Effects of shading on biomass production and N-dynamics in winter oilseed rape (*Brassica napus* L.)

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Abstract

Oilseed rape is a crop with a complex aerial architecture which is determinant for the absorption of the Photosynthetically Active Radiation (PAR). This study aims to (i) describe the architectural modifications of shoot triggered by shading and (ii) analyze the impacts on biomass production and Nitrogen (N) fluxes and allocation patterns. Two shading treatments were applied in order to characterize plant's behaviors as adaptive or failing responses to light restriction. Results showed that moderate shading induced a leaf growth oriented strategy to ensure an increase photosynthetic area for light capture. A delay in leaf senescence was observed suggesting that leaf carbohydrates remobilization was not favored. N uptake increased and N remobilization decreased under moderate shading as a consequence of N sink and source strengths which were determined by plant organ's size. Characterizations of molecular indicators (*SAG12/Cab*, *GS* and *GDH* genes) of foliar senescence and N assimilation processes were consistent with the early adaptive strategy observed under moderate shading i.e. leaf growth associated to a delay in senescence.

Key words: Oilseed rape, shading, Photosynthetically Active Radiation (PAR), N dynamics, sink/source relationships

Introduction

Oilseed rape (*Brassica napus* L.) is a crop which is subjected to a high sowing density pressure to reach high yield objectives. Throughout growth, the absorbed PAR can be affected at the plant level, by self shading as a consequence of the appearance of upper leaves, flowers and pods, and also at the canopy level, by mutual shading between plants because of high sowing density or competitive growing conditions. Under light restricting environments, plants respond by developing organs which are the most prone to capture light i.e. shoots (Tilman, 1988). Adaptive strategies encompassed (i) structural adjustments i.e. expansion in leaf area associated to a decrease in leaf thickness (Björkman, 1981; Poorter *et al*., 2006; Jullien *et al*., 2009), production of leaves with low construction metabolic costs (Smith 1982) and (ii) physiological processes i.e. decrease in photosynthetic rate per unit leaf area, lower N concentration per unit leaf area, changes in N leaf partitioning towards light harvesting components (Evans and Poorter, 2001; Frak *et al*., 2002). In the present work, shading treatments on the canopy of winter oilseed rape were applied to simulate PAR limiting conditions and to analyze the effects of carbon (C) restricting source on major ecophysiological processes related to (i) C uses i.e. biomass allocation, leaf senescence and shoot morphology, and (ii) N dynamic fluxes i.e. uptake, utilization and remobilisation. We attempted to characterize molecular mechanisms underlying these ecophysiological processes through the measurements of photosynthetic activity, chlorophyll content and genes expression patterns i.e. *SAG12/Cab* which were shown to be associated with leaf senescence (Gombert *et al.*, 2006), *GS* (Gluthamine Synthase) and *GDH* (Glutamate Deshydrogenase) involved in N assimilation and remobilization pathways (Masclaux-Daubresse *et al*., 2010).

Material and Methods

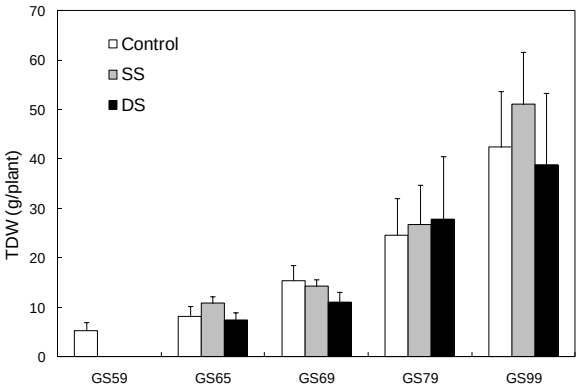
Plants (cv. Capitol) were collected from field at the end of the vegetative rest period in February 2003, transferred into greenhouses and grown in hydroponic conditions until pod maturity. After their introduction into greenhouses, plants were submitted to shading treatments (ST) and supplied with a labeled $K^{15}NO_3$ solution (1mM, 1%atom excess) in order to measure N amount (Q_N) derived from uptake and remobilization of unlabeled N. Shading effects were mimicked by a reduction in PAR with shade cloths set above the plants. Two contrasting ST were applied : *Simple Shading (SS)* with one layer of cloth and *Double Shading* (*DS)* with two layers enabling 43% and 65% of PAR extinction

