

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; LHClI, 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.

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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.

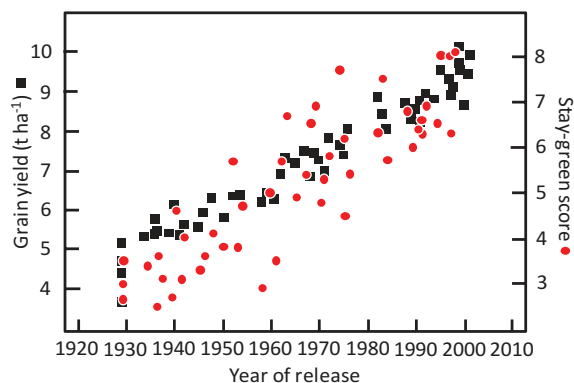


Fig. 1. Progressive increases in yields and stay-green scores of modern maize varieties since 1930. Data from Duvick DN, Smith JSC, Cooper M. Long-term selection in a commercial hybrid maize breeding program. In *Plant breeding reviews: long-term selection: crops, animals, and bacteria*, Volume 24, Part 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	<i>SGR</i> <i>NYE1</i> <i>SID</i>	<i>sgr</i> = stay-green	Binding LHCII and catabolic enzymes, stabilising catabolic complex
Chlorophyll b reductase	<i>NYC</i> <i>NOL</i> <i>HCAR</i>	<i>nyc</i> (rice and <i>Arabidopsis</i>) = stay-green <i>nol</i> (rice, but not <i>Arabidopsis</i>) = stay-green <i>hcar</i> = cell death, not stay-green	Ferredoxin/NADPH-dependent two-step conversion of chlorophyll <i>b</i> to chlorophyll <i>a</i>
'Mg dechelatase'	Identity not yet resolved	?	Removal of Mg from the macrocycle (not known whether reaction is enzymic or chemical)
Phaeophytinase	<i>PPH</i> <i>CRN1</i> <i>NCY3</i>	<i>pph</i> = stay-green	Dephnylation of phaeophytin
Phaeophorbide a oxygenase	<i>PAO</i> <i>ACD1</i> <i>LLS1</i>	<i>acd1</i> = cell death, not stay-green	Ferredoxin-dependent oxidative opening of macrocycle to form RCC
RCC reductase	<i>RCCR</i> <i>ACD2</i>	<i>acd2</i> = 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: greening 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.*

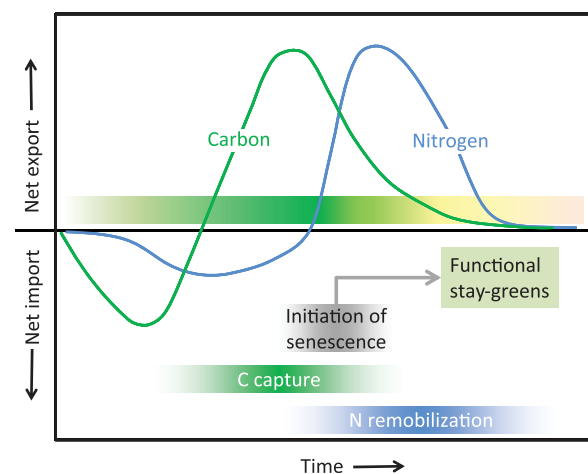


Fig. 2. The functional stay-green trait is associated with the transition from the carbon (C) capture to the nitrogen (N) mobilization phase of foliar development.

(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

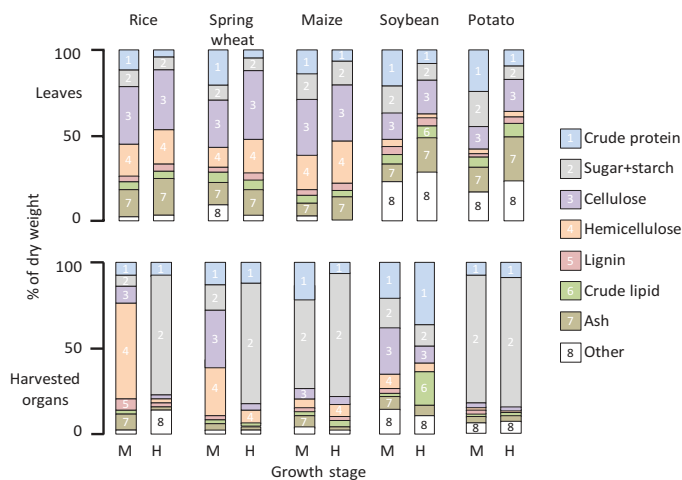


Fig. 3. Compositional profiles for leaves and harvested organs of five crop species, determined at the stage of maximal vegetative growth (M) and harvest (H). Data from Osaki M, Shinano T, Tadano T. 1991. Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation 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.

