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SIGNIFICANCE OF STAY-GREEN TO FOSTER CROP PRODUCTION UNDER STRESS ENVIRONMENT - A MINI-REVIEW

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ABSTRACT

An extended foliar greenness even under post-anthesis drought can be simply called as the stay-green phenotype. The maintenance of a balance between nitrogen (N) demand and supply during grain filling stage is the key to stay-green phenotype. Chlorophyll catabolic enzymes (CCEs) are responsible for the degradation of chlorophyll. When a mutant disrupts the activity of these CCEs, it leads to stay-green phenotype. There are five classes of stay-green identified as A, B, C, D and E. The genotype possessing these traits can retain more photosynthetically active leaves under water shortages at the grain filling stage. Maintaining the greenness of leaves for longer time is the fundamental strategy for increasing crop yield and market value. Understanding the physiological and genetic basis of stay-green in relation to drought-resistance mechanisms are fundamental to the development of new strains that are better adapted to dry conditions.

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1 Introduction

Developing crops having high water use efficiency is one of the greatest challenges that crop scientists are facing today. The crops especially of semiarid origin such as millet and sorghum are found to possess critical adaptive mechanism which help them to escape and/or resist acute drought conditions. Maintaining the greenness of leaves for longer period is a principle strategy for increasing crop production, particularly under water-limited conditions (Abdelrahman et al., 2017). The pigment composition of plants is controlled by genetic and environmental stimuli and is the consequence of a balance between *de novo* synthesis and degradation (Barry, 2009). Stay-green is the ability of a plant to remain green and maintain photosynthesis for longer period of time, thereby contributing photosynthates for an extended time towards grain development (Borrell et al., 2001). Stay-green means heritable delayed yellowing or delayed foliar senescence in crop plant species (Thomas & Howarth, 2000; Thomas & Ougham, 2014), but this trait can actually be undesirable in certain circumstance when plants compete for resources (Antonietta et al., 2014). The stay-green phenotype is measured as green leaf area duration after anthesis, and is highly influenced by the time of anthesis, with earliness tending to give an increased duration for seed filling depending on environment condition (Gregersen et al., 2013).

Stay-green is categorized into two groups: one is 'functional stay-green' where plants photosynthesize for a longer period, which can result in higher yield; second is 'non-functional/ cosmetic stay-green' where plants remain green due to lesions in chl catabolism, but lack photosynthetic competence (Hortensteiner, 2009; Tian et al., 2013). Due to its high importance, stay-green has been examined in many plants (rice, sorghum, barley, soybean, maize, wheat, french bean, tobacco, *Arabidopsis*, lettuce, broccoli, tomato and *Capsicum* etc.) which are being reviewed by Thomas & Smart (1993) and Luche et al. (2015). The relationship of stay-green with useful traits such as greater number of fertile tillers (Ahlawat et al., 2008), higher number of grains/ear (Luche et al., 2017), tolerance to abiotic (Kassahun et al., 2010; Velasco-Arroyo et al., 2016) and biotic (Sun et al., 2017) stresses have been reported.

Mutants disrupting chlorophyll (chl) degradation, lead to stay-green phenotypes which ultimately enhance grain yield especially under water stressed environmental condition (Hortensteiner & Krautler, 2011). Stay-green mutants have been categorized into five groups viz., A, B, C, D and E (Figure 1), using both temporal and biochemical characteristics (Thomas & Howarth, 2000). Type A arises when leaves and stems prolongs their photosynthetic activity, experiencing a delay in plant senescence, but later senescence proceeds at a usual rate. Senescence in B type appears in normal way, but proceeds at relatively slow rate. Even though a
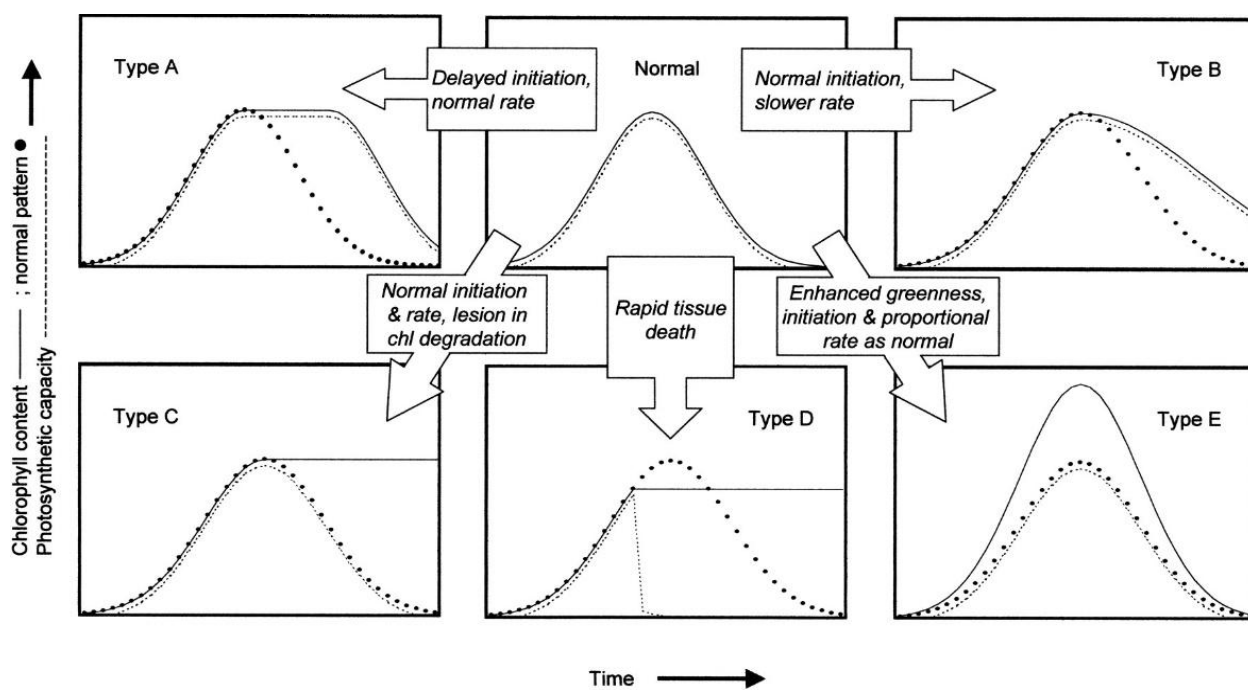

