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# Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns

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## Abstract

**Oilseed rape (*Brassica napus* L.) is commonly grown for oil or bio-fuel production, while the seed residues can be used for animal feed. It can also be grown as a catch crop because of its efficiency in extracting mineral N from the soil profile. However, the N harvest index is usually low, due in part to a low ability to remobilize N from leaves and to the fall of N-rich leaves which allows a significant amount of N to return to the environment. In order to understand how N filling of pods occurs, experiments were undertaken to quantify N flows within the plant by $^{15}N$ labelling and to follow the changes in soluble protein profiles of tissues presumed to store and subsequently to remobilize N. Whereas N uptake increased as a function of growth, N uptake capacity decreased at flowering to a non-significant level during pod filling. However, large amounts of endogenous N were transferred from the leaves to the stems and to taproots which acted as a buffering storage compartment later used to supply the reproductive tissue. About 15% of the total N cycling through the plant were lost through leaf fall and 48%, nearly all of which had been remobilized from vegetative tissues, were finally recovered in the mature pods. SDS-PAGE analysis revealed that large amounts of a 23 kDa polypeptide accumulated in the taproots during flowering and was later fully hydrolysed. Its putative function of storage protein is further supported by the fact that when plants were grown at lower temperature, both flowering, its accumulation and further mobilization were delayed. The overall results are discussed in relation to plant strategies which optimize N cycling to reproductive sinks by means of buffering vegetative tissues such as stems and taproots.**

Key words: Brassica, flowering, nitrogen mobilization, nitrogen storage, nitrate uptake, soluble proteins.

## Introduction

Oilseed rape (*Brassica napus* L.) is an important agricultural crop, grown primarily for oil production, but also as a valuable break-crop in cereal crop rotation. After oil extraction, the high protein seed residue can be used as animal feed providing that the effects of toxic products such as glucosinolates are eliminated by heat treatment. Given its high capacity to absorb mineral N from the soil profile in autumn and early winter, oilseed rape is also widely grown as a catch crop to reduce nitrate leaching from arable cropping systems. The maximum rate of nitrate uptake by *Brassica napus* L. roots is higher on a root weight basis than for many other cultivated species (Lainé *et al*., 1993). Oilseed rape roots are also able to increase their uptake rate capacities rapidly in order to compensate shoot N requirement (Lainé *et al*., 1995). However, about 50% of applied N fertilizer is recovered in the harvested seeds (Schjoerring *et al*., 1995), which, in view of the high N rates usually applied to this crop, implies an inefficient utilization and the potential risk of significant losses of the applied N to the environment.

Several reports suggest that this relative inefficiency of oilseed rape to export N to harvestable tissues results from loss of N through frozen leaf fall in winter (Dejoux *et al*., 2000) as well as through loss of leaves with a high N content after flowering. The dead leaves found on the soil surface in spring contain a significant amount of N, usually exceeding 2% of the dry weight and suggesting a low ability of oilseed rape to remobilize leaf N to the developing pods. This low efficiency with which *Brassica*

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*napus* L. translocates N to reproductive tissues is rather surprising in view of its capacity to take up mineral N from the soil. The low N harvest index found for this species may therefore be a result of sink strength limitation rather than an unavailability of N within the plant to sustain significant N mobilization to reproductive tissues. Similarly, the efficiency with which leaf N is mobilized to the seeds might be improved by increasing N storage within the plant through alternative sinks. It has, for example, been shown in both herbaceous (Staswick, 1990, 1994; Volenec *et al*., 1996) and woody species (Stépien *et al*., 1994) that N can be transiently stored as vegetative storage proteins, which can be hydrolysed to supply developing sinks with N. Although considerable improvement of crop cultivars has been obtained over the last five decades, mostly under high yield/high N input conditions, some results suggest that this selection strategy may have had a negative effect on N storage capacity. For example, in a comparison of different grass species, it was found that species in a low-N environment relied more heavily on N storage than species usually found in N-rich environments (Thornton *et al*., 1994). It has also been hypothesized (Chapin *et al*., 1990) that, from an ecological point of view, N cycling and storage within the plant and its environment, is required in species adapted to low-N environments. A better understanding of N storage and remobilization, which act as a buffer system between the uptake of N by roots and its use for growth, is needed, especially in a species such as oilseed rape, whose ancestors were probably ruderal with high requirements for soil N availability. Furthermore, even if genetic manipulation of N carriers toward over-expression might seem a sensible objective, it must be kept in mind that their activity is usually subjected to complex regulation (Crawford, 1995; Forde and Clarkson, 1999) in order to match N acquisition and N demand for growth. Alternative sinks for driving uptake may be found such as N storage which can be growth-uncoupled, and may have a positive influence on N-use efficiency in the long term (Clarkson and Hawkesford, 1993; Imsande and Touraine, 1994).

Although a few rapeseed models exist in the literature (Petersen *et al*., 1995; Gabrielle *et al*., 1998) for the prediction of dry matter production and N uptake, these are incomplete as regards the above process, as they do not account for N partitioning between pods and vegetative parts after flowering. Moreover, shortcomings have been found in the ability of a model such as DAISY (Petersen *et al*., 1995) to simulate plant N dynamics late in the growth period, which also highlight the necessity to understand more precisely how N flows within the plants are controlled after the flowering stage.

The objectives of these studies were first, to quantify and distinguish by $^{15}N$ labelling between the origin of the N used for pod filling, namely from uptake or from vegetative tissue mobilization, secondly to describe the kinetic behaviour of different source tissues and, lastly, to identify some proteins that might be involved in N storage.

