

REVIEW PAPER

Delayed senescence and crop performance under stress: always a functional couple?

Mariana Antonietta[†], Dana Martinez[†], and Juan J. Guiamet^{*†}

Instituto de Fisiología Vegetal, CONICET-UNLP, Argentina

[†] These authors contributed equally to this work.

* Correspondence: jguiamet@fcnym.unlp.edu.ar

Received 2 January 2024; Editorial decision 15 April 2024; Accepted 17 April 2024

Editor: Diego Gomez-Casati, Universidad Nacional de Rosario, Argentina

Abstract

Exposure to abiotic stresses accelerates leaf senescence in most crop plant species, thereby reducing photosynthesis and other assimilatory processes. In some cases, genotypes with delayed leaf senescence (i.e. ‘stay-green’) show stress resistance, particularly in cases of water deficit, and this has led to the proposal that senescence delay improves crop performance under some abiotic stresses. In this review, we summarize the evidence for increased resistance to abiotic stress, mostly water deficit, in genotypes with delayed senescence, and specifically focus on the physiological mechanisms and agronomic conditions under which the stay-green trait may ameliorate grain yield under stress.

Keywords: Abiotic stress, cytokinins, drought, senescence, stay-green, yield.

Introduction

Environmental conditions are defined as stressful if they cause a decrease in growth or fitness in a plant species. Biotic (e.g. pathogens, herbivores, competing plants) or abiotic (e.g. shortage or excess of water or minerals, high temperature) factors can impact negatively on plants and constitute important stress factors that limit yields of agricultural crops. Worldwide, it has been estimated that cereal production of different countries decreased by 10% and 9% due to drought and heat stress, respectively, during the 1964–2007 period (Lesk *et al.*, 2016); for soybeans, grain yields decreased by about 11% in dry years during the period 1961–2014 (Matiu *et al.*, 2017). This provides a rough estimate of the impact of stress factors reducing global agricultural productivity. In the present scenario of climate change, most models predict more frequent episodes of drought or heat waves (Lopez *et al.*, 2018), and

overall greater intensity of stress (Strzepek *et al.*, 2010). The forecasted increased incidence of several types of stress in agricultural ecosystems will pose an additional threat to food security in large parts of the world. The mechanisms whereby stress factors impair growth or agricultural performance are varied, depending on the type and intensity of stress and the species subjected to stress. For example, the primary deleterious effect of waterlogging may be to reduce levels of oxygen in the soil, thus limiting root respiration. This will affect energy generation for root functioning, with further impacts on above-ground physiology via decreased nutrient acquisition (Board, 2008). Similar cascades of deleterious effects might apply to almost all types of stressful conditions, and this highlights the complexity faced by studies of plant responses to stress factors.

A common phenomenon under different types of stress is physiological and morphological remodeling of the shoot via accelerated senescence and leaf abscission (Munné-Bosch and Alegre, 2004). Accelerated senescence of leaves releases mineral elements previously allocated to chloroplasts that can be redistributed to other organs (e.g. younger leaves, fruits), while, at the same time, reducing the potential demand for scarce resources (e.g. water under drought). These adjustments come at the cost of a decrease in the photosynthetic potential of the shoot. Therefore, senescence acceleration may promote survival of plants under drought by reducing the overall plant demand for water, thereby limiting the chances of severe dehydration and cell death (Munné-Bosch and Alegre, 2004; Wolfe *et al.*, 2016). However, in agricultural settings and for herbaceous annual plants, crop breeding may not seek enhanced survival but increased production under stress conditions (Blum, 2009). In this review, we will emphasize the relationship between leaf senescence and stress resistance in an agronomic sense, that is, as maintenance of crop production under stressful conditions, and not merely survival. Since most studies at the genetic and metabolic levels have been made under controlled conditions, these may not be easily extrapolated to field conditions, but still they can throw light on the possible mechanisms behind the stay-green phenotype. Our focus will be mostly on water deficit, since this is one of the stress factors most frequently faced by crops, and on grain crops given that physiological processes related to delayed senescence (mainly nutrient and carbon remobilization) could have very different implications in other types of crops where seeds are not the harvestable organ.

Plant responses to environmental stress

Environmental stresses promote responses that range from physiological adjustments to changes in growth and development. The array of responses elicited under stress depends on the species involved, the period in the life cycle, and the type and severity (i.e. intensity, duration) of the stress. Water and nitrogen (N) deficit are major limitations to crop production, although plants under field conditions are usually exposed to a combination of other factors, such as extreme temperatures, high irradiance, etc., which tend to exacerbate the effects of each single stress (Albrizio *et al.*, 2010; Ergo *et al.*, 2021). Here we analyse the interrelation between senescence progression and stress responses in the context of gradually imposed limitations of main resources (e.g. water). Extremely severe environmental episodes and artificially imposed stresses that provoke irreversible damage or death before any possibility of acclimation are not considered here, as these may not be of common occurrence under most agricultural conditions.

Plants develop three main adaptive mechanisms to resist stress: escape, avoidance, and tolerance (Kooyers, 2015; Shavrukov *et al.*, 2017). Regarding water stress, escape refers

to the synchronization of the plant life cycle to prevent sensitive phases of development from experiencing severe water deficit. Avoidance strategies allow the plant to minimize the chances of experiencing tissue water depletion: examples of avoidance mechanisms are increased root growth and hydraulic conductivity to improve water absorption; osmotic adjustment to maintain tissue water potentials; and stomatal closure, leaf rolling, and decreased canopy area (through leaf area reduction, senescence, and abscission) to prevent water loss. Tolerance refers to the ability to display resilience under low water availability, maintaining physiological activities or tissue viability through metabolic adjustment and repair mechanisms. An extreme example of water deficit tolerance is seen in resurrection plants, where leaf water content can decrease to very low levels without compromising plant survival (Scott, 2000; Leprince and Buitink, 2015). In this review, we will use the term resistance to refer to both avoidance and tolerance mechanisms related to delayed senescence, whereas escape mechanisms will not be included since these would change also the length of the plant cycle, making it difficult to compare different senescence phenotypes.

A major deleterious effect of water deficit is the decline in the photosynthetic capacity of the canopy as a consequence of reduced development of photosynthetic area, decreased photosynthetic activity per unit leaf area, and/or the shortening of the lifespan of photosynthetic organs (Munné-Bosch and Alegre, 2004). Drought also imposes limitations to mineral absorption, and nutrient deficit usually becomes an intrinsic feature of water deficit (Weber *et al.*, 2012). Either under drought or under well-irrigated conditions, N deficiency induces proteolysis to supply the demand for N from other organs. Leaf photosynthetic proteins, mainly Rubisco, which represents around one-third of remobilized N in C₃ plants, are degraded to N-containing compounds, predominantly amino acids, which are mobilized out of the leaf to growing organs (Sakuraba, 2022). As a consequence, the amounts of Rubisco and of other proteins decrease drastically under N-deficient conditions, reducing the photosynthetic capacity and accelerating leaf senescence.

