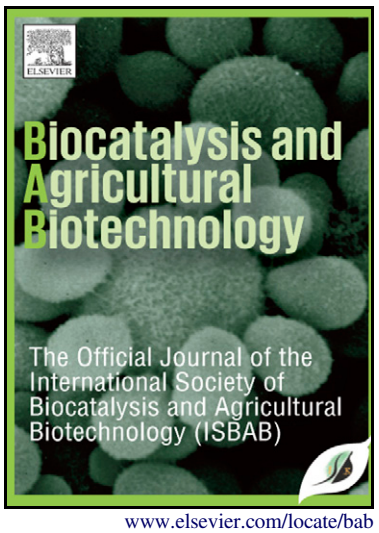

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^{13}C and ^{15}N partitioning among shoots, roots, and soil in *Brassica napus* genotypes varying in seed oil content potential

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Abstract

Four genotypes of *B. napus* (cultivars Topas, Sentry and Polo, and the experimental line 04C204) varying in seed oil content (SOC) potential (from 42.2% to 50.0% seed DW) were used to study C and N partitioning among shoots, roots and soil, as well as nitrogen use efficiency (NUE). Topas, with lowest SOC potential, had higher biomass accumulation in shoots and roots and higher NUE than 04C204 with the highest SOC potential. Although the absolute amounts of biomass and N accumulated in Topas were greater than in 04C204, the percentage of ^{13}C partitioned to shoots was lower, and the percentage of ^{15}N partitioned to roots was higher. These results imply that SOC may be more associated with the proportions of C and N partitioned between shoots and roots, than with absolute amounts accumulated in these organs. This study also indicates that while Topas fixed the most CO_2, it also had the greatest nitrogen fertilizer demand, while 04C204, with the highest SOC potential, had the greatest proportion of whole plant C retained within shoots of all the genotypes. These results suggest that a breeding strategy to optimize *B. napus* as a biodiesel feedstock may well be selection for maximum SOC at a minimal investment in vegetative biomass (and hence a lower N fertilizer requirement).

Keywords: carbon, nitrogen, partitioning, seed oil content, *Brassica napus.*

1. Introduction

In recent decades, the rising cost of petroleum and the increased concern about greenhouse gas emissions has led to increase in the demand for plant-derived oils as renewable alternatives to fossil oil for use as biofuels and in industrial applications. The world production of vegetable oil increased by 61% between 1997–2007 (FAOSTAT, 2008). Replacing petroleum diesel with biodiesel could reduce the accumulation of CO_2 in the atmosphere (Peterson and Hustrulid, 1998). The production and subsequent consumption of biodiesel results in less greenhouse gas emission compared to conventional diesel (Hill et al., 2006; Hirel et al., 2007; Peterson and Hustrulid, 1998). However, the supply of vegetable oils today relies upon only a few crops, such as soybean, oilseed rape and palm. The restricted supply of feedstock for biodiesel production is one of the biggest challenges in the industry. There is thus a considerable interest to improve oil productivity and plant growth efficiency in the oilseed crops specifically for biodiesel feedstocks.

Oilseed rape or canola (*Brassica napus* L.) is a crop that is grown mainly for its high quality oil for the human diet. It also is a promising crop for further development into a major feedstock for biofuel production because of its high seed oil content (30 - 50%) (Sana et al., 2003) compared to some other seed oil crops, e.g., soybean (18-20%) (Liu, 1997). The production of oilseed rape has become second only to soybean oil in the world supply (Rayner, 2002). However, it is well known that the nitrogen (N) requirement per unit of yield in oilseed rape is

relatively high compared to many other crops, such a cereals (Chamorro et al., 2002; Hocking and Strapper, 2001; Scott et al., 1973). The application of N fertilizer can have negative and unpredictable effects on the environment (Chamorro et al., 2002; Erisman et al., 2007). It has been reported that N_2O emitted from N fertilizer applied to crops has made a great contribution to the increased concentrations of N_2O in the atmosphere (Bøckman and Hans-Werner, 1998). Thus, optimizing N fertilizer utilization by oilseed rape is an important factor regarding its use as a biodiesel feedstock crop. Another important factor in this regard is the distribution of carbon (C) and N among plant parts (e.g. seed, shoot and root) and how much of these assimilates are exuded from the roots into the soil. Crops that contribute to long-term C pools in the soil can have a positive effect on the global C balance and this factor is often an important component of lifecycle analysis of biofuel feedstock (Lal, 2010; Tilman et al., 2006). Changes in C exudation from roots can also affect the composition and activity of the microflora in the rhizosphere (Hartmann et al., 2009) which might affect N_2O emissions from the soil (Sey et al., 2010).

In the present work, four genotypes of *B. napus* (three cultivars and one experimental line) varying in seed oil content (SOC) potential, i.e., cv. Topas (42.2% ± 0.3% SOC), (Agriculture Canada, 1987); cv. Sentry (44.6% ± 0.2% SOC), (Rimmer et al., 1998); cv. Polo (47.2% ± 0.1% SOC), (Rahman et al., 2001) and experimental line 04C204 (50.0 ± 0.6% % SOC), (P. McVetty, *unpublished data*); were studied. For this study, SOC potential refers to the mean oil contents and ranges provided in the above previous reports. The range of SOC for these genotypes is

wide, (7.8%) and each genotype is significantly different from all other genotypes used in this study. This study focused on C and N partitioning during vegetative and early reproductive growth of these lines and plants were not grown to maturity. The objective of this study was to determine if SOC potential influences carbon and nitrogen partitioning, as well as nitrogen use efficiency (NUE), among these genotypes in ways that might influence improving the plant's potential as a biodiesel feedstock crop. While this study does not address actual oil yield (i.e. SOC multiplied by the seed yield per ha), experimental line 04C204 with the highest SOC potential, has been shown in field studies to have had the highest seed oil yield of the four *B. napus* genotypes used in this study). In three separate greenhouse experiments, these genotypes were grown to assess dry weight, total N, and ^{13}C and ^{15}N partitioning at three growth stages to determine if genotypes with different SOC potential also have differences in assimilate partitioning between shoot and root and between the plant and the soil.

