SEMINAR REPORT

Stay green genes: A potential tool in plant breeding

By

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DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF HORTICULTURE

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CERTIFICATE

This is to certify that the seminar report entitled **"Stay green genes: A potential tool in plant breeding"** has been solely prepared by **Alfiya A R (2018-11-161)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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I, Alfiya A R (2018-11-161) declare that the seminar entitled **"Stay green genes: A potential tool in plant breeding"** has been prepared by me, after going through various references cited at the end and has not been copied from any of my fellow students.

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1. Introduction

Plant breeding has made great contribution through supply of food to the growing human population by the release of more efficient cultivars, which show adaptation to varying environmental conditions. In current agricultural scenario, there is a continuous increase in demand for agricultural production coupled with rapid climate change. Hence, plant breeders are rethinking of investing not only in the traditional criteria such as yield, but also in the selection of genotypes with high productivity unaffected by the climate change. This can only be achieved by a thorough understanding of crop physiology and stress adaptations. 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.

The association between stay-green and desirable traits such as greater number of fertile tillers, higher number of grains per ear, higher industrial quality, tolerance to abiotic and biotic stresses have been reported. However, maintenance of grain filling in the last stage of plant maturity has been considered as key to the success of stay-green genotypes. A greater capacity for grain filling in maintaining photosynthetic tissues of stay green wheat genotypes has been observed, resulting in increased average weight of grains.

What is stay green?

Stay-green is the term given to a variant in which senescence is delayed in comparison to a standard reference genotype. 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 loose photosynthetic ability, providing greater production of sugars from photosynthesis. 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. Moreover, greater ability to tolerate stresses such as high temperatures and drought was identified in stay-green genotypes, as a result of protection of photosynthetic structures to reactive oxygen species, such as superoxide and peroxide.

2. Classes and types of stay-greens

2.1 Classes of stay greens

Five distinct classes 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 & 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.

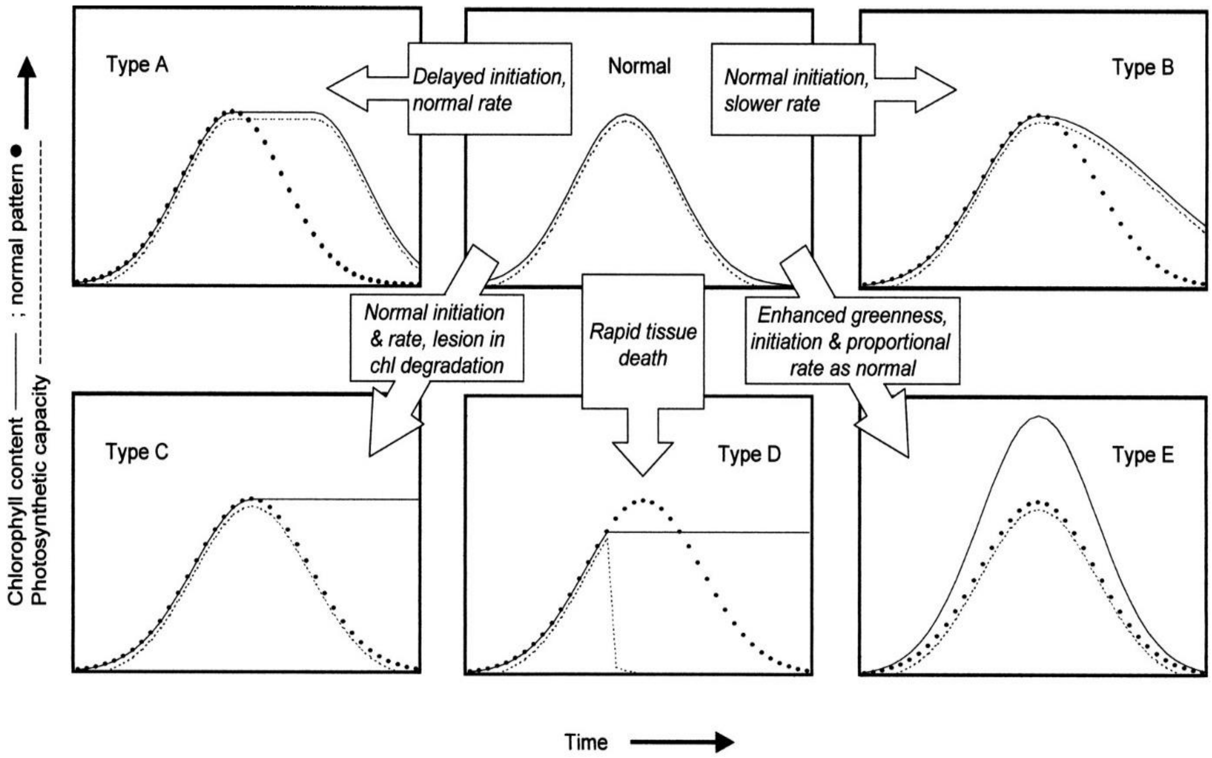


Fig 1: Classes of stay-greens

2.2 Types of stay-greens

- **Functional stay-green :** Alteration of genetic processes determining the initiation of senescence and results in a phenotype which photosynthesize for longer than normal **(**Type A, B & E).
- **Non functional stay-green :** Leaves remain green due to retention of chlorophyll resulting from lesions in its catabolism, but lack photosynthetic competence (Type C & D)

3. Physiology of stay-green character

Senescence is a physiological process where there is a mobilization of nutrient reserves and cytokinins into fruits and seeds. This translocation leads to reduced RNA synthesis, causing changes in gene expression and thereby reducing protein synthesis, resulting in the decline of the photosynthetic capacity and cellular disorganization, leading to plant death. The main objective of senescence is the remobilization and recycling, so that developing tissues are resourced in the detriment of senescent tissues.

4. Physiological features of stay-green plants

Kamal *et al* (2019) described the physiological features of stay- green plants that make them more adaptable to the adverse conditions.

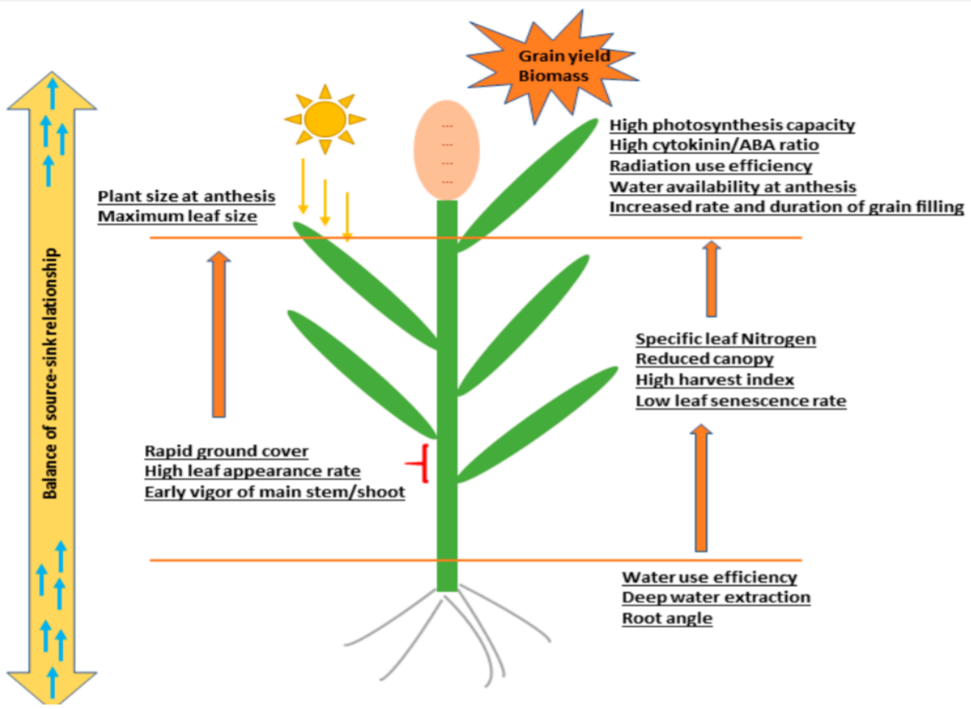


Fig 2: Physiological features of stay-green plants

5. Other factors influencing stay-green nature

Apart from these, factors like hormones, low accumulation of reactive oxygen species and chlorophyll metabolism also contribute to the expression of stay-green nature.

