

¹Huzhou Academy of Agricultural Sciences, Huzhou, China

²Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

³Huzhou Bureau of Agriculture, Huzhou, China

Seed weight and oil content response to branch position reveals the importance of carbohydrate and nitrogen allocation among branches in canola (*Brassica napus* L.) genotypes

Yun Ren¹, Jianfang Zhu¹, Yichun Wang³, Shanlin Ma¹, Genru Ye¹, Shuijin Hua^{2*}

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Summary

Increasing seed weight and oil content are the main pathways to increase canola seed oil yield. The effect of branch position on seed weight and oil content has not been previously reported in canola. Field experiments were conducted to explore the impact of branch position on seed weight and oil content. Four canola genotypes, Zheyou 50, Zhongshuang 11, Zheyou 18, and Zheshuang 8, were used to evaluate seed weight, oil content, carbohydrate profile, and nitrogen content in the main inflorescence and branches from the top to the bottom of the main stem. Seed weight and oil content decreased from the main inflorescence to the lower branches in the four genotypes. Lower carbohydrate and nitrogen content in the seed and low transport efficiency of the two chemical compounds in the siliques and branches were responsible for the lower seed weight and oil content in Zheshuang 8 and Zheyou 18, respectively. However, the decreasing seed weight and oil content in the branches did not correspond with decreasing carbohydrate and nitrogen content in the branches from the top to the bottom. The result suggested complex carbohydrate and nitrogen metabolism in the canola seed in the different branches.

Introduction

Seed weight and oil content are two essential traits for canola (*Brassica napus* L.) seed oil yield production. BERRY and SPINK (2006) estimated that canola seed weight should reach 5.0 mg or more per seed to achieve a yield of 6.5 t ha⁻¹ of seed in the UK. Canola germplasm with heavy seed exist in the natural accessions. KENNEDY et al. (2011) evaluated the performance of seed traits including seed weight in 1500 rapeseed accessions and the result revealed that the seed weight ranged from 1.3 to 7.0 mg per seed. Consequently, there is a high potential for substantial improvement of canola seed weight in breeding programs. Seed weight is one of the three yield components, namely, siliques per plant, seeds per silique, and seed weight in canola. In recent years, canola seed weight has been received more and more attentions by researchers and breeders. Many researchers have investigated the genetic control of canola seed weight (CAI et al., 2012; CAI et al., 2014; LI et al., 2014a; LI et al., 2014b; LI et al., 2015; LIU et al., 2015). Multiple QTLs controlling canola seed weight have been identified. For example, more than 100 QTLs affecting seed weight were found in genetically different canola populations and environments; while one QTL (*qSW.A7-2*) was detected in all environments (SHI et al., 2009). These results indicate that canola seed weight is a complicated quantitative trait and governed not only by the genetic factors with different pathways but also the interactions with different environments. More recently, a gene encoding an auxin response factor, *ARF18*, was found to regulate seed weight and silique length (LIU et al., 2015). However, in addition to genetic and environmental factors, less attention is paid to the developmental issues modulating seed weight, for example, canola branch position.

Canola seed oil content is another important trait and is regulated by many factors as well. Numerous reports revealed that canola seed oil content is controlled by many QTLs (ZHAO et al., 2005; JIANG et al., 2014; JAVED et al., 2016). Although the pathway governing seed oil biosynthesis is well described, a key gene for seed oil content has not been isolated, possibly due to the lack of major effect QTLs (BAUD and LEPINIEC, 2009). Furthermore, seed oil content is heavily affected by crop management. Nitrogen application has a positive effect on seed yield, yet, seed oil content can be negatively affected (ZUO et al., 2016). Planting date can also markedly affect seed oil content (ROBERTSON and GREEN, 1981). However, there is no report on the relationship between canola seed oil content and branch position.

After budding, branches establish and elongate rapidly in canola. The flowering sequence in the different branch positions is usually from the top to the bottom of the main stem, namely, from the main inflorescence, the first branch, second branch and so on. However, the flowering sequence on the branch begins from the bottom to the top on the main inflorescence and from the adaxial region on other branches. Consequently, a gap of flowering time and seed and silique development among branches occurs in canola. However, regardless of this gap, canola is harvested at the same time and the whole plant is used as a harvesting unit. As a result, the differences of seed matter and oil accumulation potentially exist in different branches in canola.

During silique wall and seed development, nitrogen-containing compounds and carbohydrates are relocated from the main stem and are also produced through silique wall photosynthesis (BENNETT et al., 2011). Furthermore, the canopy of canola consists of siliques with different branches; levels of irradiation will differ depending on branch location. The resultant photosynthetic activity of siliques on different branches would lead to varying seed weight and oil content since carbohydrates are essential for seed matter accumulation and oil biosynthesis (HUA et al., 2014).

The aim of this study was to evaluate seed weight and oil content at different branch locations in four canola cultivars. Moreover, carbohydrate profiling and nitrogen content were determined in the seed, silique, and the branch stem to investigate the effect of the distribution of the compounds on seed weight and oil content.

Materials and Methods

Plant materials and crop management

The field experiments were carried out over two growth seasons in 2012-2013 and 2013-2014 at Huzhou Academy of Agricultural Sciences Experimental Station. Four canola (*Brassica napus* L.) cultivars, Zheyou 50, Zhongshuang 11, Zheyou 18, and Zheshuang 8, were selected as plant materials. Zheyou 50 and Zhongshuang 11 are two high seed oil content cultivars in China whose oil content can reach 49.00% and 49.04%, respectively. The two cultivars were bred by Zhejiang Academy of Agricultural Sciences and Institute of Oil Crops, Chinese Academy of Agricultural Sciences, respectively.

* Corresponding author

Zheyong 18 and Zheshuang 8 were released by Zhejiang Province, China. The cultivars were bred by Zhejiang Academy of Agricultural Sciences. Zheyong 18 was the first cultivar successfully bred for mechanical harvesting in Zhejiang Province. Three to five seeds of the four cultivars were directly sown in a shallow hole (about 3 cm) and covered by a layer of soil on 1 October 2012–2013 and 2013–2014. At the five-true-leaf stage, seedlings were thinned to one plant in each hole. Disease and pest control were performed to maximize the canola seed yield and quality. Urea was used as a nitrogen fertilizer and 150 kg N ha⁻¹ of urea was applied into the soil before sowing. A second nitrogen application was performed as a top dressing with 75 kg N ha⁻¹ nitrogen fertilizer (urea) at the end of January in each growth season. Calcium superphosphate, potassium oxide, and borax were applied at a rate of 375, 120, and 15 kg ha⁻¹, respectively, as a basal fertilizer. No irrigation was applied during two growing seasons.