2. Materials and Methods

2.1. Plant materials and growth conditions:

Seeds of *B. napus* cv. Topas, cv. Sentry, cv. Polo and the canola line 04C204 (courtesy of P. McVetty, University of Manitoba) were sown in 3 L plastic pots containing 2.5 kg sandy loam soil (68% sand, 23.4% silt and 8.6% clay) from the Annapolis Valley, Nova Scotia. The soil pH

was 6.9 (soil:water ratio of 1:2). Soil analysis indicated the following availability of nutrients in the soil: 14 mg kg^{-1} N (Nitrate), >60 mg.kg^{-1} P, 106 mg kg^{-1} K, 3 mg kg^{-1} S, 55.4 mg kg^{-1} Fe, 1200 mg kg^{-1} Ca, 236 mg kg^{-1} Mg, 24.7 mg kg^{-1} Cu and 5.5 mg kg^{-1} Zn.

The soil in each pot was covered with black plastic disk in which there were five holes for watering and ventilation (these holes were sealed during ^{13}C labeling; see below). Seeds were sown in the center of the pot and a single plant was grown out of a central hole in the disk. Plants were grown in greenhouse located on the campus of Saint Mary's University in Halifax, NS, Canada (44°38' N, 63°35' W) with supplemental lighting to maintain a photoperiod of 16/8 h (day/night). The minimum photosynthetic photon flux density (PPFD) at plant height supplied by the supplemental lighting was 300 µmol m^{-2} s^{-1} provided by 600 W SON-T Green Power lamps (Philips, Belgium). Temperature was set at 25/18^{o}C (day/night) and controlled by heating and ventilation. In the first two weeks, each pot was watered with 100 mL of one-half strength of Hoagland's nutrient solution daily (Hoagland and Arnon, 1950). From the 4th week on, each pot was watered with 200 mL per plant daily.

2.2. Experiment 1 – Biomass accumulation

B. napus cv. Topas, cv. Sentry, cv. Polo and line 04C204 were grown under the conditions described above. The plants of each genetic line were harvested at the three growth stages (Tab. 1). At harvesting, shoots were separated from roots, soil was removed from the roots by gently

washing, and the plant materials were then dried separately at 80°C for 3 days. There were 6 replicates for each sample (1 plant/sample).

2.3. Experiment 2 - ^{13}C and ^{15}N labeling and partitioning at three growth stages

In Experiment 2, *B. napus* cv. Topas, cv. Sentry, cv. Polo and line 04C204 were labeled with ^{13}C and ^{15}N at the three growth stages used in Experiment 1 and partitioning of these stable isotopes was monitored to observe short-term effects (3 and 10 days, respectively) on C and N partitioning Due to the limitation of the size of the labeling chamber (see below), the replicate number for each *B. napus* genetic line was limited to three.

Plants were labeled with ^{15}N by applying 200 mg of $K^{15}NO_3$ (15 atom % excess) in 200 mL of water to each plant 7 days before ^{13}C-labeling.

Before ^{13}C labeling, the disks covering the soil in each pot were sealed with UHU® Tac Adhesive Putty (Saunders Mfg. Co., Readfield, ME, USA) to prevent access of $^{13}CO_2$ to the soil and root zone during labeling. Twenty four pots of plants were placed into a custom -designed ^{13}C-labeling chamber (Fig. 1) with light, temperature, and ventilation control. Light was supplied with two 1000 W high pressure sodium lamps (Philips, Belgium). The PPFD in the chamber was 350-370 μmol m^{-2} s^{-1} at the level of top of the pots. Temperature inside of chamber was maintained at ~28°C by pumping the air in the chamber at a controlled rate through a brass coil immersed in an ice bath. Moisture which condensed in the cooling coil was

removed via gravity feed through a T-piece, thereby partially dehumidifying the air before it was returned to the chamber. Hence, temperature and relative humidity (~95%) in the chamber were regulated by controlling the pumping speed of air through the cooling/dehumidifying system. Under these conditions, the CO_2 compensation point of the plant was determined in preliminary tests to be approximately 75 µl L^{-1}. The CO_2 concentration in the labeling chamber was monitored by a LiCor-820 CO_2 gas analyzer (LI-COR Environmental, Lincoln, NE, USA). After the plants were placed into the chamber and CO_2 had declined to approximately 75 µl L^{-1}, 200 mL of 1 M $Na_2{}^{13}CO_3$ (99 atom % excess; Cambridge Isotope Laboratories, Inc., US) solution was injected into 200 mL of 3 M H_2SO_4 solution in a flask at the bottom of the chamber by a peristaltic pump at a controlled rate to maintain a CO_2 concentration of approximately 400 µl L^{-1} in the chamber. Once 200 mL of 1 M $Na_2{}^{13}CO_3$ solution had been completely injected into the chamber, the plants were given time to photosynthesize until the concentration of CO_2 in the chamber had declined to approximately 75 µl L^{-1}, previously determined to be the CO_2 compensation plants for the plants within the chamber. Then, injecting 1 M Na_2CO_3 to increase CO_2 concentration in the chamber to around 400 µl L^{-1}, and letting the plants assimilate CO_2 down to 75 µl L^{-1}. This process was repeated twice. The total labeling time was approximately 5 h. After labeling, the plants were removed from the labeling chamber and, except for the immediately harvested plants (see below), were placed in a greenhouse and were grown under the conditions described above (section 2.1). Three control

plants for each of the *B. napus* genotypes for the labeling experiments were grown under exactly the same conditions, but were not labeled with ^{13}C and ^{15}N.

Immediately after ^{13}C labeling, three labeled and three control plants from each of the *B. napus* genotypes were harvested separately to provide "time 0" samples. There were three replicates for each sample (1 plant/sample). Shoots were removed from roots and then, each pot was immersed into 4 L of water. Soil was gently removed from the root systems, and the roots were washed another two times in 1 L of clean water. The shoots and the clean roots were dried at 80^{o}C for 3 days and weighed (as dry weight patterns in labeling Experiments 2 and 3 were the same as in Experiment 1, data is not presented). Dried shoots and roots were pulverized into a fine powder with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, US) with a 60 mesh delivery tube. Subsamples of approximately 20 mg for each plant part were analyzed for ^{13}C and ^{15}N (see below).

