Senescence

Leaf senescence is a type of programmed cell death, which is mainly related to the development process. Leaf senescence is the process of leaf turning from green to yellow and gradually toward functional exhaustion and death. Chlorophyll degradation is the most obvious feature in the process of leaf senescence. Leaf senescence is accompanied by a series of physiological and biochemical changes, including decrease of photosynthetic capacity, respiration rate, chlorophyll content, protein content and nucleic acid content, and changes of endogenous hormones, soluble sugar contents, reactive oxygen and free radical metabolism. In this process, plants can reuse energy materials and accumulate nutrients for new leaves and seeds.

2.2. Mechanisms of Plant Leaf Senescence

Leaf senescence is a complex process consisting of many physiological and molecular events. Nooden et al. put forward the three stage theory of leaf senescence, and divided leaf senescence into three stages: initiation, degeneration and terminal [7]. Once the process of leaf senescence is activated, it enters a degeneration stage. The main characteristics of degeneration stage are cell component degradation and nutrient recycling, and various protein degradation systems are activated [8]. After a series of decay and degradation, the leaves that completely turned yellow entered the final stage of leaf senescence. The significant characteristics of apoptosis can be detected at the terminal stage, such as chromatin concentration and DNA fragmentation, indicating that there is a process like cell apoptosis in leaf senescence [9].

2.3. Factors Affecting Plant Leaf Senescence

Leaf senescence as the final stage of plant growth and development is affected by both internal and external factors, including plant hormones, metabolic status, and environmental factors.

2.3.1. Internal Factor

The internal factors are mainly the regulation of all kinds of plant hormones which have been shown to play a key role in leaf senescence [10]. The effect of plant hormones on leaf senescence is cytokinins, auxin and gibberellins can delay leaf senescence; abscisic acid and ethylene can promote leaf senescence. Cytokinins (CKs) can inhibit leaf senescence and prolong leaf life. The cytokinins content in leaves gradually decreases with senescence process. Exogenous CKs and overexpression a key gene for cytokinin synthesis *IPT* can retard plant senescence [11, 12]. Type A ARRs which cytokinins biosynthesis genes and signaling genes have been shown have reduced transcription during leaf senescence [13]. CKs receptor AHK2 and AHK3 double mutant lines showed premature senescence and could not be retard to wild type phenotype when stimulated by CKs [14]. Early studies showed that exogenous application of auxin delays leaf senescence [15]. RNA-Seq analysis performed in cotton showed that most auxin pathway related genes were downregulated, suggesting that auxin may act as a negative regulator of leaf senescence [16]. Ethylene plays a regulatory role in many processes such as seed germination, seedling growth, fruit ripening and flower senescence. It also responds to several external signals such as injury, pathogen invasion and stress caused by ozone. Ethylene accelerates leaf and flower senescence, while inhibitors of ethylene biosynthesis delay leaf senescence, indicating that ethylene is a key senescence promoting hormone [17]. Abscisic acid (ABA) as a very important regulating hormone can make plants overcome and adapt to all kinds of disgusting factors. ABA can regulate stomata, seed dormancy and germination, root growth and tolerance to biotic and abiotic stresses. Spraying appropriate concentration of ABA can induce the expression of *SAGs*, and effectively accelerate leaf senescence [18, 19]. The regulation of hormones on leaf senescence is not independent. Extensive cross talks exist between senescence regulating hormones.

Sugar as a signal has different physiological functions at different stages of plant development and plays a key role in the initial stage of leaf senescence. It was first discovered that the decrease of sugar concentration induced the initiation of senescence, because the decrease of sugar concentration induced the expression of some senescence associated genes (*SAGs*), while the increase of sugar concentration inhibit the expression of some *SAGs* [20, 21]. Further studies have shown that increasing sugar concentration can also induce senescence [22-24]. High concentration of sugar can reduce photosynthetic activity, when the sugar level exceeds the acceptable threshold, it will trigger leaf senescence [21]. Delayed senescence was a consequence of decreased sugar [25]. The level of sugar during leaf senescence is also affected by other factor such as nitrogen concentration, light conditions and developmental stages [9].

