Journal of Experimental Botany, Vol. 57, No. 9, pp. 1949–1956, 2006
doi:10.1093/jxb/erj142 Advance Access publication 23 May, 2006

Journal of Experimental Botany

RESEARCH PAPER

The expression patterns of *SAG12/Cab* genes reveal the spatial and temporal progression of leaf senescence in *Brassica napus* L. with sensitivity to the environment

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Received 15 November 2005; Accepted 26 January 2006

Abstract

Despite a high nitrate uptake capacity, the nitrogen use efficiency (NUE) of oilseed rape is weak due to a relatively low N remobilization from vegetative (mostly leaves) to growing parts of the plant. Thus, this crop requires a high rate of N fertilization and leaves fall with a high N content. In order to reduce the rate of N fertilization and to improve the environmental impact of oilseed rape, new genotypes could be selected on their capacity to mobilize the foliar N. Various indicators of leaf senescence in oilseed rape were analysed during plant growth, as well as during senescence induced by N deprivation. Metabolic changes in leaves of increasing age were followed in N-supplied and N-deprived rosettes by measuring chlorophyll, total N, and soluble protein contents. Similarly, the expression of genes known to be up-regulated (*SAG12*) or down-regulated (*Cab*) during leaf senescence was monitored. The amount of soluble proteins per leaf was a better indicator of leaf senescence than chlorophyll or total N content, but was not evaluated as an accurate indicator under conditions of N deprivation. On the other hand, up-regulation of *SAG12* concomitantly with down-regulation of *Cab* in the leaf revealed the spatial and temporal progression of leaf senescence in oilseed rape. This study shows, for the first time at the whole plant level, that the *SAG12/Cab* gene expressions match the sink/source transition for N during both developmental and nutrient stress-induced leaf senescence.

Key words: *Brassica napus* L., chlorophyll, leaf senescence, senescence-associated genes, senescence-down-regulated genes, soluble proteins.

Introduction

Nitrogen (N) and other mobile elements are redistributed in higher plants from vegetative plant parts (sources) to growing organs or storage tissues (sinks). One of the major features of the progression of leaf senescence is the mobilization of N products that are readily translocated as nutrients to N sinks (Feller and Fischer, 1994; Masclaux *et al.*, 2000).

During the development of reproductive tissues, the N demand by the reproductive sink organs greatly increases. In senescing leaves, the export of N products is not compensated by the N import and then leads to the decrease of the N content. By contrast, when the reproductive organs have been removed in *Brassica napus*, foliar senescence is slowed down due to the absence of a significant sink demand (Noquet *et al.*, 2004). In this case, the export of N from the senescing leaves is reduced and therefore leads to a higher N content in the fallen leaves. In oilseed rape, the low N demand at the vegetative stage of development (i.e. before the development of reproductive organs) may explain the high N content remaining in the fallen leaves (Triboï-Blondel, 1988; Hocking *et al.*, 1997; Rossato *et al.*, 2001).

High N use efficiency (NUE: ratio of harvested N to N fertilization) is partly a question of highly co-ordinated timing of N distribution to the locations of greatest N demand within the plant (Masclaux *et al.*, 2000). Therefore, leaf fall is of considerable interest because N remaining in prematurely fallen leaves is a loss for dry matter production but it may also increase nitrate leaching following the mineralization of leaf organic N. Due to a low NUE, oilseed rape requires a high rate of N fertilization, which is too high with regard to the N content of harvested tissues. Whatever

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the rate of N fertilization, the oilseed rape N harvest index is low compared with cereals (Dreccer *et al.*, 1997). The fall of the leaves with a high N content (up to 4.5% of the dry matter) leads to a return of N to the soil that can reach 100 kg N ha^{-1} (Dejoux *et al.*, 2000). Therefore, new genotypes of oilseed rape selected for their capacity to mobilize the foliar N would require lower N fertilization with a potential improvement of the environmental impact of this crop.

Leaf senescence progresses in an age-dependent manner. In *B. napus* and other annual plants, such as wheat and rice (Feller and Keist, 1986), the lower old leaves wilt first and the uppermost younger leaves remain active for a longer time so that senescence starts at the bottom of the canopy and moves upwards. Along the axis of the young rosette of *B. napus*, each leaf can easily be ranked and a single plant provides a large set of leaves with a gradation of N mobilization rate. Based on a weekly ^{15}N-labelling in field conditions during the oilseed rape growth cycle, Malagoli *et al.* (2005) gave an accurate description of N flows from or to each individual leaf rank. Although a general description can be obtained for all leaf ranks, this study revealed a complex system of sink/source transitions for all leaves. N for leaves located at the bottom of the canopy comes mostly from concurrent nitrate uptake, before being remobilized to upper leaves during the plant's life span. A single N atom can therefore be mobilized several times from leaf to leaf, being finally used for seed filling. Overall, these results showed that lower leaf ranks were, during vegetative growth, less efficient in term of N mobilization, having therefore a different behaviour than leaves in the upper canopy, for which a large proportion of their N content is translocated to reproductive sinks. However, this approach is far too heavy to use if a comparison between numerous genotypes under different environments is expected, for which more easily accessible indicators of N mobilization during leaf senescence are requested.

