Identification of QTLs for Seed Yield and Yield-Related Traits in Brassica Napus Grown with Contrasting Nitrogen Supplies

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Abstract

Oilseed rape (*Brassica napus* L.; *B. napus*) is the main oil crop in China as well as in the world. Nitrogen (N) deficiency significantly reduces the seed yield of *B. napus*. However, a very few studies involved in the genetic mechanism of seed yield (SY) and SY-related traits of *B. napus* in response to N deficiency. In this study, plant height (PH), branch number (BN), pod number (PN), seed number (SN), 1000-seed weight (SW) and SY were investigated using a *B. napus* double haploid (*BnaTNDH*) population derived from a cross between cultivars 'Tapidor' and 'Ningyou7' grown at an optimal (ON) and a low N (LN) supplies in three-year field trials. Great variations of SY and related traits were observed in *BnaTNDH* population under contrasting N supplies. A total of 106 and 110 significant quantitative trait loci (QTLs) were detected for six traits at ON and LN in three field trials, respectively. All of these significant QTLs for the same trait were integrated into 191 consensus QTLs. Nine and eleven consensus QTLs at ON and LN were detected in two or three trials, respectively, and the remaining were environment-specific. One hundred and three unique QTLs were integrated from 191 consensus QTLs, including 29 low N specific QTLs, 35 optimal N specific QTLs and 39 constitutive QTLs. *uqA3q* was integrated from four consensus QTLs for PN, PH, SN, SY at LN, *uqC9f* was integrated from consensus QTLs for BN, SY, PN at ON and LN. Both were detected in three trials. This result may help to better understand the genetic mechanism of yield traits in response to low N and promote the breeding of N efficient varieties.

Introduction

Nitrogen (N) is component of nucleic acids, proteins, chlorophyll, alkaloids, vitamins, and hormones, which is essential for plants growth and development (Hawkesford et al., 2012). Oilseed rape (*Brassica napus* L.; *B. napus*) is one of the most important oil crops worldwide, which acquires nitrate and ammonium and recycles organic nitrogen (Masclaux-Daubresse et al., 2010). The application rate of N fertilizer in *B. napus* ranged from 65 to 325 kg / hm² in China, which depend on the field fertility, SY target, varieties and other factors (Zhang et al., 2020). Rational application of N fertilizer can significantly promote the seed yield (SY), oil production, protein content and polysaturated fatty acid content of *B. napus* (Gao et al., 2019). On the contrary, irrational fertilization not only decreases the crop yield and quality, but also causes soil acidification and eutrophication (Liu et al., 2013b; Guo et al., 2010; Hirel et al., 2011). Breeding N-efficient *B. napus* cultivars is an important strategy to improve the SY in a sub-optimal N supply and reduce the application of N fertilizers.

SY is a complex trait, which is mainly related to the potential of *B. napus* for growth and branching after flowering which enable the crop to use one yield component to compensate for limitations in another one (Bouchet et al., 2014). SY of *B. napus* is directly related to pod number per plant, seed number per pod and 1000-seed weight, and also indirectly associated with plant height and branch number (Ding et al., 2012). N deficiency significantly decreased SY components such as plant density, branch number, pod number per plant, seed number per pod, except for 1000-seed weight (Cong et al., 2020).

Quantitative trait loci (QTL) analysis based on high-density genetic linkage map can provide basic information on the genetic architecture of quantitative traits (Agrama 2006). Bouchet et al. (2016) mapped 17 low-N specific QTLs, 18 optimal-N specific QTLs for flowering days, seed protein content, SY, seed number per pod, 1000-seed weight, oil content and oil/protein in a double haploid (DH) population of *B. napus* through three-year field trials, and homologous QTLs for SY were found on A3/C3, A5/C5, A9/C9 chromosomes. Wang et al. (2017) find that all major QTLs and some stable QTLs for N use efficiency were associated with root morphology traits in *B. napus* at ON and/or LN. At present, many QTLs have been mapped for SY or N use efficiency in *B. napus* at ON, but limited QTLs were detected at LN.

In this study, a *B. napus* DH population (*BnaTNDH* population) was employed to conduct field trials at ON and LN for three years. The QTLs for SY and SY-related traits of *B. napus* under contrasting N supplies were identified. Some major QTLs in response to low N were obtained.

Materials And Methods

Plant materials and field trials

A *BnaTNDH* population with 182 lines was used in this study, which was derived from a cross between a European winter type cultivar 'Tapidor' and a Chinese semi-winter type cultivar 'Ningyou7' by microspore culture (Qiu et al., 2006).

Three field trials were conducted in sandy paddy soil in Qichun county, Hubei Province, China (115°45′N latitude, 30°19′E longitude), during *B. napus* growing seasons the 2008–2009 (Tri.1), 2009–2010 (Tri.2) and 2010–2011 (Tri.3). Soil properties were as follows: pH (1:1 H₂O) 4.8, organic matter 34.9 g·kg⁻¹, total N 0.22 g·kg⁻¹, available N 0.074 g·kg⁻¹, Olsen-phosphorus 3.32 mg·kg⁻¹, available potassium 42 mg·kg⁻¹, and available boron 0.09 mg·kg⁻¹. The basal fertilizers included P 38.7 kg·ha⁻¹, K 124.5 kg·ha⁻¹, ZnSO₄·7H₂O 45 kg·ha⁻¹ and Borax (Na₂B₄O₇·10H₂O) 15 kg·ha⁻¹. 60% of 120 and 40 kg·ha⁻¹ N were applied to create ON and LN conditions before transplantation, and rest of the urea was applied before the overwinter stage. Three replications for 182 *BnaTNDH* lines and their parents were planted in a randomized complete-plot design with each plot comprising 18 plants, separated by a distance of 0.20 m between plants and 0.28 m between rows. Seeds were sown in a nursery bed in the field in middle September and seedlings were transplanted 30 d after sowing. Plants were harvested in the following middle May. Standard agricultural practices were followed for field management.