## Materials and methods

### *Plant culture*

*Brassica napus* L. cv. Capitol plants were taken from a field plot in December when they were at the 6 leaf stage. The roots were gently rinsed with distilled water before transferring the plants to a hydroponic system (27 seedlings per 15 $dm^3$ plastic tank) in a growth room. The aerated nutrient solution contained 1 mM $KNO_3$, 0.40 mM $KH_2PO_4$, 1.0 mM $K_2SO_4$, 3.0 mM $CaCl_2$, 0.50 mM $MgSO_4$, 0.15 mM $K_2HPO_4$, 0.20 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 $(NH_4)_6Mo_7O_{24}$, and 0.1 μM $CoCl_2$, and was renewed every 2 d. $CaCO_3$ was then given in excess at a final concentration of 2 mM in order to maintain the solution pH at $6.5 \pm 0.2$. Light was provided by high-pressure sodium lamps (250 μmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation at the height of canopy) for 16 h $d^{-1}$. The thermoperiod was 20 °C (day) and 15 °C (night). The plants were used for experiments when the lateral roots (partly damaged during collection of the plants from field plots) had been growing for 3 weeks.

### *Chemical analysis and calculation of N remobilization*

The total N and $^{15}N$ in the plant samples was determined with a continuous flow isotope mass spectrometer (Twenty-twenty, PDZ Europa Scientific Ltd, Crewe, UK) linked to a C/N analyser (Roboprep CN, PDZ Europa Scientific Ltd, Crewe, UK). As all the mineral N taken up from the nutrient solutions was $^{15}N$ labelled, the accumulated uptake and further translocation into plant parts could be calculated from the excess $^{15}N$ in each tissue. Consequently, the patterns of net translocation of endogenous unlabelled N ($^{14}N$ absorbed prior to the beginning of the experiment) between plant parts could be used to calculate N remobilization within the plant. The N in growing leaves derived from the mobilization of endogenous unlabelled N was calculated by subtracting from total N ($^{14}N + {}^{15}N$), firstly, the $^{15}N$ content derived from uptake of $^{15}NO_3^-$ and, secondly, the initial $^{14}N$ content found in this tissue at the beginning of the experiment.

*Electrophoretic analysis of soluble proteins.* Soluble proteins were extracted from 1 g of fresh tissue at 4 °C with 7 ml of 50 mM TRIS buffer (pH 7.5) containing 2 mM phenylmethylsulphonyl fluoride, 10 μM leupeptin, 1 mM EDTA, and 0.1% (v/v) β-mercaptoethanol. After centrifugation (3200 *g*, 10 min), nucleic acids in the remaining supernatant were precipitated with protamine sulphate (1 mg $ml^{-1}$), except in the case of leaves and stems so as to avoid RubisCO precipitation. The nucleic acid pellet was discarded after centrifugation (18 000 *g*, 10 min). The supernatant was separated into 11 subsamples, each receiving 0.015% (w/v) sodium deoxycholate. After 10 min, the soluble proteins were precipitated at 4 °C with 7.2% (w/v) trichloroacetic acid. The protein pellets were rinsed with acetone and their content determined (Lowry *et al*., 1951). BSA was used as the standard protein. For SDS-PAGE analysis, one pellet of soluble protein was resuspended in Laemmli lysis buffer (Laemmli, 1970), denatured for 4 min at

100 °C, and centrifuged (10 000 *g*, 2 min). For SDS-electrophoresis, a 15% duracryl running gel was used with a stacking gel containing 5.8% duracryl. Two wells were used for loading proteins with known molecular masses. The gels were run for 2 h at constant 200 V, and silver stained as described previously (Lopez *et al.*, 1991).

For two-dimensional gel electrophoresis, one pellet of soluble protein was resuspended in the buffer described earlier (O'Farrell, 1975) and used to run 2-D SDS-PAGE gels, loaded with 150 µg of protein, according to a modified procedure from O'Farrell (O'Farrell, 1975) on an Investigator System (Millipore Corporation, Saint-Quentin en Yvelines, France). First-dimension isoelectric focusing was carried out in a 4.1% acrylamide tube gel containing 9.5 M urea, 2% (v/v) Triton X100, 5 mM (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate), and 5.8% (v/v) Millipore 2 D optimized carrier ampholytes (pH 3–10). After 2 h of prefocusing to 1500 V with the current limited to 110 µA $tube^{-1}$, protein samples (21 µl) were loaded on the basic end of the tube gel and focused with 2000 V $tube^{-1}$ for 17.5 h. The gels were removed and pre-equilibrated for 2 min in a 375 mM TRIS-HCl buffer, pH 8.6, containing 3% (w/v) sodium dodecyl sulphate, 50 mM dithiothreitol and 0.01% (w/v) bromophenol blue. The isoelectrofocused proteins in the second dimension were, like the one-dimensional gels separated using a 15% duracryl running gel, run for 5.5 h at 500 V. The gels were subsequently silver stained as described previously (Lopez *et al.*, 1991).

All gels were analysed by computerized image analysis (Biocapt, Bio1-D, Bio2-D, Vilber Lourmat, Marne-la-Vallée, France). The gels were scanned, and the individual staining intensities of each polypeptide expressed as a percentage of the total staining intensity of the gel.