Leaf senescence is a succession of many physiological and molecular events that arise according a three-stage process (Noodén *et al.*, 1997), comprising an initiation phase (sink/source transition), a degenerative phase (disassembly of cellular components), and a terminal phase (loss of cell integrity and cell death) (Yoshida, 2003). But leaf senescence is not only an essential step for plant development since it can also be induced prematurely by numerous environmental stresses (Gan and Amasino, 1997). Nutrient stress, such as N limitation, induces premature senescence in the N-deprived plants (Smart *et al.*, 1994). During leaf ageing or under environmental stress, various physiological and molecular indicators have been used to characterize the initiation phase of leaf senescence that reflects the status of source or sink of the leaf for N (Yoshida, 2003). Because one of the earliest features of leaf senescence is the decline of photosynthesis, yellowing is often used as an obvious macroscopic symptom of senescence. Nevertheless, visible yellowing is a relatively late event and many senescence-enhanced genes show increased expression before a leaf yellows (Buchanan-Wollaston and Ainsworth, 1997). Senescence-enhanced genes that encode different types of proteins, including proteases, lipases, nucleases, and N-metabolizing enzymes, have been isolated from *B. napus* leaves and expression analyses have shown that the induction of expression does not occur at the same stage of senescence (Buchanan-Wollaston and Ainsworth, 1997; Buchanan-Wollaston *et al.*, 2005). Among these, senescence-associated genes (SAG) are expressed only during developmentally controlled senescence. For example, in *B. napus*, *SAG12*, a putative cysteine protease, is encoded by two genes *BnSAG12-1* and *BnSAG12-2* (Noh and Amasino, 1999), which are orthologues of *SAG12* from *Arabidopsis thaliana* (Lohman *et al.*, 1994). Both genes show the same temporal expression pattern during the senescence process: no expression of these genes in young or mature leaves followed by an expression level of both genes that reaches a maximum at an early stage of senescence and then a slight decrease in the degenerative phase that remains at the same level in the terminal phase of senescence. Other genes, such as *LSC650* and *LSC760* (leaf senescence clone encoding a catalase protein and an aspartic protease, respectively), are expressed during all stages of *B. napus* leaf development but their expression increased significantly during the senescence process (Buchanan-Wollaston and Ainsworth, 1997). On the other hand, the expression of senescence-down-regulated genes (SDG) such as those involved in photosynthesis and also in the structure of the chloroplast, decreases during leaf senescence (Hensel *et al.*, 1993). For example, the expression of the *Cab* gene (chlorophyll *a/b*-binding protein) decreases in the fully expanded leaves and is expressed no more in the senescing leaves (Buchanan-Wollaston, 1994).

The aim of this work was to compare various indicators of senescence in order to find the ones that are able spatially and temporally to characterize the sink/source transition of an oilseed rape leaf during growth as well as during an induced senescence. Thus, metabolic changes in leaves of increasing age from N-supplied or N-deprived rosettes were examined by measuring chlorophyll, total N, and soluble protein contents. In addition, the expression pattern of genes known to be up-regulated (*SAG12*) or down-regulated (*Cab*) during leaf senescence was established.

Materials and methods

Plant culture

Seeds of *Brassica napus* L. cv. Capitol were germinated and grown in hydroponic solution (30 seedlings per 15 l plastic tank) in a greenhouse. The aerated nutrient solution contained 1 mM KNO_3, 0.40 mM KH_2PO_4, 1.0 mM K_2SO_4, 3.0 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.15 mM K_2HPO_4, 0.2 mM Fe-Na EDTA, 14 μM H_3BO_3, 5.0 μM $MnSO_4$, 3.0 μM $ZnSO_4$, 0.7 μM $CuSO_4$, 0.7 μM $(NH_4)_6Mo_7O_{24}$, 0.1 μM $CoCl_2$, and was renewed every 2 d. pH was maintained at

6.5±0.5 by adding $CaCO_3$ (200 mg l^{-1}). The natural light was supplemented with phytor lamps (200 μmol m^{-2} s^{-1} of photosynthetically active radiation at the height of the canopy) for 16 h d^{-1}. The thermoperiod was 20 °C (day) and 15 °C (night). Time was expressed in cumulative degree days after sowing (°C DAS).

