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ORIGINAL PAPER

Quantitative trait loci that control the oil content variation of rapeseed (*Brassica napus* L.)

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Abstract

Key message **This report describes an integrative analysis of seed-oil-content quantitative trait loci (QTL) in *Brassica napus*, using a high-density genetic map to align QTL among different populations.**

Abstract Rapeseed (*Brassica napus*) is an important source of edible oil and sustainable energy. Given the challenge involved in using only a few genes to substantially increase the oil content of rapeseed without affecting the fatty acid composition, exploitation of a greater number of genetic loci that regulate the oil content variation among rapeseed germplasm is of fundamental importance. In this study, we investigated variation in the seed-oil content among two related genetic populations of *Brassica napus*, the TN double-haploid population and its derivative reconstructed-F_2 population. Each population was grown in multiple experiments under different environmental conditions. Mapping of quantitative trait loci (QTL) identified 41 QTL in the TN populations. Furthermore, of the 20 pairs of epistatic interaction loci detected, approximately one-third were located within the QTL intervals. The use of common markers on different genetic maps and the TN genetic map as a reference enabled us to project QTL from an additional three genetic populations onto the TN genetic map. In summary, we used the TN genetic map of the *B. napus* genome to identify 46 distinct QTL regions that control seed-oil content on 16 of the 19 linkage groups of *B. napus*. Of these, 18 were each detected in multiple populations. The present results are of value for ongoing efforts to breed rapeseed with high oil content, and alignment of the QTL makes an important contribution to the development of an integrative system for genetic studies of rapeseed.

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Introduction

Oilseed *Brassica*, especially *Brassica napus*, is one of the most widely planted oil crops. Production of approximately 60 million tons of rapeseed worldwide supplies 20 million tons of oil to the edible-oil and biodiesel industries annually (USDA ERS 2010). Over the past two decades, the increase in oil content has lagged behind increases in the seed yield of *B. napus*, with the seed-oil content of the majority of commercial cultivars remaining at 40–50 %. Given the present capacity for worldwide rapeseed production, an overall elevation in seed-oil content by even a few percent would enable a remarkable improvement in levels of oil production.

The seed-oil content varies quantitatively among germplasm of *B. napus*; this variation is attributed to the complex regulation of multiple genes that are involved in various aspects of seed-storage-oil metabolism (Barker et al.

2007; Mekhedov et al. 2000; Ohlrogge and Browse 1995). Thus, mapping the genetic loci that control the quantitative variation is a preliminary step to disclose the complex regulation of this trait. Given the limited map density and mapping algorithms available at the time, the earliest report of quantitative trait loci (QTL) that control seed-oil content variation in *B. napus* detected only three discrete loci (Ecke et al. 1995). Of these, two showed a close association with qualitative variation in erucic acid content. Both parental lines used in the study produced moderate amounts of seed oil (Ecke et al. 1995). Subsequent studies mapped QTL for seed-oil content from different types of parental-line combinations. These included combinations where both parental lines had a high oil content (Zhao et al. 2005), both had a moderate oil content (Burns et al. 2003; Qiu et al. 2006), or one parent had a high, and the other a moderate, oil content (Delourme et al. 2006). However, the failure to identify any QTL that accounts for the full extent of the variation in seed-oil content suggests that a pyramiding approach that involves multiple genes might be helpful in realizing this goal. Although previous studies intended to compare QTL results with those of other studies, only a few loci that were found in different populations could be confirmed because of the lack of a reference system. Establishment of a reference system that readily enables mapping and alignment of QTL among populations will be valuable for the genetic improvement of rapeseed.

In the present study, we updated seed-oil-content QTL in the TN population, which was used to identify seed-oil content and erucic acid content QTL (Qiu et al. 2006), as well as QTL for many other important agronomic traits (Feng et al. 2012; Long et al. 2007; Shi et al. 2009). Using the TN genetic map as a reference, we then projected the majority of the seed-oil-content QTL, which were previously detected in an additional three populations, onto the TN map. This increases the convenience with which the distribution of QTL in different backgrounds can be compared.

Materials and methods

Plant materials and field experiments

The double-haploid (DH) parental lines, Tapidor and Ningyou7, were used to generate two related genetic populations of *B. napus*. Whereas the TN DH population (Qiu et al. 2006) comprised 202 DH lines, the TN reconstructed-F_2 (TN RC-F_2) population comprised 404 F_2 lines, with each F_2 line derived from the crossing of two randomly selected DH lines (Shi et al. 2009). Both populations were used to investigate variation in the seed-oil content in multiple field experiments.

We defined each particular combination of experimental year × location × population as an independent experiment. In total, 15 experiments were carried out (Table 1; Electronic Supplementary Material 1). In every experiment, the population and parents were planted with three replications, and each line was grown in a three-row plot with a randomized complete-block design in each replication. All individual plants in every block were pooled at harvest to collect a single sample to measure seed quality. The threshed seeds were desiccated in an oven at 35 °C for 24 h in order to minimize the moisture content. The oil content of the desiccated seeds was measured by near-infrared reflectance spectroscopy for each sample, and was expressed as a percentage of the total seed dry weight.

Descriptive statistical analysis of phenotypic variance

For each independent experiment, the phenotypic variance was resolved, with a general linear model (GLM) using SAS 8.0 (SAS Institute 1999), into components of genotypic effect (σ^2_G) and error (σ^2_e). For TN DH and TN RC-F_2 population, respectively, multiple experiments were jointly analyzed as well; and the phenotypic variance was further resolved into genotypic effect (σ^2_G), environmental effect (σ^2_E), genotype × environment interaction (σ^2_{GE}), and error (σ^2_e). The broad-sense heritability (H^2) of seed-oil content was calculated as the proportion of the genotype-contributed variance among the total phenotypic variance. Meanwhile, the adjusted mean (least square mean) of each line across multiple experiments was obtained from the joint analysis, and used for both QTL mapping and epistatic interaction analysis, in order to make a comparison with the genetic loci that identified in each independent experiment.

