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QTL identification for nine seed-related traits in *Brassica juncea* using a multiparent advanced generation intercross (MAGIC) population

Haifei Zhao, Wei Yan, Kunjiang Yu, Tianya Wang,
*Aimal Nawaz Khattak, Entang Tian**

Agricultural College of Guizhou University, Guizhou University, Guiyang, P.R. China

**Corresponding author: erictian121@163.com*

Haifei Zhao and Wei Yan contributed equally to the work.

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Abstract: Agronomic traits are usually determined by multiple quantitative trait loci (QTLs) that can have pleiotropic effects. A multiparent advanced generation intercross (MAGIC) population is well suited for genetically analysing the effects of multiple QTLs on the traits of interest because it contains more QTL alleles than a biparental population and can overcome the problem of confounding the population structure of the natural germplasm population. We previously developed the *B. juncea* MAGIC population, derived from eight *B. juncea* lines with great diversity in agronomic and quality traits. In this study, we show that the *B. juncea* MAGIC population is also effective for the evaluation of multiple QTLs for complex agronomic traits in *B. juncea*. A total of twenty-two QTLs for nine seed-related traits were identified, including one QTL for each oil content, seed number per silique and thousand-seed weight; two QTLs for each acid detergent lignin and neutral detergent fibre; three QTLs for each acid detergent fibre and protein content; four QTLs for the seed maturity time; and five QTLs for the white index. Some of these QTLs overlapped. These results should be helpful for further fine mapping, gene cloning, plant breeding and marker-assisted selection (MAS) in *B. juncea*.

Keywords: *Brassica juncea*; MAGIC population; plant breeding; QTL mapping; seed-related traits

Brassica juncea (AABB, $2n = 36$) is becoming the oilseed crop of choice worldwide as a result of its increased blackleg resistance (Roy 1984), oil stability to oxidation (Wijesundera et al. 2008), and heat and drought tolerance (Burton et al. 2003; Østergaard et al. 2006) in comparison with *Brassica napus*. Especially after the canola-quality, *B. juncea*, with less than 2% erucic acid in the seed oil and less than 30-μM glucosinolates (GSLs)/g of the de-oiled cake, was developed through a cross between a zero-erucic *B. juncea* line and a low-GSL *B. juncea* line (Love et al. 1991). For heterosis utilisation, the Ogura cytoplasmic male sterility (cms) and its restorer gene (*Rfo*) were introduced (Delourme et al. 1998). Then, the *B. juncea* Ogura cms restorer line (*RfoRfo*) was improved, with drastically reduced linkage drag, good seed setting and high agronomic performance by hybridisation with resynthesised *B. juncea* lines and the subsequent molecular marker-assisted selection in *B. juncea* (Tian et al. 2014).

The advent of molecular markers enables variation in a complex trait to be dissected into the effects of

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the QTLs and assists the transfer of these QTLs into the desired cultivars or lines to our benefit (Yin et al. 2003). Two-parent populations, natural germplasm populations, and MAGIC populations are the most popular platforms for association mapping and QTL mapping. The MAGIC population is developed from multiple parents, such as eight and six founders, and has great diversity among the generated lines whose genomes are fine-scale mosaics of the contributions from all the founders (Cavanagh et al. 2013). The MAGIC lines are suitable for high-resolution trait mapping (Huang et al. 2015) and can also provide excellent materials for plant breeding due to their features of having a high recombination and a resulting diverse phenotypic diversity (Yan et al. 2020). The first MAGIC population was developed in *Arabidopsis thaliana* (L.) (Kover et al. 2009), and then dozens of MAGIC populations were established in a wide range of various crops, including rice (Bandillo et al. 2013), the tomato (Pascual et al. 2015), the fava bean (Sallam & Martsch 2015), the cowpea (Huynh et al. 2018), maize (Dell'Acqua et al. 2015), barley (Sannemann et al. 2015), the strawberry (Wada et al. 2017), sorghum (Ongom et al. 2018), and wheat (Yao et al. 2019). The durations required to construct MAGIC populations can be long, depending on the number of founders involved, making the process logistically challenging and labour intensive (Ongom & Ejeta 2018). In *B. juncea*, the use of fast-cycling lines in our earlier study of the construction of a MAGIC population significantly reduced the challenges described above. The newly developed *B. juncea* MAGIC population has been used for the association mapping of the GSL contents, and three major GSL consensus QTLs, *QGsl.ig01.1* on J01 for indole GSLs and *QGsl.atg09.1* on J09 and *QGsl.atg11.1* on J11 for aliphatic GSLs and total GSLs were identified (Yan et al. 2020). The results verified the better usefulness of the *B. juncea* MAGIC population in identifying the associated QTLs of complex traits.

In the present study, the phenotypic variation in nine seed-related traits, acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent fibre (NDF), oil content (OIL), protein content (PRO), seed maturity time (SMT), seed number per silique (SN), thousand-seed weight (TSW), and white index (WI), was studied. The whole genome-wide association analysis (GWAS) was conducted for the nine seed-related traits using the *B. juncea* MAGIC mapping population first developed in Brassicaceae in our earlier study (Yan et al. 2020). The results can provide important genetic information for the genetic mechanism research and elite lines for the breeding of these nine seed-related traits in *B. juncea*.

MATERIAL AND METHODS

Plant materials and field experiments. The MAGIC population was developed from eight diverse founders through three rounds of intercrossing and the subsequent single-seed descent methods in our earlier study (Tian et al. 2016; Yan et al. 2020). In this study, we further investigated the potential utilisation of the population in associating the mapping of the nine seed-related traits. The studied traits included ADF, ADL, NDF, OIL, PRO, SMT, SN, TSW and WI. A randomised block design with three replications for each entry was adopted. To measure these phenotypic traits, two rows were planted for each replication of each of the 170 lines in the MAGIC population in 2018 and 2019. Each plot consisted of two rows with one size of 3.66 m^2 (3 × 1.22 m). All the materials were planted on the farm of Guizhou University, Guiyang, China.