Measurement of phenotypic traits

In each plot, six individuals from the middle row were used to determine plant height (PH) measured from ground level to the tip of the main inflorescence, number of primary branches (BN) measured as the number of primary branches arising from main shoot and seed number per pod (SN) measured as the average number of well filled seeds from 100 well-developed pods sampled from the primary branch in the middle of each plant studied. All representative individuals from each plot were harvested by hand at maturity stage to investigate seed yield per plant (SY) and seed weight of 1,000 seeds (SW). Pod number per plant (PN) was calculated using the following formula: PN = (SY × 1000) / (SW × SN).
Statistical analysis and QTL detection

Data analysis was conducted using SPSS 20.0 (IBM, USA) and Microsoft Excel 2019 (Microsoft, USA). Duncan multiple-range test was used for multiple comparison of different traits between two parents. Three-way ANOVA with F test was used at \( P < 0.05 \) level. Different growth environments (years) and N treatments were treated as fixed factors, and genotypes were treated as random factor. Correlation analysis was conducted to determine the relationship between the tested traits. The broad-sense heritability (\( h^2 \)) for each trait was calculated at both N levels as follows: 
\[
h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/n + \sigma_e^2/nr},
\]
where \( \sigma_g^2 \) is the genotypic variance, \( \sigma_{ge}^2 \) is the interaction variance of genotype with environment, \( \sigma_e^2 \) is the error variance, \( n \) is the number of environments and \( r \) is the number of replicates.

The \textit{BnaTNDH} linkage map contained a total of 2041 molecular marker and the average marker density was from 0.39 to 0.97 per cM (Zhang et al., 2016). QTLs were detected by composite interval mapping (CIM) using WinQTL cartographer 2.5 software (http://statgen.ncsu.edu/qtlcar/WQTLCart.htm) (Wang et al., 2006). For each trait, QTL threshold (\( P < 0.05 \)) was estimated from 1,000 permutations (Silva et al., 2012). Biomercator v4.2 was used to integrate consensus QTL and unique QTL (Arcade et al., 2004). The significant QTLs for the same trait identified in the different trials were integrated into consensus QTLs by meta-analysis. Then the consensus QTLs for different traits that overlapped were integrated into unique QTL. The consensus QTLs detected in at least two trials were considered as major consensus QTLs. Each QTL was denominated as "q" (abbreviation of QTL) + trait name + trial number + chromosome name + the serial letter (a,b,c...). For example, \textit{qPHON3-A3b} denoted the second QTL for plant height on chromosome A3 at ON in Tri.3. Each consensus QTL was denominated as "cq" (abbreviation of consensus QTL) + trait name + chromosome name + the serial letter. For example, \textit{cqPHON-A3c} indicated the third consensus QTL for PH at ON located on A3. Each unique QTL was denominated as "uq" (abbreviation of unique QTL) + chromosome name + the serial letter. For example, \textit{uqA2d} indicated the fourth unique QTL on A2.

Results

Differences in the six tested traits between cultivars Tapidor and Ningyou 7, and among \textit{BnaTNDH} population

At ON, SY of Tapidor was significantly lower than that of Ningyou7 in the three trials; BN of Tapidor was lower than that of Ningyou7 in Tri.2 and Tri.3; SN of Tapidor was obviously more than that of Ningyou7 in Tri.1; PN of Tapidor was lower than that of Ningyou7 in Tri.3 (Fig. 1; Table 1). At LN, BN of Tapidor was less than that of Ningyou7 in Tri.1 and Tri.2, and SY of Tapidor was less than that of Ningyou7 in Tri.3 (Fig. 1; Table 1). There was no significant difference in PH between Tapidor and Ningyou7 at two nitrogen supplies in three trials.
Table 1
Means and ranges of the seed yield (SY) and SY-related traits in the parental lines and the BnaTNDH population grown at an optimal (ON) and a low N supply (LN) in three field trials.