To sample the amount of ^{13}C and ^{15}N labeled materials that may have been exuded from the roots, the slurries from the root washing were combined for each plant and mixed for 10 min. A sample of 200 mL of the combined slurries was filtered through Whatman #1 filter paper. A sub-sample of 100 mL of the filtrate was dried at 80^{o}C in oven. The dried matter containing water-soluble solutes and filtrate particulates was re-suspended in 5 mL water and kept -20^{o}C until analysis for ^{13}C and ^{15}N content.

Three days after ^{13}C labeling (and hence 10 days after initiation of ^{15}N labeling), control and labeled plants were harvested to determine the heavy isotope partitioning during the "chase" period (i.e. the time between labeling and harvest) among shoot, roots and the soil. Samples were handled as described above for the materials harvested immediately after labeling.

Total N content and ^{13}C and ^{15}N analysis of plant samples were conducted at the Agriculture and Agri-Food Canada Research Centre at Lethbridge, AB, Canada using a Carlo Erba NA1500 combustion analyzer (Carlo Erba Strumentazoine, Milan, Italy) interfaced to an Optima Mass Spectrometer (V.G. Isotech, Middlewich, United Kingdom). Analysis of ^{13}C and ^{15}N analysis of soil extracts were carried out at Stable Isotope Laboratory, University of Saskatchewan, Saskatoon, SK, Canada, using a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer.

Amounts of ^{13}C and ^{15}N (in µg) among shoots, roots and soil at each sampling time was calculated from the measured atom % of the heavy isotopes in these tissues as follows:

i) atom % excess = (atom % in labeled sample) – (atom % in unlabeled sample)

ii) ug ^{13}C or ^{15}N excess = atom % excess x (C or N% in sample) x (mg DW of component) ÷10

Partitioning of ^{13}C and ^{15}N in shoot, roots and soil is expressed as a percent of the total of the amount of labeled isotope recovered and was calculated as follows:

iii) (μg of ^{13}C or ^{15}N in component) ÷ (μg of ^{13}C or ^{15}N shoot + root + soil) x 100

2.4. Experiment 3 - ^{13}C and ^{15}N partitioning at three separate growth stages in plants labeled once at growth stage I

In Experiment 2, plants were labeled at three growth stages and the "chase" period was the same after each labeling (i.e. 3 days for ^{13}C and 10 days for ^{15}N). In Experiment 3, the same genotypes of *B. napus* were used (cv. Topas, cv. Sentry, cv. Polo and line 04C204), but the ^{13}C and ^{15}N labeling was carried out only once (hence the chase period varied with when the plants were harvested). Experiment 3 allows longer term observation of genotype effects on C and N partitioning than Experiment 2. The plants were cultured under the same conditions as in Experiment 2 and the methods for labeling and sampling were also the same as in Experiment 2. There were three replicates for each sample. The ^{13}C label was applied at growth stage I (Tab. 1). The ^{15}N label was applied 3 days before the ^{13}C labeling. Aside from harvesting immediately after the application of the ^{13}C label, samples were also collected at growth stages II and III (Tab.1).

2.5. Nitrogen use efficiency (NUE)

NUE in the present work was defined as units of plant biomass produced per unit of N in shoot and root tissues, i.e., NUE = mg DW mg^{-1} N.

3. Results

3.1 Experiment 1 - Biomass accumulation

Biomass accumulation in shoots or roots was not different among genotypes at growth stage I (Fig. 2). However, at growth stages II and III, biomass accumulation was significantly higher in the shoots and roots in cv. Topas compared to other genotypes. The ratio of root to shoot was also significantly higher in cv. Topas than in other genotypes at these growth stages. The lowest ratio of root to shoot occurred in line 04C204 at growth stage III (Fig. 2).

3.2. ^{13}C and ^{15}N partitioning in experiment 2

In experiment 2, ^{13}C labeling was conducted at growth stages I, II and III, respectively. Following ^{13}C labeling, ^{13}C was "chased" for only 3 days before harvesting shoots, roots and soil at each growth stage. At growth stage I, there was no significant difference in ^{13}C excess in shoots, roots and soil in all genotypes (Fig. 3). The proportions of ^{13}C partitioned to shoots, roots and soil was approximately 85-86%, 13-14% and 0.15-0.21%, respectively (Tab. 2). At growth stages II and III, ^{13}C excess was much higher in shoots, roots and soil in cv. Topas compared to other genotypes, particularly line 04C204 (Fig. 3). However, the proportion of total ^{13}C partitioned to shoots (84.65%) was much lower, and that partitioned to roots (14.94%) and soil (0.40%) was much higher in cv. Topas compared to line 04C204 (90.99%, 8.74% and 0.27% respectively) at growth stage II (Tab. 2). The pattern in differences in ^{13}C partitioning among shoot, roots and soil between these genotypes were similar at growth stages III as

growth stage II. Cultivars Sentry and Polo had intermediate levels of ^{13}C excess (Fig. 3) and ^{13}C partitioning (Tab. 2) compared to the other two genotypes.

^{15}N labeling was carried out by application of $K^{15}NO_3$ to plants at each growth stage 10 days before plants were harvested. It was more difficult to establish trends in ^{15}N accumulation and partitioning among genotypes compared to that seen for ^{13}C. There were significant differences among genotypes in ^{15}N excess in shoots at stage I and in roots at stage II and III (Fig. 4). ^{15}N residue in soil was very low in all growth stages and in all genotypes (Fig. 4). In terms of partitioning of ^{15}N among shoot, root and soil (Tab. 3), there were no differences among genotypes at growth stage I, but by growth stage II and at growth stage III cv. Topas had a smaller proportion of ^{15}N in shoots, and greater in roots, than line 04C204 (similar to the pattern seen in the proportions of ^{13}C distributed between shoots and roots).

3.3. ^{13}C and ^{15}N partitioning in experiment 3

In experiment 3, ^{13}C and ^{15}N were labeled only once at the growth stage I. Immediately after ^{13}C labeling, there were no significant differences in^{13}C excess in shoots and roots in all genotypes (Fig. 5A and 5B). ^{13}C excess in soil at growth stage I was higher in cv. Topas than in other genotypes (Fig. 5C), but the amount of ^{13}C excess in soil was small compared to that contained within shoots and roots. Between growth stages I and II, there was a small decline in ^{13}C in shoots in all genotypes, but a large increase in ^{13}C in roots. At growth stage III, there was a significant difference in ^{13}C excess in shoots between cv. Topas and cv. Sentry and line

04C204 (Fig. 5A). In roots at both growth stages II and III, cv. Topas generally had higher ^{13}C than the other genotypes, and this was most apparent at stage III, in which ^{13}C excess was approximately 2 times greater in cv. Topas than in line 04C204 (Fig. 5B). ^{13}C excess in soil trended downward with time in all genotypes (Fig. 5C).

