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Breeding to Enhance Stay Green Traits

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Introduction

What is Stay green? The ability of a plant to postpone/delayed senescence and retain their leaves in the active photosynthetic condition. The extended foliar greenness during grain filling and delayed senescence will maintain more photo synthetically active leaves (Xu *et al.*, 2000). Therefore, we expected to give a higher production and productivity of grain as well biomass under biotic and abiotic stress condition.

Senescence and Stay green: The term **'stay green' (SG)** is given to a variant in which transition from the period of C capture to that of N remobilization corresponds to the functional initiation of senescence is delayed. So, that rate of senescence determines the maintenance of chlorophyll and hence photosynthates for sink formation.

Stay green traits refer to a set of characteristics exhibited by certain plant varieties that enable them to maintain their green color and delay the process of senescence, or aging, even under adverse environmental conditions. These traits are crucial for plants as they allow them to sustain photosynthesis and maintain physiological functions for a longer period, even when facing stresses like drought, heat, or disease.

The stay-green character is characterized by a longer green state of the plant in the late period of grain filling, establishing a senescence pattern in which leaves and stem are the last parts to lose photosynthetic ability, providing greater production of sugars from photosynthesis (Silva *et al.*, 2008). Based on the increase of grain filling ability and improvement of desirable traits, it is suggested that the character results in yield gains. These gains are a consequence of increased plant photosynthetic efficiency and ability, making it an important tool (Parry *et al.*, 2010).

Where, when and why stay green traits is required?

- Stay green required specially in a drought (Rosenow *et al.* 1983) and heat stress (Wahid *et al.* 2007) environmental condition.
- To keep greenness of leaves alive for longer period of time, especially during the grain filling stage (Spano *et al.* 2003).
- To maintain or increase higher grain yield.

Key features associated with stay green traits include:

1. Delayed Senescence: Stay green plants exhibit a slower rate of leaf aging compared to non-stay green varieties. This delayed senescence allows the plants to retain functional leaves for an extended period, contributing to sustained photosynthesis and higher productivity.

2. Chlorophyll Retention: Stay green plants maintain higher levels of chlorophyll in their leaves during senescence. Chlorophyll is essential for photosynthesis, and its retention helps to maintain the plant's ability to capture light energy and convert it into chemical energy.

3. Sustained Photosynthetic Activity: Despite facing environmental stresses, stay green plants are capable of sustaining photosynthetic activity in their leaves for longer durations.

This allows them to continue producing sugars and other metabolites necessary for growth and development.

4. Improved Stress Tolerance: Stay green traits are often associated with enhanced stress tolerance, including tolerance to drought, heat, salinity, and various biotic stresses. The ability to maintain greenness and physiological activity under stress conditions contributes to the plant's survival and productivity.

5. Yield Stability: Stay green varieties tend to exhibit greater yield stability across variable environmental conditions. By maintaining photosynthetic activity and functional leaves, these plants can mitigate yield losses caused by environmental stresses, resulting in more consistent yields over time.

Overall, stay green traits play a significant role in plant adaptation to environmental challenges and can contribute to the development of more resilient and productive crop varieties. Breeding programs often target the enhancement of stay green traits to improve crop performance, stress tolerance and agricultural sustainability.

Physiological basis of stay green traits

- Leaf greenness depends on the concentration of chlorophyll pigment absorbing sunlight energy for photosynthesis.
- Leaf yellowing generally results from progressive breakdown of chlorophyll during senescence.
- Plants usually assimilate carbohydrates and N_2 in vegetative organs (source) and remobilize them to newly developing tissue or to reproductive organs (sink) during senescence.
- Senescence is a physiological process where there is a mobilization of nutrient reserves and cytokinin into fruits and seeds.
- Persistence of high photosynthetic capacity and efficient N_2 remobilization during grain filling have been considered as key factors in increasing grain yield.
- Genotypes possessing stay green trait maintain more photosynthetically active leaves than those not possessing this trait.

Types of stay green

1. Functional stay green: Alteration of the genetic processes determining the initiation of senescence and its rate of progress results in a phenotype which continues to photosynthesize for longer than normal (*i.e.* is **functional stay green**) and which might therefore be expected to result in a higher yield.