Trait measurements. The nine seed-related traits were measured in the seed maturity time. The SMT was determined when the seeds of each plot became black. Three randomly chosen plants of each plot were used for the SN measurement, and the seed number of ten pods in the middle of the main stem for each plant was counted. In addition, five grams of seeds from each of three randomly chosen plants in each plot were harvested at maturity and analysed for the remaining traits, ADF, ADL, NDF, OIL, PRO, TSW and WI. The *B. juncea* seed fibre components were obtained by near-infrared reflectance spectroscopy (NIRS) using an NIR System 6500 with WinISI II software FOSS (NIRS System DS2500F, Germany) according to the previous reports (Liu et al. 2015). The contents of the ADF, ADL and NDF were expressed in % seed dry weight. In addition, OIL and PRO were also estimated by the NIRS and expressed as % (Liu et al. 2015). The TSW was measured by the weight of 1 000 seeds for each selected plant. For the seed colour, an SC-G automatic seed test analysis system (Wanshen company, Hangzhou, China) was used, and the light intensity was 6504K. All the phenotype data were averaged over the three technical replicates.

Marker-trait association analyses. The marker-trait association analyses between the three GSL quality traits of two consecutive years and the ILP genotyping data were conducted using a general linear model (GLM) with the TASSEL 3.0 software package (Bradbury et al. 2007). The significance threshold for the associations between the ILP markers and the traits was set as $P < 0.001$ ($-\log10(p) = 3$). The ILP markers prefixed with "At" were mapped on the chromosomes of the *B. juncea* genome (Panjabi et al. 2008). In addition, the ILP markers prefixed with "Bnap" and "Brap" were mapped on the chromosomes of the *B. juncea* genome by blasting their original DNA sequence against the *B. juncea* genome (Brassica_juncea_v1.5,http://brassicadb.org/brad/datasets/pub/Genomes/Brassica_juncea/V1.5/Bju.genome.fasta.gz) and assigned a genome location using a BLAST-like alignment tool (Kent 2002; Yan et al. 2020).

RESULTS AND DISSCUSSION

Phenotypic variation. The MAGIC population was developed in our earlier study, displaying great trait variation and potential utilisation in the QTL mapping of complex traits (Yan et al. 2020). In the present study, we have investigated the diversity of nine seed-related traits in the MAGIC population in 2018 and 2019, respectively. The measured seed-related traits include ADF, ADL, NDF, OIL, PRO, SMT, SN, TSW and WI, the data of which are significantly correlated between 2018 and 2019, with coefficients of correlation from 0.617 to 0.992 (Figure 1). ADF, ADL and NDF are three fibre components in *B. juncea* seeds that have a range from 5.22–10.24% (mean: 7.79 ± 1.15%), 0.00–1.90% (0.76 ± 0.46%) and 9.69–16.97% (13.00 ± 1.21%), respectively. OIL and PRO are two important quality traits of oil crops. OIL has a 1.41-fold variation, ranging from 30.71 to 43.25%, with an average of 38.05 ± 2.41%. PRO has a 1.41-fold variation, ranging from 30.71 to 43.25%, with an average of 38.05 ± 2.41%. SMT has 12 days of variation among the MAGIC lines, with an average of 180.07 ± 2.83 days. SN has a 2.43-fold variation, ranging from 9.10 to 22.09, with an average of 17.52 ± 2.03. TSW has a 2.08-fold variation, ranging from 1.66 to 3.45 g, with an average of 2.35 ± 0.29 g. WI has a 2.36-fold variation, ranging from −44.63 to −18.9, with an average of −29.34 ± 5.59. the broad-sense heritability (H^2) was calculated for

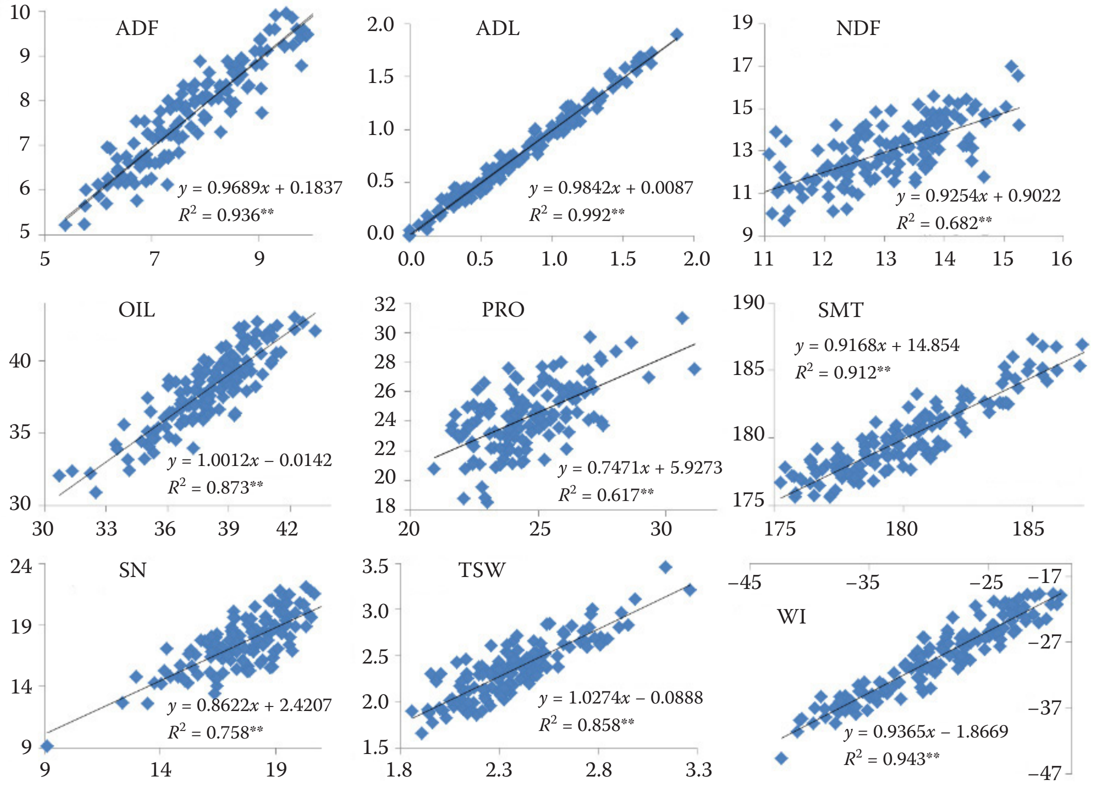

Figure 1. The correlation coefficients between 2018 and 2019 for the nine seed-related traits: acid detergent fibre (ADF); acid detergent lignin (ADL); neutral detergent fibre (NDF); oil content (OIL); protein content (PRO); seed maturity time (SMT); seed number per silique (SN); thousand-seed weight (TSW) and white index (WI)