5.1 Stay green and hormones

It is a fact that senescence is associated with the balance between hormones such as cytokinins and ethylene, and the over expression or suppression of these hormones showed changes in the timing of senescence, accelerating and retarding the process . In transgenic tobacco plants, which were induced for high cytokinin production, an association between higher hormone contents and higher chlorophyll and nutrient contents and maintenance of photosynthesis in older tissues, reduced the degenerating effects of senescence (Fig 3) (Jordi *et al.*, 2000). The application of cytokinin 6-benzylaminopurine (BAP) to wheat plants, promoted delay in plant senescence, associated with a reduced chlorophyll and soluble protein degradation rate, besides sustaining stomatal aperture and conductance and guaranteeing the maintenance of the absorption rates of CO2 .

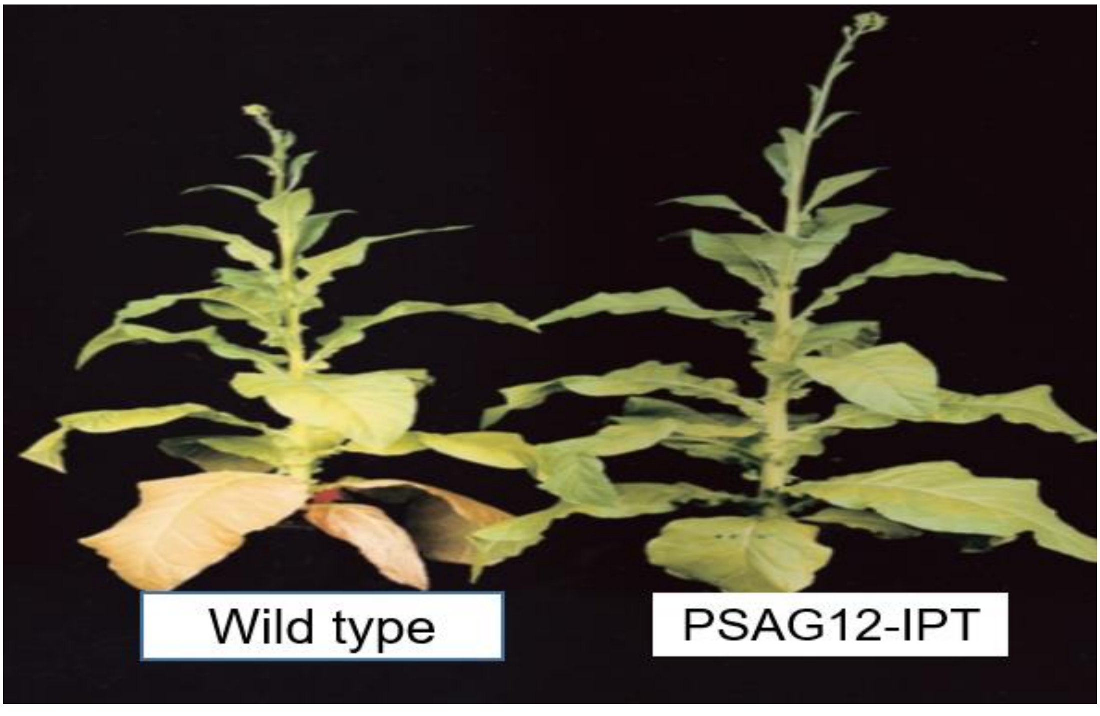


Fig 3: Wild type & transgenic tobacco plant (induced with high cytokinin production)

5.2 Reactive oxygen species and stay-green

The senescence of tissues along with the remobilization of nutrients to younger or strategic tissues for survival / perpetuation of the plant, decreases photosystem II (PSII) activity and is associated with an increased content of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), a marker molecule for senescence. Low accumulation of reactive oxygen species such as superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) were found in mutant stay-green wheat (tasg1) compared with wild types subjected to drought stress, providing greater stability of the proteins found in the walls of thylakoids (Tian *et al*., 2013) (Fig 4). The protection of the photosynthetic apparatus of chloroplasts, such as the maintenance of photosystem II (PSII) and control of content of reactive oxygen species, was also indicated as a major contribution to slowing the degeneration of tissues in genotypes wheat with functional stay-green character .

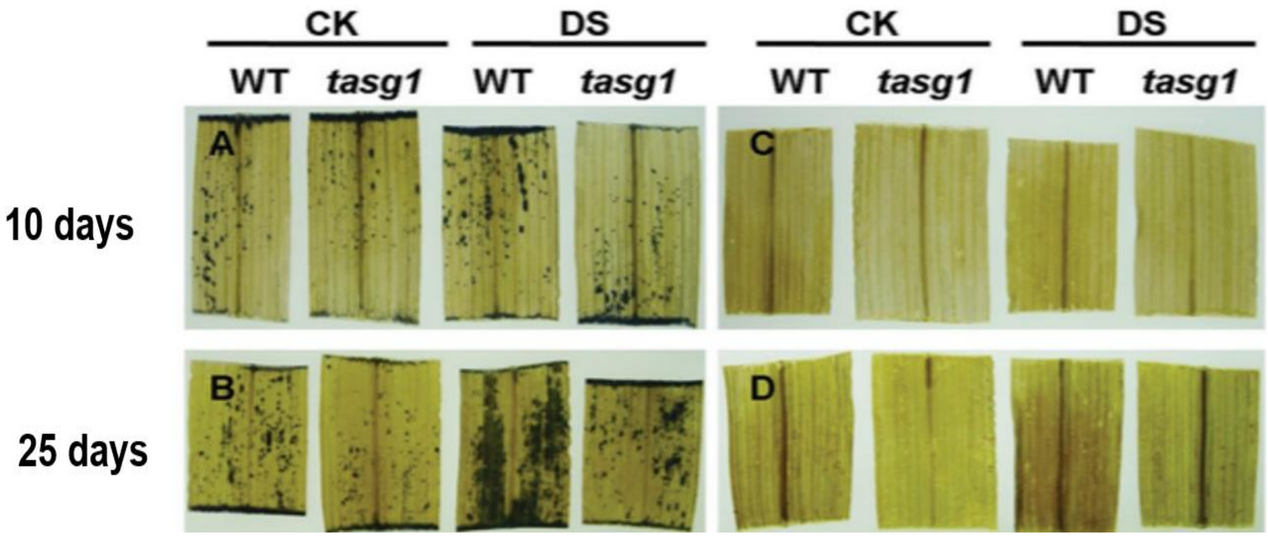


Fig 4: Change in superoxide and peroxide accumulation in wild and stay-green mutant in wheat

CK: Control

DS: Drought stress

WT: Wild type

tasg1: Wheat stay-green mutant

A & B: Change in O_2^- at early and later stages of drought

C & D: Change in H_2O_2 at early and later stages of drought

5.3 Chlorophyll metabolism and stay-green

Chlorophyll is the major pigment in plants that is responsible for the greenness of leaves that are prime site for photosynthesis that ultimately results in biomass production. During chlorophyll catabolism, chlorophyll b will be converted to chlorophyll a which will be further reduced to phein aby the metal chelating substance(MCS) and further to pheide a by pheophytinase (PPH) enzyme that is further reduced to primary fluorescent chlorophyll catabolite(pFCC) by pheide a oxygenase (PAO) and red chlorophyll catabolite reductase (RCCR). pFCC will be further modified or hyper modified into non fluorescent chlorophyll catabolite(NCC) and stored in vacuole. When stay green genes like SGR, NYC1 or NOL are present they interfere with any of these steps in degradation pathway and causes delayed senescence (Horstensteiner and Krautler, 2011).