respectively. The ^{15}N labeling experiment was performed as described in Rossato *et al.* (2001) from GS59 to pod maturity (following the BBCH decimal system). At five sampling dates, four plants per treatment were collected. Their biomass and Q_N were measured for each organ. Total N and ^{15}N allowed the determination of N uptake and consequently the calculation of N remobilized by each organ between GS59 and GS65. Chlorophyll content and photosynthetic activity were performed for each leaf rank at each sampling dates. At GS69, patterns of genes expression (*SAG12/Cab*, *GS1*, *GS2*, *GDH*) were also characterized, following procedures as in Gombert *et al.* (2006). A single factor ANOVA was performed to test the effect of the shading treatment on measured variables at each harvest date (STATGRAPHICS Plus 3.1 Software).

Results

Effects of shading on biomass production, biomass partitioning and shoot morphology

ST affected differently the total biomass with higher TDW for *SS* plants compared to control and *DS* plants at seed maturity (Fig. 1). Differences were only significant at GS69 mainly due to a decrease in taproot, root and stem DW for *DS* plants and an increase in leaves DW for *SS* plants (data not shown *P*<0.05). The main differences between control and shaded plants were observed for biomass partitioning. At GS79, shaded plants increased the relative biomass allocation to the leaves, suggesting a delay in leaf senescence and an increase in the vegetative stage length (Fig. 2).



ig. 1: Evolution of total dry weight (TDW) for *SS, DS* and the control at the different growth stages. * *P<0.05*.

The allocation of biomass for pods production was delayed for shaded plants as a consequence of an increase in the vegetative phase length. Relative biomass allocation to fallen leaves was similar between shaded and control plants whatever the stage (Fig. 2) although the number of fallen leaves was slightly lower for shaded plants at GS79 (13.5 and 12.7 and 16 for *SS* and *DS* and control plants, not significant). Differences in final biomass performances at GS99 between *SS* and *DS* plants were not explained by early biomass partitioning nor leaf senescence. The analysis of shoot morphology showed that at GS79 (i) the leaf number on the main stem for *SS* and control plants differed significantly (28.5 and 25.2 respectively, *P*<0.05, data not shown) and (ii) the number of ramifications was significantly higher for shaded plants than for control plants (65.7, 132.2 and 131.5 for control, *SS* and *DS* plants, *P*<0.01, data not shown).

Photosynthetic activity and chlorophyll content in response to light restriction

The CO_2 leaf exchange rate was significantly lower for *DS* plants at the dates prior to GS79 (data not shown, $P< 0.01$). The photosynthetic activity was modulated by the shading intensity :a moderate shading stress enhanced or kept photosynthesis activity while higher light restriction decreased this process. Chlorophyll contents started decreasing at GS79 only for control plants (data not shown, $P<0.01$), suggesting that mobilization of N containing pigments did not occur for shaded plants which was consistent with a observed delay in senescing and N leaf remobilization processes.

