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Full Length Article

Nitrogen Rate and Planting Density Effects on Yield and Nitrogen Utilization Efficiency of Direct Seeded Rape (*Brassica napus*)

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Abstract

The high yield of crops mainly depends on the interaction between nitrogen (N) fertilization and planting density. The present study evaluated the influence of different N application rates and planting density on the yield and N utilization efficiency of direct seeded rape during the 2016–2017 and 2017–2018 growing seasons. The three N application rates including 108 kg N ha^{-1}, 144 kg N ha^{-1} and 180 kg N ha^{-1} were laid out into main plots while planting density including 15.0 × 10^4, 22.5 × 10^4, 30.0 × 10^4 and 37.5 × 10^4 plants ha^{-1} respectively into sub-plots. The results showed that suitable planting density was the premise to gain high rapeseed yield and the contribution of planting density to rapeseed yield was small when it surpassed a certain value. The highest yield in the two growing seasons was achieved at 144 kg N ha^{-1} with planting density of 30.0 × 10^4 plants ha^{-1}. The rapeseed yield increased linearly as N uptake of the seed part increased, but at high planting density, there was no increase in yield with the increase N uptake of non-seed parts (stem + husk) when rapeseed yield exceeded a certain value. High planting densities raised shoot N uptake and N transfer to rapeseed seeds with the increase of density and N utilization efficiency was also improved. The too much N application resulted in more N accumulation in non-seed parts without increasing production. Under the same target yield, the increased planting density can save 32.4–65.7% of N fertilization compared with the conventional planting density. The combination of different N fertilization rates and planting densities is helpful to increase rapeseed yield. The best N management strategy is to achieve high yield and reduce the environmental risk to reduce the N fertilization at suitable high density.

Keywords: Directed sowing rape; Nitrogen fertilization; Planting density; Yield; Nitrogen utilization efficiency

Introduction

Nitrogen (N) fertilizer has been widely used to improve the yield of crops, but inadequate N inputs resulted in low yields and food shortages (Sims *et al.* 2013). In the modern intensive agricultural production system, up to 50% of the N fertilizer applied into farmlands is lost to the environment (Cameron *et al.* 2013). Managing agricultural nutrients to provide secure and reliable food supply while protecting the ecological environment remains one of the major challenges today (Zhang *et al.* 2007; Cui *et al.* 2014).

Optimization of the N application rate is an important direction to achieve food and environmental safety (Helmers *et al*. 2012). The agronomic indicators are necessary to consider in determining the optimal N application rate (Jin *et al.* 2012). For instance, plant density management has a crucial impact on the N application rate (Gao *et al*. 2009), while higher plant densities can significantly improve the N uptake and utilization efficiency in crops (Cong *et al*. 2020). Studies have reported that for achieving the target yield, high-density planting can reduce the N application rate by 22.8–25.4% compared with low-density planting (Li *et al.* 2014).

With urbanization, the rural labor engaged in agriculture is seriously insufficient and the labor cost is rising (Hui 2013). In order to coordinate the relationship among crop production, labor cost and target yield, increased N fertilizer application under low-density planting to improve the growth of individual plant or decreased N fertilizer application to reduce the competition among plants under high-density planting was advocated (Ren *et al.* 2017). Previous studies have shown that the change of N fertilizer rates and planting densities would affect N loss and N cycling between plants and soil, resulting in environmental costs (Gao *et al*. 2009). Consequently, further understanding of the interaction between the N fertilizer rate and the planting density on crop yield and N utilization is of great significance to optimize N application

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and achieve high levels of output with low environmental costs (Abdul *et al*. 2018; Fang *et al*. 2018).

As one of the major oil crops in China, rape is widely planted in most regions because of its high oil content and economic value. Rapeseed oil accounts for 55% of the total output of oil crops in China, which shows that rape plays an important role in oil crops (Scarisbrick *et al*. 1982). However, with the increase of labor cost and the low mechanization of rape production compared with other crop productions, farmers grow less rape, resulting in strong demand for oil products. At present, the self-sufficiency rate of oil is only about 40% and the safety of edible vegetable oil is still under serious threat (Özcan *et al*. 2014). Since the short growth period and large population of direct seeding rape, the increase of population number makes the branch site move up which is more conducive to mechanized harvesting. The individual development of direct seeding rape is relatively poor, and the formation of yield needs to give full play to the group advantage. Therefore, the establishment of appropriate group structure is an effective way to ensure the high yield of direct seeding rape.

With the purpose of save labor, low-density planting is always adopting in practice, which produces more branches and a great number of pods per plant (Scarisbrick *et al*. 1977). Whereas, with the planting density increasing, the number of branches and pods per plant decreased, which led to the decrease of yield per plant (Scarisbrick *et al*. 1977; Velázquez *et al*. 2018). As one of major factors contributing to rapeseed yield, N fertilizer could efficiently control the number of pods per plant. The optimized management of N fertilizer can promote the growth of plants and increase the number of pods per plant, and partly balance the number of pods reduced due to the increase of planting density (Ahmadi and Bahrani 2009; Ren *et al*. 2017; Zheng *et al*. 2020).

