Differences of nitrogen efficiency of rapeseed cultivars and their physiological properties

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Abstract

Differences of N efficiency of oilseed rape (*Brassica napus* L.)varieties and their physiological properties were studied using a pot experiment and the ratio of seed yield with stressed nitrogen to that with normal N supply was adopted as nitrogen efficiency coefficient. Results showed that the determined nitrogen efficiency coefficient of 8 rapeseed varieties varied from 0.37 to 0.69, the ratio of nitrogen uptake amounts per plant under N stressed condition to that with normal N supply were different. The higher the nitrogen efficiency, the higher their ratio. Under low nitrogen-supplying condition, high nitrogen efficiency varieties had longer roots, more lateral roots, higher nitrate reuse amounts in stem and leaves and nitrate reductase activities in leaves.

Key words: *Brassica napus* L, Nitrogen efficiency, Nitrogen efficient coefficient, Physiological fundamentals

Introduction

Oilseed rape is an important crop of oil-bearing plants and it needs a large amount of nutrients. Due to its low application efficiency of N fertilizers (Schjoerring et al., 1995), a large amount of N fertilizer has been applied. It is estimated that the rate of N fertilizer to seed rape has reached 200-300 kg/ha N (Schjoerring et al., 1995; Wiesler et al., 2001a), and it is still increased year by year. In contrast, study on the difference of genotypes and selection as well as breeding of seed rape is behind other crops. Some results from other countries have shown that a great difference exists in the genotypes of rapeseed for N efficiency (Wiesler et al., 2001b), and the contribution of N uptake or absorbing efficiency is much higher than N use efficiency (Mahmoud et al.,2004); dropped leaves contain less nitrogen, and the variety with high N harvest index has high N efficiency (Bettina et al.,1999). Rise of activities of asparaginate synthetase and glutamine synthetase (GS) of rapeseed through gene transformation can raise N use efficiency (Beatrix et al., 2004). However, few reports were found for the physiological basis of the difference of the N efficiency for genotypes. This paper compared the difference of N efficiency of some rapeseed genotypes and preliminarily studied their mechanism for providing a scientific basis in the management of N nutrient and selection and breeding of high N efficiency varieties.

Materials and methods

Variety tested: Eight cultivars of rapeseed were used in the experiment, including 742, 231, bin270, Xiangyou 13, Xiangyou 15, Zhongyou 821, Huiyou 50 and Wesbreak. All the varieties were provided by Hunan Sub-center of the Improvement Center of the National Oil Crop.

Soil and Fertilizer: The experiment was conducted in the Resource and Environmental Base of Hunan Agricultural University. The soil used was an alluvial for vegetable cultivation derived from river flow alluvial material, containing organic matter 29.06 g/kg, total N 5.2g/kg, and total P 10.74 g/kg, total K 19.9 g/kg. The NaOH hydrolyzed N was 125 mg/kg, Olsen-P 59 mg/kg, available K 91mg/kg with pH 5.15. Urea was used as N fertilizer, calcium magnesium phosphate as P fertilizer (containing P_2O_5 12%) and potassium chloride (containing K_2O 60%) as K fertilizer.

Pot exzperiment: For collection of dropped leaves, a pot experiment was used for the study. The pot was made from porcelain or china, 22 cm×40cm in size and brown in color. Nine kg air-dried soil was filled in each pot. Two treatments were designed: applying N fertilizer and non-applying N with 3 replicates. For the N treatment, 0.2g/kg N, 0.1g/kg P_2O_5 and 0.1g/kg K_2O were applied while for the non-N treatment, only P and K fertilizer were added. All fertilizers were applied as basal. For each variety, 30 pots were planted with half as N treatment and half as non-N treatment. Pots were arranged in a randomized block. Careful management was conducted during plant growing period.

