Contribution of the amino acid and sucrose transporters to assimilate partitioning in response to sink manipulations in oilseed rape (*Brassica napus* L.)

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ABSTRACT

In plants, photosynthesis is active primarily in mature leaf mesophyll cells, and photosynthates are transported, mainly as sucrose and amino acids, to meristems and developing organs such as growing young leaves, roots, flowers, fruits, and seeds. Phloem loading of sucrose and amino acids has been shown to be catalysed by active, energy-dependent transporters.

One field experiment was conducted on oilseed rape (*Brassica napus* L.) to evaluate the contribution of the amino acid and sucrose transporters to assimilate partitioning in response to sink manipulations : removing flowers or developing siliques. All the organs were collected each week and analysed for sucrose and amino acid contents. The rate of response is quite rapid, amino acids and sucrose quantities increase in leaves and stems 2 weeks after removal of the sinks because of high contents and dry matter. This accumulation is more pronounced in both organs until which correspond to siliques development in normal growth. Then, amino acids and sucrose levels decrease in leaves whereas they rise in stems. The results suggest modifications in transport processes. So, levels of amino acid and sucrose transporters mRNAs (RT-PCR) were examined in mature leaves and stems. Expression profiles suggest a coordinated regulation between amino acid and sucrose transporters. Investigations on transport activities of these carriers are in progress.

INTRODUCTION

In most photosynthetically active mesophyll cells, primary products of photosynthesis surplus which are trioses phosphate is converted into amino acids or into sucrose. In order to growth and development of other parts of the plant (sink organs), sucrose and amino acids are exported from source leaves and are loaded into the sieve element-companion cell complex. The accumulation of sucrose and amino acids in the sieve tube requires the presence of transporters to drive this active accumulation (Lemoine, 2000 ; Ortiz-Lopez *et al.*, 2000). Long-distance transport of amino acids is mediated by several families of differentially expressed amino acid transporters whereas sucrose transporters families are more restricted. Amino acid and sucrose carriers are localised at branch points in the translocation of assimilated nitrogen and carbon. So, these integral membrane proteins are potential sites that regulate partitioning.

The goal of this study is to investigate the contribution of the amino acid and sucrose transporters to assimilate partitioning in oilseed rape (*Brassica napus* L.) subject to source-sink manipulations. These treatments were consisted in removing flowers or developing siliques. Here we report on the distribution of amino acids and sucrose in mature leaves and stems in combination with expression profiles of amino acid and sucrose transporters genes.

MATERIALS AND METHODS

Plant material and sink manipulations

Winter oilseed rape (*B. napus* L. cv Capitol) was sown on the 10th of September 2000 on a field plot located on a clay soil near Ouistreham, Calvados, France. Fertilisation followed the current recommendations and pest and diseases were controlled such that crop damage was negligible. The experiment took place in the growing season of 2001 and consisted in two treatments with three replicates, each containing three plants. Flowers were continuously

removed before the flowering stage every two days for 14 weeks whereas developing siliques were cut after the flowering stage every two days for 8 weeks.
Mature leaves and stems samples were collected every week from plants with flowers or developing pods present (controls) and from plants at the same age without flowers (flower removal) or developing siliques (depod). Each plant fraction was weighted for dry weight determination, and then, ground.

Analysis of sugars and amino acids

Sugars and amino acids were extracted from 40 to 50 mg dried powder with hot ethanol:water (80/20, v/v, and 50:50, v/v at 80°C). Glucose and sucrose (after hydrolysis by invertase) were determined by using enzymatic assay according to the manufacturer (Trinder, Sigma, France). Amino acids contents were measured by using ninhydrine method.

Semi-quantitative RT-PCR

Total RNA from mature leaves and stems was extracted according to Kay et al. (1987). For reverse transcription-mediated PCR analysis, RNAs were converted to cDNAs by reverse transcriptase using the manufacturer's protocol (Promega, France). cDNA fragments, *BnAAP4* (AY188955) and *BnSUC1* (AY190281), were amplified using specific primers. To normalise each PCR reaction, a riboprobe was amplified simultaneously.

RESULTS

In oilseed rape subject to source-sink manipulations, amino acids and sucrose contents in mature leaves and stems and expression profiles of amino acid and sucrose transporters genes were analysed. In stems of control plants, amino acids content decreases constantly between inflorescences development and siliques formation (from 28 to 10 mg/g DW) (Fig. 1). Then, an increase in amino acids concentration is observed during the early siliques development (week 6). During pods filling, amino acids content falls and is stabilised to 3 mg/g DW when pods are mature. On the other hand, in stems of flower removal plants, amino acids content decreases more slowly and is stabilised to 15 mg/g DW (week 4). Thereafter, amino acids content reduction is related to the strong increase in dry matter of stems of flower removal plants. In stems of control plants, *BnAAP4* transcripts are expressed at the time of inflorescences development (week 0) and in pods filling (weeks 8 and 9) (Fig. 1). In stems of flower removal plants, the rate of *BnAAP4* transcripts remains stable during the 14 weeks.