### *Experimental treatments, labelling and harvests*

At day 0, plants were supplied with $K^{15}NO_3$ ($^{15}N$ excess of 1.00%), and then sampled after 0, 3, 10, 17, 24, 39, and 70 d. The roots were first rinsed with a 1 mM solution of $CaSO_4$ to remove any superficial $^{15}N$. Six plants were harvested at each date and separated into lateral roots, taproots, green leaves, dead leaves, stems, flowers, and pods. One subsample of each plant fraction was used for fresh weight determination, dried at 60 °C, reweighed for dry weight determination and then ground to a fine powder for isotopic analysis. The second subsample was weighed, and kept at −80 °C until SDS-PAGE analysis of the soluble proteins.

Another experiment was performed to delay the flowering process by decreasing the growth temperature. Plants were grown from day 0 under the same above-described conditions except that the temperature was set at 4 °C for 15 d, then at 20 °C until the end of the experiment. Control plants were grown constantly at 20 °C. The plants were harvested after 0, 3, 6, 9, 15, 21, 27, and 39 d as described above.

## Results

### *Growth and nitrogen flows*

Growth analysis can be seen as the first step to integrate changes in source–sink relationships within the plant (Fig. 1). Plant growth was significant until day 39, after which it remained at about 8.8 g DW $plant^{-1}$. The growth of each plant fraction was sequential and occurred in the following order: roots and leaves (until day 24), followed by stem (from day 10–39), and reproductive tissues (flowers and pods, from day 24–70). Leaf senescence started 10 d after the beginning of the experiment, which explains the subsequent decrease in biomass of photosynthetic leaves (from 2.06 g at day 24 down to 0.21 g DW $plant^{-1}$ at day 70). The highest root biomass (lateral roots + taproots) was recorded on day 24, while only the taproot weight decreased thereafter. Reproductive tissue growth occurred as the dry weight of vegetative tissues decreased.

All of the N in the plant acquired before the experiment was called endogenous, while that acquired during the experiment was called labelled N. Cumulative nitrate uptake calculated from $^{15}N$ labelling (Fig. 2A) was nearly linear during the first 24 d, then exhibited an inflexion during flowering, to plateau at day 39, after which no significant uptake of $^{15}NO_3^-$ was recorded. An overview of nitrogen flows within the plant was obtained (Fig. 2B) by expressing the N content of each tissue as a percentage of the maximum value recorded during the experiment. Both lateral roots and taproots behaved as sink tissues until day 39, but only the taproots showed a decrease in N content thereafter, the relative decrease being similar to that of the stems. The N content in green leaves significantly decreased after 24 d. It can therefore be hypothesized that N filling of the flowers and pods

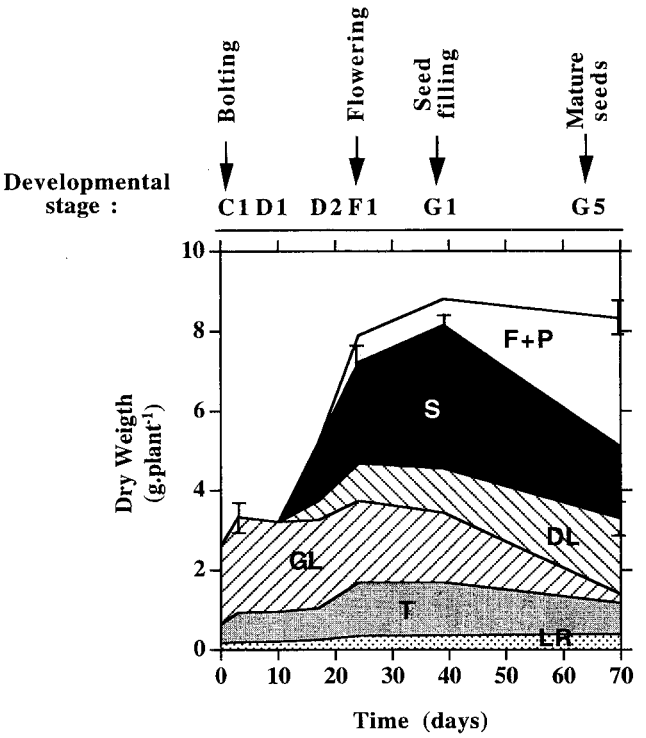


**Fig. 1.** Biomass changes in *Brassica napus* L. plants further separated into lateral roots (LR), taproot (T), green leaves (GL), dead leaves (DL), stem (S), and flowers plus pods (F + P). Vertical bars when large enough indicate ± SE of the mean for $n = 6$.

