The stay-green trait

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Received 24 October 2013; Revised 13 December 2013; Accepted 7 January 2014

Abstract

Stay-green (sometimes staygreen) refers to the heritable delayed foliar senescence character in model and crop plant species. In a cosmetic stay-green, a lesion interferes with an early step in chlorophyll catabolism. The possible contribution of synthesis to chlorophyll turnover in cosmetic stay-greens is considered. In functional stay-greens, the transition from the carbon capture period to the nitrogen mobilization (senescence) phase of canopy development is delayed, and/or the senescence syndrome proceeds slowly. Yield and composition in high-carbon (C) crops such as cereals, and in high-nitrogen (N) species such as legumes, reflect the source–sink relationship with canopy C capture and N remobilization. Quantitative trait loci studies show that functional stay-green is a valuable trait for improving crop stress tolerance, and is associated with the domestication syndrome in cereals. Stay-green variants reveal how autumnal senescence and dormancy are coordinated in trees. The stay-green phenotype can be the result of alterations in hormone metabolism and signalling, particularly affecting networks involving cytokinins and ethylene. Members of the WRKY and NAC families, and an ever-expanding cast of additional senescence-associated transcription factors, are identifiable by mutations that result in stay-green. Empirical selection for functional stay-green has contributed to increasing crop yields, particularly where it is part of a strategy that also targets other traits such as sink capacity and environmental sensitivity and is associated with appropriate crop management methodology. The onset and progress of senescence are phenological metrics that show climate change sensitivity, indicating that understanding stay-green can contribute to the design of appropriate crop types for future environments.

Key words: Carbon, chlorophyll, hormone, leaf, nitrogen, protein, QTL, senescence, stress, transcription factor, yield.

Introduction

The term stay-green (sometimes staygreen) applied to plants is relatively recent in origin. The earliest record we have been able to find is a 1962 publication of Proefstation voor de Akker-en Weidebouw, Wageningen by E. Steinbuch, W.S. Poelstra and T.C. van der Kamp, entitled ‘Investigation into the cultivation and processing of 5 broad bean varieties in 1961. Effect of variety and degree of ripeness on yield, grading and quality’. Herein it is stated that ‘staygreen lines of broad bean had a uniform seed size and could be harvested at a more mature stage than the very late white varieties’. Appropriately for a character studied by Mendel (Thomas et al., 1996), stay-green seems originally to have been a phenotype descriptor used by legume breeders. For example, an early journal paper describes stay-green as a character in Vicia faba (Sjödin, 1971). Intensive selection had been steadily increasing both yield and the duration of greenness in a range of agricultural species since the early decades of the 20th century (Fig. 1; Duvick et al., 2004), and by the end of the 1970s, stay-green was becoming established explicitly as a superior characteristic and marketing feature of commercially bred grain crops, particularly maize. This development coincided with the first physiological analyses of the stay-green phenotype and a growing realization that there are multiple routes to delayed foliar yellowing (Thomas and Smart, 1993; Thomas and Howarth, 2000). Major advances in understanding the origins and implications of stay-green...
followed from the discovery of the pathway of chlorophyll catabolism and associated genes (Hörtensteiner and Kräutler, 2011), growing awareness of the functional significance of the photosynthetic and nitrogen mobilization phases of leaf development (Gregersen, 2011), increasing knowledge of the role of leaf senescence in stress responses (Guo and Gan, 2012), and the identification of system-wide regulators of the timing and rate of the senescence syndrome (Breeze et al., 2011; Guo, 2013). The present review will selectively discuss each of these aspects, with particular emphasis on the physiological consequences of staying green. It ends with some thoughts about the part stay-green might play in research that addresses present and future global challenges.

**Chlorophyll metabolism and stay-green**

**Chlorophyll catabolism**

Loss of chlorophyll is the visible symptom of leaf senescence and, by definition, the stay-green trait reflects impaired or delayed chlorophyll catabolism. Stay-greens have been broadly divided into cosmetic, where the primary lesion is confined to pigment catabolism, and functional, in which the entire senescence syndrome, of which chlorophyll catabolism is only one component, is delayed or slowed down, or both (Thomas and Howarth, 2000). Many of the non-visible components of the senescence syndrome are more or less unaffected by the mutant genotype in cosmetic stay-greens. In an early study, Thomas and Stoddart (1975) showed that soluble protein is mobilized normally during senescence of a *Festuca* cosmetic stay-green, and that proteolysis could be inhibited by treatment with cytokinin or accelerated with abscisic acid (ABA), just as in the wild type, without an appreciable effect on pigment retention. The pathway of chlorophyll catabolism is now known in considerable detail, and the points at which mutation leads to a cosmetic stay-green phenotype can be identified (Table 1). The lesion originally described in *Festuca* was eventually identified as the consequence of an insertion in the gene *SGR* (Armstead et al., 2006). Subsequently stay-greens defective at the *SGR* locus have been reported for pea (Armstead et al., 2007), *Arabidopsis* (Ren et al., 2007), rice (Jiang et al., 2007), and tomato and pepper (Barry et al., 2008), among other species.

The SGR step stands at the origin of the chlorophyll catabolism pathway and is one of the points at which transcriptional regulation of senescence-related pigment loss is exerted

![Fig. 1. Progressive increases in yields and stay-green scores of modern maize varieties since 1930. Data from Duvick DN, Smith JSC, Cooper M. Long-term selection in a commercial hybrid maize breeding program. In Plant breeding reviews: long-term selection: crops, animals, and bacteria, Volume 24, Part 2. Copyright © 2004 John Wiley & Sons, Inc.](http://jxb.oxfordjournals.org/)

**Table 1.** 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 | sgr = stay-green | Binding LHCl 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 | nal (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 | pph = stay-green | Dephytylation of phaeophytin |
| | CRN1 | | |
| | NCYS | | |
| Phaeophorbide a oxygenase | PAO | acd1 = cell death, not stay-green | Ferredoxin-dependent oxidative opening of macrocycle to form RCC |
| | ACD1 | | |
| | LLS1 | | |
| RCC reductase | RCCR | acd2 = cell death, not stay-green | Ferredoxin-dependent reduction of RCC to pFCC |
| | ACD2 | | |
(Armstead et al., 2007; Ougham et al., 2008; Hörtensteiner, 2009; Sakuraba et al., 2012). Another early reaction in chlorophyll breakdown is the conversion of chlorophyll b to a via a two-step reductase reaction, catalysed by the products of the genes NYC1NOL and HCAR. Mutational suppression of either NYC or NOL in rice, but only of NYC in Arabidopsis, results in a cosmetic stay-green phenotype (Sato et al., 2009; Horrie et al., 2009). In the hcar mutant, senescence is disrupted by cell death. Leaf tissue in the dark loses viability while retaining pigment, but in the light it bleaches rapidly (Meguro et al., 2011; Sakuraba et al., 2012).

Arabidopsis and rice mutants lacking phaeophytinase, the enzyme that removes phytol from phaeophytin, are phenotypically similar at the level of chlorophyll degradation but do overproduce chlorophyll—for example by overexpression of the genes encoding chlorophyllide a oxygenase and red chlorophyll catabolite (RCC) reductase, the acd1 and acd2 genotypes, respectively, of Arabidopsis) results in a photosensitive cell-death phenotype, but such mutants are not true senescence stay-greens (Tanaka et al., 2003; Pružinská et al., 2007). SGR, NYC1, PPH, and ACD2 are coordinately regulated at the transcriptional level in Arabidopsis (Hörtensteiner, 2013). On activation of SGR at the initiation of senescence, its translation product, the SGR protein, binds to the light-harvesting complex II (LHCII), the major (but not exclusive) location of chlorophyll a and b in the thylakoid membrane. All enzymes in the catabolic pathway up to and including RCC reductase assemble into a complex with SGR–LHCII. The resulting macromolecular machine converts chlorophyll to the photodynamically inert product pFCC by channelling the photoactive intermediate catabolites, thereby minimizing the risk that the subcellular apparatus necessary for orderly senescence will be exposed to photo-oxidative damage (Sakuraba et al., 2012). Interference with the assembly of this machine results in a cosmetic stay-green phenotype.