Thermal stress, particularly heat, affects membrane stability, protein conformation and enzymatic activity, thereby triggering ROS production and oxidative stress (Ergo *et al.*, 2021). Chloroplasts are the main target of these metabolic impairments, i.e. ATP production and ribulose biphosphate regeneration decrease with the concomitant drop in CO₂ fixation. Water deficit and/or high irradiance exacerbate the detrimental effects of heat. High temperature episodes also reduce assimilate transport to the grains (Harding *et al.*, 1990). Overall, photosynthetic decline and accelerated canopy senescence represent a common symptom observed under these stresses. Senescence plays a pivotal role in crop yield as its occurrence limits the C available to feed growing organs (e.g. immature seeds), but senescence also provides sink tissues with remobilized N to sustain their growth.

Photosynthesis decrease and chloroplast dismantling are accelerated under stress

The negative impact of water deficit on photosynthesis occurs through stomatal limitations, reducing CO₂ diffusion into the leaf, or non-stomatal limitations that affect metabolic and photochemical reactions (Ouyang *et al.*, 2017). Metabolic impairment includes decreased carboxylation and regeneration of ribulose-1,5-bisphosphate, and decreased chlorophyll content and photosystem II quantum efficiency (Guan *et al.*, 2015; Chen *et al.*, 2016). Severe damage caused by drought, usually in combination with high temperatures and/or high irradiance, leads to the over-reduction of the electron transport chain components, alterations in energy dissipation mechanisms, photoinhibition, and disassembly of the photosynthetic apparatus (i.e. dissociation of light-harvesting complexes (LHC) from photosynthetic reaction centers, degradation of photosystem II, etc.) (Ergo *et al.*, 2021).

Water deficit and/or high N-demand conditions accelerate the natural (age-related) decline of photosynthetic rate and chloroplast dismantling in mature leaves, promoting leaf senescence (Bielczynski *et al.*, 2017). Younger leaves are not competent to senesce, tend to be more resistant to drought than older leaves, and represent high-N-demand organs. Some evidence shows that the natural disassembly of photosystems leads to an abscisic acid (ABA)-mediated retrograde signaling from the chloroplast to the nucleus, suggesting that the decrease in photosynthesis might trigger the expression of the genetic program involved in chloroplast dismantling and N remobilization (Krieger-Liszkay *et al.*, 2019). In addition, LHCII degradation might provide substrates for synthesis of ABA, the main hormone involved in abiotic stress responses. Xanthophylls bound to LHCs dissociate from the antennas under increased temperatures or drought and might serve as substrates for ABA synthesis (Hobe *et al.*, 2006).

Furthermore, downregulation of any LHC chlorophyll *a/b* binding protein (LHCb) provokes reduced stomatal response to ABA leading to increased sensitivity to drought in Arabidopsis, whereas the overexpression of one LHCb results in enhanced ABA-mediated stomatal response (Xu *et al.*, 2012). From the above-mentioned, drought resistance traits could allow the maintenance of photosynthesis under water deficit leading to a delay in senescence (thus, this is a consequence rather than the primary cause of resistance). This possibility does not exclude opposite scenarios, where delayed senescence per se could confer drought resistance. Experiments performed in rice with Ethyl methane sulfonate-induced mutants show that senescence-delayed phenotypes first characterized under dark-induced senescence conditions (i.e. not an output of drought tolerance) display drought resistance (Ramkumar *et al.*, 2019).

Even though leaves are the main source of C for crop yield, crop productivity depends also on photoassimilates produced in other organs of the plant. When leaf senescence is accelerated in response to terminal stress (mainly during the reproductive

period) other photosynthetic organs play critical roles in maintaining yields (Sanchez-Bragado *et al.*, 2020). These organs include wheat ears (lemma, palea, awns) (Martinez *et al.*, 2003; Maydup *et al.*, 2010), rice panicles (Zhang *et al.*, 2023), sterile spikelets of sorghum (AuBuchon-Elder, 2020), and pods and seeds of alfalfa and soybean (Zhang *et al.*, 2017; Cho *et al.*, 2023). Compared with leaves, non-foliar organs often show higher rates of photosynthesis, slower post-anthesis senescence, higher tolerance to environmental stresses, and also higher capacity to re-fix CO₂ respired by the heterotrophic tissues of fruits. The photosynthetic contribution of non-foliar organs to grain yield ranges from 10% to 55% (Lawson and Milliken, 2023). In wheat, when measured for the whole organ, photosynthesis in the ear is higher than that of the flag leaf, under drought and well-watered conditions, revealing a potential central role of this organ in grain filling and yield (Abbad *et al.*, 2004; Maydup *et al.*, 2014).

Crosstalk between genetic regulation of senescence and stress

As the leaf ages, the integration of internal (i.e. hormone balance, plant growth) and external (environmental conditions, biotic interactions) signals leads to the execution of a distinct gene expression program. A decline in expression of photosynthetic genes and particularly an up-regulation of hundreds to thousands of senescence associated genes (SAGs) define the developmental transition from maturity to senescence (Buchanan-Wollaston *et al.*, 2003; Ahmad and Guo, 2019). The leaf senescence syndrome, observed macroscopically as leaf yellowing, is characterized by a metabolic shift from nutrient assimilation to nutrient remobilization. This shift takes place through the coordinated execution of physiological and molecular changes. For example, prior to cell death there is a decline in photosynthesis; chloroplast dismantling; and massive degradation of proteins, lipids, nucleic acids and other macromolecules; and concomitant release of catabolites (nutrients), i.e. N and P, and their export. Therefore, far from being just an age-related degenerative process, leaf senescence governs nutrients, particularly dynamics of N-rich compounds, affecting plant fitness and productivity profoundly (Havé *et al.*, 2017; Fan *et al.*, 2023). Excess or limiting availability of water, nutrients, and light and/or extreme temperatures exerts a strong influence on the onset and the progression of leaf senescence. Indeed, most transcriptome studies reveal an overlap between natural (developmental, age-related) and stress-induced leaf senescence (Buchanan-Wollaston *et al.*, 2003; Ahmad and Guo, 2019; Tan *et al.*, 2023). A meta-analysis comprising 28 microarrays of gene expression profiles associated to developmental and induced senescence (provoked by a variety of biotic and abiotic stresses, such as pathogen, wounding, salt or sugar excess, and others) revealed distinctive signaling pathways triggering senescence, which coalesce in common execution

programs. Interestingly, the main differences in gene expression under different scenarios were found among those genes that are down-regulated during senescence (Guo and Gan, 2012).