The change on planting density could affect the crop morphology, alter the utilization of resources and induce different yields react to different N application rates (Zheng *et al*. 2020). At low density, increasing N fertilizer rates will increase the number of pods per plant and improve the yield, but the potential N losses will also increase at the same time. By contrast, under high density, the relationship between plant population and yield per plant would be better coordinated by reducing the application rates of N fertilizer without affecting the seed yield and increasing the environmental cost (Zhang *et al*. 2014).

Therefore, this study mainly analyzed the influences of the N fertilizer rate and the planting density on yield and N utilization of direct seeding rape while providing the basis for the reasonable N application under the condition of increasing density.

Materials and Methods

Site characteristics

The experiment was carried out from September 2016 to May 2017 and September 2017 to May 2018 in Jianyang city (30°16′26.32″ N, 104°26′30.38″ E), the west of Sichuan Basin, Sichuan province, southwest China with subtropical monsoon climate and annual precipitation of 600–1200 mm. The annual average temperature ranges between -5 to 37.5°C with about 300 days of frost-free period. The soils of the experimental site are purple. The chemical properties of the soil were shown in table (Table 1).

Experimental design and management

A two-factor split plot experiment was designed using a random arrangement with three replicates. The individual plot area was 20 m^2 (4.0 m × 5.0 m). There were three fertilization treatments in main plots, including 108, 144 and 180 kg N ha^{-1}. The sub-plot planting densities treatments included were four 15.0 × 10^4 plants ha^{-1} (plant and row spacing: 20 cm × 33.3 cm), 22.5 × 10^4 plants ha^{-1} (plant and row spacing: 13.3 cm × 33.3 cm), 30.0 × 10^4 plants ha^{-1} (plant and row spacing: 10 cm × 33.3 cm), 37.5 × 10^4 plants ha^{-1} (plant and row spacing: 8 cm × 33.3 cm).

Except for the different dosage of N fertilizer, K, P and B fertilizer was the same under each treatment at 90 kg K_2O ha^{-1} and 90 kg P_2O_5 ha^{-1} using urea (46% N), potassium chloride (60% K_2O) and calcium superphosphate (12% P_2O_5) as fertilizer sources. The basal fertilizers, including 70% of the N fertilizer and whole K, P fertilizers were incorporated one day before transplanting. The remaining 30% N fertilizer was top dressed at the stem elongation period.

Chuanyou 36, a leading rape variety in Sichuan province, was used as the material in this experiment. In late September, the seedlings were raised and thinned at first leaf stage. Final singling was carried out in the three-leaf stage. During the experiment, plant protection measures such as pest and disease control and herbicide application were followed as local practice. Nonetheless, no obvious problems of weeds and pests in these two growing seasons was observed.

Soil sample collection

The soil-sampling scheme was the same during two seasons. Soil samples were taken from the 0–20 cm and 20–40 cm depth per plot by five points sampling method before transplanting. The content of total N and N, including available P and K, organic matter and pH were measured.

Plant sample collection

During both growing seasons, plants were harvested separately and all the plants per plot were gleaned to evaluate rapeseed yield; 10 samples per plot were randomly selected to count the number of pods per plant, the number of seeds per pod and 1000-seed weight at maturity.

Dry matter determination

The mature plant samples were classified and put into the sample bag according to the organs; the enzymes were deactivated at 105°C in the oven for 30 min, then dried the plant organs at 80°C and measured for dry weight (Zhang *et al*. 2019).

N content determination and nitrogen use efficiency

The dried plant samples were ground and the N content was determined by elemental analyzer. Dry weight and N content of different plant parts were used to calculate the amount of N shoot uptake of each plot (Zhang *et al*. 2019).
The N harvest index was calculated as follows:
N harvest index (NHI) = Rapeseed N accumulation/Total plant N accumulation (Tirol-Padre *et al.* 1996; Dong *et al*. 2007).
N internal utilization efficiency (NUE kg kg^{-1}) = Yield (kg ha^{-1}) / Total plant N accumulation (kg N ha^{-1}) (Tollenaar *et al*. 2006; Dong *et al*. 2007; Caviglia *et al*. 2014).
N partial factor productivity (PFP kg kg^{-1}) = Yield (kg ha^{-1}) / N fertilizer rates (kg N ha^{-1}) (Shapiro and Wortmann 2006; Zheng *et al.* 2020).

Statistical analysis

The data of both seasons were analyzed using the Microsoft Excel 2007 and SPSS 17.0 (SPSS Inc. Chicago, IL, USA) software. The means were compared using Duncan's test at a 0.05 probability level.

Results

Rapeseed yield and its components

The contribution of N application to rapeseed yield was higher than planting density (Table 2). With N fertilization increased, rapeseed yield improved under low-density planting treatments. Under low planting density (15.0×10^4 plants ha^{-1}, 22.5×10^4 plants ha^{-1}) and 180 kg N ha^{-1} rapeseed yields was highest; and at high planting density (30.0×10^4 plants ha^{-1}, 37.5×10^4 plants ha^{-1}) with 144 kg N ha^{-1}, rapeseed yields were highest.

Between years, rapeseed yield were higher during 2016–2017 growing season than 2017–2018, similar response of rapeseed yield to N application and planting density were observed (Table 2). The high-density planting greatly increased the rapeseed yield. Compared to 108 kg N ha^{-1} and 15.0×10^4 plants ha^{-1}, the rapeseed yield increased by 51.3 and 45.9% for 108 kg N ha^{-1} and 37.5×10^4 plants ha^{-1} in 2016–2017 and 2017–2018 growing seasons, respectively.

