

# Pollen number and vigor contribute to effective ovule numbers among contrasting nitrogen utilization efficiency oilseed rapeseed genotypes before heart stage

Xiao Guo (✉ [guoxiao19890910@outlook.com](mailto:guoxiao19890910@outlook.com))

Queensland Government <https://orcid.org/0000-0002-0366-5572>

Yuyu Xie

Northwest Agriculture and Forestry University

Yunyou Nan

Northwest Agriculture and Forestry University

Xiaoming Wu

Oil Crops Research Institute Chinese Academy of Agricultural Sciences

Biyun Chen

Oil Crops Research Institute Chinese Academy of Agricultural Sciences

Bao-Luo Ma

Ottawa Research and Development Centre

Yajun Gao

Northwest Agriculture and Forestry University

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## Research article

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# Abstract

**Background:** While no significant differences in initial ovule number were found among oilseed rape genotypes, there was a large variation in effective ovule number (EON), which determines the final seeds per silique (SPS), a critical component of yield. Up to date, on study has been focused on unraveling the pre-flowering main factors to restrict EON and identifying the critical period of EON formation among contrasting nitrogen utilization efficiency (NUE) oilseed rape genotypes.

**Results:** In this study, we selected 18 oilseed rape genotypes with different NUE to identify the main factors that contribute to EON, and determine if genotypes differed in the critical period of EON formation under both field and pot experiments from 2016-2018. Our results showed the high NUE genotypes also showed 14.3% higher NUE, accompanied with 29.4% higher yield per plant and 21.1% higher SPS. The greater productivity of the high NUE oilseed rape genotypes was associated with 44.1% greater pollen number, 23.5% higher pollen vigor, and 39.3% lower ovule abortion rate, compared to the low NUE genotypes. In addition, at the heart stage, the high NUE genotypes displayed higher silique net photosynthetic rate, surface area, biomass, and RNA expression levels, compared to the low NUE ones. Taken together, this study indicated the pollen number, pollen vigor and ovule abortion rate contributed to the final EON of diverse oilseed rape genotypes; the critical period of determining EON among contrasting NUE genotypes was at the heart stage.

**Conclusion:** Increasing pollen number and vigor, and decreasing ovule abortion rate before the heart stage should be the prerequisite for breeders to improve yield and NUE of oilseed rape genotypes.

## Background

Oilseed rape (*Brassica napus* L.) is the world's second largest crop source of vegetable oil following soybean, with increasing significance in the international market. Yield, one of the most important traits in oilseed rape breeding, is composed of seeds per silique (SPS), siliques per plant (SPP) and thousand seed weight [1]. As with other crops, yield components are critical to yield formation and are often compensated each other, depending on genotypes and interaction with environments [2, 3]. SPS, which is usually negatively correlated with the other two yield components for competition among sinks, has always received much attention [2, 4-7]. On the other hand, the SPS was positively correlated with nitrogen utilization efficiency (NUE; seed dry weight / shoot N accumulation), which indicated SPS might work as a simple and applicable indicator for the evaluation of NUE in oilseed rape [8].

During the pollination process, pollen grains of oilseed rape fall and germinate on the stigma, forming pollen tube, and sending the male gametes to meet the female gametophyte within the ovule to initialize fertilization. Thus, successful reproduction relies on the number of pollen grains and female ovule number [9, 10]. Following fertilization, embryogenesis is a developmental process that can be divided into five distinct stages: pre-embryo, globular, heart, torpedo and maturation stages [11-13]. The fertilized ovules may fail to produce seeds at any stages, due to the internal and external environmental

conditions. The final remaining ovules (effective ovule number, EON) eventually develop into viable seeds (SPS). Therefore, to some degree, the EON determines the SPS, the critical component of the seed yield in oilseed rape plants. While no significant difference in the initial ovule number was observed among the diverse oilseed rape genotypes [5, 10], it was reported large variations in the final SPS (from 5 to 35) [5]. Based on this, we hypothesized that there was a critical period from the pre-embryo to maturation stages that determines the differences in EON among contrasting NUtE genotypes.

Biologically, the EON of oilseed rape genotypes is primarily determined by the number of ovules per placenta as well as the frequency of embryonic abortions [5, 14]. The number of ovules per placenta was reported to be determined by the process of ovule differentiation and development [15]. The frequency of embryonic abortions of oilseed rape was determined by the fertilization process, which is dependent on many factors such as pollen grain germination [16], silique photosynthesis [17, 18], nutrition accumulation [19] and the number of flowers [20, 21]. Although from the ovule fertilization to seed maturity, the developmental processes among oilseed rape genotypes could be largely clear, there is little knowledge of the main factors that determine the final EON before fertilization. Identification of these reasons will be more meaningful for breeders to take steps to reduce ovule abortion and increase SPS and seed yield of oilseed rape genotypes.

From a preliminary study on 50 oilseed rape genotypes, we demonstrated that the EON was strongly and positively correlated with NUtE, and nitrogen utilization-efficient genotypes had higher EON than the inefficient genotypes [8]. Therefore, the main objectives of the current study were to: i) unravel the main factors to restrict EON of diverse oilseed rape genotypes, and ii) determine the critical period of EON formation among contrasting NUtE oilseed rape genotypes.

## Methods

### Plant materials

The results from a previous study on 50 oilseed rape genotypes allowed us to categorize the tested genotypes into four groups, based on their NUtE values: Nt-responder, Nt-nonresponder, Nt-efficiency, and Nt-inefficiency. Nt-responder referred to genotypes with an NUtE value above the mean at high N, while genotypes with a NUtE value below the mean were categorized into the Nt-nonresponder group. At the low N supply, genotypes displayed a NUtE value above the mean were called Nt-efficient, and Nt-inefficient genotypes were those genotypes with a NUtE value below the mean [8]. According to the rank of responses reported previously, we chose 18 genotypes with distinctive NUtE for this study. These included 5 Nt-responder, 5 Nt-nonresponder, 4 Nt-efficiency and 4 Nt-inefficiency and are described in Table 1.