Experimental design and sampling

The experiment used a randomized complete block design with four canola cultivars as experimental treatments. Three replications were conducted in the experiment. The plot was 20 m in length and 3.2 m wide. Canola plants were planted in eight rows with a 0.2 m space between plants and 0.35 m space between rows. During harvesting, ten plants were randomly selected in each plot excluding boarder plants. The ten plants were dug with root and soil and rinsed by tapped water to carefully remove soil from the root. The whole plants were quickly transported to the lab and separated into main inflorescence, the first branch (from the top to the bottom regarding the sequential number of branch) until the tenth branch. The branches below the tenth were mixed. The main stem and root were separately reserved. Each branch was further divided into branch stem, silique wall, and seed. The seeds were dried in the sunlight until the seed water content reached 8 g 100 g⁻¹. Silique wall, branch stem, main stem, and root were dried in an oven at 80 °C until the dry weight became constant.

Seed weight, oil content, carbohydrate profiling, and nitrogen (N) determination

The one-thousand seed weight was recorded using a balance. Seed oil content was determined by near-infrared (ANATARIS II, FT-NIR Analyzer, Thermo Fisher Scientific Inc., Madison, WI, USA). Carbohydrates including total soluble sugar, sucrose, and starch content were determined. The dried sample was ground to a powder. Briefly for carbohydrate profiling, about 50 mg of powder was boiled

twice in a water bath using 10 mL of 800 mL L⁻¹ ethanol for 30 min and then cooled to room temperature. The extract was centrifuged at 10000 × g for 10 min. One hundred milligrams of activated charcoal was added to the supernatant and kept at 80 °C in a water bath to remove the chlorophyll. The chlorophyll-free supernatant was then used for the determination of total soluble sugar and sucrose content according to HENDRIX (1993). The starch content in the pellets was digested with amyloglucosidase for 100 min at 55 °C and then determined according to HENDRIX (1993). N content was measured using the standard Kjeldahl method.

Statistical analysis

Mean data of one thousand seed weight, seed oil content, total soluble sugar, sucrose, starch, and nitrogen content in branch stem, silique wall, and seed with different branches and genotypes were analyzed using the MIXED procedure of SAS (SAS INSTITUTE, 2004). Branch position, genotype, and the interaction between branch position and genotype were considered as fixed effects. Year, block, and all interactions among these effects were considered as random effects. When the main effect of branch position was significant, mean comparisons were further conducted using Duncan's method ($\alpha = 0.05$).

Results

Seed weight in different branch and genotype

Seed weight was significantly affected by branch position, genotype, and year with a strong interaction between these factors (Tab. 1). Genotypic variation of seed weight revealed that Zhongshuang 11 had heavier seed weight than other genotypes. On average, the seed weight in Zhongshuang 11 was 7.9%, 23.4%, 20.9% in 2012 and 6.2%, 16.2%, 16.3% in 2013 higher than Zheyong 50, Zheyong 18, and Zheshuang 8, respectively (Fig. 1A and B). The upper position of branches had the greatest seed weight in all genotypes. Seed weight of the main inflorescence and the first branch were on average 24.3% and 21.4% higher, respectively, than that of the tenth and below the tenth branches in Zhongshuang 11 and Zheyong 50 (Fig. 1A and B). Generally, variations of seed weight in branches located in the middle position of the stalk were less because of the close seed weight among these branches (Fig. 1A and B).

Seed oil content in different branch and genotype

Seed oil content was also significantly influenced by genotype,

Tab. 1: Analysis of variance (branch position, genotype, and year) of seed weight (SW), seed oil content (SOC), silique total soluble sugar content (Si-TSSC), seed soluble sugar content (Se-TSSC), branch soluble sugar content (B-TSSC), silique sucrose content (Si-SU), seed sucrose content (Se-SU), branch sucrose content (B-SU), silique starch content (Si-ST), seed starch content (Se-ST), branch starch content (B-ST), silique N content (Si-N), seed N content (Se-N), and branch N content (B-N)

Source	SW	SOC	Si-TSSC	Se-TSSC	B-TSSC	Si-SU	Se-SU	B-SU	Si-ST	Se-ST	B-ST	Si-N	Se-N	B-N
	<i>P</i> value													
Branch position (BP)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype (G)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Year (Y)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.032	<0.001	0.265	<0.001	0.906	0.214
BP*G	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BP*Y	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.034	0.214
G*Y	0.018	<0.001	<0.001	<0.001	0.005	<0.001	<0.001	0.099	0.003	<0.001	0.003	<0.001	0.020	0.776
BP*G*Y	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001

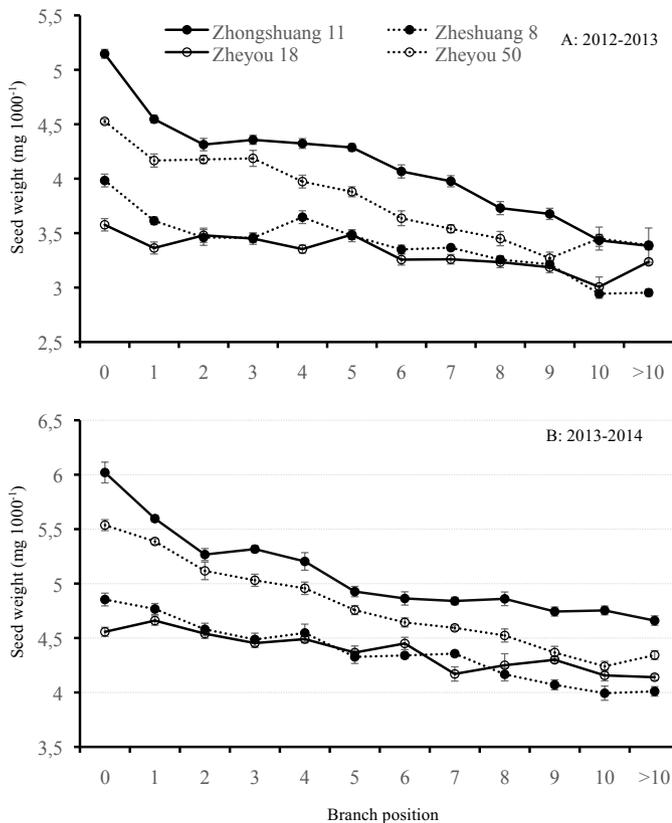


Fig. 1: Seed weight in the main inflorescence and different branches from the top to the bottom of the main stem in the four cultivars, Zheyou 50, Zhongshuang 11, Zheyou 18, and Zheshuang 8 in (A) 2012-2013 and (B) 2013-2014 growth seasons.