chloroplast (Thomas, 1977; Hilditch *et al.*, 1989; Bachmann *et al.*, 1994; Guimét 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 *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 (Hausmann *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

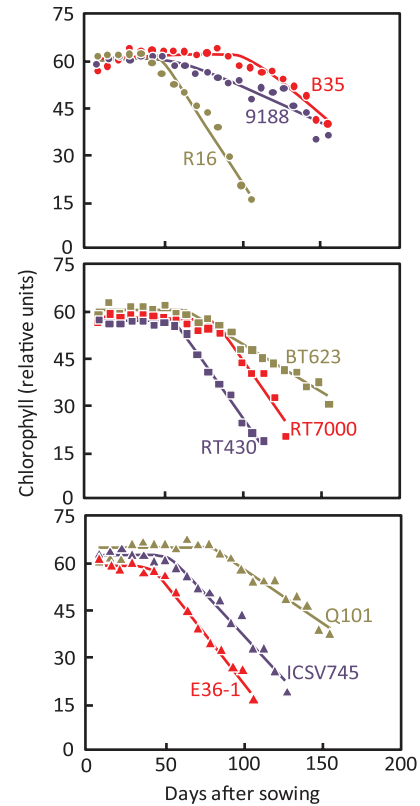


Fig. 4. Relative chlorophyll content, measured with a Minolta SPAD meter, in leaf 6 of nine sorghum genotypes grown to harvest under well-watered greenhouse conditions. From Thomas and Howarth (2000). Five ways to stay green. *Journal of Experimental Botany* **51**, 329–337. With permission from the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