The total proportion of ^{13}C partitioned to shoots tended to decrease over time in all genotypes (Tab. 4). However, it is interesting that the magnitude of the decrease between growth stage I and III was much greater in cv. Topas (i.e., a decline of 12.9%) compared to line 04C204 (i.e., declined only 6.52%). Correspondingly, the proportion of ^{13}C in roots tended to increase between growth stages I and III, but to a much greater degree in cv. Topas (i.e., an increase of 13.09%) compared to the other genotypes, and especially line 04C204 (i.e., an increase of only 6.53%). These data indicate that line 04C204 retained a greater proportion of its labelled ^{13}C in shoots over time compared to cv. Topas.

Regarding ^{15}N excess among components in experiment 3, there were no significant differences in ^{15}N excess in shoots among genotypes at the growth stages I and II, however, there was a significantly higher ^{15}N excess in shoots in cv. Topas than in line 04C204 at growth stage III (Fig. 6A). There was also significantly higher ^{15}N excess in roots of cv. Topas compared to the other genotypes at growth stage II and III (Fig. 6B). ^{15}N excess in soil declined dramatically after the initial labeling at growth stage I (Fig. 6C).

In terms of the proportion of ^{15}N partitioned among components, there was a greater proportion of ^{15}N in shoots and a significantly lower proportion in roots in line 04C204 compared to cv. Topas, at the growth stages II and III (Tab. 5).

3.4. Total nitrogen content

There were no significant differences in total nitrogen content in shoots among genotypes at the growth stage I. However, at the growth stage II, total N in the shoots in cv. Topas was significantly higher than that in cv. Polo and line 04C204 (Fig. 7A). In roots, total N tended to be higher in cv. Topas compared to the other genotypes at the growth stages II and III (Fig. 7B).

3.5. Nitrogen use efficiency

There were not many differences in NUE in shoots among cultivars and no consistent patterns (Fig. 8A). At the growth stage I, cv. Topas showed a lower NUE in shoots than the other genotypes. However, by growth stages II and II, cv. Topas had higher levels of NUE in shoots compared to some of the other genotypes. The pattern of NUE changes in roots was similar to that in shoots (Fig. 8B).

4. Discussion

Seed oil content in *Brassica* species varies significantly with genetic line and environmental conditions (Frick et al., 1994; Sana et al., 2003). With the growing importance of agriculturally-based feedstock for biofuel production, it is of great interest to identify genotypes of *Brassica* superior in SOC and relatively low in greenhouse gas emissions in their production. To our knowledge, the current study is the first to look at C and N partitioning among shoots, roots and soil in genotypes of *B. napus* varying in SOC potential. In this study, it was found that the four genotypes of *B. napus* varying in SOC potential showed different patterns of biomass accumulation, C and N partitioning, and NUE.

4.1. Biomass accumulation

By growth stage III, cv. Topas, the genetic line with the lowest SOC potential had much greater biomass accumulation than genotypes with higher SOC potential (Fig. 2). In fact, there appeared to be a negative correlation between genetic line SOC potential and biomass accumulation (especially in roots). This may seem counterintuitive, but it has been previously reported that while CO_2 enrichment and increased N fertilizer increased plant height and the dry weight of reproductive organs in *Brassica* species, SOC actually decreased or did not change (Franzaring et al., 2008; Frick et al., 1994; Hocking and Strapper, 2001; Jackson, 2000; Taylor et al., 1991).

It is interesting that there also appeared to be a negative correlation between genetic line SOC potential and root to shoot ratios. For example, line 04C204 with the highest SOC potential

had the lowest root to shoot ratio, indicating that this genetic line retains relatively more of its biomass in shoots rather than partitioning it to roots. Although line 04C204 had the lowest absolute amount of biomass accumulate among the four genotypes, the root to shoot ratios may be indicating that biomass partitioning among plant components is more important in determining SOC than the absolute amount of biomass accumulated (Addo-Quaye et al., 1985; Rood et al., 1984). This may be related to the fact that activity of key enzymes involved in triacylglycerol synthesis (e.g. diacyglycerol acyltransferase; DGAT) has been shown to be highly influential in determining SOC (Weselake et al., 2009), and is perhaps more important than the absolute amount of assimilate available for seed filling in *B. napus*.

The current study focused on C and N partitioning during vegetative and early reproductive growth (i.e. up to the 20% flowering stage) and SOC and seed oil yield of the four genotypes were not assessed. A set of control plants were grown to maturity and siliques were harvested. Unfortunately, these samples were destroyed by vermin so determination of SOC for plants grown under these experimental conditions was impossible. However, recent field studies involving these four genotypes conducted in eastern Canada confirm that the SOC and seed oil yield of Polo and 04C204 were higher than that of Topas (Beaudette et al., 2010). Similarly, field studies by El-Ali (2011), using the four genotypes used in the current study and three levels of N fertility, found that Polo and 04C204 had the highest SOC and that 04C204 had the highest seed oil yield at all N fertility levels.

4.2. ^{13}C partitioning

The similarity of patterns between ^{13}C excess levels among shoots, roots and soil (Fig. 3 and 5) and that of biomass accumulation (Fig. 2), is not unexpected because the ^{13}C in these pools is simply a fraction of the total C in the biomass accumulated in the tissue; a fraction originating from photosynthesis during the approximate 5-hour labeling period, minus that lost in respiration and the net accumulation/loss of ^{13}C into/out of the tissue/soil during the chase period. Although previous reports of labeled C partitioning in *B. napus* are rare, it has been previously reported that approximately 17-19% of fixed ^{14}C was translocated to the roots in *B. napus* over a 2 week period (Shepherd and Davies, 1993). This compares favorably with our results in experiment 3 which indicated that by growth stage III approximately 81-89% in of ^{13}C fixed at growth stage I was partitioned to shoots and 11-19% to roots (Tab. 2).