5.4 Chlorophyll catabolism

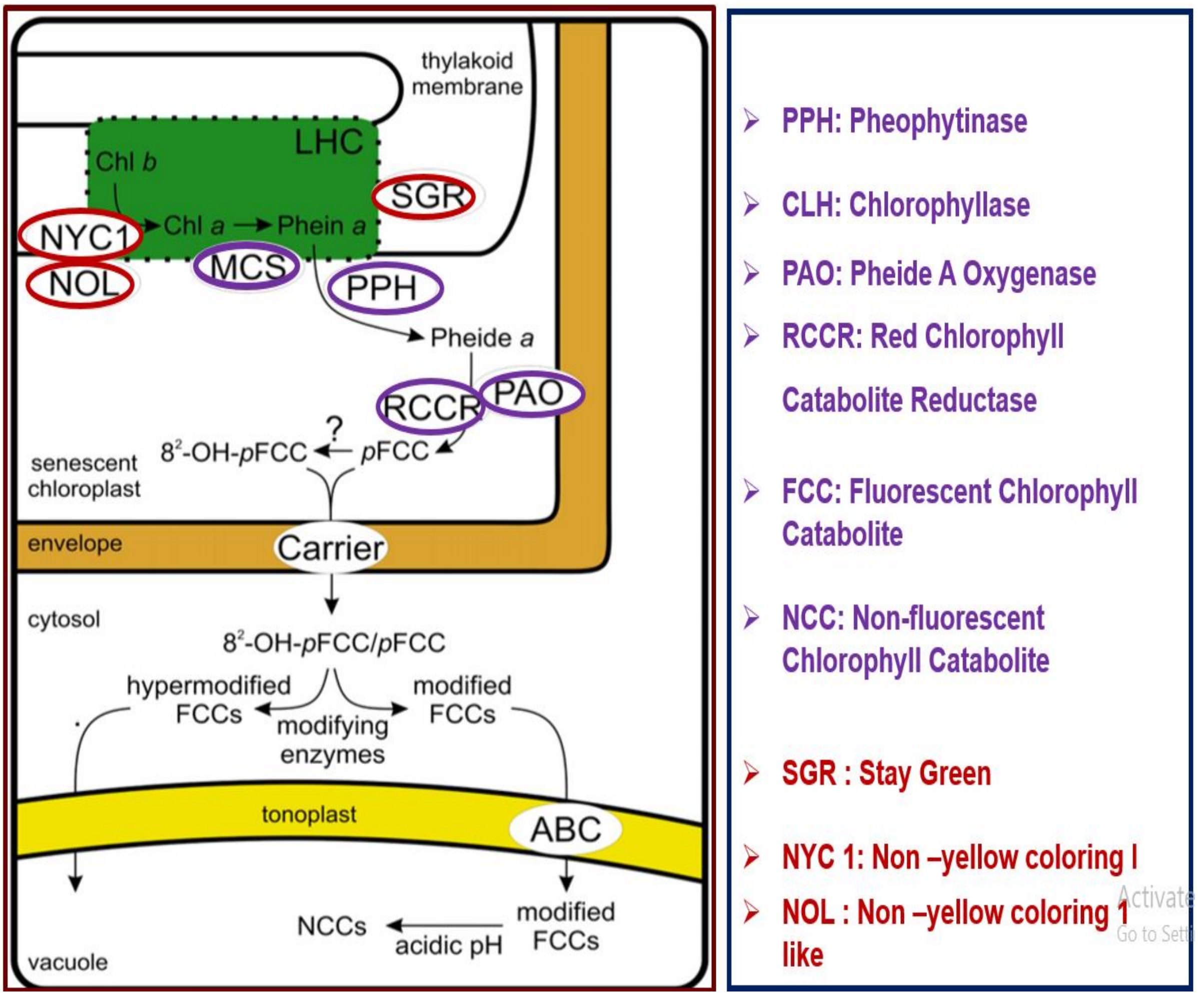


Fig 5: Chlorophyll degradation pathway

5.41 Known plastid-located components of the chlorophyll degradation pathway and consequence of their disruption for expression of the stay-green character

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 NOL HCAR	nyc (rice and Arabidopsis) = stay-green nol (rice, but not Arabidopsis) = stay-green hcar = cell death, not stay-green	Ferredoxin/NADPH-dependent two-step conversion of chlorophyll b to chlorophyll a
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

Table 1: Known plastid-located components of the chlorophyll degradation pathway and consequence of their disruption for expression of the stay-green character

Jiang *et al* (2007) studied molecular cloning and functional analysis of stay green gene in rice and found that there is higher pigment retention in the stay-green mutant than the wild type after five days of dark induced senescence.

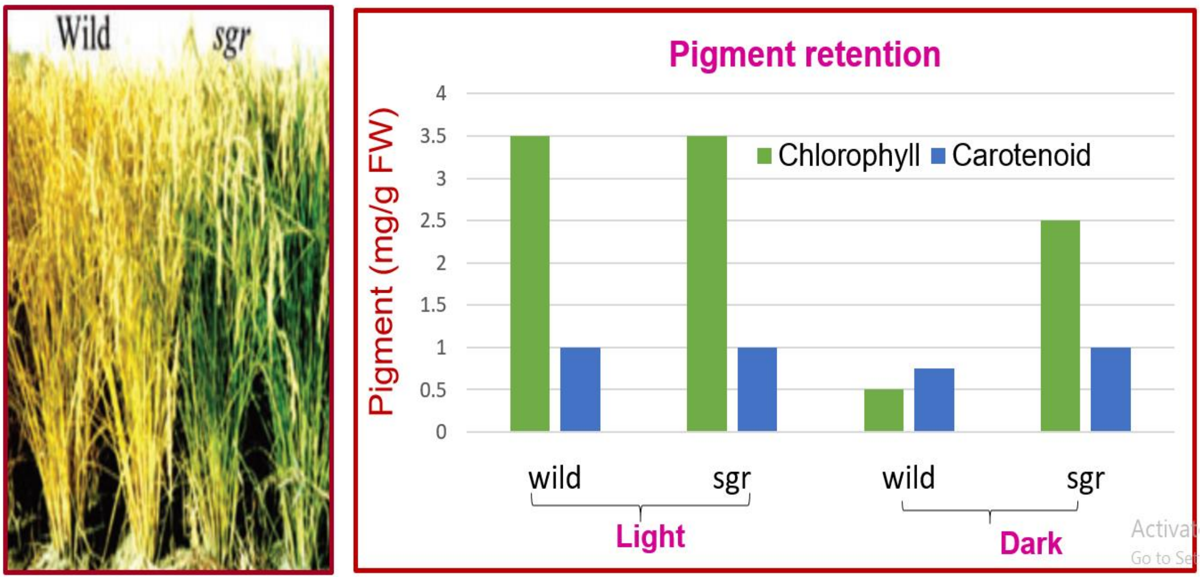


Fig 6: Pigment retention in wild and stay-green mutant in rice in normal condition and after five days of dark induced senescence

6. Stay green genes/QTLs identified

Crop	**Identified genes/QTL**	**Reference**
Rice	46 main QTLs localized in 25 chromosomal regions	Jiang *et al.* (2004)
Garden pea	*PsSGR*	Sato *et al.* (2007)
Maize	14 QTLs	Zheng *et al*. (2009)
Wheat	*QSg.bhu-1A,QSg.bhu-3B* and *QS g.bhu-7D*	Kumar *et al*. (2010)
Sorghum	*Stg1,Stg2, Stg 3* and *Stg4*	Reddy *et al.* (2014)
Soyabean	*Gm SGR 1*	Shi *et al*. (2016)
Pakchoi	*Brnye1*	Wang *et al.*(2018)

Table 2: Stay-green genes or QTLs identified

7. Exploitation of stay green genes

Stay-green character can be utilized in crop improvement due to their contribution to following characters

- Higher yield
- Higher biomass production
- Increased drought tolerance
- Increased disease resistance
- High temperature resistance
- High salt tolerance
- Reduced incidence of postharvest yellowing
- Extended shelf-life

8. Case studies

8.1 Stay green for higher yield under drought

Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns

Borrell *et al* (2014) studied the influence of stay-green allele in enhancing grain yield in sorghum under drought conditions. Stay-green is an integrated drought adaptation trait characterized by a distinct green leaf phenotype during grain filling under terminal drought. They used sorghum (*Sorghum bicolor*), a repository of drought adaptation mechanisms, to elucidate the physiological and genetic mechanisms underpinning stay-green. Near-isogenic sorghum lines (cv RTx7000) were characterized in a series of field and managed-environment trials (seven experiments and 14 environments) to determine the influence of four individual stay-green (Stg1–4) quantitative trait loci (QTLs) on canopy development, water use and grain yield under post-anthesis drought. The Stg QTL decreased tillering and the size of upper leaves, which reduced canopy size at anthesis. This reduction in transpirational leaf area conserved soil water before anthesis for use during grain filling. Increased water uptake during grain filling of Stg near-isogenic lines (NILs) relative to RTx7000 resulted in higher post-anthesis biomass production, grain number and yield. Importantly, there was no consistent yield penalty associated with the Stg QTL in the irrigated control. These results establish a link between the role of the Stg QTL in modifying canopy development and the subsequent impact on crop water use patterns and grain yield under terminal drought.