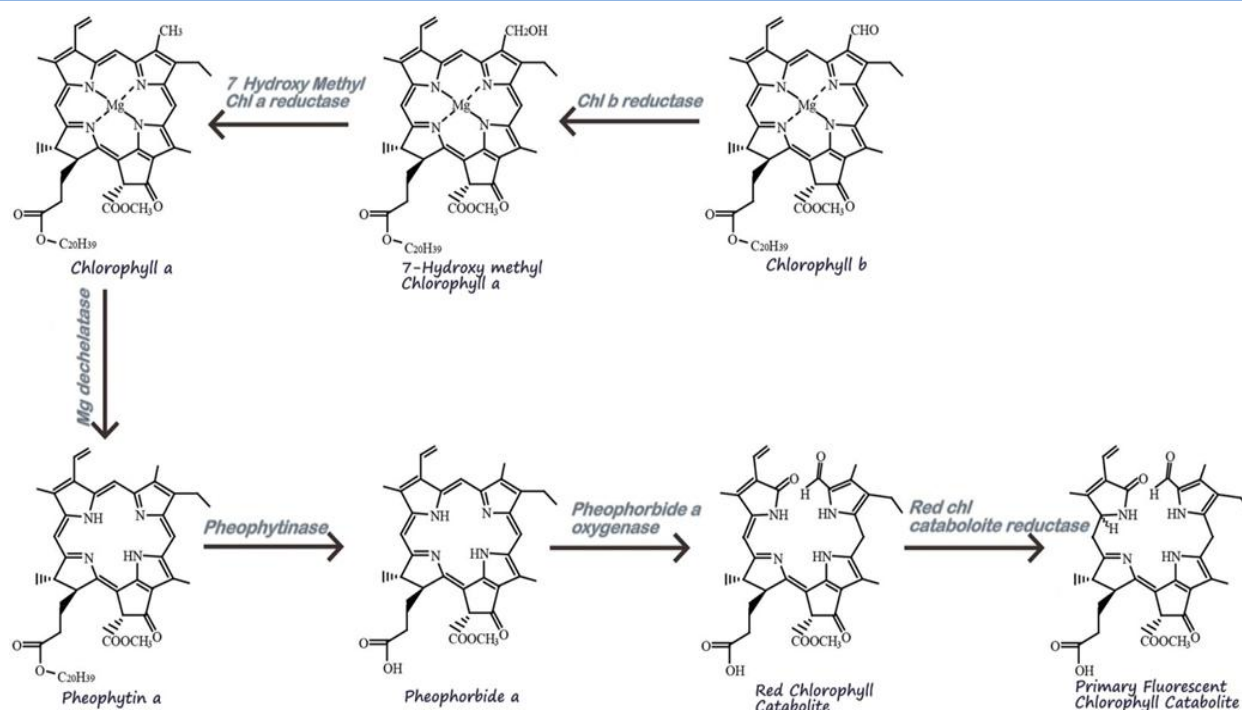Figure 1: Five ways to stay-green. (Adopted from Thomas & Howarth, 2000).

Figure 2: The chlorophyll degradation pathway

normal rate of senescence with declining photosynthetic activity follows in type C (cosmetic stay-green), the chl degradation pathway failure retains the greenness. In type D stay-green, green colour is retained forever due to killing of the leaf tissue by freezing, boiling or drying. Type E is due to highest accumulation of chl in photosynthetic tissues, results in delaying of senescence and maintains green tissue, even with the reduced ability of fixing CO_2.