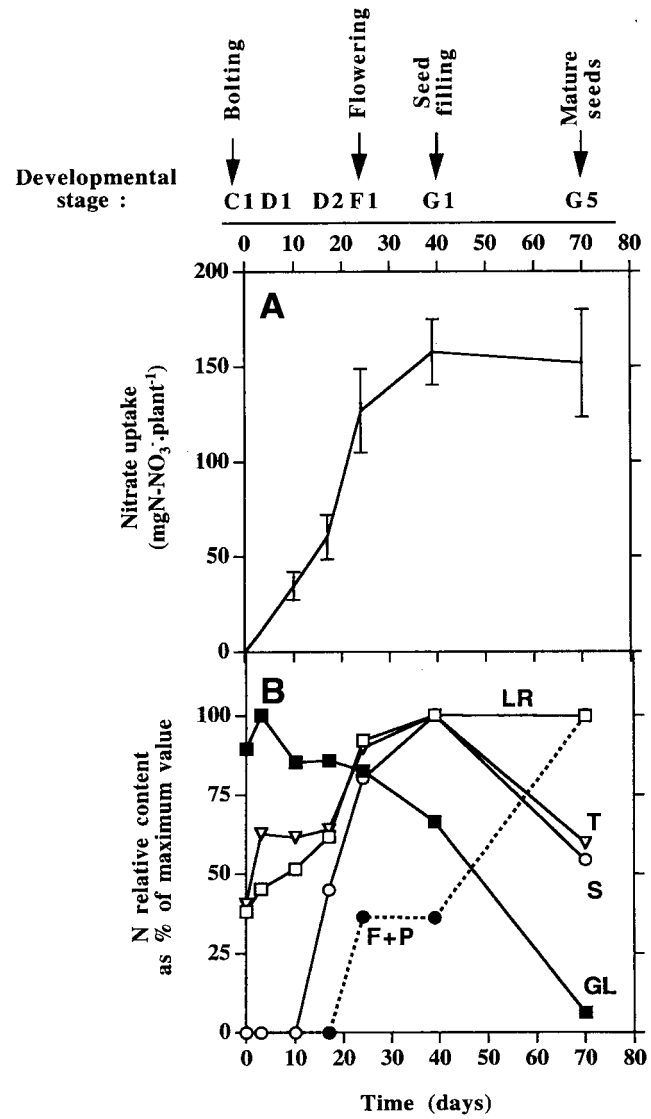


**Fig. 2.** (A) Cumulative net nitrate uptake in *Brassica napus* L. plants estimated by $^{15}N$ labelling. (B) N relative content in lateral roots (LR), taproot (T), green leaves (GL), dead leaves (DL), stem (S), and flowers plus pods (F + P), expressed as a percentage of maximum N content for each tissue. Vertical bars when larger than the symbol indicate ± SE of the mean for $n = 6$.

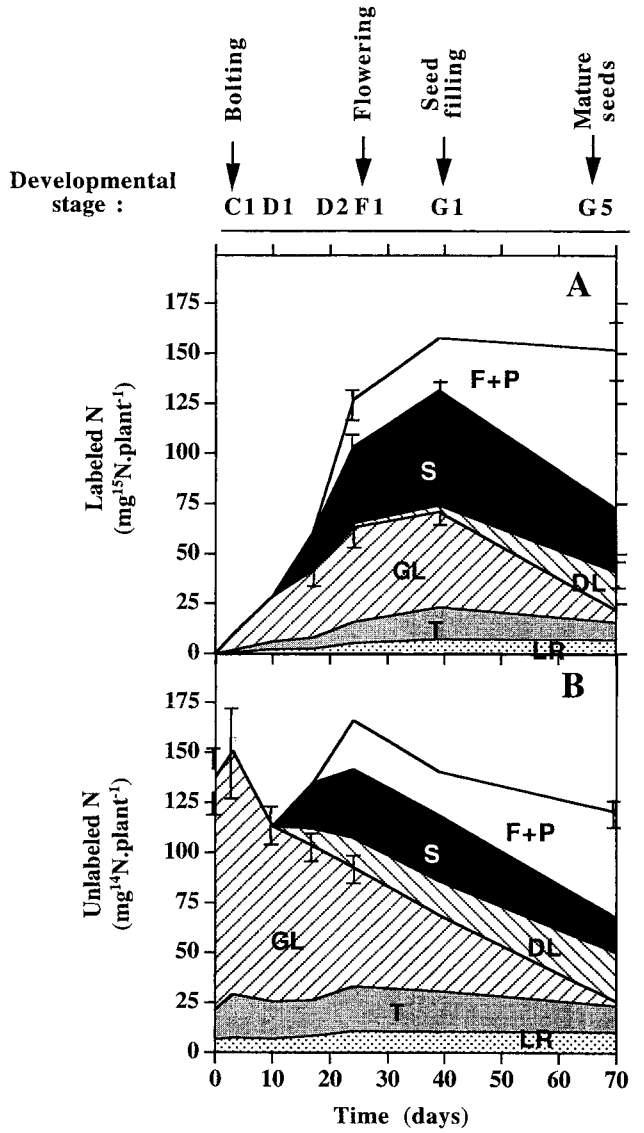


**Fig. 3.** Changes in the distribution within *Brassica napus* L. plants of (A) labelled N derived from the $^{15}N$-nitrate taken up, and of (B) unlabelled endogenous $^{14}N$ content in lateral roots (LR), taproot (T), green leaves (GL), dead leaves (DL), stem (S), and flowers plus pods (F + P). Vertical bars when larger than the symbol indicate ± SE of the mean for $n = 6$.

occurred between days 24 and 39 at the expense of N uptake and from leaf N remobilization, while at a later stage, the N in pods was mostly derived from N mobilized from the taproots, stems, and leaves, with no significant contribution of N uptake (Fig. 2A). It can also be suggested that the N of stems, which at day 39 accounted for nearly 41% of the total plant biomass (Fig. 1), was derived both from N uptake and from N mobilization in leaves.