The alterations of pods per plant with N application and planting density were similar during these two growing seasons (Fig. 1). High-density planting suppressed the number of pods per plant. The number of pods per plant under 15.0×10^4 plants ha^{-1} was higher than other planting density treatments. The N fertilization remarkably increased the number of pods per plant, and the number of pods per plant at the treatment of 180 kg N ha^{-1} was always the largest no matter what density treatment was.

There was no significant difference in seeds per pod and 1000-seed weight under different treatments (Fig. 1). As the number of pods per plant was significantly higher in 2016–2017, the number of seeds per pod in 2016–2017 was significantly greater than 2017–2018 (Fig. 1). The average number of seeds per pod was 21.2 and 15.3 in the 2016–2017 and 2017–2018 seasons, respectively.

The relationship between rapeseed yield and yield components showed a significant positive correlation between N fertilization rate, planting density and rapeseed yield, the correlation coefficient between N fertilizer rates and rapeseed yields was higher than of densities and rapeseed yields (Fig. 2). The number of pods per plant and seeds per pod were positively correlated with rapeseed yield, while the 1000-seed weight negatively correlated with rapeseed yield. Compared with the correlation between rapeseed yield and group yield components, the correlation between rapeseed yield and individual yield components was relatively weak.

Shoot N Uptake

Similar to rapeseed yield, the N application and density had a significant impact on shoot N uptake of rapeseed at maturity (Table 3).

Under the same N treatment, high-density planting obviously increased the shoot N uptake of rapes. Compared with 15.0×10^4 plants ha^{-1} density, shoot N uptake increased by an average of 12.5, 32.6, 33.0% and 13.5, 36.9, 37.6% at 22.5×10^4 plants ha^{-1}, 30.0×10^4 plants ha^{-1} and 37.5×10^4 plants ha^{-1} in 2016–2017 and 2017–2018, respectively.

Compared with planting density, N fertilizer application has more effect on shoot N uptake and it was generally the highest under 180 kg N ha^{-1}. Nevertheless, there was no obvious difference of shoot N uptake at 180 kg N ha^{-1} under high-density planting.

The relationships between shoot N uptake and rapeseed yield at different planting densities was improved linearly as seed N uptake, and slope at different densities was similar, which indicated that the yield value of N uptake per seed under different densities was approximately equal (Fig. 3).

At the two growing seasons, N uptake of non-seed parts (stem + husk) was changed at different densities. Under low planting densities, rapeseed yield improved linearly as the N uptake of non-seed parts increased. However, rapeseed yield no longer improved with the N uptake of non-seed parts increasing when the rapeseed yield reached a certain value under the high-density treatment. These indicate that the non-seed part of rape could not form higher rapeseed yield when it absorbed more N under high density treatment.

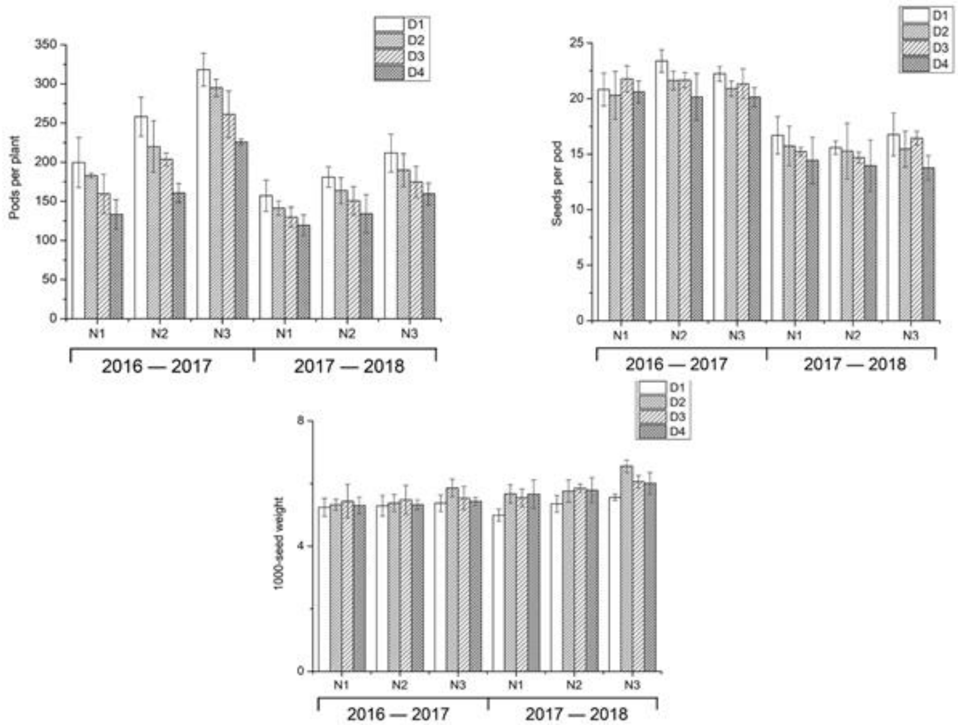

Fig. 1: Yield components under different N fertilizer rates and planting densities during 2016-2017 and 2017-2018 growing seasons in Jianyang city, southwest China