2.3.2. External Factor

Besides age dependent developmental progress and plant hormones, senescence of plant can be induced by external factors such as extreme temperature, nutrient deficiency, water stress, pathogen infection, and oxidative stresses which induced by ozone or UV-B [26]. Salinity stress is a major abiotic stress limiting plant growth and development. The leaf senescence induced by salt was shown to be accompanied by a decrease in chlorophyll content, reduction of photosynthetic efficiency, an elevation of H_2O_2 level, and expression of *SAGs*. Drought stress may lead to oxidative stress, which could cause leaf wilting, cell membrane damage and premature leaf senescence under severe conditions due to excessive ROS generation [27].

2.4. Plant Leaf Senescence Related Genes

Leaf senescence is a type of programmed cell death, which is associated with a series of genes activation, expression and regulation [4]. During this process, the expression of many genes which involved in metabolism and signal, especially

transcription factors and their downstream genes will be changed. A large number of senescence related genes have been cloned from Arabidopsis and rice. According to the expression during leaf senescence, they were classified as senescence-down regulated genes (*SDGs*), such as genes encoding photosynthesis related proteins, chlorophyll a/b binding proteins, and senescence-up regulated genes, also called senescence-associated genes (*SAGs*), such as genes encoding proteases and genes involved in nutrient catabolism and transporters [28]. Most of the *SAGs* could be detected at the early stage of leaf growth, and their expression increased with the development of leaf senescence. There are small parts of *SAGs*, which can be detected only at senescence stage, such as *SAG12*, *SAG13*, *LSC54* [4, 29, 30]. Arabidopsis leaf senescence transcriptome data revealed that many transcription factor families play important roles in regulation of leaf senescence, including NAC family, WRKY family, C2H2 zinc finger protein family, C3H zinc finger protein family, AP2/ERFs family, MYBs family, homebox protein family, bZIPs family and bHLHs family [31]. At present, many achievements have been obtained on the molecular mechanism of plant leaf senescence regulation. Most of them focused on the identification and functional analysis of leaf senescence related genes. Genetic engineering has also been used to delay plant leaf senescence. The researches of leaf senescence signal regulatory networks have become hotspots.

3. Leaf Senescence Related Genes in Tobacco

NtCP1 and *NtCP2* were genes encoding cysteine proteinase in tobacco. *NtCP1* was a highly senescence specific gene, which was expressed only in natural aging tobacco leaves, and cannot be induced by adverse environmental conditions. *NtCP2* was expressed in mature tobacco leaves and down regulated in senescence leaves. It was significantly down regulated after drought and high temperature treatment [32]. *NtCP23* and *MC* were also tobacco cysteine proteinase encoding genes. The expression of *NtCP23* was similar to *NtCP1*, except that it can be detected at the early stage of leaf growth and development. *MC* showed the highest expression at the early stage of leaf development, and decreased gradually with leaf senescence [33]. These protease encoding genes may be involved in the degradation of proteins during leaf senescence. Nitrogen in aging leaf cells was generally converted into glutamine through the glutamic acid synthesis cycle and transported to young leaves and reproductive organs through vascular bundle in the form of glutamine to achieve the purpose of nitrogen reuse. *NtGln1-3* was related to the reutilization of nitrogen in tobacco, encoding glutamyl ammonia synthetase, transcript of which was higher in young leaves and aging leaves, but cannot detected in mature leaves [33, 34]. In the process of senescence, the expression level of *NtPSA1*, which encoding non-catalytic subunits of 26S proteasome, was higher in activity growing tissues, and lower in aging leaves and flowers [33, 35]. *NtHIN1* and *NtH1N18* were the response factors of polyamine, a substance that can lead to mitochondrial dysfunction in tobacco. The expression level of *NtHIN1* and *NtH1N18* were significantly increased during leaf and flower senescence [36]. The Ndh complex, which was encoded by the *ndhF* gene, can increase the reduction of the electron transport protein and promote the generation of reactive oxygen species, causing the dysfunction of the chloroplasts and thus promoting senescence of the leaves. Compared with wild type tobacco, leaf senescence of *ndhF* gene knockout tobacco line was more than 30days delayed [37]. The expression of *CYP82E4*, which was a member of P450 family, encoding a protein controlling the conversion of nicotine and demethylation nicotine, was significantly up-regulated with leaf senescence [38].