QTL mapping to each independent experiment

The TN genetic map, which contains 786 molecular makers and was mapped with yield (Shi et al. 2009) and glucosinolate (Feng et al. 2012) QTL, was used for QTL mapping (Electronic Supplementary Material 2). Composite interval mapping with WinQTL Cartographer 2.5 software (Wang et al. 2006; Zeng 1994) was used to detect QTL in each experiment independently. Permutation test (Doerge and Churchill 1996) with 500 times was performed to obtain a logarithm of the odds (LOD) threshold of significance for each experiment, and putative QTL were extracted with a significance threshold of $p \leq 0.05$. Likelihood results were extracted using a distance of 10 cM to define two separate QTL. The genetic distance that spanned the decrease of 2 LOD scores on both sides of the peak position of each QTL was used as the confidence interval for each QTL.

Table 1 Phenotypic variation and variance components in the multiple experiments based on TN population

Experiment code	Seed-oil content (%)					ANOVA[c]					Genetic contribution of detected QTL (%)	
	Tapidor	Ningyou7	Population			σ_G^2	σ_e^2	σ_E^2	σ_{GE}^2	H^2	Additive (dominant)	Epistatic
			Max	Min	Mean							
03N	44.5	41.3	48.1	37.5	43.3 ± 1.02	3.62	1.05				56	5
03S	43.2	44.2	48.5	38.9	44.0 ± 1.17	2.73	1.38				33	
04N	44.5	44.1	50.7	37.9	44.3 ± 1.37	2.87	1.87				25	
04S	42.1	41.3	46.9	33.8	41.5 ± 1.17	3.16	1.37				18	
05S′	42.0	39.1	45.9	36.3	40.1 ± 0.80	2.43	0.63				30	4
06N	45.2	41.4	49.2	37.7	43.7 ± 0.76	3.67	0.57				43	16
06S′	42.4	39.6	47.6	35.2	41.0 ± 1.03	3.68	1.05				27	13
06S	39.3	38.1	47.9	36.3	41.9 ± 1.40	2.16	1.95				27	16
06W	NA[b]	41.9	50.3	32.9	41.7 ± 2.10	6.52	4.42				7	6
07E	42.6	41.8	47.1	36.4	42.2 ± 1.03	3.12	1.06				27	7
07N	42.6	41.6	47.2	35.3	42.1 ± 1.09	3.65	1.20				20	
07S	45.1	45.5	50.9	39.9	46.1 ± 0.91	2.85	0.83				25	
TN DH jointly[a]	43.4	41.6	47.5	37.2	42.7 ± 1.21	2.09	1.47	2.20	0.79	0.95	65	2
05S′(F_2)	42.0	39.1	44.4	35.8	40.8 ± 1.18	1.30	1.38				27 (23)	9
06N(F_2)	45.2	41.4	48.2	39.6	44.4 ± 0.70	1.66	0.49				67 (20)	
06S′(F_2)	42.4	39.6	46.0	36.4	41.8 ± 1.03	1.64	1.06				67 (19)	10
TN RC-F_2 jointly	42.2	40.5	45.9	37.4	42.3 ± 1.02	1.15	1.05	2.14	0.28	0.85	85 (24)	

[a] The 12 experiments based on TN DH population, and the 3 experiments based on TN RC-F_2 population, were jointly analyzed, respectively; and the adjusted mean of each line across multiple experiments was obtained for QTL analysis as well

[b] Materials planted in experiment 06W were sown in May and harvested in September. As a spring-type environment, data were not obtained for Tapidor and 57 lines that require strong vernalization

[c] All variances were significant at $p < 0.0001$, except in experiments 06S ($p = 0.0045$) and 06W ($p = 0.0382$)

Pairwise identification of epistatic loci in each independent experiment

The epistatic interactions of pairwise loci that affect seed-oil content variation in each independent experiment were identified with QTLmapper 2.0 software (Wang et al. 1999). First, the main-effect markers and putative interaction markers were selected sequentially by stepwise regression at the $p \leq 0.05$ significance level. Then, the putative epistatic loci were analyzed using the background genetic variation control method.

Projection of QTL from other populations onto the TN genetic map

The *Map projection* package in BioMercator 2.1 software (Arcade et al. 2004) was used to project the QTL that control seed-oil content in three previously reported DH populations onto the TN genetic map. These were the DY, RNSL (Delourme et al. 2006) and SG (Zhao et al. 2012) populations.

First, the corresponding linkage groups in different populations were paralleled, the top–bottom direction of the linkage group in different populations was adjusted for consistency, and common genetic loci (represented by common molecular markers) among the different populations were identified. For each population, the whole spanning interval of overlapping QTL that were detected in different experiments was taken as a single QTL in this projection.

Between each pair of corresponding linkage groups, at least two common loci and the absence of inversion are required to implement the projection of the map/QTL from one population onto another. Therefore, in the present study, two procedures of QTL projection on different linkage groups were applied. One procedure was direct projection from a population onto the TN map when it has enough loci in common with the TN linkage group (e.g., map X and Y in Fig. 1); the second procedure used a third population as an intermediate to achieve projection when the population has insufficient loci in common with the TN linkage group (e.g., map Z in Fig. 1).

