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Nitrogen Efficiency in Oilseed Rape and Its Physiological Mechanism

Zhen-hua Zhang[1,2,3,4], Hai-xing Song[1,2,3,4]* and Chunyun Guan[5]
*[1]Department of Plant Nutrition,
College of Resources and Environment,
Hunan Agricultural University,
[2]Hunan Provincial Key Laboratory of Plant Nutrition in
Common University, Changsha,
[3]Hunan Provincial Key Laboratories of Farmland Pollution Control and
Agricultural Resources Use, Changsha,
[4]National Engineering Laboratory of High Efficiency Utilization of Soil and
Fertilizer Resoureces, Changsha,
[5]National Center of Oilseed Crops Improvement,
Hunan Branch, Changsha,
China*

1. Introduction

N fertilizer application can guarantee the high yield of crop; it is a general method to improve the yield of crop (Zhang et al., 2010). However, not only N use efficiency was declined, but also environment contamination was serious day by day because of N fertilizer over application (Zhang et al., 2010). So, control and reduction in the amount of N fertilizer application is very important, it is necessary to dredge up the potential of N absorption and N use efficiency of crop. Oilseed rape (Brassica napus L.) is an important oil production crop in China. However, the N use efficiency and N efficiency of oilseed rape are very low (Schjoerring et al., 1995). The N application amount has reached 200-330kgN per ha (Schjoerring et al., 1995; Wiesler et al., 2001a) and it is increasing year by year. But the studies on differences of N efficiency in different oilseed rape varieties and breeding research of the oilseed rape with high N efficiency were relatively slower than other cereal (Liu et al., 2009). Oilseed rape requires high amounts of N for growth, but the N efficiency (seed yield per unit of accumulated N in plant) is very low. Consequently, it is necessary to improve the N efficiency (Rathke et al., 2006). In generally, N efficiency has two components: N uptake efficiency and N utilization efficiency (Sattelmacher et al., 1994). The differences of N efficiency between oilseed rape genotypes are significant (Wiesler et al., 2001b; Christian et al., 1999). Kessel et al., 1999 has been measured N concentration of organs in different oilseed rape genotypes, results suggested that oilseed rape possess the 2 physiological characteristics (low N concentration in dropped leaves, high N harvesting

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* Corresponding Author

index) has higher N efficiency also. The development of N efficient genotypes and improvement of N management will require to understand the relationship between physiological processes and biomass, yield formation of crop under no N application conditions (Dreccer et al., 2000). Mahmoud et al., (2005) compared the differences of root growth and nitrate N exhaust in cultivar culture between high N efficiency and low N efficiency oilseed rape; larger amount of root biomass, higher root uptake activity and higher exhausted nitrate N amount were found in the high N efficiency oilseed rape; Nitrate-N uptake from soil depends on root growth and uptake activity, the amount of N depleted from the compartments significantly correlated with root-length density (Mahmoud et al., 2005). Seiffert et al., (2004) suggested that, the N use efficiency can be increased significantly by strengthen the activities of asparagine synthetase and glutamine synthetase through transgenic methods. Obviously, it is necessary to 'system study' on N uptake and N use efficiency in different oilseed rape. The differences of yield, N absorption and N use efficiency in different oilseed rape genotypes should be compared, and preliminarily discussion the contribution of N absorption efficiency and N use efficiency to N efficiency under different N application levels, in order to supply scientific basic and plant materials for the future study.

The differences of concentration and distribution of N in crop is depending on the differences of organs and growth stages, and N redistribution in different organs will occur at different growth stages; these differences are related to the transfer of growth center (Peoples et al., 1998; Zhang et al., 2008). Studies in wheat (Andersson et al., 2005), oilseed rape (Malagoli et al., 2005) and pea (Séverine et al., 2005) showed that the leaves and stems of crop have become important N "sources" after the flowering stage, grain N does not only come from root N uptake during the later growing period, but also from that redistributed from the vegetative organs (Zhang et al., 2010). The latter was one of the key factors that guarantee crop N requirement during the later growing period. Nutrient competition between different organs of the crop during the later growing period is fierce; the uptake ability of the root declines at this time and soil mineral nutrients are exhausted (Dong et al., 2009). In oilseed rape, all leaves drop off at harvesting stage and N in the leaves is lost. To achieve high yield and high N use efficiency in crop production, N must be redistributed from the roots to the grains, residue N in uneconomic tissues must be reduced, and N harvesting index of economic tissues must be increased (Zhang et al., 2010). Research on N redistribution in cereals reached the following conclusions. The redistribution proportion of N from the leaves, stems and reproductive organs to the grains was more than 60% in wheat (Palta et al., 1995a, 1995b). Dalling et al., (1985) showed that, although the soil can supply adequate N after the crop flowering stage, at least 50% of the N in grains is still redistributed from the vegetative organs. The effect of environmental conditions on N redistribution was considerable e.g., low N in barley (Przulj et al., 2001) and drought stress in wheat (Barbottin et al., 2005; Palta et al., 1994) can accelerate N redistribution in plants, but the process can be restrained when plants are damaged by diseases and insects e.g., wheat (Dimmock et al., 2002). However, only a few studies looked at N redistribution in oilseed rape. Malagoli et al., (2005) found that in oilseed rape the N of flowers and pods was mainly from endogenous N, comprising 64 and 73% of total N content in these tissues, respectively. The N requirements of seed filling were mainly satisfied by N mobilized from the vegetative parts. Rossato et al.,