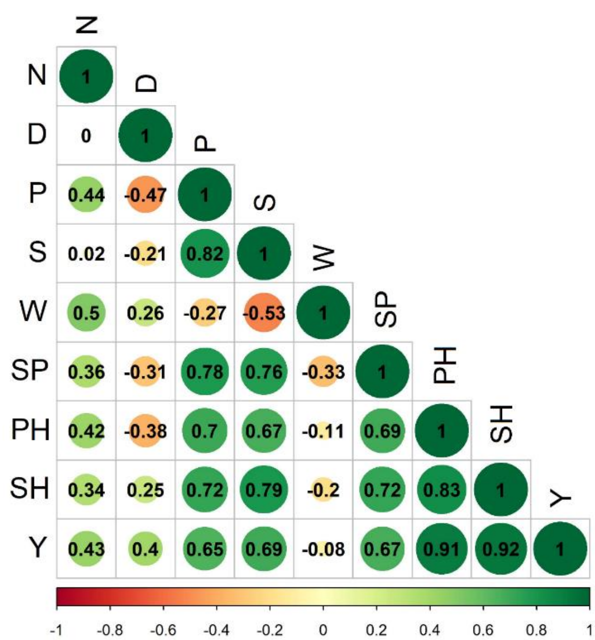

Fig. 2: Correlation matrix between different parameters of rapeseed yield. N represents N fertilizer rate, D represents planting density, P represents pods per plant, S represents seeds per pod, W represent 1000-seed weight, SP represents seeds per plant, PH represent pods per ha, SH represents seeds per ha, Y represents rapeseed yield. The number in the circle represents the determination coefficient

N Utilization Rate

Reducing the application rates of N fertilizer significantly improved the N harvest index (NHI), internal utilization efficiency (NUE) and its partial factor productivity (PFP). All indicators were maximum at 108 kg N ha^{-1}, which were significantly higher than 144 kg N ha^{-1} and 180 kg N ha^{-1} in the two growing seasons.

The NHI and the PFP increased with the increasing of planting density, with the highest for 30.0 × 10^4 plants ha^{-1} and 37.5 × 10^4 plants ha^{-1}, significantly higher than 15.0 × 10^4 plants ha^{-1}. However, the NUE decreased with the increase of density.

During both growing seasons, the NHI at the level of 108 kg N ha^{-1} was 18.7 and 14.9% higher on average than 180 kg N ha^{-1}. NUE was 34.4 and 39.0% higher, respectively While PFP was 21.3 and 39.5% higher, respectively (Table 4–6).

In the 2016–2017 growing season, the NHI, NUE and PFP under low N and high planting density (108 kg N ha^{-1} and 37.5 × 10^4 plants ha^{-1}) were 43.9, 16.2 and 79.0% higher than under high N and conventional planting density (180 kg N ha^{-1} and 15.0 × 10^4 plants ha^{-1}) and 37.5, 4.0 and 74.8% higher than 2017–2018 growing season, respectively. In the 2016–2017 growing season, the NHI, NUE and PFP under medium N and high planting density (144 kg N ha^{-1} and 37.5 × 10^4 plants ha^{-1}) were 29.3, 6.9 and 50.0% higher than under high N and conventional

Table 1: Soil chemical properties at the experimental sites during both growing seasons

Year	Organic matter (g kg^{-1})	pH	Total N (g kg^{-1})	available N (mg kg^{-1})	available P (mg kg^{-1})	available K (mg kg^{-1})
2016–2017（0–20 cm）	6.65	7.22	1.14	68.45	13.48	84.75
2016–2017（20–40 cm）	6.86	7.35	1.29	55.46	14.38	85.64
2017–2018（0–20 cm）	7.78	7.12	0.07	93.47	27.30	86.50
2017–2018（20–40 cm）	8.82	7.23	0.07	77.08	29.27	88.50

Table 2: Rapeseed yields (kg ha^{-1}) under different N fertilizer rates and planting densities during 2016–2017 and 2017–2018 growing seasons

	2016–2017				2017–2018			
Treatments	Plant density (× 10^4 plants ha^{-1})				Plant density (× 10^4 plants ha^{-1})			
N application (kg N ha^{-1})	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
108	1835f	2339e	2499de	2776c	1514g	1826f	2262d	2209d
144	2365e	2806c	3221a	3103ab	1882f	2221d	2699a	2502b
180	2585d	3013b	3145ab	3097ab	2107e	2401c	2453bc	2371c
ANOVA	F value	P value			F value	P value		
N	125.52	*< 0.01***			168.58	*< 0.01***		
Density (D)	101.81	*< 0.01***			139.44	*< 0.01***		
N × D	4.28	*< 0.01***			14.58	*< 0.01***		

Note: Different lowercase letters in each group (2016–2017 and 2017–2018) indicate significant ($P < 0.05$) differences basing on Duncan's multiple range test. ** $P \leq 0.01$; * $P \leq 0.05$.

