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Metabolic Regulation of Leaf Senescence in Sunflower (*Helianthus annuus* L.) Plants

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1. Introduction

The leaf is the main photosynthetic organ of plants and its development a complex process governed by a combination of environmental factors and intrinsic and genetically regulated signals (Van Lijsebettens & Clarke, 1998). Usually, leaf ontogeny includes an early phase of increasing photosynthetic rates while the leaf is actively expanding, a mature phase where such rates peak and a senescence phase where they decline (Gepstein, 1988; Miller et al., 2000). During early development, the leaf is a sink receiving nutrients from the rest of the plant; however, as soon as it reaches full photosynthetic capacity, it becomes the main source organ of the plant. After this productive period, the leaf enters the senescence phase, during which most compounds present in it are removed and reused (Hörtensteiner & Feller, 2002; Buchanan-Wollaston et al., 2003a). Leaf senescence, which is last stage in leaf development, is a highly regulated and programmed degeneration process governed by a variety of developmental and environmental signals (Lim et al., 2003). This important phase in the leaf lifespan period may last as long as leaf maturation and involves a shift from nutrient assimilation to nutrient remobilization and recycling (Guiboileau et al., 2010). In senescent leaf metabolism, carbon and nitrogen assimilation are replaced by catabolism of chlorophyll and macromolecules such as proteins, RNA and membrane lipids, the degradation of which marks the senescence phase. Unsurprisingly, senescence alters the expression of many genes. These senescence-associated genes include regulatory genes encoding transcription factors; genes involved in degradative processes that code for hydrolytic enzymes such as proteases, lipases and ribonucleases; and genes with secondary functions in senescence that code for proteins involved in nutrient remobilization (e.g. glutamine synthetase, which catalyses the conversion of ammonium into glutamine to enable nitrogen recycling in senescing cells) (Taiz & Zeiger, 2010). Environmental cues such as day length and temperature, and various biotic and abiotic sources of stress, can also affect the initiation and progress of such a high complex as leaf senescence.

During senescence, some metabolic pathways are triggered and others turned off. These dramatic metabolic changes result in orderly degradation of cellular structures, starting with chloroplasts (Wiedemuth et al., 2005), and also in the subsequent remobilization of the resulting materials. Chloroplasts play a dual role; thus, they are the main source of nitrogen

and also the regulators of their own degradation during senescence (Zapata et al., 2005). Most of the protein in green cells is located in chloroplasts, which thus constitute their main reserves of organic nitrogen. Efficient recycling of nitrogen from the photosynthetic apparatus during early senescence requires the presence of intact mitochondrial, nuclear and cellular membranes (Gan & Amasino, 1997; Nam, 1997; Noodén et al., 1997; Hörtensteiner & Feller, 2002; Cabello et al., 2006). Leaf proteins (particularly photosynthetic proteins) are extensively degraded during senescence (Martínez et al., 2008), which confirms that one of the primary functions of leaf senescence is to recycle nutrients (especially through nitrogen remobilization) (Himelblau & Amasino, 2001). Protein breakdown starts early in senescence and proteolysis is believed to start within chloroplasts. Some proteins (e.g. chlorophyll-binding light-harvesting proteins LHCII) seem to be entirely degraded within chloroplasts, whereas Rubisco and other chloroplastic proteins may be broken down via a hybrid pathway involving both chloroplasts and extraplastidic compartments such as the central vacuole and small senescence-associated vacuoles (SAVs), which are absent from mature, non-senescing leaves but present in large numbers during senescence (Otegui et al., 2005; Martínez et al., 2008). Degradation of chloroplastic proteins releases potentially phototoxic chlorophylls that necessitate degradation. Therefore, leaf senescence is characterized by a decline in photosynthetic activity and chlorophyll content, and the rapid chlorophyll loss associated with chloroplast degeneration is frequently used as a biomarker for the start of senescence. Although chlorophyll degradation is an early senescence signal, leaf yellowing is not an appropriate marker of early senescence because it is observed when senescence has progressed to a great extent (Diaz et al., 2005). Nitrogen and carbon metabolism plays a crucial role in the senescence process, which is seemingly governed by both external and internal factors. Thus, leaf senescence induction involves the joint action of external (nitrogen availability, light) and internal signals (regulating metabolites, C/N ratio) (Wingler et al., 2006; Wingler & Roitsch, 2008).

Other important signals for induction or progression of senescence include the redox status of leaf cells and the production of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide radical (Kukavica & Veljovic-Jovanovic, 2004; Zimmermann & Zentgraf, 2005). There are many sources of reactive oxygen species, which are produced during aerobic metabolism in chloroplasts, mitochondria and peroxisomes in both photosynthetically active and senescent cells. The toxicity of these reactive species is dictated by various enzymatic and non-enzymatic protective antioxidant defences. Superoxide dismutases, catalases, peroxidases and the ascorbate–glutathione cycle enzymes are the primary antioxidant enzymes. Plant ageing increases oxidative stress and the levels of reactive oxygen species, which may additionally diminish antioxidant protection (Buchanan-Wollaston et al., 2003b; Zimmermann & Zentgraf, 2005). Chloroplasts are probably the main target of age-associated oxidative stress in plants (Munné-Bosch & Alegre, 2002). Therefore, a plausible model for regulation of leaf senescence is a shifted balance between the production of reactive oxygen species and their removal by antioxidant systems.