(2001) studies of *Brassica napus* L. suggested that large amounts of endogenous N were redistributed from the leaves to the stems to the roots tap tissues which acted as a buffering storage compartment and later used to supply the reproductive tissues. About 15% of total N cycling through the plant was lost through leaf fall and 48% had been remobilized from vegetative tissues and finally recovered in the mature pods (Thomas et al., 2007). It can be concluded that, the accumulation and distribution characters of N in vegetative and reproductive organs are important factors that affected the crop production (Zhang et al., 2006), the higher yield production is not only dependent on higher N absorption, but also depend on higher N distribution efficiency (Wang et al., 2003). Studies on the regular of N absorption, distribution and redistribution at different growth stages will supply scientific foundation for rational application of N fertilizer, improving N harvesting index and increasing crop production. However, most of these studies mainly focused on the redistribution of N in cereal crops such as wheat (Anderson et al., 2005; Dalling et al., 1985), maize (Pommel et al., 2006) and rice (He et al., 2002). A few of them (Rossato et al., 2002; Malagoli et al., 2005) looked at oilseed rape. Moreover, studies on the relationship between N redistribution and crop production were fewer. In this study, two oilseed rape varieties (X-36 and X-50) with different N use efficiencies were grown in sand culture with complete nutrient solution and normal N supply. To better understand the N dynamics during later growth stages, the relationship between N redistributed from the vegetative organs to the grain and grain yield was studied using the ^{15}N labeling method.

2. Materials and methods

2.1 Comparison of Nitrogen (N) efficiency between oilseed rape genotypes

2.1.1 Plant materials and experiment design

The experiment was conducted at Agricultural Resources and Environmental College Experiment Field in HuNan Agricultural University during Sep. 2004 to May. 2005. 16 oilseed rape varieties were used as plant materials, the numbers are shown in Table 2, plant materials were supplied by China National Oilseed Crop Improvement Center, Hunan Branch. The soil used was an alluvial for vegetable cultivation derived from river flow alluvial material, containing organic matter 27.79 g/kg, total N 1.90 g/kg, and total P 0.79 g/kg, total K 19.76 g/kg. The NaOH hydrolyzed N was 108.31 mg/kg of soil, Olsen-P 15.85 mg/kg of soil, available K 19.76 g/kg of soil with pH 5.67. 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.

The experiment had two N treatments: N application and no N application, 16 oilseed rape varieties used as plant materials, 32 treatments, 3 replicates, 96 districts, 10.5 m² per district, randomized block. N application treatment: 225kg N, 75kg P_2O_5 and150 kg K_2O per hectare, 50% N fertilizer used as basal fertilizer, 20% N fertilizer used as added fertilizer during winter, 30% N fertilizer used at stem elongation stage, all P and K fertilizer were used as basal fertilizer; no N application treatment: the same with N application treatment except of non-N fertilizer applied. Seedling on Sep. 25 2004, transplanted on Nov. 2 2004, transplanted density was 100 thousand plants per ha, management as normal.

2.1.2 Sampling, determination and calculation

Sampled whole plant at harvesting stage, separated it according to organs after clear, dried until constant weight, measured dry weight and total N. The measurement methods followed by the general agricultural chemistry analysis methods; the plant total N measured by Kjedahl method (Hardy et al., 1968). Below formula was used to calculate N use efficiency based on total N, N use efficiency=grain yield / plant total N. Data has been analysis by T-test using SPSS statistic software (Norusis, 1990), the significant differences between the two varieties (p<0.05) are figured by stars (*) in Table 6.

2.2 Vegetative N redistribution during later growing period and its contribution to N efficiency

2.2.1 Variety tested and crop culture

High N use efficiency variety X-36 and low N use efficiency variety X-50 were used as plant materials and grown in a glasshouse in the field experiment station of Hunan Agricultural University (Southern China) with average temperature 19 °C and natural light. These materials were selected from 30 varieties that have been evaluated in the last 10 years in Hunan Province. All the varieties were provided by the Hunan Sub-center of the Improvement Center of the National Oil Crop.

The sand culture and ^{15}N labeling method were used in this experiment. On a 30 cm × 30 cm brown plastic bowl, sand culture (water and dilute hydrochloric acid were used to clean the growth matrix) with complete nutrient solution and normal N supply was used as growth medium. The culture solution had these components: KNO_3 5 mmol·L^{-1}, KH_2PO_4 1 mmol·L^{-1}, $MgSO_4$ 7 mmol·L^{-1}, $Ca(NO_3)_2$·4H$_2$O 5 mmol·L^{-1}, Fe-EDTA 3 mmol·L^{-1}, B 0.5 mg·L^{-1}, Mn 0.5 mg·L^{-1}, Zn 0.05 mg·L^{-1}, Cu 0.02 mg·L^{-1}, Mo 0.01 mg·L^{-1}, $Ca(^{15}NO_3)_2$ and $K^{15}NO_3$ (Shanghai Chemical Engineering Corporation Research Institute) with ^{15}N excess = 20.28% were used as N sources for the labeling treatment.

