Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle

Laurence Rossato, Carine Noquet, Philippe Malagoli and Alain Ourry

UMR INRA/UCBN 950 EVA et Nutritions N, C et S, Esplanade de la Paix, Universit? de Caen, 14032 CAEN cedex, FRANCE,
Fax: +33 2 31 56 53 60, Email rossato@ibfa.unicaen.fr

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. 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 tissues. About 15% of the total N cycling through the plant was lost through leaf fall and 48%, nearly all of which had been remobilized from vegetative tissues, was finally recovered in the mature pods. A 23 k Da polypeptide, accumulated in the taproots during flowering and later fully hydrolyzed to sustain grain filling, has been characterized as a vegetative storage protein (VSP). A mechanistic N uptake model based on the functioning of NO_3^- transport system has also been proposed. 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.

Media summary

Optimizing N cycling to reproductive sinks by means of buffering vegetative tissues might improve the N harvest index and reduce the risk of significant losses of the applied N to the environment.

Key words

Brassica napus – N fluxes – VSP – source/sink relationships – high and low affinity nitrate transport systems – mechanistic model of N uptake

Introduction

Oilseed rape (*Brassica napus* L.) is an important agricultural crop, grown primarily for oil production. After oil extraction, the high protein seed residue can be used as animal feed. 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. In spite of relative high efficiency to take up mineral N from the soil, oilseed rape (*Brassica napus* L.) possesses a low capacity to use N. Thus, high rates of N fertilizer are usually applied to oilseed rape crops in order to obtain maximum seed yields but the contribution of fertilizer derived N to total N content is higher in vegetative than in reproductive shoot components (Schjoerring *et al*., 1995). Dropped leaves before flowering contain a significant amount of N usually exceeding 2 % of the dry weight which therefore induces potential risk of losses of applied N fertilizer to the environment (Rossato *et al.*, 2001). The low N harvest index found for this species may consequently 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. For example, in herbaceous or woody species, N can be transiently stored as specific storage protein (VSP). Thus, two vacuolar glycoproteins, VSPα and VSPβ, are accumulated in soybean leaves. These VSPs are developmentally regulated, and respond to source/sink status changes for N induced by sink organ removal (Staswick 1994).

At field level, N uptake is usually modelled by a N supply/demand scheme (CERES-N model, Gabrielle *et al.*, 1998). N demand is generally defined by a N dilution curve for rape (Colnenne *et al.*, 1998). This concept relies on the assumption that N uptake is mainly driven by crop biomass. Given this assumption is likely to be violated when N soil availability is high, an alternative concept is proposed to improve N uptake modelling, which considers physiological processes of root nitrate transport systems. Indeed, it is now well established that at least two nitrate uptake transport systems are involved in nitrate uptake, their activities being affected by substrate concentrations. The low (LATS) and high (HATS) affinity transport systems have two components, one constitutive (CHATS, CLATS) and the other inducible (IHATS, ILATS). The objectives of this work were i) to quantify and distinguish by ^{15}N labelling the origin of the N used for pod filling, namely from uptake or from vegetative tissue mobilization ii) to describe the kinetic behaviour of different source tissues iii) to identify some proteins that might be involved in N storage iv) to study the influence of reproductive sink tissue removal (i.e. young flowers and pods) on N storage along developmental stages of oilseed rape and lastly v) to develop a mechanistic model of NO_3^- uptake based on the functioning of NO_3^- transport systems.

Methods

In experiment 1, *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 in a growth room. 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. At day 0, plants were supplied with $K^{15}NO3$ (^{15}N excess of 1.00%), and then sampled after 0, 3, 10, 17, 24, 39, and 70 d.

In experiment 2, *Brassica napus* L. cv. Capitol, grown under field conditions were submitted to different treatments (flower or pod removal) through developmental stages in order to modify N source/sink relationships within the plant. Plants were harvested on 22, 29 March, 6, 20, 26 April, 10, 17, 23 May and 26 June. Each harvest date corresponded to a developmental stage change in control plants. Details about plant harvest and sample preparation for N, ^{15}N, and protein analysis of experiment 1 and 2 have been described by Rossato *et al.* (2001; 2002).

