QTL mapping and epistasis analysis for plant height and height to the first branch in rapeseed (*Brassica napus* L.)

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Abstract

Since plant height and height to 1-st branch are complicated quantitative traits. QTL analysis can help us to understand the basis of heterosis and improve the selecting efficiency. A genetic linkage map consisting of 244 molecular markers was constructed based on F_2 population derived from a cross between double low CMS maintainer 1141B and double high restorer KenC_1. The markers in the linkage map distributed on all the 20 main linkage groups and 1 triplet and covered 2769.5 cM of the rapeseed genome. The statistic software of Windows QTL Cartographer Version2.0 and Composite Internal Mapping (CIM) were applied to detect QTLs for plant height and height to 1-st branch. A total of 7 QTLs located in 5 different linkage groups were identified for these two traits. The QTLs explained the phenotypic variation from 8.54% to 54.59% individually , and all of them showed that 1141B decreased the plant height and the height to 1-st branch. Furthermore, fifteen two-locus combinations showed significant epistatic effects, which separated in the entire genome for both traits. The epistatic interactions had pleiotropic effects between two traits. Interactions were detected between QTL and non-QTL (non-significant effect loci) and between two non-QTLs. The later was the majority. AA was the most common type of interactions in plant height. DD and DA were the most common type of interactions for height to 1-st branch. This indicated that the genes controlling plant height and height to 1-st branch were complicated. Epistasis interactions might play an important role. Some questions were also discussed.

Key words : *Brassica napus* L. ; Plant height ;Height to 1-st branch ;Genetic linkage map ;Molecular marker; QTL maping ; Epistatic effect

Introduction

The heritabilities in plant height and height to 1-st branch (Distance from ground to the emergence of first branch) are high (Lou, 2002; Huang et al., 2004), while both of them are complicated quantitative traits which have closely related with seed yield and other important traits in *Brassica napus* L (Hu et al., 1997;Wang et al., 2004; Wang et al., 2004; Zhou et al., 2004). Since plant height and height to 1-st branch are quantitative traits. They are dominated by multiple genes and influenced by environments. QTL mapping for agronomically important traits were carried out by molecular markers, and applied in genetic improvement for many crops (Paterson et al., 1988). At present, QTL mapping studies mainly focus on development (Pilet et al., 1988), resistance(Pilet, 2001; Howell et al., 2003) and quality traits (Mahmood, 2003; Sabharwal et al., 2004) etc. in rapeseed. QTL analysis for plant height was performed using mixed model approach (Zhao, 2005). In the present report, the linkage map was constructed with 3 kinds of molecular markers of SRAP(sequence relatived amplified polymorphism), AFLP (amplified fragment length polymorphism)& SSR(simple sequence repeat polymorphisms) by F_2 populations of 1141B×Ken C_1 in the paper. The purpose of the study is to dissect the genetic components involved in plant height and height to 1-st branch.

Material and Methods

Development of mapping population *B.napus* lines 1141B and Ken C_1-1 with large difference (Ma et al.,2003)were used as parents, in which 1141B was double low maintainer line of hybrid Huaza No.4, which was bred by Huazhong Agricultural University. Ken C_1 was a restorer line of Qinyou No.2, which was bred by Rapeseed Hybrid Research Center of Shanaxi. Materials were provided by Rapeseed Laboratory of Huazhong Agricultural University. 136 F_2 plants derived from F_1 self-pollinating, and $F_{2:3}$ family populations were obtained. Linkage map was constructed by F_2 populations with three types of molecular markers: SRAP, AFLP and SSR.

Molecular markers and laboratory assay Three types of markers were employed to assay DNA polymorphisms: SRAP,AFLP and SSR. Taq DNA polymerase, MseI, PstI, EcoRI and T4 DNA, dNTPs, adapters and the primers were made by Sangon company in Shanghai.