2.2.2 Experimental treatment and labeling

The seedlings were planted on Sept 25, 2005; transplanted on Nov 2, 2005; one plant per bowl, 72 bowls per variety and harvested on May 5, 2006. ^{15}N labeled days and time of sampling are shown in Table 1. Labeling was done at the seedling stage (Dec 2-22, 2005; 21 d), stem elongation stage (Jan 21-25, 2006; 5 d), flowering stage (Mar 3-7, 2006; 5 d) and siliquing stage (Apr 15-23, 2006; 9 d) . Samples were taken at the end of each labeling stage (Dec 23, 2005; Jan 26, 2006; Mar 8, 2006; Apr 24, 2006). The other plants were transplanted into sand culture with no ^{15}N nutrient and sampled during later growth stages (flowering stage, Mar 8, 2006; siliquing stage, Apr 24, 2006; harvesting stage, May 5, 2006; Table 1), in order to distinguish between N redistributed and N taken up.

Labeling end stage in Table 1 means the end of each labeling treatment at four growth stages. Take seedling stage for instance. Labeling treatment was done at the seedling stage (Dec 2-22, 2005; 21 d); labeling treatment application on Dec 2, 2005; and finished on Dec 22, 2005. Sampling time at labeling end stage means 6 plants per variety were sampled on Dec 23, 2005. The other plants (12) were transplanted into sand culture with no ^{15}N nutrient and sampled at siliquing stage (Apr 24, 2006) and harvesting stage (May 5, 2006), in order to

distinguish between N redistributed and N taken up. Sampling schedule for the other stages are shown in the text and this table.

Days for ^{15}N labeling	Pot number	Sampling time			
		Labeling end stage	Flowering stage	Siliquing stage	Harvesting stage
Seedling stage, 21 days	3×6=18	Root, stem, leaf		Root, stem, leaf, Silique	Root, stem, leaf, silique husk, grain
Stem elongation stage, 5 days	4×6=24	Root, stem, leaf	Root, Stem, Leaf, Flower	Root, stem, leaf, Silique	Root, stem, leaf, silique husk, grain
Flowering stage, 5 days	3×6=18	Root, stem, leaf, flower		Root, stem, leaf, silique	Root, stem, leaf, silique husk, grain
Siliquing stage, 9 days	2×6=12	Root, stem, leaf, silique			Root, stem, leaf, silique husk, grain

Table 1. Time of labeling (^{15}N) and sampling scheme at each growth stages

2.2.3 Sampling, determination and calculation

Whole plants were sampled according to the design indicated in Table 1. There were 6 replications (randomly selected were 6 plants per sampling time per variety), subsamples were taken from different tissues after clearing and drying. Biomass dried to a constant weight was measured. Dry samples were ground and sifted for isotope analysis. To measure biomass and calculate total N exactly, fallen leaves were collected in time. The concentration of N in the plant was measured using the Kjeldahl method (Kamprath et al., 1982); plant total N, grain total N, harvesting index and N harvesting index were calculated using the following formulas:

$$\text{Total N in plant} = \text{plant biomass} \times \text{N concentration in plant tissues}$$

$$\text{Grain total N} = \text{grain yield} \times \text{N concentration in grain}$$

$$\text{Harvesting index} = \text{grain yield/biomass}$$

$$\text{N harvesting index} = \text{grain total N/ total N in plant}$$

The samples were pre-processed by semi micro determination (Brenna et al., 1998). The excess ^{15}N in each tissue was measured by the use of a continuous flow isotope mass spectrograph at the Institute of Genetics, Hebei Academy of Agricultural Science. The amount and proportion of redistributed N from the vegetative organs to the reproductive organs were calculated as follows:

N redistribution proportion = ^{15}N accumulated amount in reproductive organs/^{15}N accumulated amount in the plant at the end of the labeling treatment×100 % (Because the flower and silique are reproductive organs of the plant, labeling was done at the flowering and siliquing stages, the ^{15}N accumulated amount in the reproductive organs should be

subtracted from the ^{15}N accumulated amount in the reproductive organs at the end of the labeling treatment.);

N redistribution amount = N redistribution proportion × N accumulated amount in the plant at the end of labeling treatment;

N loss proportion = [^{15}N accumulated amount in the plant at the end of the labeling treatment - [15]N accumulated amount in the plant at harvesting stage (or siliquing stage)]/^{15}N accumulated amount in the plant at the end of labeling treatment×100 %;

N loss amount = proportion of N loss × N accumulated amount in the plant at the end of the labeling treatment

The partitioning of absorbed N was calculated from the excess ^{15}N in each tissue combined with the calculated total plant N uptake. This is based on the assumption that unlabeled N from the sand culture was taken up and allocated in the different plant tissues in a way similar to that of labeled N. All data were analyzed using the T-test by SPSS statistic software. The significant differences between the two varieties (p<0.05) were indicated by asterisks (*) in the table.