### Experimental design

This study contained a field trial and a controlled pot experiment, and conducted at Yangling district, Shaanxi province (34° 24' N, 108° 08' E) from 2016-2018. In the field experiment, the soil was alluvial texture with a pH of 7.65, containing 13.7 g kg<sup>-1</sup> organic matter, 1.19 g of total N kg<sup>-1</sup>, 24.7 mg of

available N kg<sup>-1</sup>, 15.7 mg of Olsen-P kg<sup>-1</sup> and 76.9 mg of available K kg<sup>-1</sup>. In the pot experiment, the red loess soil had the following properties: 9.21 g kg<sup>-1</sup> organic matter, 0.92 g of total N kg<sup>-1</sup>, 22.0 mg of available N kg<sup>-1</sup>, 10.7 mg of Olsen-P kg<sup>-1</sup> and 73.6 mg of available K kg<sup>-1</sup>, with a pH of 7.5.

Our previous study indicated that the high NUtE of N-efficient genotypes expressed only under high N supply conditions, while the low NUtE of N-inefficient genotypes expressed under low N supply conditions. Therefore, the high N efficient genotypes were planted with high N level (150 kg N ha<sup>-1</sup> in the field experiment and 0.30 g of N kg<sup>-1</sup> dry soil in the pot experiment), while the 8 low N genotypes were tested with low N level (0 kg N ha<sup>-1</sup> in field experiment and 0.10 g of N kg<sup>-1</sup> dry soil in pot experiment). Each experiment was arranged in a randomized complete block design with four replicates. In both experiments, sufficient phosphorus as superphosphate (P<sub>2</sub>O<sub>5</sub> 135 kg ha<sup>-1</sup> and 0.20 g kg<sup>-1</sup> dry soil) and potassium (K<sub>2</sub>O 150 kg ha<sup>-1</sup> and 0.30 g kg<sup>-1</sup> dry soil) were supplied.

### **Observations and measurements**

In order to monitor the dynamics of silique development, flowering and fertilization process of the genotypes were categorized into five stages: pre-embryo (20-80 / °C, 1-4 growing degree days after flowering), globular (95-136 / °C, 5-8 growing degree days after flowering), heart (153-224 / °C, 9-14 growing degree days after flowering), torpedo (240-396 / °C, 15-22 growing degree days after flowering), and maturation (391-540 / °C, 23-30 growing degree days after flowering) stages. For accurate monitoring, fully-opened flowers on the main inflorescence of each plant were marked with color strings around the peduncles in each day. In the pot experiment, five marked siliques of each plant at each developmental stage were chosen for the determination of net photosynthetic rate, silique length, width, surface area, EON, biomass and RNA expression levels.

At the flowering stage, after the sampled buds were removed from the petals, we recorded the stamen and anther number. The number of pollens was counted under light microscope (Axioplan 2; Zeiss), pollen vigor was measured according to HY Zheng, HM Wu, XY Pan, WW Jin and XX Li [22]. The initiation ovule number and the abortion rate were determined according to XJ Wang, A Mathieu, PH Cournede, JM Allirand, A Jullien, PD Reffye and BG Zhang [20]. The pollen vigor was expressed as the total number of germinated pollen grains divided by the total number of pollen grains. The abortion rate was calculated as the aborted ovules over the total number of ovules.

### **Measurement of agronomic traits**

At maturation stage, plant height was measured from the base of the stem to the tip of the main inflorescence [23]. The point of measurement of stem diameter was set at 10 cm from basal of the main stem [24]. First valid branch height was measured as the height from the base of the stem to the effective primary branches at the bottom of the main stem. The number of the first valid branches was measured according to JS Xu, X Song, Y Cheng, XL Zou, L Zeng, X Qiao, GY Lu, GP Fu, Z Qu and XK Zhang [25].

## Measurement of yield and N use efficiency

Number of siliques per plant was measured as the number of effective pods on the main inflorescence, branch inflorescence and the whole plant, respectively [3]. Seed weight of each plant was measured by weighing 500 fully developed seeds with four replications, and then was converted to 1,000-seed weight for easy comparison with other studies [26]. Seed yield per plant was measured as the average dry weight of seeds of the 4 randomly selected plants from each genotype [27]. According to HY He, R Yang, YJ Li, A Ma, LQ Cao, XM Wu, BY Chen, H Tian and YJ Gao [8], N utilization efficiency (NUtE) and N uptake efficiency (NUpE) were estimated with the following equations:  $NUtE = \text{Seed yield} / \text{The N accumulation (shoot)}$ ;  $NUpE = \text{The N accumulation (shoot)} / \text{The N supply}$ .

## Measurement of siliques indexes

At each stage (pre-embryo, globular, heart, torpedo, and maturation), silique gas exchange rate was measured on the marked siliques between 09:00 and 11:00, using a Portable Photosynthesis System (Li-6400, LI-COR, Lincoln, NE, USA). Afterwards, the marked siliques were picked from the main inflorescence. Half of each of sampled siliques was rapidly frozen in liquid N, and stored at -80 °C for later RNA transcript analysis, and the other half was used for the determination of surface area [28] and the normal ovule numbers, which was counted under a light microscope. The sampled siliques were oven-dried at 105 °C for 30 min and then at 70 °C until a constant dry weight was reached and weighed.