Regarding ^{13}C partitioning to soil, some differences among genotypes were evident at some growth stages (Figs. 3 and 5), and the partitioning pattern generally reflected the aforementioned inverse relationship between SOC potential and ^{13}C excess accumulation. However, the relative amounts of ^{13}C in this fraction were quite low. The ^{13}C in the soil is attributed to non-decomposed exudates and microbial biomass. C fluxes from living plant roots to soil may account for 10-40% of total net assimilation (Van-Veen et al., 1991; Whipps, 1987). However, it has been reported that a large proportion of plant photoassimilates transported below ground were quickly transformed to CO_2 and returned to the atmosphere (Kuzyakov and

Domanski, 2000; Leake et al., 2006). In the Brassicas, 30-34% of carbon transported to roots was released into the rhizosphere, but 35-51% of this carbon was assimilated and respired by rhizospheric microorganisms (Shepherd and Davies, 1993).

From experiment 2, it is interesting that there is a positive relationship between SOC potential and the percentage of ^{13}C allocated to shoots (i.e., there are higher percentages of ^{13}C partitioning to shoots and lower percentage in roots in line 04C204 compared to cv. Topas (Tab. 2). This again suggests that SOC may be more associated with the partitioning of carbon between shoots and roots, rather than the absolute amount of carbon accumulated. This partitioning pattern was also observed in experiment 3 (Tab. 4). In fact, since labeling was done only once in experiment 3, but monitored over multiple growth stages, it also became clear that not only is there a positive relationship between SOC potential and the percentage of ^{13}C allocated to shoots, but that genotypes with higher SOC potential also better retained relative amounts of ^{13}C in shoots over time. For example, in line 04C204 with the highest SOC potential, ^{13}C in shoots decreased by only 6.52% (from 95.07 to 88.55%) between growth stages I and III, while in cv. Topas with the lowest SOC potential, ^{13}C in the shoots decreased by 12.9% (Tab. 4). This further supports the suggestion that SOC may be more associated with the partitioning of carbon between shoots and roots, rather than the absolute amount of carbon accumulated.

4.3. Nitrogen partitioning

Oilseed rape has a higher requirement for N per unit yield than cereal crops (Hocking and Strapper, 2001). It also has a higher capacity to absorb N from the soil (Laine et al., 1993) and thus to accumulate larger quantities of N in vegetative parts of the plant. Despite the very high capacity of oilseed rape to absorb N, uptake efficiency (calculated as amount of N in the crop relative to the nitrogen supplied) is very low. Nitrogen uptake efficiency often does not exceed 50–60 % of the level of N fertilizer applied (Chamorro et al., 2002; Hocking et al., 1997; Leleu et al., 2000; Schjoerring et al., 1995). Previous studies have indicated that N absorbed by the crop has been preferentially partitioned to leaves and stem during stem extension and flowering (Jensen et al., 1997; Rossato et al., 2001). Experiment 2 in our study indicated that more than 85-92% of recovered ^{15}N was in shoots, 7-14% in roots, and less than 1% in soil one week after application (Fig. 4, Tab. 3), but there were no clear trends in ^{15}N accumulation among the four genotypes. However, in experiment 3 which had a single labeling event and longer chase times, significant differences in ^{15}N excess in shoots was apparent at growth stage III and in the roots at both growth stages II and III (Fig. 6, Tab. 5) These data indicate a negative relationship between genetic line SOC potential and ^{15}N accumulation (Fig. 6), but a positive relationship between SOC potential and the proportion of ^{15}N retained in shoots over time (Tab. 5), similar to the pattern seen in ^{13}C partitioning. However, unlike C, the total amount of N accumulated by the plant that ends up in seed is very small (i.e. 3% on average) (Chamorro et al., 2002; Hirel et al., 2007). Hence, an important consideration in terms of N partitioning in *B. napus* for use as a biodiesel feedstock is the amount of N left in the crop residue at harvest. This represents N that

will be returned to the soil, and, in addition to the fertilizer N applied to the crop, represent considerable pools of soil N that may contribute to N_2O emissions in the field (Hirel et al., 2007; Malagoli et al., 2005).

4.4. Nitrogen use efficiency

A previous study has shown that canola genotypes differed in NUE despite similar amounts of N being absorbed into plant biomass; genetic line-specific responses in NUE were mainly attributed to the differences in the root to shoot ratio and harvest index (Svecnjak and Rengel, 2006). It is interesting that in our study, that although at the growth stages II and III cv. Topas had a significantly higher biomass accumulation in both shoots and roots than line 04C204 (Fig. 2), there were smaller to no differences in total N content (Fig. 7). This resulted in a higher NUE in both shoots and roots in cv. Topas relative to line 04C204 (Fig. 8). The higher NUE in cv. Topas would suggest to some a greater efficiency in the production of the crop in the field. However, the fact that cv. Topas in general accumulated the greatest amount of N (implying a great requirement for available N in the soil under field conditions), yet had the lowest SOC potential, may suggest that it may not be the most efficient for production of a biodiesel feedstock when N fertilizer requirements and greenhouse gas emission are taken into consideration.

5. Conclusion

Our study indicates that, in general, and especially at the later growth stages, there was an inverse relationship between genetic line SOC potential and biomass, C, and N accumulation in both shoots and roots. This may seem counterintuitive (i.e. genotypes with greater potential for growth have a lower potential for SOC) and suggests that C remobilization from vegetative biomass and partitioning to seed is more important a factor controlling SOC than the absolute amount of C available within *B. napus* plants. This suggestion is supported by the fact C partitioning clearly differed among genotypes. Line 04C204, with the highest SOC potential, retained a greater proportion of its biomass in shoots (i.e. had the lowest root to shoot ratio; Fig. 2) and retained a greater proportion of ^{13}C in shoots relative to roots over time compared to the other genotypes (Tab. 4). It is interesting that line 04C204 has previously been shown in field studies (Beaudette et al., 2010; El-Ali, 2011) to have had the highest seed oil yield of the four *B. napus* genotypes used in this study. It is also noteworthy that despite a higher NUE, cv. Topas, with the lowest SOC potential, had a greater N requirement to achieve its greater biomass accumulation. Given that N fertilizer usage is one of the greatest contributors to greenhouse gas emission in agriculture (Woods et al., 2010), if greater biomass is only achieved with much great N requirements, breeding for maximum biomass accumulation may not be the optimal strategy for biofuel feedstock. In fact, a better breeding strategy to optimize *B. napus* as a biodiesel feedstock may well be selection for maximum SOC at a minimal investment in vegetative biomass (and hence a lower N fertilizer requirement). The above being said, further study with

near isogenic lines for high and low oil content, or a larger number of high and low oil lines with different genetic background are desirable to further substantiate the results obtained from this study

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Table 1. *Brassica napus* growth stages used in the present work.