2. Non-functional/Cosmetic stay green: In nonfunctional stay green mutants, senescence occurs at normal rate and photosynthetic capacity is lost, but leaf colour is retained due to defects in chlorophyll degradation pathway (Thomas and Howarth, 2000).

Classes of stay green

Five distinct types of stay-green plants have been reported where the occurrence of distinct physiological and genetic modifications can be detected, but commonly two or more types of stay green can be observed combined (Thomas and Howarth, 2000).

Type A occurs when the leaves and stems maintain their photosynthetic area active for a longer period of time, experiencing a delay in plant senescence.

For **type B**, senescence occurs in the normal period of plant development, but it occurs relatively slowly.

For **type C**, also known as cosmetic stay-green, there is an accumulation of pigments on the surface of the organ, giving the impression that there is a reduction of senescence. However, the rate of degradation of protein and chlorophyll occurs normally below the green surface.

Type D is recurrent in the herbaria and freezing of vegetables, in which the green color is maintained with leaf death via freezing, boiling or drying.

Type E is described as the one with the highest content of chlorophyll in photosynthetic tissues, and that increased concentration results in a delay in yellowing of leaves and stems (similar to type A) and maintenance of green tissue, even with the reduced ability of fixing carbon dioxide.

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

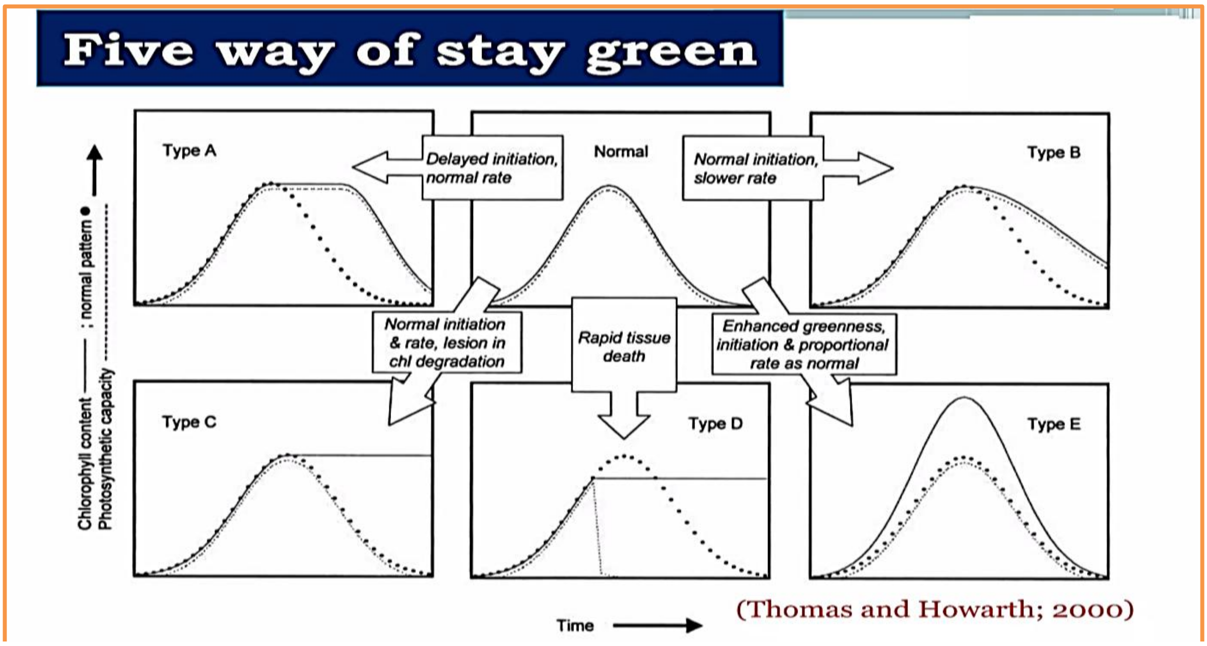
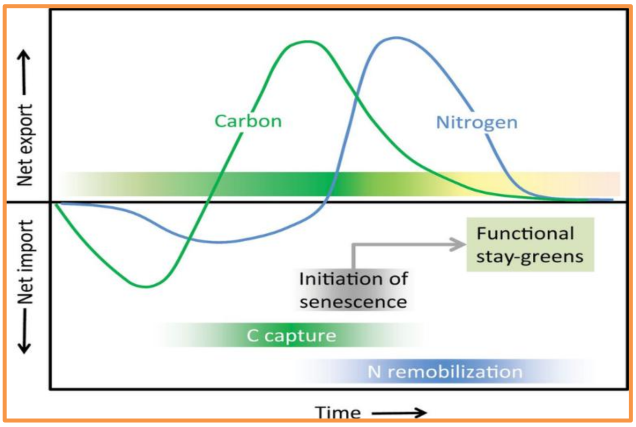