## Quantitative RT-PCR analysis

Silique samples of the 18 diverse NUtE genotypes were frozen in liquid nitrogen immediately after collection and stored at -80°C. Approximately 100 mg of tissue was ground in liquid nitrogen and total RNA was extracted using an EZNATM Plant RNA Kit (Omega Bio-Tek, USA). The sample was used for cDNA synthesis using TransScript® First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Quantitative PCR analyses were performed in an QuantStudio® Design and Analysis (QuantStudio 5, Life Technologies, USA), using an TransStart Tip Green qPCR SuperMix (TransGen Biotech, China). All the primers used in the analysis are listed in Table S1. The low NUtE genotypes (Nt-nonresponder and Nt-inefficiency) were used as references (Nt-nonresponder for Nt-responder and Nt-inefficiency for Nt-efficiency, respectively). Data were expressed as the mean of four biological replicates  $\pm$  SD.

## Statistical Analysis

All the data from both the field and pot experiments, were subjected to analysis of variance. Estimates of correlation coefficients and tests of significance, were performed using the SPSS version 17.0. Principal component analysis was performed using the Canoco 5.0 software. When the analysis showed significant, mean comparisons were made according to the least-significant difference test at  $P \leq 0.05$  ( $LSD_{0.05}$ ). All the figures were created using Origin 9.0 software, Microsoft Excel and PowerPoint 2016.

# Results

## Stamen and ovule characteristics of contrasting NUtE genotypes

Our results showed that the stamen of the contrasting NUtE genotypes was identical and normal in the field and pot experiments (Fig. 1A-D). There was also no difference in anther and filament number (Fig. 2E-H, Supplementary Fig. 1). On average, the high NUtE (Nt-responder and Nt-efficiency) genotypes had 44.1% (52.7% and 33.1%) greater pollen number, with 23.5% (21.2% and 26.2%) higher pollen vigor than the low NUtE (Nt-nonresponder and Nt-inefficiency) genotypes (Fig. 1I-P). There was no significant difference in the initial ovule number among the high and low NUtE genotypes under both the field and pot experiments (Fig. 2A-H).

## Silique indexes of contrasting NUtE genotypes

Silique net photosynthetic rate of Nt-efficient genotypes was higher than the Nt-inefficient genotypes from the heart to maturation stages (Fig. 3B). We also observed a higher silique surface area for the high NUtE genotypes than for the low NUtE ones from the heart to torpedo stages. The same trend was found in the silique length and width (Supplementary Fig. 2). Finally, higher effective ovule number and silique biomass were recorded for the high NUtE than for the low NUtE genotypes at the heart stage (Fig. 3E-H).

## Silique RNA transcript levels of contrasting NUtE genotypes

In order to understand the observed differences in silique development, 18 of the contrasting NUtE genotypes were selected for measurement of the silique transcript levels (*BnARF18*, *BnLCR*, *BnCLV3* and *BnDA1*). There was no significant difference in the expression level of the four genes among the high and low NUtE genotypes at the globular stage (Fig. 4). However, from the heart to torpedo stages, the RNA expression levels were 15-40 folds in *BnARF18*, 5-21 folds in *BnLCR*, 5-15 folds in *BnCLV3* and 4-18 folds in *BnDA1*, respectively higher for the high NUtE than for the low NUtE genotypes.

## Ovule abortion rate of contrasting NUtE genotypes

Differences in ovule abortion rate among the contrasting NUtE genotypes also existed in both experiments, with 39.3% (45.4% in Nt-responder and 34.3% in Nt-efficiency) lower aborted ovules of the high NUtE than of the low NUtE genotypes (Fig. 5A-H).

## Agronomic traits, yield and NUE of contrasting NUtE genotypes

There was no significant difference of agronomic traits (plant height, stem diameter, first valid branch height and number of first valid branches) among the diverse NUtE genotypes in both the field and pot experiments (Table S2).

The number of siliques per plant, seeds per silique and yield per plant were 12.4%, 21.1% and 29.4% greater for the high NUtE genotypes than for the low NUtE genotypes. In contrast, the low NUtE genotypes displayed 14.3% higher 1000-seed weight than the high NUtE genotypes in both experiments (Table 2).

Compared to the low NUtE, the high NUtE genotypes showed 30.9% and 14.3% higher NU<sub>p</sub>E and NUtE, respectively (Fig. 6D).

### **Correlations between effective ovule number and the agronomic traits**

Principal component analysis showed that the first two principal components explained about 66%-83% of the total variation (Fig. 7A-B). The first principal component was mainly spanned by traits of EON, anther number, filament number, pollen number, pollen vigor, initial ovule number, stem diameter, first valid branch height, 1000-seed weight, yield per plant, and NUtE on the positive side of the scale, while ovule abortion rate on the negative side. The vectors indicated NUtE, the yield per plant, pollen number and pollen vigor exhibiting obviously positive correlations with EON, while there was a strong and negative correlation between ovule abortion rate and EON in both the pot and the field experiments.

Using the path coefficient analysis, we illustrated the direct and indirect effects of the three traits (pollen number, pollen vigor and ovule abortion rate) on EON and their interrelationships (Fig. 7C-D). The pollen number and pollen vigor displayed a positive direct effect on EON, while the ovule abortion rate displayed a negative correlation with EON. There exhibited a positive indirect effect of pollen number (or pollen vigor) on EON via the pollen vigor (pollen number), however a negative correlation between EON and ovule abortion rate.

## **Discussion**

Our study showed that the pollen number, pollen vigor and ovule abortion rate contributed to different EON of contrasting NUtE oilseed rape genotypes was before heart stage. In order to explain this, a simplified model (Fig.8) was drawn to map a mechanistic understanding for the pre-flowering main reasons and critical period of EON formation among contrasting NUtE oilseed rape genotypes. This explanation is discussed in detail below.