| Trait                  | N Treatment | N Treatment | Trial | Tapidor | Ningyou7 | Mean | Range | CV (%) \(^{a}\) | \(h^{2b}\) |
|------------------------|-------------|-------------|-------|---------|-----------|------|-------|----------------|-----------|
| PH (cm)                | ON          | ON          | Tri.1 | 129.6 ± 5.6a | 127.6 ± 3.9a | 140.2a | 109.3–169.2 | 8.5           | 0.75       |
|                        | ON          | ON          | Tri.2 | 103.1 ± 5.9d | 109.6 ± 3.4cd | 122.8c | 94.1–153.9 | 10.4          |            |
|                        | ON          | ON          | Tri.3 | 124.2 ± 1.8ab | 130.6 ± 5.1a | 136.5b | 84.0–174.5 | 9.4           |            |
|                        | LN          | LN          | Tri.1 | 116.4 ± 9.4bc | 123.5 ± 8.1ab | 122.4c | 97.0–154.3 | 9.2           | 0.56       |
|                        | LN          | LN          | Tri.2 | 104.2 ± 3.7d | 105.1 ± 1.3d | 117.7d | 84.4–154.3 | 10.5          |            |
|                        | LN          | LN          | Tri.3 | 105.3 ± 12.1d | 113.0 ± 7.3cd | 114.5e | 87.0–135.9 | 9.0           |            |
| BN (N)                 | ON          | ON          | Tri.1 | 4.5 ± 0.3c   | 6.9 ± 0.4a   | 5.9b   | 4.3–7.6   | 12.4          | 0.59       |
|                        | ON          | ON          | Tri.2 | 4.5 ± 0.8c   | 6.5 ± 0.4a   | 5.7c   | 2.8–8.8   | 18.0          |            |
|                        | ON          | ON          | Tri.3 | 6.3 ± 0.3ab  | 7.1 ± 0.3a   | 6.7a   | 2.5–9.5   | 15.3          |            |
|                        | LN          | LN          | Tri.1 | 4.0 ± 0.7c   | 5.5 ± 0.2b   | 4.7e   | 2.0–6.2   | 15.9          | 0.51       |
|                        | LN          | LN          | Tri.2 | 4.4 ± 0.2c   | 5.5 ± 0.5b   | 5.1d   | 2.7–7.7   | 16.9          |            |
|                        | LN          | LN          | Tri.3 | 4.4 ± 1.1c   | 4.4 ± 0.4c   | 4.4f   | 1.9–6.7   | 18.0          |            |
| PN (N)                 | ON          | ON          | Tri.1 | 179.7 ± 58.0a | 124.8 ± 11.0bc | 164.3a | 54.4–406.1 | 27.3          | 0.59       |
|                        | ON          | ON          | Tri.2 | 100.4 ± 13.8bcd | 118.5 ± 17.0bcd | 140.5b | 10.0–312.9 | 36.7          |            |
|                        | ON          | ON          | Tri.3 | 105.4 ± 19.8bcd | 132.2 ± 9.5b | 124.0c | 18.5–244.4 | 38.2          |            |
|                        | LN          | LN          | Tri.1 | 82.7 ± 34.3cdef | 63.0 ± 11.8ef | 88.8d | 31.7–174.5 | 26.2          | 0.55       |
|                        | LN          | LN          | Tri.2 | 86.8 ± 6.0cdef | 81.0 ± 3.8def | 95.1d | 25.5–222.4 | 33.3          |            |
|                        | LN          | LN          | Tri.3 | 56.5 ± 6.7f   | 57.4 ± 13.1f | 46.8e | 5.8–106.3 | 38.6          |            |
| SN (N)                 | ON          | ON          | Tri.1 | 15.7 ± 2.0ab  | 16.3 ± 0.9a  | 17.7a | 11.0–25.0 | 17.4          | 0.69       |
|                        | ON          | ON          | Tri.2 | 14.0 ± 1.0abcd | 15.4 ± 1.2abc | 14.1c | 4.5–21.6 | 20.9          |            |
|                        | ON          | ON          | Tri.3 | 12.7 ± 0.8de  | 15.5 ± 0.8abc | 15.0b | 2.2–22.2 | 24.4          |            |
|                        | LN          | LN          | Tri.1 | 14.3 ± 0.3abcd | 13.9 ± 1.0abcd | 14.8b | 7.1–22.1 | 22.9          | 0.67       |
|                        | LN          | LN          | Tri.2 | 13.1 ± 1.5cde | 13.1 ± 1.4cde | 13.4d | 6.6–23.1 | 19.5          |            |
|                        | LN          | LN          | Tri.3 | 11.0 ± 2.0e   | 13.2 ± 1.7bcde | 12.3e | 4.2–22.8 | 29.0          |            |
| SW (g/1000 seeds)      | ON          | ON          | Tri.1 | 2.3 ± 0.1e    | 3.8 ± 0.1b   | 2.9c   | 2.1–4.1   | 13.3          | 0.84       |
|                        | ON          | ON          | Tri.2 | 2.5 ± 0.1d    | 4.0 ± 0.1ab  | 3.1b   | 2.0–4.8   | 14.2          |            |
|                        | ON          | ON          | Tri.3 | 2.1 ± 0.1f    | 3.6 ± 0.2c   | 2.7d   | 1.4–4.0   | 15.8          |            |
|                        | LN          | LN          | Tri.1 | 2.3 ± 0.1e    | 3.9 ± 0.1b   | 2.9c   | 1.9–4.1   | 15.0          | 0.80       |
|                        | LN          | LN          | Tri.2 | 2.5 ± 0.1d    | 4.1 ± 0.2a   | 3.3a   | 2.1–5.0   | 14.7          |            |
|                        | LN          | LN          | Tri.3 | 2.1 ± 0.1f    | 3.5 ± 0.1c   | 2.7d   | 1.1–4.7   | 20.8          |            |
| SY (kg/ha)             | ON          | ON          | Tri.1 | 1194.8 ± 308.5b | 1465.7 ± 167.9a | 1509.8a | 455.7–2297.4 | 24.7          | 0.76       |
|                        | ON          | ON          | Tri.2 | 603.1 ± 140.0cd | 1355.3 ± 79.6ab | 1120.0b | 263.1–2210.0 | 36.9         |            |
|                        | ON          | ON          | Tri.3 | 470.1 ± 73.0d | 1332.8 ± 168.5ab | 974.8c | 50.4–2029.6 | 49.2         |            |
|                        | LN          | LN          | Tri.1 | 489.9 ± 183.0d | 651.6 ± 167.5cd | 703.0d | 168.3–1353.7 | 32.4         | 0.73       |
|                        | LN          | LN          | Tri.2 | 535.3 ± 79.1cd | 807.1 ± 108.8c | 747.7d | 153.4–1708.6 | 36.8         |            |
|                        | LN          | LN          | Tri.3 | 208 ± 35.9e   | 501.4 ± 92.1d | 303.5e | 13.1–979.9 | 57.1          |            |

\(^{a}\)CV, coefficient of variation. Different letters indicated the significant difference at \(P = 0.05\) level. Data are mean ± SD, \(n = 3\). \(^{b}\)heritability

Compared with at ON, BN and SY of Ningyou7 were significantly decreased at LN. SW of Tapidor was significantly less than that of Ningyou7 at both ON and LN in the three trials (Fig. 1; Table 1). Compared with ON, SY, PH, BN, PN and SN of the BnaTNDH population were significantly decreased at LN. Among them, SY at LN in Tri.1, Tri.2 and Tri.3 decreased by 53.4%, 33.2% and 68.9%, respectively; SN decreased by 45.9%, 32.3% and 62.2%, respectively (Table 1). The \(h^{2}\)
observed in the BnaTNDH population for the six traits, ranged from 0.51 for BN at LN to 0.84 for SY at ON. In general, a higher $h^2$ was observed at ON than at LN (Table 1).

In the three trials, all traits showed approximately normal distributions and transgressive segregations at two N supplies (Table 1; Fig. 2). The results of ANOVA showed that environment, N treatment, genotype and the interactions between these factors had significant effects on all the tested traits (Table 2). SY was highly positively correlated with PN and SN at both ON and LN across three field trials (Table 3). There was a weak correlation between PH and BN at both ON and LN across three field trials. While no significant correlation and weak correlation was observed between SY and SW at both ON and LN in Tri.1 and Tri.2, and Tri.3, respectively (Table 3).