Table 1. Descriptive statistics of the nine seed-related traits

Traits	Mean ± SD	Range	P^a	CV (%)	H^2 (%)^b
ADF (%)	7.79 ± 1.15	5.22–10.24	0.038	15.37	93.60
ADL (%)	0.76 ± 0.46	0.00–1.90	0.004	59.77	99.20
NDF (%)	13.00 ± 1.21	9.69–16.97	0.748	10.11	73.00
OIL (%)	38.05 ± 2.41	30.71–43.25	0.049	6.54	87.60
PRO (%)	24.41 ± 2.04	18.50–31.14	0.041	8.69	84.04
SMT (day)	180.07 ± 2.83	175.25–187.36	0.000	1.57	91.90
SN	17.52 ± 2.03	9.10–22.09	0.002	11.92	79.00
TSW (g)	2.35 ± 0.29	1.66–3.45	0.005	12.42	87.30
WI	−29.34 ± 5.59	−44.63–18.9	0.011	19.18	94.30

ADF – acid detergent fibre; ADL – acid detergent lignin; NDF – neutral detergent fibre; OIL – oil content; PRO – protein content; SMT – seed maturity time; SN – seed number per silique; TSW – thousand-seed weight; WI – white index; SD – standard deviation; CV – coefficient of variance; $^a P$ values of the Shapiro-Wilk test; $^b H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_E^2)$; H^2 – broad-sense heritability; σ_G – genotypic variance; σ_E – environmental variance

the nine traits (Table 1). All the traits indicated a higher H^2 than 70%, suggesting that all these traits are stably inherited. The Shapiro-Wilk normality test indicated that the NDF is normally distributed (P = 0.748), and the other eight traits did not fit a normal distribution (P < 0.05) (Figure 2; Table 1).

Principal component analysis. To characterise the *B. juncea* MAGIC population, a principal com-

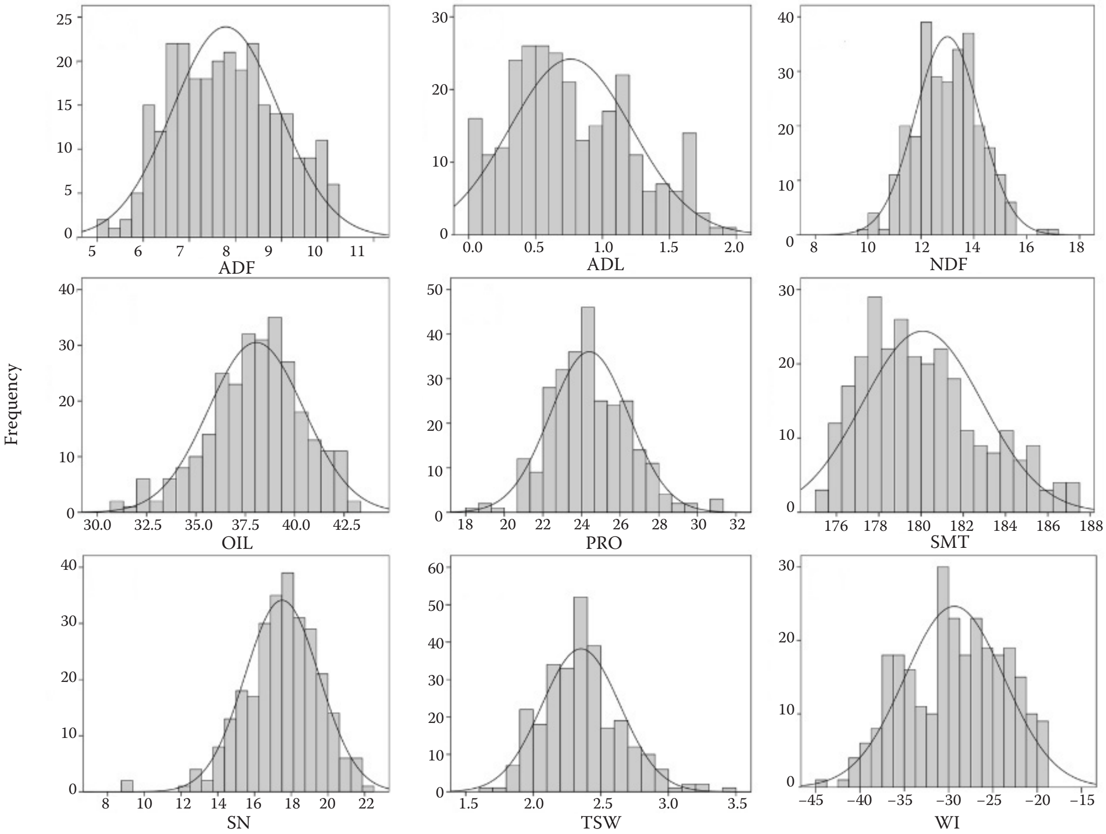

Figure 2. The frequency distribution histogram of the: acid detergent fibre content(ADF, %); acid detergent lignin content (ADL, %); neutral detergent fibre content (NDF, %); oil content (OIL, %); protein content (PRO, %); seed maturity time (SMT, days); seed number per silique (SN); thousand-seed weight (TSW, g) and white index (WI)

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Table 2. Factor loading, proportion variance, and cumulative proportion of the principal component analysis based on the nine seed-related traits of the *Brassica juncea* MAGIC population