SRAP markers and laboratory assay SRAP(sequence relatived amplified polymorphism) analysis followed the methods suggested by Li and Quiros et.al.(2001). Polymorphism was detected by 170 primer pairs using two parents 1141B and KenC_1. The polymorphic marker loci were tested in F_2 individuals by 24 primer pairs. The primers were divided into 2 types, positive and negative. They are represented by A1～Q10.

AFLP assay AFLP (amplified fragment length polymorphism) analysis followed the methods by Lu (2003). The

polymorphic markers detected between the parents were used to assay the entire F_2 population based on 158 pairs of PstI/MseI and 256 pairs of EcoRI/MseI. The polymorphic marker loci were tested in F_2 individuals by 33 AFLP primer pairs

SSR **assay** SSR(simple sequence repeat polymorphisms) analysis followed the methods by Lu(2003).The SSR primer pair sequences were obtained at http://ukcrop.net/perl/ace/search/Brassica DB. They have different genomic sources (*B.rapa* AA 2n=20, *B.napus* AACC 2n=38 and *B.oleracea* CC 2n=18, respectively, prefixed by "Ra", "Na" and "O". Prefixed by "P" were provided by Dr. Tu Jinxing from Huazhong Agricultural University.

Field trial and data analysis The field trial was carried out in the experimental field of Henan Academy of Agricultural Sciences (Zhengzhou, Henan). The field trial was in random block design with 3 replications.

QTL mapping and epistasis analysis QTLs (quantitative trait loci) were detected using Windows QTL Cartographer Version2.0(North Carolina State University,WANG Sheng Chu,BASTEN C.J.,ZENG Z.D.2003). Gene action was considered by D/A (dominance effect / additive effect) ratio as additive (A) if D/A ratio ranged from 0 to 0.20; partial-dominance (PD) if D/A ratio ranged from 0.21 to 0.80; dominance (D) if D/A ratio ranged from 0.81 to 1.20; and over-dominance (OD) if D/A ratio was larger than 1.20 (Yue et al., 2001). The digenic interaction between 2 loci was searched by co-dominant loci for each trait with two-way analysis of variance. The interaction can be further parti-tioned into four terms each specified by a single degree of freedom: additive (A locus)×additive(B locus) (AA), additive×dominance (AD), dominance×additive (DA), and dominance×dominance (DD). Statistical significance for each term was assessed using an orthogonal contrast test with the statistical package StatSoft Inc.(1991).

Results

Constrction of linkage map

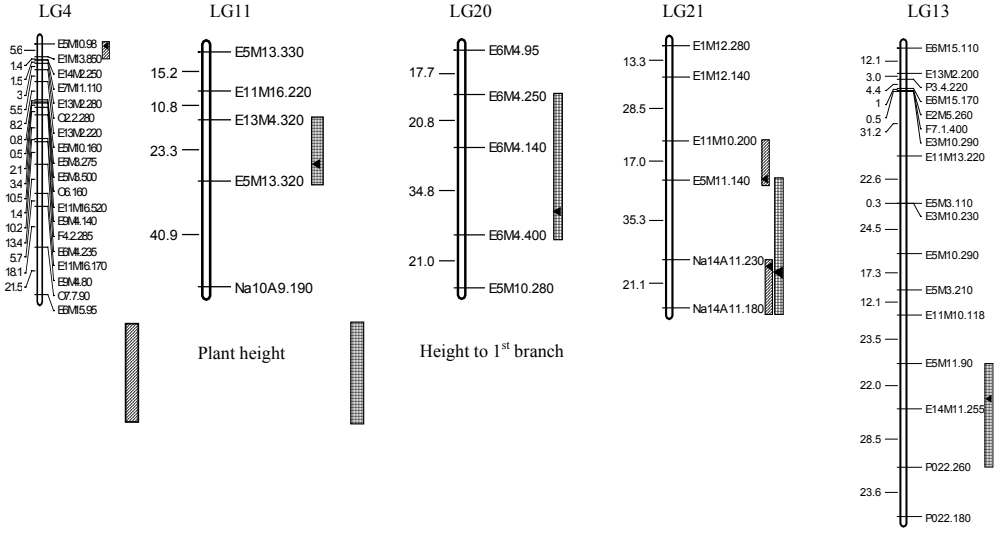
A genetic linkage map consisting of 244 DNA markers was constructed based on F_2 population derived from a cross between double low CMS maintainer 1141B and double high restorer $KenC_1$. The markers in the linkage map distributed on all the 20 main linkage groups and 1 triplet and covered 2769.5 cM of the rapeseed genome in length with average interval of 11.35 cM.

