

# Genetics and Genomic basis of Stay-green Trait

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## ABSTRACT

Staygreen is one such trait in which genotypes possessing this trait maintain more photosynthetically active leaves (& less senescent) than genotypes not possessing this trait. Delay of leaf senescence also known as stay-green character, has been identified as an important component in the genetic improvement of several crops to promote stress tolerance and yield gain. Although the stay-green phenotype is superficially similar in all species and genotypes, the genetic and physiological routes the traits are diverse. Photosynthetically active leaves for longer period depends on the concentration of chlorophyll pigment absorbing sunlight for photosynthesis. A multi dimensional approach for studying the senescence pathway rather than studying only the physiological role made a significant role in improvement. Hence new approaches like genomics, proteomics and metabolomics studies are necessary to understand the various transcription factors involved in regulating the leaf senescence process. Therefore, this review has aimed to bring light to major aspects of the stay-green character, showing its potential use in plant breeding.

## HIGHLIGHTS

- ① Green Traits are required especially in a drought environmental condition.
- ② To keep the greenness of leaves alive for a longer period of time, especially during the grain filling stage.
- ③ To maintain or increase higher grain yield.
- ④ Stay-green results when the plant's normal process of senescence is disrupted.

**Keywords:** Stay-green, Photosynthesis, Genomics, Senescence

Plant breeding has made significant progress in providing food to the world's increasing human population by releasing more productive cultivars that demonstrate adaptation to environmental changes. Plant breeders are rethinking, investing not only in the traditional criteria, such as yield, but also in the selection of genotypes with high productive efficiency through the understanding of crop physiology and stress adaptation. Stay-green character, or the delay of leaf senescence, has been described as a key component in the genetic improvement of many crops to enhance stress tolerance and yield. The connection between stay-green and desirable traits like more fertile tillers, more grains per ear, higher industrial efficiency, and resistance to abiotic and biotic stresses has been established (Rakshit *et al.* 2020).

Stay-green genotypes have a higher potential for grain filling in preserving photosynthetic tissues, resulting in higher grain average weight. As a result of extending the ability to produce photoassimilates towards the end of maturation, the delay of senescence was also established as a major factor in increasing the average weight of grains of durum wheat mutants. As a result, the aim of this review was to shed light on key aspects of the stay-green character demonstrating its potential for use in plant breeding. Stay-green refers to a genotype in which senescence is delayed compared to a normal reference genotype. The plant's stay-

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green character is described by a longer green state in the late stages of grain filling, resulting in a senescence character. It is hypothesised that the character increases yield by increasing grain filling capacity and improving desirable traits. Increased plant photosynthetic efficiency and capacity result in these improvements, making it a valuable tool. (Thomas and Howarth 2000).

### Senescence and Stay-green

Senescence is a physiological mechanism in which nutrient stores are mobilised into fruits and seeds. Stay-green is a key trait that helps plants to keep their leaves in an active photosynthetic state even when they are stressed. Senescence influences crop plant assimilation and grain filling at an early stage. Increased green leaf area at maturity, leaf nitrogen status, and transpiration efficiency were all linked to higher grain yield in stay-green genotypes compared to senescent genotypes. The emergence and progression of senescence are phenological indicators of climate change vulnerability, implying that a better understanding of stay-green will aid in the development of potential crop forms. As a result, any defence mechanisms that delay the onset of senescence and hold leaves green are expected to increase crop yield (Hiremath *et al.* 2017). Stay-green is also one of the important parameter to assess the yield under drought condition with good root system (Hilli 2021).

### Physiological basis of the stay-green

While the stay-green phenotype appears to be the same in all organisms and genotypes, the genetic and physiological pathways through which the traits are transmitted are complex. The concentration of chlorophyll pigment that absorbs sunlight for photosynthesis determines how long photosynthetically active leaves stay active. Leaf yellowing generally results from progressive breakdown of chlorophyll during senescence. Plants assimilate carbohydrates & N<sub>2</sub> in vegetative organs (source) and remobilize them to newly developing tissue or to reproductive organs (sink) during senescence. High photosynthetic ability and efficient nitrogen remobilization during grain filling are thought to be important factors in rising grain yield. Senescence is a physiological mechanism in which

nutrient reserves and cytokinins are mobilised into fruits and seeds.

### Importance of Chlorophyll in stay-green traits

The pigment chlorophyll is responsible for the leaves' green colour. Chlorophyll is involved in photosynthesis, which results in the creation of biomass. Stay green genotypes have more photosynthetically active leaves than genotypes that do not have this trait.

Chlorophyllase (CLH) is the enzyme that converts chlorophyll a to chlorophyllide a. (Chlide a) The final biomass production would be lower if there is depletion during the reproductive stage. In addition to the catabolic chlorophyll enzymes already mentioned, the breakdown of chlorophyll necessitates an additional protein known as stay green (SGR).

Overexpression of the SGR gene causes chlorophyll degradation, suggesting that this protein is a "master" regulator of chlorophyll degradation (Armstead *et al.* 2007). Mutants that interrupt the function of chlorophyllase (CLH) cause stay-green phenotypes.

Since a leaf's durability and photosynthetic ability are linked to its nitrogen status, it's crucial to understand the role of nitrogen in extending leaf greenness in stay-green hybrids. N<sub>2</sub>, Mg<sup>+</sup><sub>2</sub>, and other constituents make up the pigment chlorophyll.