Figure 1. Five ways to stay-green. Curves show chlorophyll content and photosynthetic capacity (arbitrary scale) for a representative leaf, whole plant or canopy

Carbon capture/ Nitrogen remobilization and stay green

Figure 2: The functional stay green traits is associated with the transition from the carbon capture to the nitrogen mobilization phase of foliar development.



An individual leaf starts life as a sink for organic carbon (C), nitrogen (N) and other nutrients as its structure is built and its assimilatory apparatus is developed. It then becomes a net contributor of photosynthate to the plant as a whole. The C-capture phase of leaf function is succeeded by a phase of net organic N remobilization. C and N export cease in the terminal phase of leaf death (Fig. 2). The transition from the period of C capture to that of N remobilization corresponds to the functional initiation of senescence. The leaves of a plant population, aggregated into a canopy, also go through C-capture and N-remobilization phases, although there are scaling issues that need to be considered when extrapolating results from laboratory to field (Thomas and Ougham, 2014). Functional stay-greens are genotypes in which the C–N transition point is delayed, or the transition occurs on time but subsequent yellowing and N remobilization run slowly (Thomas and Howarth, 2000, Fig. 2).

Genetics of the stay green traits

- The wheat stay green character govern by four recessive genes that are segregated independently and interacted in an additive manner (Joshi *et al.*, 2007).
- In rice, this is governed by recessive mutant gene *sgr*(t) on chromosome 9 (Cha *et al.*, 2002).
- In Arabidopsis it is also govern by recessive gene *fiw* on chromosome 4 (Nakamura *et al.* 2000).

Conclusion

Genes related with stay-green traits

Protein	Gene	Mutant phenotype	Function
Stay-green	*SGR* *NYE1* *SID*	*sgr* = stay-green	Binding LHCII and catabolic enzymes, stabilising catabolic complex
Chlorophyll b reductase	*NYC*	*nyc* (rice and *Arabidopsis*) = stay-green	Ferredoxin/NADPH-dependent two-step conversion of chlorophyll *b* to chlorophyll *a*
	NOL	*nol* (rice, but not *Arabidopsis*) = stay-green	
	HCAR	*hcar* = cell death, not stay-green	
'Mg dechelatase'	Identity not yet resolved	?	Removal of Mg from the macrocycle (not known whether reaction is enzymic or chemical)
Phaeophytinase	*PPH* *CRN1* *NCY3*	*pph* = stay-green	Dephytylation of phaeophytin
Phaeophorbide a oxygenase	*PAO* *ACD1* *LLS1*	*acd1* = cell death, not stay-green	Ferredoxin-dependent oxidative opening of macrocycle to form RCC
RCC reductase	*RCCR* *ACD2*	*acd2* = cell death, not stay-green	Ferredoxin-dependent reduction of RCC to pFCC

Source: Thomas and Oughum (2014)

Breeding to enhance stay green traits in crops is crucial for improving drought tolerance and ensuring stable yields under stress conditions. By maintaining green leaf area during critical growth periods, stay green varieties can optimize photosynthesis, leading to better resource use and enhanced resilience against climate challenges. Advances in molecular breeding techniques enable efficient selection for these traits, offering a pathway to develop varieties that can survive in increasingly variable environments. Ultimately, stay green traits supports sustainable agriculture and food security, making it essential for future breeding programs.

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