2 Chl degradation pathway in higher plants

Chl degradation is a highly controlled sequential process that converts fluorescent chl molecules into non-fluorescent catabolites, via a highly conserved multistep pathway termed as the 'PAO (pheophorbide a oxygenase)' pathway, that are stored within vacuole (Figure 2). Chl degradation is of vital importance to plant development for its detoxifying activity of the photo-toxicity of chl molecules once they are freed from their binding proteins (Li et al., 2017). In higher plants, there are two forms of chl molecules, chl *a* and chl *b*, out of which chl *a* is the degradable form. Chlorophyllide *a,* pheophorbide *a*, red chl catabolite and primary fluorescent chl catabolites are the intermediates responsible for this conversion. There are six types of CCEs encoded by chlorophyll catabolic genes (CCGs) involved in chl catabolism, viz. chlorophyll *b* reductase (CBR), 7-hydroxymethyl chlorophyll *a* reductase (HCAR), Mg^{2+}-dechelatase, pheophytinase (PPH), PAO and red chlorophyll catabolite reductase (RCCR) (Hortensteiner & Krautler, 2011).

Chl *b* is synthesized from Chl *a* by oxygenation of its C-7 methyl group into a formyl group. In the degradation process of Chl, Chl *b* has to be converted to Chl *a*, because chl derivatives with C-7 formyl groups are not catalyzed in the later steps of chl degradation (Hortensteiner et al., 1995). The conversion of chl *b* to chl *a* proceeds by two successive reductions. The formyl group of chl *b* is reduced by CBR to a hydroxymethyl group to produce 7-hydroxymethyl chlorophyll *a*. Then the reduction of 7-Hydroxymethyl chlorophyll *a* by HCAR produces chl *a*. Mg from chl *a* is then removed by Mg^{2+}-dechelatase to convert chl *a* to pheophytin *a*. The phytyl group of pheophytin *a* is then hydrolyzed by PPH to produce pheophorbide *a* and the tetrapyrrole ring structure of pheophorbide *a* is opened by PAO, resulting in oxidized red chl catabolite (RCC), which is subsequently catalyzed by catabolite reductase RCCR to generate primary fluorescent chl catabolite (pFCC) (Krautler, 2016). This pFCC is further modified and transported into the vacuole where the acidic pH isomerize it to non-fluorescent products (Suzuki et al., 2005; Tanaka & Tanaka, 2006; Balazadeh, 2014).

For the expression of stay-green, three steps in the chl degradation pathway is mainly known to be affected. Conversion of chl *b* to chl *a* is the first step in the pathway affected in which the activity of the CBR and HCAR are reduced to an extent that the greenness of the leaf remains unaffected. The second step to be affected is the conversion of pheophorbide *a* into RCC by PAO enzyme (Hortensteiner, 2009). And the final step which is thought to be

affected is the conversion of RCC to pFCC catalyzed by the enzyme RCCR. Change in the level of activity of these enzymes (downregulation) in the stay-green mutant is responsible for expression of the trait.

3 The stay-green gene

Defects in chl degradation are a cause of delayed leaf senescence. In many plant species, stay-green mutants have been identified that show retention of Chl during senescence. Mutations in NYC1 [(NON-YELLOW COLORING1) show such stay-green phenotypes in *Arabidopsis* and rice. CBR enzyme, a CCE is encoded by 2 isozyme genes NYC1 and NYC1-LIKE (NOL). NYC1 expression has consistent correlation with leaf senescence (Kusaba et al., 2007). Rice or *Arabidopsis* NYC1 mutants exhibit a stay-green phenotype with high retention of light harvesting complex II (LHCII) subunits and Chl *b* during senescence. An increasing number of natural or experimentally created stay-green mutants have been identified in plants such as bell pepper, tomato (Barry et al., 2008), rice(Park et al., 2007), *Arabidopsis* (Ren et al., 2007) termed as NON-YELLOWING 1 (NYE1)/ STAY-GREEN1(SGR1) as well as Gregor Mendel's famous green cotyledon mutant of pea (Sato et al., 2007). Even though the functional activity of SGR proteins in *sgr* missense mutants are critically dependent on several amino acid residues, but they may not affect the expression level of SGR genes (Barry et al., 2008). The SGR proteins are generally classified into two distinct subfamilies named as SGR and SGR-like (SGRL), both of which exist in both monocotyledonous and dicotyledonous plants (Barry, 2009). The *Arabidopsis thaliana* genome encodes 3 SGRs namely SGR1/NYE1, SGR2/NYE2 and SGRL. They contain a variable N-terminal domain including the putative chloroplast transit peptide, a conserved central domain called the SGR domain, and a variable C-terminal region. A cysteine rich motif of unknown function is present in the C-terminal part of SGR which distinguishes it from SGRL proteins (Aubry et al., 2008). Shimoda et al. (2016) reported about the photosystem degradation capacity of NYE1/SGR1 during senecsence by producing a Mg^{2+} dechelatase that means the CCE Mg^{2+}-dechelatase is encoded by SGR1. The products of all these genes exert an important regulatory role in chl degradation during senescence, destabilizing protein-pigment complexes and increasing availability of chl for cleavage by CCEs (Qian et al., 2016). The physical interactions between CCEs and light-harvesting complexes (LHCs) is the main reason for degradation of Chl (Sakuraba et al., 2012). Sakuraba et al. (2014a) and Sakuraba et al. (2014b) also reported about the positive regulation (enhances leaf senescence) of Chl degradation by SGR1 and SGRL during senescence, whereas the process is negatively regulated (limits leaf senescence) by SGR2. SGRL is expressed in the developmental stage and catalyzes a reaction from chl *a* and chlorophyllide *a* to pheophytin *a* and pheophorbide