### Table 2

|                      | PH | BN | PN | SN | SW | SY |
|----------------------|----|----|----|----|----|----|
| **Environment**      | d.f. | 2  | 2  | 2  | 2  | 2  |
| sig.                 | *** | *** | *** | *** | *** | *** |
| **N treatment**      | d.f. | 1  | 1  | 1  | 1  | 1  |
| sig.                 | *** | *** | *** | *** | ** | *** |
| **Genotype**         | d.f. | 181| 181| 181| 181| 181| 181|
| sig.                 | *** | *** | *** | *** | *** | *** |
| **Environment × N treatment** | d.f. | 2  | 2  | 2  | 2  | 2  |
| sig.                 | *** | *** | *** | *** | *** | *** |
| **Environment × Genotype** | d.f. | 337| 337| 344| 341| 341| 344|
| sig.                 | *** | *** | *** | *** | *** | *** |
| **N treatment × Genotype** | d.f. | 181| 181| 181| 181| 181| 181|
| sig.                 | ** | ** | *** | *** | *** | *** |

*a*d.f., degrees of freedom. *b*sig., significance. ns, no significance. **$P<0.01$, ***$P<0.001$. 
### Table 3

Correlation coefficients among seed yield (SY) and SY-related traits in the *BnaTNDH* population grown at an optimal (above diagonal) and a low N supply (below diagonal) in three field trials

| Environments | PH   | BN   | PN   | SN   | SW   | SY   |
|--------------|------|------|------|------|------|------|
| **Tri.1**    |      |      |      |      |      |      |
| PH           | 0.19*| 0.20**| 0.18*| -0.24**| 0.26**|
| BN           | 0.13 | 0.34**| 0.17*| -0.02 | 0.45**|
| PN           | 0.42**| 0.15*| -0.15*| -0.43**| 0.61**|
| SN           | 0.28**| 0.05 | 0.09 | 0.07 | 0.55**|
| SW           | -0.26**| 0.07 | -0.47**| 0.06 | -0.02 |
| SY           | 0.39**| 0.23**| 0.57**| 0.77**| 0.07  |
| **Tri.2**    |      |      |      |      |      |      |
| PH           | 0.34**| 0.12 | 0.23**| -0.17*| 0.26**|
| BN           | 0.26**| 0.57**| 0.07 | 0.02 | 0.57**|
| PN           | 0.25**| 0.36**| -0.08 | -0.18*| 0.68**|
| SN           | 0.17*| -0.03 | 0.06 | -0.05 | 0.53**|
| SW           | -0.19*| -0.06 | -0.23**| -0.01| 0.06  |
| SY           | 0.25**| 0.30**| 0.80**| 0.52**| 0.08  |
| **Tri.3**    |      |      |      |      |      |      |
| PH           | 0.45**| 0.31**| 0.46**| 0.01 | 0.41**|
| BN           | 0.49**| 0.34**| 0.29**| 0.16*| 0.40**|
| PN           | 0.29**| 0.32**| 0.43**| -0.11 | 0.83**|
| SN           | 0.29**| 0.13| 0.34**| 0.08 | 0.73**|
| SW           | 0.10 | 0.11 | 0.00 | 0.16*| 0.23**|
| SY           | 0.38**| 0.32**| 0.77**| 0.75**| 0.34**|

**P<0.01, *P<0.05.**

QTL detection and consensus QTL

A total of 216 significant QTLs for SY and SY-related traits were detected at two N supplies in three trials (Fig. 2; Supplementary Table 1). These QTLs were mainly located on chromosomes A2, A3, A9, C6 and C9, and the phenotypic variation (PVE) ranged from 4.0–23.5%. They were subjected to the first round of QTL meta-analysis trait-by-trait, and 191 consensus QTL were obtained. Among them, 20 consensus QTLs could be detected in two or three trials (Table 4)
### Table 4
Summary of major consensus QTL and their corresponding identified QTL