Sampling, determination and calculation

Sampling: Entire plant was sampled at seedling stage (70d after transplanting), peduncle-growing stage (111d after transplanting), anthesis stage (152d after transplanting), siliqua development stage (169d after transplanting) and harvest stage (189d after transplanting). During sampling, all organs dropped down were collected separately, and then washed, and dried at oven until the weight being constant. However, for root sample, conducted first was length measurement and lateral root number counting before oven-dried process. The samples were then ground, sieved and total N in plants was determined. For the first 3 samplings, the vigorous leaves at the middle part of the plants were chosen, and cut along the middle vein into two parts, half of the leaf cut down being used for determination of nitrate retactase while the other half being oven-dried for estimation of the moisture. The half leaf for determination of enzyme activities was changed into oven-dried weight according to the weight changes before and after oven-dried process.

Determination and calculation: Soil basic properties were determined by normal methods, the plant total N by Kjedahl method, nitrate reductase by vital method (Zou, 1995). Root length by grid counting method (Liu et al., 1997), lateral root number of the first order roots was directly counted. Some methods for calculation of the results are listed as follows: N efficiency coefficient = seed yield without N/seed yield with N; Reuse rate of nitrate N in stem and leaf = the maximum amount of nitrate N in each plant – the minimum amount of nitrate N (the highest period of nitrate accumulation for each plant was at the seedling stage for N stressed treatment while it was at the anthesis stage for the normal supply of N treatment. In contrast, the minimum period for nitrate accumulation was at harvest stage for both N addition and no N addition treatments). Data obtained either from field or from determination in laboratory were subject to variance analysis using SPSS software

Results

Differences of seed yield of rape varieties and N efficient coefficient

Seed yields and N efficiency coefficients were listed in Table 1 for the 8 varieties of rapeseed with different N rate. It was clear from Table 1 that whether N fertilizer was applied or not, difference of the seed yield for the 8 varieties was significant. The range of seed yield without N addition varied from 8.9 g to 15.5 g per plant while from 19.7 g to 24.8 g per plant for that with N application. The variation of yield was higher for that without N than that with N. Without N addition, variety 231 was the highest in yield while variety 742 was the highest with N addition. The lowest yield was found for variety bin270 without addition of N and for variety Huiyou 50 with N addition.

N efficient coefficient could reflect the resistance of rapeseed to N stress. The higher the coefficient, the higher the resistance. Calculated results showed that variety 231 had the highest coefficient followed by Xiangyou 15 whereas the lowest was found for bin270. This indicated that variety 231 and Xiangyou 15 was stronger in resistance to N stress and therefore was relatively a high N efficient variety, whereas bin270 was low in resistance to N stress, and thereby a low N efficient variety.

Table 1 Grain yields and nitrogen efficiency coefficients of different varieties of rapeseed

Treatment	742	231	Bin270	Xiangyou 13	Xiangyou 15	Zhongyou 821	Huiyou50	Wesbreak
No N application (g/plant)	12.2c	15.5a	8.9e	12.3c	13.5b	12.1c	11.6c	10.5d
N application (g/plant)	24.8a	22.4bc	23.8ab	20.9cd	21.4cd	22.9abc	19.7d	20.1d
N efficient coefficient	0.49	0.69	0.37	0.59	0.63	0.53	0.59	0.52

Table 2 N uptake amount of different rapeseed varieties

	742	231	Bin270	Xiangyou 13	Xiangyou 15	Zhongyou 821	Huiyou 50	Wesbreak
				No N application (g/plant)				
Seedling	0.319bc	0.354a	0.303c	0.326bc	0.339ab	0.311c	0.317bc	0.307c
Stem Enlongation	0.533bc	0.667a	0.492c	0.605abc	0.631ab	0.596abc	0.502c	0.503c
Flowing	0.594bc	0.687a	0.535c	0.621abc	0.648ab	0.604abc	0.599abc	0.557bc
siliquing	0.685c	0.826a	0.659c	0.752b	0.790ab	0.697c	0.797a	0.703c
harvesting	0.749c	0.857a	0.648d	0.822ab	0.827ab	0.757c	0.818b	0.753c
				N application (g/plant)				
Seedling	0.857a	0.805abc	0.849ab	0.756abc	0.768abc	0.847ab	0.726bc	0.710c
Stem Enlongation	1.495a	1.484a	1.481a	1.402a	1.433a	1.484a	1.452a	1.420a
Flowing	1.522a	1.552a	1.537a	1.524a	1.534a	1.516a	1.543a	1.513a
Siliquing	1.556a	1.564a	1.545a	1.530a	1.543a	1.537a	1.556a	1524a
Harvesting	1.655a	1.673a	1.719a	1.653a	1.724a	1.651a	1.713a	1.686a
				Ratio of no N application to N application				
Seedling	0.372	0.452	0.357	0.431	0.441	0.367	0.437	0.432
Stem Enlongation	0.357	0.449	0.332	0.432	0.440	0.402	0.346	0.354
Flowing	0.390	0.443	0.348	0.407	0.422	0.398	0.388	0.368
Siliquing	0.440	0.528	0.427	0.492	0.512	0.453	0.512	0.461
harvesting	0.453	0.512	0.377	0.497	0.480	0.459	0.478	0.447
Average	0.372	0.452	0.357	0.431	0.441	0.367	0.437	0.432