a, respectively, whereas SGR is expressed in the senescent stage and catalyzes a reaction only from chl *a* to pheophytin *a*. Both subfamilies do not use chl *b* as substrate. Chl *b* tightly holds Mg (Saga & Tamiaki, 2012), and SGR is thus not able to extract Mg from chl *b*. Conversion of chl *b* to chl *a* is thus important to loosen the Mg^{2+} and eventually the Mg is extracted by SGR. The overexpression of SGR1 in *Arabidopsis* resulted early leaf yellowing but the "STAY-GREEN" phenotype was expressed in *sgr1* mutants (Sakuraba et al., 2014a). On the contrary, SGR2 over expression resulted in "STAY-GREEN" phenotype whereas premature leaf senescence phenotype was expressed by by *sgr2* null mutants under both the dark and abiotic stress conditions in *Arabidopsis*. Expression of SGR1 is weak in pre-senescent leaves, but is induced during developmental and dark-induced senescence (Ren et al., 2007). SGR proteins from different species are highly similar and localize to the chloroplast's thylakoid membrane (Park et al., 2007). In *Arabidopsis*, the interaction between SGR1, six plastid-localized CCEs and LHCII proteins results in SGR1–CCE–LHCII protein complexes required for chl degradation (Sakuraba et al. 2015). However, the binding affinity of SGR2 for CCEs is much lower than for SGR1 (Sakuraba et al. 2014b). So, the hetero-dimerization of SGR1 and SGR2 can obstruct SGR1 and CCEs interactions, limiting the SGR1–CCE–LHCII protein complex formation and finally chl degradation.

4 Mechanism of stay-green trait

In order to improve stay-green traits, understanding the mechanism of the traits has received much attention in plant science world. Chl content and level of photosynthetic activity of the leaves in water limiting conditions determines the expression of stay-green traits. Any mutation in the steps of chl biosynthesis and degradation pathway leads to expression of the trait.

4.1 Carbon-Nitrogen Transition

The Carbon (C)-capture phase of leaf function is succeeded by a phase of net organic N remobilization (Thomas & Ougham, 2014). This C-N transitional period is actually the functional initiation of senescence and it is either delayed or runs slowly in functional stay-green (Thomas & Howarth, 2000). Chl is made up of C, N, Mg and other constituents. Therefore, C: N ratio is very important in the regulation of heat induced leaf senescence. For example, Borrell & Hammer (2000) showed that senescent and stay-green sorghum hybrids are differed for N demand: supply balance, where stay-green having a shortfall in N that is about 25% lower than that in senescent hybrids, and explaining a slower rate of leaf senescence in the stay-green genotypes. Jespersen et al. (2015) reported about the improved protein level, photosynthesis and delayed senescence in creeping bent grass (*Agrostis stolonifera*) by spraying of carbonyldiamide as a source

of N. The stay-green expression can be considered as the equilibrium of N demand and supply during the stage of grain filling. The current research revealed that an increase in N uptake during grain filling maintain high photosynthetic activity in the leaves for longer period. Until and unless the C accumulation remains lesser than the sink capacity, prolonged photosynthesis keep on contributing C in crops by functional stay-green trait (Thomas & Ougham, 2014).

4.2 Chlorophyll Biosynthesis pathway

Chl biosynthesis pathway can be divided into 4 phases (Hortensteiner, 2009; Figure 3). In the first phase, Glutamic acid is converted to 5-Aminolevulinic acid (ALA) in the presence of Glutamyl tRNA reductase (GluTR). Two molecules of ALA are condensed to form porphobilinogen (PBG). Again four PBG molecules are linked to form protoporphyrin IX, which converted into Monovinyl protochlorophyllide *a* by the action of enzyme Mg^{2+}-chelatase. A light dependent reaction takes place in the presence of NADPH and Protochlorophyllide oxidoreductase (POR) that lead to the formation of chlorophyllide. Attachment of phytol tail by the action of Chl synthetase completes the process by forming chl *a*.