| Trait | N Treatment | QTL     | Chro. | Position (cM) | CI (cM)<sup>b</sup> | QTL     | LOD | R<sup>2</sup> | Position (cM) | CI (cM) | AE<sup>c</sup> |
|-------|-------------|---------|-------|---------------|---------------------|---------|-----|-------------|---------------|---------|----------------|
| PH    | LN          | cqPHLN–C6c | C6    | 42.6          | 41.6–43.5           | qPHLN2–C6c | 4.5 | 7.5        | 42.8          | 41.3–45.3 | +              |
|       |             |         |       |               |                     | qPHLN1–C6d | 3.3 | 5.2        | 42.5          | 41.3–43.5 | +              |
| BN    | ON          | cqBNON–C9 | C9    | 116.7         | 108.1–125.3         | qBNON2–C9a | 3.3 | 7.4        | 114.0         | 106.0–137.6 | +              |
|       |             |         |       |               |                     | qBNON3–C9b | 3.7 | 7.4        | 117.3         | 107.2–137.2 | +              |
| LN    |             | cqBNLN–C9b | C9    | 114.6         | 110.6–118.6        | qBNLN2–C9b | 3.7 | 7.4        | 116.5         | 106.7–125.4 | +              |
|       |             |         |       |               |                     | qBNLN1–C9c | 4.1 | 7.9        | 113.0         | 106.1–116.5 | +              |
| PN    | ON          | cqPNON–A3d | A3    | 97.7          | 96.3–99.0           | qPNON1–A3 | 5.6 | 11.2       | 98.7          | 95.3–104.4 | –              |
|       |             |         |       |               |                     | qPNON3–A3a | 6.6 | 12.7       | 95.7          | 95.3–98.8  | –              |
|       |             |         |       |               |                     |         |     |            |               | 100.9     | 100–104.7     |
| LN    |             | cqPNLN–A3a | A3    | 83.5          | 82.0–84.9           | qPNLN1–A3a | 4.5 | 9.1        | 77.9          | 74.0–81.0  | –              |
|       |             |         |       |               |                     | qPNLN2–A3b | 2.8 | 5.5        | 84.3          | 77.1–84.7  | –              |
|       |             |         |       |               |                     | qPNLN1–A3c | 7.5 | 14.7       | 84.7          | 82.2–85.7  | –              |
|       |             |         |       | cqPNLN–C9    | C9                  | qPNLN3–C9a | 7.1 | 15.2       | 124.3         | 111.8–137.8| +              |
|       |             |         |       |               |                     | qPNLN2–C9b | 3.0 | 5.8        | 127.4         | 113.1–140.4| +              |
| SN    | ON          | cqSNON–A2a | A2    | 48.1          | 47.1–49.1           | qSNON1–A2a | 5.5 | 10.4       | 46.2          | 39.9–48.6  | +              |
|       |             |         |       |               |                     | qSNON3–A2a | 3.7 | 7.2        | 48.2          | 46.5–48.6  | +              |
|       |             |         |       | cqSNON–A9b   | A9                  | qSNON1–A9b | 3.3 | 6.4        | 26.1          | 23.9–26.7  | –              |
|       |             |         |       |               |                     | qSNON2–A9c | 3.2 | 5.4        | 28.9          | 25.4–35.1  | –              |
|       |             |         |       | cqSNON–C1a   | C1                  | qSNON1–C1a | 2.8 | 5.3        | 3.6           | 1.1–6.1    | +              |
|       |             |         |       |               |                     | qSNON2–C1b | 4.3 | 8.2        | 1.0           | 0.0–6.1    | +              |
| LN    |             | cqSNLN–A9c | A9    | 37.4          | 35.3–39.5           | qSNLN2–A9c | 3.1 | 6.1        | 35.2          | 31.9–38.0  | –              |
|       |             |         |       |               |                     | qSNLN1–A9d | 4.5 | 8.7        | 39.4          | 35.2–41.0  | –              |
| SW    | ON          | cqSWON–A2c | A2    | 82.9          | 81.0–84.9           | qSWON1–A2b | 5.9 | 8.5        | 80.8          | 78.3–83.8  | +              |
|       |             |         |       |               |                     | qSWON2–A2a | 3.7 | 6.8        | 84.7          | 80.8–86.9  | +              |
|       |             |         |       |               |                     | qSWON3–A2b | 5.1 | 10.1       | 84.0          | 79.9–91.2  | +              |
|       |             |         |       | cqSWON–A4b   | A4                  | qSWON1–A4b | 4.1 | 5.7        | 12.7          | 9.8–14.8   | +              |

<sup>a</sup>Chrom., chromosome. <sup>b</sup>Ci, confidence interval. <sup>c</sup>Ae, additive effect.
A total of 49 significant QTLs for SW were detected at both N supplies. The PVE of 24 QTLs at ON ranged from 4.0–23.5%, while that of 25 QTLs at LN ranged from 4.0–13.8%. These significant QTLs were integrated into 40 consensus QTLs. Six consensus QTLs were considered to be major consensus QTL (Table 4). Among of them, three QTLs were identified at ON. cqSWON-A2c was detected in all the trials with PVE of 8.5%. cqSWON-A4d and cqSWON-A4c were identified in Tri.1 and Tri.2 with PVE of 7.4% and 7.5%, respectively. At LN, there were three QTLs, cqSWLN-A2d, cqSWLN-A2e, cqSWLN-A3f. Among of them, cqSWLN-
A2d and cqSWLN/A2e were detected in Tri.1 and Tri.2, with an average PVE of 11.2% and 12.8%, respectively. cqSWLN/A3f was detected simultaneously in Tri.2 and Tri.3, which accounted for an average PVE of 7.6%.

A total of 36 significant QTLs for SY were detected at two N supplies. Among of them, the PVE of 16 QTLs at ON ranged from 4.4–11.0%, and that of 20 QTLs at LN ranged from 4.1–15.3%. A total of 31 consensus QTLs were identified by QTL integration. cqSYON/C8c was detected in Tri.2 and Tri.3, with an average PVE of 6.8%. cqSYLN/A2c, cqSYLN-A3f and cqSYLN-C9 were detected in Tri.2 and Tri.3 and the average PVE were 6.5%, 7.0% and 10.9%, respectively.

Most of the consensus QTLs associated with various traits overlapped on A2, A3, C6 and C9 chromosomes. A total of 103 unique QTLs were obtained from 191 consensus QTLs, including 33 ON-specific QTLs, 27 LN-specific QTLs and 39 constitutive QTLs detected at both ON and LN (Fig. 2; Supplementary Table 2).

Among 33 ON-specific QTLs, uqA2e, uqA3a and uqA9b for two traits were clustered on A2, A3, A9, respectively (Table 5). uqA2e for two traits of PH and SN was located in the interval of 47.1–49.0 cM on A2. uqA3a was integrated from two consensus QTLs, cqPHON/A3a and cqBNON/A3a, and was clustered in the interval of 0.0–8.3 cM on A3. uqA9b was obtained from two consensus QTLs, cqBNON/A9a and cqSNON/A9a, and clustered in the interval of 10.9–20.4 cM on A9.
Table 5
ON and LN-specific unique QTLs associated with at least two traits and constitutive QTLs associated with at least three traits.

