

Responses of rapeseed to sulphur restriction: contribution of sulphur reserves and role of sulphate transporters for sustain seed yield

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Abstract

Our goal was to investigate the impact of sulphur (S) limitation applied at different phases of development of rapeseed on the S use efficiency (SUE), the nitrogen use efficiency (NUE), the seed yield components and grain quality. At the rosette stage, 28 days of S limitation do not impact on the total biomass. This is related to the enhancement of sulphate remobilization from mature leaves that is concomitant to the up-regulation expression of *BnSultr4* (encoding tonoplasmic transporters implied in sulphate efflux from vacuole). The SUE is strongly increased by a S limitation. When S limitation is applied at early seed formation, the increase of S remobilization from leaves is sufficient to maintain the seed yield and grain quality. If S restriction appears at the early flowering stage or at the early bolting stage, the improvement of SUE *via* the increase of S remobilization is not sufficient because these S-limitation treatments lead to reduce seed quality, and NUE, seed yield and seed quality, respectively. These data highlight (i) the importance of sulphate availability in soil on the SUE, NUE and yield components and (ii) the ability of rapeseed to increase its leaf S remobilization efficiency (mainly *via* the sulphate remobilization) to compensate for low soil S availability.

Introduction

Because the energy cost of fertilizers is one of the highest budget items at the expense of farmers, the research of genotypes having high nutrient use efficiency in association with a more rational management of fertilizer inputs is a priority. In the case of rapeseed (*Brassica napus* L.), a crop plant very demanding of nitrogen (N), the improvement of Nitrogen Use Efficiency (NUE) is particularly required. Indeed, despite its high capacity of nitrate uptake, this crop plant is characterized by low NUE with only 50% of the N absorbed by the plant which is found in seeds at harvest. Among the factors that impact on the NUE, the level of sulphur (S) fertilization could interact with the N metabolism and lead to affect the NUE of rapeseed [1]. Moreover, the importance of S nutrition for agricultural production will inevitably grow in response to depletion of S in soil caused mainly by the reduction of atmospheric deposition from industrial sources [2, 3]. To assess the impact on the availability of inorganic S (sulphate) on rapeseed, our goals were (i) to determine the developmental stages the most sensitive to the restriction of S, (ii) to establish the laws of responses of S metabolism (absorption/storage/mobilization) to the S limitation, and (iii) to examine the impact of S restriction on NUE and its further consequences on seed yield and grain quality.

Materials and methods

After sowing, seedlings (cv. Capitol) were grown under a thermoperiod of 20°C (day-16h) and 15°C (night-8h), on 25% Hoagland nutrient solution (containing 508.7 µM SO₄) renewed twice a week. A first set of seedlings of 5 days-old were transplanted under hydroponic conditions and the 25% Hoagland nutrient solution was supplied with ³⁴SO₄²⁻ (1 atom% excess) during 51 days in order to obtain plants with homogeneous ³⁴S labelling. Afterward the plants were cultivated for the chase period in a 4 l tank of the aerated nutrient solution without addition of ³⁴S. Two different levels of mineral S were applied during 28 days: 300 µM MgSO₄ corresponding to control plants or 15 µM MgSO₄ corresponding to Low S treatment. A second set of plants of 36 days-old after sowing were submitted to vernalization at 8°C (day-10h) and 4°C (night-14h) for 46 days with the same nutrient solution renewed twice a week. After vernalization, plants were transplanted into a pot containing a mixture of 1/3 vermiculite and 2/3 perlite and submitted to a thermoperiod of 20°C (day) and 15°C (night) to initiate flowering. During different periods of growth (from rosette to bolting, to visible bud or to early grain formation stages), plants were supplied with ³⁴SO₄²⁻ (1 atom% excess) and ¹⁵NO₃⁻ (2 atom% excess). Mineral S restriction corresponding to 8.7 µM SO₄²⁻ (versus 508.7 µM SO₄²⁻ for control plants) was applied at early bolting, flowering, or grain formation stages, until the end of the growth cycle (GS 99).

The seed yield and number of seeds per plant were determined at harvest (GS 99) for 8 replicates per treatment. At GS 99, the different parts of plants (lateral roots, taproot, leaves, stem, floral stem, pod walls and seeds) were weighed, freeze-dried and ground to a fine powder for elemental and isotope analyses. The mature seeds were used for analyses of oil and glucosinolate contents and the determination of fatty acids composition. The expression of *BnSultr4;1* and *4;2*, two genes encoding tonoplasmic sulphate transporters implied in sulphate remobilization in leaf [4] was also followed in order to determine the physiological mechanisms induced by S depletion. The use of stable isotope ^{34}S and ^{15}N allowed (i) to quantify the flux of S at the whole plant level during the ontogenetic cycle, (ii) to determine the origin of S found in seeds, (iii) to identify the source tissues and the nature of remobilized S reserves, and (iv) to evaluate the impact of S limitation on SUE and NUE [5, 6].