Difference of N uptake and accumulation in plants of rape variety

N amount taken up and accumulaed in plants as well as their ratio are listed in Table 2 for both N addition and non-addition treatments. Under the condition without N addition, N amounts absorbed and accumulated in plants were greatly significant while only was the seedling stages found significant for N addition treatment. Without N addition, variety 231 was the highest in N uptake at any stage during the entire growing period while variety Bin270 the lowest. With application of N fertilizer, variety 742 was the highest while Wesbreak the lowest in N uptake at seedling. Variety 231 was the highest in the ratio of plant uptake N for treatment without N addition to that with N addition during the entire growing period, showing this variety having a relatively higher ability of absorbing N under N stressed condition. This might be one of the important, physiological bases for rape's resistance to N stress.

Differences of root length, lateral root number, NR activity, and the reuse of N amount in plants

Using the average of seedling, peduncle and anthesis stages as criteria to analyze the root length, lateral root number and NR activity of leaf, results showed that with N stress, all the variables were significant among varieties (Table 3) while with normal supply of N, only was the significance of root length and NR activity found. Seen from the ratio of no N addition to that with N addition, it is clear that under the N stressed condition, the root length for all verities was increased, the increased degree depending on variety. The highest increase was found for Xiangyou 15 followed by 231, while the lowest was for Bin 70. For the lateral root number, 231, Xiangyou 15 and Xiangyou 13 were increased, whereby Huiyou 50 kept stable and the other varieties decreased. The NR activity in leaf and stem was increased only for 231 and Xiangyou 15 with other varieties being decreased. The reuse of N in stems and leaves were all decreased in a large amount except 231 and Xiangyou 15 that decreased much less than others.

Table 3 The root length, number of lateral root, nitrate reductase activity and reuse amount of nitrates in stem and leaf of different rapeseed varieties

	742	231	Bin270	Xiangyou 13	Xiangyou 15	Zhongyou 821	Huiyou 50	Wesbreak
	No N application							
Root length (m/plant)	235c	272a	185e	246bc	261ab	213d	199de	202de
Number of lateral root (number/plant)	40cd	52a	32e	45bc	48ab	36de	40cd	38de
Nitrate reductase activity (g/gFW)	12.0ab	14.7a	9.9b	12.5ab	13.9a	12.4ab	10.6b	10.3b
Reuse amount of nitrate in stem and leaf (μg/gDW)	91.9b	105.6a	81.7c	91.4b	100.2a	90.6b	82.3c	85.0bc
	N application							
Root length (m/plant)	233a	222ab	180d	230a	203bc	186cd	181d	174d
Number of lateral root (number/plant)	45a	45a	41a	42a	44a	38a	40a	40a
Nitrate reductase activity (g/gFW)	14.1a	13.2a	13.8a	12.7a	13.2a	13.3a	11.8a	11.4a
Reuse amount of nitrate in stem and leaf (μg/gDW)	340a	327ab	324ab	309b	317ab	322ab	311ab	309b
	Ratio of no N application to N application							
Root length	1.01	1.23	1.03	1.07	1.29	1.15	1.10	1.16
Number of lateral root	0.89	1.16	0.78	1.07	1.09	0.95	1.00	0.95
Nitrate reductase activity	0.85	1.11	0.72	0.98	1.05	0.93	0.90	0.90
Reuse amount of nitrate in stem and leaf	0.27	0.32	0.25	0.30	0.32	0.28	0.26	0.28