**Chlorophyll synthesis**

In principle, another route to stay-green via pigment metabolism is the continued biosynthesis of chlorophyll in excess of the activity of the catabolic pathway. Plants engineered to overproduce chlorophyll—for example by overexpression of the gene encoding chlorophyllide a oxygenase (Kusaba et al., 2013)—have a delayed yellowing phenotype of the kind classified as type E by Thomas and Howarth (2000). It is clear that the potential to make chlorophyll persists until quite a late stage in leaf development: regreening of yellow leaves, in which gerontoplasts redifferentiate into chloroplasts, demonstrates this (Zavaleta-Mancera et al., 1999). Moreover, senescent leaf tissues fed aminolaevulinic acid are photosensitized, indicating that the steps in pigment synthesis are intact at least as far as the closed-cycle tetrapyrrole intermediates (Hukmani and Tripathy, 1994). The activity of porphobilinogen deaminase, an enzyme near the beginning of the pathway, declines to a low level before senescence commences (Frydman and Frydman, 1979), and aminolaevulinic acid formation is suppressed in darkness by post-translational feedback in response to accumulation of protochlorophyllide (Richter et al., 2010). Protochlorophyllide oxidoreductase, the key enzyme in chlorophyll biosynthesis, is obligately light dependent (Paddock et al., 2012). As stay-greens of the cosmetic type described above retain pigment in darkness, it seems certain that persistence of chlorophyll biosynthesis does not contribute to the phenotype. And yet there remains a long-standing and unresolved mystery: could it be that the leaves of at least some angiosperms have the capacity to synthesize chlorophyll independently of light, as some older publications (e.g. Adamson et al., 1980) suggest? Future discoveries about the contribution of the synthesis side of the chlorophyll turnover equation to expression of the stay-green trait may well spring new surprises.

**Carbon capture, nitrogen remobilization, and stay-green**

The carbon–nitrogen transition

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; Yoo et al., 2007; Fig. 2).

The physiological regulation of the transition point is a long-standing issue in senescence research. Hensel et al.
(1993) proposed that the switch to nutrient salvage and yellowing is a direct response to the decline in photosynthetic capacity. Based on the comparative behaviour of cosmetic stay-green and normal genotypes, Hilditch et al. (1989) concluded that the C–N shift cannot be a consequence of simply turning off synthesis or maintenance of chloroplast structure and function—there must also be positive induction of senescence-specific degradation processes. Changes in the transition point have implications for crop yield and composition. Fig. 3 compares cereal grains and potato tubers (which are rich in starch and other C compounds relative to N content) with the high-N legume soybean (Osaki et al., 1991). As long as sink capacity provides somewhere to put the additional C, the functional stay-green character, by prolonging photosynthesis, will generally contribute to increased yield in high-C crops (Gregerse et al., 2013). However, delaying the C–N transition and/or slowing foliar senescence may compromise yield in high-N species, and the published evidence suggests that, under field conditions, the functional stay-green trait can be of limited or even negative value for soybean (Kumudini et al., 2002) and cowpea (Ismail et al., 2000). Moreover, as discussed below in connection with the role of NAC transcription factors, stay-green in cereals can have negative consequences for crop quality (the ‘dilution effect’: Simmonds, 1995) by interfering with the supply of N for grain protein synthesis and the import from senescing leaves of nutritionally important minerals (Uauy et al., 2006).

N mobilization in cosmetic stay-greens

Cosmetic stay-greens have little significant influence on the C-capture phase of foliar development, but the extended lifespan of chlorophyll in these plants is accompanied by retention of the membrane proteins with which they are associated in the chloroplast (Thomas, 1977; Hilditch et al., 1989; Bachmann et al., 1994; Guiamet and Giannibelli, 1994; Kusaba et al., 2007; Schelbert et al., 2009). Thylakoid proteins are second only to ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) as a source of remobilized N during senescence (Morita, 1980). Steps in chlorophyll catabolism are the primary sites of the genetic lesion in cosmetic stay-greens; disruption of membrane organization and of protein recycling are pleiotropic consequences of this class of mutation (Thomas et al., 2002). Experiments on Lolium perenne failed to identify a significant effect of sgr on vegetative growth (Macduff et al., 2002), and grain yield components and N-protein content in rice plants carrying the sgr mutation were reported to be not significantly different from wild-type values (Cha et al., 2002). It seems that, in contrast to species with high-N sinks, the demands of developing vegetative tissue or grains can be met by N recycled from Rubisco and other soluble proteins, without recourse to the N immobilized in thylakoids as a consequence of pigment retention. This may be an aspect of domestication in cereal, forage, and root crops, leading to gigantism in both sources and sinks (Lester, 1989; Evans, 1996; Ross-Ibarra et al., 2007). The relatively high proportion of N in pre-harvest foliage, particularly evident in wheat, soybean, and potato (Fig. 3), is indicative of selection for hypertrophy and source abundance. In natural ecosystems, however, evergreen shrubs and trees have adopted the stay-green strategy as a fitness attribute related to low rates of internal N recycling in response to nutrient-poor environments (Aerts, 1995).

Proteolysis and stay-greens

Progress towards understanding the biochemistry and regulation of protein catabolism and N recycling in senescence has lagged behind chlorophyll catabolism but is gathering pace (Feller et al., 2007; Roberts et al., 2012; Ono et al., 2013). There are a few observations of stay-green arising from interference with proteolytic enzymes. Antisense suppression of tobacco CND41, which encodes an aspartic protease thought to function in Rubisco degradation, delays senescence in lower leaves, whereas overexpression of CND41 accelerates yellowing (Kato et al., 2005). Older leaves of mutant maize plants with a transposon insertion in the senescence-associated legumain gene Seeβ retain chlorophyll and photosynthetic activity for longer than those of the wild type (Donnison et al., 2007). However, where there is an effect at all, suppressing the activities of proteolytic enzymes generally accelerates senescence (Roberts et al., 2012); this is particularly true of the components of autophagic and ubiquitin–proteasome pathways, which are often suggested to have causative roles in the senescence syndrome (Thompson et al., 2005; Deprost et al., 2007; Katsiarimpa et al., 2013).

Environmental responses, stress, and stay-green

Functional stay-green and drought resistance in sorghum

Stay-green and stress response traits are closely associated. This relationship is particularly apparent in genetic studies
that show quantitative trait loci (QTLs) for temperature and drought responses coinciding with loci for leaf senescence, and in numerous examples of improvements in stress tolerance achieved by simultaneous selection for stay-green (Ougham et al., 2000; Vijayalakshmi et al., 2010; Jordan et al., 2012; Emebiri, 2013). Here, we discuss the example of sorghum, where stay-green has been targeted as a valuable agronomic trait. In this species, water limitation during the grain development stage can cause premature leaf death and poor yield of seed and stover. Retention of green leaf area in stay-green genotypes is associated with enhanced capacity to continue normal grain fill under drought conditions, reduced lodging, high stem carbohydrate content and grain weight, and resilience against charcoal stem rot (McBee et al., 1983; Borrell et al., 2000; Burgess et al., 2002).

