Responses of Seed Yield and Quality to Nitrogen Application Levels in Two Oilseed Rape (Brassica napus L.) Varieties Differing in Nitrogen Efficiency

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Abstract: The relationship between nitrogen efficiency (NE), defined as seed yield per unit nitrogen (N) application, and seed quality was examined in two oilseed rape (Brassica napus L.) varieties at 5 N application levels, 0.6, 3, 6, 12, 15 mmol L\(^{-1}\), N1, N2, N3, N4 and N5, respectively. Seed yield, oil yield and protein content were increased with the increase in N application level, but NE and oil content were decreased, and the fatty acid composition in seed was hardly changed. Analysis of seven fatty acids revealed a slight decrease in the contents of erucic acid and arachidonic acid with the increase in N application level, but no obvious change in the contents of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Compared with the low NE variety H29, the seed yield and contents of erucic acid and arachidonic acid in the high NE variety bin270 were more markedly increased with the increase in N application level, and the oil content was hardly changed. The seed yield, oil content and oil yield were higher in the high NE variety than in the low NE variety at all 5 N application levels. There were no significant differences in protein, palmitic acid, stearic acid and oleic acid contents between the varieties at any of the 5 N application levels, but there were slight differences in the linoleic acid and linolenic acid contents between the two varieties. In brief, N application improved oil yield more greatly in the high NE variety than in the low NE variety, but hardly affected the fatty acid composition. Therefore, the seed quality and oil content of oilseed rape may not be decreased by breeding of a high NE variety with a high N absorption efficiency and high N use efficiency.

Key words: Nitrogen efficiency, Oilseed rape (Brassica napus L.), Quality, Yield.

Oilseed rape needs a large amount of N during growth, and the N application level significantly influences the growth and seed quality of oilseed rape (Liu, 1987; Sun et al., 2010; Zou et al., 2009). Nitrogen efficiency (NE) is defined as seed yield per unit N application (Moll et al., 1982). For resource conservation, improvement of environment, selection of stress tolerant crops, and cultivation of high NE oilseed rape varieties, and maintaining seed yield under reduced N application condition are now gathering concern around the world. NE differs with the genotype significantly, and is decreased by the increased N application level (Wiesler et al., 2001; Christian et al., 1999). N use efficiency (NUE) and N absorption efficiency (NAE) are important resources of NE changes under a normal N application and N-deficient condition, but the contribution of NUE was decreased and that of NAE was increased under an N-deficient condition (Liu et al., 2009). A higher NE has been reported in varieties with a lower N concentration in dropping leaves (Kessel et al., 1999), larger amount of N accumulation (Liu et al., 2008) and higher N harvesting index (Kessel et al., 1999; Song et al., 2008). Root biomass is probably an important index for evaluation of oilseed rape NAE (Cao et al., 2010). The NUE of oilseed rape was increased by increasing the GOGAT (glutamine oxoglutarate aminotransferase) and GS (glutamine synthetase) activities...
by a transgenic method (Seiffert et al., 2004).

The contents of oil, protein and fatty acid in seed of oilseed rape are important indexes of seed quality. A better N nutrient condition was found to increase protein content, but also to decrease oil content (Zhao et al., 2007; Li et al., 2007). However, there are few detailed studies on the oil content and quality at different N application levels. In the present study, we determined the changes in NE, seed yield, protein and oil contents of seed of various oilseed rape varieties at different N application levels to reveal the relationship between NE and seed quality, and to provide a scientific basis for rational N application that relieves the incompatibility of protein content with oil content in seed quality improvement.

Materials and Methods

1. Plant materials and treatments

A trial experiment was conducted at the Agricultural Resources and Environment Department, Hunan Agricultural University using bin270 a typical high NE variety, and H29 a typical low NE variety. The two varieties were selected from 30 oilseed rape varieties during the past 10 years in Hunan province, which is the main growing area of oilseed rape cultivar in China. All varieties were provided by the Hunan Subcenter of the Improvement Center of the National Oil Crop, and required vernalization. According to Moll et al. (1982), the soil N supply is difficult to calculate. In this study, NE was defined as the crop yield at a unified N application level (at the same growth stage) as reported by Zhang et al. (2010). The mean yield of bin270 is significantly higher than that of H29 under the field experiment condition. Plants were grown in perlite sand (washed with water and diluted HCL) in plastic pots (30 cm x 30 cm) with a brown color in a glasshouse at the field experiment station of Hunan Agricultural University (Southern China), under natural light and water applied in combination with culture solution.