3. Results

3.1 Grain yield between different oilseed rape varieties

The results of grain yield showed that, the differences of grain yield between different oilseed rape varieties were significant, regardless N application level (Table2). The rang of yield variation in 16 varieties were 1060-1913 kg per hm[2] under no N application condition; the yields of Xy1, Xy7 and Xh20 were higher (≥2720kg per hm[2]), the yields of Xy8, Xy9, Xy11

Variety No.	No N application	N application	N application - No N application
Xy1	1913a	2490ab	577bc
Xy6	1132de	1630e	498bcd
Xy7	1908a	2290bc	382cd
Xy8	1105e	1620e	515bcd
Xy9	1060e	1550e	490bcd
Xy11	1063e	1650e	587bc
Xy12	1730ab	2070cd	340d
Xy13	1381cd	2429abc	1048a
Xy14	1659ab	1770de	111e
Xy15	1742ab	1720de	-22e
Xy16	1696ab	2290bc	594bc
Xy17	1813ab	2445ab	632b
Xh19	1801ab	2720a	918a
Xh20	1908a	2786a	878a
Xy21	1837ab	2480ab	643b
Xy24	1588bc	1920de	332d

Different letters in the same row indicated that differences between varieties in t-test are significant at p<0.05 level. The same as below.

Table 2. Grain yield of different oilseed rape varieties

were lower (≤1105 kg per hm^2). N response can be expressed by ratio of (yield with N application – yield with no N application) / N application amount. Because the N application amount of all varieties under N application treatment were the same in this study; so, the N response can be indirectly expressed by the differences of yield under N application condition and no N application condition. N responses of Xy13, Xh19 and Xh20 were higher than other varieties significantly; N responses of Xy14, Xy15 were lower than other varieties significantly. In the case of Xy15, grain yield can be increased by N application significantly, average increased of yield in sixteen cultivars were 35%.

3.2 N absorption amount and N use efficiency in different oilseed rape varieties

Results of table3 showed the relationship between N absorption amount and grain yield. N absorbed amount of different oilseed rape varieties were different under different N application level conditions. The differences of N absorption amount between varieties were significant under no N application condition; however, the differences were smaller under N application condition. N absorption amount of Xy1, Xy21 and Xy24 (≥1.688g per plant) were more than other varieties Xy8, Xy9 and Xy13 (≤1.293g per plant) significantly. The relationship between N absorption amount and grain yield showed that, the correlation between N absorption amount and grain yield was significant under no N application condition, correlation coefficient was 0.685** ($R_{0.01}$=0.623); but under N application condition, there was no significant correlation, and the correlation coefficient was 0.415($R_{0.05}$=0.497). This indicates that, only under the no N application condition, grain yield can be increased significantly by increased N absorption amount. The differences of N use efficiency between varieties were significant according to the differences of N absorption amount, regardless of N application level. The changes scope of N use efficiency in sixteen varieties was 7.1-12.6, the highest was Xh20 of 12.6, and the lowest was Xy9 of 7.9.

Variety	N absorption (g/plant)		N use efficiency (g/g)	
No.	No N application	N application	No N application	N application
Xy1	1.744a	2.200a	11.0bc	11.4abc
Xy6	1.467d	2.207a	7.7ef	7.4e
Xy7	1.448d	2.209a	13.2a	10.4bc
Xy8	1.254e	2.151a	8.8def	7.6e
Xy9	1.285e	2.186a	8.2ef	7.1e
Xy11	1.456d	2.154a	7.3f	7.7e
Xy12	1.545bc	2.149a	11.2abc	9.6cd
Xy13	1.293e	2.245a	10.7bcd	10.8abc
Xy14	1.462d	2.192a	11.4abc	8.1de
Xy15	1.518cd	2.177a	11.5ab	7.9de
Xy16	1.489cd	2.177a	11.4abc	10.5bc
Xy17	1.500cd	2.158a	12.1ab	11.4abc
Xh19	1.550bc	2.206a	11.6ab	12.3ab
Xh20	1.603b	2.224a	11.9ab	12.6a
Xy21	1.688a	2.224a	10.9bc	11.2abc
Xy24	1.688a	2.242a	9.4cde	8.6de

Table 3. N absorption and use efficiency of different oilseed rape varieties

3.3 The differences of N efficiency in different varieties and its analysis

Results of Table 2 and Table 3 showed that, the differences of N efficiency, N absorption efficiency and N use efficiency between varieties were significant under no N application condition; and the differences of N efficiency and N use efficiency between varieties were significant under N application condition; while there were no differences of N absorption efficiency between varieties. Grain yield of high N efficiency variety is higher than average yield of 16 varieties under no N application condition; otherwise it was low N efficiency variety. D-value of grain yield of high N response variety between no N application and N application conditions is higher than average D-value of 16 varieties; otherwise it is low N response variety. The 16 varieties can be divided into 4 genotypes according to the definitions (Table4): (1) N high efficiency – N high response; yield was higher under no N application condition; yield was increased significantly under N application condition (see varieties Xy1,Xy16,Xy17,Xh19,Xh20 and Xy21); (2) N low efficiency – N low response; yield was lower under no N application condition; yield increase was not distinct under N application condition; (see varieties Xy6, Xy8 and Xy9); (3) N high efficiency – N low response, yield was higher under no N application condition; but the yield increase was not distinct under N application condition (see varieties Xy7,Xy12,Xy14,Xy15 and Xy24); (4) N low efficiency – N high response; yield was lower under no N application condition, but the yield was increased significantly under N application condition (see varieties Xy11 and Xy13).