Results and Discussion

Impact of S limitation at the rosette stage:

In response to a moderate S restriction (28 days) applied to an early vegetative stage of development (rosette), rapeseed maintains a growth rate similar to plants well supplied with sulphate (control) [5]. This is concomitant with an efficient remobilization of S reserves from mature and old leaves (Fig. 1A). The remobilization of S reserves is not due to an acceleration of leaf senescence but is largely associated to a vacuolar efflux of sulphate implying *BnSultr4* transporters (Fig. 1B) [4, 5].

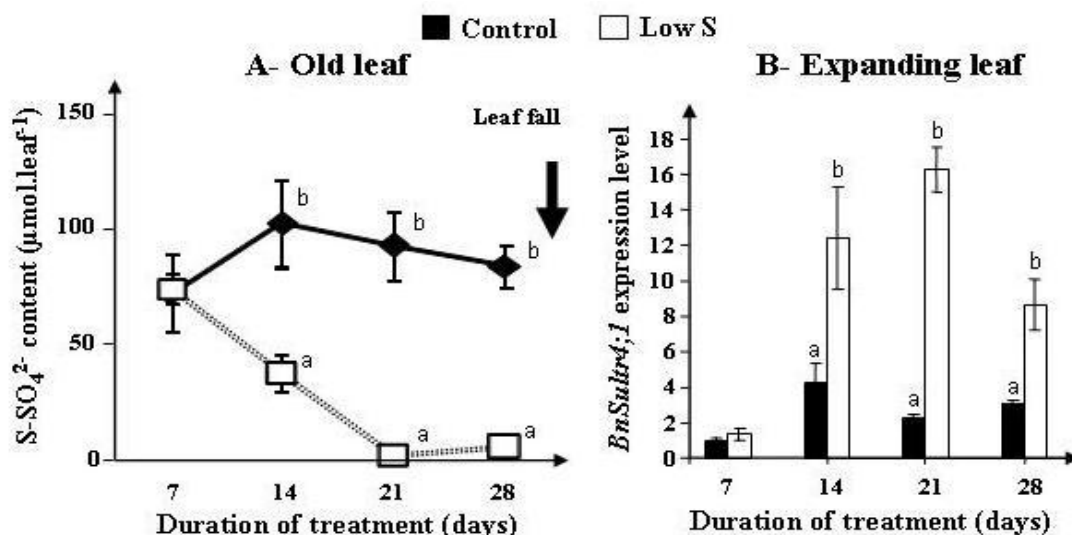


Fig. 1. Changes in the S-sulphate amount in the old leaf (A) and relative expression of *BnSultr4;1* gene expression in the expanding leaf (B) of rapeseed in control plants and, sulphate limited plants (Low S) for 28 days at the rosette stage. Vertical bars indicate \pm s.e. of the mean ($n=4$) when larger than the symbol. Different letters indicate that mean values are significantly different at a given date ($p<0.05$).

At rosette stage, our data suggest that to sustain the S demand for growth under S restriction, vacuolar SO_4^{2-} is specifically remobilized in Low S conditions without any acceleration of leaf senescence, and SO_4^{2-} mobilization is related to an up-regulation of *BnSultr4;1* and *BnSultr4;2* expression [4, 5].

Impact of S restriction applied at the early bolting, flowering or seed formation stages:

S harvest index (SHI, Table 1) is largely enhanced when S limitation is applied at early seed formation while N harvest index (NHI, Table 1) is only reduced by a S restriction occurring at the beginning of bolting stage.

Table 1. S harvest index (SHI, % of plant S in seeds), N harvest index (NHI, % of plant N in seeds), S utilization efficiency (SUE) and N utilization efficiency (NUE) expressed as mg of mature seed DM /mg of S or N in shoots at GS99 in control plants and plants subjected to S limitation at different stages. The values correspond to the mean \pm SE ($n=4$) and different letters indicate that mean values are significantly different ($p<0.05$).