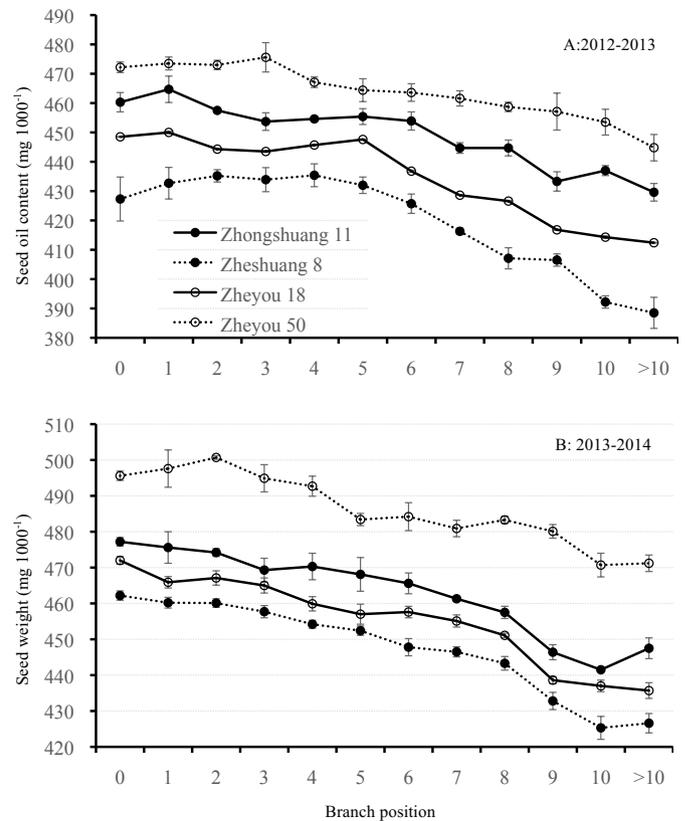


Fig. 2: Seed oil content in the main inflorescence and different branches from the top to the bottom of the main stem in the four cultivars, Zheyou 50, Zhongshuang 11, Zheyou 18, and Zheshuang 8 in (A) 2012-2013 and (B) 2013-2014 growth seasons.

branch position, growth season, and their interactions among these factors (Tab. 1). Seed oil content of the four genotypes in 2012 was significantly lower than that in 2013 and the gap of the seed oil content between two years averagely ranged from 13.8 to 28.0 mg g⁻¹ with different genotypes (Fig. 2A and B). Furthermore, seed oil content among the four genotypes differed significantly. Zheyou 50 contained far more oils than other genotypes. On average, it was 4.2%, 9.6%, and 6.8% more oils than that in Zhongshuang 11, Zheshuang 8, and Zheyou 18, respectively (Fig. 2A and B). As for the branch position, seed oil content decreased significantly from top to bottom in all genotypes. In general, the gap of seed oil content between the main inflorescence and the lowest branch ranged from 24.4 (Zheyou 50 in 2013) to 38.8 (Zheshuang 8 in 2012) mg g⁻¹ (Fig. 2A and B). The difference in seed oil content was much smaller on the upper branches yet dropped dramatically below the fifth branch in the four genotypes (Fig. 2A and B).

Total soluble sugar content (TSSC) in seed, silique, and branch stem in different branch and genotype

Generally, TSSC in each branch stem was below 2 mg g⁻¹ in all genotypes. Silique wall TSSC was much higher as compared with that in branch stem and ranged from 1.5 to 5 mg g⁻¹. The highest TSSC was found in the seed which was greater than 14 mg g⁻¹ (Fig. 3A to F).

In the branch stem, Zheyou 18 had the highest TSSC while Zheshuang 8 possessed the lowest content. The mean TSSC of each branch in Zheyou 18 was 43.75% and 43.94% higher than that in Zheshuang 8 in two years, respectively (Fig. 3A and B). Close TSSC values were observed in Zhongshuang 11 and Zheyou 50 at each branch. Regarding branch position, TSSC at the higher stalk position was

generally less than at the lower position.

In the seed, Zhongshuang 11 and Zheyou 50 had similar TSSC, which were on average 19.30 mg g⁻¹ and 19.38 mg g⁻¹, respectively (Fig. 3 C and D). Seed TSSC in Zheshuang 8 was significantly higher than that in Zheyou 18 below the second branch. The mean seed TSSC in the branches below the second branch in Zheshuang 8 was 8.36% and 9.96% higher than that in Zheyou 18 over the two growth seasons (Fig. 3C and D). Zhongshuang 11 had significantly lower TSSC in the main inflorescence than other branches except the branch below the tenth. A stable TSSC was observed from the first to the sixth and the first to the ninth branches in the two growth seasons, respectively, and then decreased rapidly in Zhongshuang 11. Seed TSSC from the second branch onwards remained relatively stable in Zheshuang 8. Conversely, Zheyou 18 showed decreasing seed TSSC from the main inflorescence. However, the TSSC from the sixth to the ninth branch showed very few variations in the 2012-2013 growth season in Zheyou 18 (Fig. 3C and D).