In experiment 3, uptake kinetics of nitrate transporters by 16 days old plants grown previously with or without NO_3^- have been determined by measurements of $^{15}NO_3^-$ influx for a range of concentrations from 0 to 10 mM KNO_3 (Faure-Rabasse *et al.*, 2002). Effects of different factors such as day/night cycle (16h/8h), PAR, ontogenetic stages, or root temperature (from 24 to 4 ?C) have been studied on the activities of HATS and LATS by measuring influx at 100 ?M and 5 mM respectively. $^{15}NO_3^-$ influx was assessed by transferring plants on a solution containing $K^{15}NO_3$ (^{15}N excess of 99,8 %) during five minutes. The model simulates total nitrogen taken up by rape culture from the root transport processes formalised by kinetic equations of the different nitrate transport systems (equation of Michaelis-Menten type for CHATS and IHATS, and linear for CLATS and ILATS). Input variables (nitrate concentrations in different soil depths, soil temperature, root biomass and PAR) needed to run the model were obtained from Grignon-Ch?lons rape databank (www-bioclim.grignon.inra.fr; Gosse *et al.*, 1999). Four auxiliary variables (temperature, day/night cycle, PAR and ontogenetic stages) were introduced to integrate environmental and endogenous factors regulations on N uptake and their effects were deduced from the above described experiments (Malagoli *et al*., 2004). This model was developed with Model Maker software (Cherwell Scientific).

Results

Nitrogen flows within the plant and changes in soluble protein SDS-PAGE profiles (Experiment 1 and 2)

Flows of N from N taken up and from endogenous N mobilization before and during pod filling were quantified (Experiment 1; figure 1). Before pod filling, the N taken up (157.5 mg $N\text{-}NO3^-.plant^{-1}$) was mostly allocated to the stems (57.1 mg), leaves (47.8 mg), flowers (26.1 mg) and taproots (15.9 mg). 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%).

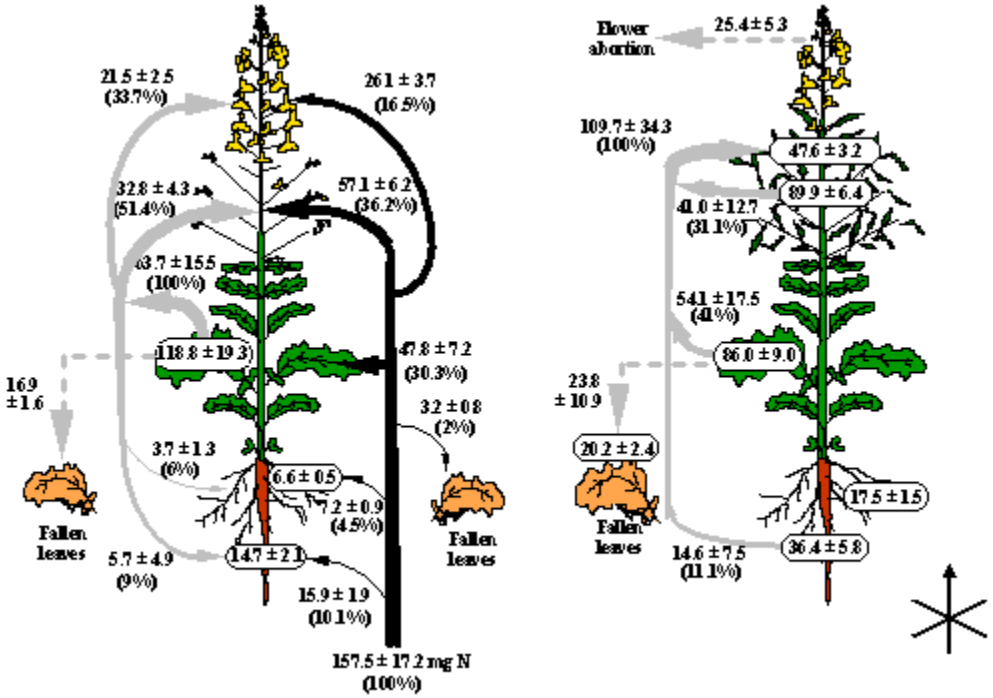

Figure 1. Nitrogen flows within *Brassica napus* L. plant, expressed in mg N plant^{-1} from internal cycling of unlabelled endogenous N (left arrows) and from allocation of ^{15}N-nitrate taken up (right arrows) before (left scheme, between day 0 and day 39) and after flowering (right scheme, between 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.