Experimental treatments and sampling

The period of growth that was studied began at 308 °C DAS and ended at 548 °C DAS. At 308 °C DAS, one set of plants was transferred to a solution without KNO_3 and was grown under N-deprivation conditions until the end of the experiment.

On each date (308, 357, 436, and 548 °C DAS), three plants of both sets (N-supplied and N-deprived plants) were harvested and divided into the whole root system, the stem, and individual leaves. The relative chlorophyll content of each leaf was determined using a SPAD analyser (see below). The plant fractions were randomly subsampled. One subsample of each plant fraction was weighed, dried at 60 °C, reweighed for dry weight determination, and then ground to a fine powder for nitrogen analyses. A second subsample was immediately frozen in liquid nitrogen after harvest and then stored at −80 °C until protein and RNA extractions.

Nitrogen analysis

The total N in the plant samples was determined with a continuous flow isotope mass spectrometer (Isoprime, GV Instruments, Manchester, UK) linked to a C/N analyser (EA3000, EuroVector, Milan, Italy).

Chlorophyll content

Relative chlorophyll content per unit of leaf area was determined using a non-destructive chlorophyll meter SPAD (Soil Plant Analysis Development) analyser (Minolta, model SPAD-502), which measures leaf transmission at two wavelengths (650 and 940 nm). It has been demonstrated that relative SPAD values depend on chlorophyll content in a linear manner (Monje and Bugbee, 1992). Each data point represents the average of eight independent measurements on each leaf.

Soluble protein extraction and quantification

Soluble proteins were extracted from 500 mg FW of frozen leaf tissue at 4 °C with 2 ml of 100 mM potassium phosphate buffer, pH 7.5. The homogenate was centrifuged (2000 *g*, 4 °C, 5 min) and supernatant was collected. Protein quantification was carried out on aliquots of this supernatant using Bradford's method (Bradford, 1976). BSA was used as the standard for the quantification. For each sample, three quantifications were made.

RNA extraction

Total RNA was extracted from 500 mg FW of frozen leaf tissue. Leaf material was ground to a powder with a pestle in a mortar containing liquid nitrogen. The resulting powder was mixed with 750 μl extraction buffer (0.1 M TRIS, 0.1 LiCl, 0.01 M EDTA, 1% SDS (w/v), pH 8) and 750 μl of hot phenol (80 °C, pH 4). This mixture was spun for 30 s. After addition of 750 μl of chloroform/isoamylalcohol (24:1), the homogenate was centrifuged at 20 000 *g* for 5 min at 4 °C. One volume of 4 M LiCl solution was added to the supernatant and the mix was incubated overnight at 4 °C. After centrifugation (20 000 *g*, 30 min, 4 °C), the pellet was suspended in 250 μl of sterile water. To precipitate total RNA, 50 μl of 3 M sodium acetate (pH 5.6) and 1 ml of 96% ethanol were added for 1 h at −80 °C. After centrifugation (20 000 *g*, 20 min, 4 °C), the pellet was washed with 1 ml of 70% ethanol (v/v), then centrifuged at 20 000 *g* for 5 min at 4 °C. The resulting pellet was dried and resuspended in sterile water containing 0.1% SDS and 20 mM EDTA. Quantification of total RNA was realized by spectrophometry at 260 nm (Optiquant, Pharmacia Biotech, France) before being used in RT-PCR analyses.

RT-PCR analysis

For RT-PCR, 3 μg of total RNA was converted to cDNA with MLV reverse transcriptase using the manufacturer's protocol (Promega, France). From each sample, two RT reactions were made and followed by two independent PCRs using specific primers for *Brassica napus Cab* gene LHCII type I (AY288914) (forward primer 5′-GGCAGCCCATGGTACGGATC-3′ and reverse primer 5′-CCT CCITCGCTGAAGATCTGT-3′) and primers shared by *SAG12-1* (AF089848) and *SAG12-2* (AF089849) genes of *B. napus* (forward primer 5′-GGCAGTGGCACACCAICCGGTTAG-3′ and reverse primer 5′-AGAAGCITTCATGGCAAGACCAC-3′). All of the PCRs were performed with Qiagen Taq polymerase (France) for 35 or 18 cycles, respectively, for *SAG12* and *Cab* genes: one cycle at 94 °C for 10 min, 35 (*SAG12*) or 18 (*Cab*) at 94 °C for 1 min, 58 °C for 45 s, 72° C for 1 min, and one cycle at 72 °C for 10 min. Two single 267 bp and 290 bp cDNAs were amplified using, respectively, specific primers of *SAG12* and *Cab* genes. The identity of the amplified fragments was checked by sequencing (Genome Express, France). For all RT-PCR reactions, 25S rRNA (D10840) was used as a cDNA synthesis and amplification control. RT-PCR products were separated by electrophoresis through 1% agarose gel. Agarose gel was visualized and photographed using a Biocapt (Vilber Lourmat, France). Each PCR product was quantified using bio 1-D software and normalized using intensity of 25S rRNA signal.