Functional stay-green in sorghum is expressed as different combinations of delayed onset and a reduced rate of senescence across the range of genotypes (Thomas and Howarth, 2000; Fig. 4). The source of stay-green used in most of the genetic studies and associated breeding programmes is the line B35, a derivative of Ethiopian durra and Nigerian landraces (Mahalakshmi and Bidinger, 2002). Genetic mapping in populations based on crosses with B35 have identified four major stay-green QTLs (Stg1, StgI, Stg3, and Stg4 in decreasing order of importance), together accounting for up to 54% of the phenotypic variance. Stg1 and Stg2 are both located on chromosome 3, Stg3 on chromosome 2, and Stg4 on chromosome 5 (Subudhi et al., 2000; Xu et al., 2000; Kim et al., 2005). Several minor stay-green loci have also been reported, but these are generally unstable across environments (Crasta et al., 1999). The line E36-1, derived from Ethiopian zera-zera germplasm and unrelated to B35, expresses a stay-green phenotype when grown under drought conditions in the field (Van Oosterom et al., 1996) but not under well-watered conditions (Fig. 4). Three of the major stay-green QTLs in mapping populations based on crosses with E36-1 are shared with those derived from B35 (Haussmann et al., 2002).

Mechanism of drought tolerance in stay-greens

Numerous studies have mapped stay-green in sorghum and associated the trait and its genetic loci with responses to drought, a phenotypic connection that is broadly maintained under field conditions (Kassahun et al., 2010; Jordan et al., 2012), but there is little information available on the underlying physiological mechanism of this relationship. Tuinstra et al. (1998) showed a positive association between xylem pressure potential on the one hand, and grain yield and stay-green on the other, indicating that the QTL for xylem pressure potential influences differences in drought tolerance by maintaining plant water status. Other hypotheses propose that delayed loss of photosynthetic capacity, or enhanced uptake of N in the post-anthesis period, somehow lead to better drought tolerance (Vadez et al., 2013). Thomas et al. (2000) pointed out that the principal sources of stay-green in cultivated sorghum are East African land-race types that tend to be perennials. Because perennials generally have low harvest indices and reproductive sink strengths, the monocarpic influence that would trigger and sustain wholesale foliar senescence in an annual is weak. It is also possible that vegetative tissues of perennials are intrinsically less sensitive to senescence signals than those of annuals. Progress towards deeper insights into stay-green sorghum physiology is likely to come from advances in understanding the source–sink basis of annuality and perenniality (Thomas, 2013) and expanding knowledge of senescence regulatory networks, as described later in this review, together with developments in sorghum genomics and synteny relationships with model species such as rice (Ramu et al., 2009; Mace et al., 2013). As an example, Srinivas et al. (2008) used conserved markers to identify the segment of rice chromosome 1 collinear with the region of sorghum chromosome 3 containing Stg1, the major stay-green QTL described above. Of the many QTLs that have been mapped to this region of the rice genome (Fig. 5), one controls leaf chlorophyll content and others are associated with the quality of the leaf as a source tissue and with drought tolerance.

Photoperiod sensitivity and stay-green

Monocarpic senescence is influenced by photoperiod in annual species with a daylength requirement for floral initiation, but senescence is also under the control of a pathway
independent of flowering (Wingler et al., 2009; Parrott et al., 2012). There is evidence that the stay-green tendency, dwarf habit, and daylength insensitivity have moved as linked phenotypes during the selection of modern, highly productive, short-stemmed, non-lodging bread wheat varieties. For example, a QTL for delayed flag leaf senescence maps onto wheat chromosome 2D, close to an allele of the Ppd-D1 locus for photoperiod insensitivity and the stature gene Rht8 (Pestsova and Röder, 2002; Verma et al., 2004). The senescence of deciduous tree leaves is also embedded in a complex of developmental responses to environmental cues. Thus, overexpression of the phytochrome A gene in hybrid aspen (Populus tremula × tremuloides) resulted in daylength-insensitive plants that, unlike wild-type aspen, did not cease growth, acclimate to cold, develop dormancy, or undergo leaf senescence and abscission in response to short days (Olsen et al., 1997).

Hormones, transcription factors, and stay-green

Hormonal regulation of senescence

The initiation and progression of senescence are under hormonal control. Cytokinins are the most potent general antagonists of senescence (Zwack and Rashotte, 2013), and there are numerous examples of cytokinin-mediated stay-greens. Studies on a range of species, beginning with the classic experiments of Gan and Amasino (1995) on tobacco, have created stay-greens by engineering autoregulated production of endogenous cytokinin through transformation with a gene encoding isopentenyl transferase fused with the promoter region of a senescence-associated gene (SAG). The Arabidopsis stay-green ore12-1 is a gain-of-function mutant in which the cytokinin receptor gene AHK3 is expressed constitutively (Kim et al., 2006). Overexpression of a proteolysis-insensitive version of the type B response regulator ARR2, another component of the cytokinin signalling pathway, also results in a stay-green phenotype (Kim et al., 2012). Such analyses of stay-green genotypes reveal a central role for the AHK3–ARR2 interaction in the hormonal regulation of senescence. Cytokinin-mediated delay of leaf senescence is inhibited by downregulating extra-cellular invertase associated with transfer of translocated carbon from the vascular system to the sink (Moore et al., 2003; Lara et al., 2004). This represents a point of contact between cytokinin action and the sugar-sensing/autophagy network that regulates development, nutritional responses, and lifespan (Liu and Bassham, 2012; Thomas, 2013). Some pathogens that infect leaves stimulate cytokinin production and delayed senescence in a zone surrounding the infection site. The result is a ‘green island’, an area of zombified stay-green tissue that benefits the pathogen by continuing to supply it with photosynthate (Walters and McRoberts, 2006).

Other hormones have been implicated in senescence and stay-green in some species and tissues (Kusaba et al., 2013). Roles for ABA in oxidative regulation are described below. Senescence in Arabidopsis and a number of other plants is altered by chemical and genetic interference with ethylene physiology (Pierik et al., 2006; Graham et al., 2012). The dominant ethylene-insensitive receptor mutant of Arabidopsis, etr1-1, is stay-green (Grbic Fig. 5. Region of the rice genome (from 28 to 37.1 Mb on chromosome 1) corresponding to the sorghum stay-green QTL Stg1 (Srinivas et al., 2008), showing several rice QTLs affecting source quality. QCC-1 is a QTL for leaf chlorophyll content (Teng et al., 2004). Image from the QTL Genome Viewer provided by the Q-TARO Database (Yonemaru et al., 2010), http://qtaro.abr.afrc.go.jp/cgi-bin/gbrowse/Oryza_sativa/.
and Bleeker, 1995), as is the ethylene signalling mutant ore3 (Oh et al., 1997) and acs family mutants defective in ethylene biosynthesis (Tsuchisaka et al., 2009). Competence to respond to developmental or environmental cues inducing senescence is age dependent. Studies in Arabidopsis plants exposed to ethylene over the course of development have established that there is a window of maturity-related ethylene sensitivity, during which plants acquire the competence to senesce but do not initiate and execute senescence because ethylene and/or some other endogenous regulator are limiting (Graham et al., 2012). Several Arabidopsis stay-green mutant lines, designated old, have been identified in which the timing of the response to ethylene is delayed (Shirzadian-Khorramabad et al., 2008). The mechanistic basis of such responses is tied up with the phenomena of juvenility, maturity, and phase change, in which microRNAs, epigenetic regulation, and cell-death pathways interact in a complex fashion that is not yet well understood (Thomas, 2013).

Reactive oxygen species (ROS) and WRKY

ROS, which generally accumulate in tissues with age, are common factors in the signalling pathways by which leaf senescence responds to hormones and environmental factors. Initiation and execution of leaf yellowing have been associated with the timing and interaction of $H_2O_2$ build-up and expression of genes encoding antioxidants, notably catalases and ascorbate peroxidases. The balance between ROS formation and its removal by antioxidant systems determines the cellular redox state, which has further consequences for metabolism and gene expression (Zimmermann et al., 2006).