Like branch stem, Zheyou 18 had the highest amount of TSSC in the silique of each branch. Zhongshuang 11 exhibited the lowest TSSC above the sixth branch, however, it showed much higher TSSC below the sixth branch as compared with Zheshuang 8. Zheyou 50 had significantly higher TSSC in comparison with Zheshuang 8. On average, silique TSSC of each branch in Zheyou 18 was 15.23%, 31.73%, and 35.09% higher than that in Zheyou 50, Zheshuang 8, and Zhongshuang 11, respectively (Fig. 4E and F). Branches in the middle position presented higher TSSC than other ones in general (Fig. 3E and F). For example, silique TSSC peaked at the fourth branch in Zheyou 18 in both years while, Zheyou 50 and Zheshuang 8 obtained the maximum content moving to the second or lower branch.

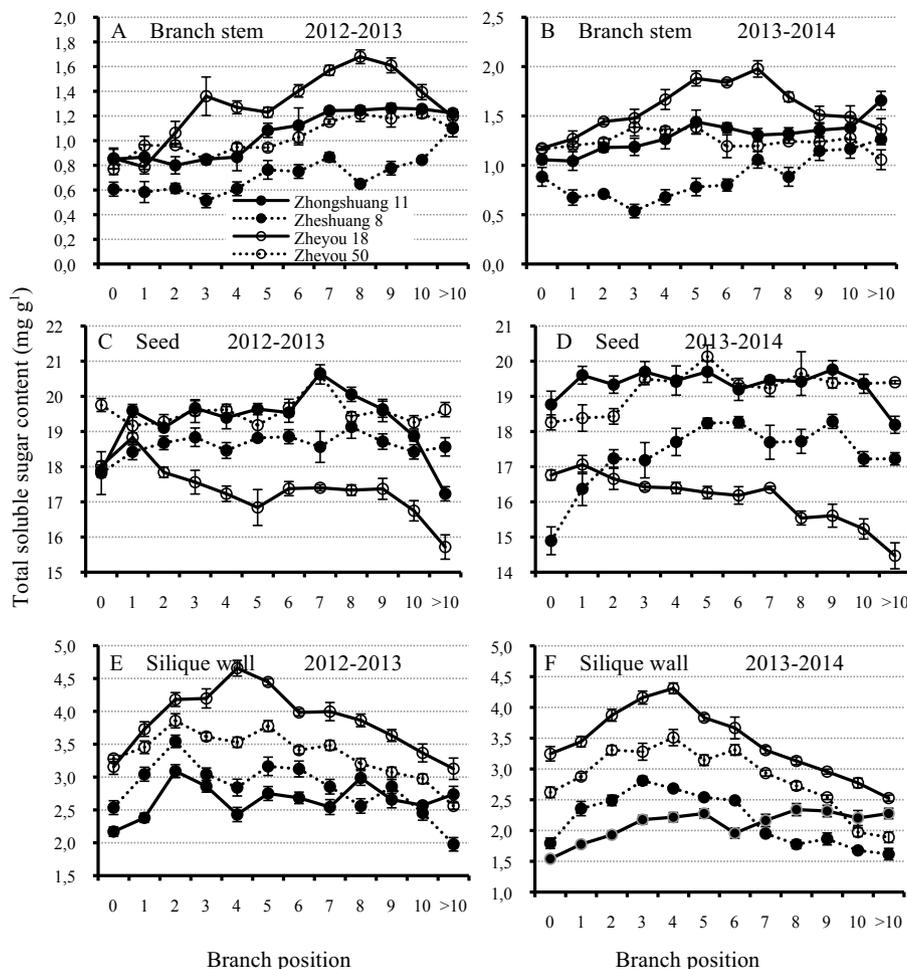


Fig. 3: Total soluble sugar content in the main inflorescence and different branches from the top to the bottom of the main stem in the branch stem in (A) 2012-2013 and (B) 2013-2014, seed in (C) 2012-2013 and (D) 2013-2014, and silique wall in (E) 2012-2013 and (F) 2013-2014 growth seasons in the four cultivars, Zheyou 50, Zhongshuang 11, Zheyou 18, and Zheshuang 8.

Sucrose content in branch stem, seed, and silique wall in different branch and genotype

In the branch stem, two genotypes, Zheshuang 8 and Zhongshuang 11, showed higher sucrose content than Zheyou 18 and Zheyou 50 except at the tenth or below tenth branches in both growth seasons. Average sucrose content of each branch in Zhongshuang 11 and Zheshuang 8 was 7.2 mg g^{-1} and 7.8 mg g^{-1} , respectively, while Zheyou 18 and Zheyou 50 was 5.4 mg g^{-1} and 4.4 mg g^{-1} (Fig. 4A and B). Sucrose content peaked at the second to the fourth branch in Zhongshuang 11 and Zheshuang 8 in the two growth seasons, respectively (Fig. 4A and B). For Zheyou 18, branch sucrose content increased and peaked at the sixth and fourth branch in the two growth seasons, respectively (Fig. 4A and B). For Zheyou 50, sucrose content remained relatively stable from the main inflorescence to the eighth branch and then increased quickly in 2012-2013 while, few fluctuations were observed from the second to the branch below the tenth in 2013-2014 (Fig. 4A and B).

Significant genotypic variation of sucrose content in the seed was observed (Fig. 4C and D). Zheyou 18 had the lowest seed sucrose content in each branch. Zheyou 50 and Zhongshuang 11 had the highest seed sucrose content. The mean seed sucrose content of each branch in Zheyou 18 was 9.69%, 16.75%, and 17.78% lower than that in Zheshuang 8, Zheyou 50, and Zhongshuang 11, respectively (Fig. 4C and D). Sucrose content was relatively stable above the ninth and eighth branch in the two growth seasons, respectively, and then decreased rapidly in Zheyou 18. The sucrose content below the tenth

branch was on average 12.70% less than that in the ninth or eighth branch in Zheyou 18 (Fig. 4C and D). A similar trend of sucrose content was found in Zheshuang 8 as compared with Zheyou 18 in 2012-2013 in each branch, however, the content was almost evenly distributed in the 2013-2014 growth season (Fig. 4C and D). For Zhongshuang 11, seed sucrose content slightly decreased from the main inflorescence to the ninth branch, and then decreased dramatically in the 2012-2013 growth season. Sucrose content increased from the main inflorescence to the second branch until the ninth branch, then decreased rapidly again in the 2013-2014 growth season (Fig. 4C and D). Changes of seed sucrose content in Zheyou 50 were similar to Zhongshuang 11 (Fig. 4C and D).