Many SAGs that conjoin natural senescence and stress related regulatory networks are modulated by hormonal signaling. Reverse genetics reveals the central role of transcription factors (TF) in the association between leaf longevity and plant fitness under abiotic stresses. NAC and WRKY are two of the most representative TF families, with members having dual positive and negative regulatory roles in senescence (Kim *et al.*, 2016; Liu *et al.*, 2016). For instance, the NAC TF ORESARA 1 (ORE1), also called ANAC092/NAC2, acts as a positive regulator of natural leaf senescence and a negative regulator of stress resistance (Balazadeh *et al.*, 2010). Direct targets of ORE1 include chlorophyll catabolic genes (Gao *et al.*, 2016; Swida-Barteczka and Szweykowska-Kulinska, 2019), and ORE1 antagonizes the function of GOLDEN2-LIKE TFs, which are associated with photosynthesis and chloroplast maintenance (Rauf *et al.*, 2013). Studies in rice show that NAC2 is induced by ABA and, in turn, regulates ABA-mediated stress responses. *OsNAC2* RNAi plants display enhanced resistance to drought and high salinity throughout vegetative and reproductive development, maintaining yield after the drought episode, whereas *OsNAC2* overexpressors show lower resistance to these conditions (Shen *et al.*, 2017). *NtNAC028* represents another example of cross regulation between senescence and stress resistance, in that *NtNAC028* is up-regulated during leaf senescence and under high salinity and leaf dehydration. *NtNAC028* loss of function tobacco plants show delayed senescence and increased hypersensitivity to these stresses, whereas the constitutive expression of *NtNAC028* in Arabidopsis leads to plants with early senescence and enhanced resistance to abiotic stresses (Wen *et al.*, 2022).

Many other regulatory pathways seem to modulate leaf and plant longevity in a crosstalk with ABA-mediated responses. For example, the expression of the NAC TF *VND-INTERACTING2* (*VNI2*) increases with leaf longevity, and it is also induced by ABA and salt stress. *VNI2* integrates responses to these factors, directly activating *COLD-REGULATED* (*COR*) and *RESPONSE TO DEHYDRATION* (*RD*) genes, which confers stress resistance and also delays leaf senescence (Yang *et al.*, 2011). Furthermore, the overexpression of *VNI2*, *COR*, or *RD* leads to delayed leaf senescence. *VNI2* might serve as an integrator of developmental and environmental signals, extending leaf longevity through the promotion of stress-responsive genes. More recently it was shown that *VNI2* delays senescence through inhibition of another NAC TF, *ACTIVATION FACTOR2* (*ATAF2*), a positive regulator of *ORE1* (Nagahage *et al.*, 2023). Differential phenotypes obtained as an outcome of manipulating NAC TFs and/or target genes might be extrapolated to field conditions. For instance, *NAC7* was identified within a stay-green (SG) QTL derived from mapping *High Protein 1* (*IHP1*) and *Low Protein 1* (*ILP1*) Illinois maize lines with different senescence rates (Zhang *et al.*, 2019). Under field conditions *nac7* RNAi plants

displayed a noticeable functional SG phenotype, i.e. delayed senescence, increased biomass, and N accumulation with respect to null plants. In a wide phylogenetic analysis performed in the same study, the authors identified the closest ortholog to *ZmNAC7* to be *OsNAC60*, a negative regulator of drought resistance (Zhang *et al.*, 2019).

WRKY53 is another central player in senescence and stress-related programs. This TF is a positive regulator of senescence, and promotes the expression of genes involved in transport and remobilization (Zentgraf and Doll, 2019). Overexpression and down-regulation of *WRKY53* leads to accelerated and delayed senescence phenotypes, respectively (Miao *et al.*, 2004). Among other targets, the direct interaction between WRKY53 and the receptor kinase CR5, a negative regulator of senescence and osmotic stress resistance, leads to an antagonistic WRKY53–CR5 regulation of chlorophyll content and stomatal conductance (Burdiaik *et al.*, 2022).

Many downstream SAGs code for hydrolytic enzymes and for components of autophagy, cellular machinery involved in bulk and targeted degradation pathways (Marshall and Vierstra, 2018; Janse van Rensburg *et al.*, 2019). Natural and stress-related senescence converge on the up-regulation of autophagy related (*ATG*) genes. Autophagy serves diverse functions related to enhanced water and N use efficiency, i.e. post-translational down-regulation of aquaporins, and of ABA suppressors, which confers resistance to water deficit, and chloroplast protein degradation (for a detailed review of autophagy and drought, see Tang and Bassham, 2022). Most *ATG* loss of function phenotypes characterized in different species, such as Arabidopsis, maize, and rice, display accelerated senescence and high sensitivity to abiotic stresses (Thompson *et al.*, 2005; Li *et al.*, 2015; Fan *et al.*, 2020). Furthermore, ¹⁵N pulse–chase analysis in rice *ATG8* overexpression and RNAi lines show that increasing autophagic activity leads to higher recycling efficiency to grains, pointing to the relevance of this ubiquitous mechanism in N mobilization (Zhen *et al.*, 2019), even under field conditions (Fan *et al.*, 2020).

Chloroplast dismantling also relies on autophagy-independent, extraplastidial vesicular pathways, such as senescence associated vacuoles (SAVs) and chloroplast vesiculation containing vesicles (CCVs) (Otegui *et al.*, 2005; Wang and Blumwald, 2014). SAVs are lytic compartments themselves that harbor the cysteine protease SAG12 (the most used senescence specific marker gene), among other proteases (Otegui *et al.*, 2005; Carrión *et al.*, 2013). They develop specifically during natural and ethylene/dark-induced senescence of Arabidopsis, soybean, and tobacco. Rubisco and glutamine synthetase 2, specific thylakoid proteins (LHCa, PSA, but not LHCb or D1), and chlorophyll have been detected in SAVs (Martínez *et al.*, 2008; Gomez *et al.*, 2019). Senescence is not notably affected in Arabidopsis *sag12*-KO plants, but these knockouts produce seeds with lower N content and decreased yield when grown under N deficit (James *et al.*, 2018). CCVs carry chloroplast proteins to the central vacuole for degradation. CCVs involve a chloroplast-targeted protein named CV, and they participate