N uptake capacity decreased at flowering to a non significant level during pod filling. The source/sink relationships for endogenous N were largely modified during pod filling (the only sink), as the status of stems and taproots (acting as buffering tissues) changed from sink to source. 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. Taproot soluble protein profiles were analysed by SDS-PAGE (Experiment 2; figure 2). A 23 k Da protein was accumulated during the flowering stage at such a rate that it became the most prominent polypeptide of this tissue in mid-April, before being nearly fully hydrolysed during pod development (Figure 2). N source/sink disruption within the plant such as flower or pod removal induced a late massive 23 k Da protein accumulation in the end of June (Experiment 2; figure 2).

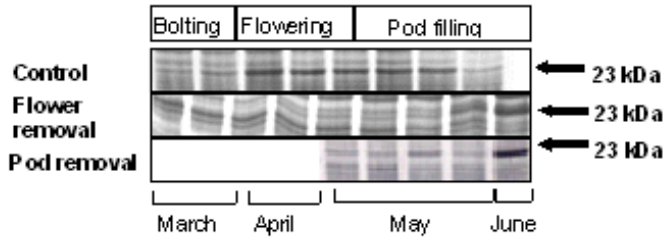

Figure 2. Changes in SDS-PAGE profiles of soluble proteins extracted from taproots of *Brassica napus* L. control plants grown in field conditions and effect of flower or pod removal on the accumulation of the 23 k Da protein in taproot. Arrows indicate protein of 23 kDa.

Modelling N uptake at field level (Experiment 3)

When total N uptake was only controlled by soil NO_3^- concentrations (unregulated uptake), model outputs were largely overestimated (17-fold higher) compared with the observed data (Experiment 3; figure 3A). When no fertilization was applied (N0 treatment), integration of temperature, day/night cycle and PAR factors decreased by 36, 32 and 19% the amount of the predicted total N taken up during the plant growth cycle, respectively (Figure 3A). Ontogenetic effect was only observed at the end of the growth cycle (-24%).

Integration of the four factors in the model reduced the estimation of the total N taken up by rape crop by 5.5-fold (Figure 3A). Comparison between the measured and the predicted N uptake at harvest showed that the simulated N uptake, defined as the regulated uptake, was still 3-fold higher than the observed uptake. When the soil N availability was considered, the model matched better to the observed data for N0. When the model was run with inputs coming from N1 (135 kg $N.ha^{-1}$) and N2 (270 kg $N.ha^{-1}$) treatments, outputs showed that the model was responsive to the N application compared to the N0 treatment (Figure 3B).