Silique sucrose content in Zheshuang 8 was the highest while that in Zheyou 50 was the lowest (Fig. 4E and F). Zheyou 18 had significantly higher silique sucrose content than that of Zhongshuang 11 at most branches. On average, silique sucrose content at each branch of Zheshuang 8 was 15.95%, 28.32%, and 26.59% higher than that in Zheyou 18, Zhongshuang 11, and Zheyou 50, respectively (Fig. 4E and F). Silique sucrose content increased from the main inflorescence and peaked at the third branch and then decreased in Zheshuang 8 in both growth seasons. However, the content increased again from the ninth branch in 2012-2013 yet, decreased drastically from the eighth branch in 2013-2014 in Zheshuang 8 (Fig. 4E and F). For Zheyou 18, silique sucrose content showed few variations except at the first branch in 2012-2013 and the main inflorescence in 2013-2014. For Zhongshuang 11 and Zheyou 50, silique sucrose content

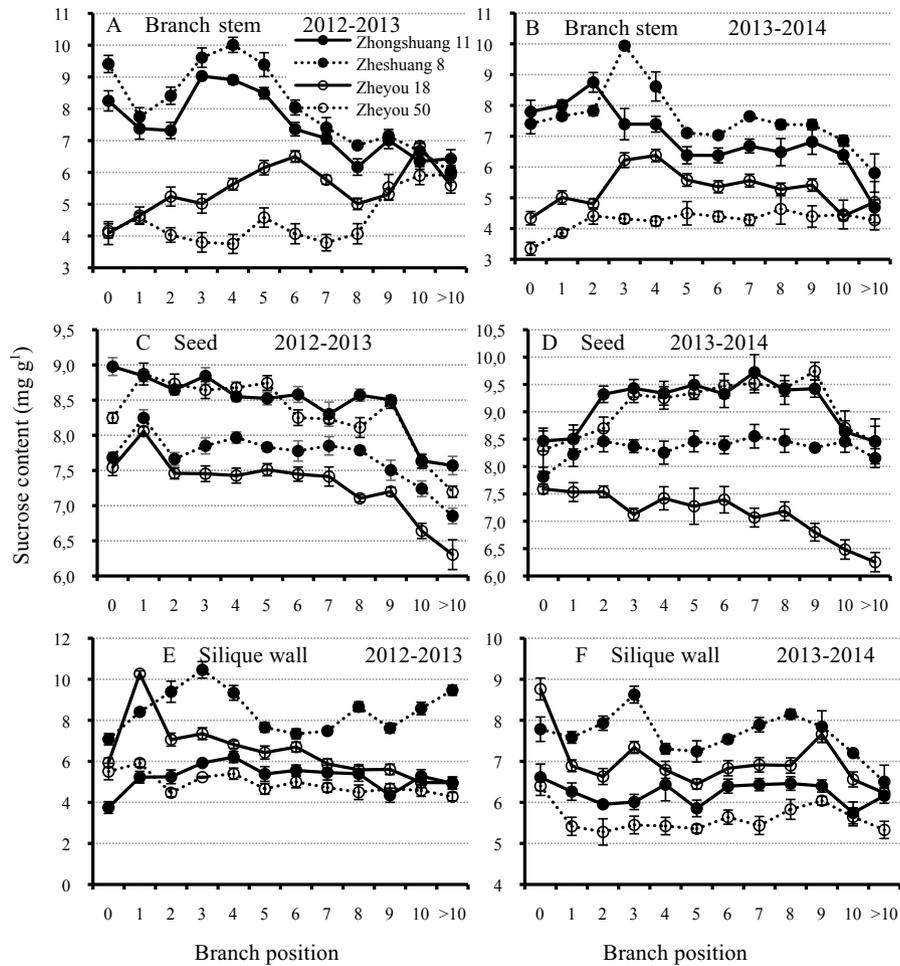


Fig. 4: Sucrose content in the main inflorescence and different branches from the top to the bottom of the main stem in the branch stem in (A) 2012-2013 and (B) 2013-2014, seed in (C) 2012-2013 and (D) 2013-2014, and silique wall in (E) 2012-2013 and (F) 2013-2014 growth seasons in the four cultivars, Zheyu 50, Zhongshuang 11, Zheyu 18, and Zhesuang 8.

was much more stable than other genotypes at each branch (Fig. 4E and F).

Starch content in branch stem, seed, and silique wall in different branch and genotype

Starch content in different organs showed that seed starch content was significantly lower than branch stem (Fig. 5A to F) and silique. For Branch stem, Zheyu 18 had the lowest starch content in each branch. Zheyu 50 had the highest starch content at the main inflorescence and the first branch and then decreased quickly, which was significantly lower than Zhesuang 8 and Zhongshuang 11 (Fig. 5A and B). Compared with Zhesuang 8, Zhongshuang 11 had higher starch content from the main inflorescence to the fourth branch while, the opposite trend was found at lower branch position (Fig. 5A and B). Zhongshuang 11 peaked at the second and fourth branch in the two growth seasons, respectively. For Zhesuang 8, starch content increased from the main inflorescence to the seventh or eighth then declined sharply in the two growth seasons (Fig. 5A and B). For Zheyu 50, starch content decreased with decreasing branch height. Unlike the other three genotypes, starch content in Zheyu 18 was relatively stable at each branch position (Fig. 5A and B).

Like branch stem starch content, seed starch content in Zheyu 18 was the lowest (Fig. 5C and D). For Zhongshuang 11, seed starch content was significantly lower than Zhesuang 8 and Zheyu 50 above the sixth branch and then sharply increased below the sixth branch,

which exceeded the two genotypes (Fig. 5C and D). The maximum seed starch content was observed at the main inflorescence and the first branch in Zheyu 50, which was higher than other genotypes. However, the seed sucrose content from the second to the sixth branch ranked the second, which was lower than Zhesuang 8. The seed sucrose content below the sixth branch was ranked third in Zheyu 50 (Fig. 5C and D). Seed starch was relatively stable in each branch in Zheyu 18 in both growth seasons as compared with other genotypes (Fig. 5C and D). For Zhongshuang 11, branches at the middle positions, which was from the second to the sixth branch in 2012-2013 growth season and from fourth to the sixth branch position in 2013-2014 growth season, showed the lowest content compared with other branches (Fig. 5C and D). The seed starch content increased from the sixth branch and peaked at the ninth and the seventh branch in the two growth seasons, respectively in Zhongshuang 11 (Fig. 5C and D). For Zhesuang 8, the seed starch content increased from the main inflorescence to the second branch and then decreased yet, increased at the sixth and seventh branch in 2012-2013 growth season (Fig. 5C and D). For Zheyu 50, seed starch content continuously decreased from the main inflorescence (Fig. 5C and D).