in both natural and stress-induced senescence (Sade *et al.*, 2018; Ahouvi *et al.*, 2022). Soybean CCVs contain two highly similar CV proteins, encoded by paralogous genes, *CV1* and *CV2*. Interestingly, *CV1* expression is differentially regulated in soybean genotypes with contrasting slow- (tolerant) or fast- (sensitive) wilting phenotypes under drought, whereas *CV2* expression is promoted during natural senescence and by the senescence-accelerating hormones ABA, salicylic acid, and jasmonic acid. Arabidopsis plants with overexpression of soybean *CV2* contain reduced amounts of thylakoid proteins PsaB, D1, and PsbO (Fleitas *et al.*, 2023). CCVs and SAVs might coalesce with the central vacuole, the main proteolytic compartment in the cell, which harbors most of the senescence and stress-induced proteases (Martínez *et al.*, 2007; Esteban-García *et al.*, 2010). Hydrolytic and other execution pathways can clearly modulate senescence. *Stay-Green* (*SGR*) codes for a protein involved in dismantling of the chlorophyll-protein complexes (Fang *et al.*, 2014). Natural polymorphisms in the promoter region of *OsSGR* lead to different senescence rates in rice cultivars. Earlier and higher induction of *OsSGR* relates to accelerated senescence in Indica cultivars. The introgression of Japonica alleles of *OsSGR* into Indica type cultivars led to delayed senescence, enhanced photosynthesis, and increased grain yield under field conditions (Shin *et al.*, 2020).

Senescence delay, stay-greens, and tolerance to abiotic stresses?

Since very often abiotic stress factors accelerate senescence, as reviewed above, stress resistant genotypes might display delayed

senescence under stress conditions merely as a consequence of their stress resistance. Therefore, the impact of senescence delay on stress resistance must be tested with genotypes where senescence symptoms are mitigated under both stressful and non-stressful conditions, i.e. where the primary phenotype is the delay of senescence. Spontaneous or induced mutations (transgenic or not) can delay canopy senescence in several crop species (Thomas and Howarth, 2000). These variants with delayed senescence are commonly referred to as ‘stay-green’ (SG). While some of these genetic variants may be merely cosmetic (i.e. they retain chlorophyll without interfering with the loss of assimilatory capacities), others are functional, and these plants maintain photosynthesis and other assimilatory processes for longer periods of time (e.g. Acciaresi *et al.*, 2014), even though phenological development (i.e. date of seed maturity) is not noticeably affected.

Stay-green phenotypes related to changes in cytokinin levels

Cytokinins (CKs) are very powerful inhibitors of leaf senescence (Noodén, 2004). There are a number of SG mutants and genotypes where the delay of senescence is associated with greater leaf CK levels (Fig. 1). Hybrids of sunflower with contrasting dynamics of green leaf area (i.e. SG versus non-SG) differed in the persistence of live roots during grain filling, with the SG hybrids showing longer-lived roots than the normally senescing hybrids (Lisanti *et al.*, 2013; Mangieri *et al.*, 2017), which translated into higher CK content in the xylem sap (Mangieri *et al.*, 2020) and leaves (Mangieri *et al.*, 2017).

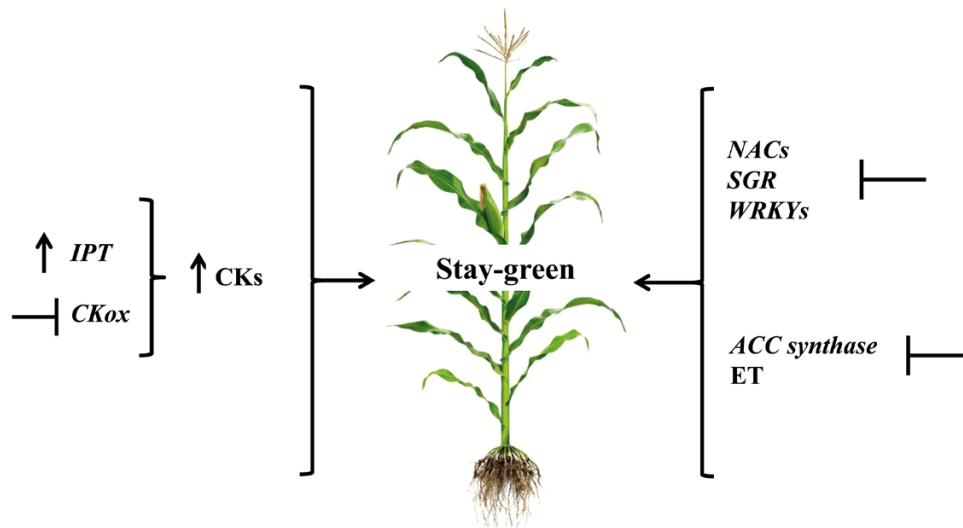


Fig. 1. A schematic representation of possible molecular and physiological mechanisms underlying the stay-green trait in crops. The image represents an idealized plant, not any specific crop species, growing under non-limiting environmental conditions. Two basic hypothetical mechanisms resulting in a stay-green phenotype are presented: in one (left side) increased expression of cytokinin (CK) biosynthetic genes results in higher amounts of CKs leading to delayed senescence. Alternatively, this might be due to decreased expression of genes involved in CK degradation. On the right side, reduced expression of ethylene biosynthetic genes or of senescence-associated genes results in a stay-green phenotype. ACC, aminocyclopropane-1-carboxylic acid; *CKox*, cytokinin oxidase; ET, ethylene; *IPT*, isopentenyl transferase; *SGR*, stay-green.

Likewise, the concentrations of zeatin and zeatin riboside, two important CKs, are higher in the xylem sap of non-senescent than in normally senescing hybrids of sorghum (Ambler *et al.*, 1992). In maize, compared with earlier senescing counterparts, SG genotypes show higher concentrations of CKs in leaves (He *et al.*, 2005; Zhou *et al.*, 2016) whereas in roots, zeatin riboside concentrations also remain higher but reduced levels of isopentenyladenosine have led to the proposal of a faster rate of CK transport from roots to shoots in SG genotypes (He *et al.*, 2005).

Leaf CK levels can be increased through expression of the gene for isopentenyl transferase (IPT), which catalyses the first step in the biosynthesis of CKs (Kamada-Nobusada and Sakakibara, 2009) (Fig. 1). Expression of the *ipt* gene under control of the promoter of *senescence-associated gene 12* (*SAG12*), which codes for a cysteine type protease (Lohman *et al.*, 1994), increases CK production in leaves starting to senesce (Gan and Amasino, 1995), thus retarding leaf senescence of many plant species in an autoregulated fashion (e.g. Jordi *et al.*, 2000; Clark *et al.*, 2004; Robson *et al.*, 2004; Merewitz *et al.*, 2011; Xiao *et al.*, 2017). Cytokinin levels increased quite significantly in *P_{SAG12}-IPT* plants of *Petunia × hybrida* subjected to water shortage (e.g. Clark *et al.*, 2004). Both in *Petunia × hybrida* and *Solanum melongea*, *P_{SAG12}-IPT* lines retained green leaves for longer after the start of a water deficit treatment (Clark *et al.*, 2004; Xiao *et al.*, 2017). Although *SAG12* was first described as a senescence-associated gene expressed in leaves, there is evidence that it is also transcribed in roots, where it can have an important role in remobilization of root N to the shoot (James *et al.*, 2018). In line with these observations, CK contents also increased in roots of *Agrostis stolonifera* *P_{SAG12}-IPT* lines subjected to drought, apparently counteracting the water-deficit induced loss of root viability (Merewitz *et al.*, 2011).