Translocation within the plant of $^{15}N$ derived from the $NO_3^-$ taken up during the 70 d (Fig. 3A) was clearly related to the dry weight distribution between organs (Fig. 1). $^{15}N$ amounts increased in all tissues until day 39, firstly in green leaves, stems, reproductive organs and, to a lesser extent, in the roots. While no $^{15}NO_3^-$ uptake was detected between day 39 and day 70 (Fig. 2A), the amount of N isotope continued to increase in the pods and flowers, showing that significant cycling of the previously absorbed $^{15}N$ occurred towards these tissues. This was further supported by the change in endogenous unlabelled N distribution within the plant (Fig. 3B). Total endogenous $^{14}N$ per plant was kept significantly constant during the experiment at about 139.3 ± 5.7 mg endogenous $^{14}N$ plant$^{-1}$, indicating that no significant loss of N occurred either by efflux in the nutrient solution or by volatilization. The endogenous unlabelled N

distribution between organs was greatly modified with time, demonstrating that a significant amount of endogenous unlabelled N was translocated from green leaves to taproots and stems during an initial phase, and from green leaves, stems and taproots to the flowers and pods later on. All the previous data taken together were used to calculate flows of N from N taken up and from endogenous N mobilization before and during pod filling (Fig. 4). Whereas 157.5 mg N-$NO_3^-$ plant$^{-1}$ were absorbed before pod filling, i.e. before day 39, none was taken up later. Before pod filling, the N taken up was mostly allocated to the stems (57.1 mg), leaves (47.8 mg), flowers (26.1 mg) and taproots (15.9 mg). A smaller proportion (3.2 mg, 2% of total N taken up) was lost through leaf fall. At this stage, large amounts of endogenous unlabelled N were mobilized from leaves (63.7 mg N corresponding to more than half of their initial N content) to stems (51%), flowers (33%) and taproots (9%), while 16.9 mg N were lost through the fall of dead leaves. The source–sink relationships for endogenous N were largely modified during pod filling (the only sink), as the status of stems and taproots changed from sink to source (Fig. 4). About 109.7 mg N were mobilized to the pods, 41, 31.1 and 11.1% being remobilized from leaves, stems and taproots, respectively. During the 70 d, 44 mg of N were lost through the death of leaves, which corresponded to 16% of total N cycling through the plant (272.2 mg N), while 25.4 mg N were lost by flower abortion. In the meantime, more than 48% of the N entering the plant was finally found in the pods (131.9 mg N), nearly all of which was directly derived from the remobilization of N from other tissues.

### *Changes in soluble protein SDS-PAGE profiles*

During growth, little change was found in the soluble protein profiles analysed by SDS-PAGE (data not shown), provided that the same amount of proteins were visualized. For example, the relative abundance of both the small and large subunits of RubisCO in green leaves remained the same for 39 d (i.e. from the bolting stage C1 to the end of the flowering stage G1, CETIOM source;