Silique starch content of Zheyu 18 was the lowest while Zhongshuang 11 was the highest in both growth seasons (Fig. 5E and F). Silique starch content in Zheyu 50 ranked second, however, the content was similar to Zhongshuang 11 (Fig. 5E and F). Silique starch content decreased from the main inflorescence to the second and the third branch and then remained relatively stable in Zheyu 18

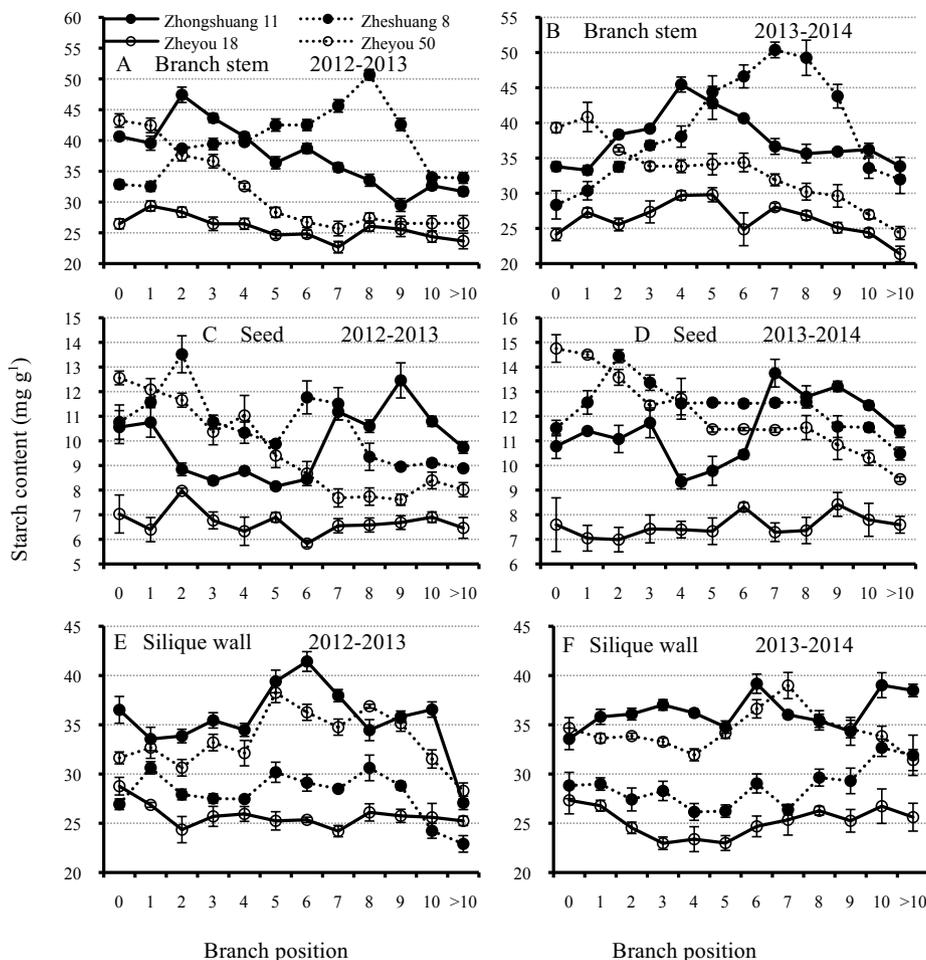


Fig. 5: Starch content in the main inflorescence and different branches from the top to the bottom of the main stem in the branch stem in (A) 2012-2013 and (B) 2013-2014, seed in (C) 2012-2013 and (D) 2013-2014, and silique wall in (E) 2012-2013 and (F) 2013-2014 growth seasons in the four cultivars, Zheyong 50, Zhongshuang 11, Zheyong 18, and Zheshuang 8.

in both growth seasons (Fig. 5D and E). For Zheshuang 8, silique starch content above the eighth or seventh showed small variations in two growth seasons, respectively. However, the content from the eighth branch decreased in 2012-2013 growth season while, the opposite trend was observed in 2013-2014 in Zheshuang 8 (Fig. 5D and E). For Zheyong 50, the middle position branches had much higher silique starch content. For example, silique starch content was higher than other branches from the fifth to eighth branch in the 2012-2013 growth season and that from the sixth to the eighth branch in the 2013-2014 growth season (Fig. 5D and E). For Zhongshuang 11, large variation of silique starch content in different branches was found in 2012-2013 especially below the fourth branch. The starch content increased from the fourth branch and then decreased quickly in Zhongshuang 11 (Fig. 5D and E).

Total N content in branch stem, seed, and silique wall in different branch position with genotypes

In general, N content in the branch stem was the lowest while that was the highest in the seed (Fig. 6 A, B, C, D, E, and F). The result indicated that a majority of N in the stem and silique shifted to the seed for its development.

Significantly genotypic variation of N content was found in branch stems (Fig. 6A and B). Branch stem N content in Zheyong 18 was significantly higher than other genotypes from the main inflorescence to the sixth branch. However, the content below the sixth

branch in Zheyong 18 was lower than that in Zhongshuang 11 (Fig. 6A and B). Branch stem N content in Zheshuang 8 and Zheyong 50 showed far lower amount as a comparison with Zhongshuang 11 and Zheyong 18 (Fig. 6A and B). The upper position branches in Zheyong 50 had lower N content as compared with Zheshuang 8. However, the reverse trend was found after the seventh branch (Fig. 6A and B). Branch stem N content in Zheshuang 8 was very stable in each branch except the lowest branch in the 2013-2014 growth season (Fig. 6A and B). For Zheyong 50, higher N content was found as the branch position lowered (Fig. 6A and B). Branch stem N content in Zhongshuang 11 increased from the main inflorescence to the ninth and sixth branch in the two growth seasons, respectively (Fig. 6A and B). A similar trend in N content was observed in Zheyong 18 (Fig. 6A and B). However, the peak N value occurred earlier, at the fourth and the fifth branch in the two growth seasons, respectively (Fig. 6A and B).