Results

Changes in fresh weight of leaves and roots

During plant development, new leaves appeared at the top of the plant while lower leaves entered into senescence. In N-supplied plants, the total fresh weight of leaves increased with time, as well as the root fresh weight (Fig. 1A). The fresh weight of the middle and top leaves (leaf ranks 6–9) increased with time, whereas it decreased at the base, thus underlying the senescence process in the lowest part of the canopy, resulting from a concomitant decrease in water content and dry weight. In N-deprived plants, the total fresh weight of leaves remained at the same value until 357 °C DAS (Fig. 1B) and then decreased as a result of leaves being smaller and the progressive fall of leaf ranks 1 and 2 (between 357 and 436 °C DAS), and leaf rank 3 (between 436 and 548 °C DAS). By contrast, the fresh weight of roots increased until the end of the experiment (548 °C DAS). Consequently, the shoot:root ratio remained at about 6 in N-supplied plants, whereas it decreased from about 6 to 2 in N-deprived plants mostly due to root growth (Fig. 1C). Thus, N-deprivation reduced leaf more than root growth compared with N-supplied plants.

Changes in chlorophyll content in individual leaves

Data of chlorophyll content in individual leaves, commonly used as an indicator of leaf senescence, are shown in Fig. 2. In N-supplied plants, leaf chlorophyll content stayed relatively constant during the entire experiment for all leaves, except for older leaves (leaf ranks 1–3) and newly formed

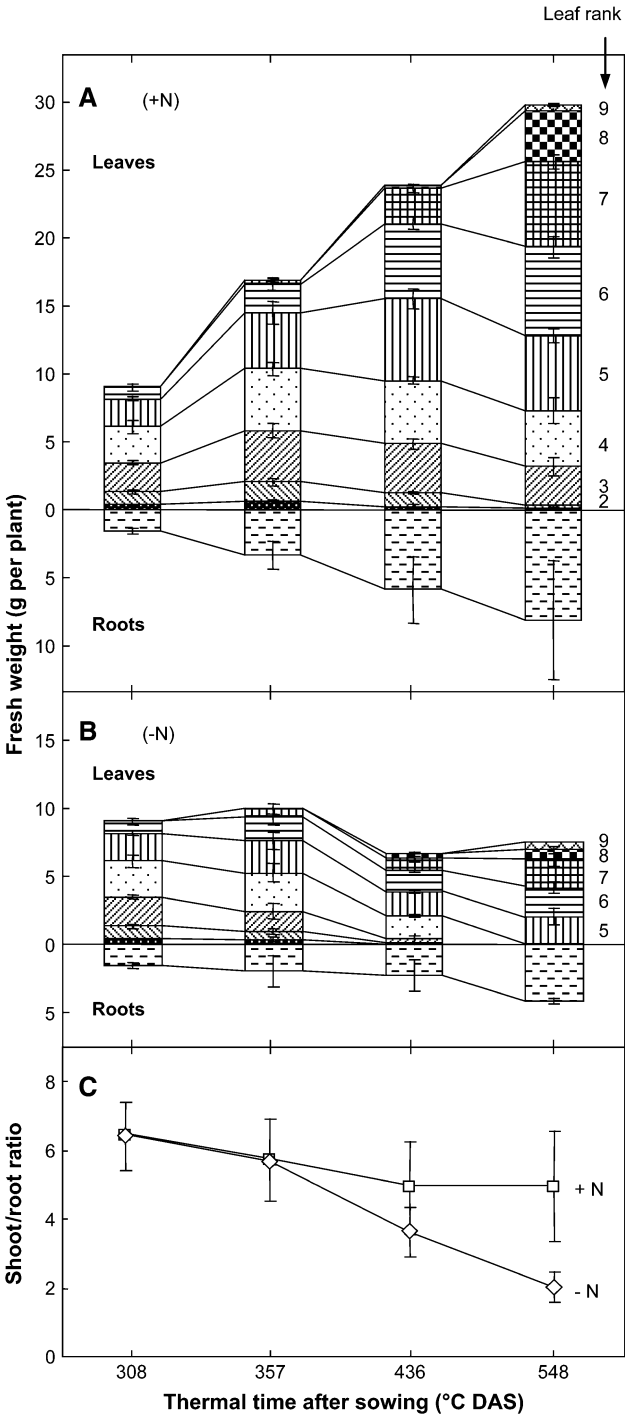

Fig. 1. Leaf and root fresh weights in N-supplied (A) and in N-deprived (B) plants further separated into root (below the rule) and leaf rank (above the rule in ascending order of rank). Shoot:root ratio (C) in N-supplied (squares) and N-deprived (diamonds) plants. Each box represents the fresh weight of each individual leaf and the root system per plant. Error bars represent standard deviation for n=3 when larger than the symbol.