In this chapter, we describe various aspects of leaf senescence in sunflower plants, with special emphasis on changes in the contents of some nitrogen and carbon metabolites potentially acting as regulators or markers of senescence during sunflower leaf development, and also on the role of oxidative stress in this process and the influence of external factors such nitrogen supply and irradiance exposition on it.

2. Growth-related parameters and photosynthetic activity during sunflower leaf senescence

We examined various markers widely used to monitor leaf development (viz. photosynthetic pigment level, protein content and CO_2 fixation rate) in primary leaves of sunflower plants grown for 42 days. The start of senescence in sunflower plants was associated with a considerable decrease in protein content and specific leaf masses referred as weight (Table 1).

Leaf age (days)	Soluble protein (mg g^{-1} DW)	Specific leaf mass (mg DW cm^{-2})
16	152.3 ± 9.4	2.2 ± 0.11
22	178.5 ± 7.7	3.1 ± 0.28
28	108.1 ± 4.6	3.0 ± 0.27
36	89.6 ± 1.9	2.5 ± 0.23
42	62.2 ± 1.4	2.2 ± 0.29

Table 1. Changes in soluble protein and specific leaf mass during sunflower primary leaf ageing. Data are means ± SD for duplicate determinations in three separated experiments.

These changes may reflect alterations in N and C compound distributions as a consequence of N remobilization, the efficiency of which is related to the ratio between biomass in the sink and source organs (Wiedemuth et al., 2005; Diaz et al., 2008). Since chloroplasts contain the largest amounts of protein in leaves, their breakdown releases most of the nitrogen that is reused by other plant organs. The mechanisms behind chloroplast degradation in senescing leaves are poorly understood (especially those for the degradation of Rubisco and chlorophyll-binding light-harvesting proteins, which are the most abundant chloroplastic proteins) (Martínez et al., 2008). Chloroplasts contain a large number of proteases, some of which are encoded by senescence-associated genes, which are up-regulated during senescence. Degradation of some thylakoid proteins such as LHCII seemingly occurs exclusively within chloroplasts and requires the prior release and breakdown of pigments (Hörtensteiner & Feller, 2002; Buchanan-Wollaston et al., 2003a). CND41 protease is believed to be involved in Rubisco degradation and in the translocation of nitrogen during senescence in tobacco leaves (Kato et al., 2004, 2005). However, the central vacuole and SAVs also play a role here, as they help complete the degradation of Rubisco and other stromal proteins (Martínez et al., 2008). The relative rates of degradation of some photosynthetic components may be altered by the environmental conditions. Thus, LHCII degradation in rice is delayed by low irradiances (Hidema et al., 1991). Also, the protein content in senescing sunflower leaves was found to drop earlier in nitrogen-deficient plants than in high-nitrogen plants (Agüera et al., 2010). Changes in photosynthetic pigment contents also indicate progress of leaf senescence (Yoo et al., 2003; Guo & Gan, 2005; Ougham et al., 2008). The chlorophyll breakdown pathways operating during leaf senescence are well-known and require pigment degradation and avoiding photodamage in order to maintain the ability to export released nutrients to other plant parts (Hörtensteiner,

2006; Ougham et al., 2008). Chlorophylls in sunflower plants are more susceptible to degradation than are carotenoids during leaf senescence, and both total chlorophyll and carotenoid contents are high in young and mature leaves, their levels peaking at 22 days and decreasing afterwards during senescence (Fig. 1). Carotenoid degradation is usually slower than chlorophyll breakdown and can be especially complex depending on the particular pigment species (Suzuki & Shioi, 2004).