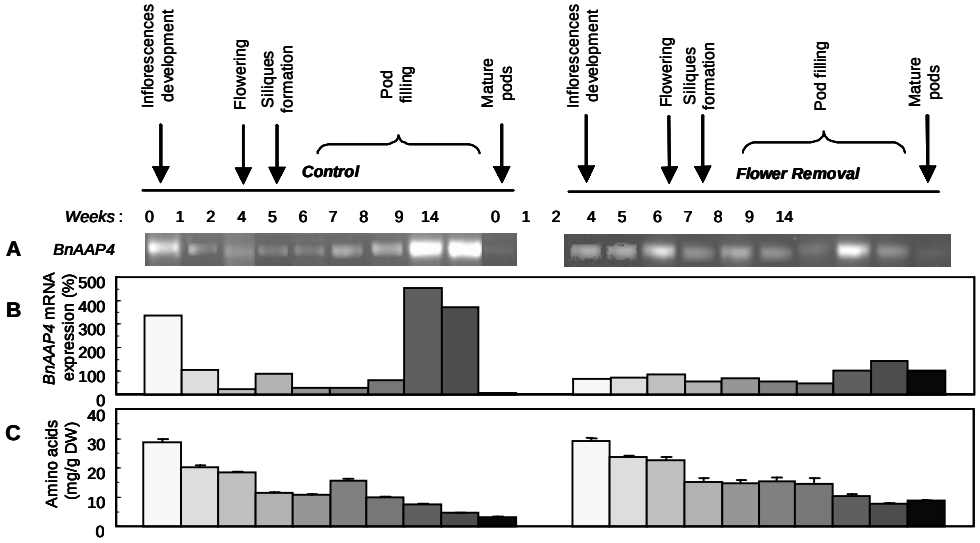

Fig. 1. Evolution of *BnAAP4* mRNA levels, amino acids contents in oilseed rape stems from plants control plants and from plants with flowers removed every two days for 14 weeks. **A**, Semi-quantitative RT-PCR of *BnAAP4* amino acid transporter. **B**, Quantitation of *BnAAP4* after normalisation by a riboprobe. **C**, Amino acids contents (n=9).

In mature leaves of control plants, amino acids content rises until flowering (week 2, 40 mg/g DW) before decreasing during pods development (Fig. 2). The level of expression of *BnAAP4* transcripts remains unchanged. Unlike, in mature leaves of flower removal plants,

amino acids content remains constant. The rates of *BnAAP4* transcripts fluctuate (weeks 4 and 9) (Fig. 2). The same tendency was observed for sucrose content and rates of *BnSUC1* transcripts in mature leaves and in stems of the same plants. A rather similar behaviour was obtained for depod treatment.

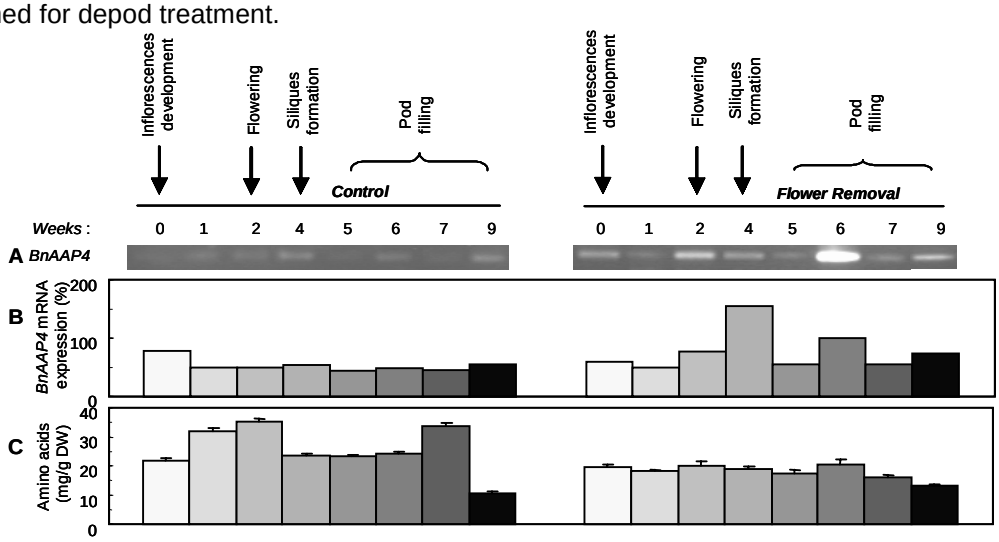

Fig. 2. Evolution of *BnAAP4* mRNA levels, amino acids contents in oilseed rape leaves from plants control plants and from plants with flowers removed every two days for 14 weeks. **A**, Semi-quantitative RT-PCR of *BnAAP4* amino acid transporter. **B**, Quantitation of *BnAAP4* after normalisation by a riboprobe. **C**, Amino acids contents (n=9).

DISCUSSION

In *Brassica* napus, such as in *Arabidopsis*, AAP4 and SUC1, amino acid and sucrose carriers, are expressed in mature leaves and in stems. These transporters might be involved in phloem loading of amino acids and sucrose in leaves, and storage and retrieval along the translocation pathway in stems. Sink organs which are flowers and siliques require a massive importation of amino acids and sucrose for their development (Herbers and Sonnewald, 1998). So, in mature leaves of control plants, there is constant export of these photoassimilates into stems during the normal ontogenetic cycle until pods filling. There, the important foliar senescence leads to a quasi complete export in amino acids and sucrose without the level of expression of BnAAP4 and BnSUC1 carriers genes increases. In parallel, in stems of control plants, transitory storage organ, the expression of *BnAAP4* and *BnSUC1* transcripts increase in order to ensure a role of redistribution towards pods. Removal of reproductive tissue (flowers or siliques) leads to modify the distribution of the amino acids and sucrose in plant as well as expression profiles of the transporters implied in this partitioning. In mature leaves, the synthesis of the photoassimilates continues and even is accentuated and export to stems pursues without leaves not being emptied. Stems, unable to redistribute the amino acids and sucrose towards pods, ensure mainly a role of storage organ as shows it the constant rate of expression of *BnAAP4* and *BnSUC1* transcripts. Similar expression profiles of BnAAP4 and BnSUC1 transporters genes let suppose a coordinated regulation of these carriers on the transcriptional level. Measurements of amino acids and sucrose transport activities from leaves of removal reproductive organs could provide new insights into a possible coordinated regulation of these carriers.

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