For the projection algorithm, a specific distance ratio was first computed for each interval bounded by two common loci (i.e., the ratio between the interval length of the two common loci on maps A and B). Then, a global ratio was computed for projection of loci that were located

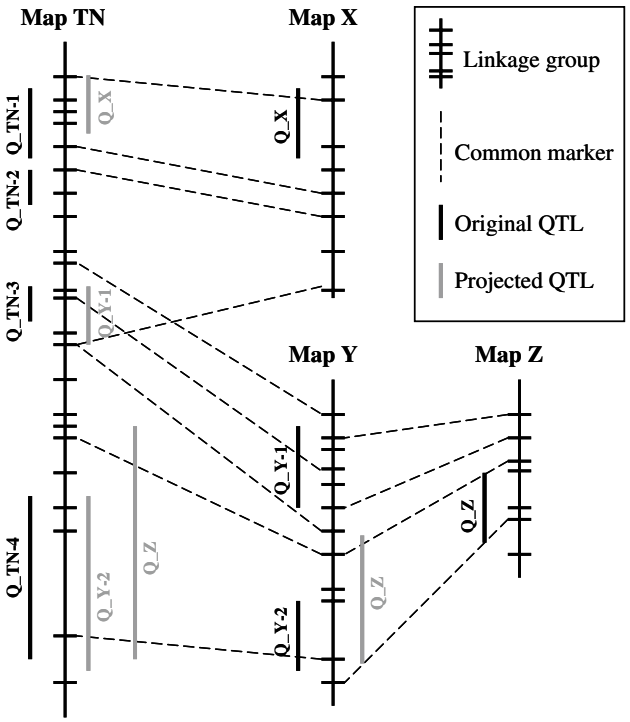


Fig. 1 Projection of QTL from other populations onto the TN genetic map

above the first interval of common loci and below the last interval of common loci. Finally, the remaining loci positions and/or QTL intervals were projected from map B onto map A by application of the appropriate distance ratio (Arcade et al. 2004).

Results

Variation in the seed-oil content of the TN population of *B. napus*

As in the four independent experiments described by Qiu et al. (2006), the percentage of oil content in seeds was similar in the two parental lines in all 15 experiments we performed (43.0 ± 1.7 % in Tapidor and 41.7 ± 2.1 % in Ningyou7). Transgressive segregation was observed in each experiment; whereas, the mean minimal content of seed oil in the TN DH population was 37.2 % and the mean maximal content was 47.5 %, the extent of variation was slightly smaller in the TN RC-F_2 population (Table 1). The analysis of variance revealed that genotypic variance contributed 70–95 % of the phenotypic variation in the independent experiments (Table 1). Joint analysis of the 12 experiments based on TN DH population, and the 3 experiments based on TN RC-F_2 population, showed that the genotype × environment interaction were significant in the phenotypic

Fig. 2 Distribution of seed-oil-content QTL in the *B. napus* genome, using the TN genetic map as a reference. Linkage groups represent the TN genetic map. To facilitate viewing, only markers from the *Brassica* public resource (http://www.brassica.info/resource/markers.php) are shown. *Bold horizontal sticks* represent separate QTL intervals that have been identified in different populations (DY, RNSL, SG, and TN) and projected onto the TN genetic map; and the darkness of the sticks, which is *light gray*, *gray*, or *black*, indicates the QTL was detected in single, double, or three or more experiments, respectively. *Patterned sticks* under the QTL intervals in TN population are QTL that identified with adjusted mean of each line across multiple experiments of TH DH or TN RC-F_2 population. *Triangles* attached to the linkage groups indicate associated markers that have been identified in an association study with breeding lines of *B. napus* (Zou et al. 2010)

variations ($p < 0.0001$); however, the broad-sense heritability of seed-oil content can be as high as 90 %.

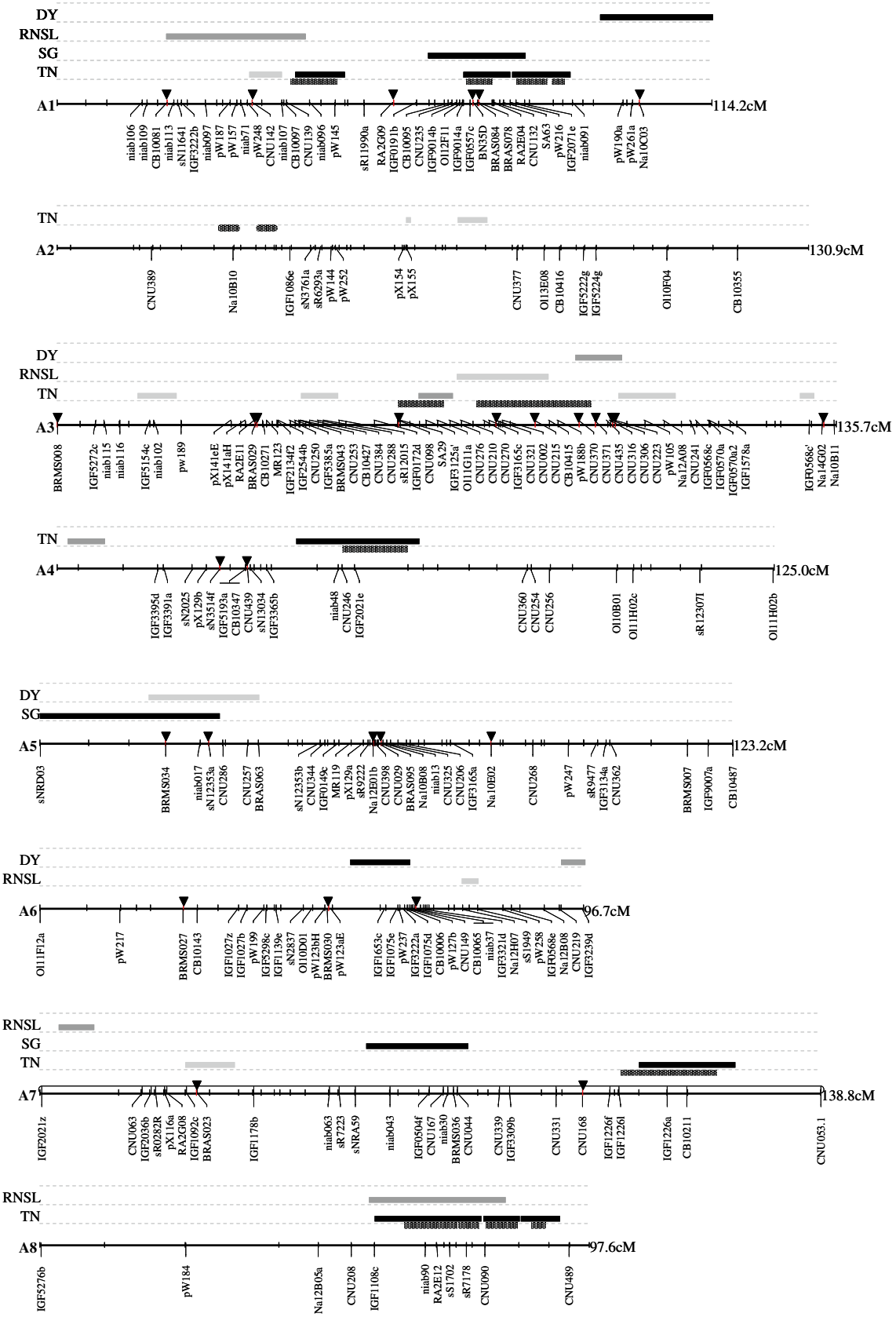
Genetic loci that control variation in the seed-oil content of the TN population