	SHI	NHI	SUE	NUE
Control	26 \pm 1.3 ^a	49 \pm2.1^b	81 \pm 5.8 ^a	32 \pm 2.1 ^b
S limitation at early bolting	25 \pm 2.2 ^a	35 \pm 3.9 ^a	203 \pm 26 ^b	18 \pm 2.7 ^a
S limitation at early flowering	45 \pm 1.8 ^b	54 \pm1.9^b	461 \pm24^c	48 \pm5.6^c
S limitation at early seed formation	55 \pm1.7^c	53 \pm2.7^b	379 \pm24^c	39 \pm 3.9 ^{bc}

In all S limitation treatments, the S use efficiency (SUE, Table 1) was strongly increased and finally the number of seeds per plant (Fig. 2A) was not affected compared to control. However, S restriction applied at the beginning of the bolting stage led to a decline of seed yield (-33%), viability and seed quality resulting in a decrease in protein content (-10%), oil (-30%) (Fig. 2B) and fatty acid ω 3 and ω 6 (Fig. 2C). At bolting stage, the S limitation also causes a decrease in NUE (Table 1) despite sufficient availability of mineral N.

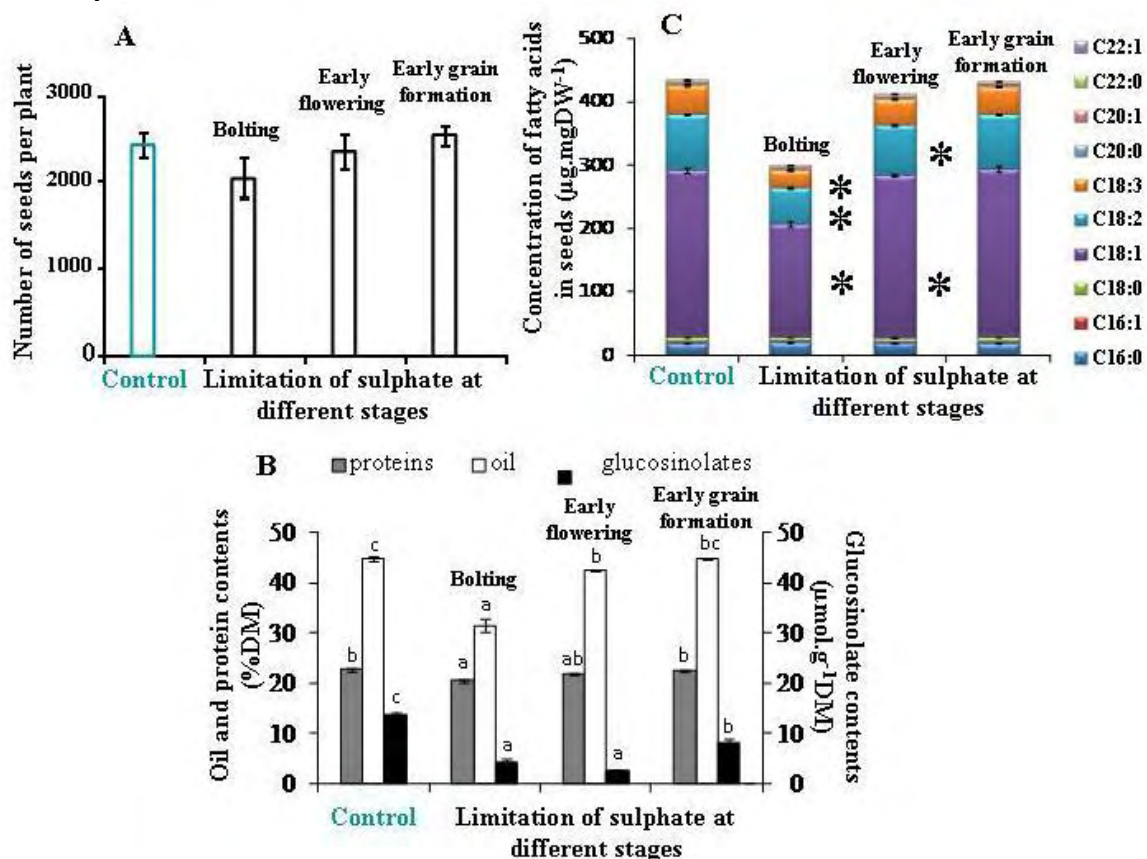


Fig. 2. Number of seeds per plants (A), seed composition in protein, oil and glucosinolates determined by NIRS (B), and fatty acids composition (C) in seeds harvested at GS 99 from plants supplied with ample sulphate (Control) or S-limited at the beginning of bolting, flowering or grain formation stages. The values correspond to the mean \pm SE ($n=4$). Different letters indicate that mean values are significantly different ($p<0.05$). C18:3= ω 3, C18:2= ω 6, C18:1= oleic acid.