The experiment was conducted in a completely random design with 5 replications each. The concentration of N applied was 0.6, 3, 6, 12 and 15 mmol L\(^{-1}\) respectively, shown as N1, N2, N3, N4 and N5, respectively. N5 treatment was Hoagland (Gao, 2000) culture solution with complete nutrients, the nutrient solution was composed of 5 mmol L\(^{-1}\) KNO\(_3\), 1 mmol L\(^{-1}\) KH\(_2\)PO\(_4\), 7 mmol L\(^{-1}\) MgSO\(_4\)\(_{5}\)H\(_2\)O, 3 mmol L\(^{-1}\) Ca (NO\(_3\))\(_2\)-4H\(_2\)O, 0.5 mg L\(^{-1}\) Fe-EDTA, 0.5 mg L\(^{-1}\) B, 0.5 mg L\(^{-1}\) Mn, 0.05 mg L\(^{-1}\) Zn, 0.02 mg L\(^{-1}\) Cu and 0.01mg L\(^{-1}\) Mo, respectively. The concentration of other nutrients in N1, N2, N3, N4 treatments were the same as those in N5 treatment, but the concentration of N in KNO\(_3\) and Ca(NO\(_3\))\(_2\)-4H\(_2\)O was 0.2, 1, 2 and 4 mmol L\(^{-1}\), respectively. In seedling-transplanting cultivation, oilseed rape was sown on quaternary red paddy soil in a field on 30 September 2007, transplanted 1 month later, 1 plant pot\(^{-1}\) in a randomized block arrangement with 5 replications. The plants were harvested on 2 May, 2008 (average temperature was 25.1ºC/10.3ºC day/night, and the average irradiance 39000 lux).

2. Methods of measurement

Seeds of oilseed rape were dried and weighed after harvest. The protein content, oil content and fatty acid composition were then measured by the following methods. Oil yield was calculated as seed yield and oil content:

Protein content was calculated as 6.25 x total nitrogen (TN), measured by the Kjeldahl method (Bao, 2005). Oil was extracted with a fat analyzer (SZF-06A), and the oil content was calculated by a method reported previously (Guan, 1985). Fatty acid composition was measured after digestion by a mixture of diethyl-ether/petroleum ether (1:1), and measured by gas chromatography (Agilent 1200) (Guan, 1985).

3. Data analysis and calculation

NE was calculated (Moll et al., 1982; Zhang et al., 2010) as seed yield (g) divided by N supply (g). N supply was calculated from the nutrient solution volume and N concentration during all growth stages. The data was analyzed using SPSS software and Microsoft Excel 2003 software; the differences between varieties were analyzed by t-test. Differences between N application levels were analyzed using SPSS software, SSR Multi-comparison was further applied for differences considered significant in the variance analysis between different N application levels. The t-test for varietal difference was performed for each N treatment; analysis of variance for treatment effect was performed for each variety.

Fig. 1. Seed yield (g plant\(^{-1}\)) in different oilseed rape varieties at different N application levels. Different letters indicate significant differences in the five N treatments at the 5% level. Asterisks indicate significant difference between the two varieties at the same N level according to t-test (P<0.05).
Results

1. Responses of seed yield to N application levels
As Fig. 1 shows, the seed yield of the two oilseed rape varieties increased with the increase in N application level, and the rate of increase was higher in the high NE variety, bin270, than in the low NE variety, H29. The difference in seed yield with the N application level was significant in bin270. However, there was no significant difference in seed yield between N1 and N2 levels in H29. Obviously, bin270 responded better to the increase in N application. The seed yield of bin270 was always higher than that of H29 under different N application levels, and the differences between the 2 varieties were significant. The seed yield of bin270 was 17.5, 31.1, 25.7, 20.1 and 38.1% higher than that of H29 at the N1, N2, N3, N4 and N5 level, respectively.