In this pathway, mainly two steps i.e. conversion of Glutamic acid to ALA and Monovinyl Protochlorophyllide *a* to Chlorophyllide *a* are influenced for the expression of stay-green trait (Thomas & Ougham, 2014). In both the steps, the action of enzymes responsible for the conversion is over expressed or maintained for a longer period of time for the expression of stay-green phenotype.

5 Relation of phyto-hormones with stay-green trait

The initiation and progression of senescence in leaves involve complicated hormonal crosstalk (Kim et al., 2011 a; Khan et al., 2014; Kim et al., 2015; Schippers et al., 2015). Plant senescence is influenced by some major hormones such as Abscisic acid (ABA), Cytokinins (CKs), ethylene and strigolactones (SLs) which induces antagonistic and synergistic signaling effects (Thomas & Ougham 2014). Leaf senescence is regulated by nuclear and chloroplast signals. Expression of senescence-associated transcription factors (TFs) are induced by positive and negative stimuli in the nucleus. Positive stimuli are ethylene, ABA, jasmonic acid (JA), salicylic acid (SA), brassinosteroids (BR), SLs, stress, dark and ageing, whereas the only negative stimuli are the CKs (Kusaba et al., 2013).''Positive TFs'' induce the expression of senescence associated genes (SAGs) that promote leaf senescence whereas ''negative TFs'' induce SAGs that repress leaf senescence. Thus nucleus born senescence-associated TFs regulates chloroplast senescence. On the other hand, retention of chloroplast activity represses the expression of senescence inducible genes, indicating that signals from the chloroplast can regulate nuclear derived programs of senescence.

5.1 Ethylene

Ethylene acts as a positive regulator of senescence in ethylene insensitive 2 (EIN2) mutants by controlling the timing of leaf senescence (Pierik et al., 2006). The N-terminal transmembrane domain (a type of endoplasmic reticulum membrane-bound receptor), which includes ethylene response sensors (ERS1 and ERS2), ethylene response factors (ETR1 and ETR2) and ethylene
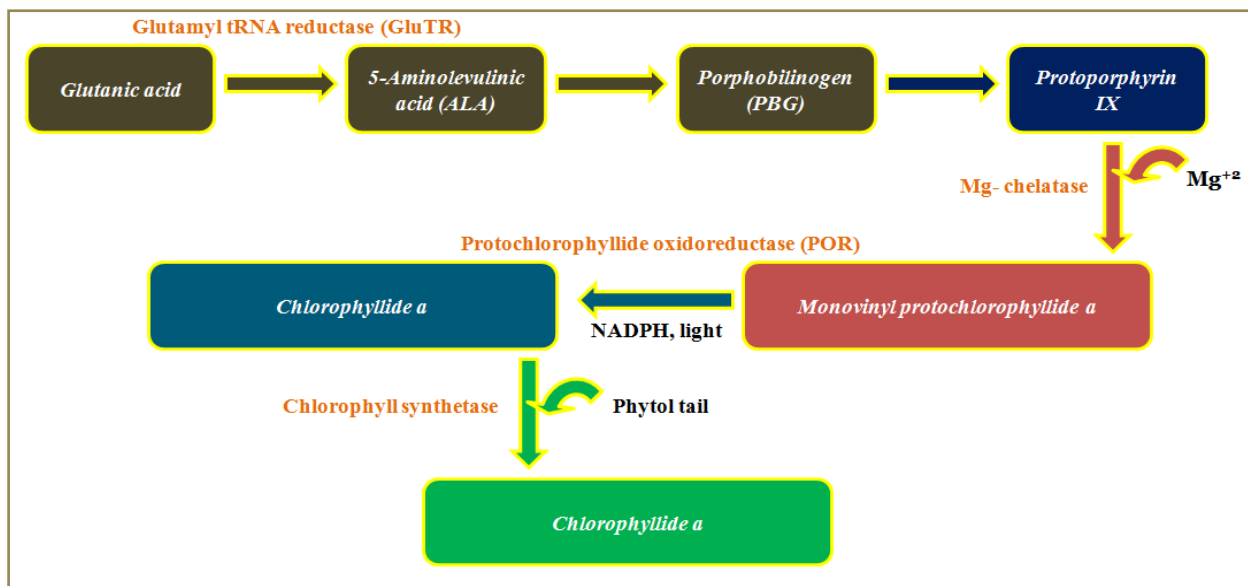

