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REVIEW PAPER

The stay-green trait

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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

Abbreviations: ABA, abscisic acid; C, carbon; GPC, grain protein content; LHCII, light-harvesting complex II; N, nitrogen; QTL, quantitative trait locus; RCC, red chlorophyll catabolite; ROS, reactive oxygen species; Rubisco, ribulose-1,5-bisphosphate carboxylase-oxygenase.

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 remobilization 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.

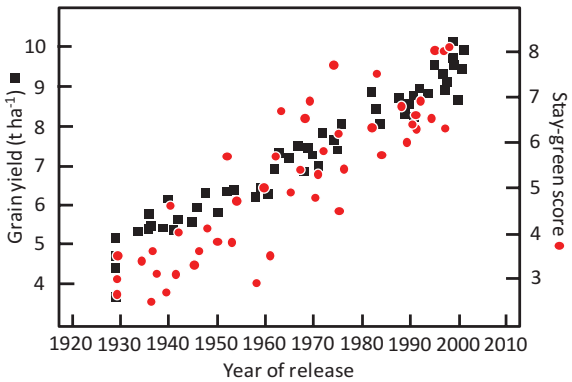

Fig. 1. Progressive increases in yields and stay-green scores of modern maize varieties since 1930. Data from Duvick DN, Smith JSC, Cooper M. Long-term selection in a commercial hybrid maize breeding program. In *Plant breeding reviews: long-term selection: crops, animals, and bacteria*, Volume 24, Part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

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

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

Where a gene has been identified more than once by different groups, the alternative names are given.

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

(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 *NYC*/*NOL* 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; Horie *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 cosmetic stay-greens (Morita *et al.*, 2009; Schelbert *et al.*, 2009). Knocking out either of the two reactions that open the tetrapyrrole macrocycle (phaeophorbide *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 photoreactive 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.*

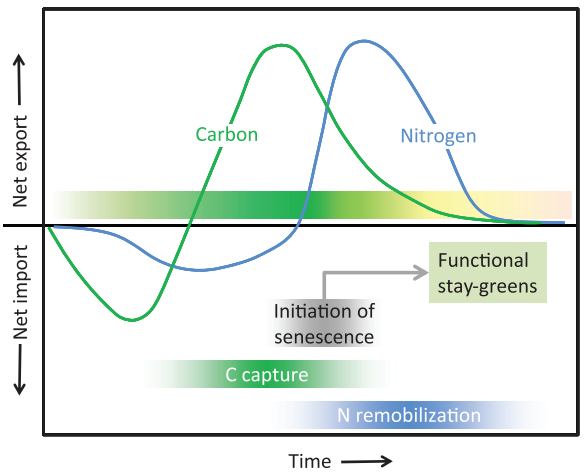

Fig. 2. The functional stay-green trait is associated with the transition from the carbon (C) capture to the nitrogen (N) mobilization phase of foliar development.

(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 (Gregersen *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, 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; Guiamét 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).