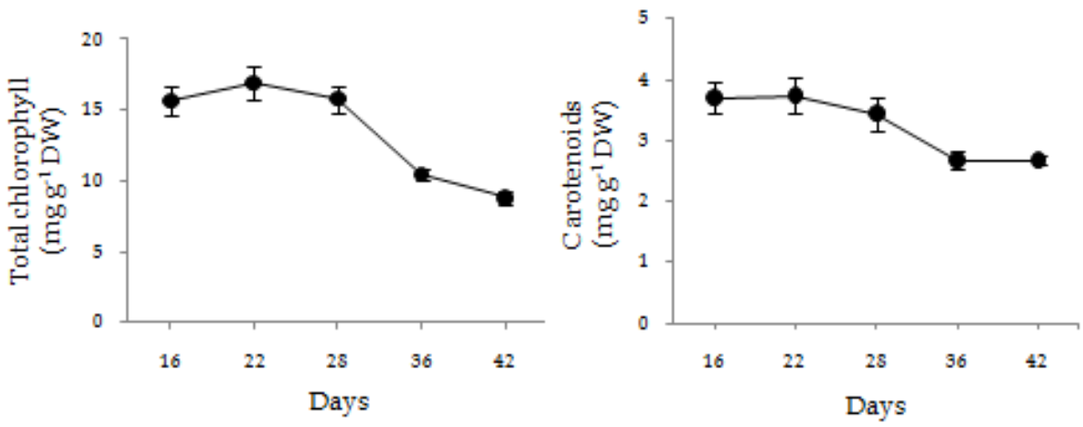

Fig. 1. Changes in pigment levels during ageing of sunflower primary leaves. Data are means ± SD for duplicate determinations in three separated experiments.

Chlorophyll loss in sunflower plants is also a typical phenomenon of leaf senescence of potential use as an indicator. The marked decrease in total chlorophyll observed after 28 days is mainly due to the loss of chlorophyll *a*, which is the form most strongly affected by leaf ageing as revealed by a significant decrease in Chl *a*/Chl *b* ratio in senescent leaves (Cabello et al., 2006). In radish cotyledons, however, the ratio of Chl *a* to Chl *b* increases slightly during senescence, which suggests that Chl *b* is degraded faster than is Chl *a* (Suzuki & Shioi, 2004).

Other typical changes observed during senescence are a rapid decline in photosynthetic activity, which may be a senescence-inducing signal (Bleecker & Patterson, 1997; Quirino et al., 2000), and a reduction in transpiration rate, which is probably due to an increase in abscisic acid levels inducing stomatal closure, although this is not a direct induction factor for senescence (Weaver & Amasino, 2001). A marked decrease in CO_2 fixation rate and transpiration in sunflower plants was observed during natural leaf senescence, a process that starts and develops in plants aged 28–42 days (Fig. 2).

Although natural senescence is the final stage of leaf development, it may start prematurely by effect of exposure to environmental stress or nutrient deprivation (Quirino et al., 2000; Lim et al., 2003, 2007, Wingler et al., 2009). In fact, poor nitrogen nutrition and exposure to high irradiance are known to lead to early senescence in sunflower leaves (Agüera et al., 2010). Thus, the decrease in chlorophyll content associated to leaf senescence starts earlier in sunflower plants grown with low nitrogen, which suggests that leaf senescence is accelerated under these conditions. In addition, the decline in photosynthetic activity is more apparent with nitrogen deficiency (Agüera et al., 2010). Similarly, the loss of photosynthetic activity is more marked in leaves of sunflower plants grown at high

irradiance than in others grown at a low photon flux density, also indicating that an increased irradiance may accelerate leaf senescence.

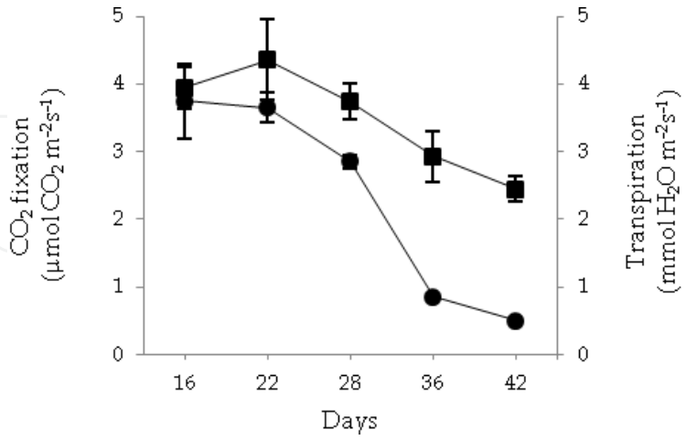

Fig. 2. Carbon dioxide fixation rates and transpiration in sunflower primary leaves of different age. Data are means ± SD of measured values on primary leaves of ten plants randomly selected for each age.