As for seed N content, Zhongshuang 11 exhibited the highest N amount in each branch position. Zheyong 18 showed the lowest seed N content. Furthermore, seed N content in the four genotypes showed relatively stable in each branch (Fig. 6C and D). Silique N content in Zhongshuang 11 and Zheshuang 8 was significantly higher than that in Zheyong 50 and Zheyong 18 (Fig. 6E and F). Small fluctuations of silique N content were observed in each branch in the four genotypes in general. However, a drastic increase of silique N content from the eighth branch in Zheshuang 8 was found in the 2012-2013 growth season (Fig. 6E and F).

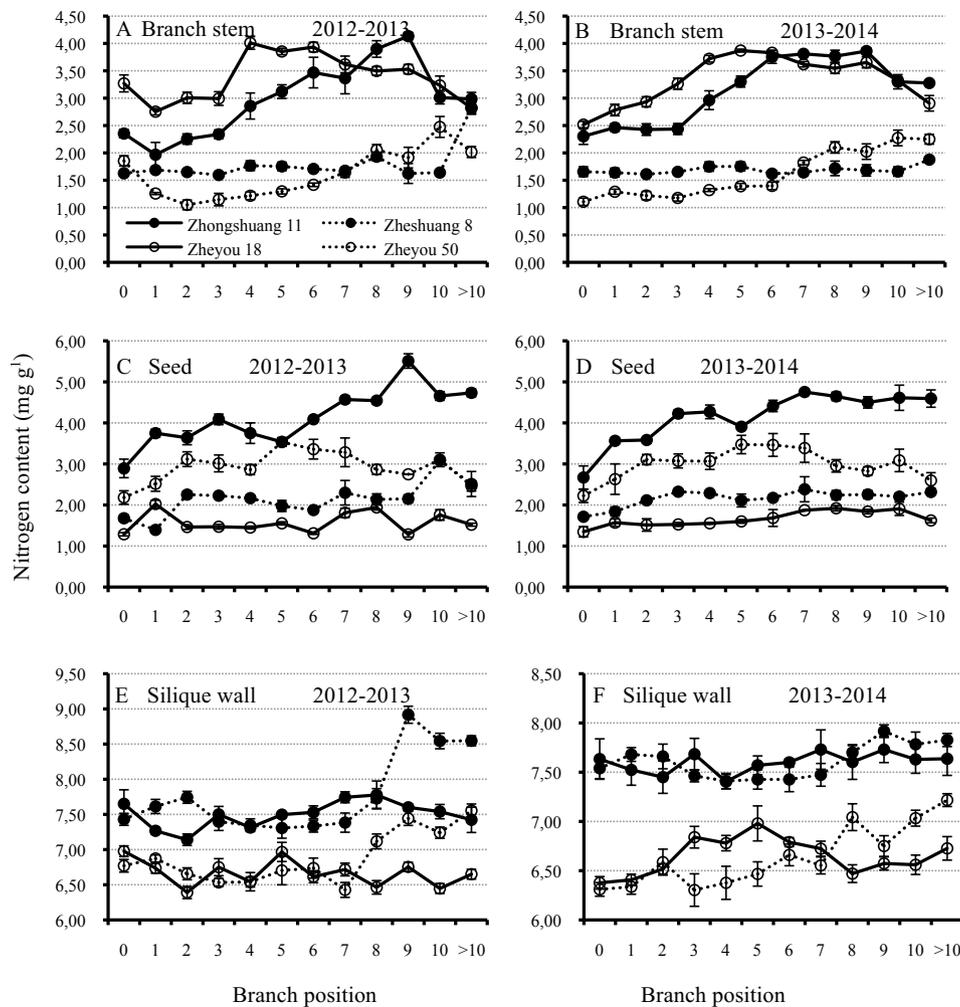


Fig. 6: Nitrogen content in the main inflorescence and different branches from the top to the bottom of the main stem in the branch stem in (A) 2012-2013 and (B) 2013-2014, seed in (C) 2012-2013 and (D) 2013-2014, and silique wall in (E) 2012-2013 and (F) 2013-2014 growth seasons in the four cultivars, Zheyong 50, Zhongshuang 11, Zheyong 18, and Zheshuang 8.

Discussion

Seed traits including weight and oil content are important for canola oil production. Although there are numerous studies on seed weight and oil content of canola (FAFAJI, 2014; RAD et al., 2014; LI et al., 2015), few have investigated the effect of canola developmental issues on seed weight and oil content such as branch position. Therefore, the objective of the current study was to understand the effect of branch position on seed weight and oil content.

In this study, we found significant differences in canola seed weight and oil content with different branches. Both the seed weight and oil content decreased from the top to the bottom branch suggesting a difference in the vertical allocation of carbohydrate and nitrogen for dry matter accumulation and lipid biosynthesis in different branches. In wheat, seeds on the head of the main tiller were heavier than any other position (NIK et al., 2012). In rice, the phenomenon exists as well and leads to the appearance of superior and inferior grains with different positions in the same spikelet. Consequently, the development of grains with different position results in a large difference of seed mass accumulation and quality formation between the two types of grain (TAN et al., 2009; FU et al., 2011; DONG et al., 2012; DAS et al., 2016).

In canola, the sequence of branch establishment initiates from the main inflorescence and then from the top to bottom of the stalk. The flowering sequence initiates at the bottom of the main inflorescence

and the proximal upper position branch as the middle and lower position branches develop. As a result, temporal and spatial differences in seed development follow. Consequently, the hypotheses for the difference in seed weight and oil content are (1) the photosynthetic product and nitrogen in the elongating stem is preferentially supplied to the main inflorescence and then from the upper to the lower branches; and (2) photosynthetic products in the silique decrease from the main inflorescence and the upper to the lower branches.