leaves (leaf ranks 7–9) which decreased and increased, respectively (Fig. 2A). Chlorophyll content was at its maximum value in the middle and mature leaves. Leaf chlorophyll content in older leaves decreased earlier in N-deprived plants than in N-supplied plants (Fig. 2B). Chlorophyll content in leaf ranks 5–9 was quite similar in N-supplied and N-deprived plants. With regard to chlorophyll content, senescence of leaf ranks 1–3 was accelerated by N deprivation and, in addition, this treatment promoted senescence in leaf rank 4.

Changes in the amount of protein in individual leaves

In N-supplied plants, the amount of soluble proteins always displayed a symmetrical repartition around the median leaf ranks (Fig. 3), with lower values in senescing (N sources) and newly formed (N sinks) leaves and a maximum in mature leaves. Thus, these median leaves constituted the most important reserve of endogenous N (soluble proteins) of the plant. In N-deprived plants, the pattern of protein amount was similar to that in N-supplied plants until 436 °C DAS. Nevertheless, protein amounts were drastically reduced in all leaves, including newly formed ones, beyond approximately 50%, 75%, and 90% of N-supplied values at 357 °C DAS, 436 °C DAS, and 548 °C DAS, respectively. These reduced amounts in proteins were due, at least in part, to the lower leaf biomasses (Fig. 1) and also to the lower N content in N-deprived plant leaves (data not shown).

Changes in expression of genes affected by leaf senescence

The expression of two genes, *SAG12* (cysteine protease) and *Cab* (chlorophyll *a*/*b*-binding protein), that reflect the stage of leaf senescence was monitored in *Brassica*. The kinetics of gene expression (Fig. 4) was studied in leaf rank 4 that was chosen because of the changes in physiological (Fig. 1) and biochemical (Figs 2, 3) indicators of senescence previously observed. *Cab* was constitutively expressed at 308 °C DAS in both plants, but down-regulation occurred earlier in N-deprived plants (436 °C DAS versus 548 °C DAS). On the contrary, the other gene, *SAG12*, was only expressed during leaf senescence. In leaf rank 4, *SAG12* was expressed at 548 °C DAS and 436 °C DAS in N-supplied and N-deprived plants, respectively. In addition, the up-regulation of *SAG12* occurred concomitantly with the down-regulation of *Cab* whatever the N treatment, but earlier under the N-deprivation condition. The up-regulation of *SAG12* and down-regulation of *Cab* indicated that leaf rank 4 senesced earlier under N-deprivation conditions. This new indicator followed a drastic decrease in the amount of N per leaf (Fig. 4) suggesting that it could be used, whatever the developmental stage, to identify leaf ranks subjected to sink/source transition for N (Fig. 5A). Thus, an attempt was made to identify at each harvest date, the leaf rank characterized by both up-regulation of *SAG12* and down-regulation of *Cab* (Fig. 5B). *SAG12*/*Cab* gene expression allowed a theoretical leaf rank located on node 2.2 to be identified as a leaf which mobilized N at the beginning of the experiment