Between 4 and 11 QTL were detected by composite interval mapping in independent experiments, with 4–6 detected in most experiments. In total, 88 original QTL were detected from the 15 experiments; these comprised 36 non-overlapping QTL intervals on 13 linkage groups of the *B. napus* genome (Electronic Supplementary Material 3). The average marker interval of the TN genetic map used for this study was 2.7 cM, the average confidence interval of the original oil content QTL was 7 cM, and each of the non-overlapping QTL intervals spanned 5–20 cM (Fig. 2).

Single QTL contributed 2–20 % of the oil content variation in particular experiments (Electronic Supplementary Material 3). In addition to the two QTL intervals on linkage groups A8 and C3 (which were shown to be associated with qualitative variation in erucic acid content in previous studies), the QTL on linkage groups A1, A3, A4, A9, A10, C2, and C6 also had significant additive effects of 0.6–0.8 on the variation in oil content.

Using the TN RC-F_2 population, dominant effects were identified for 20 QTL, of which more than half were also detected in the DH population (Electronic Supplementary Material 3). The heterozygous genotype increased the oil content above that of the mid-parent value for 12 of these 20 QTL. By contrast, for the other eight QTL, the heterozygous genotype had oil content lower than that of the mid-parent value. Although most of these QTL showed only a partially dominant effect, four QTL (on linkage groups A1, A4, C2, and C6) showed positive complete- or over-dominance (D/A 1.1–1.3).

Nineteen pairs of epistatic interactions were detected by stepwise regression with the QTLmapper software in 9 of the 15 independent experiments (Table 2). The interactions involved 36 separate loci, of which two interacted


DY
RNSL
SG
TN
A1
114.2cM
niab106 niab109 CB10081 niab113 sN11641 IGF3222b niab097 pW187 pW157 niab71 pW248 CNU142 niab107 CB10097 CNU139 niab096 pW145 sR11990a RA2G09 IGF0191b CB10095 CNU235 IGF9014b OI12F11 IGF9014a IGF0557c BN35D BRAS084 BRAS078 RA2E04 CNU132 SA63 pW216 IGF2071e niab091 pW190a pW261a Na10C03
TN
A2
130.9cM
CNU389 Na10B10 IGF1086e sN3761a sR6293a pW144 pW252 pX154 pX155 CNU377 OI13E08 CB10416 IGF5222g IGF5224g OI10F04 CB10355
DY
RNSL
TN
A3
135.7cM
BRMS008 IGF5272c niab115 niab116 IGF5154c niab102 pw189 pX141eE pX141aH RA2E11 BRAS029 CB10271 MR123 IGF2134f2 IGF2544b CNU250 IGF5385a BRMS043 CNU253 CB10427 CNU384 CNU288 sR12015 IGF0172d CNU098 SA29 IGF3125a' OI11G11a CNU276 CNU210 CNU270 IGF3165c CNU321 CNU002 CNU215 CB10415 pW188b CNU370 CNU371 CNU435 CNU316 CNU306 CNU223 pW105 Na12A08 CNU241 IGF0568c IGF0570a IGF0570a2 IGF1578a IGF0568c' Na14G02 Na10B11
TN
A4
125.0cM
IGF3395d IGF3391a sN2025 pX129b sN3514f IGF5193a CB10347 CNU439 sN13034 IGF3365b niab48 CNU246 IGF2021e CNU360 CNU254 CNU256 OI10B01 OI11H02c sR12307I OI11H02b
DY
SG
A5
123.2cM
sNRD03 BRMS034 niab017 sN12353a CNU286 CNU257 BRAS063 sN12353b CNU344 IGF0149c MR119 pX129a sR9222 Na12E01b CNU398 CNU029 BRAS095 Na10B08 niab13 CNU325 CNU206 IGF3165a Na10E02 CNU268 pW247 sR9477 IGF3134a CNU362 BRMS007 IGF9007a CB10487
DY
RNSL
A6
96.7cM
OI11F12a pW217 BRMS027 CB10143 IGF1027z IGF1027b pW199 IGF5298c IGF1139e sN2837 OI10D01 pW123bH BRMS030 pW123aE IGF1653c IGF1075e pW237 IGF3222a IGF1075d CB10006 pW127b CNU149 CB10065 niab37 IGF3321d Na12H07 sS1949 pW258 IGF0568e Na12B08 CNU219 IGF3239d
RNSL
SG
TN
A7
138.8cM
IGF2021z CNU063 IGF2036b sR0282R pX116a RA2G08 IGF1092c BRAS023 IGF1178b niab063 sR7223 sNRA59 niab043 IGF0504f CNU167 niab30 BRMS036 CNU044 CNU339 IGF3309b CNU331 CNU168 IGF1226f IGF1226l IGF1226a CB10211 CNU053.1
RNSL
TN
A8
97.6cM
IGF5276b pW184 Na12B05a CNU208 IGF1108c niab90 RA2E12 sS1702 sR7178 CNU090 CNU489