| Unique QTL | Consensus QTL |
|------------|---------------|
| Chro. a | N treatment | Position (cM) | CI (cM) | Position (cM) | CI (cM) |
| A2 | uqA2c | LN | 29.9 | 28.7-31.0 | cqSNLN–A2c | 29.4 | 27.8-30.4 |
| | | | | | cqPNLN–A2a | 31.6 | 27.8-32.9 |
| | uqA2d | ON&LN | 42.6 | 40.6-44.6 | cqPNLN–A2b | 41.0 | 32.9-44.3 |
| | | | | | cqPHLN–A2a | 42.0 | 39.8-45.2 |
| | | | | | cqSWLN–A2a | 44.4 | 37.8-45.2 |
| | uqA2e | ON | 48 | 47.1-49.0 | cqPHLN–A2b | 47.2 | 45.4-52.8 |
| | | | | | cqSNON–A2a | 48.1 | 47.1-49.1 |
| | uqA2i | LN | 77.6 | 76.2-78.8 | cqSYLN–A2a | 76.0 | 75.6-79.9 |
| | | | | | cqSWLN–A2c | 76.5 | 75.1-80.1 |
| | | | | | cqSNLN–A2d | 80.1 | 77.5-82.0 |
| | uqA2j | ON&LN | 83 | 82.3-83.7 | cqSWLN–A2c | 81.4 | 80.1-82.7 |
| | | | | | cqSWON–A2c | 82.9 | 81.0-84.9 |
| | | | | | cqSYON–A2b | 83.0 | 82.0-85.0 |
| | | | | | cqSNON–A2c | 84.0 | 82.0-86.0 |
| | | | | | cqSYLN–A2c | 84.8 | 83.3-86.4 |
| A3 | uqA3a | ON | 3.3 | 0.0-8.3 | cqPHON–A3a | 20.4 | 0.6-27.8 |
| | | | | | cqBNON–A3a | 0.6 | 0.0-10.8 |
| | uqA3i | ON&LN | 59.4 | 59.1-59.7 | cqSWLN–A3f | 58.4 | 57.6-59.3 |
| | | | | | cqPHLN–A3b | 59.4 | 58.8-59.5 |
| | | | | | cqSYLN–A3b | 59.5 | 58.9-62.7 |
| | | | | | cqPHON–A3d | 62.7 | 58.8-66.5 |
| | uqA3j | LN | 65.6 | 65.2-66.0 | cqSNLN–A3a | 65.5 | 62.7-66.5 |
| | | | | | cqPHLN–A3c | 65.6 | 65.5-66.3 |
| | uqA3m | ON&LN | 76.7 | 76.0-77.4 | cqSYLN–A3d | 76.7 | 75.4-76.9 |
| | | | | | cqSWON–A3e | 76.9 | 75.4-81.0 |
| | | | | | cqSNLN–A3b | 76.4 | 75.4-80.0 |
| | | | | | cqSWLN–A3d | 76.4 | 73.8-84.0 |
| | uqA3n | ON&LN | 84.9 | 84.4-85.5 | cqSYON–A3a | 81.6 | 81.0-84.3 |
| | | | | | cccPNLN–A3a | 83.5 | 82.0-84.9 |
| | | | | | cqSWLN–A3e | 85.7 | 84.0-85.7 |
| | | | | | cqSNON–A3a | 86.3 | 84.7-87.2 |
| | uqA3o | ON&LN | 92 | 91.2-92.9 | cqSYON–A3b | 92.0 | 87.2-92.8 |
| | | | | | cqSYLN–A3e | 92.0 | 88.7-92.8 |
| | | | | | cqSNON–A3b | 91.8 | 87.2-93.1 |
| | | | | | cccPNLN–A3d | 92.0 | 86.6-92.1 |
| | | | | | cqBNON–A3b | 84.7 | 84.3-91.5 |
| | | | | | cccPNLN–A3e | 92.8 | 92.3-94.6 |

aChro., chromosome. bCI, confidence interval.
| **Unique QTL** | **Consensus QTL** |
|----------------|------------------|
| uqA3p          | ON&LN 97.5       |
|                | 96.8-98.3        |
|                | cqSYON–A3c 95.7  |
|                | 95.3–97.5        |
|                | cqSNLN–A3c 95.7  |
|                | 96.8-98.3        |
|                | cqPNON–A3d 97.7  |
|                | 96.3–99.0        |
| uqA3q          | LN 101.8         |
|                | 100.6-103.1      |
|                | cqPNLN–A3f 100.9 |
|                | 99.7–102.2       |
|                | cqPHEL–A3e 104.7 |
|                | 100.0–108.8      |
|                | cqSNLN–A3d 100.9 |
|                | 100.0–104.7      |
|                | cqSYLN–A3f 102.6 |
|                | 100.5–104.7      |
| A4             | uqA4h ON&LN 31   |
|                | 30.4-31.7        |
|                | cqSWLN–A4d 31.0  |
|                | 30.4–35.8        |
|                | cqPNLN–A4c 31.0  |
|                | 30.4–31.7        |
| uqA4i          | LN 39.3          |
|                | 37.1-41.6        |
|                | cqPHEL–A4e 39.8  |
|                | 37.4-42.8        |
| A5             | uqA5d ON&LN 54.5 |
|                | 52.9–56.0        |
|                | cqSYLN–A5 53.3   |
|                | 50.6–55.0        |
|                | cqPNLN–A5 53.5   |
|                | 53.3–63.6        |
|                | cqPNON–A5 56.0   |
|                | 54.3–59.0        |
| A8             | uqA8b ON&LN 69.9 |
|                | 68.0–71.8        |
|                | cqPHON–A8 76.6   |
|                | 68.5–79.3        |
|                | cqBNLN–A8 68.0   |
|                | 66.0–70.9        |
|                | cqSNLN–A8b 70.9  |
|                | 68.0–74.9        |
| A9             | uqA9b ON 15.6    |
|                | 10.9-20.4        |
|                | cqBNON–A9a 10.8  |
|                | 6.6–19.8         |
|                | cqSNON–A9a 20.8  |
|                | 8.6–22.3         |
| uqA9e          | LN 37.2          |
|                | 36.0-38.4        |
|                | cqSWLN–A9 29.9   |
|                | 28.9–38.0        |
|                | cqSNLN–A9c 37.4  |
|                | 35.3–39.5        |
|                | cqBNLN–A9 38.0   |
|                | 35.2–38.4        |
| C8             | uqC8c ON&LN 18.7 |
|                | 15.5-22.0        |
|                | cqPNLN–C8 9.3    |
|                | 1.9–26.1         |
|                | cqSYON–C8c 15.3  |
|                | 10.7–19.9        |
|                | cqSNLN–C8a 24.2  |
|                | 15.2–28.5        |
|                | cqSYLN–C8c 25.0  |
|                | 15.2–30.7        |
| C9             | uqC9e ON&LN 102.2|
|                | 98.5–105.9       |
|                | cqBNLN–C9a 102.9 |
|                | 93.4–105.3       |
|                | cqSNON–C9e 101.7 |
|                | 93.7–103.1       |
|                | cqSYON–C9a 107.2 |
|                | 107.1–109.5      |
| uqC9f          | ON&LN 122.42     |
|                | 117.8-127.0      |
|                | cqBNLN–C9b 114.6 |
|                | 110.6–118.6      |
|                | cqBNON–C9 116.7  |
|                | 108.1–125.3      |
|                | cqSYON–C9b 117.3 |
|                | 113.3–130.5      |
|                | cqSNON–C9b 118.3 |
|                | 113.5–130.8      |
|                | cqSYLN–C9 124.6  |
|                | 117.0–132.1      |
|                | cqPNLN–C9 125.8  |
|                | 116.4–135.2      |