Growth Stage	Developmental stage description	BBCH stage code (Lancashire et al., 1991)
I	6-7 leaves unfolded	16-17
II	8-9 visibly extended internodes	38-39
III	approximately 20% flowering	61-62

Table 2. ^{13}C partitioning (%) in shoots, roots and soil 3 days after labeling in four *Brassica napus* genotypes at different growth stages in experiment 2.

B. napus	Growth stage I			Growth stage II			Growth stage III		
Cultivar/line	Shoot	Root	Soil	Shoot	Root	Soil	Shoot	Root	Soil
cv. Topaz	86.12±1.66	13.67±1.67	0.21±0.03 a	84.65±2.95 b	14.94±2.91 a	0.40±0.04 a	87.50±0.84 c	12.26±0.81 a	0.24±0.03 a
cv. Sentry	86.56±1.15	13.28±1.15	0.16±0.01 ab	88.07±0.59 ab	11.67±0.59 ab	0.26±0.01 b	91.12±0.47 b	8.69±0.44 b	0.19±0.04 ab
cv. Polo	86.25±1.81	13.61±1.81	0.15±0.01 b	86.98±2.22 ab	12.74±2.16 ab	0.28±0.07 b	90.54±0.75 b	9.30±0.76 b	0.16±0.02 bc
04C204	85.60±2.15	14.24±2.14	0.15±0.02 b	90.99±0.10 a	8.74±0.11 b	0.27±0.02 b	93.13±0.60 a	6.78±0.62 c	0.09±0.01 c

Note: Each value is a mean of three replicates. The means in each column were analyzed by Student's t-test at the α=0.05 significance level. Values in each column with the same letter are not significantly different.

Table 3. ^{15}N partitioning (%) in shoots, roots and soil 7 days after applying the label in four *Brassica napus* genotypes at different growth stages in experiment 2.

Brassica	Growth stage I			Growth stage II			Growth stage III		
Cultivar/line	Shoot	Root	Soil	Shoot	Root	Soil	Shoot	Root	Soil
cv. Topaz	90.21±2.26	9.49±2.25	0.30±0.03	84.59±1.23 b	14.99±1.18 a	0.43±0.07	85.22±1.71 b	14.26±1.77 a	0.52±0.06 a
cv. Sentry	87.84±3.34	11.98±3.33	0.17±0.02	90.20±1.78 a	9.36±1.68 b	0.44±0.16	87.80±1.37 ab	11.73±1.42 ab	0.47±0.08 ab
cv. Polo	92.25±1.29	7.53±1.32	0.22±0.02	85.94±0.42 b	13.37±0.28 a	0.69±0.14	88.73±1.60 ab	10.85±1.54 5 ab	0.42±0.05 ab
04C204	89.42±0.87	10.38±0.81	0.20±0.08	89.63±0.04 a	9.99±0.02 b	0.39±0.06	90.90±1.73 a	8.80±1.68 b	0.30±0.05 b

Note: Each value is a mean of three replicates. The means in each column were analyzed by Student's t-test at the α=0.05 significance level. Values in each column with the same letter are not significantly different.

Table 4. ^{13}C partitioning (%) in shoots, roots and soil of four *Brassica napus* genotypes in experiment 3. ^{13}C labeling was carried out only at stage I.

B. napus Cultivar/ line	Growth stage I			Growth stage II			Growth stage III		
	Shoot	Root	Soil	Shoot	Root	Soil	Shoot	Root	Soil
cv. Topas	93.66±0.72	6.04±0.76	0.29±0.04	84.72±1.00	15.12±0.99	0.15±0.02	80.76±1.35 c	19.13±1.36 a	0.11±0.00
cv. Sentry	94.43±0.83	5.39±0.77	0.18±0.07	88.61±0.65	11.25±0.66	0.14±0.02	85.02±1.30 ab	14.83±1.30 bc	0.15±0.01
cv. Polo	92.95±1.44	6.81±1.41	0.24±0.03	85.02±1.37	14.86±1.38	0.12±0.01	84.41±0.73 bc	15.47±0.76 ab	0.12±0.04
04C204	95.07±1.61	4.75±1.57	0.17±0.04	88.39±5.01	11.49±4.98	0.11±0.04	88.55±2.04 a	11.28±2.04 c	0.17±0.01

Note: Each value is a mean of three replicates. The means in each column were analyzed by Student's t-test at the α=0.05 significance level. Values in each column with the same letter are not significantly different.

Table 5. ^{15}N partitioning (%) in shoots, roots and soil of four cultivars of *Brassica napus* in experiment 3. ^{15}N labeling was carried out only at 3 days before growth stage I.

B. napus	Growth stage I			Growth stage II			Growth stage III		
Cultivar /line	Shoot	Root	Soil	Shoot	Root	Soil	Shoot	Root	Soil
cv. Topas	81.57±2.00 ab	6.64±0.12 a	11.79±2.13 ab	88.56±0.31 c	11.04±0.46 a	0.04±0.16	86.32±3.01 b	13.53±3.01 a	0.15±0.02 b
cv. Sentry	84.79±1.14 a	6.55±0.83 a	8.66±1.94 b	90.36±0.57 ab	9.33±0.54 ab	0.32±0.03	88.63±0.27 ab	11.08±0.24 ab	0.29±0.05 ab
cv. Polo	80.51±0.58 b	6.36±0.26 a	13.13±0.32 ab	89.31±0.52 bc	10.05±0.30 bc	0.64±0.34	87.72±1.61 ab	11.88±1.65 ab	0.30±0.08 ab
04C204	82.06±1.93 ab	4.15±0.60 b	13.78±1.85a	91.21±0.23 a	8.54±0.24 c	0.25±0.03	90.98±0.31 a	8.57±0.21 b	0.45±0.18 a

Note: Each value is a mean of three replicates. The means in each column were analyzed by Student's t-test at the α=0.05 significance level. Values in each column with the same letter are not significantly different.