### Classes of stay green

There are five classes of stay green on the basis of time and duration of occurrence of senescence. (Zhou *et al.* 2011)

**Type A stay-greens:** Delayed initiation of senescence but then proceeds at normal rate)

**Type B stay-greens:** Initiate senescence on schedule, but there after comparatively slow)

**Type C stay-greens:** Arise due to specific defects in chlorophyll degradation pathway)

**Type D stay-greens:** Green color is maintained with leaf death)

**Type E stay-greens:** Chlorophyll content remain same but enzyme activity is reduced)

Among these, type C and D are non functional form of stay-green. And first two classes are functionally stay green.



## Non-Functional Stay-green

Senescence occurs at a normal rate, and photosynthetic potential is lost, but due to defects in the chlorophyll degradation pathway, leaf colour is preserved (Thomas and Howarth 2000). The luxury form of stay green is bad for the plants because it promotes the growth of vegetative sections of the plant rather than grain yield. Functional stay green, on the other hand, is beneficial when photosynthetic products are moving from source (leaf) to sink (grain) under stress conditions.

Variable measurements to differentiate stay-green and non stay-green lines:

- ♦ Live root length density(LRLD) – max length/unit soil volume-SG genotype
- ♦ Green leaf area index (GLAI) - depicts delay in senescence
- ♦ Leaf Zeatin contents – involved in control of senescence
- ♦ Intensity of light incident on leaves to know time of onset of senescence

## Genetics and genomic basis :-

Multi dimensional approach to understand leaf senescence:

- ♦ Leaf senescence is caused by both external and internal causes, resulting in a typical senescence or degradation pathway. As a result, rather than focusing solely on the physiological role, a multi-dimensional approach to studying the senescence pathway is needed. Hence new approaches like genomics, proteomics and metabolomics studies are necessary to understand the various transcription factors involved in regulating the leaf senescence process.

## The NAC Domain and DNA Binding

- ♦ X-ray crystallography helps to determine NAC Domain structure from Arabidopsis ANAC019.
- ♦ In the major groove of binding site have a conserved amino sequence by forming beta sheet.
- ♦ DNA binding specificity is based on microarray binding for NAC proteins.
- ♦ NAC proteins further be separated into clusters

on the basis of DNA binding preferences which largely match the phylogenetic relationship of the proteins

## Regulation of leaf senescence by nuclear and chloroplast signals:-

The nucleus and chloroplasts are involved in controlling the leaf senescence process, as seen in this diagram. Positive transcription factors induce SAGs to trigger senescence by sending information to chloroplast, while negative transcription factors prevent SAGs from causing senescence. Similarly the retention of this chlorophyll activity is due to inhibition of these SAG's to cause senescence by transferring signals to nucleus. Thus both nucleus and chloroplast signals are involved in regulating the senescence process.

- ♦ The leaf senescence process is highly regulated genetic program which is associated with drastic changes of gene expression.
- ♦ Many senescence-associated genes (SAGs) are transcriptionally upregulated during leaf senescence.
- ♦ Arabidopsis DNAs has shown changes in the expression of about 20% of the genes during leaf senescence (Li and Guo 2014).

## The Zip transcription factor HAHB4 is up-regulated in darkness

HAHB4 is a member of the sunflower Zip protein family and is involved in drought tolerance and ethylene-mediated senescence (the genes ATHB7 and ATHB12 in Arabidopsis bear the most resemblance). The expression of HAHB4 is induced in darkness and quickly disappears when plants are exposed to light. To find out whether this transcription factor is involved in the transcriptional down-regulation of a large group of photosynthesis-related genes, researchers expressed HAHB4 DNA in sunflower leaf discs and used iRNA to knock it out (Moschen *et al.* 2017).

## Importance of stay green trait

- ♦ Delayed senescence is useful only when it contributes to increase yield.
- ♦ Carbohydrate assimilation is higher in stay-green genotype than senescent hybrids.



- ◆ In some crop it leads to drought resistance.
- ◆ Sometimes it also leads to disease resistance (e.g., CsSGR gene- anthracnose resistance in cucumber due to mutation).
- ◆ It leads to lodging resistance.
- ◆ Physical and chemical treatment of mutagen & silencing of gene approaches for development of stay green trait in crops for better yield under stressed environment.

### Limitations in Utilization of Stay-green trait

- ◆ Genetic mechanisms of Stay-green trait in crop plants are poorly understood.
- ◆ Evaluation of the stay green response is difficult.
- ◆ Progress in improving drought tolerance by conventional breeding methods is slow.
- ◆ Variation in flowering time complicates conventional selection.
- ◆ Selecting stay-green with retaining local adaption( grainqlty, pest/disease adaption).

### CONCLUSION

Several stay-green QTL have been identified in Several crops like sorghum, wheat, rice etc. Incorporation of stay-green trait in a genotype will increase the ultimate grain yield and market value which will contribute to our national economy. The use of stay green trait in breeding programmes may result in significant genetic progress for attributes such as high yield, industrial quality, disease resistance and tolerance to abiotic stresses. There is a need to integrate genomic, transcriptomic, proteomic, metabolomic, and phenomic studies for in-depth understanding of the molecular nature of leaf senescence rather than only physiological analyses.

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