QTL analysis for plant height and height to 1-st branch

3 QTLs were detected for plant height by Composite Internal Mapping (Table1). They were located on linkage group 21and 4, respectively. Each QTL explained 28.60%, 23.85% and 11.11% of the phenotypic variation, respectively with an average of 21.19%. Table1 indicated that alleles from female parent 1141B decreased plant height. Genetic effects of the three main QTLs showed partial-dominant effects.

Table 1 Putative QTLs detected by CIM for plant height and height to the first branch

Traits	LG	QTL	flanking	Marker	Max LOD	Additive effect	Dominant effect	D/A ratio	Variation %
Plant height	21	ph21a	E11M10.200	E5M11.140	7.57	-6.92	-5.93	0. 86	23.85
	21	ph21b	Na14A11.230	Na14A11.180	7.73	-4.47	4.86	−1. 09	28.60
	4	ph4a	E5M10.98	E1M13.850	2.55	-4.36	4.22	−0. 97	11.11
Height to 1-st branch	21	bh21	E5M11.140	Na14A11.180	6.95	-6.66	8.95	−1. 34	20.68
	11	bh11	E13M4.320	E05M13.320	2.93	-4.32	5.04	−1. 17	8.54
	20	bh20	E6M4.250	E6M4.400	2.76	-11.63	-1.24	0. 11	54.59
	13	bh13	E05M11.90	P022.260	2.10	-4.51	7.30	−1. 62	10.22



4 QTLs were detected for height to 1-st branch (Table1). They were located on linkage group 21, 11, 20 and 13, respectively. These QTLs accounted for 8.54%-54.59% phenotypic variation individually with an average of 23.51%. Table 1 showed that alleles from female parent 1141B decreased the effects of height to 1-st branch, while QTLs of bh21 and bh13 showed opposite direction over-dominant effects, bh11 showed dominant effect,and QTL of bh20 showed partial-dominant effect.

Epistasis analysis of plant height and height to 1-st branch

Eight significant two-locus combinations controlling plant height were detected by two-way ANOVAs (table 2). The phenotypic variation of one -locus was 0.04%-10.65% with an average of 2.10%. The phenotypic variation of two-locus interaction was 7.90%-11.82%, with an average of 9.38%. AA was the most dominant type of interactions in plant height.

Seven significant two-locus combinations controlling height to 1-st branch were also detected (table 2). The phenotypic variation of one-locus was 0. 1%-8.21% and the average was 2.89%. The phenotypic variation of two-locus interaction was 7.18%-12.90% and the average value was 9.74%. DD and DA were the most significant type of interactions in height of 1-st branch.

Table 2 Digenic interactions significantly detected for plant height and height to the first branch