Autoregulated CK production can be achieved also by expressing the *ipt* gene under control of the *senescence-associated receptor kinase* (*SARK*) promoter (Hajouj *et al.*, 2000). Basal (older) leaves of *P_{SARK}-IPT* lines of tobacco contain higher levels of free CK, CK-nucleotides, CK-nucleosides, and CK-glucosides than their corresponding wild type counterparts, particularly under water deficit treatments (Rivero *et al.*, 2007). *P_{SARK}-IPT* plants remained viable after 2 weeks of drought treatment, and fully recovered when watered after dehydration whereas wild type plants were completely and irreversibly dehydrated. Compared to wild type, *P_{SARK}-IPT* plants substantially reduced the degradation of chloroplast components, maintained photosynthetic electron transport during drought (Rivero *et al.*, 2010), and accumulated more biomass (Rivero *et al.*, 2007). Similarly, the expression of *P_{SARK}-IPT* in maize increased CK levels under drought, and this translated into preservation of leaf area and gas exchange parameters, e.g. photosynthesis and stomatal conductance (Oneto *et al.*, 2016). However, the impact of *P_{SARK}-IPT* on maize grain yield was not quite clearcut. Drought resistance of the above-mentioned senescence-delayed transgenics was revealed in experiments

where drought was imposed in different ways, but stomatal conductance and transpiration were maintained higher than in the wild type in all cases where this was measured. In some experiments, soil water content was maintained at a constant, albeit low, level through controlled irrigation for the duration of the experiment (Oneto *et al.*, 2016), whereas in other experiments drought was imposed by withholding irrigation until the end of the measurement period (e.g. Rivero *et al.*, 2007). Detailed analysis of leaf water dynamics in such conditions seems warranted. Both *P_{SARK}-IPT* and *P_{SAG12}-IPT* plants have higher CK contents and delayed senescence, which raises the possibility that enhanced drought resistance is not directly related to senescence delay but to higher levels of CKs per se. For example, CKs may modulate stomatal aperture in some species (Dodd, 2003), which might alleviate stress-related stomatal limitations to photosynthesis. Autoregulated increase of CK levels can also ameliorate the negative effects of salt stress (Shan *et al.*, 2019), but there are cases where *P_{SAG12}-IPT* lines may be more susceptible to stress. For example, in tobacco, a *P_{SAG12}-IPT* line shows reduced growth in dense plant canopies, i.e. under light deprivation (Boonman *et al.*, 2006), highlighting the adaptive importance of shade-induced senescence.

Other genetic variants resulting in stay-green phenotypes

Overexpression of the Arabidopsis 14-3-3 protein GF14 λ in cotton caused a dramatic preservation of chlorophyll and gas exchange in plants subjected to water deficit (Yan *et al.*, 2004). As in the case of senescence delay through autoregulated expression of the *ipt* gene, the GF14 λ cotton plants also maintained higher stomatal conductance, and therefore photosynthesis, under drought (Yan *et al.*, 2004).

In soybeans, a number of spontaneous mutations impair chlorophyll degradation and several other processes involved in chloroplast breakdown during senescence (Guamet *et al.*, 1990). A genotype harboring two recessive alleles at different genetic loci (i.e. *d1d1d2d2*) completely inhibits chlorophyll, thylakoid protein, and Rubisco degradation during senescence (Guamet *et al.*, 1990; Guamét and Gianibelli, 1994, 1996; Fang *et al.*, 2014; Nakano *et al.*, 2014). These genes are the duplicated orthologs of *SGR* of Arabidopsis (Fang *et al.*, 2014) (Fig. 1). In combination with the dominant gene *G*, which by itself causes chlorophyll retention only in the seed coat (i.e. the *GGd1d1d2d2* genotype), these alleles can delay the senescence-related decline in photosynthesis, at least under growth chamber conditions (Guamet *et al.*, 1990). However, when grown outdoors under natural temperature, irradiance, and vapor pressure deficit conditions, *GGd1d1d2d2* does not outperform its wild type counterpart nor display higher photosynthesis or stomatal conductance (Luquez and Guamet, 2001). Moreover, in spite of its SG phenotype, *GGd1d1d2d2* displays a quite severe drought sensitive phenotype under water deficit (Luquez

and Guamet, 2002). When subjected to a moderate water stress treatment (soil water potential of -0.7 MPa), leaves of *GGd1d1d2d2* reached lower leaf water potentials and lower relative water contents, and showed severe visible symptoms of dehydration, but no noticeable degradation of chlorophyll. The stomatal behavior of these plants was normal (i.e. stomatal conductance decreased to a similar extent in water-stressed plants of both genotypes) and both were equally sensitive to exogenous applications of ABA for stomatal closure. The study of this mutant genotype underscores an antagonistic pleiotropic link between degradation of chloroplast components and drought resistance, which might be taken as evidence that in some cases accelerated senescence, not senescence delay, might play a protective role under stress conditions.

Stay-greens have also been obtained by manipulative approaches that reduce ethylene production (e.g. in maize, Young *et al.*, 2004; in rice, Fukao *et al.*, 2012), in line with reports about lower ethylene production in naturally occurring SGs (in wheat, Kumar *et al.*, 2021) or lower sensitivity to exogenous applications of etephon (Zhang *et al.*, 2012) (Fig. 1). In maize, aminocyclopropane-1-carboxylic acid synthase mutants showed delayed senescence, lower ethylene production, and higher stomatal conductance and photosynthesis under drought but also a reduced responsiveness to dark-induced senescence (Young *et al.*, 2004). Nonetheless, to our knowledge, ethylene-related SGs have not been tested for stress resistance under field conditions.