### **Pollen number, pollen vigor and ovule abortion rate contribute to diverse EON in oilseed rape genotypes**

Compared to the low NUtE genotypes, the high NUtE genotypes produced a higher seed yield per plant and higher NUtE (Table 2 and Fig. 6) mainly resulted from larger filled pod number (EON), which associated with higher pollen number, better pollen vigor, and a low success ratio of developed pods (ovule abortion rate). The process from the initial ovule to final seeds is affected by many factors, such as pollen sterility [5], pollen tube growth [29], floral bud quantity [30], inflorescence position [16], planting density [17], shading [18] and silique photosynthesis [31]. In this study, we explored the main factors that may have caused diverse EON in two perspectives: before and after ovule fertilization. After ovule fertilization, we observed a negative correlation between EON and the ovule abortion rate (Fig. 7C-D). Previous studies [32, 33] provided some evidence in support of our findings. Study reported that the ovule abortion is frequently associated with selection of superior genotypes [34]. Our results showed the ovule abortion rate of the high NUtE was significant lower than of the low NUtE genotypes (Fig. 5), which indicated breeding towards the lower ovule abortion rate genotypes can be a relatively quick trait-based

strategy to reduce ovule abortion and improve seed yield and NUtE. The significant and negative correlations between EON and the abortion rate suggest that ovule abortion rate may have acted as the “upstream” of EON. It is therefore, implementation of strategies targeting to reduce ovule abortion rate, such as sufficient supply of water [35], appropriate temperature [36] and light [20], may increase EON, yield and NUtE in oilseed rape genotypes.

It is noteworthy that while there was no significant differences in the number of anthers and the initial ovule number between the high and low NUtE genotypes (Fig. 1A-H and Fig. 2), there was 44.1% larger pollen number with 23.5% higher pollen vigor for the high NUtE than for the low NUtE genotypes (Fig. 1I-P). Furthermore, both the pollen number and pollen vigor displayed significant and positive correlations with EON (Fig. 7C-D), indicating before ovule fertilization, pollen number and pollen vigor may be the vital contributors to EON, seed yield per plant and NUtE. In oilseed rape plants, sometimes, ovules fail to develop into mature seeds because of fewer viable pollens available to fertilize all the ovules, leading to eventually aborted ovules. The viable pollen number could therefore, be the “upstream” of ovule abortion [37], regulating EON through adjusting ovule abortion. Pollen grains are produced within the anther and released to the environment after maturation [38]. During pollen development, nitrogen is essential to ensure efficient growth. Inadequate N supply significantly reduced pollen number and pollen vigor in maize [22, 39]. O Nikolic, T Zivanovic, M Jelic and I Djalovic [40] demonstrated in field experiment, there was a significant correlation between the plant N accumulation and grain yield in wheat. In our study, we found 19.2% higher shoot N accumulation at pre-flowering for the high NUtE than for the low NUtE genotypes (data not shown). Accordingly, we speculated that the accumulation of shoot or whole-plant N before flowering may have served as the reserves that allowed the plant to produce more pollen number with higher pollen vigor, leading to less ovule abortion in the high NUtE genotypes.

### **Larger effective ovule number for the high NUtE than for the low NUtE genotypes at the heart stage**

Because of the complex conditions, especially its variable germplasm resources, the EON of diverse oilseed rape genotypes showed great variation [5]. Therefore, identifying the critical period of variation could help breeders or farmer take steps to decrease ovule abortion, increase SPS and seed yield. In the present study, we firstly tested the initial ovule number, and found there was no significant difference among contrasting NUtE genotypes (Fig. 2), similar to those reported in S Li, L Chen, L Zhang, X Li, Y Liu, Z Wu, F Dong, L Wan, K Liu, D Hong, et al. [10] and Y Yang, J Shi, X Wang, G Liu and H Wang [5]. Our results indicated the critical period may occur after fertilization: pre-embryo, globular, heart, torpedo and maturation stages. [11-13]. Through carefully examining the results, we clearly identified the heart stage as the critical stage at which the difference in dynamic change of EON formation became significant among the contrasting NUtE genotypes remained till the maturity stage (Fig. 3E-F). Previously, WJ Xu, Y Fu, HL Dong, ZF Chen, QY Zhang, SS Mao, YJ He and W Qian [41] and Y Yang, J Shi, X Wang, G Liu and H Wang [5] respectively claimed the globular or torpedo stage as the critical period for EON formation among diverse oilseed rape genotypes. It was inconsistent with our results, which may be our fine turning procedure with more frequent samples followed in the current study that allowed us to pinpoint the critical stage may explain the inconsistency with the literature.

The rapeseed silique is not only one of the main photosynthetic organs formed [42], but also serves as an important sink organ that stores carbohydrates for filling the developing seeds [31, 43, 44]. Results suggest that an increase in silique biomass accumulation and sustained photosynthesis are the major physiological determinants of EON increases [45]. In this study, the significantly higher silique net photosynthetic rate and larger biomass for the high NUtE genotypes than for the low NUtE genotypes at the heart stage (Fig. 3A-B and 3G-H), served as the physiological reasons for the difference in EON among contrasting NUtE genotypes. It is expected that plants with greater silique surface area would have higher silique photosynthesis and thus would increase seed yield [46]. In this study, the high NUtE genotypes displayed significantly larger silique surface area, silique length and width compared to the low NUtE oilseed rape genotypes at heart stage, implying the heart stage is the critical period of EON development among contrasting NUtE oilseed rape genotypes, which was consistent with the results of silique net photosynthetic ratio, biomass and EON.