8.11 Materials and methods

- Materials : 36 genotypes[34 NILs & parents *i.e.*, BTX642 (stay-green type) and RTX7000 (non stay-green type)]
- Only 4 NILs each with *stg 1*,*stg 2*,*stg 3* ,*stg 4* QTLs considered
- Method : Biomass sampling

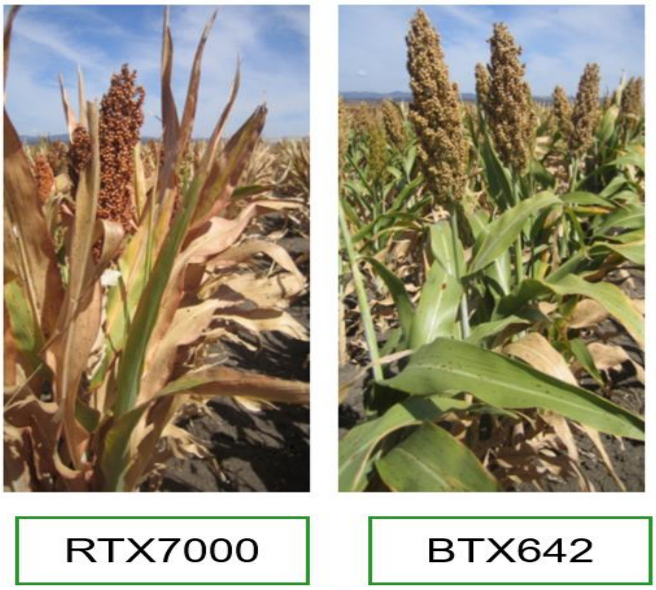


Fig 7 : Parental plants under study *i.e.*, RTX7000 (non stay-green type) and BTX642 (stay-green type)

Fig 8 : Field view under study location

When drought stress was imposed, wild type (non stay-green type) dried off without completing its grain formation while stay-green lines were able to survive and resulted in grain formation.

8.12 Cumulative water use pattern

It was found that the stay-green lines conserved the water before anthesis and utilised them during grain filling period during the drought stress.

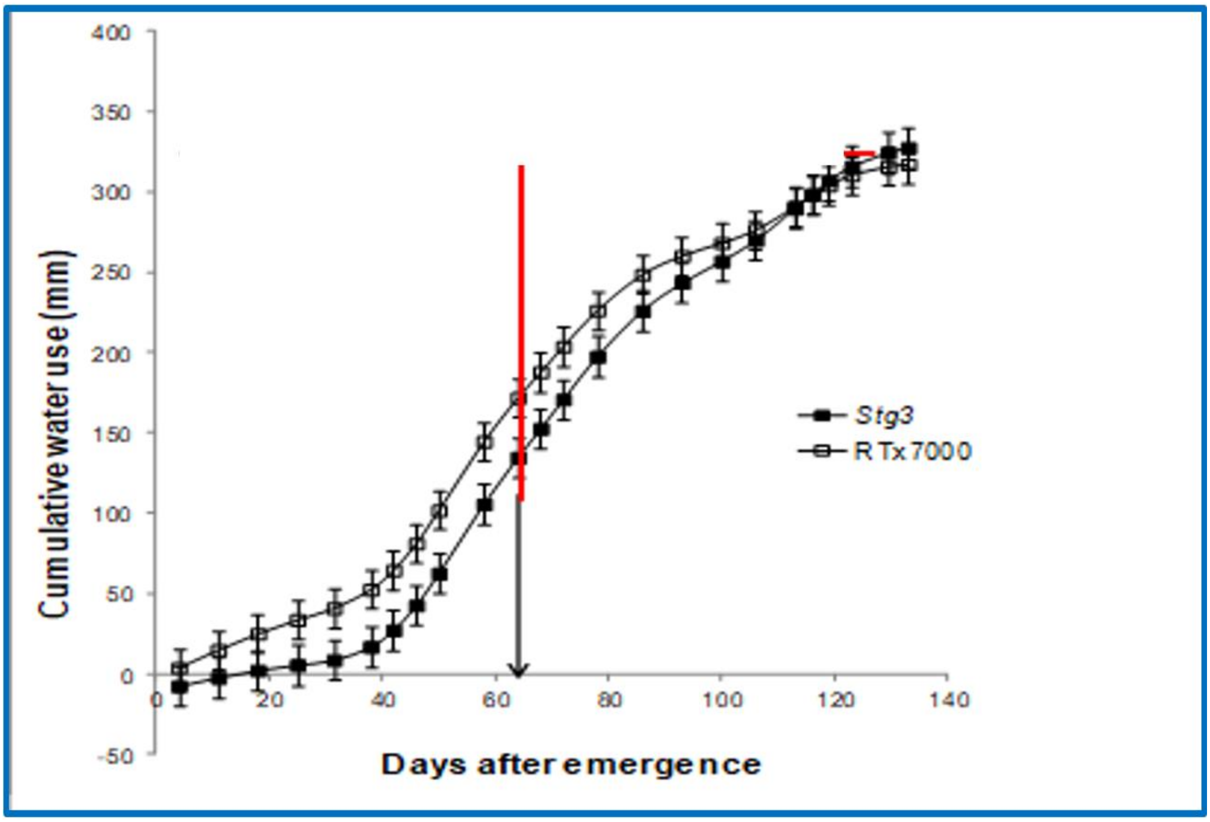


Fig 9 : Cumulative water use pattern of stay-green line and non stay-green parental line

Stg3 uses less water than RTx7000 before anthesis and more water after anthesis. The temporal pattern of cumulative crop water use for RTx7000 (open squares) and *Stg3* (filled squares) grown under a low water high-density treatment in a rain-out shelter study. The black vertical arrow marks anthesis.

8.13 Grain yield under drought stress

The study revealed that all the stay-green NILS produced higher yield compared to the non stay-green parental line.

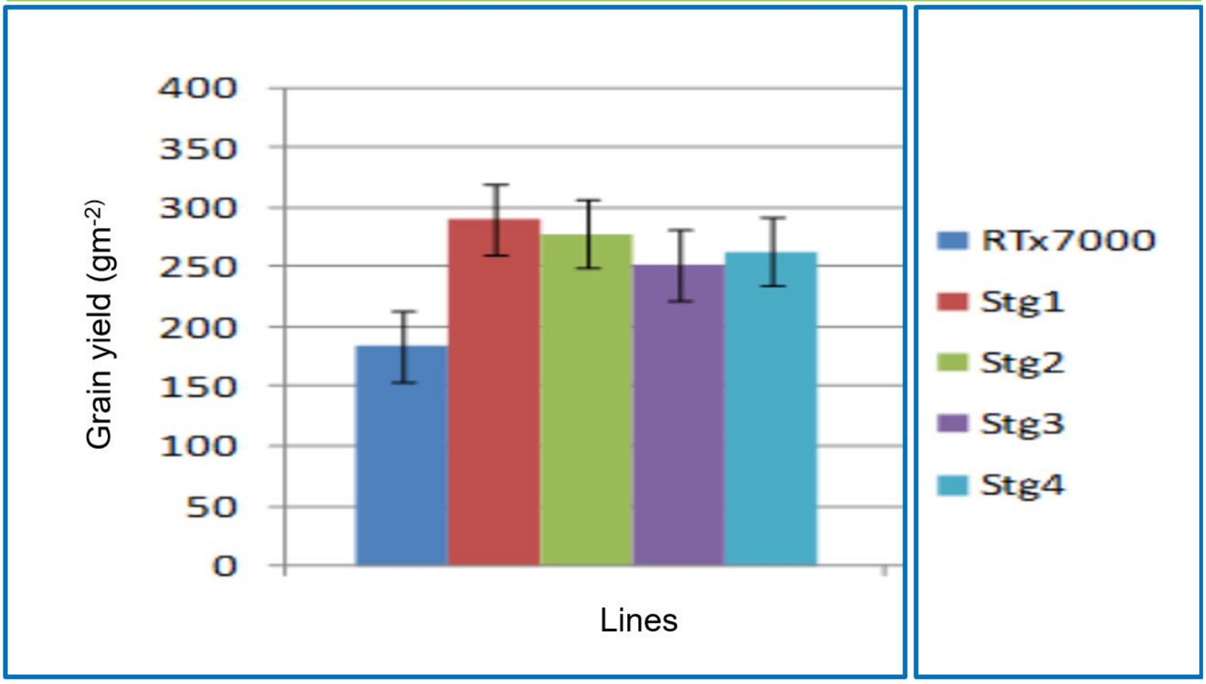


Fig 10 : Yield comparison between the stay-green lines and the non stay-green parental line under drought condition