WRKY53 is a redox-sensitive gene induced by $H_2O_2$. It encodes a transcription factor that autoregulates its own synthesis by feedback inhibition. WRKY53 interacts with a large number of genes of various kinds, including other members of the WRKY family, genes encoding catalases, various SAGs, and components of the salicylate and jasmonate signalling networks (Miao and Zentgraf, 2007). Downregulating WRKY53 expression delays functional senescence (Miao et al., 2004), and application of the analytical tools of systems biology has identified this transcription factor as an early-acting component in the senescence regulatory network (Guo, 2013).

NAC family transcription factors

The case of the NAC family of transcription factors is of particular interest because it provides a rare example of a mechanistic explanation for a type of functional stay-green that has been exploited for crop improvement (Uauy et al., 2006; Brevis et al., 2010). Studies of NACs also give insights into how domestication and selection for agronomic performance lead to a tendency to functional stay-green as a result of shifting the C capture–N mobilization transition (Fig. 2).

Grain protein content (GPC) in wheat maps to a single locus on chromosome 6, encoding an NAC transcription factor. The protein encoded by a related Arabidopsis gene, AtNAP, is a positive regulator of senescence initiation (Guo and Gan, 2006). A number of other members of the NAC family in this species are implicated in the control of senescence, although they are not close homologues of GPC/AtNAP (Kim et al., 2009; Balazadeh et al., 2011; Lee et al., 2012). Downregulating GPC transcript levels resulted in stay-green wheat plants in which leaf senescence was delayed by 24 d, protein content in the grain was reduced by 5.8%, and grains were 30% deficient in zinc and 24% in iron (Uauy et al., 2006). The corresponding GPC phenotype in barley has been shown to be due to allelic variation in a senescence-associated NAC gene that is also a component of a network controlling vernalization, photo-induction, and flowering time (Parrott et al., 2012). By virtue of the critical roles GPC-related NACs play in regulating cereal leaf senescence and determining the partitioning of N and minerals between the grain and crop residue, variations in such NAC genes are likely to account for a range of agronomically important stay-green phenotypes.

NAC transcription factors are networked to ROS and pathogen signalling pathways (Balazadeh et al., 2011; Lee et al., 2012; Hickman et al., 2013). SAG113, a regulator of ABA-mediated stomatal function, is one of AtNAP’s targets (Zhang et al., 2012). The phenotype of sag113 mutant plants induced to senesce by ABA treatment is stay-green (Zhang and Gan, 2012). The high degree of interactivity between nodes of transcriptional regulation, hormone- and ROS-mediated signalling pathways, and sensors of environmental cues and stresses (Guo, 2013; Hickman et al., 2013) offers an almost unlimited number of junctures at which genetically determined modification can result in a stay-green phenotype. The upshot is a rich source of variation for targeted or empirical crop improvement.

The future for the stay-green trait

Senescence and crop improvement

Crop breeding and management techniques have enhanced plant resistance to early- and late-season stresses such as chilling and short daylengths and, in doing so, have explicitly or incidentally altered the timing and rate of senescence (Gregersen et al., 2013). The success of these measures can be seen, for example, in the geographical range of maize grown in Europe, where a succession of new adapted cultivars of this subtropical species has allowed it to be grown as far north as Scandinavia (Odgaard et al., 2011). In the extreme case of winter-sown temperate cereals such as wheat and barley, planting and germination take place in the autumn, and the crop is already established and ready to rapidly develop a full canopy as soon as spring temperatures permit. Their yields, reflecting the canopy’s prolonged C-capture phase, are correspondingly higher than those of spring-sown varieties (Ellis and Russell, 1984).

After a century of intensive improvement, maize and rice have probably arrived at the limit of what can be achieved by breeding for delayed senescence, and the focus is currently on traits related to sink capacity, plant architecture, and resistance to pests, diseases, and stress (Lee and Tollenaar, 2007; Wu, 2009; Fischer and Edmeades,
However, as we have seen, QTL and marker-assisted breeding in sorghum show that stay-green retains its effectiveness as an improvement trait if it is associated with selection for stress tolerance (Vadez et al., 2013). Other crops are benefitting from this approach, for example wheat (Elshafei et al., 2013), cowpea (Muchero et al., 2013), and barley (Emebiri, 2013). Submergence tolerance in rice represents a trait of major agronomic importance (Laurentius et al., 2009) that turns out to have a stay-green aspect: conditional and ectopic overexpression of the submergence tolerance regulator SUBMERGENCE1A results in constrained ethylene production and responsiveness to jasmonate and salicylate, and consequently postpones dark-induced senescence through the maintenance of chlorophyll and carbohydrate reserves in photosynthetic tissue (Fukao et al., 2012).

**Agronomic problems associated with the stay-green trait**

For certain cultivated species, there can be serious agronomic downsides associated with a protracted C-capture phase and retention of a lush green canopy that compromise the nutrient and water economy of the crop. For example, the economic case for Miscanthus as a combustible or fermentable energy source depends on minimizing the amounts of residual elements other than C, H, and O remaining after shoot senescence at the end of the growing season. A prolonged C-capture phase is highly desirable, and stay-green is certainly valuable in Miscanthus for biomass yield, particularly under conditions where growth may be water limited (Clifton-Brown et al., 2002), but this should not be at the cost of incomplete nutrient transfer from senescing green tissue to underground rhizomes (Fig. 6). High-efficiency salvage of N, phosphorus (P), potassium (K), and other nutrients is essential for growth of the following season’s biomass to be supported almost entirely by recycling from rhizomes, thereby avoiding the need for the external application of any further fertilizer. The sustainability of perennial grasses as a source of renewable energy is in part a consequence of the timely and efficient way they integrate C capture and the movement of nutrients between shoots and rhizomes in their growth cycles (Propheter and Staggenborg, 2010). Moreover, completely draining aboveground biomass of everything except lignocellulose is necessary for dry-down, large-scale desiccation that maximizes the economic yield of dry matter for harvest and transport. Dry-down is also desirable during the harvesting of grain maize because too much residual moisture in the shoot can clog the cutting mechanisms of combine harvesters (Yang et al., 2010). In these cases, and others such as development of cereal crops providing both high yield and high protein, the idiootype will comprise late initiation of canopy senescence, to maximize C capture, followed by fast and complete mobilization of N and other nutrients. As exemplified by the small sample of sorghum genotypes shown in Fig. 4, there is plenty of genetic variation out there for senescence initiation, independent of rate, available for exploitation.

**Fig. 6.** In the energy crop Miscanthus, the transition from the C-capture (green) phase of crop development to terminal senescence and dry-down is ideally associated with high-efficiency transfer of N and other minerals (NPK) to the rhizome. Pictures courtesy of John Clifton-Brown, Aberystwyth University, UK.