Type	NHE-NHR	NLE-NLR	NHE-NLR	NLE-NHR
Variety	Xy1 Xy16 Xy17 Xy19 Xy20 Xy21	Xy6 Xy8 Xy9	Xy7 Xy12 Xy14 Xy15 Xy24	Xy11 Xy13

Table 4. N efficiency and response type of oilseed rape

Results of Table5 showed that, variation coefficient of grain yield were almost the same regardless of N application level. Variation coefficient of N use efficiency was higher than variation coefficient of N absorption amount under the two N application level conditions. It was suggested that, the contribution of N use efficiency to N efficiency was higher than N

N Levels		Mean	S	CV(%)
No N application	Grain yield (kg/hm²)	1584	323	20.4
	N absorption (g/Plant)	1.499	0.142	9.5
	N use efficiency (g/g)	10.5	1.7	16.2
N application	Grain yield (kg/hm²)	2116	424	20.0
	N absorption (g/Plant)	2.194	0.031	1.4
	N use efficiency (g/g)	9.7	1.9	19.5
Subtraction of both		533	277	52.1

Table 5. Variation of Grain yield, N absorption and use efficiency of different oilseed rape varieties

absorption efficiency in oilseed rape under the field condition, regardless of N application level. The differences of variation coefficient between N absorption amount and N use efficiency were smaller under no N application condition, compared with N application condition. It was suggested that variation coefficient of N use efficiency was declined, but variation coefficient of N absorption amount was increased under N stress condition. This indicates that, the contribution of N absorption efficiency to N efficiency was increased with N application level, but the contribution of N use efficiency to N efficiency was decreased.

3.4 Biomass and N absorption of the two oilseed rape varieties

Table 6 results showed that there is no significant difference of N content in plant and grain between X-36 and X-50, in addition, biomass, harvesting indexes and total N amount of X-36 are almost the same with X-50. However, grain yield and N harvesting indexes of X-36 are higher than X-50 significantly. This indicates that the reasons for grain yield of X-36 being higher than X-50 was not greater N absorption and harvesting indexes but rather higher N redistribution amount and higher N harvesting index.

Variety	Biomass (g⊙plant^1)	Grain yield (g⊙plant^1)	N concentration of plant (mg⊙g^{-1}DW)	N concentration of grain (mg⊙g^{-1}DW)	Total N in plant (g⊙plant^1)	Total N in grain (g⊙plant^1)	Harvesting index	N harvesting index
X-36	119.8±9.5	31.0±2.6*	27.0±0.9	44.2±1.8	3.22±0.16	1.37±0.07	0.26±0.024	0.43±0.029*
X-50	111.4±8.7	25.7±2.9	28.1±1.8	45.5±1.7	3.12±0.23	1.17±0.14	0.23±0.018	0.37±0.029

*Indicated that differences between varieties in t-test are significant at p<0.05 level. The same as below.

Table 6. Physiological parameters of two oilseed rape varieties

3.5 Distribution characters of N absorbed at different growth stages

Fig.1 results showed that N absorbed at seedling and stem elongation stages are mainly distributed into leaves, average proportions of transferred-N/total-N in two varieties are accounting for 83.5% and 66.3% respectively. N absorbed at flowering stage is mainly distributed into leaves and stem, proportions are accounting for 42.8% and 36.3% respectively. While 42% (average values of two cultivars) of N absorbed at siliquing stage is directly distributed into silique. Proportion of N distributed into leaves is declined on a large scale with senescence of plant, proportion of N distributed into stem and root are declined also, however proportion of N distributed into silique and grain are increased at the same time. Proportion of N absorbed at seedling stage is redistributed from vegetative organs to grain in X-50 is the lowest, but still 43% of N is distributed into grain at harvesting stage.