Table 3: Shoot N uptake (kg N ha^{-1}) under different N fertilizer rates and planting densities during both growing seasons

	2016–2017				2017–2018			
Treatments	Plant density (× 10^4 plants ha^{-1})				Plant density (× 10^4 plants ha^{-1})			
N application (kg N ha^{-1})	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
108	87.1g	101.8fg	118.2def	124.5cd	73.4f	81.3f	100.8d	106.6c
144	105.6ef	122.8cde	151.7ab	148.1ab	82.9e	95.7de	115.4b	112.3b
180	130.7cd	139.2bc	158.9a	157.6a	101.1cd	115.1bc	136.1a	135.4a
ANOVA	F value	P value			F value	P value		
N	43.90	*< 0.01***			42.23	*< 0.01***		
Density (D)	26.16	*< 0.01***			31.32	*< 0.01***		
N × D	0.61	0.72			0.25	0.95		

Note: Different lowercase letters in each group (2016–2017 and 2017–2018) indicate significant ($P < 0.05$) differences basing on Duncan's multiple range test. ** $P \leq 0.01$; * $P \leq 0.05$.

Table 4: N harvest index (NHI) under different N fertilizer rates and planting densities during both growing seasons

	2016–2017				2017–2018			
Treatments	Plant density (× 10^4 plants ha^{-1})				Plant density (× 10^4 plants ha^{-1})			
N application (kg N ha^{-1})	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
108	0.50c	0.53bc	0.54b	0.59a	0.46d	0.50bc	0.51b	0.55a
144	0.45d	0.48cd	0.52bc	0.53bc	0.42e	0.45de	0.47cd	0.50bc
180	0.41e	0.44de	0.49cd	0.48cd	0.40f	0.44de	0.46d	0.46d
ANOVA	F value	P value			F value	P value		
N	32.15	*< 0.01***			27.52	*< 0.01***		
Density (D)	16.89	*< 0.01***			19.38	*< 0.01***		
N × D	0.74	0.62			0.55	0.76		

Note: Different lowercase letters in each group (2016-2017 and 2017-2018) indicate significant ($P < 0.05$) differences basing on Duncan's multiple range test. ** $P \leq 0.01$; * $P \leq 0.05$

Table 5: N internal utilization efficiency (NUE kg kg^{-1}) under different N fertilizer rates and planting densities during both growing seasons

	2016–2017				2017–2018			
Treatments	Plant density (× 10^4 plants ha^{-1})				Plant density (× 10^4 plants ha^{-1})			
N application (kg N ha^{-1})	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
108	23.67a	24.47a	21.68b	20.90b	21.29a	15.23b	14.30c	15.20b
144	20.82b	20.17c	20.62bc	19.24cd	17.32ab	14.32c	14.50bc	15.11b
180	17.99d	17.82de	16.01de	15.69e	14.62bc	10.38d	11.42d	11.07d
ANOVA	F value	P value			F value	P value		
N	41.25	*< 0.01***			39.97	*< 0.01***		
Density (D)	4.36	*< 0.05**			21.84	*< 0.01***		
N × D	0.69	0.66			0.67	0.68		

Note: Different lowercase letters in each group (2016–2017 and 2017–2018) indicate significant ($P < 0.05$) differences basing on Duncan's multiple range test. ** $P \leq 0.01$; * $P \leq 0.05$

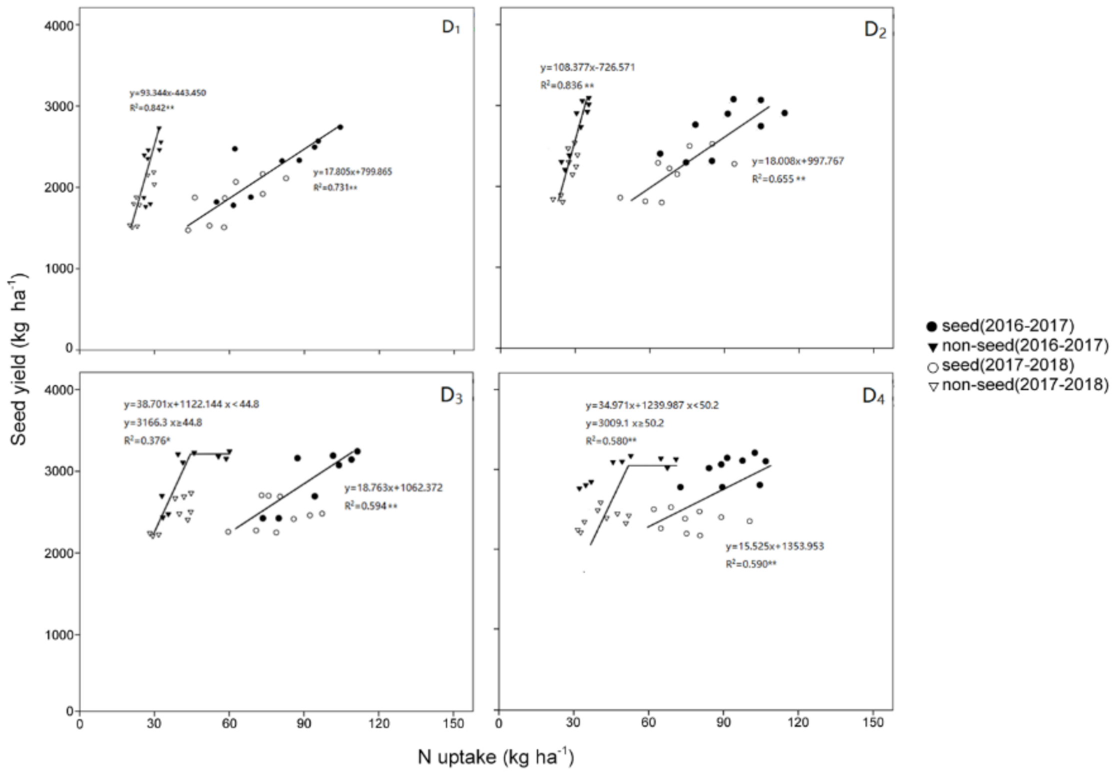

Fig. 3: The relationship between rapeseed yield and N uptake of seed and non-seed parts under different planting density treatments during growing seasons