The silique is a main organ in which seeds are formed and filled, and silique traits of oilseed rape are all complex quantitative traits controlled by polygenes [6, 47]. Recent literature have reported the identification of genes that regulate seed development [48], seed weight [49], seeds per silique [10], silique development [50] and silique length [51]. Here, we examined four genes that related to silique development to understand the molecular mechanism of EON variation. Our results clearly showed higher silique RNA transcript levels of the high NUtE than of the low NUtE genotypes from the heart to torpedo stages (Fig. 4), providing a molecular evidence for the difference in EON development between the high and low NUtE oilseed rape genotypes. After flowering, green siliques of oilseed rape functioned as photosynthesis center, and may contribute assimilates and nutrients into the silique biomass and developing seeds [28, 31, 43]. Therefore, the higher silique net photosynthetic rate of the high NUtE genotypes resulting in higher silique surface area, greater biomass and larger EON, compared to the low NUtE genotypes. With these results as the supporting evidence, it was concluded that the heart stage is the critical period of determining the EON among contrasting NUtE genotypes in silique development.

## Conclusion

In summary, compared to the low NUtE oilseed rape genotypes, the high NUtE genotypes produced a larger number of pollens with higher pollen vigor before flowering. These plants also displayed a lower ovule abortion rate after ovule fertilization. At the heart stage, the high NUtE genotypes formed a larger number of EON than the low NUtE genotypes. The dynamic difference in EON between the genotypes maintained until the maturation phase. Consequently, the high NUtE genotypes produced more SPS and greater yield with larger NUtE than the low NUtE genotypes. With this point of view, we suggest that the necessary measures that needed for oilseed rape improvement to achieve higher NUtE and seed yield should be targeting to increase pollen number and vigor, and decrease the ovule abortion rate before the heart stage.

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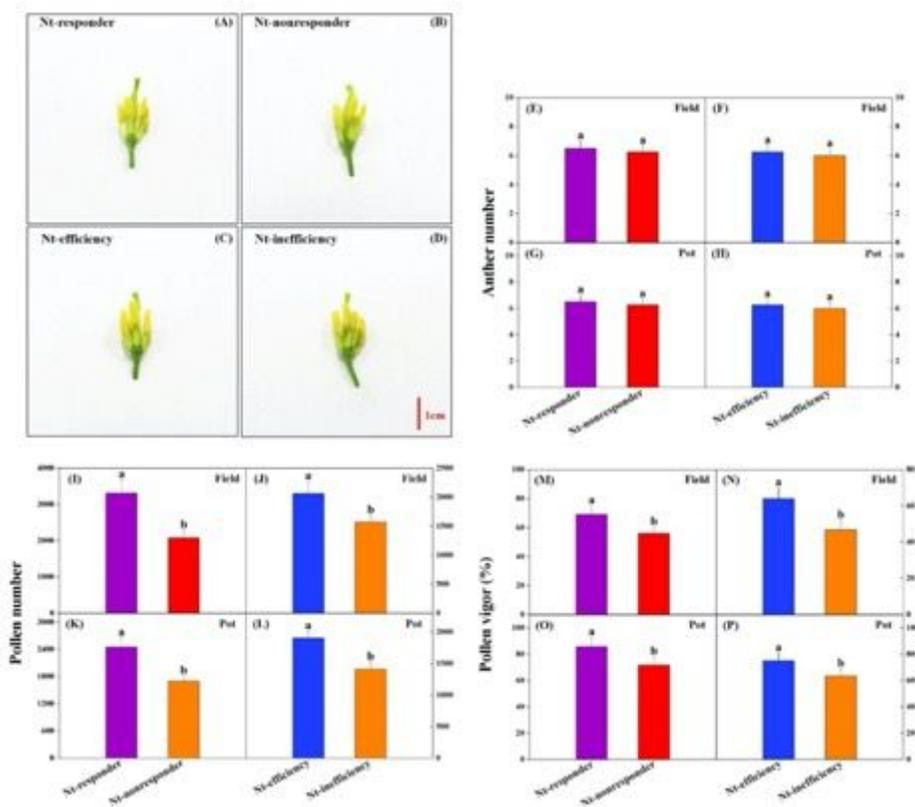
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## Tables

**Table 1** The name, origin and ecotype of the oilseed rape genotypes used in this study.

	Name of genotype	Origin	Ecotype
Nt-responder	6020-1	China	Semi-winter
	71-8	China	Semi-winter
	Moneta	Canada	Spring
	Jian 72	China	Semi-winter
	Zheyou 18	China	Semi-winter
Nt-nonresponder	Wesbery-1	Australia	Spring
	28960	Germany	Winter
	Bridger	Germany	Winter
	Sollux	Germany	Winter
	H 49	Soviet Union	Winter
Nt-efficiency	Guiyou 3	China	Semi-winter
	Zhongshuang 10	China	Semi-winter
	Zhongshuang 9	China	Semi-winter
	Zheyou 18	China	Semi-winter
Nt-inefficiency	77023	China	Semi-winter
	85-110	China	Semi-winter
	Zhongshuang 4	China	Semi-winter
	Sollux	Germany	Winter