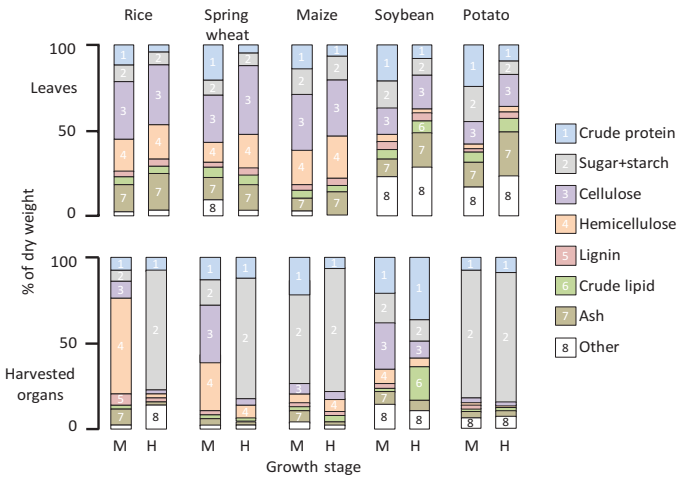

Fig. 3. Compositional profiles for leaves and harvested organs of five crop species, determined at the stage of maximal vegetative growth (M) and harvest (H). Data from Osaki M, Shinano T, Tadano T. 1991. Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation in field crops. *Soil Science and Plant Nutrition* **37,** 117–128. Copyright © Japanese Society of Soil Science and Plant Nutrition, reprinted by permission of Taylor & Francis Ltd, www.tandfonline.com on behalf of The Japanese Society of Soil Science and Plant Nutrition.

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 *See2β* 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.*, 2007; 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 resistance to 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 (*Stg2*, *Stg1*, *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).

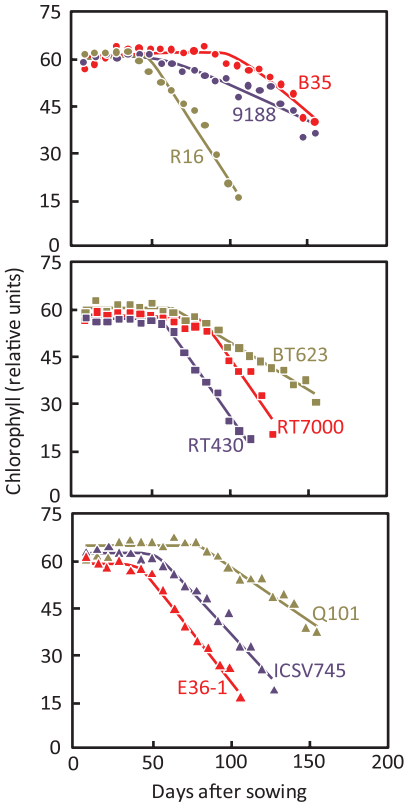

Fig. 4. Relative chlorophyll content, measured with a Minolta SPAD meter, in leaf 6 of nine sorghum genotypes grown to harvest under well-watered greenhouse conditions. From Thomas and Howarth (2000). Five ways to stay green. *Journal of Experimental Botany* **51,** 329–337. With permission from the Society for Experimental Biology.

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 syntenic 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 extracellular 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 (Grbić