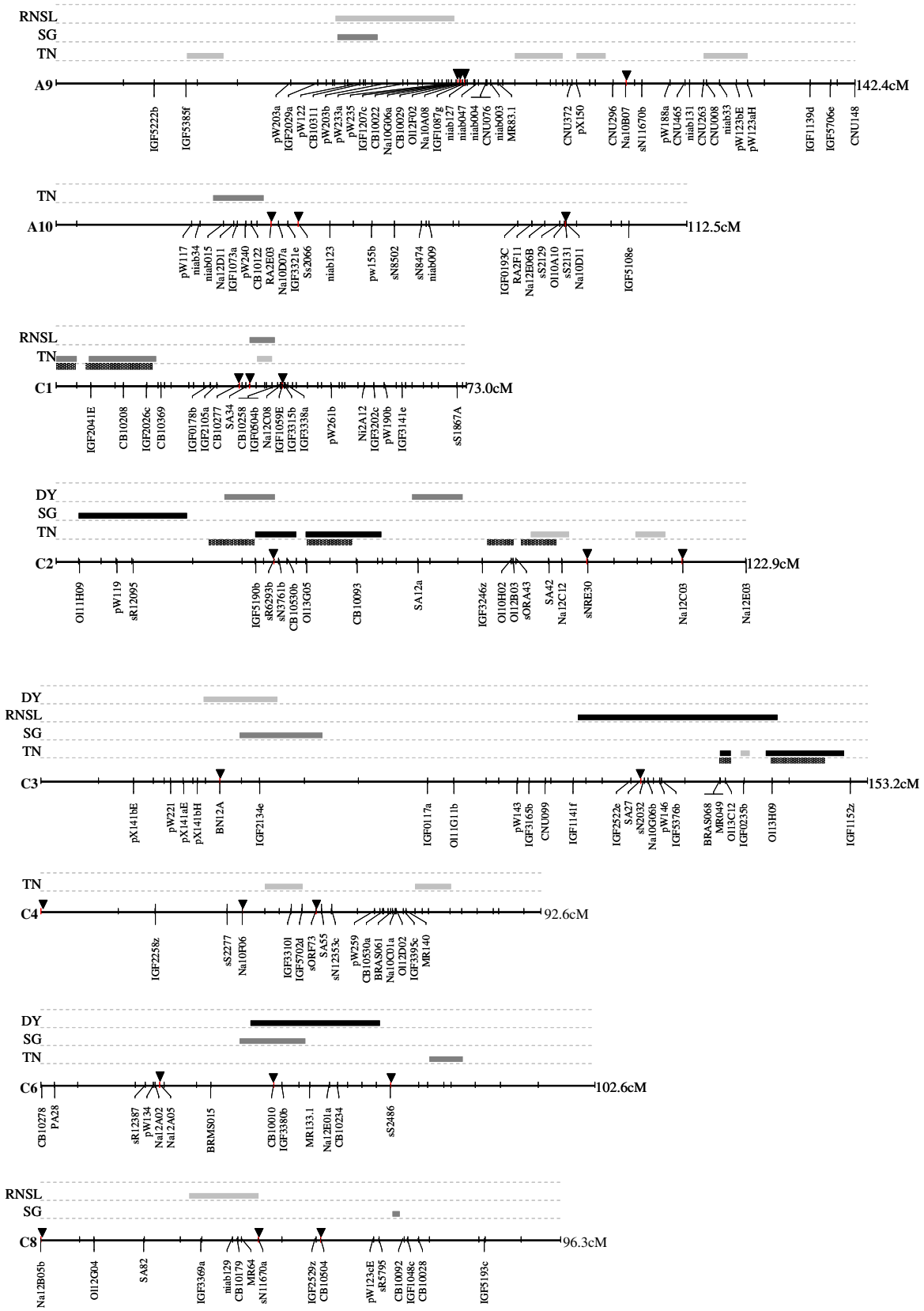


Fig. 2 continued

Table 2 Epistatic interactions detected in the TN population

Interval i	Marker interval	Interval j	Marker interval	Epistatic effect	Experiment
A1–25	**EST156a/pW145**	A2–48	Ol10F04/E5HM40-310	0.24**	06S′
A1–31	IGF0191b/E5HM31-505	C3–19	E6HM40-280/pW143	−0.28***	07E
A2–47	35RXTRAP10-3/Ol10F04	A8–5	Na12B05a/CNU208	−0.5***	06N
A3–27	**CNU288/sR12015**	C4–4	sS2277/Na10F06	−0.48***	07E
A3–29	E4HM31-190/IGF0172d	A9–67	P13M10-265/IGF1139d	0.4***	05S′
A3–67	S14M08-2-180/P07M21-490	**C1–6**	**IGF2026c/TrAPG_4**	−0.56**	06N
A3–71	**IGF0568c′/Na14G02**	C1–34	S13M08-1-190/S04M03-1-210	0.32**	06S′
A3–72	**Na14G02/Na10B11**	**C1–5**	**CB10208/IGF2026c**	−0.41***	06S′
A4–7	pX129b-sN3514f	A6-5	EST92a-BRMS027	0.21**	TN DH jointly
A5–13	E1HM31-130/sN12353b	A6–5	EST92a/BRMS027	−0.28***	06N
A5–13	E1HM31-130/sN12353b	C4–4	sS2277/Na10F06	0.47***	06S
A6–1	Ol11F12a/P13M9-150	A9–58	sN11670b/pW188a	−0.41***	06S
A6–10	IGF1027b/pW199	**A7–43**	**CB10211/CNU053.1**	−0.59***	06S′
A7–41	**FTin1-a/IGF1226a**	C8–18	CB10028/S10-170	−0.42***	06N
A8–18	**CNU489/P7M5-170**	**C3–40**	**Au8/IGF1152z**	−0.45*** (A_iA_j), −1.00*** (D_iD_j)	06S′(F_2)
A9–2	E8HM31-460/IGF5222b	C7–8	Au36/IGF3138z	−0.39***	06S
A9–61	niab131/CNU263	**C2–21**	**P5M5-1000/P7M7-490**	0.52***	06S
C2–5	**sR12095/S13-50**	C7–9	IGF3138z/IGF5702e	0.65** (A_iD_j), 0.84** (D_iD_j)	05S′(F_2)
C2–34	Na12C03/Na12E03	C3–21	IGF3165b/CNU099	−1.12***	06W
C3–39	**Ol13H09/Au8**	C6–11	BRMS015/CB10010	−0.46***	03N