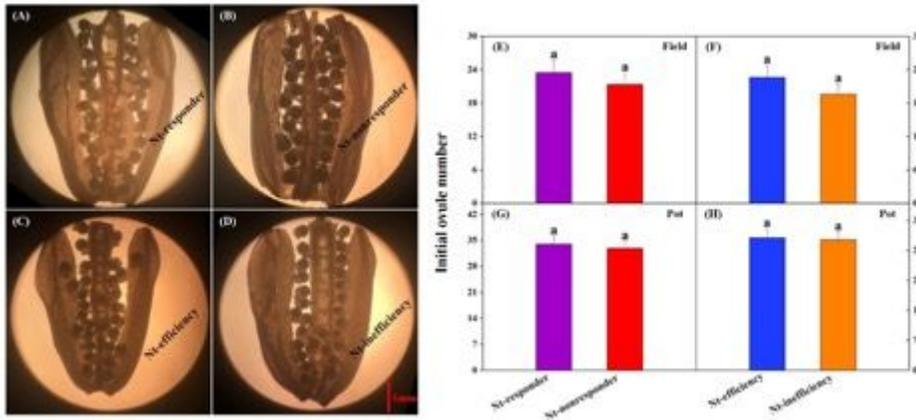
## Figures



**Figure 1**

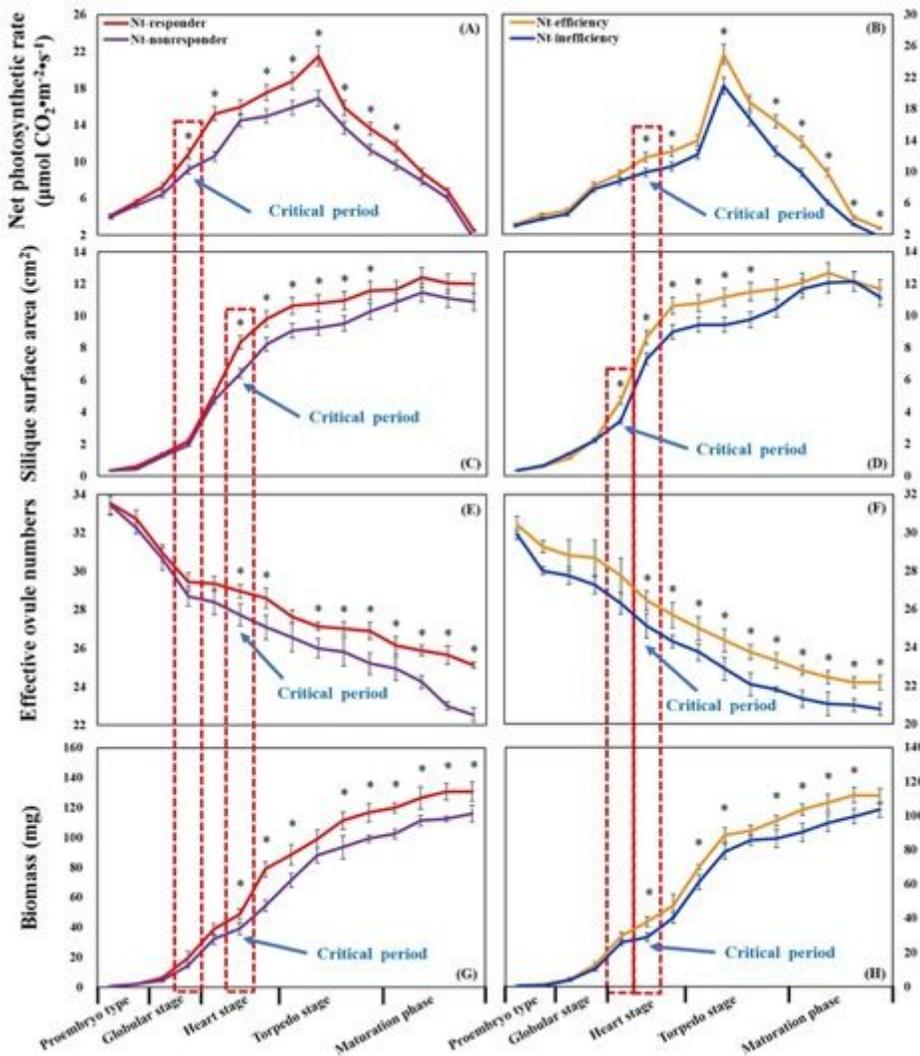
Stamen traits under field and pot experiments. (A-D) the stamen phenotype among the high and low NUtE genotypes, scale bars = 1 cm; anther number (E-H), pollen number (I-L) and pollen vigor (M-P) of contrasting NUtE genotypes. Each bar represented a mean plus SE of four biological replicates, and

different letters above the bars within a figure indicate significant differences according to ANOVA-protected LSD0.05 test.