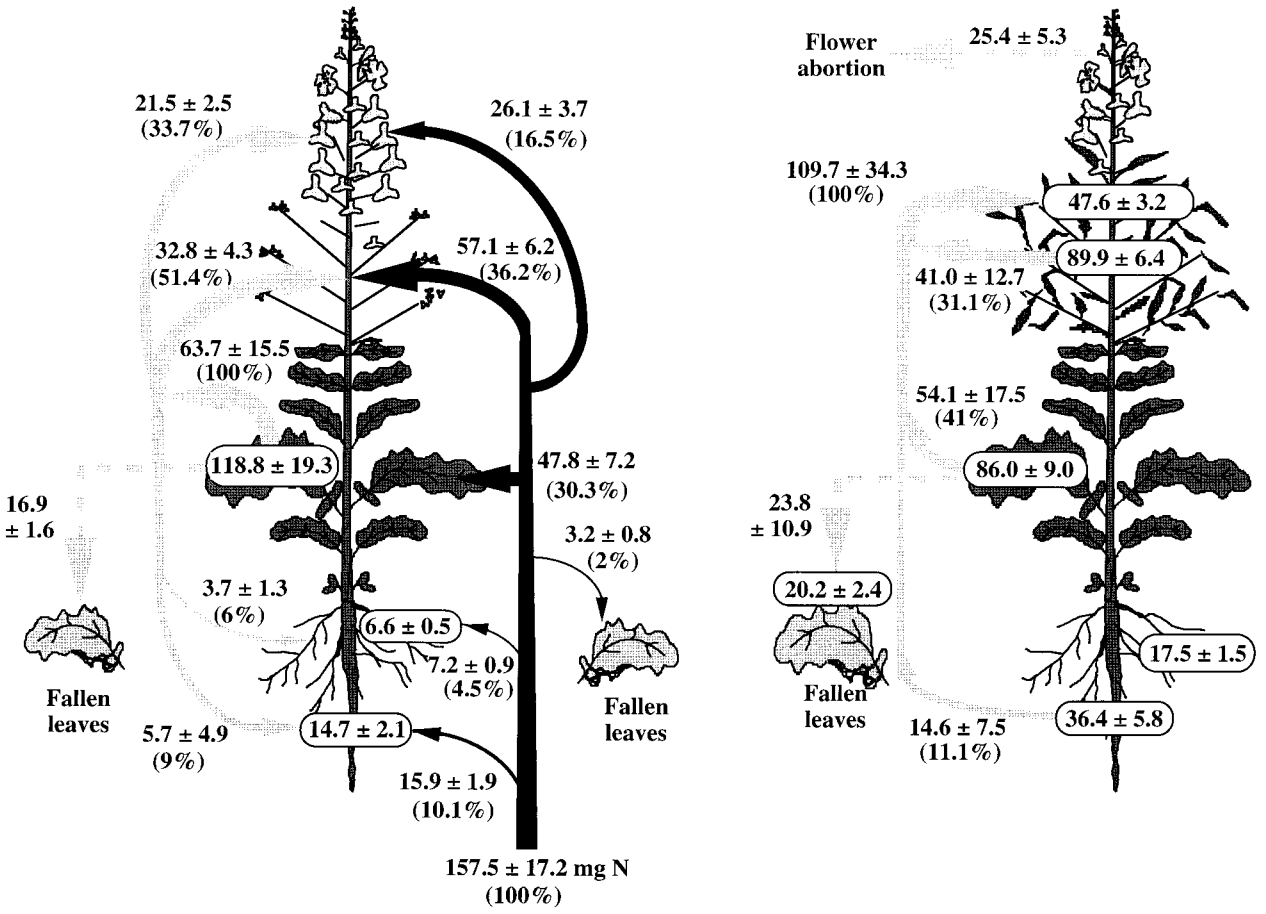


**Fig. 4.** Nitrogen flows within *Brassica napus* L. plant, expressed in mg N plant$^{-1}$ from internal cycling of unlabelled endogenous N (left arrows) and from allocation of $^{15}$N-nitrate taken up (right arrows) before (left scheme, between day 0 and day 39) and after flowering (right scheme, between day 39 and day 70). Each value is given as the mean ± SE of the mean for $n = 6$. Numbers between brackets indicate the percentage of total N taken up, and the total unlabelled endogenous N mobilized from leaves or to flowers and pods.

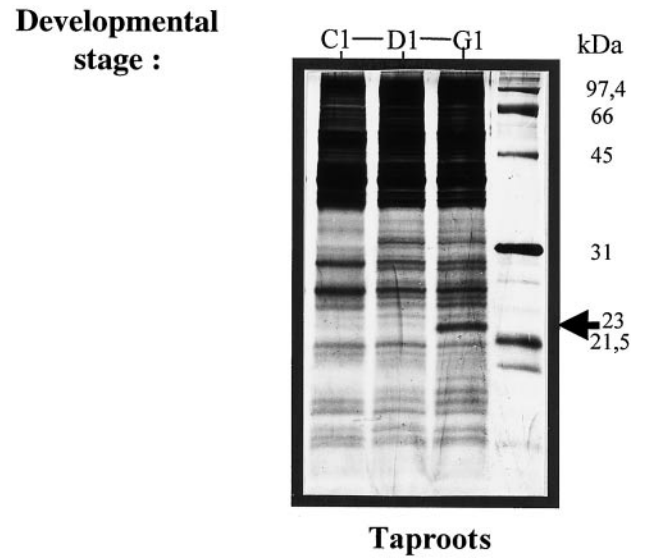


**Fig. 5.** Changes in SDS-PAGE profiles of soluble proteins extracted from taproots during the growth of *Brassica napus* L. Each well was loaded with a constant amount of soluble proteins (3.4 μg) and the position of molecular weight markers is indicated on the right side. Arrows indicate polypeptides of 23 kDa.

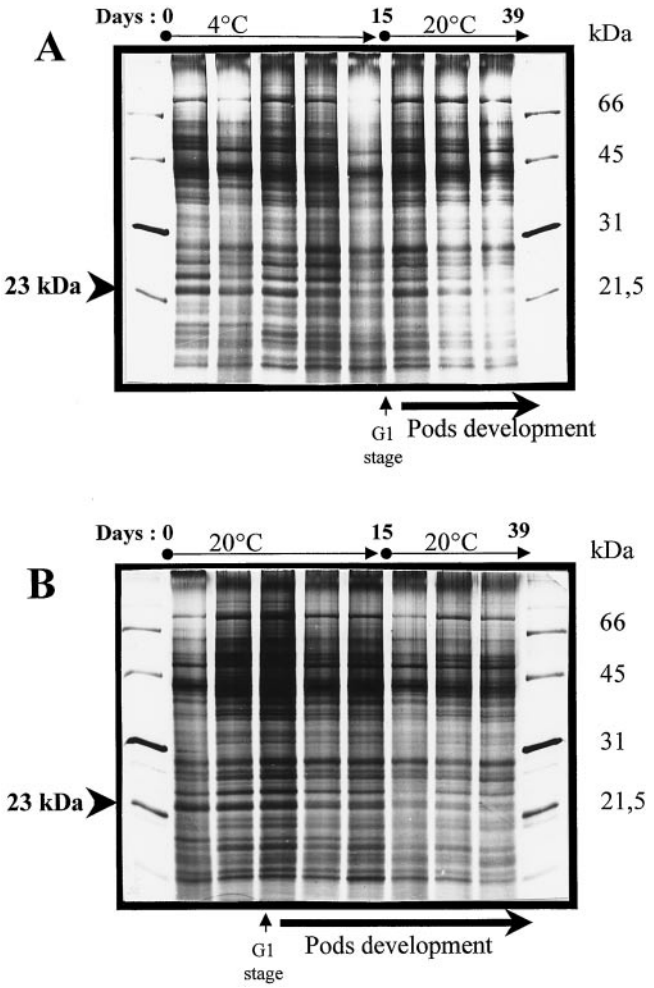


**Fig. 6.** Changes in SDS-PAGE profiles of soluble proteins extracted from taproots of *Brassica napus* L. grown for the first 15 d at either 4 °C (A) or 20 °C (B). Each well was loaded with a constant amount of soluble proteins (3.4 μg) and the position of molecular weight markers is indicated on each side. Arrows indicate polypeptides of 23 kDa.