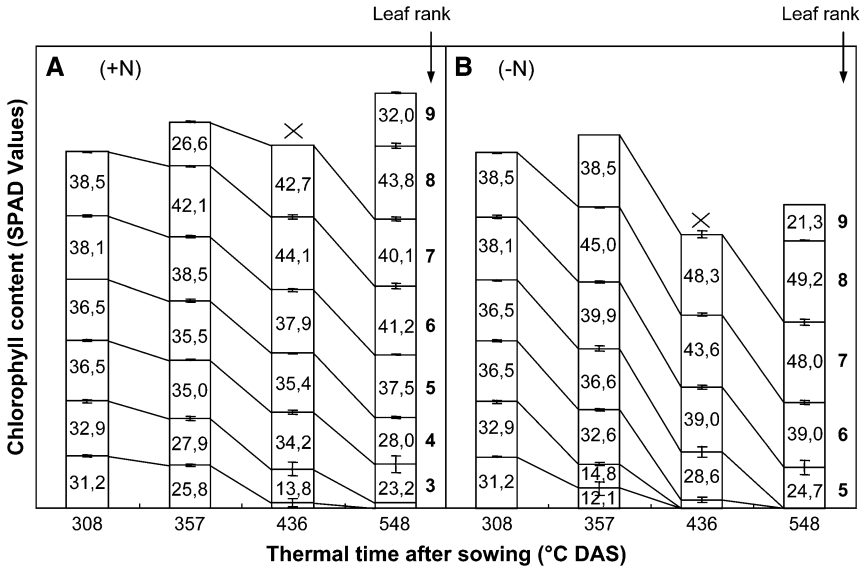

Fig. 2. Leaf chlorophyll content in N-supplied (A) and N-deprived (B) plants. Total chlorophyll was analysed with a SPAD analyser as described in the Materials and methods. Each box represents the chlorophyll content of each individual leaf. Error bars represent standard deviation for n=3 when larger than the symbol. The cross at the top of two columns indicates that the surfaces of the last leaf ranks were too small to be measured.

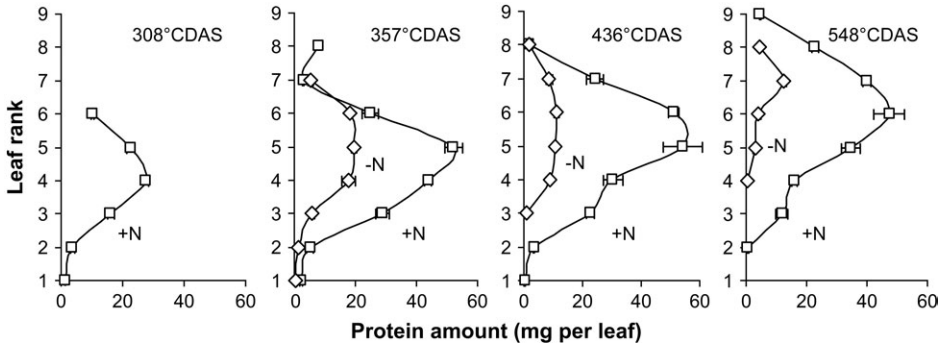

Fig. 3. The amount of soluble protein in individual leaf rank in N-supplied (squares) and N-deprived (diamonds) plants during time after sowing. Error bars represent standard deviation for n=3 when larger than the symbol.

(308 °C DAS). It appeared that, with time, foliar senescence of N-supplied plants progressed spatially toward the apex of the plant to reach the theoretical leaf rank 3.3 at 548 °C DAS. In N-deprived plants, the spatial progression of leaf senescence was accelerated and reached the theoretical leaf rank 5.5 at 548 °C DAS. From the linear correlations (Fig. 5B) observed during early vegetative growth, the temporal senescence progression of one leaf ranked every 169.49 °CD for N-supplied plants while it progressed at a faster rate under N-deprivation conditions (one leaf rank every 71.42 °CD).

Discussion

Metabolic indicators of leaf senescence

In most studies of leaf senescence, chlorophyll content is used as a relevant metabolic indicator of the late stages of senescence (Buchanan-Wollaston, 1994; Lohman *et al*., 1994; Buchanan-Wollaston and Ainsworth, 1997; Lee *et al*., 2001). By contrast to many other investigations, the chlorophyll content was measured in each individual leaf of the plant. Whatever the rate of N fertilization, the chlorophyll content was lower in the lowest leaves of the plant and gradually increased to the top leaves. This confirms that in rape, as in other annual plants such as wheat, rice, and tobacco (Dalling *et al*., 1976; Mae and Ohira, 1981; Masclaux *et al*., 2000), leaf senescence during vegetative development is a sequential process, starting at the bottom of the canopy and moving upwards. By contrast to the obvious differences in dry matter production between N-supplied and N-deprived plants, very few changes were observed in the chlorophyll content under N-deprivation conditions. This result is in agreement with the observation of Kappen *et al.* (1998) that reports the absence of an effect