When S limitation was applied at early flowering stage, SUE, NUE (Table 1) and the sulphur remobilization efficiency (SRE) to seeds increased, but not enough to maintain seed quality,

especially oil content (Fig. 2B) and fatty acid composition (decrease of fatty acid ω_6 and oleic acid; Fig. 2C).

At a later stage (beginning of seed formation), a limitation of S does not significantly affect grain yield or lipid and protein quality of seeds. Indeed, determination of S flows coming from the remobilization of endogenous S or from the S absorption (using ^{34}S tracer labelling method) reveals that the remobilization of S reserves accumulated mainly in leaves and roots is effective enough to compensate for the low availability of inorganic S (Fig. 3).

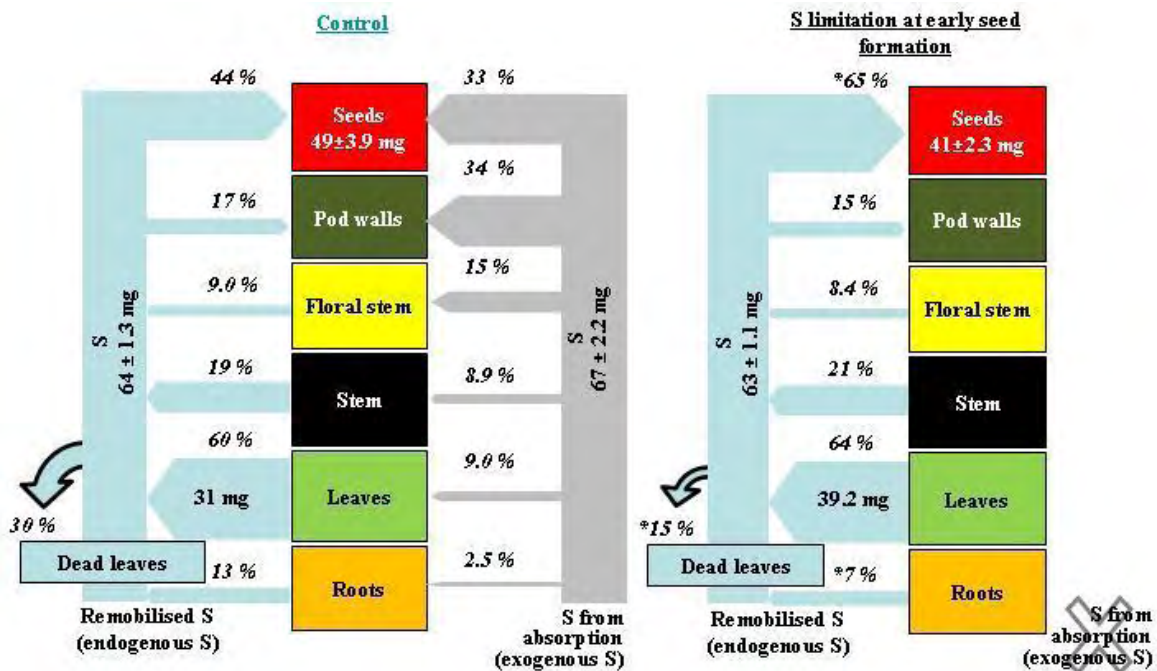


Fig. 3. Flows in % of remobilized S (determined on the basis of ^{34}S enrichment, [6]) and S taken up (estimated from the unlabelled S, [6]), and the S amount present at GS 70 and GS 81 in seeds (in mg in seeds), pod walls, floral stems, stems, leaves, and roots of oilseed rape for control plants (Control, **A**) and S deficient plants (Limitation of S applied at early formation of seeds, **B**). Values are given as the mean \pm s.e. ($n = 4$). In S deficient plants (B), the uptake of sulphate was nil during the duration of the experiment. The thickness of the arrows represents the relative importance of each flow to or from a tissue related to the S taken up or S remobilized, and asterisks indicate that mean values are significantly different from control ($p < 0.05$).

Conclusion

These preliminary results underline the importance of the level of mineral S on the NUE and yield components. Data also highlight the importance of sulphate reserves in leaves as well as the ability of rapeseed to increase its foliar S remobilization efficiency to compensate for low soil S availability. However, under non-limiting S fertilization, recent data obtained under greenhouse or field conditions indicate that the leaves fall with high levels of residual S (see dead leaves in Fig. 3). These results indicate that the SUE may be much smaller than the NUE and that a better management of S fertilizer is feasible to improve both NUE and SUE.

References

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