					Variation explained %			P	Interaction
Traits	Locus A	LG	Locus B	LG	A	B	A×B		Types
PH	Na14A11.180	21	E3M11.250	16	8.83	0.35	11.82	0.0083	DD
	Na14A11.180	21	O6.160	4	3.01	1.53	10.78	0.0088	AA
	E3M11.250	16	B5.140		2.17	0.08	11.45	0.0089	AD
	E1M13.315	12	Na10H3.195	1	1.76	0.04	9.56	0.0237	DD
	Na14A11.230	21	Na12E9.320	6	2.05	0.35	7.9	0.0254	AA
	Na14A11.230	21	O6.160	4	10.65	0.05	8.22	0.0293	AA
	Na10F3.220	3	M4.1.180	7	0.96	0.06	7.98	0.0304	AA
	B5. 140		E1M13.315	12	1. 20	0. 55	7. 29	0. 0313	AA
BH	Na14A11.230	21	Na14A11.180	21	4.97	2.86	9.58	0.0069	DD
	Ra2D4.190	10	E14M2.250	4	0.8	2.57	12.9	0.0101	DA
	E1M13.315	12	Na10H3.195	1	3.98	0.1	10.27	0.0131	DD
	Na14A11.180	21	Ra2D4.190	10	8.21	0.65	10.27	0.0159	DA
	Na14A11.180	21	O6.160	4	6.29	1.04	9.43	0.0221	DA
	Na10F3.220	3	E5M3.265	17	2.13	3.15	8.58	0.0291	DD
	Na14A11.230	21	O6.160	4	3.35	0.29	7.18	0.0408	AA

Discussions

QTL mapping and epistatic effects 3 QTLs were detected for plant height by Composite Internal Mapping. Gene effects of the three main QTLs showed partial-dominant effects. It demonstrated that alleles from female parent 1141B decreased plant height. Gene effects of the three main QTLs showed partial-dominant effects. Two main effects QTLs located on linkage group 21. Each QTL explained 28.60% and 23.85% of the phenotypic variation, respectively with an average of 26.23%.The result indicated that linkage group 21 was very important for plant height. It consistent with the result of Zhao et al.(2005) that main effects QTLs located on linkage group 16 or 19.Maybe the reason is that there were different DNA markers and differen linkage group between Zhao et al.(2005) and the paper. 4 QTLs were detected for height to 1-st branch. It showed that alleles from female parent 1141B decreased the effects of height to 1-st branch. Gene effects of the three main QTLs were complicated. QTLs of bh21 and bh13 showed opposite direction over-dominant effects, bh11 showed dominant effect,and QTL of bh20 showed partial-dominant effect. It indicated that over-dominance and dominance effects might play an important role in height to 1-st branch. The QTL effect of bh20 was very high,it was explained 54.59% of the phenotypic variation. It might be used as marker-assisted selection in breeding.

Digenic interactions were detected for plant height and height to the first branch. It showed that there were very large numbers of two-locus combinations controlling plant height and height of 1-st branch, in which 15 significant two-locus combinations were detected. The results indicated that the epistasis interactive effects for two-locus combinations detected was greater than that of one-locus. It also indicated that all the epistatic interactions were detected between two background markers without identified QTL. AA was the most significant type of interactions in plant height. The results agreed with the previous reported by Zhao et al.(2005), which indicated that plant height was controlled by many QTLs. Additive effects were predominant, totally explained 75% of the phenotypic variation and often combined with digenic epistasis.

Molecular markers and density of linkage map RFLP, RAPD, AFLP, SSR, ISSR are frequently used for map

construction in many crops, The new kind of marker SRAP is advantaged with its low cost, stable marker pattern and high in polymorphism (Li et al. 2001). On the other hand, we found that the ratio of distorded segregation is quite low, it is only 1.33% in this research. The experimental process is simple and the price is much low than AFLP. However, AFLP has the highest efficiency for examing polymorphism and can detect many micro-differences in level of DNA. The results indicated that AFLP is the highest precision marker and the polymorphism is the richest marker after comparing the markers among 774 AFLP, 262 RAPD, 185 RFLP and 68 SSR (Garcia A F, et al 2004). Several large gaps were still found within linkage groups although AFLP markers showed powerful to detect the polymorphisms.. SRAP marker can fill up these gaps and make marker more evenly distributed on the map. So, each of 3 kinds of markers (SRAP, SSR and AFLP) had their special advantages and can complimentarily.

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