2. Responses of NE to N application levels
NE in Table 1 was calculated from seed yield and solution N supply. NE of the 2 varieties decreased with the increase in N application level. The degree of decrease was especially high at lower N application levels. The decrease in bin270 and H29 was the highest, 65.8% and 69.3%, respectively, when the N application level was increased from N1 to N2, and that in bin270 and H29 was the lowest, 3.9% and 16.4%, respectively, when the N application level was increased from N4 to N5. The results also showed that bin270 always had a higher NE than H29 regardless of N application level. This decrease was probably because the marginal physical product (MPP) decreased with the increase in N application level.

3. Responses of seed quality to N application levels

1. Protein content
As Fig. 2 shows, the seed protein content of the two varieties increased with the increase in N application level from N2 to N5, but it was higher at N1 than at N2, probably because of the "concentration effect" that was caused by a decrease in dry biomass under a serious N-deficient condition. Protein content was higher at N4 and N5 than at the other N levels in both varieties, but the protein content was lower at N2 than at other N levels. Except at N1, there were no significant differences in protein content between the two varieties, and there was no advantage of high

| Cultivar | N levels | hexadecanoic acid | Stearic acid | Oleic acid | Linoleic acid | Linolenic acid | Arachidonic acid | Erucic acid |
|----------|----------|-------------------|--------------|------------|---------------|----------------|-----------------|-------------|
| bin270   | N1       | 4.24 a            | 1.42 a       | 61.30 a    | 19.33 a       | 8.92 a         | 2.11 a          | 1.12 a      |
|          | N2       | 4.32 a            | 1.53 a       | 59.84 a    | 20.26 a       | 8.50 a         | 2.16 a          | 1.03 a      |
|          | N3       | 4.22 a            | 1.49 a       | 60.93 a    | 19.80 a       | 8.52 a         | 1.65 ab         | 0.84 ab     |
|          | N4       | 4.31 a            | 1.40 a       | 62.31 a    | 20.54 a       | 8.02 a         | 1.43 b          | 0.39 b      |
|          | N5       | 4.06 a            | 1.37 a       | 62.57 a    | 19.79 a       | 8.50 a         | 1.44 b          | 0.44 b      |
| H29      | N1       | 4.20 a            | 1.40 a       | 63.12 a    | 17.65 a       | 9.09 a         | 1.86 a          | 1.08 a      |
|          | N2       | 4.05 a            | 1.46 a       | 61.56 a    | 17.10 a       | 8.91 a         | 1.91 a          | 1.60 a      |
|          | N3       | 4.12 a            | 1.36 a       | 65.52 a    | 17.94 a       | 8.77 a         | 1.40 b          | 0.79 b      |
|          | N4       | 4.22 a            | 1.38 a       | 62.37 a    | 18.29 a       | 8.83 a         | 1.49 ab         | 0.90 ab     |
|          | N5       | 4.17 a            | 1.37 a       | 61.14 a    | 18.39 a       | 8.97 a         | 1.35 b          | 0.73 b      |

Values followed by different letters in the same column are significantly different at the 5% level.
protein content in the high NE variety.

(2) Fatty acid composition
As Table 2 shows, the N application level hardly affected the fatty acid composition of oilseed rape: erucic acid and arachidonic acid contents were decreased with the increase in N application level, but there were no significant changes in the contents of the other fatty acids.

The changes in erucic acid and arachidonic acid contents with the N application level were obviously more marked in bin270 than in H29. The erucic acid content was 60.7 and 32.4% lower in bin270 and H29, respectively, and the arachidonic acid content was 31.8 and 27.4% lower, respectively, at N5 than at N1. This was due to the higher dry biomass accumulation in the high NE variety bin 270 than in the low NE variety H29 when the N application level was high.

(3) Oil content and yield
As Fig. 3 shows, the oil content in seed was decreased with the increase in N application level. The oil content was 16.1 and 19.4% higher at N1 than at N5 in bin270 and H29, respectively, and the differences in oil content between successive N application levels were larger at a higher N application level. Oil content of bin270 was higher than that of H29 at all N application levels, and differences in oil content between successive N5, N4 and N3 treatments were significant. The oil content decreased with the increase in N application level, but oil yield was 29.1, 33.2, 36.5, 31.2 and 45.3% higher in bin270 than in H29 at N1, N2, N3, N4 and N5, respectively (Table 3).