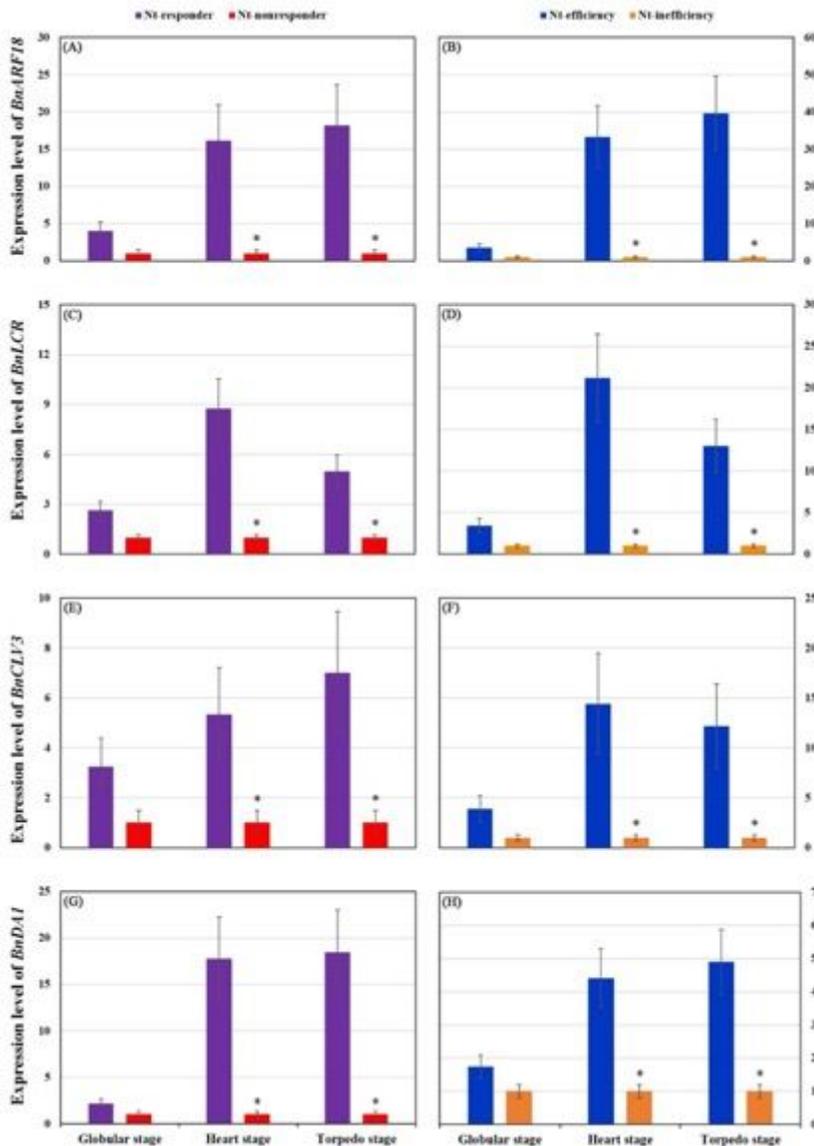
**Figure 2**

Initial ovule number under field and pot experiments. (A-D) the initial ovule phenotype under light microscopy (scale bars = 1 mm); (E-H) mean the initial ovule numbers of contrasting NUtE genotypes. Each bar represented a mean plus SE of four biological replicates, and different letters above the bars indicate significant differences according to ANOVA-protected LSD0.05 test.



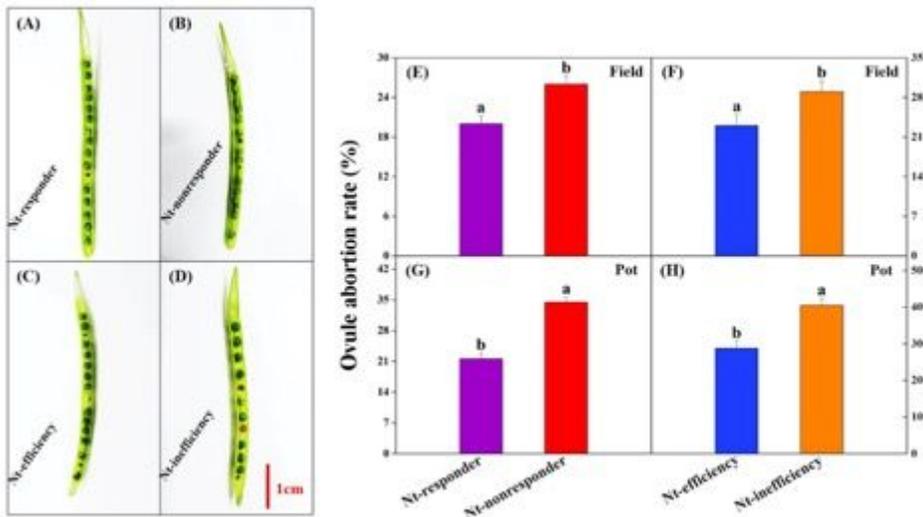
**Figure 3**

Silique net photosynthetic rate (A-B), silique surface area (C-D), effective ovule number (E-F) and silique biomass (G-H) among contrasting NUtE genotypes from pre-embryo, globular, heart, torpedo and maturation stages under pot experiment. \* indicates significant difference among contrasting NUtE genotypes ( $P < 0.05$ ). The red frame indicate the initial critical period when the significance difference in silique indexes occurred.



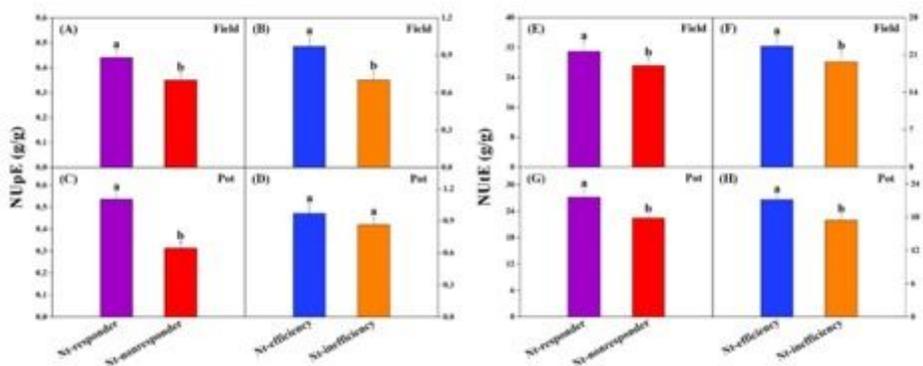
**Figure 4**

BnARF18 (A-B), BnLCR (C-D), BnCLV3 (E-F) and BnDA1 (G-H) expression levels at the globular, heart and torpedo stages. The low NUtE genotypes (Nt-nonresponder and Nt-inefficiency) were used as references (Nt-nonresponder for Nt-responder and Nt-inefficiency for Nt-efficiency, respectively). Data are expressed as the means of four biological replicates, and \* indicates significant differences between the contrasting NUtE genotypes ( $P < 0.05$ ).



**Figure 5**

Ovule development phenotypes of contrasting NUtE genotypes under field and pot experiment. (A-D) the image of ovule abortion at maturation phase (scale bars = 1 cm); (E-H) the mean and standard error of the mean of the ovule abortion rate of different NUtE oilseed rape genotypes. Each bar represented the mean of four biological replicates, and different letters above the bars indicate significant differences according to ANOVA-protected LSD0.05 test.