3. Carbon and nitrogen metabolites as regulators of leaf senescence in sunflower plants

The contents in soluble sugars of sunflower plants increase with leaf ageing, and the opposite holds for the starch content. Our results show that accumulation of soluble sugars in plants grown at high irradiance is not much greater than in plants grown at low irradiance, although a substantial increase in the monosaccharide-to-sucrose ratio is observed at the start of senescence (especially at high irradiance levels) (Fig. 3). The accumulation of soluble sugars is associated to leaf age but unrelated to photosynthetic activity because CO_2 fixation rates decrease during ageing; rather, it is due to starch hydrolysis. The increase in soluble sugars may also be ascribed to senescence causing a loss of functional and structural integrity in cell membranes, thereby boosting membrane lipid catabolism and hence sugar production by gluconeogenesis (Buchanan-Wollaston et al., 2003b; Lim et al., 2007). Leaf senescence is a plastic process triggered by a variety of external and internal factors (Weaver & Amasino, 2001; Buchanan-Wollaston et al., 2003a; Balibrea-Lara et al., 2004; Wingler et al., 2006). Senescence reduces photosynthetic carbon fixation, but is important for the recycling of nitrogen and other nutrients (Díaz et al., 2005; Wingler et al., 2005). By virtue of its lying at the crossroads of carbon and nitrogen metabolism, senescence is regulated by carbon and nitrogen signals. Increasing evidence suggests a role for hexose accumulation in ageing leaves as a signal for either senescence initiation or acceleration in annual plants (Masclaux et al., 2000; Moore et al., 2003; Díaz et al., 2005; Masclaux-Daubresse et al., 2005; Parrott et al., 2005; Pourtau et al., 2006; Wingler & Roitsch, 2008; Agüera et al., 2010). Recently, the role of sugar accumulation or starvation in leaf senescence was critically evaluated by van Doorn (2008), who pointed out that little is known about sugar concentrations and senescence regulation in different tissues and cells.

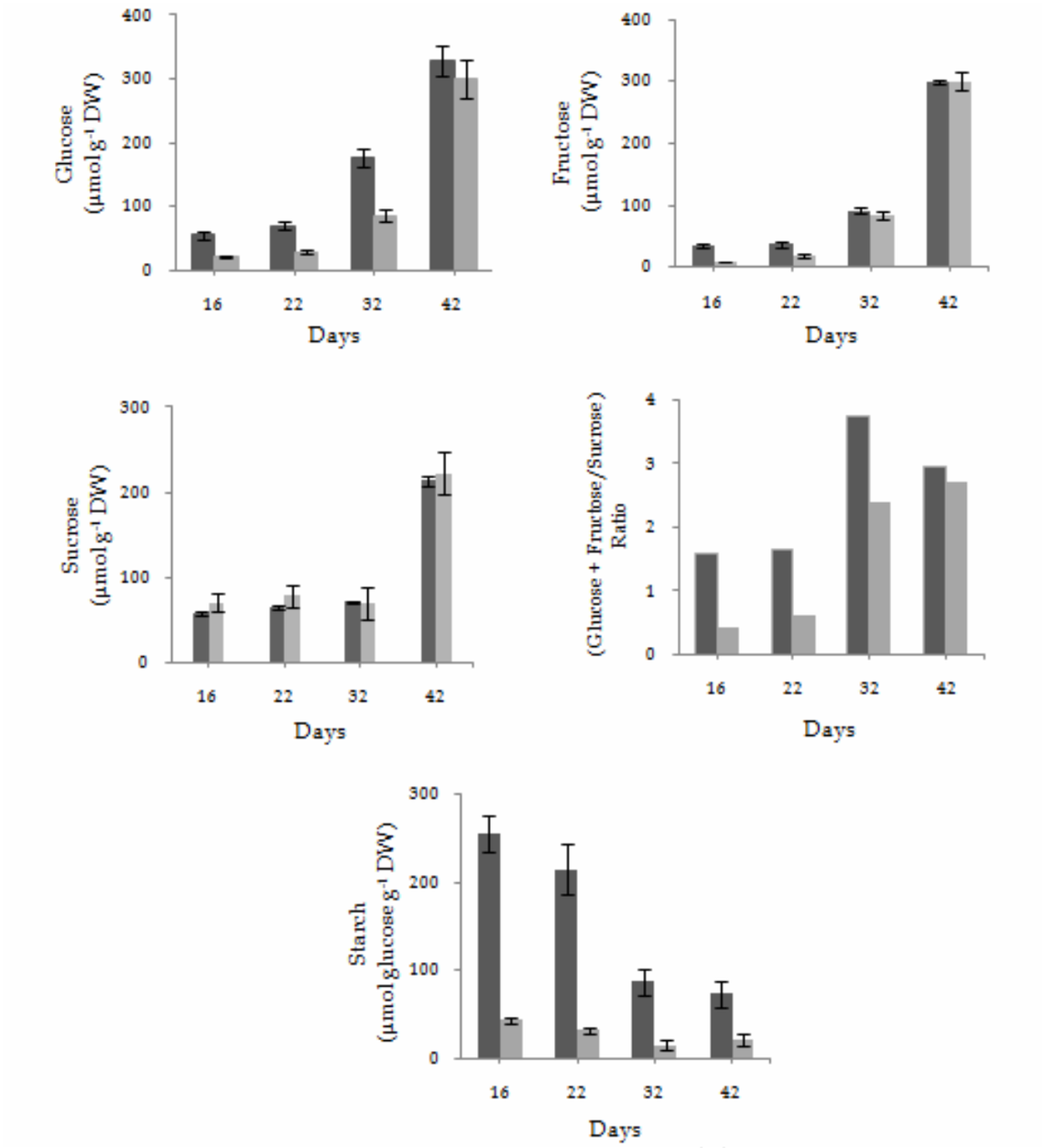

Fig. 3. Changes in glucose, fructose, sucrose and starch contents, and in hexoses-to-sucrose ratio, during development of sunflower primary leaves. Plants were grown at 125 µmol photons m^{-2} s^{-1} (grey bars) or 350 µmol photons m^{-2} s^{-1} (black bars). Data are means ± SD for duplicate determinations in three separate experiments.