Discussion
Protein content, oil content and fatty acid composition are important quality parameters of the seed of oilseed rape. The protein content of oilseed rape increased but the oil content decreased with the increase in N application level (Zhao et al., 2007; Li et al., 2007; Zhang et al., 2010). Similar results were obtained in the present study in both high NE and low NE varieties. The larger the amount of protein accumulated, the larger the amount of carbohydrate exhausted. Therefore, the increase in N application level may increase the amount of protein accumulated, but with a concomitant decrease in oil accumulation. The oil yield in the high NE variety decreased from the N1 to N5 level, but that in the low NE variety decreased from the N1 to N5 level, suggesting that the oil content in the seed of the high NE variety was not decreased by the application of less N, that the high NE variety has better oil accumulation ability than the low NE variety. However, this was not the case under a serious N-deficient condition (N1 and N5).

Generally, the effect of N application level on fatty acid composition is weaker than that on protein and oil contents (Sun et al., 2010; Zhao et al., 2007; Li et al., 2007). In this study, only erucic acid and arachidonic acid contents were negatively related to the N application level, and the other fatty acids were hardly affected by the N application level. The above results showed that the fatty acid content of oilseed rape mainly depends on genetic factors and is hardly influenced by N application level (Osborne et al., 1978; Ahmad et al., 2000). These results suggest that the fatty acid composition is not greatly influenced by breeding of high NE varieties or by changing the N application level.

In brief, the seed yield of oilseed rape can be increased by two factors, N application level and NE, but the effects of the two factors on seed yield and quality are different. Seed yield was increased, but seed oil content was decreased by the increase in N application level in the low NE variety. However, in the high NE variety, seed yield and seed oil content were greater compared with the low NE variety because of its high NAE and high NUE. Therefore, oil yield is higher in the high NE variety than in the low NE variety and there are significant differences in oil yield between the two varieties under all N conditions. However, the above results were obtained on only two varieties, and
the essential relationship between NE and seed quality needs to be studied more extensively using many more oilseed rape varieties.

Conclusions

(1) There were significant differences in seed yield, NE and oil yield between the high and low NE varieties. These values were higher in the former than in the latter at all N application levels, but there was little difference in the protein content and fatty acid composition in seed between the two varieties.

(2) Seed yield, oil yield and protein content of seed were increased with the increase in N application level, but the oil content was decreased, and fatty acid composition of seed was influenced only slightly by N application level; the contents of only erucic acid and arachidonic acid were slightly decreased by the increase in N application level.

(3) Seed yield increased, but the oil content of seed decreased more drastically with the increase in N application level in the low NE variety. However, both seed yield and oil content increased with the increase in N application. These results suggest that seed quality will not be worsened by breeding of high NE varieties.

Acknowledgement

This research was supported by the National Natural Science Foundation of China (31071851, 31101596 and 30971860), National oilseed rape production technology system of China, Open novel science foundation of Hunan province (10K034), Talent Scholar of Human Agricultural University (11Y21), FuRong Scholar Program of Hunan Province, P.R.China and Talent Science Foundation of Resources and Environment College in Hunan Agricultural University.

References

Ahmad, A., Abdin, M.Z. 2000. Interactive effect of sulphur and nitrogen on the oil and protein contents and on the fatty acid profiles of oil in the seeds of rapeseed (Brassica napus L.) and mustard (Brassica juncea L. Czern. and Coss.). J. Agro. Crop Sci. 185: 49.

Bao, S.D. 2005. Soil and aricultural chemical analysis. Beijing: China Agricultural Press. pp. 285-287*.

Berendse, F., Alerts, W. 1987. Nitrogen use efficiency: A biologically meaningful definition?. Funct. Ecol. 1: 293-296.

Birk, E.M., Vitousek, P.M. 1986. Nitrogen availability and nitrogen use efficiency in loblolly pine stands. Ecology. 67: 69-79.

Gao, L.Q., Wu, X.M., Li, Y.J. 2010. Relationship between genotypic differences of rapeseed (Brassica napus L.) nitrogen uptake efficiency and economic characteristics. Chin. J. Oil Crop Sci. 32: 270-278*.

Christian, M., Maria, K., Bettina, K. 1999. Genotypic variation for nitrogen efficiency in winter rapeseed cultivars. “New Horizons for an old crop”, Proceedings of the 10th international rapeseed congress, Canberra, Australia.