*Chro., chromosome. CI, confidence interval

Among 27 LN-specific QTLs, six LN-specific QTLs for more than two traits were clustered on A2, A3, A4 and A9 chromosomes (Table 5). uqA3h for four traits of PH, PN, SN and SY was located in the interval of 100.6-103.1 cM on A3. uqA4b for three traits of BN, SN and SW was integrated from three consensus QTLs of cqSWLN–A9, cqSNLN–A9 and cqBNLN–A9, and located in the interval of 36.0-38.4 cM on A9. uqA2a, uqA2d, uqA3c and uqA4b were all associated with two traits at LN.

Among 39 constitutive QTLs, 13 unique QTLs detected for more than three traits were located on A2, A3, A4, A5, A8, A9, C8 and C9, respectively (Table 5). Among them, uqA3f for four traits of BN, PN, SN and SY was integrated from cqSYON–A3, cqSYLN–A3, cqSNON–A3, cqPNLN–A3, cqBNON–A3 and cqPNLN–A3.
A3 and located in the interval of 91.2±92.9 cM on A3.

Candidate genes underlying QTLs associated with SY and two major unique QTLs

cqSYLN-C9, detected from qSYLN2–C9a and qSYLN3–C9b, was considered to be the major QTL among QTLs associated with SY due to the PVE of qSYLN3–

C9b was 15.4% (Table 4, Supplementary Table 1). Two candidate genes, BnaC09g46700D and BnaC09g47860D, were identified in the confidence regions of qcSYLN-C9. The orthologues of them in Arabidopsis have been reported in association with glutamate synthase and affecting N assimilation (Hanke et al., 2005, Fontaine et al. 2012). There were not SNPs and InDels in the coding sequence of two candidate genes between Tapidor and Ningyou7. However, there were two SNPs in the promoter of BnaC09g47860D between the two parents (Table 6).

Table 6

| QTL name | Gene name | Promoter (2 Kb upstream of TSS) | Coding sequence | Homologs in A. thaliana |
|----------|-----------|--------------------------------|-----------------|------------------------|
|          |           | Number of SNPs | Number of InDels | Number of SNPs | Number of InDels | Stop-frameshift | Non-frameshift |
| cqSYLN-C9 & uqC9f | BnaC09g46700D | 0 | 0 | 0 | 0 | 0 | 0 | AT5G10000 | (FD4) |
| uqA3q | BnaA03g41350D | 22 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | AT3G51520 | (DGAT2) |

Among unique QTLs, uqA3q and uqC9f were detected in three trials and associated with four traits and three traits, respectively. One candidate gene, BnaA03g41350D, was identified in the genomic region of uqA3q, whose orthologue gene in Arabidopsis affects triacylglycerol (TAG) biosynthesis in response to N deficiency (Yang et al., 2011). There were 22 SNPs and 15 InDels in the promoter but not in the coding sequence between Tapidor and Ningyou7 (Table 6).

Discussion

In the present study, at both N supplies, SW of Ningyou7 were significantly higher than that of Tapidor in three trials; at ON, BN of Ningyou7 was significantly higher than that of Tapidor in three trials, SY of Ningyou7 was considerably higher than that of Tapidor in Tri.1 and Tri.2, SY of Ningyou7 was significantly higher than that of Tapidor in Tri.2 and Tri.3. These were similar to the performance of them in pot culture experiments that SY of Ningyou7 was significantly higher than that of Tapidor at both N supplies (Shi et al., 2010).

Ninety one percent of the 191 consensus QTLs for SY and SY-related traits were detected only in one trial (Supplementary Table 1). A large number of environment-specific QTLs for SY and its related traits are identified, indicating the growth environments have important effects on the function of the genes associated with these traits (Shi et al., 2009). Twenty consensus QTLs (10.47%) were detected simultaneously in at least two trials (Table 4), and the corresponding significant QTLs of which have similar additive phenotypic effects. At ON, Luo et al. (2017) also identified the QTLs for SY and SY related traits, such as cqSYON–C9cqPNON–A3dcqSNON–A2aqcqSNON–A9bcqSNON–C1aqcqSWON–A4bcqSWON–A4cqSYON–C8c, and the intervals of these QTLs overlapped with the QTLs for the same traits at ON in this study. Bouchet et al. (2014) detected 40 QTLs for SY, SN, SW of B. napus DH population derived from a cross between cultivars Aviso and Montego at two contrasting N conditions in three grown environments. The genomic region of cqSWON–A4b and cqSWON–A4c were identified associated with SW under optimal N condition. These robust QTLs identified across different populations and/or environments could be become accessible to ongoing breeding programs.

A total of 106 and 110 QTLs for SY and its related traits were identified at ON and LN supplies, respectively (Supplementary Table 1). The QTLs detected at ON were different from that at LN for each trait (Table 4; Fig. 3; Supplementary Table 1). Luo et al. (2017) results also showed that QTLs for SY and its related traits at ON supply differed from that at LN supply. These could be attributed to that N deficiency limits dry matter production and decreases other nutrient uptake in plant (Wang et al., 2015).

