Study on C/N metabolism characteristics in yellow-seed rapeseed (*Brassica napus* L.) during seedling stage

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Abstract

Yellow-seed rapeseed (*Brassica napus* L.) is characterized with thinner seed coat and lower hull percentage, which in turn is correlated with higher oil, protein and lower crude fiber contents compared to black-seed rapeseed from the same genetic background. The breeding of yellow-seed rapeseed has become one of the main objectives in rapeseed breeding. However the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed. The growth vigor of rapeseed plants during seedling stage is in connection with its yield significantly. Two pairs of different genetic near-isogenic lines of black-seed and yellow-seed rapeseed (*Brassica napus* L.) genotypes were used to study the physiological characters of yellow-seed rapeseed in seedling stage, which mainly included photosynthetic characters and characters on C/N metabolism. The results showed that there were higher sugar content and lower protein content, nitrogen content and activity of nitrate reductase in yellow-seed rapeseed plants than in black-seed rapeseed plants regardless of various development stages or various plant organs. It was discovered that chlorophyll content and carotenoid content of yellow-seed rapeseed were lower than those of black-seed rapeseed, and their net photosynthetic rate was also lower compared to black-seed rapeseed from the same genetic background.

Key words: Yellow-seed rapeseed (*Brassica napus* L.)**,** C/N metabolism characteristics**,** seedling stage

Introduction

Yellow-seed rapeseed (*Brassica napus* L.) is characterized with thinner seed coat and lower hull percentage, which in turn is correlated with higher oil, protein and lower crude fiber contents compared to black-seed rapeseed from the same genetic background (Gan G-X et al., 1989; Liu H-L, 1992; Liang Y et al., 2003; Shi S-J and Wu J-S, 2003; Liang Y and Li J-N, 2004; Liu X-P et al., 2005; Zhang Z-L et al., 2006). The breeding of yellow-seed rapeseed has become one of the main objectives in rapeseed breeding. However the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed. These characteristics have fundamental problems in extending yellow-seed rapeseed (*Brassica napus* L.). The growth vigor of rapeseed plants during seedling stage is in connection with its yield significantly (Liu H-L, 1987). It will be possible to know the real reason why the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed after we explore the differences of physiological characteristics during seedling stage in yellow-seed and black-seed rapeseed (*Brassica napus* L.). All these are helpful for us to cultivate and breed for yellow-seed rapeseed (*Brassica napus* L.).

Mean yield per plant of yellow-seed rapeseed was lower than that of black-seed rapeseed in the same group (Liu H-L et al., 1991). The seed fastness and seed vigor of yellow-seed rapeseed were shorter than those of black-seed rapeseed (Han J-X et al., 1993; Chen L et al., 1997). However the C/N metabolism characteristics and the differences of physiological characteristics between yellow-seed and black-seed rapeseed have not been found in previous studies (Zhao H-J et al., 1994; Liang Y et al., 2000;Leng S-H et al., 2002; Liang Y and Li J-N, 2004). In this study, two pairs of near-isogenic lines of black-seed and yellow-seed rapeseed (*Brassica napus* L.) genotypes were used to study the physiological characters of yellow-seed rapeseed in seedling stage, which mainly included photosynthetic characters and characters on C/N metabolism. The results may be useful to explain why the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed.

Material and Methods

The plant materials were two pairs of near-isonegic line of rapeseed (*Brassica napus* L.). The first pair of near-isonegic line was designated L1 (yellow-seed) and L2 (black-seed), which were the decendants derived from the cross combination of *Brassica.napus* L. and *Brassica juncea* L..In 1995,an individual plant of black-seed rapeseed was identified in the tenth progeny of the yellow inbred lines, which was identical with the yellow-seed one in both growth and appearance. The desirale plants were obtained in the third progeny of the black in bred line by inbreeding the black-seed individual plant, which was identical to the yellow-seed one except that the seed color was black. The stable inbred lines of black and yellow-seed rapeseeds formed the near-isonegic pairs. In order to maintain genetic consistency of the near- isonegic lines, using the black-seed individual plant as recurrent parent and adopting the BC1 and F1 preserved the offspring. The second pair of near-isonegic line was designated L3 (yellow-seed) and L4 (black-seed), which were the decendants derived from the cross combination of *Brassica.napus* L. and *Brassica campestirs* L..The whole course of its obtaining was similar to the first pair of near-isonegic line.

The experiment was performed in the farm of the College of Agronomy and Biotechnology of Southwest University in the years 2005–2006. The soil in the farm was model purple soil and its fertility was well-proportioned. The organic matter

content in the soil was 1.69% and the contents of available nitrogen, available phosphorus, available potassium and available boron were 81.7 mg/kg, 7.25 mg/kg, 115 mg/kg and 0.45 mg/kg separately. The value of its pH was 7.12. There were 3 repetitions in this experiment and the plot area was 8 m^2. The sowing date and transplanting date were Sep. 27and Oct. 20 respectively and the transplanting density was 1.05×10^5 plant/hm^2. Fertilizer was applied before transplanting as following: N 150 kg/hm^2, P_2O_5 120 kg/hm^2, K_2O 120 kg/hm^2, B 1.5 kg/hm^2.