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
ADF	0.93	0.00	0.01	–0.05	0.02	0.00	–0.20	0.21	0.21
ADL	0.89	0.20	0.02	–0.16	0.24	0.03	–0.13	0.08	–0.24
NDF	0.82	0.09	0.13	0.03	–0.32	–0.21	0.38	0.06	–0.03
OIL	0.12	–0.79	0.23	0.17	0.48	–0.08	0.20	0.01	0.04
PRO	–0.33	0.73	–0.26	–0.32	0.26	0.18	0.27	0.12	0.05
SMT	0.25	0.01	–0.59	0.74	–0.01	0.20	0.05	0.05	–0.03
SN	0.33	–0.70	–0.11	–0.38	–0.17	0.45	0.08	–0.03	–0.01
TSW	–0.12	0.33	0.81	0.33	–0.04	0.31	0.01	0.06	–0.01
WI	0.83	0.42	0.04	0.03	0.14	0.07	0.02	–0.32	0.09
EV	3.33	1.98	1.16	0.97	0.51	0.43	0.32	0.18	0.12
PV	37.05	22.05	12.87	10.77	5.65	4.74	3.58	1.99	1.30
CP	37.05	59.10	71.97	82.74	88.39	93.13	96.71	98.70	100.00

ADF – acid detergent fibre; ADL – acid detergent lignin; NDF – neutral detergent fibre; OIL – oil content; PRO – protein content; SMT – seed maturity time; SN – seed number per silique; TSW – thousand-seed weight; WI – white index; EV – Eigenvector value; PV – proportion of variance; CP – cumulative proportion; PC1–PC9 stands for the first nine values of the principal component analysis (PCA)

ponent analysis (PCA) was performed using the mean values of the nine seed-related traits, ADF, ADL, NDF, OIL, PRO, SMT, SN, TSW, and WI, as explanatory variables. Table 2 and Figure 3 show the factor loading, proportion of variance, and cumulative proportion of the PCA. ADF, ADL, NDF, and WI primarily, and almost, evenly contributed to the differences in PC1 based on the factor loading values. The contribution of OIL, PRO, SMT, SN, and TSW to PC1 was lower than that of the other traits. In addition, OIL, PRO and SN primarily contributed to the differences in PC2 because the factor loading value for PC2 was high (from –0.79 to 0.73) compared with that for the other traits (from 0.00 to 0.42). While TSW and SMT mainly contributed to the differences in PC3 and PC4, respectively. The

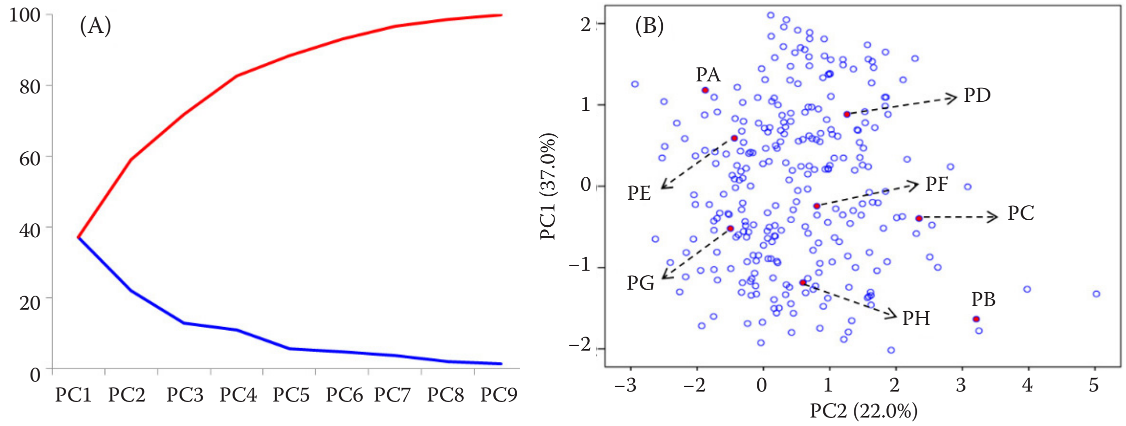

Figure 3. Line chart of the proportion of variance (blue) and cumulative proportion (red) (A) and scatter plot of the first and second principal component scores of the Principal Component Analysis (PCA) (B)

The round circles indicate the mean data from 2018 and 2019, respectively; the *X*-axis indicates the content of the different traits and the *Y*-axis indicates the distribution frequency of the different traits; the symbols with red inside indicate the data for the six founder cultivars: PA – 7H881; PB – YufengZC; PC – K100; PD – SL105; PE – SL14; PF – ShilunYC; PG – LengjiaoYC; PH – T6342

PCA results are presented in Figure 3. The scatter plot showed the first 4 principal components (PCs) before beginning to flatten (Figure 3A), with these first 4 PCs together explaining 82.8% of the total variation in the population. The first four individual PCs, 1–4, explain 37.0, 22.0, 12.8 and 10.9% of the total variation in the population, respectively, and the first two PCs explaining 59.0% of the total variation in the population were chosen for the scatter plot (Figure 3B). The lines of the *B. juncea* MAGIC population are evenly distributed and not grouped on the scatter plot, including the 8 founders.

Correlation analysis. Genetic correlations were performed among the nine seed-related traits. The correlations among most of the traits are significant (Table 3). Highly significant positive correlations were observed among the ADF, ADL and NDF contents, with the phenotypic correlation coefficients ranging from 0.690 to 0.849, suggesting a potential genetic relationship among these fibre components (Behnke et al. 2018). In addition, ADF, ADL and NDF are significantly positively correlated with SN and WI. As important quality trait in seed meal, PRO has been found to be negatively significantly correlated with ADF, NDF, OIL and SN. SMT is significantly positively correlated with ADF and negatively correlated with TSW. Furthermore, WI displayed a significantly positive correlation with ADF, ADL, NDF and SMT.

QTL identification by association mapping. To further verify the usefulness of the *B. juncea* MAGIC population for gene mapping, we conducted a GWAS for the nine seed-related traits, ADF, ADL, NDF, OIL, PRO, SMT, SN, TSW and WI. The association mapping was conducted in the sub-MAGIC population, including 170 lines, for the primary QTL identification in our earlier study (Yan et al. 2020). The TASSEL 3.0 software was used for the GWAS by a combination of the nine 2-yr seed-related traits and the genotype data generated by the 346 intron length polymorphism (ILP) polymorphic markers (Yan et al. 2020). The GWAS based on the GLM that revealed a total of twenty-two significant association signals for these seed-related traits (Table 4).