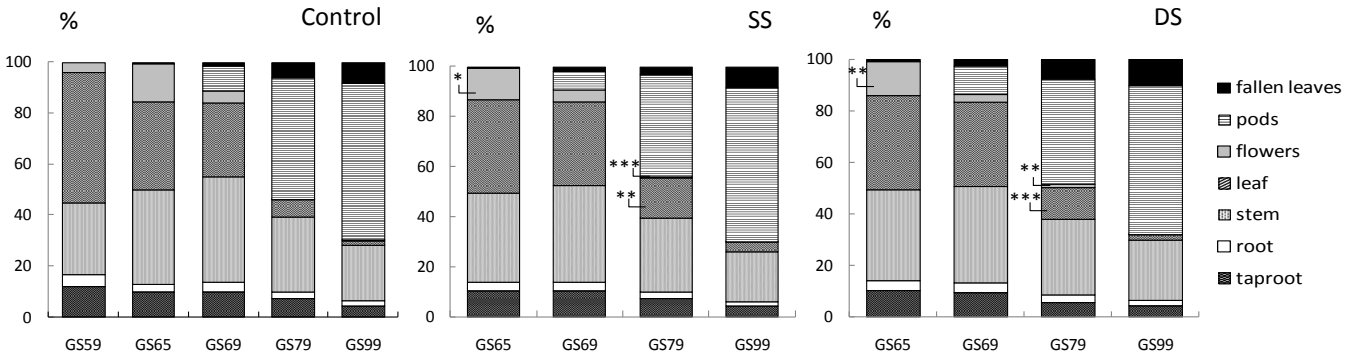


Fig. 2: Evolution of the relative allocation of the biomass of the different organs (in % of whole plant biomass) i.e. fallen leaves, pods, flowers, leaves, stem, secondary roots and taproot for control, *SS* and *DS* plants. Level of significance (when different from control plants): * *P<0.05*, ** *P<0.01*, *** *P<0.001*.

Effects of shading on total Q_N, early N uptake and remobilization

From GS59, total Q_N increased as the result of N uptake (data not shown). Differences between shading treatments affected the relative partitioning of total Q_N (rQ_N, Fig. 3). At GS65, rQ_N to the flowers was lower for shaded plants ($P< 0.05$) in favor of leaves. At GS79, the main differences in rQ_N were observed for leaves and flowers which were both higher for shaded plants as a consequence of a higher biomass partitioning of both compartments at this stage (Fig. 2). At seed maturity, rQ_N was more important for taproot of *DS* plants ($P<0.01$) since it was more prone to accumulate N compounds which were not used for growth under restriction of C sources. These observations on rQ_N were correlated to biomass partitioning (Fig. 2), meaning N requirements followed plant compartment's size.

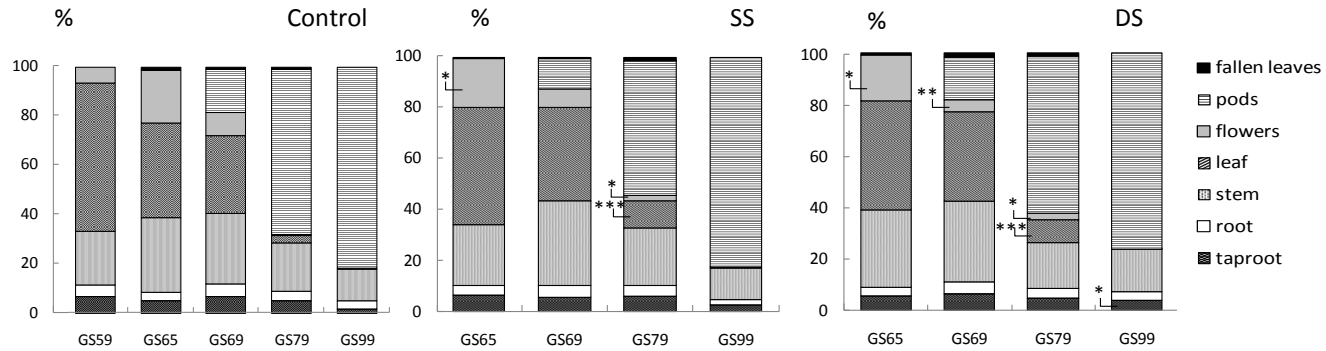


Fig. 3: Evolution of the relative allocation of total Q_N (rQ_{Ntot}) for the each organ (in % of total Q_{Ntot}) i.e. fallen leaves, pods, flowers, leaves, stem, secondary roots and taproot for control, *SS* and *DS* plants. Full legends as in Fig. 2.