Figure 3: The chlorophyll biosynthesis pathway

insensitive factors (EIN2, EIN3 and EIN4) (Iqbal et al., 2017) perceive the ethylene induced signals. In the absence of ethylene, these ethylene receptors function redundantly, negatively regulating ethylene responses via direct binding to constitutive triple response1 (CTR1), which is a key negative regulator of the ethylene signal transduction pathway. Grbic´ & Bleecker (1995) observed that the leaves of *etr1-1* mutants remain unresponsive to ethylene treatment and express stay-green phenotype. Even during leaf senescence, EIN2 and EIN3 mutants exhibit a severe stay-green phenotype. Li et al. (2013) also proved the positively regulation of EIN3 towards two important senescence regulatory genes ORE1 and NAP either directly or indirectly via negatively regulating *miR164*. Ethylene treatment may induce the expression of NYC1, NYE1 and PAO in *Arabidopsis* leaves but may repress them in *ein3eil1* double mutant (Qiu et al. 2015). Yin et al. (2016) recently identified positive regulation of an ethylene responsive factor CitERF13, for *CitPPH* (a CCG gene) promoter during citrus fruit degreening.

5.2 Abscisic Acid

Senescing leaves are characterized by an increase in ABA levels which promotes chloroplast degradation (Xue-Xuan et al., 2010; Sah et al., 2016). ABA plays a dual role by repressing chloroplast biosynthesis genes and inducing genes that promote chl degradation during senescence. ABA positively regulates degreening during leaf senescence via an AtNAP-SAG113 (a PP2C family protein phosphatase) regulatory module which is involved in the movement of stomata (Zhang & Gan, 2012). Lee et al. (2011) reported that *prk1*, an *Arabidopsis* mutant exhibited a stay-green phenotype during natural senescence, and was characterized by delayed senescence specifically in response to ABA among several senescence-inducing treatments. Liu et al. (2016a) found that after treatment with ABA, *abig1* (ABA Insensitive Growth 1, TF) mutants remain greener and produce more leaves than comparable wild-type (WT) plants, whereas when challenged with drought, *abig1* mutants have fewer yellow, senesced leaves than WT. Conclusively, ABA induced by drought increases ABIG1 transcription which promotes leaf senescence and restricts new shoot growth.

Gao et al. (2016), three ABA responsive element (ABRE) binding TFs namely ABF2, ABF3 and ABF4 act as key regulators in mediating ABA-triggered chl degradation and leaf senescence in general in *Arabidopsis*.

5.3 Jasmonic Acid

JA is another plant hormone that modulates defense responses, growt hand development, and is also proposed to mediate leaf senescence (Kim et al., 2015). Many JA biosynthesis genes are differentially regulated, as some are up-regulated and others are down regulated in the progression of leaf senescence (He et al.,

2002). Exogenous application of JA on WT *Arabidopsis* promotes leaf senescence and induces the expression of several SAGs, including JA biosynthesis genes. Castillo & Leon (2008) reported that a loss of function mutant of *COI1* [encoding the co-receptor of JA and the antisense transgenic plant of *3-ketoacyl-CoA thiolase 2 (KAT2)*] involved in JA synthesis have a stay-green phenotype in response to a dark incubation. In a recent study, Zhu et al. (2015) identified *Arabidopsis* MYC2/3/4 basic helix-loop-helix proteins (the key JA signaling components) to direct transcriptional regulation of CCGs like PAO, NYE1 and NYC1 by binding to their promoters. Further, the PAO promoter activity was found to get enhanced by over expression of MYCs in *Arabidopsis* protoplasts and also by methyl jasmonate (MeJA) treatment in WT *Arabidopsis* plants. Though, the MYCs over expression lines showed accelerated leaf yellowing, the *myc2myc3myc4* triple mutants showed a severe stay-green phenotype. These findings advocate that MYC2/3/4 proteins may directly activate CCGs for mediating JA-induced chl degradation.

5.4 Cytokinin

CKs are a class of hormones which has dominant adversary effect on senescence (Wilkinson et al., 2012; Zwack & Rashotte, 2013). Kim et al. (2006) and Kim et al. (2012) reported that AHK3 and ARR2, which are components of CK signaling pathway, are constitutively expressed in *Arabidopsis* stay-green line *ore12-1* (gain-of-function mutant) consequently supervening a stay-green phenotype. These reports unfold the influential contribution of AHK3 and ARR2 in the hormonal regulation of senescence. A wheat stay-green mutant *tasg1* exhibited a significantly delayed senescence and high photosynthetic capacity than the WT plants, the examination of which revealed that concentration of soluble sugars were higher in the flag leaves and grains of *tasg1* than in WT plant due to the altered metabolism and transport of soluble sugars regulated by CKs (Wang et al., 2016a; Wang et al., 2016b). Similarly, Yang et al. (2016) also reported an increased heat tolerance and grain yield in wheat cultivar "Wennong 6" (retaining the "STAY-GREEN" trait) with post anthesis CKs treatment.