Total sugar content in the stems and leaves of rapeseed were determined following Northwest Agricultural University' protocol in 10th leaf stage and overwintering stage. Protein content in the stems and leaves of rapeseed were determined following Hao Z-B's protocol also in 10th leaf stage and overwintering stage. Assays of nitrogen content and activity of nitrate reductase in leaves were carried out following Hao Z-B's and Zhang X-Z's protocol separately in overwintering stage. Chlorophyll content and carotenoid content of rapeseed assays were carried out in 10th leaf stage and overwintering stage following Zhang X-Z's protocol with some modification. Mixture of acetone and ethanol (99%)was adopted to extract the chlorophyll. A UV-240 ultraviolet spectrophotometer was used to determine the OD values of the extraction solutions (λ were 470 nm, 646 nm and 663 nm), these results were then used to calculate Chl a, Chl b, total Chl content and carotenoid content.The LI-COR 6400 (LI-6400) System was used to measure the net photosynthetic rates(P_n) of rapeseed leaves. The whole mensuration was finished between 10:30 a.m.and 11:00 a.m.

Results

C/N Metabolism characteristics in yellow-seed and black-seed rapeseed (Brassica napus L.) during seedling stage

The differences of total sugar/ protein content in stems between yellow-seed and black-seed rapeseed were significant, however, the differences of total sugar/ protein content in leaves between yellow-seed and black-seed rapeseed were not significant. It was found that there were higher total sugar content and lower protein content in yellow-seed rapeseed plants than in black-seed rapeseed plants regardless of various development stages or various plant organs (Table 1). Higher total sugar content and lower protein content may be an important physiological character in yellow-seed rapeseed plant.

Table 1 Total sugar and protein contents in yellow-seed and black-seed rapeseed (*B.napus* L.) at various development stages

Development stage	Genotype		Stems		Leaves	
			Total sugar (%)	Protein content (mg/g)	Total sugar (%)	Protein content (mg/g)
10th leaf stage (1/12)	Yellow seed	L1	14.56c	19.05d	8.83a	30.76a
		L3	15.74bc	20.14c	9.02a	31.04a
	Black seed	L2	10.75ab	23.87b	7.46a	30.97a
		L4	13.31a	24.77a	8.31a	32.15a
Overwintering stage (15/1)	Yellow seed	L1	20.67c	20.33d	14.89a	30.84a
		L3	20.83bc	22.02c	15.32a	30.77a
	Black seed	L2	14.73ab	25.78b	9.11a	48.65a
		L4	16.52a	26.35a	10.97a	40.32a

Notes: Values followed by a different letter are significantly different at 0.05 level, respectively, the same below.

There were lower nitrogen content and activity of nitrate reductase in yellow-seed rapeseed leaves (L1, L3) than in black-seed rapeseed leaves (L2, L4) at overwintering stage. The differences of activity of nitrate reductase in leaves between yellow-seed and black-seed rapeseed were significant (Table 2).

Table 2 Nitrogen content and nitratase activity in yellow-seed and black-seed rapeseed (*B. napus* L.) leaves at overwintering stage

Genotype		Nitrogen content (mg · g^{-1} FW)	Activity of nitrate reductase (NO_2^- μg · g^{-1} · h^{-1})
Yellow seed	L1	0.1152a	20.34b
	L3	0.1346a	21.77b
Black seed	L2	0.2075a	24.35a
	L4	0.3125a	26.02a

The differences of photosynthetic characteristic between yellow-seed and black-seed rapeseed (Brassica napus L.) during seedling stage

The differences of Chl a, Chl b, total Chl content and carotenoid content between yellow-seed and black-seed rapeseed leaves during seedling stage were significant. Chl a, Chl b content and carotenoid content in yellow-seed rapeseed were lower than those in black-seed rapeseed significantly. However the difference of total Chl content was not significant between L1 and L2 but was significant between L3 and L4 (Table 3). What the development stage affects significantly in this experiment was not the Chl a, Chl b content and carotenoid content but the total Chl content. The total Chl content in rapeseed leaves in overwintering stage was higher than that in 10th leaf stage.

The net photosynthetic rate(P_n)of leaves in yellow-seed rapeseed was different from that in black-seed rapeseed siginificantly. The former was lower than the latter regardless of 10th leaf stage or overwintering stage(Fig.1). Development stage affects the net photosynthetic rate(P_n)of rapeseed leaves significantly. The net photosynthetic rate(P_n)in overwintering stage was lower than that in 10th leaf stage(Fig.1).The possible reason may be as following: Temperature and light are two

important environmental factors that affect the photosynthetic rate. The temperature in overwintering stage is lowest in the whole year and there is no enough light in Chongqing during seedling stage.