Although sugars may not always be the direct cause of leaf senescence, there is enough evidence suggesting that sugar signalling plays a role in senescence regulation in a complex network involving a variety of other signals (Masclaux-Daubresse et al., 2007; Wingler & Roitsch, 2008; Wingler et al., 2009). Thus, cytokinin oxidase/dehydrogenase activity and senescence are positively correlated. The enzyme probably boosts senescence by destroying

cytokinins and light is known to increase cytokinin oxidase/dehydrogenase activity during senescence of barley leaf segments (Schlüter et al., 2011).

Some results also suggest that leaf senescence is regulated by the carbon–nitrogen balance (Masclaux et al., 2000). However, in spite of the drastic changes in leaf metabolism occurring during senescence, carbon and nitrogen metabolite contents have scarcely been determined (Diaz et al., 2005). Cabello et al. (2006) found sunflower leaf senescence to be associated with significant changes in the contents of carbon and nitrogen metabolites. The highest ammonium concentrations were found in young and senescent leaves, as reported in tobacco (Masclaux et al., 2000). Our results indicate that sunflower plants exhibit their peak ammonium contents in young and late senescing leaves (Table 2). The high ammonium contents of young leaves are probably a result of strong photosynthetic nitrate reduction activity and photorespiration. In addition, young leaves have low contents in soluble carbohydrates, and sugar availability is known to be a limiting factor for ammonium assimilation (Morcuende et al., 1998). The high ammonium contents of senescent leaves are mainly due to protein degradation, amino acid deamination and nucleic acid catabolism, but also to photorespiration.

Senescent leaves contain low levels of free amino acids, probably because their remobilization is essential with a view to supplying developing organs in the plant (Buchanan-Wollaston, 1997). The concentrations of glutamate (a precursor of other amino acids) and aspartate (a direct product of glutamate transamination) decrease in the final stages of senescence in *Arabidopsis*. Glutamine and asparagine, the major amino acids translocated in the phloem sap, are mobilized more efficiently during late senescence (Diaz et al., 2005). As suggested by a genome array study (Lin & Wu, 2004), the synthesis of asparagine for nitrogen remobilization during dark-induced leaf senescence in *Arabidopsis* seems to occur via a novel biochemical pathway. Cabello et al. (2006) found glutamate to be the most abundant free amino acid in sunflower leaves as previously also found in rice (Kamachi et al., 1991), tobacco (Masclaux et al., 2000; Tercé-Laforgue et al., 2004) and *Arabidopsis* (Diaz et al., 2005). The ratio (Glu + Asp)/(Gln + Asn) peaked in sunflower leaves of 22 days, but decreased gradually in leaves of 28, 36 and 42 days (Table 2), which suggests that N-rich amino acids (specially Asn, which has a lower C to N ratio) are produced for efficient export from leaves in late senescence, as proposed for *Arabidopsis* (Diaz et al., 2005).

Leaf age (days)	Ammonium (μmol g^{-1} DW)	(Glu + Asp/Gln + Asn) Ratio
16	11.40 ± 1.0	2.11
22	8.57 ± 0.9	2.29
28	7.29 ± 0.7	1.77
36	8.94 ± 0.5	1.51
42	10.91 ± 0.7	1.44

Table 2. Changes in ammonium content and glutamate + aspartate /glutamine + asparagine ratio during sunflower primary leaf ageing. Data are means ± SD for duplicate determinations in three separated experiments

We examined changes in glutamine synthetase (GS) expression and activity during leaf development (Cabello et al., 2006). GS, which is the key enzyme in ammonia assimilation, is present as chloroplastic (GS2) and cytosolic (GS1) isoforms in sunflower leaves (Cabello et al., 1991). In order to confirm whether these isoforms are differently affected by senescence in sunflower leaves, we determined their specific activity during plant development. As shown in Figure 4, total GS activity decreased with the leaf age. The decrease was consequence of a strong decline in chloroplastic GS2 activity. On the other hand, cytosolic GS1 activity increased with ageing. It should be noted that GS1 was the predominant isoform in senescent leaves of 42 days, but accounted for only 7 % of total GS activity in young leaves (16 days). As a result, the GS2/GS1 ratio decreased from 13.3 in young leaves (16 days) to 0.9 in senescent leaves (42 days) (Fig. 4). These results indicate that leaf senescence has an adverse effect on the activity of chloroplastic GS2 (the main glutamine synthetase isoform) and reduces total GS activity despite its boosting GS1 activity.