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

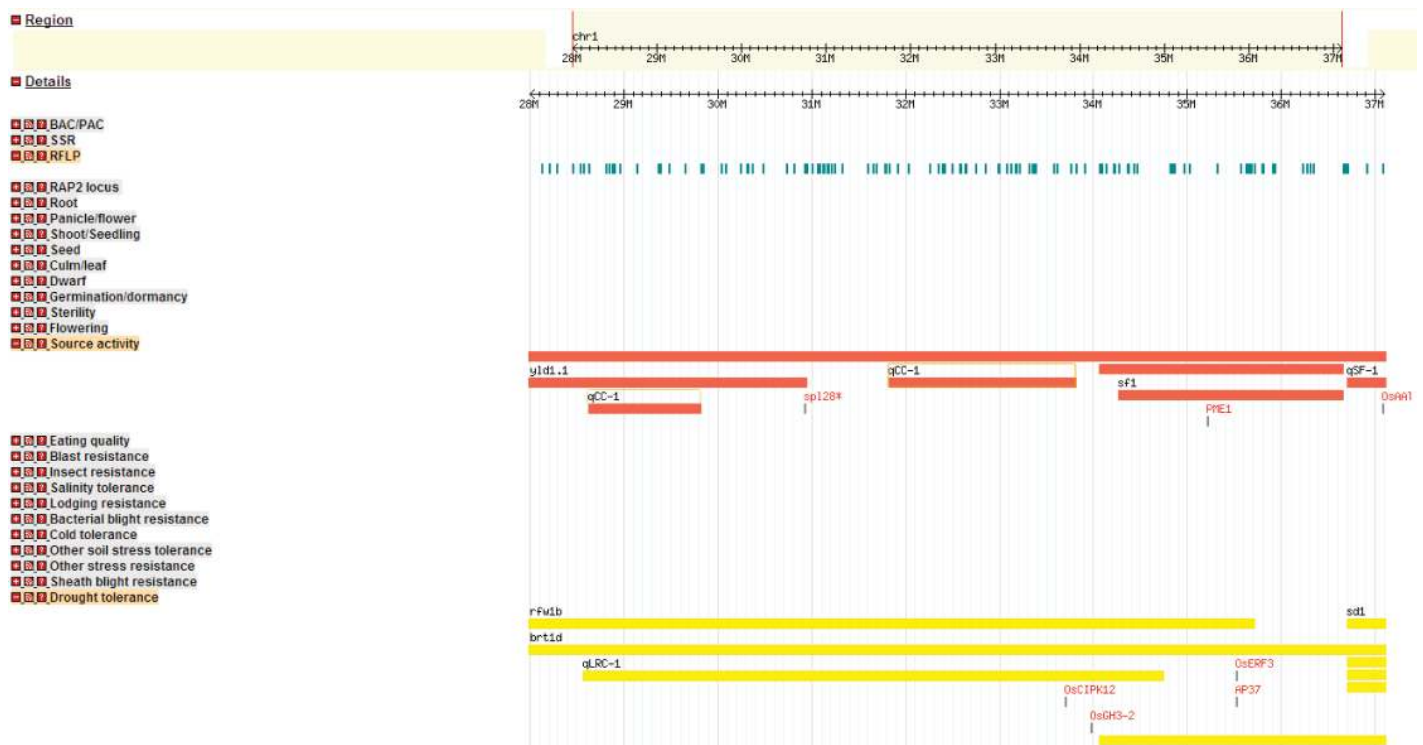


Fig. 5. Region of the rice genome (from 28 to 37.1 Mb on chromosome 1) corresponding to the sorghum stay-green QTL *Stg1* (Srinivas *et al.*, 2008), showing several rice QTLs affecting source quality. QCC-1 is a QTL for leaf chlorophyll content (Teng *et al.*, 2004). Image from the QTL Genome Viewer 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 H₂O₂ 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 H₂O₂. 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,

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 Stagenborg, 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 idiosyncrasy 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.

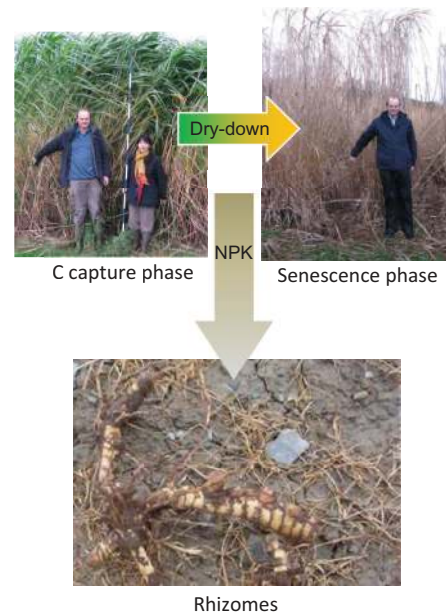


Fig. 6. In the energy crop *Miscanthus*, the transition from the C-capture (green) phase of crop development to terminal senescence and dry-down is ideally associated with high-efficiency transfer of N and other minerals (NPK) to the rhizome. Pictures courtesy of John Clifton-Brown, Aberystwyth University, 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 CO₂ 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.

References

Adamson HY, Hiller RG, Vesik 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 staygreen 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 *I* locus. *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 catabolites 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, Giovannoni 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.

Crasta 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 embryo degreening through Mendel's *I* locus. *Proceedings of the National Academy of Sciences, USA* **110**, E3888–E3894.

Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolai M, Bedu M, Robaglia C, Meyer C. 2007. The *Arabidopsis* TOR 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**, 109–152.
- Ellis RP, Russell G.** 1984. Plant development and grain yield in spring and winter barley. *Journal of Agricultural Science* **102**, 85–95.
- Elsafei AA, Saleh M, Al-Doss AA, Moustafa KA, Al-Qurainy FH, Barakat MN.** 2013. Identification of new SRAP 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. Rubiscolytics: fate of Rubisco after its enzymatic function in a cell is terminated. *Journal of Experimental Botany* **59**, 1615–1624.
- Fischer RA, Edmeades GO.** 2010. Breeding and cereal yield progress. *Crop Science* **50**, S85–S98.
- Frydman RB, Frydman B.** 1979. Disappearance of porphobilinogen deaminase activity in leaves before the onset of senescence. *Plant Physiology* **63**, 1154–1157.
- Fukao T, Yeung E, Bailey-Serres J.** 2012. The submergence tolerance gene *SUB1A* 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 Plant Reviews* **44**, 305–341.
- Grassl J, Pružinská A, Hörtensteiner S, Taylor NL, Millar AH.** 2012. Early events in plastid protein degradation in stay-green *Arabidopsis* reveal differential regulation beyond the retention of LHClI and chlorophyll. *Journal of Proteome Research* **11**, 5443–5452.
- 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, Barraclough 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.
- Guimét JJ, Giannibelli MC.** 1994. Inhibition of the degradation of chloroplast membranes during senescence in nuclear ‘stay green’ mutants of soybean. *Physiologia Plantarum* **91**, 395–402.
- Guo Y.** 2013. Towards systems biological understanding of leaf senescence. *Plant Molecular Biology* **82**, 519–528.
- Guo Y, Gan S.** 2006. AtNAP, 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.
- Hausmann BIG, 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. *Planta* **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 light-harvesting chlorophyll *a/b*-protein complexes in *Arabidopsis*. *Journal of Biological Chemistry* **284**, 17449–17456.
- Hörtensteiner S.** 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. *Trends in Plant Science* **14**, 155–162.
- Hörtensteiner S.** 2013. Update on the biochemistry of chlorophyll breakdown. *Plant Molecular Biology* **82**, 505–517.
- Hörtensteiner 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 IB 65) 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, Henzell 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, Biding FR, Hash CT, Kuruvashetti 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 CND41 protease activity in senescent tobacco leaves. *Planta* **222**, 643–651.
- Katsiarimpa A, Kalinowska K, Anzenberger F, Weis C, Ostertag M, Tsutsumi C, Schwechheimer C, Brunner F, Hüchelhoven R, Isono E.** 2013. The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2. 1 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. Trifurcate 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, Ehness 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, Voeseck 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 SISGR1 regulates lycopene and β -carotene accumulation by interacting directly with SIPSY1 during ripening processes in tomato. *New Phytologist* **198**, 442–452.
- Macduff 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 ESR/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**, 332–36.
- 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 Journal* **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 delayed senescence, biomass, and grain yield under drought stress in cowpea. *PLoS One* **8**, e70041.
- Mur LAJ, Aubry S, Mondhe M, et al. 2010. Accumulation of chlorophyll catabolites photosensitizes the hypersensitive response elicited by *Pseudomonas syringae* in *Arabidopsis*. *New Phytologist* **188**, 161–174.
- Odgaard MV, Bocher PK, Dalgaard T, Svenning J-C. 2011. Climatic and non-climatic drivers of spatiotemporal maize-area dynamics across the northern limit for maize production—a case study from Denmark. *Agriculture, Ecosystems and Environment* **142**, 291–302.
- Oh SA, Park JH, Lee GI, Paek KH, Park SK, Nam HG. 1997. Identification of three genetic loci controlling leaf senescence in *Arabidopsis thaliana*. *The Plant Journal* **12**, 527–535.
- Olsen JE, Junttila O, Nilsen J, Eriksson ME, Martinussen I, Olsson O, Sandberg G, Moritz T. 1997. Ectopic expression of oat phytochrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. *The Plant Journal* **12**, 1339–1350.
- Ono Y, Wada S, Izumi M, Makino A, Ishida H. 2013. Evidence for contribution of autophagy to Rubisco degradation during leaf senescence in *Arabidopsis thaliana*. *Plant, Cell and Environment* **36**, 1147–1159.
- 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.
- Ougham H, Armstead I, Howarth C, Galyuon I, Donnison I, Thomas H. 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 protochlorophyllide 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**, 1329–1339.
- 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, Voeseek LACJ. 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.
- Pružinská A, Anders I, Aubry S, Schenk N, Tapernoux-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, Senthilvel S, Kumar CA, Jayashree B, Folkertsma RT, Reddy LA, Kuruvinashetti MS, Haussmann BIG, Hash CT. 2009. Exploiting rice–sorghum synteny 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**, 1429–1441.
- Richter A, Peter E, Pörs Y, Lorenzen S, Grimm B, Czarnecki O. 2010. Rapid dark repression of 5-aminolevulinic acid synthesis 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. 2013. Surprising roles for bilins in a green alga. *Proceedings of the National Academy of Sciences, USA* **110**, 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.
- Sato Y, Morita R, Katsuma S, Nishimura M, Tanaka A, Kusaba M. 2009. Two short-chain dehydrogenase/reductases, NON-YELLOW COLORING 1 and 26 NYC1-LIKE, 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. Pheophytin pheophorbide hydrolase (pheophytinase) 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.

- Sjödén 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 genic-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 pheophorbide a: Inhibition of the pheophorbide a oxygenase activity does not lead to the 'staygreen' phenotype in *Arabidopsis*. *Plant and Cell Physiology* **44**, 1266–1274.
- Teng S, Qian Q, Zeng D, Kunihiko 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: Sadras 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, 75–105.
- 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, Goldsborough 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, Biding 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-TARO: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.** 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 model 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 catalases 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.