The epistatic effects were significant at ** $p < 0.05$ or *** $p < 0.005$. Epistasis was detected as additive × additive (A_iA_j) in most experiments that were based on the TN DH population, whereas some additive × dominant (A_iD_j) or dominant × dominant (D_iD_j) effects were detected in the TN RC-F_2 population. The epistatic intervals involved in the identified QTL are highlighted in bold

with different loci in different experiments. Thirteen of these interaction loci were located in the above-mentioned QTL intervals, but only two pairs of epistatic interactions involved both loci that located in a QTL interval. The epistatic interactions had effects of 0.2–1.1 on the oil content variation across experiments.

Of the genetic loci detected in each experiment, single-locus effects (additive and dominance) accounted for 18–87 % of the phenotypic variation, whereas epistatic interactions contributed 4–16 % (Table 1).

In comparison to the QTL mapping in each independent experiment, the adjusted mean of each line across multiple experiments was also used for analysis in TN DH and TN RC-F_2 population, respectively. Thus, 15 QTL were identified from the joint phenotypic data in TN DH population, while 13 QTL were identified in the TN RC-F_2 population (Electronic Supplementary Material 3); 22 of these joint QTL were involved in the non-overlapping QTL intervals which concluded from independent experiments mentioned above (Fig. 2), and totally 41 separate QTL regions were identified through the two approaches in TN populations (Electronic Supplementary Material 3).

The average confidence interval of the QTL that identified from joint analysis was smaller compare to those identified from independent experiments (*t* test, $p > 0.05$). The average contribution to phenotypic variation (R^2) of the QTL that identified from joint analysis was lower compare to those identified from independent experiments (*t* test, $p > 0.05$); as there were many QTL showed specially high contribution to phenotypic variation in particular experiments. However, there was no significant difference on the additive effects between the QTL which were identified from joint analysis or independent experiments (*t* test, $p < 0.005$).

When using the joint phenotypic data for epistatic interaction analysis, only one pair of interaction was obtained based on the TN DH population (Table 2), and it was not coincident with any pairs obtained from independent experiments.

Comparison of QTL among different populations

Fifteen, 11, and 9 separate QTL intervals that control variation in the content of seed oil were identified previously in the DY, RNSL (Delourme et al. 2006), and SG (Zhao et al. 2012) populations. The respective average marker interval and average confidence interval of the original QTL were approximately 9 and 20 cM in each of the DY and RNSL populations, and approximately 4 and 8 cM in the SG population.

Through identification of common markers in corresponding linkage groups of different populations, we derived projected locations on the TN genetic map of the oil content QTL from other population(s) on 12 linkage groups, namely linkage groups A1, A3, A5–A9, C1–C3, C6, and C8 (Fig. 2; Electronic Supplementary Material 4). Nine QTL intervals that spanned 14–40 cM on the DY genetic map were projected onto linkage groups A1, A3, A5, A6, C2, C3, and C6 of the TN genetic map, and correspondingly spanned 4–24 cM on the TN genetic map. Nine QTL intervals that spanned 6–61 cM on the RNSL genetic map were projected onto linkage groups A1, A3, A6–A9, C1, C3, and C8 of the TN genetic map; these intervals spanned 3–37 cM on the TN genetic map. All of the nine separate QTL intervals, which were identified on linkage groups A1, A5, A7, A9, C2, C3, C6, and C8 of the SG genetic map and spanned 2–25 cM, were projected onto the corresponding linkage groups of the TN genetic map; these intervals spanned 2–32 cM on the TN genetic map. However, given that the projected location of the QTL *SG_oilC8-2*, which spanned 16 cM at the end of linkage group C8 of the SG genetic map (Zhao et al. 2012), was beyond the end of linkage group C8 on the TN genetic map, it was not considered in the following comparisons.

Table 3 Comparison of oil content QTL locations among the DY, RNSL, SG, and TN populations and an association study (Z. Assoc)

Number of overlapping QTL intervals	TN (41)[a]	DY (15)	RNSL (11)	SG (9)
DY (9)[b]	2			
RNSL (9)	5	0		
SG (8)	1	3	1	
Z. Assoc (54)	12	5	5	3

[a] Number in parentheses in the column headings is the number of non-overlapping QTL intervals in each original study

[b] Number in parentheses in the row headings is the number of QTL/association markers projected onto the TN genetic map

Alignment of these projected QTL from other populations on the TN genetic map, together with those QTL identified in the TN population, identified 12 QTL intervals that were detected in different populations (Table 3). Whereas two intervals were located on each of linkage groups A1, A3, and C3, one interval was located on each of linkage groups A5, A8, A9, C1, C2, and C6 (Fig. 2). In addition to these QTL mapping studies in double parental populations, a previous association study that investigated the variation in the oil content of breeding lines assessed markers based on the TN genetic map (Zou et al. 2010; "Z. Assoc" in Table 3). This association study identified 54 markers on the TN genetic map associated with variation in the oil content across a panel of breeding lines. We found that some of these associated markers were coincident with 12 QTL intervals identified in the TN population and with 13 QTL intervals identified in the DY, RNSL, or SG populations (Table 3). Through this alignment and comparison,

we identified a total of 46 distinct QTL regions that control seed-oil content on the *B. napus* genome as represented by the TN genetic map, of which 18 QTL regions were detected in different populations including the association study panel.