**Cosmetic stay-green: more than a pretty face**

Functional stay-green clearly continues to be a valuable, albeit sometimes agronomically problematic, attribute for crop improvement. The practical usefulness of cosmetic stay-greens is more limited. Colour retention is important in plants for landscaping, decoration, or display. Stay-green is of interest for turf-grass breeding (Thorogood, 2003) and may contribute towards extending the shelf-life of green vegetables such as broccolini (Page et al., 2001). Disruption of the chlorophyll catabolism pathway is a feature of a number of pathogen interactions, such as the hypersensitive response (Mur et al., 2010), and indicates opportunities to develop new pesticide treatments. Degreening during maturation of Arabidopsis seeds is regulated by ABA, acting through ABI3, a transcription factor that activates functionally redundant SGR1 and SGR2 genes, leading to degradation of chlorophyll in the developing embryo. Failure to degreen is a serious problem for seed storage in some species, and for the quality and shelf-life of oils from canola and other oilseeds (Delmas et al., 2013). Gene expression is known to be sensitive to intermediates in tetrapyrrole metabolism—retrograde signalling between the plastid and nucleus is an example (Chi et al., 2013)—and we might therefore expect to find more or less subtle broader physiological consequences arising from a blockage in chlorophyll breakdown. It may be significant that algal bilins, which are structurally related to chlorophyll catabolites, are turning out to have important signalling functions (Rochaix, 2013). From phylogenetic analyses, it is inferred that most components of the chlorophyll catabolism pathway were present before the appearance of terrestrial plants, and in most cases they even predate evolution of multicellularity (Thomas et al., 2009). If, as seems likely,
they retain the functions they had before the ancestral vascular species recruited them into the senescence syndrome, we might expect collateral physiological alterations in cosmetic stay-green phenotypes. A recent proteomics comparison of sgr and wild-type Arabidopsis identified a number of differences in expressed protein complement beyond components of the chlorophyll breakdown pathways, some of which were already apparent before the onset of senescence (Grassl et al., 2012). Analysis of photosynthesis revealed considerable differences between senescing leaves of the wild type and those of sgr Festuca pratensis (Kingston-Smith et al., 1997). Rubisco, measured as maximum extractable activity of Rubisco, was higher in senescing leaves of the mutant than in the wild type, despite the absence of a corresponding maintenance of leaf CO₂ assimilation rate. Compared with the wild type, senescing mutant leaves had greater photochemical quenching, higher photosystem II quantum efficiency at a given irradiance, and a greater proportion of electrons directed to photorespiration. Luo et al. (2013) reported that SGR of tomato mediates lycopene and β-carotene synthesis by directly interacting with the carotenoid biosynthesis enzyme PSY1, and also influences ethylene signal transduction. A surprising observation on the phenotypic consequences of an insertion mutation in the Medicago truncatula SGR gene is that not only is leaf greenness extended but nodule senescence is also delayed (Zhou et al., 2011). It seems therefore that some, perhaps most, cosmetic stay-greens are more than merely cosmetic and may have as-yet-unexplored functional features.

Senescence and phenology

Senescence is of continuing practical value as a phenomenological metric at the crop and vegetation level for performance estimation, management, and prediction (Bannari et al., 1995). The onset of senescence is one of the four cardinal transition dates in each seasonal cycle (the others are the onset of spring greenup, the time of maximal canopy development, and the post-senescence minimum). Estimates of crop yield are obtained by modelling vegetation indices determined by satellite or airplane-based remote spectral imaging. The record of such approaches is patchy, but at their best, as in a recent study of maize and soybean yields in the central USA by Bolton and Friedl (2013), overall R² coefficients for observed versus estimated values of around 0.7 have been achieved. Phenological modelling is a sensitive tool for monitoring vegetation responses to climate change, and is revealing delayed senescence to be one of the immediate consequences of the changing relationship between temperature and photoperiod (Bauerle et al., 2012). This has implications for the design of varieties better adapted to an altered environment. Stay-green and its regulation in crops can be expected to occupy centre stage as climate change begins to bite.

References

Adamson HY, Hiller RG, Vesk M. 1980. Chloroplast development and the synthesis of chlorophyll a and b and chlorophyll protein complexes I and II in the dark in Tradescantia albiflora (Kunth). Planta 150, 269–274.
Aerts R. 1995. The advantages of being evergreen. Trends in Ecology and Evolution 10, 402–407.
Armstead I, Donnison I, Aubry S, et al. 2006. From crop to model to crop: Identifying the genetic basis of the stay-green mutation in the forage grass Festuca pratensis [Huds.] New Phytologist 172, 592–597.
Armstead I, Donnison I, Aubry S, et al. 2007. Cross-species identification of Mendel’s loci. Science 315, 73.
Bachmann A, Fernández-López J, Ginsburg S, Thomas H, Bouwkamp JC, Solomos T, Matile P. 1994. Stay-green genotypes of Phaseolus vulgaris. Chloroplast proteins and chlorophyll catabilites during foliar senescence. New Phytologist 126, 593–600.
Balazadeh S, Kwasniewski M, Caldana C, Mehrnia M, Zanor MI, Xue GP, Mueller-Roeber B. 2011. ORS1, an H₂O₂-responsive NAC transcription factor, controls senescence in Arabidopsis thaliana. Molecular Plant 4, 346–360.
Bannari AD, Morin D, Bonn F, Huete AR. 1995. A review of vegetation indices. Remote Sensing Reviews 13, 95–120.
Barry CS, McQuinn RP, Chung M-Y, Besuden A, Giovannini JJ. 2008. Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. Plant Physiology 147, 179–187.
Bauerle WL, Oren R, Way DA, Qian SS, Stoy PC, Thornton PE, Bowden JD, Hoffman FM, Reynolds RF. 2012. Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. Proceedings of the National Academy of Sciences, USA 109, 8612–8617.
Bolton DK, Friedl MA. 2013. Forecasting crop yield using remotely sensed vegetation indices and crop phenology metrics. Agricultural and Forest Meteorology 173, 74–84.
Borrell AK, Hammer GL, Henzell RG. 2000. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Science 40, 1037–1048.
Breeze E, Harrison E, McHattie S, et al. 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. Plant Cell 23, 873–894.
Brevis JC, Morris CF, Manthey F, Dubcovsky J. 2010. Effect of the grain protein content locus Gpc-B1 on bread and pasta quality. Journal of Cereal Science 51, 357–365.
Burgess MG, Rush CM, Piccinni G, Schuster G. 2002. Relationship between charcoal rot, the stay-green trait, and irrigation in grain sorghum. Phytopathology 92, S10.
Cha KW, Lee YJ, Koh HJ, Lee BM, Nam YW, Paek NC. 2002. Isolation, characterization, and mapping of the stay green mutant in rice. Theoretical and Applied Genetics 104, 526–532.
Chi W, Sun X, Zhang L. 2013. Intracellular signaling from plastid to nucleus. Annual Review of Plant Biology 64, 559–582.
Clifton-Brown JC, Lewandowski I, Bangerth F, Jones MB. 2002. Comparative responses to water stress in stay-green, rapid- and slow senescing genotypes of the biomass crop, Miscanthus. New Phytologist 154, 335–345.
Cresta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics 262, 579–588.
Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bournonville C, Bollier N, Northey JGB, McCourt P, Samuel MA. 2013. ABI3 controls senescing genotypes of the biomass crop, Miscanthus. New Phytologist 195, 81–95.
Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolai M, Bedu M, Robaglia C, Meyer C. 2007. The Arabidopsis TPR kinase links plant growth, yield, stress resistance and mRNA translation. EMBO Reports 8, 864–870.
Donnison IS, Gay AP, Thomas H, Edwards KJ, Edwards D, James CL, Thomas AM, Ougham HJ. 2007. Modification of nitrogen remobilisation, grain fill and leaf senescence in maize (Zea mays L.) by transposon insertional mutagenesis in a protease gene. New Phytologist 173, 481–494.
Duvick DN, Smith JSC, Cooper M. 2004. Long-term selection in a commercial hybrid maize breeding program. Plant Breeding Reviews 24, 109–162.

Ellis RP, Russell G. 1984. Plant development and grain yield in spring and winter barley. Journal of Agricultural Science 102, 85–95.

Elshafei AA, Saleh M, Al-Doss AA, Moustafa KA, Al-Qunayri FH, Barakat MN. 2013. Identification of new SRAF markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits in wheat under water-stressed condition. Australian Journal of Crop Science 7, 887–893.

Emebiri LC. 2013. QTL dissection of the loss of green colour during post-anthesis grain maturation in two-rowed barley. Theoretical and Applied Genetics 126, 1873–1884.

Evans LT. 1996. Crop evolution, adaptation and yield. Cambridge: Cambridge University Press.