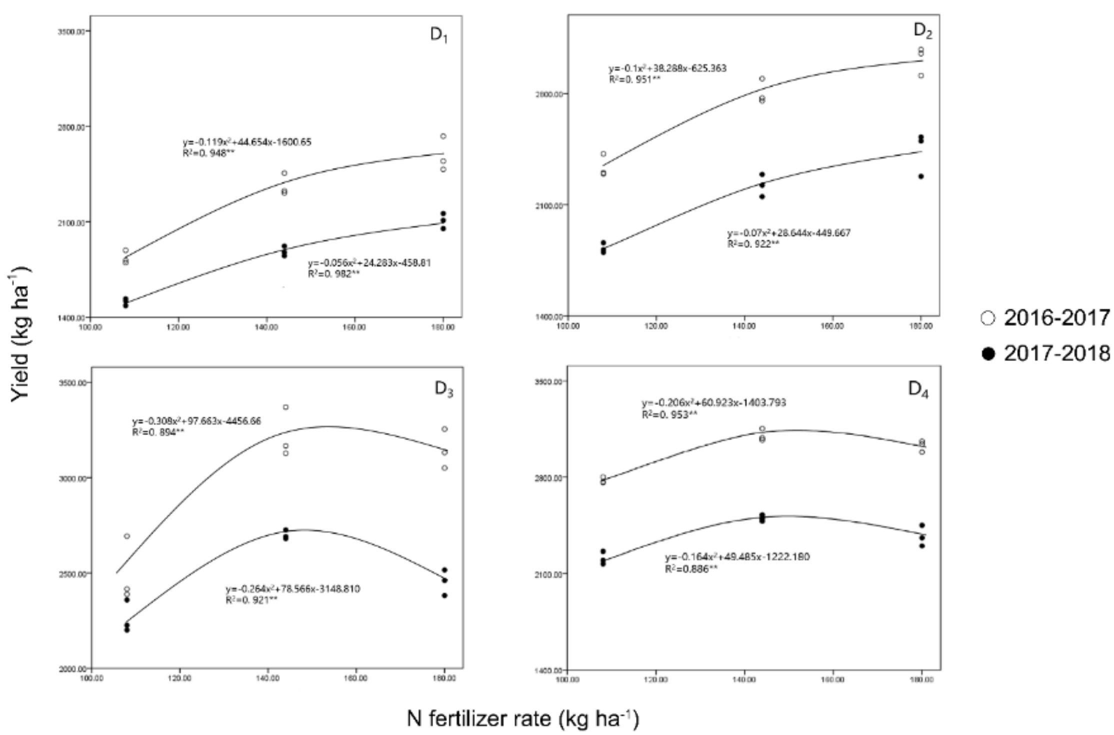

Fig. 4: Effect of N fertilizer rate and planting density on direct seeding rape yield per unit.

Table 6: Partial factor productivity (PFP kg kg^{-1}) under different N fertilizer rates and planting densities during both growing seasons

Treatments	2016–2017 Plant density (× 10^4 plants ha^{-1})				2017–2018 Plant density (× 10^4 plants ha^{-1})			
N application (kg N ha^{-1})	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
108	16.99e	21.65c	23.14b	25.71a	14.02e	16.91c	20.94a	20.46a
144	16.42e	19.49d	22.37bc	21.55c	13.07f	15.43d	18.75b	17.38c
180	14.36f	16.74e	17.47e	17.21e	11.71g	13.34f	13.63ef	13.17f
ANOVA	F value	P value			F value	P value		
N	175.33	*< 0.01***			159.54	*< 0.01***		
Density (D)	109.80	*< 0.01***			90.12	*< 0.01***		
N × D	9.99	*< 0.01***			6.75	*< 0.01***		

Note: Different lowercase letters in each group (2016–2017 and 2017–2018) indicate significant ($P < 0.05$) differences basing on Duncan's multiple range test. ** $P \leq 0.01$; * $P \leq 0.05$

Table 7: N fertilizer rates of rapeseed based on regression equations under different planting densities

Target yield (kg ha^{-1})	N fertilizer rates in 2016–2017 Plant density (× 10^4 plants ha^{-1})				N fertilizer rates in 2017–2018 Plant density (× 10^4 plants ha^{-1})			
	15.0	22.5	30.0	37.5	15.0	22.5	30.0	37.5
Average yield of Sichuan Province (2389)	146.69	110.78	88.52	89.47	-	168.42	115.18	123.63
Decrement	-	35.91	58.17	57.22	-	-	53.24	44.79
Decrease	-	32.4%	65.7%	64.0%			46.2%	36.2%

planting density (180 kg N ha^{-1} and 15.0 × 10^4 plants ha^{-1}), respectively. And the NHI, NUE and PFP were 25, 3.4 and 48.5% higher in the 2017–2018 growing season.