For three fibre components, ADF, ADL and NDF, seven consensus QTLs on three chromosomes were detected in two years. *qDj05.1* was simultaneously detected on J05 for the genetic control of ADF, ADL and NDF, explaining the phenotypic variation (PV) of 7.34–8.57%, 9.70–9.81% and 4.67–5.41%, respectively. *qADWj05.2* and *qADWj15.1*, corresponding to the significant marker of BnapPIP1160, were simultaneously detected for ADF on J05 and J15, explaining 5.62–6.0% of the PV, respectively, as reported in earlier studies (Fu et al. 2007; Miao et al. 2019). In addition, the QTLs, *qADWj15.1* and *qADLj15.1* on J15 and *qNDFj16.1* on J16, might be novel QTLs affecting the seed fibre components and were first discovered in this study. As stated in some earlier reports, the seed fibre components are highly correlated with the seed colour (Badani et al. 2006; Stein et al. 2013), *qDj05.1* was also detected to be responsible for the seed colour (WI) in this study. The QTLs of the seed fibre components and seed colour were colocalised on A05 in some earlier publications (Fu et al. 2007; Miao et al. 2019).

For OIL, one consensus QTL, *qOILj08.1*, was detected on J08, explaining 9.19–9.60% of the PV. For PRO, three specific QTLs, *qPROj01.1* on J01, *qPROj05.1*

Table 3. Pearson's correlation coefficients among the six yield-related traits

Traits	ADF	ADL	NDF	OIL	PRO	SMT	SN	TSW
ADL	0.849**[a]							
NDF	0.781**	0.690**						
OIL	0.088	0.005	–0.003					
PRO	–0.351**	–0.085	–0.314**	–0.650**				
SMT	0.185*	0.102	0.150	0.000	–0.123			
SN	0.331**	0.184*	0.203*	0.434**	–0.514**	–0.061		
TSW	–0.115	–0.075	0.022	–0.075	0.025	–0.200*	–0.379**	
WI	0.746**	0.817**	0.731**	–0.165	0.047	0.216*	–0.027	0.079

ADF – acid detergent fibre; ADL – acid detergent lignin; NDF – neutral detergent fibre; OIL – oil content; PRO – protein content; SMT – seed maturity time; SN – seed number per silique; TSW – thousand-seed weight; WI – white index; [a]the values of the phenotypic correlation coefficients based on the means across 2 years; **indicates an extremely significant correlation between two traits at $P < 0.01$; *indicates a significant correlation between two traits at $P < 0.05$

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Table 4. QTLs from the two-year data identified by the association mapping in the multiparent advanced generation intercross (MAGIC) population

Traits	QTL	Marker	Chr	Pos	$-\log_{10}(P)$	R^2 (%)	Environment
ADF	*qDj05*.1	BnapPIP1544	J05	2 111 268 bp	4.63–5.39	7.34–8.57	2018, 2019
	qADWj05.2	BnapPIP1160	J05	33 314 212 bp	3.29–3.55	5.62–6.07	2018, 2019
	qADWj15.1		J15	2 060 487 bp			
ADL	*qDj05.1*	BnapPIP1544	J05	2 111 268 bp	4.30–4.36	9.70–9.81	2018, 2019
	qADLj15.1	BnapPIP457	J15	45 398 892 bp	3.00–3.02	6.62–6.63	2018, 2019
NDF	*qDj05.1*	BnapPIP1544	J05	2 111 268 bp	3.05–3.42	4.67–5.41	2018, 2019
	qNDFj16.1	BrapPIP239	J16	5 971 093 bp	3.16–3.17	5.06–5.14	2018, 2019
OIL	*qOILj08.1*	BrapPIP12	J08	24 592 530 bp	3.55–3.67	9.19–9.60	2018, 2019
	qPROj01.1	At3g01060	J01	118.7 cM	3.36	6.30	2019
PRO	*qPROj05.1*	At1g36380	J05	36.4 cM	3.49	6.97	2019
	qPROj13.1	At2g34860	J13	62.9 cM	3.01	5.42	2019
SMT	*qSWj02.1*	At1g72890	J02	45.7 cM	3.34–3.64	8.77–9.73	2018, 2019
	qSMTj03.1	At4g18400	J03	110.3 cM	2.85–3.05	6.76–7.39	2018, 2019
	qSMTj14.1	At2g01940	J14	19.4 cM	3.08–3.48	7.82–9.04	2018, 2019
	qSMTj15.1	At4g24680	J15	69.3 cM	3.38–3.76	8.23–9.38	2018, 2019
SN	*qSNj02.1*	At5g27740	J02	75.3 cM	2.13–2.51	3.85–4.75	2018, 2019
TSW	*qTSWj09.1*	BnapPIP831	J09	51 009 053 bp	3.79–3.81	10.16–10.22	2018, 2019
WI	*qSWj02.1*	At1g72890	J02	45.7 cM	4.70–4.78	9.77–10.00	2018, 2019
	qWIj05.1	At1g36980	J05	36.4 cM	3.04–3.13	6.28–6.48	2018, 2019
	qADWj05.2	BnapPIP1160	J05	33 314 212 bp	3.34–3.58	7.19–7.75	2018, 2019
	qADWj15.1		J15	2 060 487 bp			
	qWIj15.1	BnapPIP80	J15	5 618 110 bp	3.77–3.87	7.42–7.65	2018, 2019