Feller U, Anders I, Mae T. 2007. Rubiscolectyls: fate of Rubisco after its enzymatic function in a cell is terminated. Journal of Experimental Botany 58, 1615–1624.

Fischer RA, Edmeades GO. 2010. Breeding and cereal yield progress. Crop Science 50, 595–598.

Frydman RB, Frydman B. 1979. Disappearance of porphobilinogen deaminase activity in leaves before the onset of senescence. Plant Physiology 63, 1144–1157.

Fukao T, Yeung E, Bailey-Serres J. 2012. The submergence tolerance gene SUB1 delays leaf senescence under prolonged darkness through hormonal regulations in rice. Plant Physiology 160, 1795–1807.

Gan S, Amasino RM. 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270, 1986–1988.

Graham LE, Schippers JHM, Dijkwel PP, Wagstaff C. 2012. Ethylene and senescence processes. Annual Review of Plant Biology 63, 305–341.

Grassi J, Pružinská A, Hörtenstein S, Taylor NL, Millar AH. 2012. Early events in plastid protein degradation in stay-green Arabidopsis reveal differential regulation beyond the retention of LHCII and chlorophyll. Journal of Proteome Research 11, 5434–5452.

Grbić V, Bleeker AB. 1995. Ethylene regulates the timing of leaf senescence in Arabidopsis. The Plant Journal 8, 595–602.

Gregersen PL. 2011. Senescence and nutrient remobilization in crop plants. In: Hawkesford MJ, Barracough PB, eds. The molecular and physiological basis of nutrient use efficiency in crops. New York: Blackwell, 83–102.

Gregersen PL, Culetic A, Boschian L, Krupinska K. 2013. Plant senescence and crop productivity. Plant Molecular Biology 82, 603–622.

Guilamé JJ, Giannibelli MC. 1994. Inhibition of the degradation of chloroplast membranes during senescence in nuclear ‘stay green’ mutants of soybean. Plant, Cell and Environment 17, 395–402.

Guo Y, Gan S. 2006. A small RNA, a NAC family transcription factor, has an important role in leaf senescence. The Plant Journal 46, 601–612.

Guo Y, Gan S. 2012. Convergence and divergence in gene expression profiles induced by leaf senescence and 27 senescence-promoting hormonal, pathological and environmental stress treatments. Plant, Cell and Environment 35, 644–655.

Haussmann B, Mahalakshmi V, Reddy BVS, Seetharama N, Hash CT, Geiger HH. 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. Theoretical and Applied Genetics 106, 133–142.

Hensel LL, Grbić V, Baumgarten DA, Bleeker AB. 1993. Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in Arabidopsis. Plant Cell 5, 553–564.

Hickman R, Hill C, Penfold CA, et al. 2013. A local regulatory network around three NAC transcription factors in stress responses and senescence in Arabidopsis leaves. The Plant Journal 75, 26–39.

Hilditch P, Thomas H, Thomas BJ, Rogers LJ. 1989. Leaf senescence in a non-yellowing mutant of Festuca pratensis: proteins of Photosystem II. Plantas 177, 265–272.

Horie Y, Ito H, Kusaba M, Tanaka R, Tanaka A. 2009. Participation of chlorophyll b reductase in the initial step of the degradation of lightharvesting chlorophyll a/b-protein complexes in Arabidopsis. Journal of Biological Chemistry 284, 17449–17456.

Hörtenstein S. 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends in Plant Science 14, 155–162.

Hörtenstein S. 2013. Update on the biochemistry of chlorophyll breakdown. Plant Molecular Biology 82, 505–517.

Hörtenstein S, Kräutler B. 2011. Chlorophyll breakdown in higher plants. Biochimica et Biophysica Acta 1807, 977–988.

Hukmani P, Tripathy BC. 1994. Chlorophyll biosynthetic reactions during senescence of excised barley (Hordeum vulgare L. cv IB65) leaves. Plant Physiology 105, 1295–1300.

Ismail AM, Hall AE, Ehlers JD. 2000. Delayed leaf senescence and heat tolerance traits mainly are independently expressed in cowpea. Crop Science 40, 1049–1055.

Jiang HW, Li MR, Liang NB, Yan HB, Wei YL, Xu X, Liu JF, Xu Z, Chen F, Wu GJ. 2007. Molecular cloning and function analysis of the stay green gene in rice. The Plant Journal 52, 197–209.

Jordan DR, Hunt CH, Cruickshank AW, Borrell AK, Hennell RG. 2012. The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. Crop Science 52, 1153–1161.

Kassahun B, Bидinger FR, Hash CT, Kuruvinashetti MS. 2010. Stay-green expression in early generation sorghum (Sorghum bicolor (L.) Moench) QTL introgression lines. Euphytica 172, 351–362.

Kato Y, Yamamoto Y, Murakami S, Sato F. 2005. Post-translational regulation of CND1 protease activity in senescent tobacco leaves. Planta 222, 643–651.

Katsiarimpa A, Kalinowska K, Anzenberger F, Weiss C, Ostermag T, Tsutsui C, Schwechheimer C, Brunner F, Hückelhoven R, Isono E. 2013. The deubiquitinating enzyme AMDH1 and the ESCRT-III subunit VPS21 are required for autophagic degradation in Arabidopsis. Plant Cell 25, 2236–2252.

Kim HJ, Ryu H, Hong SH, Woo HR, Lim PO, Lee IC, Sheen J, Nam HG, Hwang I. 2006. Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in Arabidopsis. Proceedings of the National Academy of Sciences, USA 103, 814–819.

Kim JH, Woo HR, Kim J, Lim PO, Lee IC, Choi SH, Hwang D, Nam HG. 2009. Tetrubic feed-forward regulation of age-dependent cell death involving miR164 in Arabidopsis. Science 323, 1053–1057.

Kim J-S, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM. 2005. Chromosome identification and nomenclature of Sorghum bicolor. Genetics 169, 1169–1173.

Kim K, Ryu H, Cho Y-H, Scacchi E, Sabatini S, Hwang I. 2012. Cytokinin facilitated proteolysis of ARABIDOPSIS RESPONSE REGULATOR 2 attenuates signaling output in two-component circuitry. The Plant Journal 69, 934–945.

Kingston-Smith AH, Thomas H, Foyer CH. 1997. Chlorophyll a fluorescence, enzyme and antioxidant analysis provide evidence for the operation of an alternative electron sink during leaf senescence in a stay-green mutant of Festuca pratensis. Plant, Cell and Environment 20, 1323–1337.

Kumudini S. 2002. Trials and tribulations: a review of the role of assimilate supply in soybean genetic yield improvement. Field Crops Research 75, 211–222.

Kusaba M, Ito H, Morita R, et al. 2007. Rice NON-YELLOW COLORING1 is involved in light-harvesting complex II and grana degradation during leaf senescence. Plant Cell 19, 1362–1375.

Kusaba M, Tanaka A, Tanaka R. 2013. Stay-green plants: what do they tell us about the molecular mechanism of leaf senescence. Photosynthesis Research 117, 221–234.

Lara MEB, Garcia M-CG, Fatima T, Ehrness R, Lee TK, Proels R, Tanner W, Roitsch T. 2004. Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. Plant Cell 16, 1276–1287.

Laurentius A, Voosenek CJ, Bailey-Serres J. 2009. Genetics of high-rise rice. Nature 460, 959–960.
Lee EA, Tollenaar M. 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Science 47, S202–S215.

Lee S, Seo PJ, Lee HJ, Park CM. 2012. A NAC transcription factor NTL4 promotes reactive oxygen species production during drought-induced leaf senescence in Arabidopsis. The Plant Journal 70, 831–844.

Lester RN. 1989. Evolution under domestication involving disturbance of genic balance. Euphytica 44, 125–132.