8.2 Stay-green for heat resistance

Association of stay-green trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.)

Kumar *et al* (2013) studied the association of stay-green trait and yield traits under terminal heat stress

8.21 Materials and methods

- Evaluation of 963 germplasm lines for stay-green trait
- Evaluation of contrasting lines for terminal heat tolerance
- Genetic association studies
- ✓ Production of F_1: Stay-green x non-stay green .

To confirm the association of the stay-green trait with the terminal heat stress, stay-green lines Chirya 3, Chirya 1, Chirya 7 and Ning 8204 were crossed with the non stay-green (NSG) cv. Sonalika.

- ✓ F_1 seeds were multiplied in an off-season nursery to obtain F_2 populations. The F_3 generation was obtained by harvesting space-sown random F_2 plants. The F_4, F_5, F_6 and F_7 generations were derived by growing small seed samples of each line and by harvesting one random plant from each line in each generation.
- ✓ Evaluation of F_7 by classifying them as stay-green & non stay-green and observed under heat stress .

Fig 11: Field view under study in wheat

8.22 Results

There was a decrease in yield when the seeds were sown in late and very late sowing dates than the timely sowing since late and very late coincided with heat stress during their anthesis period.there was a decline in grain yield but the decline was less in stay-green lines comparing to non stay-green lines. Similar trend was observed for biomass production also .

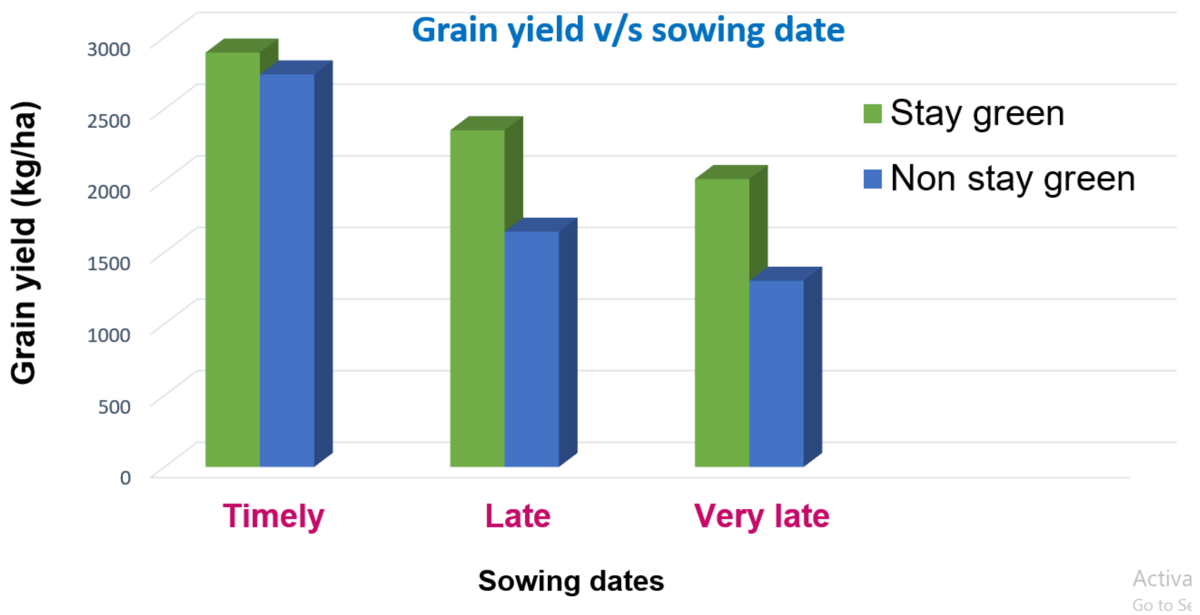


Fig 12 : Grain yield v/s sowing date

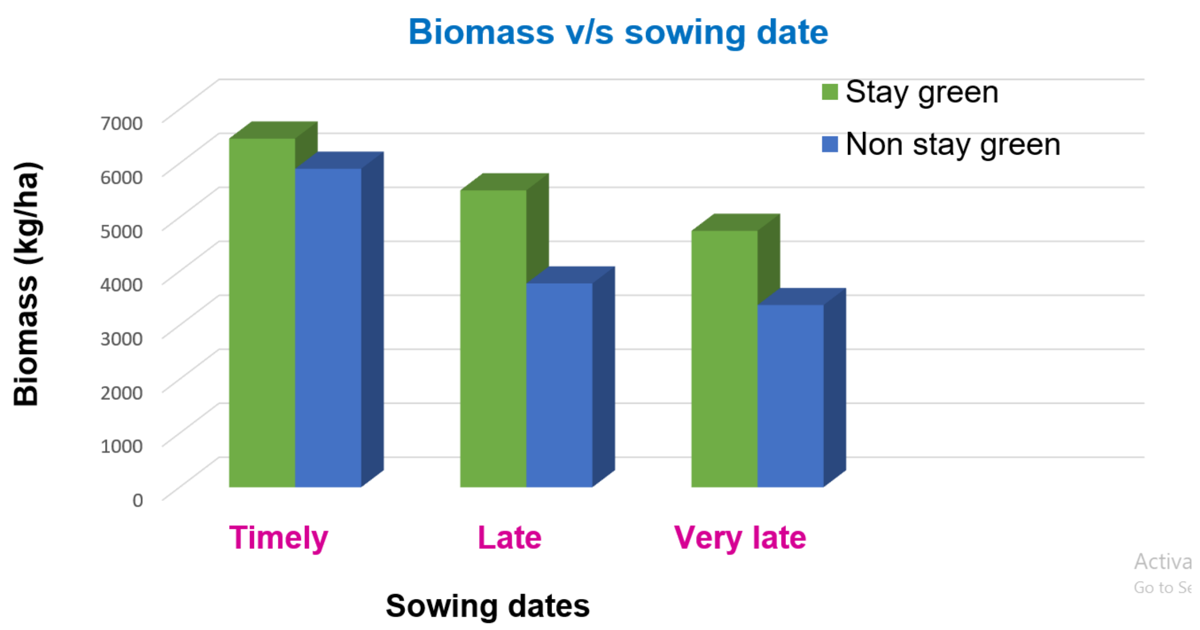


Fig 13 : Biomass v/s sowing date

8.3 Stay-green for disease resistance

STAYGREEN, STAY HEALTHY: a loss-of-susceptibility mutation in the STAYGREEN gene provides durable, broad-spectrum disease resistances for over 50 years of US cucumber production

Wang *et al* (2018) studied the influence of stay-green on disease resistance in US cucumber.