Kim et al. (2011b) found that over expression of a gene (YUCCA6) coding for the rate-limiting enzyme of auxin synthesis (Indole 3 acetic acid- IAA) results in a stay-green phenotype. Based on microarray data from rice flag leaves during early senescence, Liu et al. (2016 b) identified W-box and G-box cis-elements as positive regulators of senescence in the important rice variety Minghui 63. The W-box is the cognate cis-element for WRKY proteins, while the G-box is the cognate cis-element for bZIP, bHLH and NAC proteins. To investigate the potential relationship between flag leaf early senescence and hormone signaling, they surveyed the responses of TF genes to ABA, BR, CK, auxin, JA and gibberellic acid (GA) using data from

RiceXPro database. The result showed that the four TF families were affected by various hormones to varying degrees and also demonstrated that ABA, BR and CK-mediated signaling might converge on the same TFs. Zhang et al. (2016) reported that LpPPH gene [encoding pheophytin pheophorbide hydrolyase (PPH) that breaks down chl during leaf senescence in *Lolium perenne* L.] could be a direct downstream target of TFs in ABA and CK signaling pathways, as the breakdown could be regulated positively by ABA and ethylene, and negatively by CK.

6 Breeding for stay-green trait

Breeders have made great progress, supplying food to the growing human population through the release of more efficient cultivars, showing adaptation to environmental improvements such as irrigation and increased N input (Dias, 2015). However, the world population will be approximately 9.2 billion in 2050 (Jaggard et al., 2010) and to sufficiently feed all these people, the total food production will have to increase 60% to 70% (Tilman et al., 2011; FAO, 2012; Alexandratos & Bruinsma, 2012; Pardey et al., 2014). Thus in the context of present agriculture situation where food demand is on the surge, and there is also menacing impact of global warming and extreme environmental desecration from professional agricultural operations, plant breeders are anticipating and devoting time not only to improve yield but also to select and specify foremost genotypes having outstanding productivity, through technical know-how of stress adaptation physiology (Araus et al., 2008).

Stay-green is characterized by delaying of leaf senescence in the last stage of plant maturity. Maintenance of grain filling for a longer period of time due to delayed senescence allows plants to accumulate greater photosynthates toward the end of maturation which result in the increase of grain size. In addition, association of stay-green with different biotic (Rosenow & Clark 1981; Joshi et al., 2007) and abiotic stresses (Kassahun et al., 2010) has suggested potential for genetic improvement of several crops to promote stress tolerance and grain yield. However, breeding approaches are limited in the cases involving polygenic traits, significant environmental influences and low genetic variance. Genetic improvement for traits having high heritability is more effective. The stay-green trait is governed by only one gene having two alleles (Silva et al., 2001). The trait is positively heritable and controlled by four genes that are segregated independently, with strong contribution of additive effects (Joshi et al., 2007). Therefore, efficacious phenotypic selection and genetic manipulation for this trait will be most successful in early generations.

The stay-green genotypes in sorghum and wheat were reported have better grain filling in drought, high stem carbohydrate content, reduced lodging better grain weight and resistance to charcoal stem

rot (McBee et al., 1983; Rosenow, 1984; Borrell et al., 2000a; Silva et al., 2001; Burgess et al., 2002). Greenness during the final stage of leaf development is an essential trait in increasing source strength in grain production. And reported that stay-green japonica rice 'SNU-SG1' had a good seed setting rate and grain yield (Yoo et al., 2007). Thus, the stay-green trait is considered as an important determinant of grain yield. For increasing grain yield, persistence of high photosynthetic capacity and efficient N remobilization at the time of grain filling are the principle factors (Gentinetta et al., 1986; Thomas & Rogers, 1990; Rajcan & Tollenaar, 1999; Yamaya et al., 2002; Spano et al., 2003; Abdelkhalik et al., 2005). Stay-green phenotype and related traits has been reported to enhance grain yield especially under post-anthesis drought conditions in wheat (Christopher et al., 2008; Adu et al., 2011; Bogard et al., 2011; Lopes & Reynolds, 2012; Kipp et al., 2014; Christopher et al., 2016), sorghum (Xu et al., 2000; Borrell et al., 2000b; Borrell et al., 2012; Jordan et al., 2012; Borrell et al., 2014a; Borrell et al., 2014 b), maize (Bolanos & Edmeades, 1996; Kamara et al., 2003; Zheng et al., 2009; Wang et al., 2012) and rice (Jiang et al., 2004; Hoang & Kobata, 2009; Fu et al., 2009). Stay-green is thus widely honoured as a drought adaptation tool in cereals (Cattivelli et al., 2008; Gregersen et al, 2013). Thus, this association could be used as a basis for selection of high-yielding stay-green genotypes, especially for water-limited environments. Evangelista & Tangonan (1990) reported about the good association of stay-green phenotype with resistance to stem rot in sorghum. Similar association was also reported by Joshi et al. (2007) with resistance to spot blotch in spring wheat. Some of the stay-green wheat cultivars viz. CN12, CN17 and CN18 from southwest China are high yielding and resistant to stripe rust (Luo et al. 2009). These are the clear evidences for the persistence of stay-green phenotype which can remain photosynthetically active even at the influences of various biotic stresses.