Liu Y, Bassham DC. 2012. Autophagy: pathways for self-eating in plant cells. Annual Review of Plant Biology 63, 215–237.

Luo Z, Zhang J, Li J, Yang C, Wang T, Ouyang B, Li H, Giovannoni J, Ye Z. 2013. A STAY-GREEN protein SISG1 regulates lycopeno and β-carotene accumulation by interacting directly with SIPSY1 during ripening processes in tomato. New Phytologist 198, 442–452.

Maccuff JH, Humphreys MO, Thomas H. 2002. Effects of a stay-green mutation on plant nitrogen relations in Lolium perenne L. during N starvation and after defoliation. Annals of Botany 89, 11–21.

Mace ES, Tai S, Gilding EK, et al. 2013. Whole-genome sequencing reveals untapped genetic potential in Africa’s indigenous cereal crop sorghum. Nature Communications 4, 2320.

Mahalakshmi V, Bidinger FR. 2002. Evaluation of putative stay-green sorghum germplasm lines. Crop Science 42, 965–974.

McBee GG, Waskom RM, Miller FR, Creelman RA. 1983. Effect of senescence and nonsenescence on carbohydrates in sorghum during late kernel maturity states. Crop Science 23, 372–376.

Meguro M, Ito H, Takabayashi A, Tanaka R, Tanaka A. 2011. Identification of the 7-hydroxymethyl chlorophyll a reductase of the chlorophyll cycle in Arabidopsis. Plant Cell 23, 3442–3453.

Miao Y, Laun T, Zimmermann P, Zentgraf U. 2004. Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis. Plant Molecular Biology 55, 853–867.

Miao Y, Zentgraf U. 2007. The antagonist function of Arabidopsis WRKY53 and ESP/ESP in leaf senescence is modulated by the jasmonic and salicylic acid equilibrium. Plant Cell 19, 819–830.

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu Y-X, Hwang I, Jones T, Sheen J. 2003. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300, 332–336.

Morita K. 1980. Release of nitrogen from chloroplasts during senescence in rice (Oryza sativa L.). Annals of Botany 46, 297–302.

Morita R, Sato Y, Masuda Y, Nishimura M, Kusaba M. 2009. Defect in nonyellow coloring 3, an alpha/beta hydrolase-fold family protein, causes a staygreen phenotype during leaf senescence in rice. The Plant Cell 59, 940–952.

Muchero W, Roberts PA, Diop NN, Drabo I, Cisse N, Close TJ, Muranaka S, Boukar O, Ehlers JD. 2013. Genetic architecture of nonyellow coloring 3, an alpha/beta hydrolase-fold family protein, causes a staygreen phenotype during leaf senescence in rice. The Plant Cell 59, 940–952.

Ougham H, Armstrong I, Howarth C, Galyou I, Donnison I, Thomas J. 2007. The genetic control of senescence revealed by mapping quantitative trait loci. Annual Plant Reviews 26, 171–201.

Ougham H, Hörtensteiner S, Armstead I, Donnison I, King I, Thomas H, Mur L. 2008. The control of chlorophyll catabolism and the status of yellowing as a biomarker of leaf senescence. Plant Biology 10 (Suppl. 1), 4–14.

Paddock T, Lima D, Mason ME, Apel K, Armstrong GA. 2012. Arabidopsis light-dependent protoclorophyllide oxidoreductase A (PORA) is essential for normal plant growth and development. Plant Molecular Biology 78, 447–460.

Page T, Griffiths G, Buchanan-Wollaston V. 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. Plant Physiology 125, 718–727.

Parrott DL, Downs EP, Fischer AM. 2012. Control of barley (Hordeum vulgare L.) development and senescence by the interaction between a chromosome six grain protein content locus, day length, and vernalization. Journal of Experimental Botany 63, 1323–1339.

Pestsova E, Röder M. 2002. Microsatellite analysis of wheat chromosome 2D allows the reconstruction of chromosomal inheritance in pedigrees of breeding programmes. Theoretical and Applied Genetics 106, 84–91.

Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek LAJ. 2006. The Janus face of ethylene: growth inhibition and stimulation. Trends in Plant Science 11, 176–183.

Propheter JL, Staggenborg S. 2010. Performance of annual and perennial biofuel crops: nutrient removal during the first two years. Agronomy Journal 102, 798–805.

Průzník A, Anders I, Aubry S, Schenk N, Taperonoux-Luthi E, Müller T, Kräutler B, Hörtensteiner S. 2007. In vivo participation of red chlorophyll catabolite reductase in chlorophyll breakdown. Plant Cell 19, 369–387.

Ramu P, Kassahun B, Senthivel S, Kumar CA, Jayashree B, Folkertsa RT, Reddy LA, Kuruvinashehti MS, Haussmann BIG, Hash CT. 2009. Exploiting rice–sorghum synergety for targeted development of EST-SSRs to enrich the sorghum genetic linkage map. Theoretical and Applied Genetics 119, 1193–1204.

Ren GD, An K, Liao Y, Zhou X, Cao YJ, Zhao HF, Ge XC, Kuai BK. 2007. Identification of a novel chloroplast protein AtNYE1 regulating chlorophyll degradation during leaf senescence in Arabidopsis. Plant Physiology 144, 1420–1431.

Riechmann J, Peter E, Pörs Y, Lorenzen S, Grimm B, Czarnecki O. 2010. Rapid red repression of a 5-aminolevulinic acid synthetase in green barley leaves. Plant and Cell Physiology 51, 670–681.

Roberts IN, Caputo C, Criado MV, Funk C. 2012. Senescence-associated proteases in plants. Physiologia Plantarum 145, 130–139.

Rochaix JD. 2010. Surprising roles for bilins in a green alga. Proceedings of the National Academy of Sciences, USA 101, 3218–3219.

Ross-Ibarra J, Morrell PL, Gaut BS. 2007. Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proceedings of the National Academy of Sciences, USA 104 (Suppl. 1), 8641–8648.

Sakuraba Y, Schelbert S, Park S-Y, Han S-H, Lee B-D, Andrés CB, Kessler F, Hörtensteiner S, Paek N-C. 2012. STAY-GREEN and chlorophyll catabolic enzymes interact at light-harvesting complex II for chlorophyll detoxification during leaf senescence in Arabidopsis. Plant Cell 24, 507–518.

Sakuraba Y, Morita R, Katsumata S, Nishimura M, Tanaka A, Kusaba M. 2009. Two short-chain dehydrogenase/reductases, NON-YELLOW COLORING 1 and 26 NYCLIKE, are required for chlorophyll b and light-harvesting complex II degradation during senescence in rice. The Plant Journal 57, 120–131.

Schelbert S, Aubry S, Burla B, Agne B, Kessler F, Krupinska K, Hörtensteiner S. 2009. Phoephephytin phophorbidie hydrolase (phoephephytinas) is involved in chlorophyll breakdown during leaf senescence in Arabidopsis. Plant Cell 21, 767–785.

Shirzadian-Khorramabad R, Jing H-C, Hille J, Dijkwel PP. 2008. Identification of Arabidopsis stay green mutants with a functional ethylene-response pathway. In: McGill CR, Rowarth JS, eds. Seeds for futures. Agronomy Society of New Zealand Special Publication, No. 13, and Grassland Research and Practice Series, No. 14, 119–129.

Simmonds NW. 1995. The relation between yield and protein in cereal grain. Journal of the Science of Food and Agriculture 67, 309–315.
Sjödin J. 1971. Induced morphological variation in Vicia faba L. Hereditas 67, 155–179.

Srinivas G, Satish K, Murali Mohan S, Nagaraja Reddy R, Madhusudhana R, Balakrishna D, Venkatesh Bhat B, Howarth CJ, Seetharama N. 2008. Development of generic-microsatellite markers for sorghum staygreen QTL using a comparative genomic approach with rice. Theoretical and Applied Genetics 117, 283–296.