ADF – acid detergent fibre; ADL – acid detergent lignin; NDF – neutral detergent fibre; OIL – oil content; PRO – protein content; SMT – seed maturity time; SN – seed number per silique; TSW – thousand-seed weight; WI – white index; Chr – number of chromosomes; Pos – the position from the genetic map (cM) (Panjabi et al. 2008) or the physical position by blasting the DNA sequence of the ILP markers against the *B. juncea* genome (bp) (Yang et al. 2016); R^2 – percentage of the phenotypic variation explained by the QTL

on J05 and *qPROj13.1* on J13, explaining 6.30, 6.97 and 5.42% of the PV, were detected in 2019. For SN and TSW, two QTLs, *qSNj02.1* and *qTSWj09.1*, were detected on J02 and J09 and explained 3.85–4.75% and 10.16–10.22% of the PV, respectively. The QTLs on A02 (Ding et al. 2012; Shi et al. 2015) and A09 (Yadava et al. 2012; Bagheri et al. 2013) were also detected in some earlier studies. In A09 of *B. napus*, a known gene, *BnaA.ARF18.a*, controlling the seed weight, has been identified in the natural population by developing derived cleaved amplified polymorphic sequence (dCAPS) markers (Dong et al. 2018). In *A. thaliana*, *EOD3*/*CYP78A6* was found to be responsible for the maternal control of the seed size (Fang et al. 2012). In addition, a CACTA-like transposable element in the upstream region of *BnaA9. CYP78A9* acts as an enhancer to increase the seed weight in rapeseed (Shi et al. 2019). For SN, *BnaC9. SMG7b* functions as a positive regulator of the number of seeds per silique in *B. napus* by regulating the formation of functional female gametophytes (Li et al. 2015). All these results should be helpful in accelerating the genetic mechanism research of SN and TSW in *B. juncea*.

For SMT and WI, *qSWj02.1* was simultaneously detected on J02, explaining 8.77–9.73 and 9.77 to 10.00% of the PV, respectively; three specific QTLs, *qSMTj03.1*, *qSMTj14.1* and *qSMTj15.1*, for SMT and three specific QTLs, *qWIj05.1*, *qADWj05.2* and *qWIj15.1*, for WI were detected. Interestingly, *qADWj05.2* and *qADWj15.1* were simultaneously detected for ADF and WI. Moreover, the consensus QTLs are mapped on the corresponding physical maps (Figure 4), and the primer sequences are listed in Table 5.

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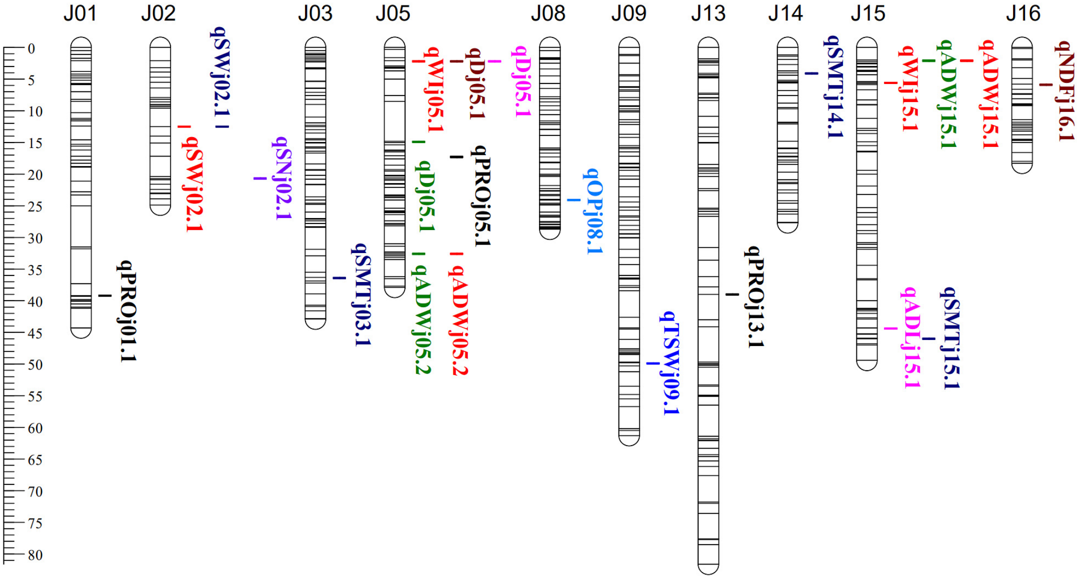

Figure 4. The consensus QTLs are mapped on the corresponding physical maps; the physical maps were obtained by blasting the DNA sequence generating the ILP primers prefixed with "BnapPIP" and "BrapPIP" against the *Brassica juncea* genome (Brassica_juncea_v1.5) using a BLAST-like alignment tool

Table 5. Primer sequence of the intron length polymorphism (ILP) primers identified by the association mapping in the present study

Marker name	Primer sequence (5'->3')		Species (reference)
	Forward	Reverse	
At1g36980	TTGATGTTCAATTG-CGCAGAA	CGACATACGCTATGAAAAGCCA	*A. thaliana* (Panjabi et al. 2008)
At1g72890	AGAGTGGCCGTCCG-ATTCC	CGACCAGCTTCGAGT-CATC(T/A)TC	*A. thaliana* (Panjabi et al. 2008)
At2g01940	CATTCTTGTGACTG-TGGTCG	TGATGTTCAATAAAAC-TCTCCACC	*A. thalian* (Panjabi et al. 2008)
At4g18400	AAACGTCTACTCCT-CCGGCTACA	TCTAACCTCGTCCT-TTCTCTTTTGG	*A. thaliana* (Panjabi et al. 2008)
At4g24680	TAGCACGACAGGA-GATCGAAG	GCATTCAGAGACGATT-TACTGCC	*A. thaliana* (Panjabi et al. 2008)
At5g27740	CTTGCCGTCTCATC-TTATGCTG	GCAACGAACTCCAAAC-TTTCAC	*A. thaliana* (Panjabi et al. 2008)
BnapPIP80	AACTTGACCCGGA-CGATGT	TCTGTTCAACCACAGCAGGA	*B. napus* (Yang et al. 2007)
BnapPIP457	AGTTCGAGTCCTCG-CCAAAT	TCCACTGTCTCCGTGACATC	*B. napus* (Yang et al. 2007)
BnapPIP831	CCAGTTTGGGAAT-CAAGAACC	TGGAGAAGTTCCACTTGATGC	*B. napus* (Yang et al. 2007)
BnapPIP1160	CTCACGGTGGTTC-ATGTTCA	ATTTGAAGTGCACGCAAGAC	*B. napus* (Yang et al. 2007)
BnapPIP1544	GCAAGTTCTGCCT-GAAACCT	CCAAGGAATCTCATCCCTCA	*B. napus* (Yang et al. 2007)
BrapPIP12	AAACTCCTAATGAT-TCTCGCCTTA	TGAGGGAAACTCGATGCTG	*B. rapa* (Yang et al. 2007)
BrapPIP239	AGAAGTTGCAGCTC-GTGGAG	GAGTTGCTTTGCAGCCTCTT	*B. rapa* (Yang et al. 2007)