The improvement of stay-green phenotype is predicted to be much higher if information about the presence of stay-green genes/QTLs (quantitative trait loci) in the promising genotypes can be gathered with the help of linked molecular markers. QTLs studies in cereals have unveiled the value of functional stay-green in improving stress tolerance. Several QTLs associated with stay-green have been identified in different cereals like, fourteen QTLs (sg1.1.1, sg1.6.1, sg2.1.1, sg2.1.2, sg2.2.1, sg2.3.1, sg2.5.1, sg2.8.1 sg3.1.1, sg3.2.1, sg3.5.1, sg3.9.1, sg4.1.1 and sg4.2.1) in maize (Zheng et al., 2009), four QTLs (StgB, Stg1, Stg3 and Stg4) in sorghum (Kassahun et al., 2010; Vadez et al., 2013), three QTLs (QSg.bhu-1A, QSg.bhu-3B and QSg.bhu-7D) in wheat (Kumar et al., 2010), six QTLs (csfl2/tcs2, tcs4, tcs5, csfl6, csfl9/tcs9 and csfl12) in rice (Fu et al., 2011) and ten QTL (HGSQ, HSPFLQ1, HSPFLQ2, HSPFL1Q, HLAUGQ1, HLAUGQ2, WGSQ, WGFL1Q1, WGFL1Q2, WLAUGQ) in

barley (Gous et al., 2016) etc. The identified QTLs affecting stay-green may be a promising target for marker-assisted introgression of the functional stay-green trait into the breeding materials for yield improvement. Wang et al. (2018) recently fine mapped a stay-green mutant in *Brassica campestris* L. ssp. *chinensis*, which they termed "*nye*". Genetic analysis revealed that the stay-green trait is controlled by a single recessive gene, *Brnye1*. Using the BSA-seq method, a 3.0-Mb candidate region identified as the *Brnye1* gene was mapped on chromosome A03. They identified 12 genes in this region, 11 of which were annotated based on the *B. rapa* annotation database. They identified *Bra019346*, a homolog of the Arabidopsis *AtNYE1* gene, as a potential candidate gene responsible for the stay-green trait. It was supposed that characterization of this stay-green mutant and cloning of the stay-green gene will provide a foundation for unraveling the molecular mechanism of the stay-green trait in *B. campestris* L. ssp. *chinensis*.

Now-a-days, high-throughput phenotyping technologies like Greenseeker®, Phenomobile®, ArduCrop® have allowed researchers to accurately select stay-green traits by using methods like Normalised Difference Vegetative Index (NDVI), Light Detection And Ranging (LiDAR), Single Photon Avalanche Diode (SPAD) etc (Kipp et al., 2014; Rebetzke et al., 2016). The most relevant phenotypic parameters in this regard, such as the deviations in leaf area, greenness and photosynthetic capacity can now be easily followed and documented thoroughly by utilizing these advanced machineries. Hence, a hefty breeding population also can be screened very easily and the most suitable (only the functionally active stay-green) germplasm can be selected precisely (Christopher et al., 2014).

7 Ideotype of stay-green genotype

To be an ideal stay-green genotype and to produce potential economic yield, genotypes are assumed to have some ideal characteristics especially under drought environmental condition. Firstly, spread and deep root system is important as it enable the plant to uptake more N to maintain active source-sink relationship during the grain filling stage. Moreover stay-green genotypes also have the ability to uptake more silicon from soil which reflected as lodging resistance (Kashiwagi et al., 2007; Luyckx et al., 2017). Genotypes with stay-green should exhibit a slow rate of chl degradation as it leads to prolong the duration of leaf senescence. As a consequence minimum chl content will be retained in the leaves for photosynthesis even during the period of senescence. The third assumption is that genotypes should have more total plant leaf area, so that the total green leaf area at the maturity stage will be more and it is an excellent indicator of stay-green. Thus, it is feasible to develop an ideal stay-green cultivar through pyramiding all these traits from potential sources using conventional or molecular breeding approaches.

Conclusion

During the last few years there have been tremendous increases in the understanding of the mechanisms and processes that control chl degradation in higher plants. Stay-green genotypes retained high photosynthetic competence, mesophyll conductance and photochemical efficiency as well as leaf chl content throughout grain filling as compared with other genotypes. From several findings, it has been suggested that a functional stay-green trait can be utilized for improving crop yield potential through the improved dry matter production during grain filling. There is a positive correlation between stay-green and yield as observed in several studies. Molecular techniques can be used to identify QTL controlling stay-green and its location in the chromosome. Finally it can be concluded that based on physiological, morphological and molecular characteristics, stay-green genotypes can be isolated and used in advanced breeding programmes for genetic crop improvement.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this review paper.

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