Discussion

Like many other important agronomic traits in crops, the oil content in rapeseed is regulated by a complex system that involves multiple genes. Such complex genetic regulation makes it difficult to realize a substantial improvement in these traits through manipulation of a single gene. Nonetheless, QTL mapping promises to provide a foundation for genetic improvement of these traits. In this study, we investigated the variation in seed-oil content among two related genetic populations of *B. napus* under multiple environments, and identified 41 separate QTL intervals and 20 epistatic interaction pairs. Integrative alignment of the seed-oil-content QTL from several different populations revealed 12 QTL that contributed to phenotypic variation under different backgrounds.

Marker density on the genetic map and the resolving power of QTL mapping

Compared with a previous study that investigated the TN DH population in four experiments and used a genetic map with fewer than 300 markers for QTL mapping (Qiu et al. 2006), the present study identified seed-oil-content QTL on an additional five linkage groups. In addition, given the increased marker density and extent of linkage on the current TN genetic map relative to the map that Qiu et al. (2006) used, a higher number of separate QTL were identified for each linkage group than in the previous study, which detected only a single QTL per linkage group. Whereas Qiu et al. (2006) identified a single QTL interval close to the marker Ra2E04 on linkage group A1 (here referred to as the QTL *TN_oilA1-3*), our comparative study showed that this QTL interval was also detected in the SG population. Extension of this linkage group and additional phenotyping experiments conducted in the current study identified another QTL interval close to the marker CB10097 (referred to as the QTL *TN_oilA1-1*); this QTL interval was also detected in the RNSL population (Fig. 2). Another example is linkage group A3; in the previous study, a QTL was detected at the top of this linkage group and close to the STS marker IGF5154c (here referred to as the QTL *TN_oilA3-1*). Subsequent studies mapped a homolog of the flowering time gene *FLC* close to this QTL and also identified a QTL that controls flowering time in the vicinity of the QTL (Long et al. 2007; Raman et al. 2013a; Zou et al. 2012). This suggests that the variation in seed-oil content caused by this QTL should be a downstream effect of variation in flowering time. In the present study, two additional seed-oil-content QTL were identified in the center (close to the marker Ol11G11a, referred to as the QTL *TN_oilA3-3*) and lower (close to the marker Na12A08, referred to as the QTL *TN_oilA3-5*) portions of linkage group A3; and our comparative study showed that a QTL was detected in the RNSL and DY populations in an interval near to the QTL *TN_oilA3-3* and *TN_oilA3-5*, respectively (Fig. 2).

These findings confirm that the improvement in marker density on a genetic map considerably facilitates the mapping of QTL. With continued progress in high-density single nucleotide polymorphism (SNP) mapping in *B. napus* (Bancroft et al. 2011; Delourme et al. 2013; Raman et al. 2013b), it can be expected that an increasing number of closely linked QTL for complex traits, such as oil content in rapeseed, will be resolved in the near future.

Dominance of seed-oil-content QTL

Most previous studies that used genetic mapping to investigate oil-content QTL in rapeseed involve DH lines or inbred lines, which allow multiple repetitions of phenotype evaluation. Therefore, only additive effects and some epistatic effects of the seed-oil-content QTL were revealed in these studies. In the present study, the use of a TN RC-F_2 population enabled determination of the dominance effects for some QTL.

Of the 20 QTL that we detected with dominance effects, the majority showed dominance of about 0.5 or less, only four QTL showed positive complete-dominance or mild over-dominance effects, and one QTL on linkage group A2 showed negative dominance of about 0.8. In both autogamous species (Frascaroli et al. 2007) and allogamous species (Radoev et al. 2008), QTL for traits that exhibit strong heterosis predominantly show dominance to over-dominance effects, whereas QTL for traits that show weaker heterosis have additive to dominance effects. The present results infer that oil content in rapeseed has weaker heterosis compared with that of other traits, such as seed yield (Radoev et al. 2008). However, the expression of dominance or heterosis relies heavily on the specific germplasm combination and environment. A recent study, which focused on yield-correlated traits in the same experiments as the present study, showed that strong dominance was not detected among the numerous QTL for 15 yield-correlated traits, even for the seed-yield trait (Shi et al. 2011).

It is also notable that eight QTL showed negative dominance effects; in other words, a heterozygous genotype for these loci would decrease oil content from the mid-parent value. Shi et al. (2011) found that heterozygosity was not

always advantageous for either heterosis or hybrid performance for 15 yield-correlated traits. Therefore, QTL that are prone to negative dominance effects should be carefully selected during rapeseed breeding programs, as hybrid breeding is a preferred manner currently.

Epistatic interactions and underlying molecular mechanisms

In addition to single-locus (additive or dominance) effects, epistatic interactions also contributed a considerable portion of the genetic variation in many experiments of the present study (Table 1), and in some previous studies of yield-related traits (Radoev et al. 2008; Shi et al. 2011). However, detection of epistasis is extremely germplasm- and environment-specific, and few identical pairs of epistatic interactions that control oil content in rapeseed have been identified in different studies. In the DY and RNSL populations (Delourme et al. 2006), only three pairs of epistatic interactions were reported, and most of the interacting loci were involved in additive QTL regions; the three interaction pairs have not been identified in other populations. In both the SG (Zhao et al. 2006) and TN populations, interactions were detected between linkage groups A1 and A2, and between linkage groups A3 and C1. In the SG population, the interacting loci were located at the bottom of linkage group A1 and the lower portion of A2, whereas in the TN population these loci were located in the center of A1 and the lower portion of A2. The shortage of common markers on A2 restricts confirmation of whether the participants on the lower portion of A2 were the same locus in both populations. For the interactions between linkage groups A3 and C1, on both of which map projection was achieved, we could confirm that the participant on A3 was on the lower portion in the SG population but at the bottom in the TN population. Moreover, the participants on C1 were located very closely for both populations (at a position close to 50 cM), and thus might act from the same locus.