Sorghum lines or hybrids with the SG trait are among the most studied lines with delayed senescence, particularly because they confer resistance to water deficit in relatively dry cropping areas of Australia (Borrell *et al.*, 2014a). Two sources of SG, B35 (which displays delayed onset of canopy senescence) and K19 (with delayed onset and reduced rates of canopy senescence) showed greater green leaf area at maturity under terminal water deficit (Borrell *et al.*, 2000a). Grain yield of SG versus non-SG hybrids correlated positively with green leaf area at maturity, and negatively with rate of leaf senescence (Borrell *et al.*, 2000b). In most of the hybrids tested (i.e. combinations of B35 and K19 with other sorghum lines) that included SG and non-SG genotypes, senescence of the canopy was not affected by genotype under well-watered conditions, i.e. delayed senescence manifested only under water deficit. Analysis of mapping populations of sorghum revealed several quantitative trait loci (QTL) associated with the SG character. Four QTLs (*Stg1*, *Stg2*, *Stg3*, and *Stg4*) accounting for 53.5% of the variability in senescence behavior, and lines harboring QTLs for the SG traits also showed enhanced drought resistance (Sanchez *et al.*, 2002; Harris *et al.*, 2007). A detailed agronomic study in the SG sorghum hybrids showed that, to a large extent, water deficit resistance in these genotypes is related to architectural changes in the shoot (Borrell *et al.*, 2014a, b; George-Jaeggli *et al.*, 2017), rather than to a direct effect on senescence. The four SG QTLs reduce tillering and, therefore, water consumption during the vegetative phase, thereby increasing the amount

of water stored in the soil for reproductive growth. Thus, retention of green leaf area at maturity is possibly a consequence of reduced water uptake earlier in the crop cycle (i.e. an avoidance mechanism), which also explains why these genotypes do not display increased persistence of green leaf area under well-watered conditions (Borrell *et al.*, 2000b). Even though delayed senescence may not be the primary cause of stress resistance, the expression of the SG phenotype still seems to be a condition needed for improved post-silking C assimilation under drought.

Although not as widely studied as the SG genotypes of sorghum, there are more studies attempting to relate senescence behavior and stress resistance. In rice, three mutant genotypes cause a delay of leaf chlorophyll degradation, both after 10 d of incubation in darkness, or *in planta* at physiological maturity, with persistence of flag leaf photosynthetic capacity after anthesis (Ramkumar *et al.*, 2019). In two of these mutants, there was no drought-induced decrease of grain yield, pointing to stress resistance, but this occurred at grain yields lower than those of the wild type genotype even under non-stress conditions. Rather than stress resistance, this suggests that at least two of the mutants exerted negative effects on maximum, non-stressed yield, which is clearly not a useful trait for breeding. In the third SG genotype, yield was indistinguishable from wild type under either well-watered or water-stress treatments, and therefore these mutants did not display enhanced stress resistance in an agronomic sense in spite of the delay of senescence.

Similarly, a comparison of contemporary commercial hybrids of maize does not support the notion that the SG trait necessarily causes stress resistance. Maize hybrids (four to six, depending on the experiment) grown in the field under rainfed or irrigated conditions after silking differed in green leaf area at maturity, but there was only a weak correlation between green leaf area at maturity and yield (Antonietta *et al.*, 2021). The SG hybrids did not have a higher yield than the non-SG under water deficit (i.e. rainfed) conditions, and there was no detectable hybrid \times water treatment interaction in two years of experimentation. Since tolerance to water deficit can also result in a better performance under nutrient stresses (Bänziger *et al.*, 1999), the behavior of these hybrids was also studied under conditions of limiting N supply. However, as in the case of water deficit, the SG hybrids did not outperform non-SGs in terms of yield under limiting N supply (Antonietta *et al.*, 2016) in line with other works reporting no advantages (Kosegy *et al.*, 2013) or even penalties for an exacerbated SG phenotype under low N (Swanckaert *et al.*, 2017; Liu *et al.*, 2021). Other work, however, has reported advantages of SGs under drought and low N conditions, but the range of leaf area indexes and yields explored were much lower (e.g. yields of 2.65 t ha^{-1} across environments exposed to heat or drought, Cerrudo *et al.*, 2017; 4.7 t ha^{-1} across N levels, Bänziger *et al.*, 1999; 4.19 t ha^{-1} under low N, Worku *et al.*, 2012). Since light interception relates asymptotically to leaf area index, it is expected that a lower leaf area index would imply a larger contribution of SG to light interception, and

ultimately to yield. Inasmuch as increases in planting density reduce the per plant availability of water and nutrients, enhanced tolerance of SGs to water or nutrient deficit, if present, might improve their growth under higher planting densities. However, yield of a very strong SG hybrid was actually lower than that of its non-SG counterparts at high plant densities, whereas one hybrid displaying the SG trait only at the upper canopy layer showed the largest grain yield (Antonieta *et al.*, 2014) in line with other reports showing negative relationships between the SG score and yield at high planting densities (Zhang *et al.*, 2019; Shao *et al.*, 2021). In brief, studies with commercial hybrids of maize do not provide conclusive evidence of an overall increased stress tolerance in delayed senescence genotypes. This seems to contrast with retrospective studies showing that breeding resulted in extended leaf area duration (Duvick, 2005) and improved resistance to high planting densities that would also bring about higher yield stability (Tollenaar and Wu, 1999; Di Matteo *et al.*, 2016). However, retrospective studies involve, among others, increases in grain number (e.g. Di Matteo *et al.*, 2016) in modern genotypes, with a concomitant advantage of prolonged leaf area duration. Also, at least for maize, these retrospective studies usually involve the transition from double- to single-cross hybrids, with a concomitant reduction in interplant variability that may bring special advantages in stressful environments where interplant variability tends to be accentuated.

Controversial findings around the stay-green trait

More inconsistencies regarding the benefits of the SG trait could be expected in crops having a larger C contribution to yield of stover dry matter or of photosynthetic organs other than leaves (e.g. ears in wheat, Maydup *et al.*, 2010). Other controversies may relate to a combination of: (i) differences in the genetic background of the SG genotypes (Vadez *et al.*, 2011a; Jordan *et al.*, 2012; Borrell *et al.*, 2014a); (ii) the environmental context where stress occurs (e.g. duration of the stress, plant density); and (iii) the different mechanisms underlying the SG trait (further addressed in the next section). In turn, the interaction among these factors will determine the degree of phenotypic plasticity with which the SG phenotype is expressed, and with this, whether timely leaf senescence occurs or not. Strong hybrid vigor (Gong *et al.*, 2005), high levels of N fertilizer (Yang and Zhang, 2006), or a small response to shade (Acciaresi *et al.*, 2014; Antonietta *et al.*, 2014) may cause undesired delays in senescence negatively affecting yields. By contrast, senescence promotion of older leaves before anthesis is associated with water saving and a SG phenotype later on in sorghum under drought (George-Jaeggli *et al.*, 2017). Similarly, under N deficiency, promoting senescence of lower leaves improves leaf N remobilization and yield within different SG genotypes (Antonieta *et al.*, 2016; Liu *et al.*, 2021).