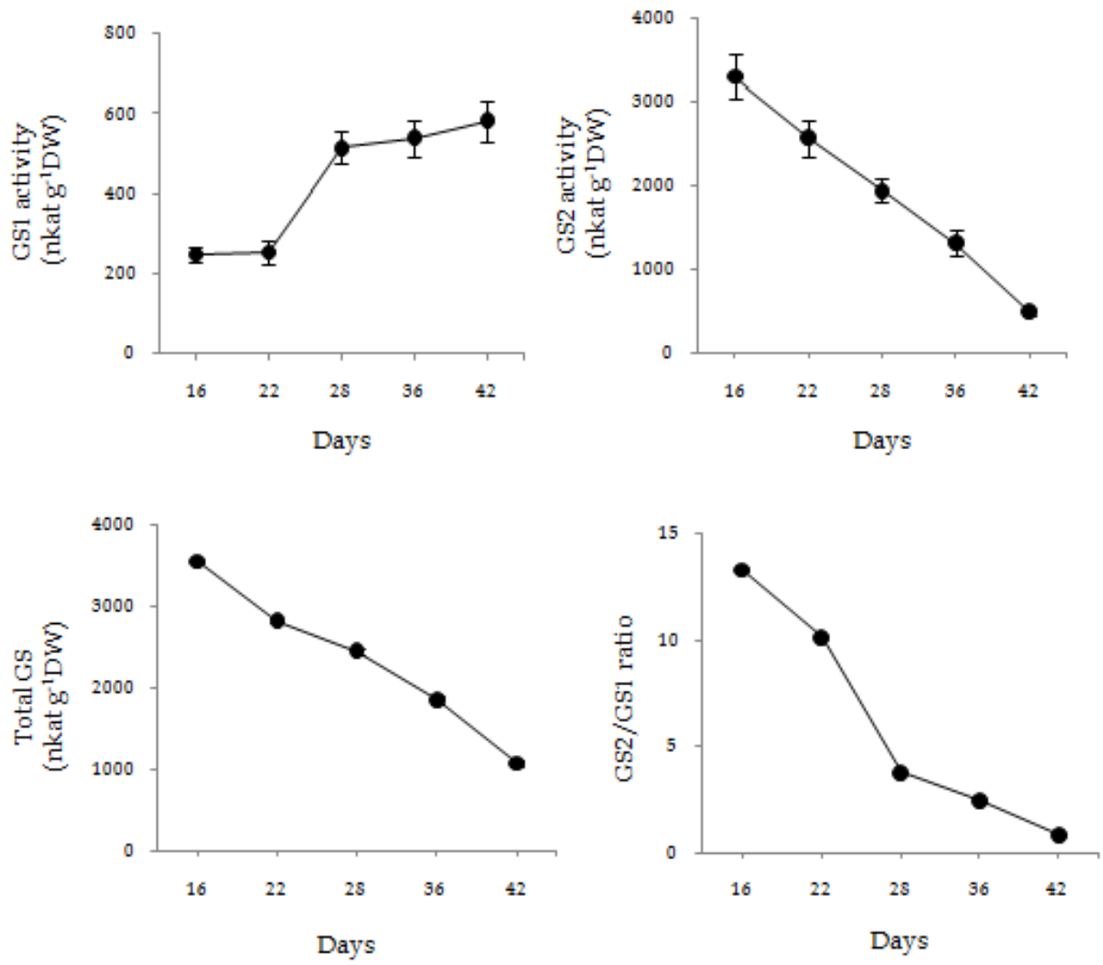

Fig. 4. Effect of ageing on total GS activity and on the activities of GS1 and GS2 isoforms in sunflower leaves. Data are means ± SD of duplicate determinations from three separated experiments.

Ageing affects glutamine synthetase activity but plays a direct role in the regulation of GS gene expression (Cabello et al., 2006). A Northern blot test using a probe corresponding to an internal fragment from *Helianthus annuus* GS2 cDNA revealed that the levels of GS2 transcripts decreased during leaf development and were very low in the late stage of senescence (42 days) (Fig. 5). Glutamine synthetase activity has been found to decrease during natural leaf senescence in a wide variety of plants including cereals, tomato and tobacco (Streit & Feller, 1983; Kamachi et al., 1991; Pérez-Rodríguez & Valpuesta, 1996; Masclaux et al., 2000). This loss of activity is mainly due to a gradual decrease in the major plastidial GS2 isoform since the cytosolic GS1 isoform remains constant or increases during leaf ageing (Pérez-Rodríguez & Valpuesta, 1996; Masclaux et al., 2000).