Discussion

Differences of N efficiency of different varieties

Currently, great concern has been given to breeding and extension of high N efficiency verities to reduce N pollution to the environment and save N resources. However, most of breeding are conducted under the condition with nutrient sufficient supply, and therefore the varieties released are those that could obtain high yield providing sufficient nutrients are supplied. It is not known what will be the results when reduction of N rate is performed. For this reason, this experiment selected 8 varieties to study the responses of the varieties when N was deficient. Results showed that deficiency of N had decreased the yield of all varieties, the degree of reduction varying with the varieties. Variety 231 was the lowest in yield reduction, highest in N efficiency coefficient and stronger in resistance to N stress, and therefore was a relatively high N efficiency variety. In contrast, bin70 was the highest in yield reduction, low in N efficiency coefficient, and weak in resistance to N stress, and therefore was relatively a low N efficiency variety. The other varieties were in between of the two. For this reason, the breeding for the future should be conducted under low N condition (Lafitte et al., 1994).

Relation of N uptake efficiency to root morphological property

Nutrient absorbing efficiency is one of the important causes of variation of nutrient efficiency (Liu et al., 2002). Root morphological and structural properties directly influence nutrient-absorbing efficiency. Plants having longer roots and vigorous lateral roots will have larger root areas to contact medium and therefore high potential to take up nutrients (Mahmoud et al., 2004; Huang et al., 2001). Just because of this, most plants extend their root length and increase the ratio of root to shoot under nutrient stressed conditions. Results showed that with N stress, varieties with a relatively high N efficiency had longer roots and higher lateral root number as well as higher N uptake during the entire growing period than those of other varieties. However, with sufficient N supply, N uptake amount for different varieties was only significantly higher at early stages, indicating the importance of supply of N to such stages. This might be caused by the confliction between oil and protein during the formation of rape seeds: at the later stage, increase of N uptake may be not beneficial to oil accumulation while at early stages, uptake of N not only benefits vegetable growth, but also provides a material basis for reproductive organ development. As a result, at later stage, part of the accumulated N could be transferred to seeds. This may alleviate the conflict between N and C metabolism during seed formation, and meet the need of N for seed development. However, it is impossible for this experiment to provide direct evidence, and further study is needed for this matter.

Relation of N use efficiency to nitrate assimilation and its reuse

Like other dryland crops, nitrate N is the major form for rapeseed uptake. Nitrate N taken up by plants must be transformed into amino acid and protein and then be used to build up plants. During the process of transformation, NR is the limiting enzyme that controls the use velocity (Abdoulaye et al., 1999) and that is affected by N supply. Results showed that under N stressed conditions, most of varieties reduced their NR activities while only did 321 and Xiangyou 15 increase its activity, resulting in the increase of NR ratio of no N addition to that with N addition, indicating the close relationship between rapeseed resistance to N stress and NR activities.

This certainly is beneficial to improvement of N use efficiency. It is reported that there existed the difference of the nitrate storage pool for different genotype varieties. However, it is not known if there is a different reuse capacity or not. In this experiment, the reuse ability is expressed by the difference between the highest and lowest accumulated nitrate amount of each plant. Results showed that under N stressed conditions, the accumulated nitrate N was decreased, and at later stages, the reused rate of nitrate N accumulated in stems and leaves reduced. However, even in this case, the amount reduced for the higher N efficient varieties was much less than other varieties, indicating that N use efficiency of rapeseed is closely related to the reuse ability of nitrate N in stems and leaves.

To sum up, under low N stress, N in rape plants was higher in assimilation and circulation, and rapid circle and higher reuse ability might be one of the important causes of high efficiency or high physiological efficiency.

Acknowledgements

This work is part of the project (30671206) supported by the National Natural Science Foundation of China (NSFC) and project 948 (2003-Q04) supported by the National Agricultural Department.

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