Gao, J.F. 2000. Experimental Technique of Plant Physiology. Xian: World Publishing Company. pp. 86-88**.

Guan, C.Y. 1985. Rapeseed quality improvement and analysis methods. Changsha: Hunan Science and Technology Press. pp. 197-198*.

Kanampiu, FK, Raun, W.R, Johnson, G.V. 1997. Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties. J. Plant Nutr. 20: 389-404.

Kessel, B., Heiko, C., Becker, K. 1999. Genetic variation of nitrogen efficiency in field experiments with oilseed rape (Brassica napus L.). “New Horizons for an old crop”, Proceedings of the 10th international rapeseed congress, Canberra, Australia.

Li, Z.Y., Guo, Q.Y., Liao, X. 2007. Effects of different amount of nitrogen on yield, quality and economics of Zhonghuang No. 9. Chin. J. Oil Crop Sci. 29: 78-82*.

Liu, Q., Song, H.X., Rong, X.M. 2009. Studies on oilseed yield and nitrogen efficiency in different cultivars of oilseed rape (Brassica napus L.). Plant Nutr. Ferti. Sci. 15: 898-903*.

Liu, Q., Song, H.X., Rong, X.M. 2008. Differences in nitrogen use efficiency among different rape varieties and their physiological basis. Plant Nutr. Ferti. Sci. 14: 113-119*.

Liu, H.L. 1987. Practical cultivation of rape seed. Shanghai: Scientific Technology Press. pp. 235-251**.

Moll, R.H., Kamprath, E.J., Jackson, W.A. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron. J. 74: 562-568.

Osborne, G.J., Batten, G.D. 1978. Oil yield and protein content of oilseed rape as affected by soil and fertilizer nitrogen and phosphorus. Aust. J. Exp. Agric. Anim. Husb. 18: 107-111.

Seiffert, B., Zhou, Z.W., Wallbraun, M., Lohaus, G., Möllers, C. 2004. Expression of a bacterial asparagine synthetase gene in oilseed rape (Brassica napus L.) and its effect on traits related to nitrogen efficiency. Plant Physiol.121: 656-665.

Song, H.X., Peng, J.W., Liu, Q. 2008. Nitrogen redistribution characteristics of oilseed rape varieties with different nitrogen physiological efficiency during later growing period. Sci. Agric. Sin. 41: 1858-1864*.

Sun, H., Zhang, J.D., Chen, P.F. 2010. Effect of different nitrogen levels on seed yield and quality of double-low rape Suyou No.4. Acta Agriculturae Jiangxi. 22: 27-29*.

Vitousek, PM, Gosz, J.R, Grier, C.C, Melillo, J.M, Reiners, W.A. 1982. A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol. Monogr. 52: 155-177.

Wiesler, F., Behrens, T., Horst, W.J. 2001. Nitrogen efficiency of contrasting rape ideotypes. Kluwer Academic Publishers, 60-61.

Zhang, Z.H., Song, H.X., Liu, Q., Rong, X.M, Peng, J.W., Guan, C.Y., Xie, G.X., Zhang, Y.P. 2010. Studies on differences of nitrogen efficiency among different rape varieties and their physiological interpretation of factors which contribute to efficiency of nitrogen utilization. J. Plant Nutr. 33: 1448-1459.

Zhang, Z.H., Liu, Q., Song, H.X., Rong, X.M., Peng, J.W., Xie, G.X., Zhang, Y.P. 2010. Nitrogen Redistribution Characteristics of Oilseed Rape (Brassica napus L.) Varieties with Different Nitrogen Use Efficiencies during Later Growth Period. Commu. Soil Sci. Plant Anal. 41: 1693-1706.

Zhou, J.X., Cheng, G.F., Ren, T.B. 2007. Effect of different nitrogen rates on yield and quality parameters of high grade yellow seed hybrid rape. Plant Nutr. Ferti. Sci. 13: 882-889*.

Zou, J., Lu, J.W., Chen, F. 2009. Effect of Nitrogen, Phosphorus, Potassium, and Boron Fertilizers on Yield and Profit of Rape seed (Brassica napus L.) in the Yangze River Basin. Acta Agronomica Sinica. 35: 87-92*.

* In Chinese with English abstract.
** In Chinese.