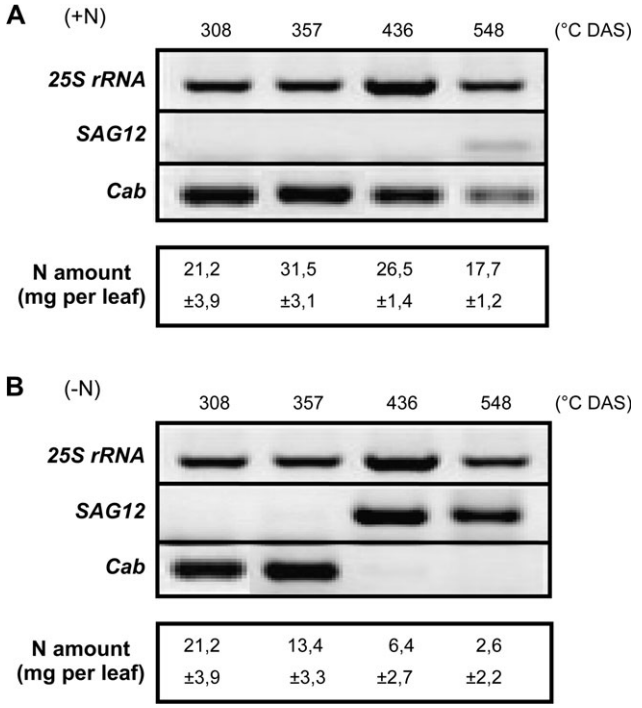

Fig. 4. RT-PCR analysis of *SAG12* and *Cab* gene expression in leaf rank 4 of N-supplied (A) and N-deprived (B) plants. Total RNA was extracted from leaf rank 4 at 308, 357, 436, or 548 °C DAS after N-supply or N-deprivation. 25S rRNA was used as a cDNA synthesis and amplification control.

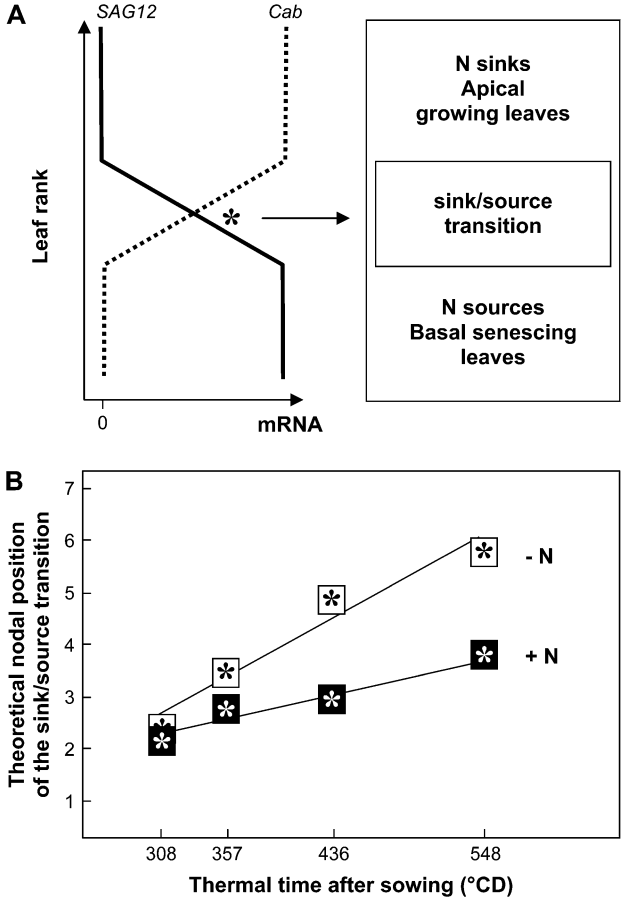

Fig. 5. Characterization of the N status of oilseed rape leaves with regard to the expression of *SAG12* and *Cab*. (A) Schematic representation of the N status of the leaves according to their nodal position along the plant axis and to *SAG12* and *Cab* expression. Sink/source transition for N (right) is thought to occur in leaves during the beginning of senescence corresponding to increased *SAG12* and decreased *Cab* expression (left). The asterisk refers to the nodal position of a theoretical leaf rank subjected to sink/source transition for N (*SAG12* up-regulation and *Cab* down-regulation, concomitantly). (B) Kinetics of the progression of leaf senescence along the axis of oilseed rape supplied (+N) or not (−N) with N. Each symbol refers to the theoretical nodal position of a leaf rank subjected to sink/source transition for N (i.e. displaying *SAG12* up-regulation and *Cab* down-regulation, concomitantly).

of N fertilization on leaf chlorophyll content of field-grown oilseed rape. Nevertheless, leaf senescence, and also leaf fall, occurred earlier in N-deprived plants than in N-supplied plants. Thus, it appears that the chlorophyll content in oilseed rape leaves is probably not a convenient metabolic indicator of leaf senescence, especially under N-deprived conditions, even if it is still often used to characterize a group of leaves at the same stage of development (Buchanan-Wollaston, 1994).

The highest quantity of soluble proteins was observed in mature median leaves, decreasing in amount in the lower leaves, and reaching a very low amount (closed to zero) in the lowest and oldest leaves. This result supports the hypothesis that, during leaf senescence, the cell metabolism is shifted toward the breakdown of macro-molecules, leading to a dramatic decrease in the amount of soluble proteins (Hensel *et al*., 1993), therefore allowing a massive mobilization of N metabolites from leaves to the growing parts of the plant such as young leaves and developing seeds (Noodén, 1988; Smart *et al.*, 1994). By contrast to the chlorophyll content, N-deprivation affected the amount of soluble proteins in leaves as well as N content. The quantity of soluble proteins in each leaf was reduced, in particular because of a lower leaf biomass in N-deprived plants. Furthermore, the decrease in the amount of soluble proteins in old senescing leaves occurred earlier than in the N-supplied plants. Because the only source of N for N-deprived plants comes from senescing leaves, the breakdown of protein will have occurred earlier. The data for N confirmed this result, showing the high total N content in median leaf rank (5 and 6) decreased in the lower leaves and the new top leaf (data not shown).