CONCLUSION

A MAGIC population is well suited for genetically analysing the effects of multiple QTLs on the complex traits of interest. In this study, we reported the continuous utilisation of the *B. juncea* MAGIC population for the association mapping of nine seed-related traits, ADF, ADL, NDF, OIL, PRO, SMT, SN, TSW and WI. The nine seed traits of the MAGIC lines displayed great diversity. A total of twenty-two QTLs were identified, including one QTL for each OIL, SN and TSW; two QTLs for each ADL and NDF; three QTLs for each ADF and PRO; four QTLs for SMT; and five QTLs for WI. These results should be helpful for further fine mapping, gene cloning, plant breeding and marker-assisted selection (MAS) in *B. juncea*.

REFERENCES

Badani A.G., Snowdon R.J., Wittkop B., Lipsa F.D., Baetzel R., Horn R., De Haro A., Font R., Lühs W., Friedt W. (2006): Colocalization of a partially dominant gene for yellow seed colour with a major QTL influencing acid detergent fibre (ADF) content in different crosses of oilseed rape (*Brassica napus*). Genome, 49: 1499–1509.

Bagheri H., Pino del Carpio D., Hanhart C.J., Bonnema A.B., Keurentjes J.J.B., Aarts M.G.M. (2013): Identification of seed-related QTL in *Brassica rapa*. Spanish Journal of Agricultural Research, 11: 1085–1093

Bandillo N., Raghavan C., Muyco P.A., Sevilla M.A.L., Lobina I.T., Dilla-Ermita C.J., Tung C-W., McCouch S., Thomson M., Mauleon R., Singh R.K., Gregorio G., Redoña E., Leung H. (2013): Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice, 6: 11.

Behnke N., Suprianto E., Möllers C. (2018): A major QTL on chromosome C05 significantly reduces acid detergent lignin (ADL) content and increases seed oil and protein content in oilseed rape (*Brassica napus* L.). Theoretical and Applied Genetics, 131: 2477–2492.

Bradbury P.J., Zhang Z., Kroon D.E., Casstevens T.M., Ramdoss Y., Buckler E.S. (2007): TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics, 23: 2633–2635.

Burton A., Salisbury P., Potts D. (2003): The potential of canola quality *Brassica juncea* as an oilseed crop for Australia. In: 13th Australian Research Assembly on Brassicas – Conf. Proc., Tamworth, Sept 8–12, 2003: 64–66.

Cavanagh C.R., Chao S., Wang S., et al. (2013): Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences of the United States of America, 110: 8057–8062.

Dell'Acqua M., Gatti D.M., Pea G., Cattonaro F., Coppens F., Magris G., Hlaing A.L., Aung H.H., Nelissen H., Baute J., Frascaroli E., Churchill G.A., Inzé D., Morgante M., Pè M.E. (2015): Genetic properties of the MAGIC maize population: a new platform for high definition QTL mapping in *Zea mays*. Genome Biology, 16: 167.

Delourme R., Foisset N., Horvais R., Barret P., Champagne G., Cheung W.Y., Landry B.S., Renard M. (1998): Characterisation of the radish introgression carrying the *Rfo* restorer gene for the Ogu-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). Theoretical and Applied Genetics, 97: 129–134.

Ding G., Zhao Z., Liao Y., Hu Y., Shi L., Long Y., Xu F. (2012): Quantitative trait loci for seed yield and yield-related traits, and their responses to reduced phosphorus supply in *Brassica napus*. Annals of Botany, 109: 747–759.

Dong H., Tan C., Li Y., He Y., Wei S., Cui Y., Chen Y., Wei D., Fu Y., He Y., Wan H., Liu Z., Xiong Q., Lu K., Li J., Qian W. (2018): Genome-wide association study reveals both overlapping and independent genetic loci to control seed weight and silique length in *Brassica napus*. Frontiers in Plant Science, 9: 921.

Fang W., Wang Z., Cui R., Li J., Li Y. (2012): Maternal control of seed size by EOD3/CYP78A6 in *Arabidopsis thaliana*. The Plant Journal, 70: 929–939.

Fu F.Y., Liu L.Z., Chai Y.R., Chen L., Yang T., Jin M.Y., Ma A.F., Yan X.Y., Zhang Z.S., Li J.N. (2007): Localization of QTLs for seed color using recombinant inbred lines of *Brassica napus* in different environments. Genome, 50: 840–854.

Huang B.E., Verbyla K.L., Verbyla A.P., Raghavan C., Singh V.K., Gaur P., Leung H., Varshney R.K., Cavanagh C.R. (2015): MAGIC populations in crops: current status and future prospects. Theoretical and Applied Genetics, 128: 999–1017.

Huynh B.L., Ehlers J.D., Huang B.E., et al. (2018): A multi-parent advanced generation inter-cross (MAGIC) population for genetic analysis and improvement of cowpea (*Vigna unguiculata* L. Walp.). The Plant Journal, 93: 1129–1142.

Kent W.J. (2002): BLAT−the BLAST-like alignment tool. Genome Research, 12: 656–664.

Kover P.X., Valdar W., Trakalo J., Scarcelli N., Ehrenreich I.M., Purugganan M.D., Durrant C., Mott R. (2009): A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. PLoS Genetics, 5: e1000551.

Li S., Chen L., Zhang L., Li X., Liu Y., Wu Z., Dong F., Wan L., Liu K., Hong D., Yang G. (2015): BnaC9.SMG7b functions as a positive regulator of the number of seeds per silique in *Brassica napus* by regulating the formation of functional female gametophytes. Plant Physiology, 169: 2744–2760.