Northern blots and immunological analyses indicate that both GS transcripts and polypeptides are affected (Pérez-Rodríguez & Valpuesta 1996). GS1 plays a major role in the synthesis of glutamine for transport and remobilization of leaf organic nitrogen (Tercé-Laforgue et al., 2004), whereas GS2 takes part in the reassimilation of ammonium from photorespiration in photosynthetic tissues (Kamachi et al., 1992). The stimulation of the cytosolic GS1 isoform during senescence can be ascribed to the need for toxic ammonium to be reassimilated in order to produce glutamine for export to sink organs; this has led some authors to assume a shift in ammonia assimilation from the chloroplast to the cytosol of leaf cells during senescence (Brugière et al., 2000). Total GS activity was found to drop by a effect of a strong decrease in GS2 activity was found during sunflower leaf ageing despite the simultaneous increase in GS1 activity. GS2 transcript levels also diminished during ageing. Our results (Figs. 4 and 5) are therefore consistent with others previously reported for tomato and tobacco.

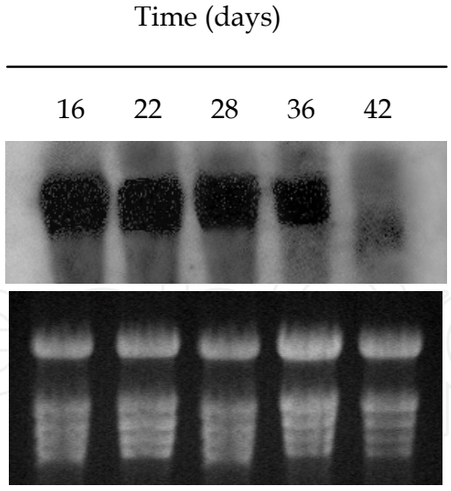

Fig. 5. Effect of ageing on GS2 mRNA accumulation in sunflower leaves.

Amino acids and other metabolites related to N metabolism deficit may act as signals to induce senescence in combination with hexose accumulation. Thus, leaf senescence in sunflower plants is induced by high sugar levels and accelerated by a low nitrogen supply, which supports the view that high sugar/low nitrogen conditions trigger senescence and facilitate its development (Wingler et al., 2009). Our results suggest that leaf senescence in sunflower plants is accelerated by nitrogen deficiency and high irradiance, and also that

some factors such the levels of soluble sugars and amino acids may interact in a complex network to promote this process.

4. Oxidative stress in sunflower plants

Leaf senescence is an oxidative process that involves degradation of cellular and subcellular structures and macromolecules, and mobilization of the degradation products to other parts of the plants (Vanacker et al., 2006). Oxidative stress during senescence may be caused or increased by a loss of antioxidant enzymatic activities (Zimmermann & Zentgraf, 2005; Zimmermann et al., 2006; Procházkova & Wilhelmova, 2007). Senescence is also accompanied by an increase in ROS, one of the origins of which is an imbalance between the production and consumption of electrons in the photosynthetic electron transport chain caused by preferential inhibition of stromal reactions in contrast with photosystem II photochemistry (Špundová et al., 2003). The inhibition of stromal reactions increases the electron flow to molecular oxygen and causes ROS to accumulate and chloroplast components to be damaged as a result (Špundová et al., 2005; Couée et al., 2006). Chloroplasts are the main source of ROS in plants (Zimmermann & Zentgraf, 2005) and also the major target of oxidative damage (Munné-Bosch & Alegre, 2002). Stromal protein degradation during leaf senescence may be initiated by oxidative processes associated with the generation of free radicals and reactive species (Procházkova et al., 2001). Like Rubisco and other chloroplastic proteins, GS2 is susceptible to degradation initiated by reactive oxygen species (Ishida et al., 2002). The chloroplastic GS2 isoform is one of the first targets of oxidative damage at high irradiation levels (Palatnik et al., 1999). Oxidized GS becomes more susceptible to proteolysis (Ortega et al., 1999); under photo-oxidative stress, GS2 cleavage occurs preferentially around the catalytic site (Ishida et al., 2002). Senescence may therefore have a direct impact on GS2 activity through enzyme degradation initiated by reactive oxygen species as reported in Rubisco (Ishida et al., 1997; Roulin & Feller, 1998). Our results indicate that the decrease in GS2/GS1 ratio during sunflower leaf ageing may be partly due to a different sensitivity to oxidative stress of the two isoforms; in fact, chloroplastic GS2 is much more sensitive to oxidative modification *in vitro* than is cytosolic GS1 (Cabello et al., 2006). Therefore, ageing induces oxidative stress in sunflower leaves and can thus have an adverse effect on chloroplastic GS2, as well as on photosynthetic pigments. Antioxidant enzyme activities in sunflower leaves were found to decline during late senescence (42 days). Similar results have been reported for tobacco (Dhindsa et al., 1981), *Arabidopsis* (Ye et al., 2000), pea (Olsson, 1995) and maize (Procházkova et al., 2001). Oxidative stress during late senescence may be caused or increased by the loss of antioxidant enzymatic activities (Zimmermann & Zentgraf, 2005). Also, the decline in antioxidant activities is believed to be a consequence rather than the origin of senescence (Dertinger et al., 2003).