Subudhi PK, Rosenow DT, Nguyen HT. 2000. Quantitative trait loci for the stay green trait in sorghum (Sorghum bicolor L. Moench): consistency across genetic backgrounds and environments. Theoretical and Applied Genetics 101, 733–741.

Tanaka R, Hirashima M, Satoh S, Tanaka A. 2003. The Arabidopsis-accelerated cell death gene ACD1 is involved in oxygenation of phosphohexide a: Inhibition of the phosphohexide oxygenase activity does not lead to the ‘staygreen’ phenotype in Arabidopsis. Plant and Cell Physiology 44, 1266–1274.

Teng S, Qian Q, Zeng D, Kunihiro Y, Fujimoto K, Huang D, Zhu L. 2004. QTL analysis of leaf photosynthetic rate and related physiological traits in rice (Oryza sativa L.) Euphytica 135, 1–7.

Thomas H. 1977. Ultrastructure, polypeptide composition and photochemical activity of chloroplasts during foliar senescence of a non-yellowing mutant genotype of Festuca pratensis. Planta 137, 53–60.

Thomas H. 2013. Senescence, ageing and death of the whole plant. New Phytologist 197, 696–711.

Thomas H, Howarth CJ. 2000. Five ways to stay green. Journal of Experimental Botany 51, 329–337.

Thomas H, Huang L, Young M, Ougham H. 2009. Evolution of plant senescence. BMC Evolutionary Biology 9, 163.

Thomas H, Ougham H. 2014. Senescence and crop performance. In: Sadrass VO, Calderini DF, eds. Crop physiology. Applications for genetic improvement, agronomy and farming systems, 2nd edn. New York: Academic Press (in press).

Thomas H, Ougham H, Canter P, Donnison I. 2002. What stay-green mutants tell us about nitrogen remobilisation in leaf senescence. Journal of Experimental Botany 53, 801–808.

Thomas H, Schellenberg M, Vicentini F, Matile P. 1996. Gregor Mendel's green and yellow pea seeds. Botanica Acta 109, 3–4.

Thomas H, Smart CM. 1993. Crops that stay green. Annals of Applied Biology 123, 193–219.

Thomas H, Stoddart JL. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (Festuca pratensis). Plant Physiology 56, 438–441.

Thomas H, Thomas HM, Ougham H. 2000. Annuality, perenniality and cell death. Journal of Experimental Botany 51, 1–8.

Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD. 2005. Autophagic nutrient recycling in Arabidopsis directed by the ATG8 and ATG12 conjugation pathways. Plant Physiology 138, 2097–2110.

Thorogood D. 2003. Perennial ryegrass (Lolium perenne L.). In: Casler MD, Duncan RR, eds. Turfgrass biology, genetics, and breeding. New York: Wiley, 125–141.

Tsuchisaka A, Yu G, Jin H, Alonso JM, Ecker JR, Zhang X, Gao S, Theologis A. 2009. A combinatorial interplay among the 1-aminocyclopropane-1-carboxylate isoforms regulates ethylene biosynthesis in Arabidopsis thaliana. Genetics 183, 979–1003.

Tuinstra MR, Ejeta G, Goldschorden PB. 1998. Evaluation of near-isogenic sorghum lines for QTL markers associated with drought tolerance. Crop Science 38, 835–842.

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314, 1298–1301.

Vadez V, Deshpande S, Kholova J, Ramu P, Hash CT. 2013. Molecular breeding for stay-green: progress and challenges in sorghum. In: Varshney R, Tuberosa R (eds) Genomic applications to crop breeding: Vol. 2. Improvement for abiotic stress, quality and yield improvement. New York: Wiley, 125–141.

Van Oosterom EJ, Jayachandran R, Bidinger FR. 1996. Diallel analysis of the stay-green trait and its components in sorghum. Crop Science 36, 549–555.

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW. 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135, 255–263.

Vijayalakshmi K, Fritz AK, Paulsen GM, Bai G, Pandravada S, Gill BS. 2010. Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Molecular Breeding 26, 163–175.

Walters DR, McRoberts N. 2006. Plants and biotrophs: a pivotal role for cytokinins? Trends in Plant Science 11, 581–586.

Wingler A, Purdy SJ, Edwards SA, Chardon F, Masclaux-Daubresse C. 2009. QTL analysis for sugar-regulated leaf senescence supports flowering-dependent and -independent senescence pathways. New Phytologist 185, 420–433.

Wu X. 2009. Prospects of developing hybrid rice with super high yield. Agronomy Journal 101, 688–695.

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (Sorghum bicolor L. Moench). Genome 43, 461–469.

Yang J, Carena MJ, Uphaus J. 2010. Area under the dry down curve (AUDDC): a method to evaluate rate of dry down in maize. Crop Science 50, 2347–2354.

Yonemaru J, Yamamoto T, Fukuoka S, Uga Y, Hori K, Yano M. 2010. Q-TAR: QTL Annotation Rice Online Database, Rice 3, 194–203.

Yoo S, Cho S, Zhang H, Paik H, Lee C, Li J, Yoo J, Lee B, Koh H, Seo H, Paek NC. 2007. Quantitative trait loci associated with functional stay-green SNU-SG1 in rice. Molecules and Cells 24, 83–94.

Zavaleta-Mancera HA, Thomas BJ, Thomas H, Scott IM. 2007. What stay-green mutants tell us about nitrogen remobilisation in leaf senescence. Journal of Experimental Botany 58, 801–808.

Zhang K, Gan SS. 2007. Regreening of senescent Nicotiana leaves. II. Redifferentiation of plastids. Journal of Experimental Botany 50, 1683–1689.

Zhang K, Gan SS. 2012. An abscisic acid-ATNAP transcription factor-SAG113 protein phosphatase 2C regulatory chain for controlling dehydration in senescing Arabidopsis leaves. Plant Physiology 158, 961–969.

Zhang K, Xia X, Zhang Y, Gan S. 2012. An ABA-regulated and Golgi-localized protein phosphatase controls water loss during leaf senescence in Arabidopsis. The Plant Journal 69, 667–678.

Zhou C, Han L, Pislariu C, Yoo S, Cho S, Zhang H, Paik H, Lee C, Li J, Yoo J, Lee B, Koh H, Seo H, Paek NC. 2007. Quantitative trait loci associated with functional stay-green SNU-SG1 in rice. Molecules and Cells 24, 83–94.

Zavaleta-Mancera HA, Thomas BJ, Thomas H, Scott IM. 1999. Regreening of senescent Nicotiana leaves. II. Redifferentiation of plastids. Journal of Experimental Botany 50, 1683–1689.

Zhang K, Gan SS. 2012. An abscisic acid-ATNAP transcription factor-SAG113 protein phosphatase 2C regulatory chain for controlling dehydration in senescing Arabidopsis leaves. Plant Physiology 158, 961–969.

Zhang K, Xia X, Zhang Y, Gan S. 2012. An ABA-regulated and Golgi-localized protein phosphatase controls water loss during leaf senescence in Arabidopsis. The Plant Journal 69, 667–678.

Zhou C, Han L, Pislariu C, et al. 2011. From model to crop: functional analysis of a STAY-GREEN gene in the legume Medicago truncatula and effective use of the gene for alfalfa improvement. Plant Physiology 157, 1483–1496.

Zimmermann P, Heinlein C, Orendi G, Zentgraf U. 2006. Senescence-specific regulation of catalasase in Arabidopsis thaliana (L.) Heynh, Plant, Cell and Environment 29, 1049–1060.

Zwack PJ, Rashotte AM. 2013. Cytokinin inhibition of leaf senescence. Plant Signaling and Behavior 8, e24737.