As flowering initiates, several important issues occur simultaneously including old leaf senescence, new leaf development, stem elongation, flowering, and silique development. Each issue, except old leaf senescing, demands considerable carbohydrate and nitrogen supplies both for the organ volume expansion and elongation. During the initial flowering, the early opened flowers commence seed (embryo) development after fertilization and the young bud is still in the state of floral organ development. The process continues to the end of flowering and this is the first gap for seed development among different branch positions. After the end of flowering, the canola plant enters silique wall and seed development. Normally, this stage ranges from 30 to 50 days (HUA et al. 2014).

There are two possible pathways leading to the difference in the seed weight and oil content. Firstly, the photosynthetic ability of the silique wall in different branches since the canopy consists of siliques (GAMMELVIND et al., 1996; KING et al., 1997). Secondly, the difference

in carbohydrate reallocation from the old leaves and stem. However, it is difficult to estimate precisely which pathway contributes more to seed mass accumulation and seed oil content presently. However, regardless of the pathways, both aim to maintain seed filling and volume expansion at seed developmental stage. In the present study, the soluble sugar content in each branch and silique wall were very low. There were two possible destinations for those sugars in branch and silique wall. First, the sugars were transformed into structural compounds such as lignin and cellulose in branch and silique wall (GÜNL and PAULY, 2011; ANDERSON et al., 2015). Second, sugars were transported to the seed since the seed is a strong sink during development (ANDRIANASOLO et al., 2017; ASSENG et al., 2017). Furthermore, the considerable carbohydrate reserves in the seed suggest the large amount of carbohydrate was deprived from those two tissues. The genotypic variation of soluble sugar content revealed that the difference of carbohydrate utilization can lead to the different seed weight and oil content. For example, Zheyou 18 had the highest branch stem and silique wall soluble sugar content while, the lowest content in the seed suggesting lower sugar transportation from these two tissues to the seed. Although Zheshuang 8 had similar seed weight to Zheyou 18 and lower seed oil content, its seed soluble sugar content was much higher than Zheshuang 8 and branch stem had the lowest soluble sugar content. The result implied that most of the soluble sugars in the branch stem and silique wall were transported into the seed in Zheshuang 8. However, the sugar was possibly used for seed mass accumulation but not for seed oil biosynthesis in Zheshuang 8. Surprisingly, Zhongshuang 11 had very low silique soluble sugar content possibly suggesting significant contribution of silique wall on seed soluble sugar content. Seed sucrose and starch content were the lowest in Zheyou 18 suggesting the lighter seed weight and lower seed oil content is due to the insufficient carbohydrate supply during seed mass accumulation and lipid biosynthesis. Zheyou 50 ranked second in seed weight and first in seed oil content in each branch. A medium amount of total soluble sugar and starch content, low sucrose content in branch and silique, and higher sucrose content in seed revealing the intensive input of sugars to the seed should be a strong indicator for seed weight and oil content accumulation. However, the sugars may flow into the seed lipid biosynthetic pathway due to the higher seed oil content. Unlike carbohydrate, seed nitrogen-derivatives occupy the cellular content following carbon-derivatives and come from silique wall and other tissues. Zhongshuang 11 had higher seed nitrogen content which might be beneficial for seed weight while, Zheyou 50 had relatively lower seed nitrogen content in comparison with Zhongshuang 11, which is helpful for increasing seed oil content as there are negative correlations between seed oil and protein content (the main nitrogen-derivative) (LI et al., 2014c; ZUO et al., 2016). The different responses of the carbohydrate type and organs to the branch position were found in present investigation. For total soluble sugar content, higher content in lower branches may suggest that soluble sugars in the lower position branch had lower transformation efficiency to secondary metabolites due to late development. Alternatively, seeds on the upper branches develop earlier than that on the lower branches; therefore, branch stem soluble sugar content was almost fully transported into the seed leading to a lower soluble sugar content. Unlike branch stem, soluble sugar content in the siliques was higher in the middle position. From canola plant morphology, siliques on the upper branches usually receive the highest irradiation due to space and earlier development. However, the middle and lower position branches rapidly develop due to higher temperatures after flowering (ZHANG et al., 2016); the soluble sugars may remain in the silique wall. Furthermore, the additive effect of the transportation of soluble sugars in the branch stem to silique wall might simultaneously lead to the higher soluble sugar content since the silique wall is an intermediate tissue between branch stem and

seed (BENNETT et al., 2011). In seed, total soluble sugar in the upper and middle positions were similar but decreased in the lower position. Seed development demands large amounts of carbohydrates, however, lower soluble sugar content in the lower branch position may not meet the requirements of development. For sucrose and starch content, both are cleaved into smaller molecules such as fructose and glucose (WEBER and ROITSCH, 2000; FALLAHI et al., 2008). Two genotypes, Zheshuang 8 and Zhongshuang 11, had higher sucrose content in the upper branches. However, in the seed and silique wall, fewer fluctuations and lower sucrose content were observed in the lower branches. This is in accordance with total soluble sugar content. A similar trend was observed in nitrogen content. Therefore, sugar content including total soluble sugar, sucrose and starch content, and nitrogen content in the different branch positions does not precisely reflect the trend of seed weight and oil content.

Conclusions

In conclusion, both seed weight and oil content decreased in the four canola genotypes from the main inflorescence to the lower branches in the main stem. The results suggest that the seed developmental schedule in the different branches affected seed dry matter accumulation and lipid biosynthesis. The mechanism of lower seed weight and oil content in Zheyou 18 and Zheshuang 8 was different. For Zheyou 18, low output efficiency of sugars from branch and silique wall to the seed was the main reason for dry matter and oil accumulation. However, for Zheshuang 8, lower sugar content in the branch and photosynthetic efficiency in the silique wall resulted in the lower seed weight and oil content. Although Zhongshuang 11 and Zheyou 50 had moderate sugar content in different organs, the destinations of the sugars were different. For Zhongshuang 11, the sugars mainly flowed into the dry matter pathway yet, into the lipid biosynthesis in Zheyou 50. Although seed weight and oil content decreased with decreasing branch height, there was no obvious correlation between seed weight (or oil content) and sugar content in the corresponding branches. A similar trend was found in the N content in different organs. Since sugar and nitrogen metabolism are both complex processes in the cell, detailed dynamic analysis on the sugar and nitrogen allocation in the different branches is further required.

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Address of the corresponding author:

E-mail: sjhual@163.com

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