Fig. 1.

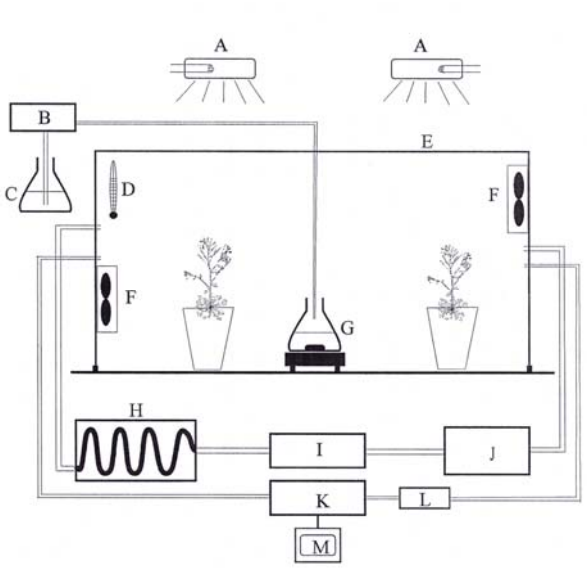
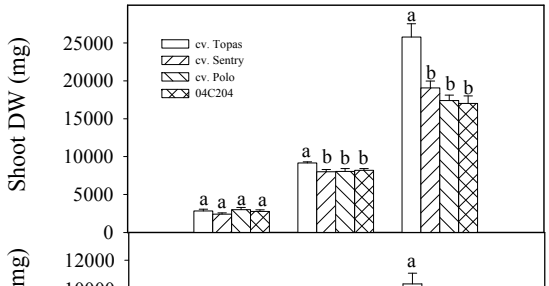


Fig. 2.



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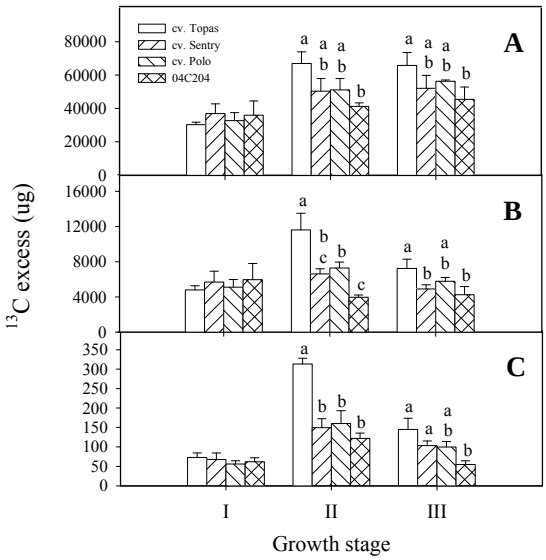

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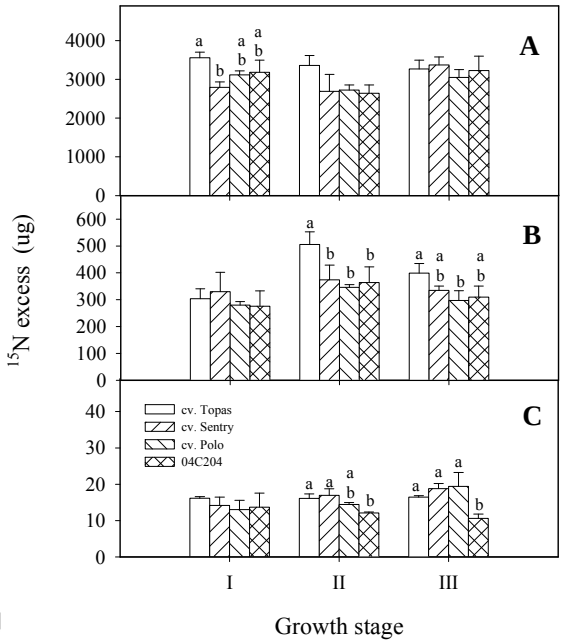

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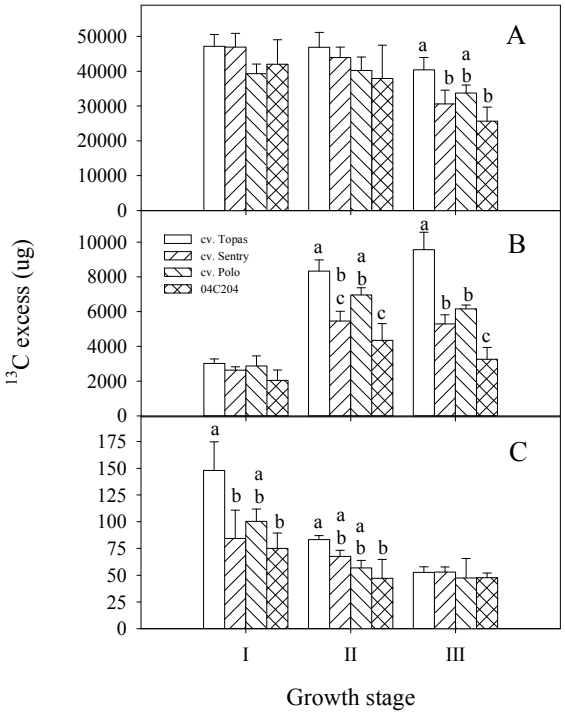