Liu J., Osbourn A., Ma P. (2015): MYB transcription factors as regulators of phenylpropanoid metabolism in plants. Molecular Plant, 8: 689–708.

https://doi.org/10.17221/73/2020-CJGPB

Love H.K., Rakow G., Ranney J.P., Downey R.K. (1991): Breeding improvements towarda canola quality *Brassica juncea*. In: Proc. 8th Int. Rapeseed Congr., Saskatoon, July 9–11, 1991: 164–169.

Miao L., Chao H., Chen L., Wang H., Zhao W., Li B., Zhang L., Li H., Wang B., Li M. (2019): Stable and novel QTL identification and new insights into the genetic networks affecting seed fiber traits in *Brassica napus*. Theoretical and Applied Genetics, 132: 1761–1775.

Ongom P.O., Ejeta G. (2018): Mating design and genetic structure of a multi-parent advanced generation intercross (MAGIC) population of sorghum (*Sorghum bicolor* (L.) Moench). G3: Genes|Genomes|Genetics, 8: 331–341.

Østergaard L., Kempin S., Bies D., Klee H., Yanofsky M. (2006): Pod shatter-resistant Brassica fruit produced by ectopic expression of the *FRUITFULL* gene. Plant Biotechnology Journal, 4: 45–51.

Panjabi P., Jagannath A., Bisht N., Padmaja K., Sharma S., Gupta V., Pradhan A., Pental D. (2008): Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using intron polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C *Brassica* genomes. BMC Genomics, 9: 1–19.

Pascual L., Desplat N., Huang B.E., Desgroux A., Bruguier L., Bouchet J.P., Le Q.H., Chauchard B., Verschave P., Causse M. (2015): Potential of a tomato MAGIC population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. Plant Biotechnology Journal, 13: 565–577.

Roy N.N. (1984): Interspecific transfer of *Brassica juncea*-type high blackleg resistance to *Brassica napus*. Euphytica, 33: 295–303.

Sallam A., Martsch R. (2015): Association mapping for frost tolerance using multi-parent advanced generation intercross (MAGIC) population in faba bean (*Vicia faba* L.). Genetica, 143: 501–514.

Sannemann W., Huang B.E., Mathew B., Léon J. (2015): Multi-parent advanced generation inter-cross in barley: high-resolution quantitative trait locus mapping for flowering time as a proof of concept. Molecular Breeding, 35: 86.

Shi J., Zhan J., Yang Y., Ye J., Huang S., Li R., Wang X., Liu G., Wang H. (2015): Linkage and regional association analysis reveal two new tightly-linked major-QTLs for pod number and seed number per pod in rapeseed (*Brassica napus* L.). Scientific Reports, 5: 14481.

Shi L., Song J., Guo C., Wang B., Guan Z., Yang P., Chen X., Zhang Q., King GJ., Wang J., Liu K. (2019): A CACTA-like transposable element in the upstream region of *BnaA9.CYP78A9* acts as an enhancer to increase silique length and seed weight in rapeseed. The Plant Journal, 98: 524–539.

Stein A., Wittkop B., Liu L., Obermeier C., Friedt W., Snowdon R.J. (2013): Dissection of a major QTL for seed colour and fibre content in *Brassica napus* reveals colocalization with candidate genes for phenylpropanoid biosynthesis and flavonoid deposition. Plant Breeding, 132: 382–389.

Tian E., Roslinsky V., Cheng B. (2014): Molecular marker-assisted breeding for improved Ogura cms restorer line (*RfoRfo*) and mapping of the restorer gene (*Rfo*) in *Brassica juncea*. Molecular Breeding, 34: 1361–1371.

Tian E., Li L., Jia S., Lin S. (2016): The variation of fatty acids of *Brassica juncea* germplasm, correlation analysis and design of specific primer of *FAE1* gene for the control of erucic acid. Guihaia, 36: 1445–1452.

Wada T., Oku K., Nagano S., Isobe S., Suzuki H., Mori M., Takata K., Hirata C., Shimomura K., Tsubone M., Katayama T., Hirashima K., Uchimura Y., Ikegami H., Sueyoshi T., Obu K.-I., Hayashida T., Shibato Y. (2017): Development and characterization of a strawberry MAGIC population derived from crosses with six strawberry cultivars. Breeding Science, 67: 370–381.

Wijesundera C., Ceccato C., Fagan P., Shen Z., Burton W., Salisbury P. (2008): Canola quality Indian mustard oil (*Brassica juncea*) is more stable to oxidation than conventional canola oil (*Brassica napus*). Journal of the American Oil Chemists' Society, 85: 693–699.

Yadava S.K., Arumugam N., Mukhopadhyay A., Sodhi Y.S., Gupta V., Pental D., Pradhan A.K. (2012): QTL mapping of yield-associated traits in *Brassica juncea*: meta-analysis and epistatic interactions using two different crosses between east European and Indian gene pool lines. Theoretical and Applied Genetics, 125: 1553–1564.

Yan W., Zhao H., Yu K., Wang T., Khattak A.N., Tian E. (2020): Development of a multiparent advanced generation intercross (MAGIC) population for genetic exploitation of complex traits in *Brassica juncea*: Glucosinolate content as an example. Plant Breeding, 139: 779–789.

Yang J., Liu D., Wang X., Ji C., Cheng F., Li B., et al. (2016): The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. Nature Genetics, 48: 1225–1232.

Yang L., Jin G., Zhao X., Zheng Y., Xu Z., Wu W. (2007): PIP: a database of potential intron polymorphism markers. Bioinformatics, 23: 2174–2177.

Yao F., Zhang X., Ye X., Li J., Long L., Yu C., Li J., Wang Y., Wu Y., Wang J., Jiang Q., Li W., Ma J., Wei Y., Zheng Y., Chen G. (2019): Characterization of molecular diversity and genome-wide association study of stripe rust resistance at the adult plant stage in Northern Chinese wheat landraces. BMC Genetics, 20: 38.

Yin X., Stam P., Kropff M.J., Schapendonk A.H.C.M. (2003): Crop modeling, QTL mapping, and their complementary role in plant breeding. Agronomy Journal, 95: 90–98.

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