data not shown). It followed that these prominent proteins were hydrolysed at similar rates to those of total soluble proteins. A parallel finding was also found for the stem and flower proteins (data not shown). The only significant change in the pattern of soluble proteins separated by SDS-PAGE was found in taproots (Fig. 5) where a protein of 23 kDa was accumulated during the flowering stage at such a rate that it became the most prominent polypeptide of this tissue, before being nearly fully hydrolysed. When plants were grown at a lower temperature (4 °C, Fig. 6A), the flowering process was delayed by about 2 weeks compared with control plants kept at 20 °C (Fig. 6B), as was the accumulation of this 23 kDa protein. Later on during pod development, this protein was fully hydrolysed with a significant delay (15 d) observed in plants kept for 15 d at 4 °C. Two-dimensional gel electrophoresis analysis of the soluble proteins (Fig. 7) extracted from the taproot of *Brassica napus* L. at the bolting stage C1 and the flowering stage G1 revealed that the previously described 23 kDa polypeptide consisted mostly of two proteins with similar molecular weights but different isoelectric points: 5.5 and 5.4.

## Discussion

### *N cycling during pod development and grain filling*

The results of this study suggested the presence of a mobile N pool in the stem and in the taproot between the C1 (bolting) and G1 (end of flowering) stages of the plant. This N was accumulated during uptake, but also included N from senescing leaves. N uptake during flowering and pod development was insignificant. This suggests that most of the N used for grain filling is derived from mobilization of N stored in vegetative tissues. Thus, more than 48% of the N entering the plant was finally found in the pods.

These observations match those reported in annual plants during monocarpic senescence. Thus, in monocarpic species, which include most agricultural crops and the model plant, *Arabidopsis thaliana*, mobilizable nutrients from the entire plant are stored ultimately in the developed seeds (Buchanan-Wollaston, 1994, 1997). A considerable percentage of nitrogen for seed filling is derived from vegetative plant parts, with only a minor portion being taken up from the soil during seed development. Therefore, remobilization of nitrogen from

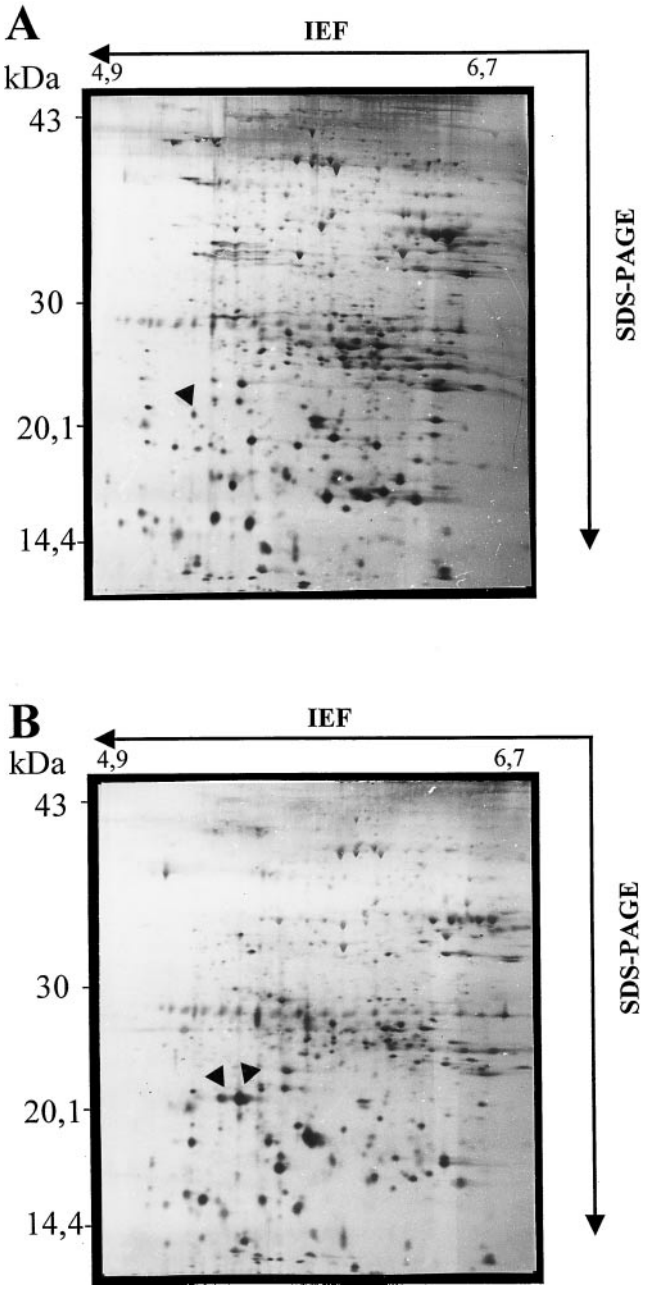


**Fig. 7.** Two-dimensional gel electrophoresis of soluble proteins extracted from taproots of *Brassica napus* L. at the bolting stage C1 (A) and the flowering stage G1 (B). Each gel was loaded with 150 µg of soluble proteins and the 23 kDa proteins are indicated by arrows. The position of isolectric points and molecular weight markers are indicated on the side of each gel.

senescing leaves (first to other parts of the plant then to seeds) is critical for the nutrient budget in seeds crops (Feller and Keist, 1986). For example, in cereals, a large proportion of the N previously accumulated in the stems and leaves is mobilized to sustain grain filling when N uptake is limited by soil N availability and/or down-regulation of the N uptake mechanisms (Reed *et al.*, 1980; Robin, 1983; Dalling, 1985; Peoples and Dalling, 1988). Using $^{15}N$ labelling during the vegetative growth phase of maize (*Zea mays* L.), it was shown that 50% of the N found in the grain at maturity had been remobilized from the stem and 20% from leaves (Crawford *et al.*, 1982), these two organs appearing to be primary N sources for the grain.