Other sources of controversy may relate to how the SG trait is defined. Most common procedures include onset of fast senescence, rate of senescence, and remaining leaf area at the end of the season, assessed either visually or through the normalized difference vegetation index in high throughput phenotyping approaches (e.g. Lopes and Reynolds, 2012; Cerrudo *et al.*, 2017). Within the same environment, very different relationship can be obtained depending on the parameter selected to represent the SG trait (e.g. Christopher *et al.*, 2016). In wheat, positive relationships between SG and yield were obtained when onset of senescence was considered, but since a delayed onset was associated with faster rates of senescence, rate was not a good proxy of SG in this case (Montazeaud *et al.*, 2016; Jocković *et al.*, 2022). By contrast, in spring wheat populations, rate of senescence appeared to be a good proxy of the SG trait, negatively relating to yield under drought and also under heat environments (Lopes and Reynolds, 2012). Remaining leaf area by the end of the season does not seem to be a good estimate of the SG in wheat (Christopher *et al.*, 2016), but it is in sorghum (Borrell *et al.*, 2000b).

Overall, the SG trait should improve canopy light interception and/or radiation use efficiency. Surprisingly, in very few cases the SG trait has been assessed in terms of its contribution to canopy light interception (as in Acciaresi *et al.*, 2014), a fundamental condition to be met in order to improve C accumulation in the reproductive period. Thus, when maximum leaf area indexes are well above critical, the contribution of the SG trait could be overestimated if this is not associated with light interception measurements. Regarding radiation use efficiency, most work identifying functional SGs is based on photosynthetic measurements made at full irradiance (e.g. He *et al.*, 2005; Oneto *et al.*, 2016; Mangieri *et al.*, 2017; Ramkumar *et al.*, 2019), which could also lead to overestimations of the SG contribution since phenotypic differences between SG and non-SG genotypes are mostly accentuated in lower canopy leaves where photosynthesis is usually light-limited.

Can delayed senescence confer cross-resistance to different types of stress?

Considering current evidence on advantages of SGs under particular stresses (e.g. drought), a further question to be asked is whether these advantages can be maintained in environments exposed to other types of stress (e.g. N deficiency) and thus SG confer cross-resistance to multiple stressors (Bänziger and Lafitte, 1997; Tan *et al.*, 2023). Extensive discussion exists around the idea that increasing yield potential could be a means for improving stress resistance; yet, yield potential and stress resistance may not be mutually exclusive but nor necessarily related (e.g. Tollenaar and Lee, 2002; Antonietta and Guamet, 2018; Du *et al.*, 2020; Wang *et al.*, 2021). Available evidence is not conclusive on SG increasing yield potential, with work suggesting advantages of SGs in control field conditions (i.e.

irrigated, fertilized, or at low planting density) (Zhang *et al.*, 2019; Kumar *et al.*, 2021) as well as no advantages (Antonietta *et al.*, 2021; Liu *et al.*, 2021) or even penalties (Ramkumar *et al.*, 2019). Multi-environment approaches are not conclusive either, with reports on advantages of the SG trait in well-watered conditions in wheat (Christopher *et al.*, 2016) as well as negative associations with the SG trait in the highest yielding environments in sorghum (Jordan *et al.*, 2012).

Retrospective studies show that the SG trait has been selected unintentionally during breeding in different crops (in maize, Tollenaar and Wu, 1999; Duvick, 2005; in barley, Mirosavljević *et al.*, 2020; in wheat, Jocković *et al.*, 2022), implying it was favored after empirical selection across multi-environment trials (which are required before releasing new commercial genotypes). The SG phenotype was also accentuated after recurrent selection cycles for drought tolerance in maize lines from CIMMYT, while also conferring an advantage under low N stress (Lafitte and Edmeades, 1995; Bänziger *et al.*, 1999) suggesting cross-resistance could be met through this trait. However, this evidence does not allow dissection of the specific gains related to the SG trait from those related to other traits that also changed during breeding (e.g. reduced anthesis-silking interval, Duvick, 2005; stand uniformity, Tollenaar and Wu, 1999) or that were dragged into the new materials during selection cycles (e.g. reduced barrenness, Lafitte and Edmeades, 1995).

Robust evidence for a positive impact of the SG trait under stress in sorghum comes from the work of Jordan *et al.* (2012) who reported a positive association between SG rating and yield in 1688 hybrids from the Queensland public breeding program tested across 23 environments with advantages found particularly in lower yielding environments (<6 t ha⁻¹), putatively where crops were exposed to different stresses. However, reports in other crops are less consistent. For example, in a population of 152 spring wheat lines, SG related to yield in environments exposed to heat and also in those exposed to drought, but in another population of 113 lines, this was only seen under heat stress (Lopes and Reynolds, 2012), ruling out the occurrence of cross resistance to drought and heat in this set of genotypes.

Yield advantages in each environment depend on the crop physiological mechanism underlying stay-green

At the crop level, the specific mechanisms behind the SG trait may determine its advantage in each type of environment. For example, increased resource capture in lower soil layers might translate into cross resistance to stresses affecting water and N availability. Under drought, the SG phenotype of some genotypes has been related to increased water availability during the grain filling period, due to a more conservative use

of water by decreased canopy size at anthesis (Borrell *et al.*, 2014a), improved transpiration efficiency (Vadez *et al.*, 2011b; Antonietta *et al.*, 2021), or increased access to deep soil moisture (Christopher *et al.*, 2008; Lisanti *et al.*, 2013; Hiremath and Nadaf, 2017). Among these, a deeper root system could also improve N capture in low N soils (Lynch, 2022), whereas reduced canopy size at anthesis or improved transpiration efficiency may not imply a direct benefit under N limitations in the absence of water deficit. At the same time, many studies have reported lower C remobilization in SG genotypes under drought (Gong *et al.*, 2005; Yang and Zhang, 2006; Antonietta *et al.*, 2021). Thus, whether the SG trait effectively translates into higher yields may depend to a large extent on the relative contribution of post-anthesis C assimilation and post-anthesis C remobilization in each crop×environment combination (Fig. 2).