Table 3 Chlorophyll and carotenoid contents in yellow-seed and black-seed rapeseed (*B.napus* L.) leaves during seedling stage

Development stage	Genotype		Chl-a (mg/g)	Chl-b (mg/g)	Total chl (mg/g)	Carotenoid (mg/g)
10th leaf stage (1/12)	Yellow seed	L1	0.6131c	0.1292c	0.7423b	0.2292c
		L3	0.7863b	0.2007b	0.9870b	0.2895bc
	Black seed	L2	0.8865b	0.2143b	1.1008ab	0.3049b
		L4	1.2765a	0.3091a	1.5856a	0.4560a
Overwintering stage (15/1)	Yellow seed	L1	0.6143c	0.1390c	0.7533b	0.2291c
		L3	0.8861b	0.2237b	1.1098b	0.3234bc
	Black seed	L2	0.9765b	0.2697b	1.2462ab	0.4023b
		L4	1.1984a	0.3116a	1.5100a	0.5085a

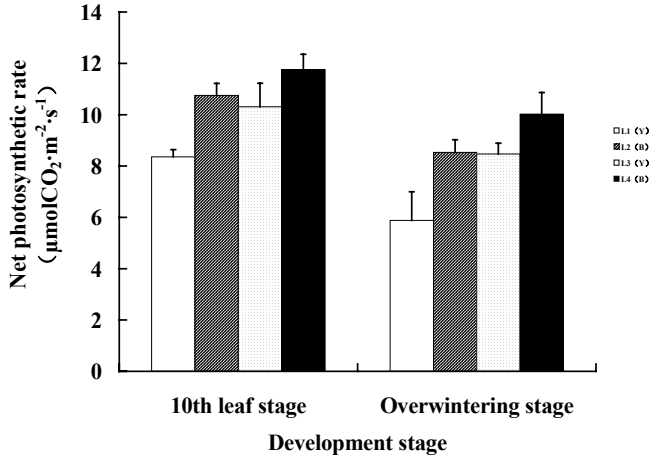

Fig. 1 P_n of yellow-seed and black-seed rapeseed (*B.napus* L.) leaves during seedling stage

Discussion

Although it is believed that yellow-seed rapeseed is more ascendent than black-seed rapeseed (Brassica napus L.) when germinating (Zhang X-K et al., 1999), the C/N metabolism characteristics and the differences of physiological characteristics between yellow-seed and black-seed rapeseed are not clearly known. In this study, we found higher total sugar content and lower protein content was an important physiological character in yellow-seed rapeseed seedling stage. There were higher total sugar content and lower protein content in yellow-seed rapeseed plants than in black-seed rapeseed plants regardless of various development stages or various plant organs. There were lower nitrogen content and activity of nitrate reductase in yellow-seed rapeseed leaves than in black-seed rapeseed leaves at overwintering stage.

Chlorophyll content, carotenoid content and the net photosynthetic rate of yellow-seed rapeseed were lower compared to black-seed rapeseed (Brassica napus L.) from the same genetic background, which meant less abilities of photosynthesis in yellow-seed rapeseed during seedling stage. These results are in accordance with the conclusion that high total sugar content and low protein content were indices of weakgrowth vigor (Zhao H-J et al., 1994). The worse growth vigor in yellow-seed rapeseed during seedling stage may affect the accumulation of dry matter in overwintering stage and affect its procreation latter and this may be one of the important reasons, which led to its lower yield.

Although breeding for yellow-seed rapeseed (Brassica napus L.) has become one of the main objectives in rapeseed breeding, the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed. These characteristics have fundamental problems in extending yellow-seed rapeseed (*Brassica napus* L.). Our study investigated the physiological characters of yellow-seed rapeseed in seedling stage and compared its major physiological characters with the ones of black-seed rapeseed. The results may be useful to explain why the growth vigor and yield of yellow-seed rapeseed were not good as black-seed rapeseed. The results also suggested we should select the new yellow-seed genotypes with better growth vigor, stronger fastness and higher yield. At the same time we should also improve their C/N metabolism characteristics in genetics way. Whether the growth vigor, fastness and yield of yellow-seed rapeseed are not good as black-seed rapeseed essentially is a complicated issue worthy worthy to be explored further. Whether the conclusion is connected with the genotypes and the plant materials used in this study is to be confirmed in continued study.

Conclusions

There were higher total sugar content and lower protein content in yellow-seed rapeseed plants than in black-seed rapeseed (Brassica napus L.) plants regardless of various development stages or various plant organs. The nitrogen content and

activity of nitrate reductase were lower in yellow-seed rapeseed leaves than in black-seed rapeseed (Brassica napus L.) leaves in overwintering stage. Chlorophyll content and carotenoid content of yellow-seed rapeseed were lower than those of black-seed rapeseed (Brassica napus L.), and their net photosynthetic rate was also lower compared to black-seed rapeseed from the same genetic background. The worse growth vigor in yellow-seed (Brassica napus L.) rapeseed during seedling stage may affect its procreation latter and this may be one of the important reasons, which led to its lower yield.

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