Nonetheless, the NHI and NUE decreased when the application of N fertilizer was high, which was very easy for plants to absorb a large amount of N, and most of N absorbed was mostly accumulated in non-economic parts, thus reducing the N application and increasing planting density can not only realize rapeseed yield and promote N to rapeseed distribution, but also effectively improve the utilization efficiency of N fertilizer at the same time.

Comparison of N Fertilizer Dosage under Four Densities

Regression analysis was carried out between the yield of rapeseed under four densities and the corresponding N fertilizer dosage (Fig. 4). The fitting degree of the four models has reached an extremely significant level.

According to the model and regression equation, the rates of N fertilizer were calculated when the rapeseed yield reaches 2389 kg ha^{-1} (Table 7). During 2016–2017 growing season, four kinds of density of rapeseed N fertilizer were 146.69 kg ha^{-1}, 110.78 kg ha^{-1}, 88.52 kg ha^{-1} and 89.47 kg ha^{-1}. During 2017–2018 growing season, 15.0 × 10^4 plants ha^{-1} level of rapeseed yield has yet to reach an average yield of Sichuan province, so it was unable to calculate the N fertilizer usage, the N fertilizer dosage of the other three-density rapeseed was 168.42 kg ha^{-1}, 115.18 kg ha^{-1}, 123.63 kg ha^{-1}, respectively. Compared with the conventional density (15.0 × 10^4 plants ha^{-1}), increasing the planting density could save 32.4–65.7% N fertilizer. Under the same target yield condition, the rape of high density requires less exogenous N and the increase of planting density could effectively reduce the rates of N fertilizer.

Discussion

The N fertilization and planting density had crucial impacts on crop yield (Liang *et al*. 2013; Fang *et al*. 2018). Previous studies have shown that crop yield frequently exhibited a curve response to N fertilizer rates or planting densities, and reaches the maximum level under the optimum N application or planting density (Roques and Berry 2016). The present study showed that rapeseed yield improved linearly with the increase of planting density at low N levels. However, planting density had a quadratic response at high N treatments. In this study, the yield of rapeseed at high-density treatments increased by 20.2–32.3% and 17.2–28.1% under the condition of constant N application compared with low-density treatment (15.0 × 10^4 plants ha^{-1}) in two growing seasons, respectively. The rapeseed yield at 180 kg N ha^{-1} were the highest under low density treatments, but rapeseed yield no longer improve while N application exceed 144 kg N ha^{-1} at high density treatments.

N application could promote crop growth, improve dry matter accumulation and leaf area per plant and reduce variation among plants at the same time (Rossini *et al*. 2011). The crop growth was poor with a relative low N supply, and the dry matter of individual plant at the stem elongation period was significantly higher at high N input treatments (Dong *et al*. 2012). The competition among plants was healthy even under high-density treatments, and individual plants could still give full play to its yield potential (Tollenaar *et al*. 2006; Ren *et al*. 2017). By contrast, high N supply increased the dry matter accumulation of individual plants (Jiang *et al*. 2002). The crowding stress among crops under suitable conditions often associated with high N application and high-density planting, which accelerated the senescence of low canopy leaves, enhancing plant competition, increasing the incidence rate of crops and resulting in low yield eventually (Tollenaar *et al.* 2006; Dong *et al*. 2012; Antonietta *et al*. 2014). Therefore, the key to high yield and high efficiency was to pay attention to the coordination of N application and planting density.

It has been pointed out in previous studies that rapeseed yield in each unit area depends on the planting density, the number of pods per plant, the seeds per pod and the individual seed weight (Diepenbrock 2000). In present study, the impacts of planting density and N application rate on the number of seeds per pod and the 1000-seed weight were not significant, which might be related to the characteristics of varieties. Consequently, the relationships between rapeseed yield and individual yield components was not very obvious.

The number of pods per plant was remarkably affected by N fertilizer rate and planting density. The planting density was negatively correlated with the number of pods per plant. The number of pods per plant decreased greatly under high-density treatments. Improving the N fertilizer rate could promote the growth of individual plants and increase the number of pods per plant (Riffkin *et al*. 2012; Ren *et al*. 2017). Studies have shown that the optimal N fertilization is closely related to the improvement of rapeseed yield components while the number of pods per plant is the most sensitive to N fertilization. The positive effect of N application on pods per plant could partially balance the decrease of pod numbers per plant at high density (Ma *et al.* 2014).

The purpose of rational close planting of rape is to cultivate a population structure with high light efficiency to improve the yield per unit area to obtain high yield (Wholey and Booth 1979; Ren *et al.* 2017). Although to some extent, the increase of planting density of rape inhibited some characters (such as pods per plant) of rape, however, with the increase of density, the leaf area index and light energy utilization rate of the population were greatly improved, which gave full play to the group effect and finally reflected in the increase of rape groups´ yield (Ozer 2003). This study showed that there was a strong correlation between rapeseed yield and yield components, and the number of pods per ha was significant positively correlated with rapeseed yield and the correlation coefficient is relatively high, which was consistent with previous study results (Ozer 2003; Ren *et al*. 2017; Zheng *et al*. 2020).