8.31 Materials and methods

8.311 Materials

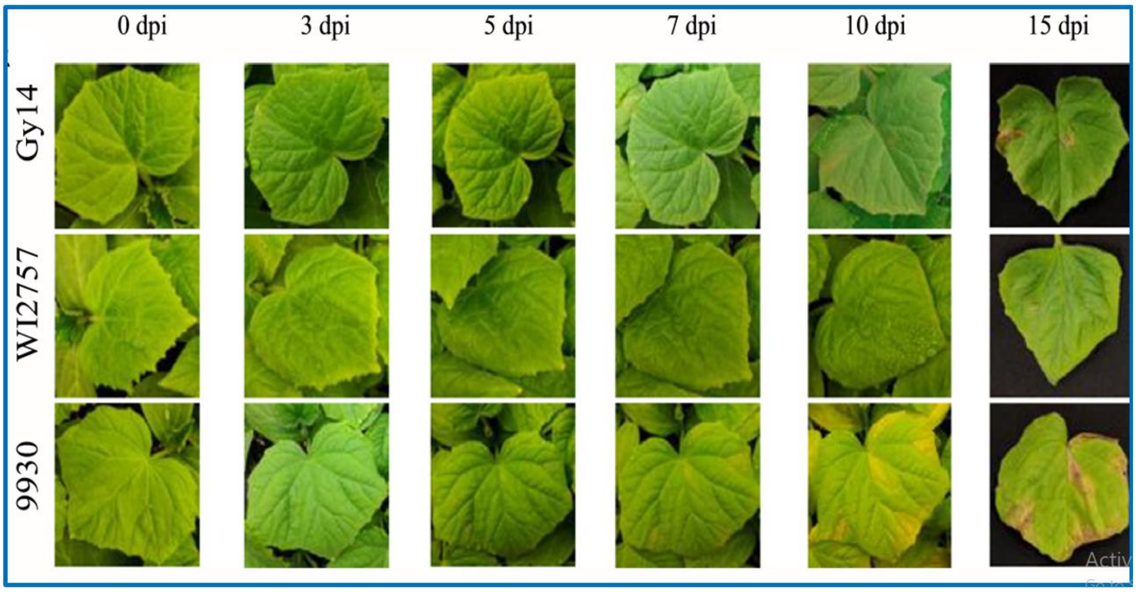
- Gly 14 and W12757: Moderately susceptible to downey mildew
- 9930: Wild type

8.312 Methods

- QTL Analysis
- Phylogenic Analysis
- Local Association Analysis
- Expression Analysis

8.32 Expression of downey mildew symptoms on inoculation

The expression of symptoms were comparatively less in the moderately resistant lines comparing to the susceptible wild type.



dpi: days post inoculation

Fig 14 : Downey mildew symptoms expression

8.33 Map-based cloning of the cucumber dm1 locus

Fine mapping with 504 cucumber Gy149 9930 recombinant inbred lines (RILs) (G9RILs) delimits the dm1 locus into a 93.7-kb region (a). Closed and open rectangles indicate alleles derived from resistant Gy14 and susceptible 9930, respectively. The nine haplotypes from 18 recombinants in the candidate gene region showed the same inoculation response to downy mildew (DM) as reflected by the mean disease scores from multiple phenotyping experiments. Amongst the 12 predicted genes in this region (b), the third one is the best candidate for dm1, which has four exons and encodes a Staygreen domain. A single nucleotide polymorphism(SNP) in the third exon results in a Q108R amino acid substitution that resulted in disease resistance.

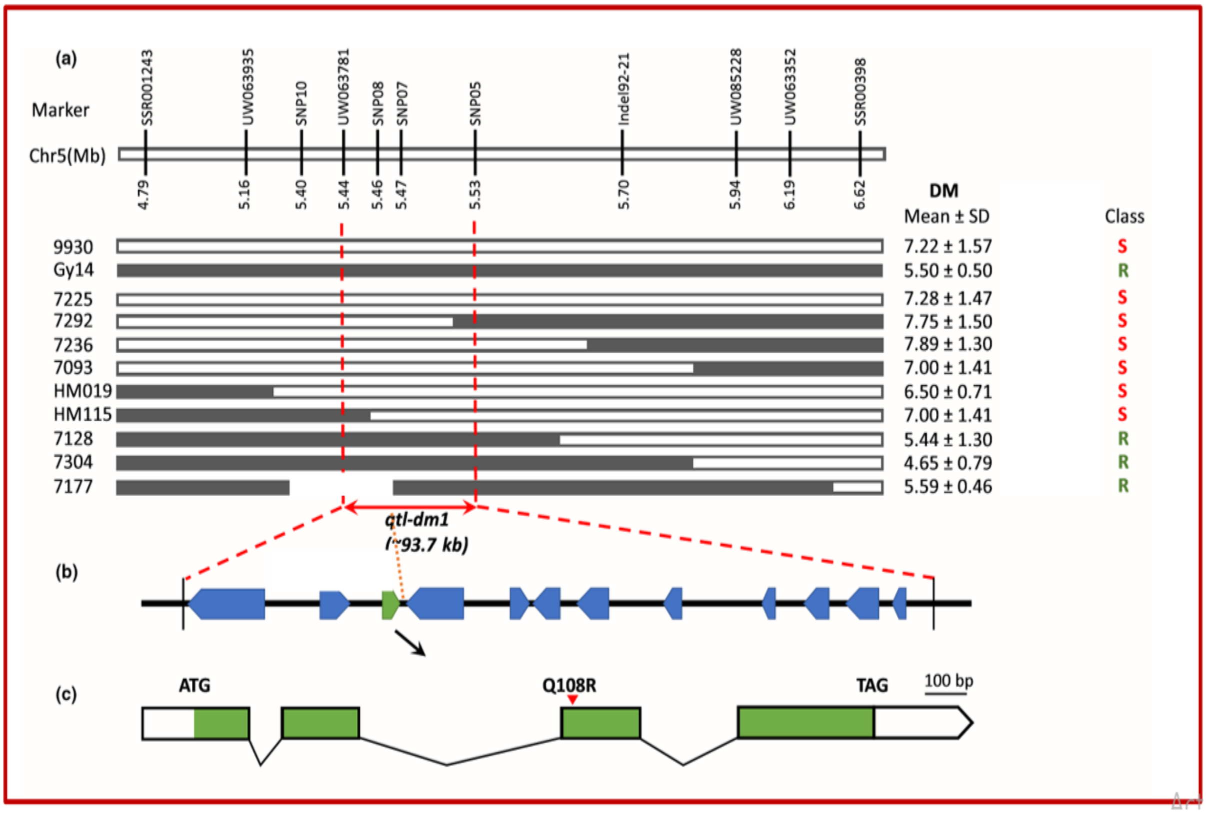


Fig 15 : Map-based cloning of the cucumber dm1 locus

8.34 Possible mechanism of disease resistance

A working model to explain the possible mechanisms of CsSGR-mediated durable and broad-spectrum disease resistance in cucumber. The responses on pathogen infection in the susceptible and resistant (green) lines are depicted to the left and right of the vertical dashed line, respectively. Cssgr is a loss-of-susceptibility mutation of CsSGR that is necessary for disease symptom development. The amino acid substitution from glutamine in the susceptible line (Q108) to arginine (R108) in the resistant line in the protein may affect the interaction of CsSGR with chlorophyll catabolism enzymes (CCEs) and light-harvesting complex subunits of photosystem II (LHCII) proteins, thus inhibiting the normal function of the chlorophyll degradation pathway genes (upregulation). It is not known whether there are interactions between mutant CsSGR protein and the pathogen to trigger active defense responses.