A recent study assessed gene-specific epistasis in a population of diverse elite rapeseed inbred lines (Würschum et al. 2013). The authors identified interactions between certain key enzymes involved in the main pathway of storage oil formation, as well as with an important transcription factor, *WRI1*, which has a general function in storage compound biosynthesis in Arabidopsis (Focks and Benning 1998; Cernac and Benning 2004). In Arabidopsis, *WRI1* regulates seed-oil formation via interaction with the promoter sites of genes that encode enzymes that catalyze the synthesis of triacylglycerols, such as the *PKp1* gene that encodes pyruvate kinase and the *BCCP2* gene that encodes the biotin carboxyl carrier protein (Baud et al. 2009; Maeo et al. 2009). In the rapeseed population, Würschum et al. (2013) also identified significant epistasis between SNPs in the *WRI1* and *BCCP* genes. On the TN genetic map, few gene-specific markers for oil formation have been mapped, but other sequence-informative markers allow a rough alignment of the *B. napus* genome with the Arabidopsis genome (Long et al. 2007; Shi et al. 2009). Consideration of the enzyme genes assessed by Würschum et al. (2013) and some transcription factors related to the biosynthesis of seed oil (Baud et al. 2007; Wang et al. 2007) suggests that three epistatic interactions detected in the present study might have identified candidate genes responsible for the observed phenotypes. The genetic interval A3–29, which might be associated with transcription factor gene *ABI5*, interacted with the genetic interval A9–67, which might be associated with the pyruvate dehydrogenase gene *PDH*. Moreover, the genetic intervals A7–41 and A7–43, which might both be associated with the beta-ketoacyl synthase III gene *KAS3*, interacted with the genetic intervals C8–18 and A6–10, respectively, both of which might be associated with the *PDH* gene. Hundreds of gene sequences associated with lipid metabolism were recently mapped in the SG population (Zhao et al. 2012); therefore, extensive assessment of gene-specific epistasis could be carried out as reported by Würschum et al. (2013).

Importance of an integrative system for QTL alignment/comparison

Some previous investigations which mapped the seed-oil-content QTL like the present study also compared seed-oil-content QTL among different populations. For example, Delourme et al. (2006) compared the seed-oil-content QTL comprehensively among six populations for which data were available, including all of the four populations we compared in the present study. However, because of the limited marker information and map quality available at the time, the authors could only identify four linkage groups (A1, A3, A8, and C3) in which QTL were detected in multiple populations, and they were unsure whether the precise locations of these QTL were the same in different populations. Subsequent updates of the genetic map and phenotyping data that involved both the SG and TN populations enabled us to identify 12 QTL intervals that were found in the different populations.

It is worth mentioning the two repeatable intervals on linkage group C3; comparison among the DY, RNSL, SG, and TN populations, as well as an association population, revealed that at least two separate QTL controlling seed-oil content are located on linkage group C3. One of these occurs on the upper portion, and the other on the lower portion of this linkage group (Fig. 2). The latter QTL was detected in the RNSL and TN populations;

whereas, the two parental lines of the RNSL population show high and moderate oil content, respectively, both parental lines of the TN population show moderate oil content. Studies of the TN population showed that this QTL might be a copy of the erucic acid regulator gene fatty acid elongase1 (*FAE1*) (Qiu et al. 2006; Wang et al. 2008). Nonetheless, given that no segregation of erucic acid content was observed in the RNSL population, this QTL also might represent a link between the independent oil content regulator and erucic acid regulator (Delourme et al. 2006). The other QTL on the upper portion of linkage group C3 was detected in the DY and SG populations, both of which were derived from parental lines with high oil content. This example showed that different lines, each with high oil content, might carry superior alleles at different loci. Therefore, exploitation of QTL through different combinations of germplasm is still required in order to uncover additional regulators of seed-oil content and superior alleles for each site; an integrative system of both markers and QTL would help to reveal the fluctuation of QTL effects among different backgrounds, from which the most effective allele for a particular locus can be selected. Currently, information for many QTL still cannot be integrated. For example, in the four populations compared in the present study, QTL on linkage group C4 were only detected in the TN population. Seed-oil-content QTL on linkage group C4 are reported in two recent studies (Chen et al. 2010; Würschum et al. 2012); nonetheless, given that only a few markers from the *Brassica* public resources were used in those studies, it is not possible to determine whether the QTL on linkage group C4 in different populations are identical.

There are several advantages to using the TN genetic map in the present study as the reference for the projection and alignment of QTL. First, the identification of many QTL in the TN population for a variety of traits (Feng et al. 2012; Long et al. 2007; Shi et al. 2009) might shed light on whether there is a correlation among these complex traits. Moreover, the co-localization of QTL for different traits would be helpful during subsequent fine-mapping studies; the trait of which phenotypic segregation is easier to monitor might assist the determination of the target trait while of which phenotypic segregation is difficult to distinguish. Second, given that the TN population has been used for SNP marker mapping and genetic map integration in several recent studies (Bancroft et al. 2011; Delourme et al. 2013; Raman et al. 2013b), the availability of a large amount of sequence information for marker development might enable more precise selection in both fine mapping and breeding.

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Conflict of interest The authors declare that they have no conflict of interest.

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