The application of N fertilizer and planting density significantly affected rapeseed yield and yield components. High-density planting improved rapeseed yield. The number of pods per plant decreased obviously as the planting density increased. The N fertilizer rate greatly increased the number of pods per plant and the yield of rapeseed, and significantly affected the response of planting density to rapeseed yield. At low density, the yield of rapeseed improved with the increase of the N fertilizer rate. Under high density, excessive N application reduced rapeseed yield. The income of rapeseed was the best under the treatment of 144 kg N ha^{-1} and 30.0×10^4 plants ha^{-1}.

One of the main measures to reduce the loss of N is to reduce the N fertilization and improve the N utilization rate. The N uptake reflects the N utilization rate (Nakamura *et al.* 2008; Caviglia *et al*. 2014).

The rapeseed yield improved linearly as N uptake increased, and the N uptake of each seed was similar under different densities. Nevertheless, for the non-seed part (stem + husk), the response of N uptake to rapeseed yield varied with changes of planting densities. The N uptake of non-seed part increased without obtaining high rapeseed yield at high density treatments, showing the obvious phenomenon of luxury N uptake. This change was consistent with previous research results, the competition among plants was invariably fierce under high density and high N supply, so much more N was distributed to the non-seed part to promote the competitiveness of plants (Bennett *et al*. 2011; Ren *et al.* 2017).

Non-leaf organs played an important role in the photosynthesis and yield formation of rape since the pod development period beginning (Beccafichi *et al*. 2003; Amanullah 2010; Zhang *et al*. 2014). High density would lead to shading between plants, so more N was distributed to the non-seed part to compensate for the decrease of light intensity and the maintenance of photosynthesis (Delagrange 2011; Ren *et al.* 2017). Although crops at high planting densities absorbed much more N, the absorbed N was used to improve the competition among plants rather than to obtain high yield. Therefore, it was necessary to optimize the population structure and reduce competition among plants by coordinating N application and planting density.

High density increased the shoot N uptake of rapeseed. Under high density and high N application treatment, the non-seed parts (stem + husk) did not make more contribution to rapeseed yield with the condition of absorbing higher N. Increasing the planting density could promote the transfer of N to rapeseeds and improve the N utilization rate. However, excessive application of N fertilizer would lead to more accumulation of N in non-economic parts of rapeseed, which could not achieve the purpose of increasing rapeseed yield.

At present, in order to give full play to the potential of increasing yield of rapeseed, the application rates of N fertilizer are too large, resulting in low N fertilizer utilization rate and surplus N fertilizer (Du *et al*. 2019). Studies have shown that crop yield was closely related to N uptake and the effects of N application on its uptake and utilization of crops are different. Instead of increasing crop yield, high N will lead to decreased N utilization efficiency, resource waste and environmental security (Anbessa and Juskiw 2012). Some studies also showed that by reducing N fertilizer and increasing planting density, high crop yield and N utilization rate can be achieved (O'Beirne and Cassidy 1990).

The results showed that dense planting could improve the N utilization efficiency, increase the NHI and the NUE of rapeseed. The NHI reflects the N transfer to the seeds. In this study, the increase of N application reduced the NHI and the NUE, which indicates that the increase of N application is not conducive to the N transfer to the rapeseeds, and more N will be used for the physiological activities of the plants themselves. The results were consistent with previous research (Tirol-Padre *et al*. 1996; Dong *et al*. 2007). Under low N fertilizer and high density, PFP is higher, it can be seen that reducing N application and density planting is the key measure to improve the utilization efficiency of N fertilizer and effectively save the N fertilizer rates (Shapiro and Wortmann 2006; Zheng *et al*. 2020).

From the relationship between rapeseed yield and N fertilizer dosage, in this study, when the same target yield was achieved, the N fertilizer dosage of high-density rapeseed was 35.9–58.2 kg per ha less than regular density (15.0×10^4 plants ha^{-1}) rapeseed, which means that increasing the planting density could save 35.1–61.2% of N application. The dense planting is a key method to improve the utilization efficiency of N fertilizer and effectively save the application of N fertilizer (Zhang *et al*. 2019). Thus, reducing the application of N fertilizer and increasing the planting density at the same time can not only achieve the high yield of rape, promote the distribution of N to seeds and slow down the N loss, but also effectively improve the efficiency of N utilization while protecting the ecological environment.

Conclusion

The application of N fertilizer and planting density significantly affected rapeseed yield and yield components. High-density planting improved rapeseed yield. At low density, the yield of rapeseed improved with the increase of the N fertilizer rate. Under high density, excessive N application reduced rapeseed yield. The income of rapeseed was the best under the treatment of 144 kg N ha^{-1} and 30.0 × 104 plants ha^{-1}. High density increased the shoot N uptake of rape. However, excessive application of N fertilizer would lead to more accumulation of N in non-economic parts of rape, which could not achieve the purpose of increasing rapeseed yield. Reducing the nitrogen rate and increasing the planting density can realize the high yield of rape and effectively improve the nitrogen use efficiency.

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Author Contributions

QZ and ZL conceived and designed the experiments. QZ, ZL and XQT performed the experiments. QZ, ZL, XQT, and YHL analyzed and interpreted the sequence data. QZ wrote the paper. All authors read and approved the manuscript.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Ethics Approvals

Not applicable.

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