Taken alone, chlorophyll content does not appear to be a reliable indicator of either the senescence stage or the N status of a leaf. The present results are also supported by those of Crafts-Brandner *et al.* (1990), who showed that in leaves the sink/source transition for N occurs earlier than chlorophyll breakdown, underlying a very early protein mobilization during leaf senescence. Thus, the quantity of soluble proteins is probably a better indicator of leaf senescence than chlorophyll or total N content in oilseed rape. Finally, the results also confirmed that the rate of leaf

senescence and the mobilization of leaf N are related to the N-nutrition status of the plant and to the sink/source transition (Crafts-Brandner *et al.*, 1996; Ono *et al.*, 1999; Masclaux *et al.*, 2000). Nevertheless, the low amount of soluble proteins in the top newly formed leaves in N-deprived plants showed that it was impossible to discriminate a young leaf from an old one which has mobilized its N. Consequently, this metabolic indicator cannot be used as an indicator of leaf senescence.

Molecular indicators of leaf senescence

Leaf senescence is associated with important changes in gene expression. Many genes, especially genes involved in photosynthesis, are down-regulated (SDGs for senescence-down-regulated genes) while specific senescence-associated genes (SAGs) are up-regulated (Hensel *et al.*, 1993; Buchanan-Wollaston, 1994; Lohman *et al.*, 1994; Noodén *et al.*, 1997). The present work focused on two genes, one down-regulated (*Cab*) and one up-regulated (*SAG12*). The chlorophyll *a*/*b*-binding protein (*Cab*) is involved in photosynthesis and its gene expression decreases during leaf development (Buchanan-Wollaston, 1994; Buchanan-Wollaston and Ainsworth, 1997). Thus, the expression of this gene was compared with the senescence-related gene expression of *SAG12*. *SAG12* encodes a cystein protease in *Arabidopsis* and *Brassica napus* and is only expressed during developmentally controlled senescence (Lohman *et al.*, 1994; Weaver *et al.*, 1998; Noh and Amasino, 1999; Grbic *et al.*, 2003).

The expression pattern of *SAG12* and *Cab* genes showed temporal and spatial regulation in relation to the progression of leaf senescence along the axis of the plant. Furthermore, the transcript levels changed earlier in N-deprived plants than in N-supplied plants confirming the sensitivity of these two genes to a senescence-promoting treatment. It is interesting to notice that *SAG12* expression was enhanced under N deprivation, although its specificity is to be controlled by a strictly developmental senescence regulatory pathway (Noh and Amasino, 1999). This result could be explained by the original approach described here of studying individual leaves of the plant instead of grouping leaves with similar chlorophyll content, and/or by an early onset of a natural phase in leaf development which therefore induces senescence. In addition, the up- and down-regulation of *SAG12* and *Cab*, respectively, immediately shows in a leaf that the sink/source transition has already occurred, whereas the opposite is true for metabolic indicators (chlorophyll, protein, and N amounts) which require kinetic measurements.

Thus, among the different indicators used in this study (metabolic and molecular), *SAG12* and *Cab* gene expressions have proven the most accurate for following the temporal and spatial progression of leaf senescence in oilseed rape. Concomitantly with the down-regulation of *Cab*, the up-regulation of *SAG12* matches the sink/source transition for N during both developmental and induced leaf senescence (N deprivation). This supports the idea that the sink/source transition for N occurs earlier than leaf chlorophyll degradation, and matches the increase of proteolysis activities (Crafts-Brandner *et al.*, 1990).

As a conclusion, the concomitant up-regulation of *SAG12* and down-regulation of *Cab* seems to be an accurate bio-indicator of leaf senescence. Furthermore, this indicator can be used to monitor the gradient of leaf senescence during the ontogenetic cycle of oilseed rape, especially during the sink/source transition of the leaves required for N filling of the grain. This discriminating indicator should be useful for relating the timing of leaf senescence with a better NUE and therefore to compare oilseed rape genotypes on their ability to mobilize foliar N.

Acknowledgements

The authors thank the Conseil Régional de Basse-Normandie and the Centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM) for Julie Gombert's PhD grant. We are grateful to O Sordet for critically reading the manuscript.

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