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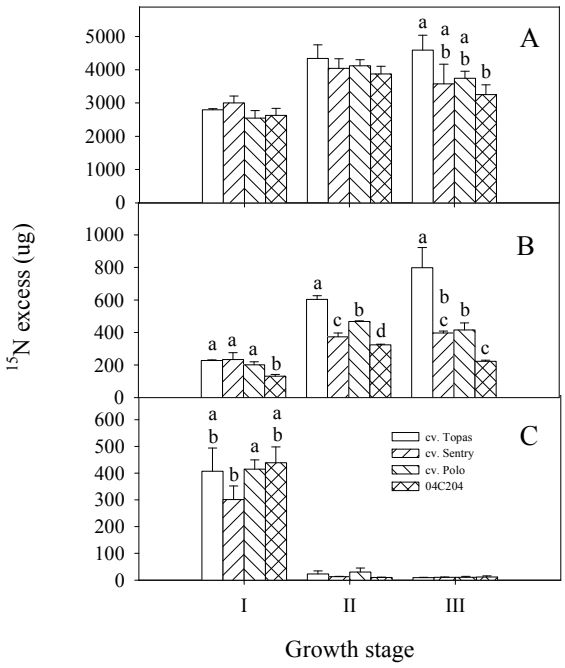

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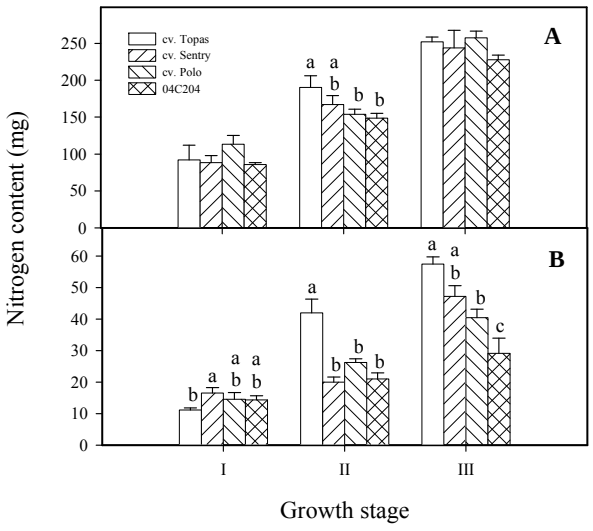

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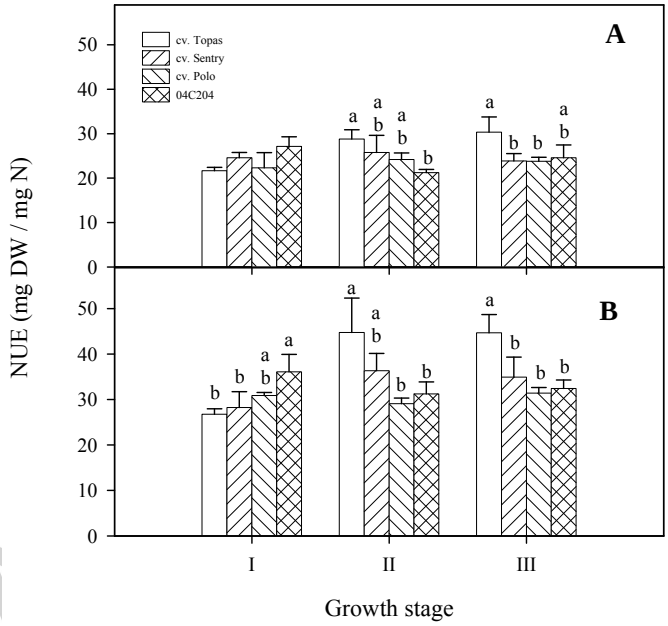

Figure legends:

Fig. 1. Diagram of the ^{13}C labeling system. A: HPS lights (1000 W each), B: peristaltic pump, C: $Na_2{}^{13}CO_3$ solution, D: temperature and relative humidity indicator, E: acrylic labeling chamber, F: circulation fans, G: H_2SO_4 solution, H: cooling system, I: condenser, J: air pump, K: CO_2 monitor, L: air pump, M: computer.

Fig. 2. Shoot and root dry weight (DW) and ratio of root to shoot in four *Brassica napus* genotypes at different growth stages (I: 6-7 unfold leaves; II: before inflorescence emergence; III: 20% flowering) in experiment 1. Each value is a mean of 6 replicates. The means in each group were analyzed by Student's t-test at the $\alpha = 0.05$ significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 3. ^{13}C excess in shoots (A), roots (B) and soil (C) in four *Brassica napus* genotypes in experiment 2. ^{13}C labeling was conducted at each growth stage (I: 6-7 leaves; II: before inflorescence emergence; III: 20% flowering). Each value is a mean of three replicates. The means in each group were analyzed by the Student's t-test at the $\alpha = 0.05$ significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 4. ^{15}N excess in shoots (A), roots (B) and soil (C) in four *Brassica napus* genotypes in experiment 2. ^{15}N was applied 7 days before ^{13}C labeling at each growth stage (I: 6-7 leaves; II: before inflorescence emergence; III: 20% flowering). Each value is a mean of three replicates. The means in each group were analyzed by the Student's t-test at the $\alpha = 0.05$ significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 5. ^{13}C excess in shoot (A), root (B) and soil (C) in four *Brassica napus* genotypes in experiment 3. (I: 6-7 leaves; II: before inflorescence emergence; III: 20% flowering). ^{13}C labeling was carried out only at stage I. Samples were taken at growth stage I, II and III, respectively. Each value is a mean of 3 replicates. The means of each group were analyzed by the Student's t-test at the $\alpha = 0.05$ significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 6. ^{15}N excess in shoot (A), root (B) and soil (C) in four *Brassica napus* genotypes in experiment 3. (I: 6-7 leaves; II: before inflorescence emergence; III: 20% flowering). ^{15}N was only applied once 3 days before ^{13}C labeling. Samples were taken at growth stage I, II and III, respectively. Each value is a three replicates. The means in each group were analyzed by the

Student's t-test at the α=0.05 significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 7. Total N content in shoots (A) and roots (B) of four *Brassica napus* genotypes at different growth stages (A: 6-7 leaves; B: before inflorescence emergence; C: 20% flowering) in experiment 2. Each value is a mean of three replicates. The means in each group were analyzed by Student's t-test at the α = 0.05 significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.

Fig. 8. Nitrogen use efficiency (NUE) in shoots (A) and roots (B) of four *Brassica napus* genotypes at different growth stages (I: 6-7 leaf; II: before inflorescence emergence; III: 20% flowering) in experiment 2. Each value is a mean of three replicates. The means in each group were analyzed by Student's t-test at the α = 0.05 significance level. Values in each group with the same letter are not significantly different. Bars represent S.E.