**Figure 6**

NUpE (A-D) and NUtE (E-H) of the contrasting NUtE genotypes under field and pot experiments. Each bar represented the mean and standard error of the mean of four biological replicates, and different letters above the bar indicate significant differences according to ANOVA-protected LSD0.05 test.

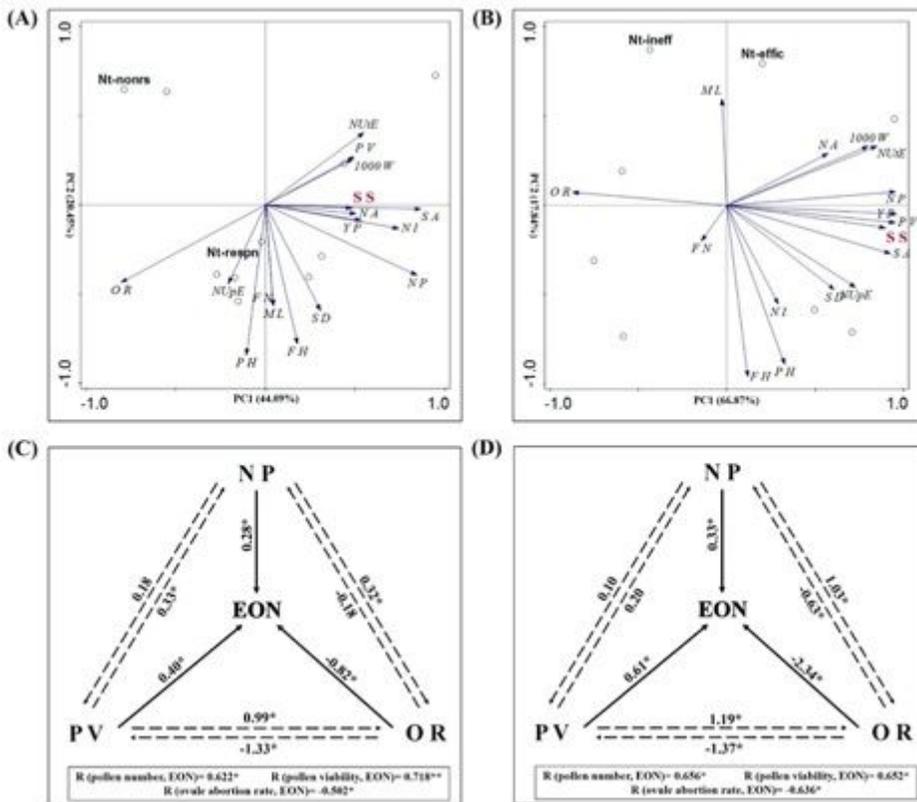


Figure 7

Principal component analysis in high N (Fig.7A) and low N (Fig.7B) and the path coefficient for EON, pollen number, pollen activity and ovule abortion rate in high N (Fig. 7C) and low N (Fig.7D) of different oilseed rape genotypes under field and pot experiments. Nt-respn, Nt-responder; Nt-nonrs, Nt-nonresponder; Nt-eflic, Nt-efficiency; Nt-ineff, Nt-inefficiency; EON, seeds per silique; N A, the number of anther; N P, the number of pollen; P V, pollen vigor; N I, the number of initial ovule; O R, ovule abortion rate; P H, plant height; S D, stem diameter; F H, first valid branch height; F N, number of first valid branches; S A, siliques surface area; 1000 W, 1000-seed weight; Y P, yield per plant.

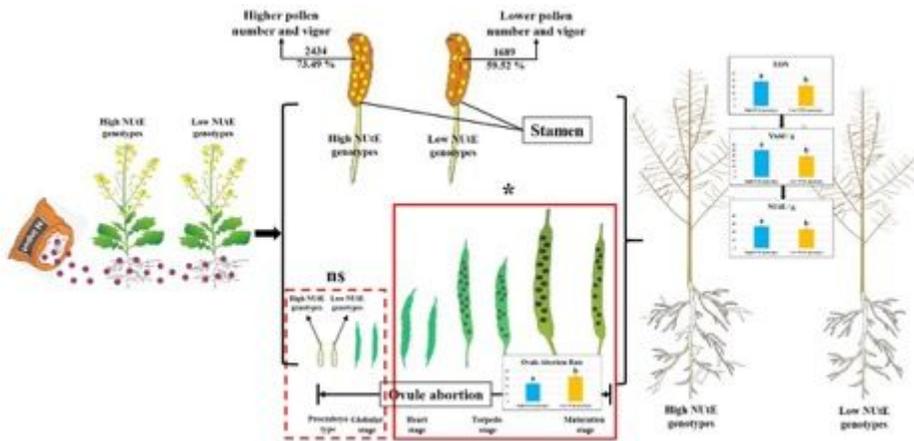


Figure 8

Simplified model for the main reasons and critical period for the difference in the effective ovule numbers among contrasting NUtE oilseed rape genotypes. Compared to the low efficient genotypes, the high NUtE genotypes produced more pollen number, with higher pollen vigor and less ovule abortion rate. After flowering, the EON of contrasting NUtE oilseed rape genotypes went through five distinct stages (proembryo, globular, heart, torpedo and maturation). There was no significant difference of EON from the proembryo to globular stages, but the high NUtE oilseed rape genotypes displayed higher EON than the low NUtE genotypes at the heart stage, and difference in the dynamic EON maintained till the maturation stage. Therefore, with higher pollen number and pollen vigor, and lower ovule abortion rate, the high NUtE genotypes produced higher EON, yield and NUtE than the low NUtE ones.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata.docx](#)