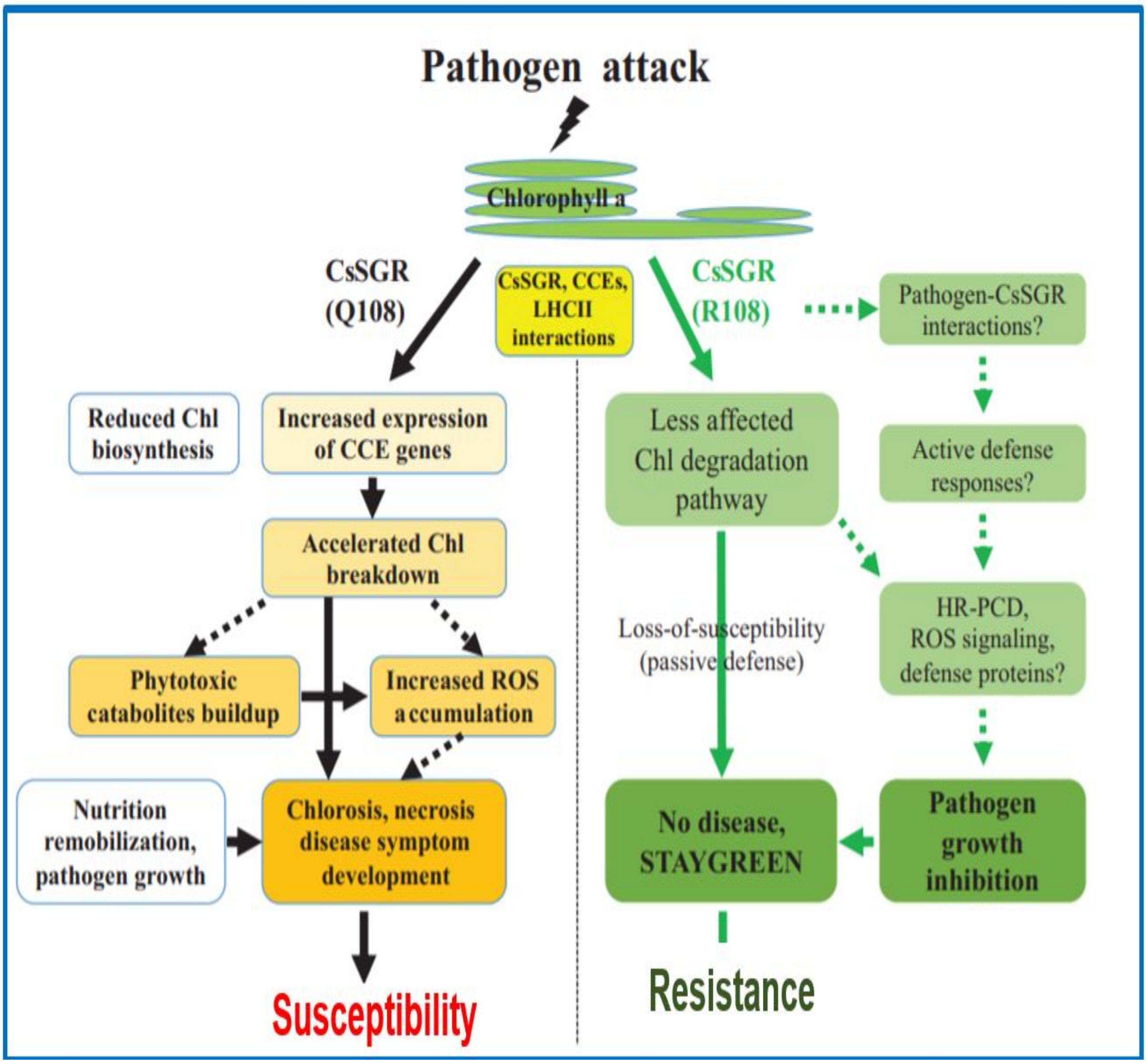


Fig 16 : Possible mechanism of disease resistance

SGR: Stay green CCE: Chlorophyll catabolism enzymes LHC II: Light harvesting complex II Chl: Chlorophyll;	HR: Hypersensitive response PCD: Programmed cell death ROS: Reactive oxygen species

8.4 Stay-green to extend shelf life

A STAY-GREEN protein SlSGR1 regulates lycopene and b-carotene accumulation by interacting directly with SlPSY1 during ripening processes in tomato

Luo *et al* (2013) studied the relation of stay green protein with the lycopene accumulation in tomato (*Solanum lycopersicum*). As a primary source of lycopene in the human diet, fleshy fruits synthesize this compound both denovo and via chlorophyll metabolism during ripening. SlSGR1 encodes a STAYGREEN protein that plays a critical role in the regulation of chlorophyll degradation in tomato leaves and fruits.

8.41 Materials and methods

Materials

- Pink tomato cultivar Zhongsu 6 (ZS 6)

Methods

- Real-time PCR analysis
- Carotenoid extraction and analysis

8.42 Results

They reported that SlSGR1 can regulate tomato (*Solanum lycopersicum*) lycopene accumulation through direct interaction with a key carotenoid synthetic enzyme SlPSY1, and can inhibit its activity. This interaction with SlSGR1 mediates lycopene accumulation during tomato fruit maturation. They confirmed this inhibitory activity in bacteria engineered to produce lycopene, where the introduction of SlSGR1 reduced dramatically lycopene biosynthesis. The repression of SlSGR1 in transgenic tomato fruits resulted in altered accumulation patterns of phytoene and lycopene, whilst simultaneously elevating SlPSY1 mRNA accumulation and plastid conversion at the early stages of fruit ripening, resulting in increased lycopene and b-carotene (four- and nine-fold, respectively) in red ripe fruits.

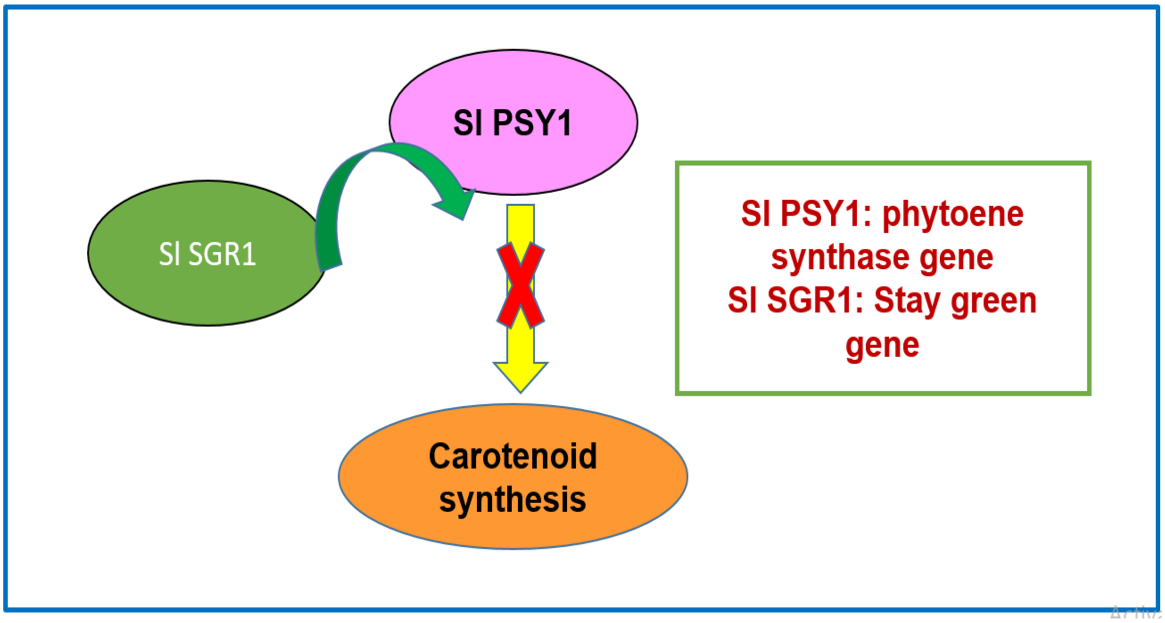


Fig 17 : Mechanism of action of inhibition by stay-green gene in carotenoid synthesis