Susceptibility to oxidative stress depends on the overall balance between production of oxidants and cell antioxidant capability. In sunflower plants, considerable oxidative stress has been observed *in vivo* during leaf senescence, as revealed by lipid peroxidation, H_2O_2 accumulation and a decrease in the levels of antioxidant enzymes such as catalase, ascorbate peroxidase and superoxide dismutase (Table 3). Lipid peroxidation only occurs during the late stage of senescence (Berger et al., 2001; Jongebloed et al., 2004; Wingler et al., 2005). High irradiance causes reversible photoinhibition of photosynthesis in pea chloroplasts and

increases ROS potentially regulating the accumulation of mRNA encoding antioxidant enzymes (Hernández et al., 2006).

Age (days)	H_2O_2 (μmol g^{-1} DW)	Catalase	Ascorbate peroxidase (U g^{-1} DW)	Superoxide dismutase	Lipid peroxidation (nmol MDA g^{-1} DW)
16	1.22 ± 0.15	1.12 ± 0.10	17.21 ± 1.22	336.4 ± 28	87.6 ± 8.2
22	1.38 ± 0.14	1.70 ± 0.12	17.99 ± 2.12	356.2 ± 39	85.6 ± 7.4
28	3.84 ± 0.42	2.25 ± 0.26	28.22 ± 3.22	538.5 ± 42	155.5 ± 12.3
36	4.76 ± 0.30	1.94 ± 0.17	21.34 ± 2.19	1450.5 ± 112	171.5 ±14.5
42	5.28 ± 0.51	1.24 ± 0.15	14.53 ± 1.17	985.2 ± 92	188.4 ± 12.8

Table 3. Hydrogen peroxide accumulation, catalase, ascorbate peroxidase and superoxide dismutase activities, and lipid peroxidation levels during sunflower primary leaf development. Data are means ± SD for duplicate determinations in three separated experiments.

The activity and expression of antioxidant enzymes are seemingly sensitive to high irradiance stress (Yoshimura et al., 2000; Hernández et al., 2004).

We found H_2O_2 accumulation in senescent sunflower to be slightly more marked in plants grown under a nitrogen deficiency; the differences, however, were not large enough to assume that H_2O_2 is a major factor regulating the induction of leaf senescence in N-deficient plants (Table 3). Interestingly, catalase and ascorbate peroxidase activity decreased steadily in plants grown with low nitrogen, but increased during early leaf development and then declined during senescence in plants grown with high nitrogen (Agüera et al., 2010). Production of ROS during leaf senescence is essentially governed by chloroplasts, which have a strong photooxidative potential (Zapata et al., 2005). A simultaneous increase in lipid peroxidation was observed. Mutations in the *Arabidopsis CPR5/OLD1* gene may cause early senescence through deregulation of the cellular redox balance (Jing et al., 2008). Also, there is evidence suggesting that inadequate oxidant and carbonyl group production are intrinsically related to plant ageing, and that low mitochondrial, superoxide dismutase and ascorbate peroxidase activities may contribute to extensive protein carbonylation (Vanacker et al., 2006; Srivalli & Khanna-Chopra, 2009).

In conclusion, during sunflower leaf development some coordinated metabolic and physiological changes are produced, and the senescence process induces significant alterations in the levels of carbon and nitrogen metabolites. Glutamine synthetase of sunflower leaves is regulated both at transcriptional and enzyme levels during leaf ontogeny. Post-translational regulation of the GS2 isoform could be due, at least partially, to oxidative processes. GS activity may be used as a biochemical marker of leaf ageing, since the beginning of senescence at about 28 days is accompanied by a drastic drop in the GS2/GS1 ratio due to the increase of the cytosolic GS1 activity and the decline of the chloroplastic GS2 activity. Our results suggest that both high irradiance and nitrogen deficiency accelerates senescence of the primary leaf, probably for maintaining the functionality of the young leaves, and that one of the reasons for this accelerated senescence

may be the high cellular oxidation and oxidative damage caused by the earlier decline of the activity of the antioxidant enzymes in these plants (Pompelli et al., 2010).

5. Acknowledgment

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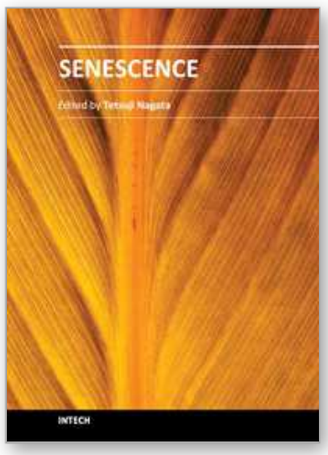

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The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

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