BnaC09g46700D and BnaC09g47860D were predicted to be the candidate genes for cqSYLN-C9. BnaC09g46700D encodes ferredoxin, which is involved in glutamate synthase (GOGAT). BnaC09g47860D encodes glutamate dehydrogenase. There are two isoforms of GOGAT—the NADH-dependent cytosolic isofrom (Iry N assimilation) and ferredoxin-dependent plastidic isofrom (Iry N assimilation) (Pathak et al., 2008). GOGAT serves as a potential target for improving N uptake efficiency and SY (Karunarathne et al., 2017). The relative expression of the homologous genes of BnaC09g46700D in Arabidopsis thaliana such as At1g10960, At1g60950 and At2g27510 were up-regulated under low NO3− treatment (Hanke et al., 2005). Moreover, the mutation of At5g18170, At5g07440 and At3g03910, the homologous genes of BnaC09g47860D in Arabidopsis thaliana, changes the primary C and N metabolic activity (Fontaine et al., 2012).
ON and LN-specific QTL were reported for flowering days and SY (Bouchet et al., 2016), and root dry weight (Liu et al., 2009) in *B. napus*. In this study, there were 103 unique QTLs, including 29 LN-specific QTLs and 39 constitutive QTLs for SY and its related traits (Supplementary Table 2). *uqA2h*, a constitutive QTL, was associated with SY and SW at ON and SY at LN (Table 5, Supplementary Table 2). Its interval was overlapped the interval of *es.A2-30* (Luo et al., 2017), which contained SY and SW at ON.

Among ON-specific QTLs, there were not QTLs co-located with the QTLs for SY. Among LN-specific QTLs, there were five unique QTLs (*uqA2i, uqA3m, uqA3q, uqC8c*) for SY overlapped with QTLs for SN (Table 5). At ON, the overlapped QTLs between for SY and for SN on A5 and C8 have also been reported in *Brassica napus* (Bouchet et al., 2014). Moreover, there was a high positive correlation between SY and SN at LN (Table 3).

Pleiotropic QTLs have also been reported for PH and spike length of wheat (Chai et al., 2019), PH and heading date of rice (Liu et al., 2013a). Xu et al. (2014) identified two genes in wheat, *Rht8* on chromosome 2D and *Rht-B1b* on chromosome 4B, which have pleiotropic effects for PH, spike length, harvest index and N utilization efficiency. QTLs for SY directly accounted for a small proportion of all identified QTLs (Chen et al., 2010; Peng et al., 2011; Luo et al., 2017). The QTLs for some plant architecture related traits, such as BN and PH, are co-located with QTLs for SY (Cai et al., 2016; Miersch et al., 2016). Plant architecture traits strongly affects light interception and photosynthesis, and plays an important role in total yield and harvest index (Sarlikti et al., 2011). Among unique QTLs, *uqA3q*, a LN specific QTL, and *uqC9f*, a constitutive QTL, were associated with the overlapped QTLs for PH, SN and SY at LN, and the overlapped QTLs for BN and SY at LN and ON, respectively (Table 5). *BnaA03g41350D* was predicted to be the candidate gene for *uqA3q*, and its homologous genes DGAT2 in *Arabidopsis thaliana* was associated with the triacylglycerol (TAG) biosynthesis. *BnaC09g46700D* and *BnaC09g47860D* were identified within the interval of *uqC9f*.

In conclusion, considerable variations of SY and SY related traits were observed among the BnaTNDH population. N deficiency reduced SY and SY-related traits except for SW. Only 20% significant QTLs were detected in more than two trials, indicating that different genetic determinants were involved in regulating SY and its related traits at ON and LN. The overlaps of the QTLs for PH and SN with SY were detected in different trials, suggesting that plant architecture had a significant effect on SY. Near-isogenic lines should be developed to fine map the major QTLs identified in this study such as *cqSYLN-C9q* and *uqA3q*. These will be helpful for a better understanding of the molecular mechanism of SY of *B. napus* under N deficiency and promote the molecular breeding of N-efficient cultivars.

**Abbreviations**

PH, plant height. BN, branch number. PN, pod number per plant. SN, seed number per pod. SW, seed weight of 1000 seeds. SY, seed yield. ON, optimal nitrogen. LN, low nitrogen. Tri.1, field trial conducted during 2008–2009. Tri.2, field trial conducted during 2009–2010. Tri.3, field trial conducted during 2010–2011. DH, double haploid. PVE, phenotypic variation

**Declarations**

**Author Contribution** MZ analyzed the data and wrote the main manuscript. TS designed and managed three-year trials. WW analyzed the data. GD and FX taught TS and MZ to complete trials and data analysis and modified the main manuscript. LS taught TS and MZ to complete trials and data analysis, reviewed the manuscript.

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**Data availability** The data sets supporting the results of this article are included within the article and its additional files

**Code availability** Not applicable

**Declarations**

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**Figures**
Figure 1
Phenotyping of Brassica napus cultivars Tapidor (left) and Ningyou7 (right) grown at an optimal N (up) and a low N (down) supply.
Figure 2

Frequency distribution of seed yield (SY) and SY-related traits in the BnaTNDH population grown at an optimal (left) and a low (right) N supplies in three field trials. Tri.1, field trial conducted during 2008-2009; Tri.2, field trial conducted during 2009-2010; Tri.3, field trial conducted during 2010-2011. The dashed and solid arrows indicated the position of phenotypic values of cultivars Tapidor and Ningyou7, respectively. The orange, blue and red arrows indicated trial 1, trial 2 and trial 3, respectively.

Figure 3

Summary of identified QTLs, consensus QTLs and unique QTLs for seed yield (SY) and SY-related trait in the BnaTNDH population grown at an optimal and a low N supply. All identified QTLs of all the traits were integrated into consensus QTLs, and then consensus QTLs were integrated into unique QTLs. The outermost circle and the second circle represented the genetic map and physical map, respectively. All SNP markers on each chromosome were corresponded to the physical position. From the third circle to the 6th circle, each color of circle stands for one trial, except green ones, which showed the positions of consensus QTLs of each trait at an optimal N or a low N supply. The innermost circle showed the positions of unique QTLs on chromosomes.