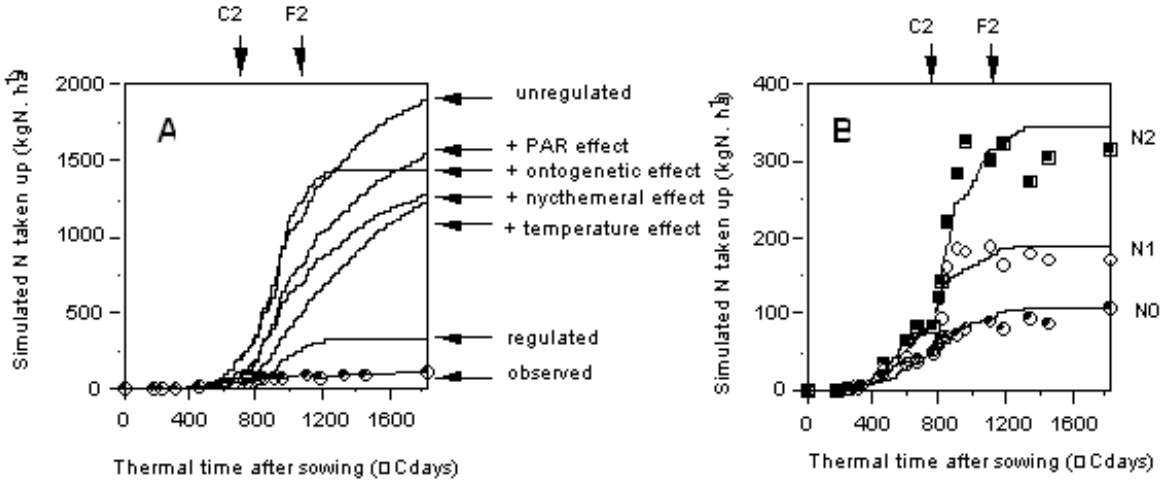

Figure 3. A: Independent or cumulative effect of endogenous or environmental factors on the simulated N taken up (kgN.ha^{-1}) by a rape culture when no fertilization was applied (N0), B: comparison between observed (N0: λ; N1: ○; N2: ■) and simulated N exported (kgN.ha^{-1}) by an oilseed rape crop receiving (N1: 135 kgN.ha^{-1}; N2: 270 kgN.ha^{-1}) or no N fertilization (N0: 0 kgN. ha^{-1}).

For the N0 treatment, model outputs showed that the inducible and the constitutive components of HATS were predominantly involved in N uptake (71 and 18% of the simulated total N uptake by IHATS and CHATS, respectively; data not shown). Despite the simulated N taken up by HATS was increased by a high fertilization level (N2), contribution of IHATS and CHATS was decreased by about 29 and 9%, respectively. On the contrary, high fertilization induced an increase of NO_3^- uptake by LATS from 12 to 166 kgN.ha^{-1}. This increase is a consequence of the extended duration (from 663 to 863 ?C days) of LATS activity and a higher contribution of the LATS components in N uptake**.**

Conclusion

Our results suggested the presence of a mobile N pool in the stem and in the taproot between bolting and end of flowering 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. 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, 1997). In oilseed rape, a 23 k Da protein accumulated during flowering in vegetative organs such as taproot and is fully hydrolysed during grain filling. Its putative function of storage protein is further supported by the fact that, like soybean VSPs (Wittenbach, 1983; Staswick, 1994), its accumulation was increased when flowers or pods were removed. As it has been shown that removal of flowers or fruits prevents senescence, one hypothesis proposed is that developing fruits produce a senescence factor (Buchanan-Wollaston, 1997). Other studies in oilseed rape have shown that a methyl-jasmonate supply induced a strong mobilization of N from senescing leaves, and a concomitant accumulation of the 23 k Da VSP, which lead to consider MeJA as a possible senescence factor, and VSP as a marker of initiation of massive senescence (Rossato *et al.*, 2002). Thus, in rape (*Brassica napus* L.), the 23 k Da VSP could be used as a storage buffer between N losses from senescing leaves promoted by MeJA (which could be produced by reproductive organs in development) and grain filling which appears later. Therefore, it can be hypothesized that increasing N storage within the plant through this VSP might improve the efficiency with which leaf N is mobilized to the seeds.

Model outputs showed that HATS was preponderant in N uptake, even if its participation decrease with the increase of N fertilization level. Conversely, the part of N taken up by LATS during the growth cycle is low even if its contribution is strongly increased by N fertilization. Simulated N uptake by the oilseed rape crop fits relatively well with data measured in field conditions, whatever the N fertilization level applied. It can therefore be hypothesized that i) the physiological description (biphasic kinetics, sensitivity to temperature and to PAR) of the differential functioning of the NO_3^- transporters is appropriate and ii) internal regulations of N transporters, whatever their complexity and putative signal involved (amino acids, organic acids, sugars; Forde, 2002) are implicitly included by the regulations presently reported occurring during photoperiod or during the plant development.

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