Nitrate uptake and nitrate reductase activity strongly decrease during the reproductive growth phase. Thus, in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), leaf nitrate reductase activity declined steadily during grain development at all leaf positions (Reed *et al.*, 1980). In soybean (*Glycine max* L.), decreases in symbiotic $N_2$ fixation and soil $NO_3^-$ utilization have been observed when leaves senesce (Harper, 1974; Thibodeau and Jaworski, 1975). It has been postulated that such senescence is caused by reproductive structures that deplete nutrients from the vegetative organs (Molisch, 1938). This concept is supported by the fact that leaf yellowing, an obvious visual characteristic of senescence, is associated with a decrease in foliar N concentration (Phillips *et al.*, 1984), which in turn is closely correlated with a decline in photosynthetic activity (Wittenbach *et al.*, 1980). This reduction in photosynthesis means that fewer photoassimilates are available for all the plant functions. As the grain is the dominant sink during the filling period, the translocation of carbohydrates to the roots is limited first when photoassimilate production is reduced (Wardlaw, 1968). Tolley-Henry *et al.* found that N uptake during grain filling was associated with the availability of photoassimilates to the roots (Tolley-Henry *et al.*, 1988). As a consequence, N uptake and assimilation probably declined or stopped due to insufficient energy. The opposite could also be true: reduced N uptake by roots led to leaf senescence. Thus, further work is under way to investigate carbon translocation from leaves to roots. In oilseed rape (*Brassica napus* L.), Merrien *et al.* suggested that the decrease of N uptake occuring during pod development could only be attributed to low nitrogen availability in soil due to water deficiency during this period (Merrien *et al.*, 1988). This seems to be in contradiction with the results of this study because the plants in these experiments were grown in a nutrient solution that was renewed every 2 d to minimize any $NO_3^-$ depletion. The present study prompts questions about the identity and the transduction pathways of a senescence signal that moves into leaves and thus may be a factor initiating a decrease in N uptake and nutrient depletion of leaves, stem and taproot to sustain grain filling.

### *Fluctuation of a 23 kDa protein in oilseed rape taproot during the growth cycle*

For the first time in the taproot of oilseed rape (*Brassica napus* L.), a protein of 23 kDa which displays the typical

accumulation pattern of a vegetative storage protein (VSP) has been identified. Very large amounts of this soluble protein accumulated in the taproot during flowering. $^{15}N$ analysis showed that its synthesis was linked to the N made available from leaf senescence, but not from direct N uptake. 2-D gel electrophoresis revealed that the single protein band at 23 kDa showed two spots corresponding to two isoforms with isoelectric points (pi) of 5.5 and 5.4. According to previous reports (Wittenbach, 1983; Staswick, 1994), this protein could act as a vegetative storage protein (VSP) as it constituted the most prominent polypeptide of the taproot at flowering (7% of the total soluble protein) and is hydrolysed completely when N filling of the grain occured. Wittenbach also found that a protein composed of two subunits (about 27 kDa and 29 kDa) accumulated to about 6% to 15% of the soluble leaf protein at flowering in soybean (*Glycine max* L.), and then declined to 1% during seed development (Wittenbach, 1983). Coleman *et al*. suggested that VSP accumulation and further remobilization are regulated by the source/sink status of the plant (Coleman *et al*., 1992). If the seed pods were removed from soybean (*Glycine max* L.), VSP continued to accumulate to about 50% of the total leaf protein (Staswick, 1990). The role of leaf VSP in temporary storage was first suggested by its preferential loss as nutrients were translocated to developing seeds and its massive accumulation when the sinks (seeds and pods) for mobilized nutrients were removed. In oilseed rape (*Brassica napus* L.), remobilization of the 23 kDa protein occurs during pod development, corresponding to the transition of the taproot from sink to source status. In potato (*Solanum tuberosum*), large amounts of patatin accumulate in tubers before being utilized during the regrowth period. Similarly, VSP accumulates in the root of leafy spurge (Cyr and Bewley, 1990). In chicory (*Cichorium intybus* L.), the 17 kDa VSP accumulates in the tuberized root during the last 3 months of the vegetative phase and is specifically utilized by the plants at the beginning of the flowering period (Limami *et al*., 1996). In rape (*Brassica napus* L.), the 23 kDa putative VSP could be used as a storage buffer between N losses from senescing leaves and grain filling which appears later. Therefore, it can be hypothesized that increasing N storage within the plant through this putative VSP might improve the efficiency with which leaf N is mobilized to the seeds. The literature as a whole suggests that many developmental and external stimuli are involved in the regulation of VSP gene expression. As this study's results are consistent with data obtained in soybean (Wittenbach, 1983; Staswick, 1990, 1994), subsequent investigations have been performed to study the effect on accumulation of the putative VSP of 23 kDa in oilseed rape (*Brassica napus* L.) of environmental factors (such as N or P availability, daylength, temperature, water deficit, wounding) or endogenous signals (methyl jasmonate, abscisic acid) which might change the N source/sink relationships within the plant.

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