4. Regulation of Tobacco Leaf Senescence

Isopentenyl-transferase plays a key role in cytokinin biosynthesis. The *IPT* gene, that encoding isopentenyl-transferasewas cloned from *Agrobacterium tumefaciens* [39]. Compared with wild type tobacco, transgenic tobacco harbored P_{SAG12}-*IPT* showed the phenotype of delayed leaf senescence, the number of flowers increased by 83.7%, the biomass increased by 40.3%, the number of seeds increased by 52.4%, but no significant difference in leaf number and plant height was observed [17]. A deeper study of P_{SAG12}-*IPT* transgenic tobacco showed that under nutrient deficient conditions, necrotic lesions were detected in old, but otherwise green leaves of unfertilized P_{SAG12}-*IPT* transgenic tobacco. The leaves of the same leaf position of wild type tobacco were yellow at the same growth stage, but no necrotic lesions appeared. The necrotic lesions were caused by the over reduction of the electron transport chain, resulting in an imbalance between light trapping and energy consumption [40]. In addition, the expression of pathogenesis-related genes *PR-1b* and *PR-Q* were significantly higher in old P_{SAG12}-*IPT* transgenic tobacco leaves with necrotic lesions [40]. Maria Pilarska et al. used the P_{SAG12}-*IPT* transgenic tobacco to prove that the lipid peroxidation was not related to leaf senescence in tobacco [41]. Christoph et al. used *Nicotiana attenuate* expressing *IPT4* from Arabidopsis, driven by the promoter of *SAG12*, proved that cytokinins in leaves were sufficient to alter ontogenic patterns of defense metabolites [42]. The maize homeobox gene *knotted1* (*kn1*) and its homologs are expressed in shoot meristems and are essential for meristem maintenance and initiation [43]. Many of the phenotypes of P_{kn1}-*IPT* transgenic tobacco were similar to those observed in P_{SAG12}-*IPT* transgenic tobacco, revealed that except inhibiting differentiation; *kn1* has effect on leaf senescence regulation [43]. Luo et al. manipulated the expression of *KN1* through a wound inducible promoter *Win3.12*, and $P_{Win3.12}$-*kn1* transgenic tobacco also showed delayed leaf senescence [44]. *BiP* gene encodes an endoplasmic reticulum binding protein.

BiP overexpressing transgenic tobacco had higher tolerance to drought stress [45]. The *CKX* gene encodes cytokinin dehydrogenase, and active CKX initially detected in tobacco tissues crude extracts. Overexpression of *AtCKX* in tobacco, the antioxidant capacity of transgenic tobacco increased significantly, even if the cytokinin level decreased significantly, the transgenic tobacco also showed obvious delayed leaf senescence [46, 47].

5. Conclusion

Tobacco is an important model plant due to its easy and high efficient transformation and regeneration, and has been used in plant biological research. Tobacco also is an economic crop for leaf use. Mature and yellow leaves are harvested for tobacco production. The yellowing process and maturity of tobacco leaves will affect the roasting and processing process after harvest, and further affect the main quality factors such as appearance quality, physical properties, chemical composition, smoke characteristics and cigarette safety. For example, precursors of tobacco aroma substances formed with the chlorophyll degradation during tobacco leaf senescence, are the material basis of tobacco leaves with different characteristics. Therefore, it is of great theoretical and practical value to further study the process and regulation mechanisms of tobacco leaf senescence. At present, the research on plant leaf senescence is mainly on the model plant Arabidopsis, as well as rice, wheat, cotton and other cash crops, but only few studies on tobacco leaf senescence. As a genetically controlled development process, leaf senescence can be regulated by gene mutation, genetic transformation and molecular marker assisted breeding. These studies have enhanced the understanding of the mechanism of tobacco leaf senescence, but there is still a long way to go to elucidate the complex mechanism of tobacco leaf senescence. Moreover, there is no in-depth study on the complex metabolic changes of tobacco leaf senescence and the changes in the expression of corresponding genes. New methods are needed to explore the senescence mechanism under special senescence mode. By identifying and cloning the key regulatory genes and molecular markers of tobacco leaf senescence, human intervention can be carried out in the process of tobacco leaf maturation, which is of great practical significance for tobacco breeding and tobacco production.

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