In addition, proportion of N that was distributed into leaves at flowering stage in X-36 and X-50 are 47.3%, 40.7% respectively, and siliquing stages are 30.6%, 25.8% respectively. Proportion of N that was distributed into grain at harvesting stage in X-36 are higher than X-50, and proportion differences between the two varieties of N absorbed at seedling and flowering stages are significant (Fig.1 and Table7). Higher proportion of N distributed into leaves is a benefit for normal functions of leaves development, higher proportion of N distributed into grain is benefit for N harvesting indexes improvement.
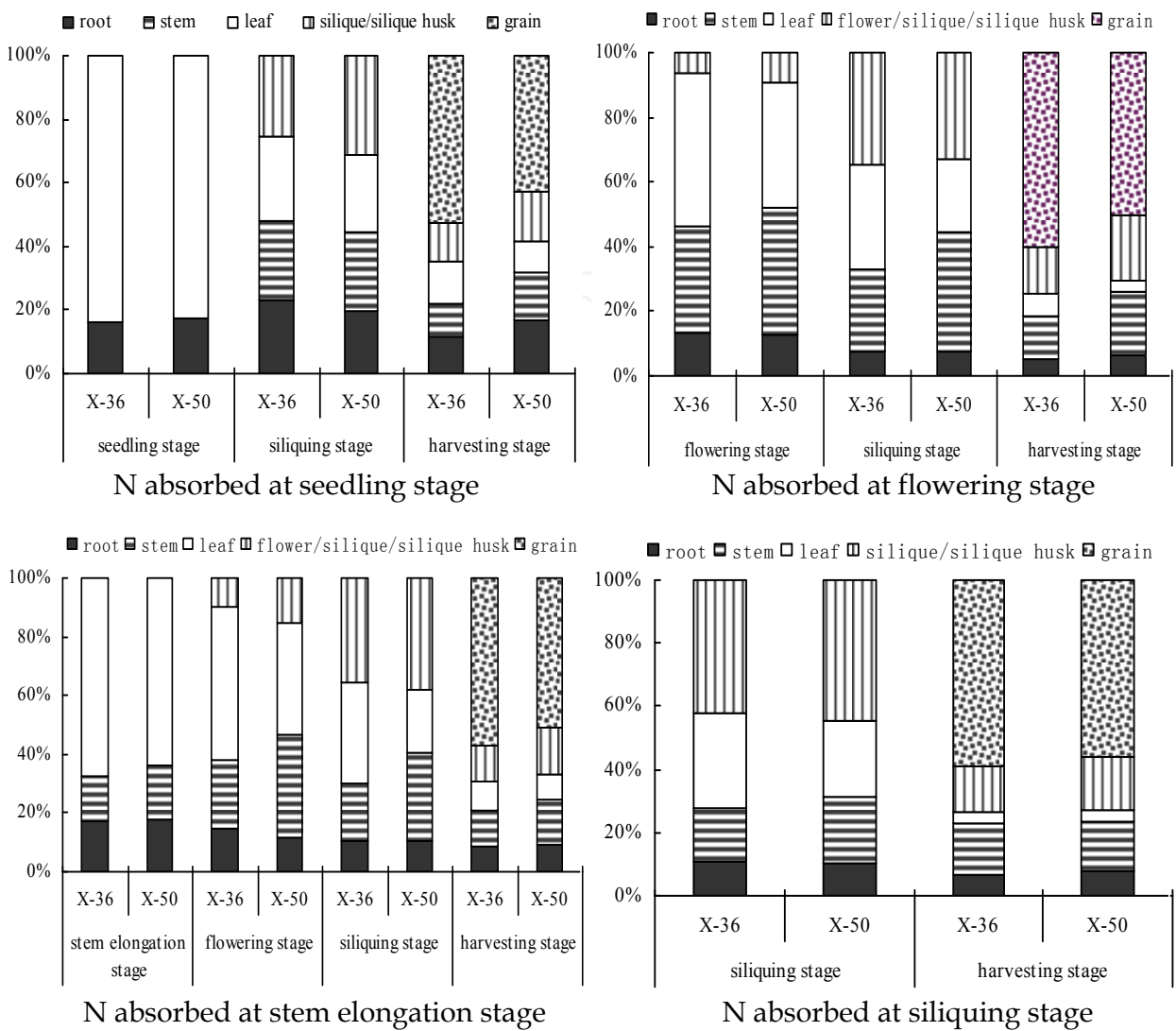
Fig. 1. Distribution proportion of N which absorbed at different growth stages in oilseed rape (vertical axis is stand for N distribution proportion among plant organs, lateral axis is stand for different growth stages)

Physiological indexes	Variety	N absorbed at seedling stage	N absorbed at stem elongation stage	N absorbed at flowering stage	N absorbed at siliquing stage	Sum
Amount of N transferred (mg)	X-36	231.2*	325.5	275.5*	88.9*	921.1
	X-50	175.1	326.0	160.4	75.0	736.5
Proportion of N transferred (%)	X-36	38.2*	46.8	49.6*	32.8	
	X-50	30.6	41.8	32.8	30.6	
Proportion of transferred-N/ grain-N (%)	X-36	16.9	23.8	20.1*	6.5	67.2
	X-50	15.0	27.9	13.7	6.4	62.9

Table 7. Translocation to grain of N which absorbed at different stages

3.6 Distribution proportion of N absorbed at different growth stages

Table7 results showed that distribution proportion of N absorbed at stem elongation and flowering stages are higher than other stages, average proportion at stem elongation and flowering stages are 44.3% , 41.2% respectively, and 34.4% , 31.7% respectively at seedling and siliquing stages. Distribution amount of N absorbed at stem elongation stage is the highest, average value is 325.8 mg, following are N absorbed at flowering and seedling stages, average values are 218.0 mg and 203.2 mg respectively, the lowest is N absorbed at siliquing stage, average value is 82.0 mg.

Average proportion of transferred-N/grain-N in two varieties is 65.1%. Distribution proportion of N absorbed at stem elongation stage is the highest, accounting for 25.8%, following are N absorbed at flowering and seedling stages, accounting for 16.9 % and 15.9% respectively, the lowest is N absorbed at siliquing stage, accounting for 6.4%. Table3 results also showed that distribution amount of N absorbed at seedling, flowering and harvesting stages in X-36 are higher than X-50 significantly, distribution proportion of N absorbed at seedling and flowering stages in X-36 are higher than X-50 significantly.