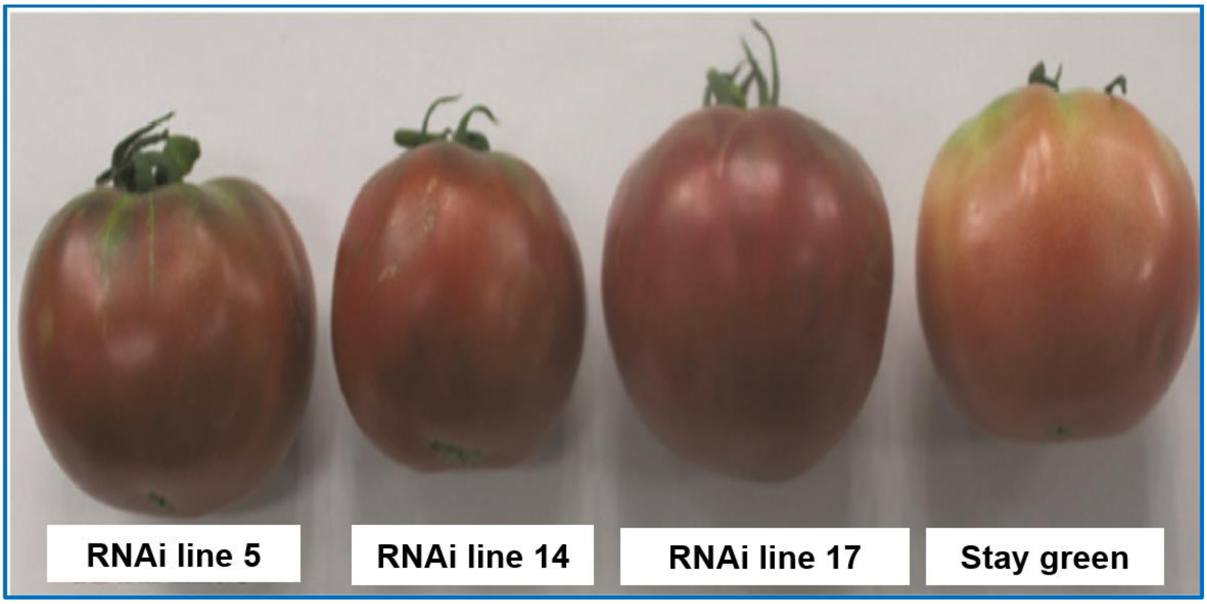


Fig 18 : Confirmation of stay-green gene mechanism in inhibiting lycopene accumulation by RNA interference of stay-green gene

9. Stay-green mutants or varieties developed

Species	Stay-green mutant or variety	Function
Arabidopsis thaliana	*nye1-1*	Cosmetic
	oreo 7	Functional
Capsicum annum	*Cl*	Cosmetic
Citrus sinensis	*Nan*	Cosmetic
Festuca pratensis	BFf993	Cosmetic
Glycine max	*cyt G*	Cosmetic
Oryza sativa	*nyc-1*	Cosmetic
	SNU-SG1	Functional
Phaseolus vulgaris	Alamo	Cosmetic
Pisum sativum	JI2775	Cosmetic
Solanum lycopersicon	*gf*	Cosmetic
Sorghum bicolor	QL 41	Cosmetic
Triticum aestivum	XN901	Functional
Zea mays	FS 854	Functional

Table 3: Stay-green mutants or varieties

10. Present status of research

- ✓ Some of the stay-green genotypes have been identified in different crops:
    - ➢ Sorghum : E 36-1

  Sorghum variety in farmer's field – SPV 2217

    - ➢ Wheat : Chirya 3
    - ➢ Rice : SNU-SG1
- ✓ These genotypes are used in transferring stay-green trait into elite varieties

11. Future thrusts

- Need to explore stay genes linked with grain yield, quality, disease resistance and tolerance to abiotic stresses in more crops
- Study on detailed information about QTLs for stay green trait in major crops that can be employed in breeding programmes for super varieties

12. Summary

Stay-green is the ability of plant to remain green for longer time than its wild type. Thomas and Howarth classified stay-greens into five classes namely type A,B,C,D and E. They also classified stay greens into functional and non-functional type. . In functional stay-greens there is alteration of genetic processes that determine the initiation of senescence and results in a phenotype which photosynthesize for longer than normal. In non-functional stay-greens, leaves remain green due to retention of chlorophyll, however lack photosynthetic competence. Stay-green genes have significant role in crop improvement since they are linked with attributes like higher yield, higher biomass production, increased drought tolerance,increased disease resistance,high temperature resistance,high salt tolerance ,reduced incidence of postharvest yellowing, extended shelf-life.

13. Conclusion

Incorporation of stay-green gene related to attributes like high yield, industrial quality, disease resistance and tolerance to abiotic stresses in economically important crops will help to tackle many burning issues. Thus more research works have to be done so that stay-green genes can be effectively utilised.

14. Discussion

1) What is the significance of cosmetic stay-green if they lack photosynthetic capacity?

   Cosmetic stay-green contribute to increased quality in number of crops including dry edible peas and beans ,garden peas ,tomatoes and peppers. The value of stay-green characteristics in these crops have positive economic impact all along the supply chain, from producer to fresh markets and processing industries.

2) How stay-green nature is achieved by sudden death of cells in type D?

By processes like freezing, drying or boiling, chlorophyll is retained by sudden death of cell eventhough they don't have photosynthetic capacity. This type can be seen in frozen spinach or in herbarium specimen.

3) What are the physiological adaptations observed in stay-green sorghum under drought condition for its better adaptability?

They reduced tillering, reduced canopy and leaf number to reduce the transpiration loss. They also conserved water before anthesis and utilised it during grain filling.

4) Which type functional or non-functional you will prefer to use in plant breeding?

Functional stay greens are more preferred in breeding programmes since they have photosynthetic capacity and the association of the stay-green nature with various attributes like higher yield, disease resistance and tolerance to stresses.

5) What type of genes are suppressed to delay senescence?

The chlorophyll catabolic enzymes are interfered. Various signal transduction pathways like ethylene induced genes are also manipulated to delay senescence.

6) How reducing ROS (Reactive oxygen species) accumulation delay senescence?

ROS at low level initiate the defence mechanism against the stress condition however at higher concentration results in oxidative stress. So maintaining ROS at lower levels is important for the stability of structures.

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GP 591: Master's Seminar

Name	: Alfiya A. R.	Venue	: Seminar Hall
Admission No.	: 2018-11-161	Date	: 09.01.2020
Major Advisor	: Dr. Minimol J. S.	Time	: 10.00 am

Stay green genes: A potential tool in plant breeding

Abstract

Plant breeding has made great contribution through supply of food to the growing human population by the release of more efficient cultivars, which show adaptation to varying environmental conditions. In current agricultural scenario, there is a continuous increase in demand for agricultural production coupled with rapid climate change. Hence, plant breeders are rethinking of investing not only in the traditional criteria such as yield, but also in the selection of genotypes with high productivity unaffected by the climate change. This can only be achieved by a thorough understanding of crop physiology and stress adaptations. 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.

"Stay-green" refers to a trait whereby plants retain leaf greenness compared to the wild type even during senescence. Thomas and Howarth (2000) classified stay-greens into two types *i.e.* functional stay-greens and non-functional stay-greens. In functional stay-greens there is alteration of genetic processes that determine the initiation of senescence and results in a phenotype which photosynthesize for longer than normal. In non-functional stay-greens, leaves remain green due to retention of chlorophyll, however lack photosynthetic competence.

Several physiological features like high photosynthetic capacity, high cytokinin/ABA ratio, high radiation use efficiency, high water availability at anthesis, increased rate and duration of grain filling, specific leaf nitrogen, reduced canopy, high harvest index and low leaf senescence rate contribute to the better adaptation of stay-greens to adverse conditions. Apart from these, factors like hormones, low accumulation of reactive oxygen species and chlorophyll metabolism also contribute to the expression of stay-green nature.

The contribution of stay-green trait have been reported in several crops, and its employment had brought an increase in grain yield, establishment of tolerance to abiotic and biotic stresses and less susceptibility to lodging. They also contribute to reduced post-harvest yellowing and extended shelf life. Kumari *et al*. (2013) identified an association of stay-green trait with canopy temperature depression and yield traits under terminal heat stress in wheat. Stay green gene was found to be inhibiting the lycopene synthesis, thus can extend shelf life in tomato (Luo *et al.*, 2013). Stay-green alleles were found to enhance grain yield in sorghum under drought condition (Borrell *et al.*, 2014). Stay-green gene was found to provide durable broad spectrum disease resistance in US cucumber (Wang *et al*., 2018).

The use of stay-green character in breeding programmes may result in significant genetic progress for attributes such as high yield, industrial quality, disease resistance and tolerance to abiotic stresses. Moreover, the pronounced additive effect in the inheritance of the stay-green character provides an incentive for the incorporation of this trait in breeding programmes. The understanding of the physiological mechanisms associated with senescence and photosynthetic efficiency in several crops may be the key to remove the plateau of productivity associated with adaptation to unfavourable environmental conditions.

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