The SG phenotype has also been associated with an improved N balance allowing plants to meet sink demand while maintaining leaf N level high enough to prevent the onset of senescence (Borrell and Hammer, 2000). This could be achieved by a higher leaf N concentration at anthesis (Borrell and Hammer, 2000; Fu *et al.*, 2009; Antonietta *et al.*, 2019) and/or larger N uptake during the reproductive period (Borrell and Hammer, 2000; Pommel *et al.*, 2006), the latter related to increased root activity (Fu *et al.*, 2009) and/or root biomass (Hou *et al.*, 2021). Adequate N nutrition can also improve drought tolerance due to higher leaf water potentials, antioxidant activity, and photosynthesis (Abid *et al.*, 2016), although it might also result in a larger canopy size increasing water demand through higher transpiration (e.g. Van Oosterom *et al.*, 2010), whereas increased root biomass or activity could promote water uptake. However, as found for C, many reports also show lower N remobilization in SG genotypes (Pommel *et al.*, 2006; Mi *et al.*, 2003; Kosgey *et al.*, 2013; Antonietta *et al.*, 2014, 2016; Swanckaert *et al.*, 2017; Shao *et al.*, 2021). If soil N is depleted during the vegetative period, a reduced kernel N supply (e.g. in maize) may affect sink strength through decreased endosperm cell number (Pico *et al.*, 2021) and activity of enzymes involved in sugar metabolism (Below *et al.*, 2000), ultimately reducing sugar export rates from leaves (Ning *et al.*, 2018). Thus, whether the SG trait brings a yield benefit under N deficiency may depend on the relative N limitations experienced by source tissues (triggering decreased photosynthetic rates and accelerated canopy senescence) and sink tissues (reducing sink strength) (Fig. 2).

Stay-green phenotypes have also been related to changes in hormone levels (addressed in a previous section). Increased CKs and reduced ABA levels found in naturally occurring SG genotypes (He *et al.*, 2005; Seiler *et al.*, 2014; Zhou *et al.*, 2016) might improve stomatal conductance, photosynthesis, and yield under temporary drought conditions (Zhou *et al.*, 2016) as seen for autoregulated *IPT*-expressing SGs (discussed previously). However, such a strategy could compromise yield under terminal drought by a faster depletion of water reserves.

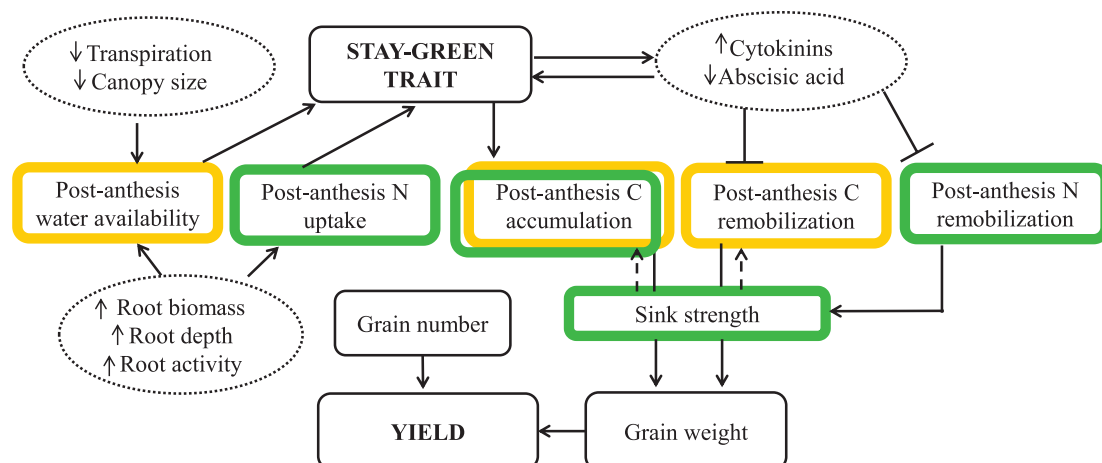


Fig. 2. Conceptual framework showing relationships between the stay-green trait and yield under non-limiting grain number. A deeper root system or higher root biomass or activity (increasing water uptake), water conservation strategies and/or hormonal balances are non-excluding mechanisms (encircled in dotted lines) leading to a stay-green phenotype. Delayed canopy senescence is expected to increase post-anthesis C accumulation, but also to reduce C and N remobilization. Increases in grain weight are mediated by sink strength, which in turn, can affect C remobilization and accumulation by feedback processes (dashed arrows). Ultimately, N taken up is assimilated in leaves and thus post-anthesis N remobilization includes N assimilated and remobilized during the post-anthesis period. Boxes denote processes that might be more relevant under drought (in orange) or under N deficiency (in green).

Increased CKs and reduced ABA could impose penalties on remobilization of soluble carbohydrates (in wheat and rice, Yang and Zhang, 2006; in rice, Fu *et al.*, 2009), so that promoting senescence through moderate use of N fertilizer or mild soil drying has positive effects on yield (Yang *et al.*, 2001). Also, a reduced responsiveness to shade-induced leaf senescence has been reported in SG genotypes of different genetic origins including *P_{SAG12}-IPT*-expressing tobacco (Boonman *et al.*, 2006), *Zmasc6* mutants of maize with reduced ethylene production (Young *et al.*, 2004), and ethyl methane sulfonate-induced mutants of rice (Ramkumar *et al.*, 2019). This negatively impacts C assimilation (Boonman *et al.*, 2006) and could be involved in the lower tolerance of SG genotypes to high planting densities (Antonietta *et al.*, 2014; Zhang *et al.*, 2019; Shao *et al.*, 2021). Thus, hormone-mediated SGs may have improved tolerance to transitory stresses (drought, heat), but with an overall lower phenotypic plasticity that could compromise yield stability under long-term drought, N deficiency, or high planting densities. Overall, this highlights the importance of understanding the mechanisms underlying the SG phenotype to adequately evaluate the possible advantages or penalties under different environments.

Conclusions

The acceleration of senescence symptoms is one of the deleterious effects of abiotic stress. However, does a delay of leaf senescence ameliorate resistance to abiotic stress? Studies with SG transgenic lines, spontaneous mutants and other genetic variants suggest that in several cases delayed senescence increases resistance to abiotic stresses, particularly water

deficit. However, in other cases the senescence behavior of genotypes does not have an effect on stress resilience. A critical point is to unravel whether stress resistance is due to senescence delay (i.e. in such a case breeding for senescence delay should be a productive strategy to gain stress resistance) or whether delayed senescence is just a consequence of stress resistance achieved through other physiological mechanisms. Even if breeding for delayed senescence offers the potential to increase stress resistance, several factors might interfere with the realization of this potential, e.g. (i) the genetic background of the SG genotypes; (ii) the environmental context where stress occurs (e.g. duration of the stress); and (iii) the impact of the specific mechanism responsible for the SG trait. Finally, whatever mechanism underlies the SG trait, to be useful in an agricultural setting, the SG genotype should exhibit a certain degree of phenotypic plasticity. Since several genes and QTLs might be targets for molecular breeding to delay senescence and increase stress tolerance, judicious testing of such variants under realistic and diverse conditions may greatly help to advance the search for superior genotypes with enhanced adaptation to harsh environments in order to secure food production in the near future.

Acknowledgements

We are grateful to Drs Eduardo Tambussi, María Lujan Maydup, and Pablo Calzadilla for stimulating discussions.

Conflict of interest

The authors declare they have no conflict of interest.

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