N absorbed at seedling and stem elongation stages that was distributed into reproductive organs, it is real N that redistributed from vegetative organs to reproductive organs. However, part of N absorbed at flowering and siliquing stages was directly transferred into reproductive organs, therefore total N in reproductive organs is not only because of N redistribution, but also because of root absorption. So, differences amount of N, which was absorbed at flowering and siliquing stages, between absorption and redistribution can not be distinguished in this study, but the main results can not be affected by these influences significantly.

3.7 Loss amount and proportion of N at harvesting stage

Table 8 results showed that loss proportion and amount of N absorbed at seedling stage are the largest, average values of the two varieties are 24.0% and 141.6 mg, the next is N absorbed at stem elongation and flowering stages, average proportion values are 10.5% and 11.7%, average amount values are 79.2 mg and 43.2 mg respectively, the smallest loss are N absorbed at siliquing stage, average values are 7.3% and 16.2 mg. Table 4 also showed that N loss proportion and amount of X-36 are lower than X-50, but there are no significant different. Loss amount of N absorbed at stem elongation and flowering stages in X-36 (63.0 and 35.0 mg) are lower than X-50 (95.3 and 51.4 mg) significantly. Total loss amount of N absorbed at the four growth stages in X-36 (252.3 mg) is 18.1% lower than X-50 (307.9 mg).

Physiological indexes	Variety	N absorbed at seedling stage	N absorbed at stem elongation stage	N absorbed at flowering stage	N absorbed at siliquing stage	Sum
N Loss amount plant $^{-1}$ (mg)	X-36	136.4	63.0	35.0	17.9	252.3
	X-50	146.7	95.3*	51.4*	14.5	307.9
N Loss proportion (%)	X-36	22.8	9.0	9.6	8.5	
	X-50	25.1	12.0	13.8	6.1	

Table 8. Loss amount and proportions of N at harvesting stage (N absorbed at different stages)

Components of N can not be reflected completely in Table8. This experiment was carried out in glass room, so possibility of rain wash can be ignored. Old and dropped leaves were carefully collected during the experiment, so N loss through senescence and leaf dropped can be neglected. N loss through water exudation and dew was little also. Therefore, only N loss through N volatilization from the aboveground organs and root secretion can not be calculated in this study.

4. Discussion

4.1 N efficiency in different oilseed rape varieties

Environmental contamination was reduced by less N fertilizer application, so, breeding and spreading new crop varieties with high N efficiency was an interesting topic recently (Xu et al., 2006; Pei et al., 2007 and Wang et al., 2003). Many studies and reports were involved in N agriculture efficiency, N physiological efficiency and N absorption efficiency. N physiological efficiency and N absorption efficiency were parts of the N agriculture efficiency, there were relationships between the three efficiency indexes. Wheat breeders identified selection standards based on physiological indexes, such as high yield and high protein content (Monaghan et al., 2001). Results showed that, the differences of N efficiency in 16 oilseed rape varieties were significant, regardless of N application level. The results obtained in the present study are consistent with previous published results (Beauchamp et al., 1976; Pollmer et al., 1979; Balko et al., 1980a; Reed et al., 1980; Russell, 1984; Moll et al., 1987; Landbeck, 1995; Bertin et al., 2000). However, few oilseed rape varieties were used in these studies, more varieties were needed to be selected and estimated in the future. 16 oilseed rape varieties were used as plant materials in this experiment, N high efficiency types were better plant materials and can tolerate low N stress. These materials have high economic benefit under low N condition; N high response types were better plant materials and can tolerate high N stress, these materials have high economic benefit under high N condition. It can be seen that, the type of "N high efficiency – N high response" varieties was the ideal variety.

4.2 N efficiency of oilseed rape under different N application level

Breeding procedure was usually conducted under N application conditions recently. So, the varieties have high yield under N application condition, it was only for N application condition. It was necessary to select high N efficiency varieties under the low N stress condition, select the varieties have high yield under no N application level (Cao et al., 2010). The correlation of various agronomic traits (grain protein, yield and its component) were different with the changes of N application level (Balko et al., 1980b; Fonzo et al., 1982; Rizzi et al., 1993; Bertin et al., 2000). Therefore, the response differences of varieties under different N application level conditions were obscured; these problems are needed to be further studied in the future. Möllers et al., (2000) studies suggested that, the differences of N efficiency between different N application levels and between varieties were big, observed significant interactions between genotype and N level suggested that the high yielding genotypes in high N supply were not necessarily high yielding in the low N supply. This study has the same results, the varieties have the same yield under N application condition, but under no N application condition, the differences of yield between varieties were big. The results of the present study is different from the results of

Grami et al. (1977) which showed that a direct relationship existed between N uptake and seed N content; they concluded that high seed N content was good for N uptake and translocation efficiency. The above results suggested that, both of N application condition and no N application conditions should be considered during the breeding and selecting procedure of crop (Liu et al., 1999; Lafitte et al., 1994).