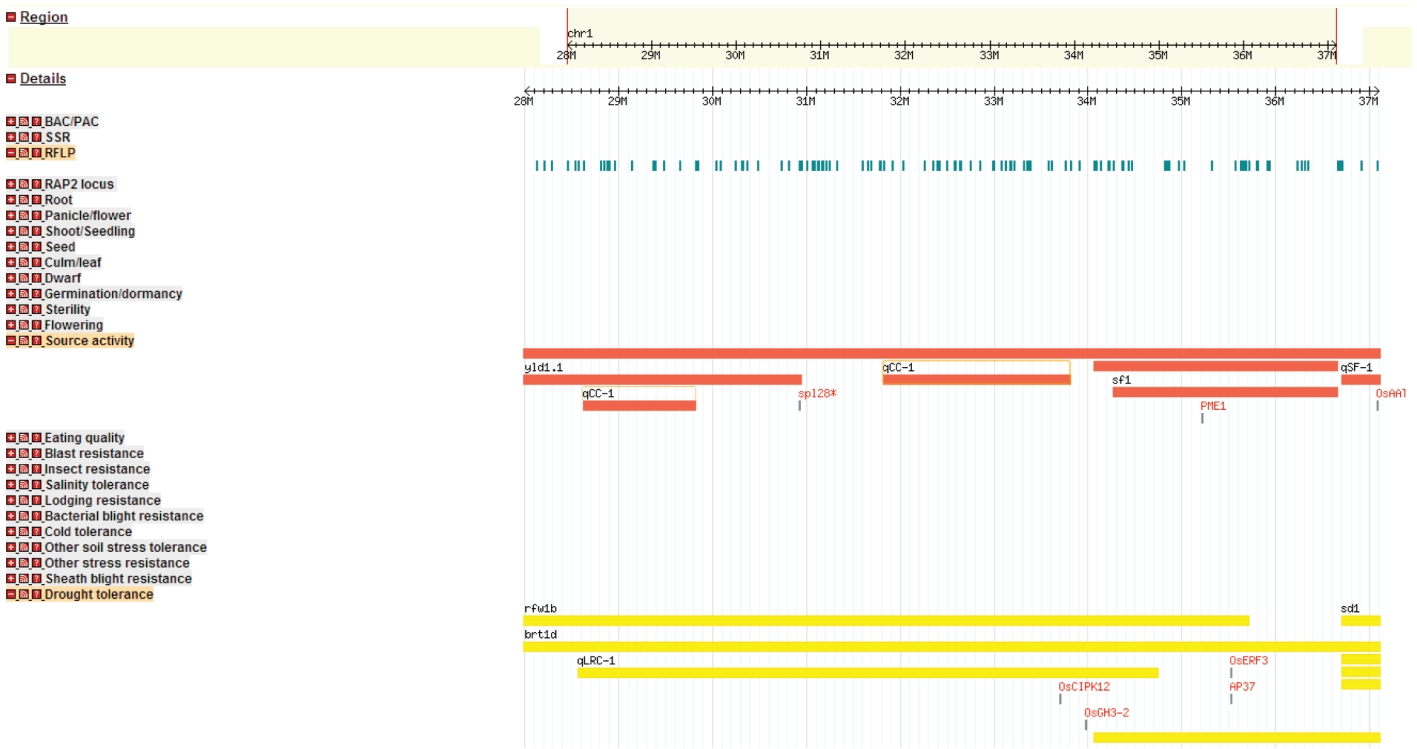

Fig. 5. Region of the rice genome (from 28 to 37.1 Mb on chromosome 1) corresponding to the sorghum stay-green QTL *Stg1* (Srinivas *et al.*, 2008), showing several rice QTLs affecting source quality. QCC-1 is a QTL for leaf chlorophyll content (Teng *et al.*, 2004). Image from the QTL Genome Viewer provided by the Q-TARO Database (Yonemaru *et al.*, 2010), http://qtaro.abr.affrc.go.jp/cgi-bin/gbrowse/Oryza_sativa/.

and Bleecker, 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 *SAG*s, 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 *NAC*s 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,

2010). 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 idiotype 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.

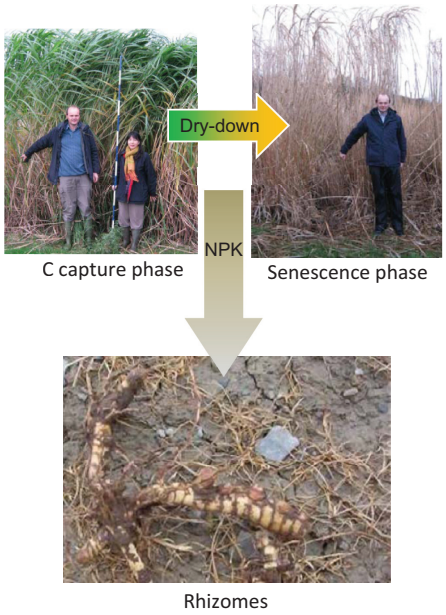

Fig. 6. In the energy crop *Miscanthus*, the transition from the C-capture (green) phase of crop development to terminal senescence and dry-down is ideally associated with high-efficiency transfer of N and other minerals (NPK) to the rhizome. Pictures courtesy of John Clifton-Brown, Aberystwyth University, 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 broccoli (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 CO_2 assimilation rate. Compared with the wild type, senescing mutant leaves had greater photochemical quenching, higher photosystem II quantum efficiency at a given irradiance, substantially increased electron flux through photosystem II, and a greater proportion of electrons directed to photorespiration. Luo *et al.* (2013) reported that SGR1 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 phenological 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 R^2 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.

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