Between GS59 and GS65, $Q_{Ntaken\ up}$ was lower for *SS* plants with leaves having the highest relative N uptake requirements (40.2, 34.2 and 29.0 % of total $Q_{Ntaken\ up}$ for *SS*, *DS* and control plants, respectively, data not shown). $Q_{Nremobilized}$ was lower for *SS* plants. Leaves allocated N whatever the ST but their N source strength greatly differed according to the ST : 62.4 and 55.8% of $Q_{Nremobilized}$ were exported from the leaves for control and *DS* plants, respectively while only 7.4% of $Q_{Nremobilized}$ contributed to remobilized N from the leaves for *SS* plants.

Gene expression analyses at GS69

Shading modulates photosynthesis and senescing processes

The gene expression patterns of *SAG12/Cab* showed a slight increase in *Cab* transcripts along with decrease in *SAG12* transcripts for leaf rank <19 of *SS* plants whereas leaves of *DS* plants had higher *SAG12* transcripts levels with no decrease in *Cab* transcripts. These observations suggested delayed and enhanced leaf senescence for *SS* and *DS* compared to the control plants.

Shading affects NH_4^+ assimilation pathways

For *DS* plants mRNA levels of *GS1* was higher along with similar levels of *GDH* transcripts to control plants while they both slightly decreased for *SS* plants meaning the expression of the cytosolic form increased only under high shading stress (Fig. 4). Concomitantly, the expression of the chloroplastic form (GS2) was less affected for *SS* plants since their expansion of foliar surface as an adaptive response to light restriction was higher than for *DS* plants. But moderate levels of *GS1/GDH* transcripts were not necessarily positively correlated to high levels of *SAG12* transcripts as observed for *SS* plants (Fig. 4) meaning the GS1/GDH pathway for NH_4^+ assimilation was also used when plants faced moderate shading stress.

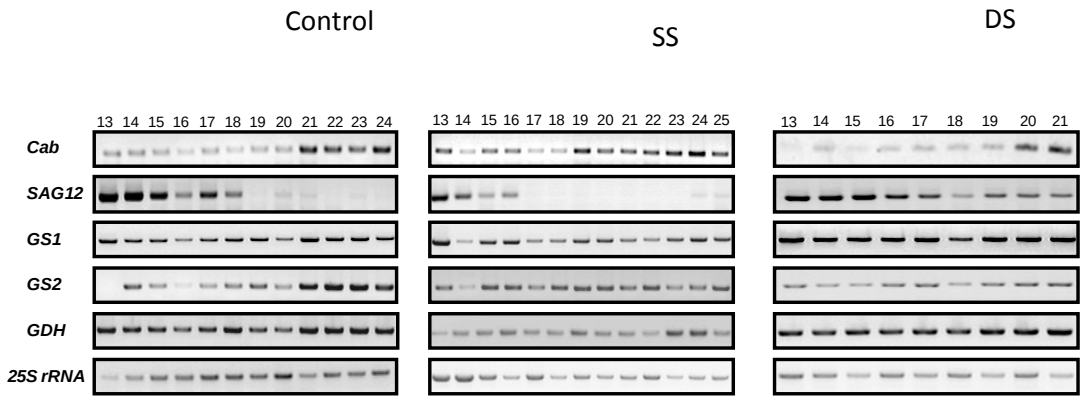


Fig. 4: RT-PCR analysis of *Cab*, *SAG12*, *GS1*, *GS2* and *GDH* gene expressions in leaf ranks 13 up to 25. Total RNA was extracted from leaves at GS69. 25rRNA was used as cDNA synthesis and amplification control.

Discussion

This study highlighted that (i) the vegetative phase was lengthened and a functional adjustment towards leaf expansion occurred under shading (ii) the shading intensity modulated shoot morphology (increase in main stem leaves and profuse branching for moderate shading vs. profuse branching only for higher light restriction) and (iii) leaf senescence process was slightly delayed but not enhanced under shading. These shoot modifications modified N dynamics as a consequence of N source and sink strength correlated to organ's size. Thus, moderate shading stress leads to a functional adjustment towards an increase in leaf expansion rather than C remobilization *via* senescing process.

The vegetative phase increased and reproductive phase was delayed, which modified N-dynamics. Profuse branching and late senescing varieties would be of interest for further selection programs aiming to maintain high yield in high sowing density context.

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