4.3 The contribution of N absorption efficiency and N use efficiency to N efficiency

Many studies were carried out on the in contribution of N absorption efficiency and N use efficiency to N efficiency, but the results were different (Zou et al., 2009). High seed protein content will cause low grain yield, this agrees with former results which observed negative relationship between protein content of seed and grain yield (Dudley et al., 1977; Simmonds, et al., 1995). Wheat breeders have reported selection standards combined with high yield and high protein content (Monaghan et al., 2001). Some results showed that N use efficiency was the main reason for changes of N efficiency under low N condition, and the main reason was N absorption efficiency under high N condition (Moll et al., 1982). The effect of N absorption efficiency on N efficiency was higher than N use efficiency significantly under low N condition, and the effect of N absorption efficiency and N use efficiency on N efficiency were almost the same under high N condition (Mi et al., 1998); the absorption efficiency was the main reason for changes of N efficiency regardless of N application level (Liu et al., 2002); N absorption efficiency and N use efficiency of oilseed rape were studied by Yan and Thurling (1987a), there were differences of N use efficiency between varieties under high N condition, and there were differences of N absorption efficiency between varieties under low N condition. The results of this study showed that, the changes of N use efficiency was the main reason of the changes of N efficiency under low and high N conditions, but Yan and Thurling (1987b) have different results. This study (Table 5) also showed that, variation coefficient of N absorption efficiency was increased under low N condition, while the variation coefficient of N use efficiency declined, it was suggested that, the contribution of N absorption efficiency to N efficiency was increased under low N condition. However, the results were only for the varieties which were used in this study, and more varieties are necessary to be used in the future.

4.4 N redistribution characteristics and relation to N efficiency

Liu, (1987) reported that N content in oilseed rape tissues at different growth stages are from 4.5% to 1.2%, N content is higher during earlier growth stages, and lower during later growth stages, N distribution and transfer in tissues can reflect plant metabolisms situation and growth center changes. The same results are showed in this study (Fig.1), N absorbed at seedling and stem elongation stages are mainly distributed into leaves, and distributed amount of N absorbed at flowering stage is lower than the former stages, 42% of N absorbed at siliquing stage was directly distributed into silique. N redistribution is happen in every part of plant tissues at different growth stages, especially after flowering stage, large amount of N are redistributed from vegetative organs to reproductive organs, these results are reported in many kinds of crops, such as wheat (Andersson et al., 2005; Palta et al., 1995a; Palta et al., 1995b; Dalling et al., 1985; Barbottin et al., 2005; Pierre et al., 2003), rice (He et al., 2002), maize (Pommel et al., 2006), pea (Séverine et al., 2005), and oilseed rape (Rossato et al., 2002; Malagoli et al., 2005). Malagoli et al., (2005) suggested that N requirements of seed filling are mainly satisfied by N mobilized from vegetative parts

(about 73% of total N in pods). Nearly all that had been remobilized from vegetative tissues were finally recovered in mature pods (Rossato et al., 2002). The results of maize (Liu et al., 2002) and wheat (Li et al., 2006) studies showed that contribution of N redistributed from vegetative organs to grain was about 50-95%, and depended on crop growth conditions, variety and N application. Table3 results also show that contribution of N redistributed from vegetative organs to grain was 65.1%, this confirmed the results of the other studies. However, a comparison of the amount of N redistribution and the proportion of N absorbed at different growth and development stages has not been done (Rossato et al., 2002; Malagoli et al., 2005; Liu et al., 2002; Li et al., 2006). Preliminary results are shown in this study (Table7), transferred proportion of N absorbed at stem elongation stage was the highest, accounting for 25.8%, followed by N absorbed at flowering and seedling stages, accounting for 16.9% and 15.9% respectively, the lowest was absorbed at siliquing stage, accounting for 6.4%.

High efficiency of N redistribution during later growth stages is physiological mechanisms for crop adapting to environmental changes for a longtime. It is important for crop to alleviate N deficiency in plant tissues, improve crop production and N efficiency, and protect environment ecology (Zhang et al., 2010). However, studies on the N redistribution and loss of oilseed rape are few recently; comparatively studies on the differences of N distribution between growth stages of oilseed rape are fewer (Zhang et al., 2010). This paper system studied on amount and proportion of N that was absorbed at different growth stages, and N distribution, loss amount during later growth stages. Results (Table8) showed that amount and proportion of N absorbed at stem elongation stage that is redistributed into grain of the two varieties was the highest, and loss proportion of N is lower than the other stages also. Loss amount and proportion of N absorbed at seedling stage is the highest, and distribution amount and proportion value is moderate. It can be concluded that fertilizer application at stem elongation stage is good for improving the N application efficiency.
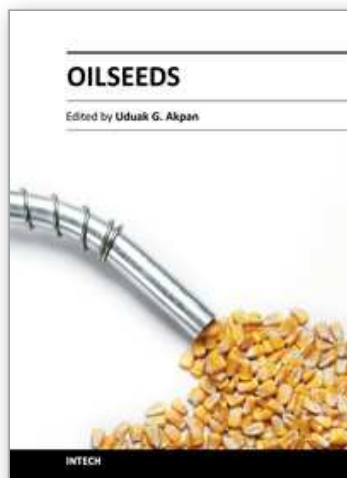
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