Mapping of QTLs Detected in a Broccoli Double Diploid Population for Plant Height and Leaf Characteristics

Jingjing Huang

Institution of Vegetables and Flowers, Chinese Academy of Agricultural Sciences <https://orcid.org/0000-0001-9420-1345>

Jifeng Sun

Weifang Academy of Agricultural Sciences

Eryan Liu

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Yumei Liu

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Zhiyuan Fang

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Limei Yang

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Mu Zhuang

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Yangyong Zhang

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Honghao Lv

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Yong Wang

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Fengqing Han

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Jialei Ji

Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers

Zhansheng Li (✉ lizhansheng@caas.cn)

<https://orcid.org/0000-0003-0132-2741>

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Abstract

Background The planting density of broccoli can directly affect the yield and overall health of plants. So there is necessary to reveal the regulatory genes of planting density in broccoli by QTL mapping. In this study, the important planting density-dependent factors of broccoli, plant height (PH), maximum outer petiole length (PL) and leaf width (LW), were investigated during 2017 and 2018. The mapping of QTLs for PH, PL and LW were performed, and the interaction between QTLs and the environment was also analyzed by a DH population constructed with 176 genotypes derived from F₁ obtained by crossing the broccoli inbred lines 86101 (P₁) and 90196 (P₂).

Results A linkage group including a total of 438 SSR markers were constructed covering a length of 1168.26 cM using QTL IciMapping 4.0 software. Finally, there were mainly four QTLs (*phc1*, *phc2*, *phc4-1*, *phc4-2*), one QTL (*plc6*), and two QTLs (*lwc1*, *lwc3*) corresponding for PH, PL and LW recurred during the two years. In three environments, inclusive composite interval mapping (ICIM) analysis showed that there was a major QTL for PH at 7.20 cM on chromosome 1 between molecular markers 8C024 and sf4482 with a high explanatory contribution rate of 20.05%. The QTL at the 11.10 cM position of chromosome 6 was located for the PL with a high explanatory contribution rate of 20.02% between the molecular markers sc2170 and sf43960. The QTL at the 147.00 cM position of chromosome 3 was located on LW with a high explanatory contribution rate of 19.97% between molecular markers of Sc52751 and RA2-E12.

Conclusions According to the QTL results of planting density in broccoli by a DH population, the possible positions of candidate genes were screened to provide a basis for further locating and cloning genes for plant height, maximum outer petiole and leaf width.

Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is a variant of *Brassica oleracea*. Broccoli is a highly popular vegetable in the international market, and it is rich in nutrition, especially the anticancer and antiviral active ingredient sulforaphane [1, 2].

Agronomic traits of plant height, plant type, petiole length, and maximum outer leaf length of broccoli are important factors affecting planting density, directly correlating with the yield and quality of broccoli [3]. It's reported that planting broccoli density had a significant effect on plant height, and density was directly proportional to plant height [4]. To improve such parameters as photosynthetic utilization efficiency, yield and lodging resistance, it was necessary to study the traits of broccoli plant height, maximum outer petiole length and leaf width, as well as QTL initial location, which had certain theoretical applications for the selection of broccoli varieties in high planting densities and revealed the regulatory mechanism [5–7].

At present, molecular marker technologies, such as RFLP, RAPD, AFLP, SSR, SCAR, SRAP, and SNP, have been widely used in the identification of important traits of *Brassica* plants, the construction of genetic maps, gene mapping, and the classification and diversity analysis of germplasms [8, 9]. Some genetic maps were constructed for some important agricultural traits, such as self-incompatibility, male sterility, floret-related traits and quality traits. Fan et al. [10] constructed a genetic linkage map containing 157 SSR markers and 15 InDel markers, covering 9 linkage groups with a total length of 830.20 cM. The map was used to map the QTL for storage stability of broccoli and obtain 8 QTLs with a contribution rate of 1.16–14.20%. Brown et al. [11] constructed a genetic linkage map containing 547 nonredundant SNP markers, which covered 9 linkage groups with a length of 984.10 cM and an average interval of 1.70 cM with only 14 intervals (approximately 3.00%) over 5.00 cM, which was used to map QTLs of carotenoids in broccoli flowers, and a total of 3 QTLs were obtained. Walley et al. [12] used a broccoli DH population combined with SSR and AFLP molecular marker technology to construct a genetic linkage map consisting of 9 linkage groups covering 946.70 cM. This linkage map has been used for QTL mapping of agronomic traits, such as broccoli ball weight, ball diameter, stem diameter, and weight loss during storage. Yu et al. [13] constructed a genetic map of the broccoli DH population based on the reference genome TO1000 and identified the loci controlling hollow stem traits in the genetic map.

In this study, a total of 176 DH genotypes obtained from the same combination of broccoli were used as the mapping DH population. The 176 DH lines were multiple discrete, repeated for two years, and came from within the species. QTL IciMapping 4.0 software was used to construct a genetic linkage map of broccoli using SSR molecular marker technology. The QTLs for broccoli plant height (PH), maximum outer petiole length (PL) and maximum outer leaf width (LW) in the three environments were preliminarily analyzed. The QTLs of the DH population in the BIP and MET modes and the QTL × environment interaction effects were also estimated. This method not only combines molecular genetic benefit estimation with QTL analysis but also fully considers the influence of environmental factors on QTL analysis, and the accuracy and efficiency of mapping have been significantly improved.

Results

Phenotypic Analysis of PH, PL and LW

From Table 1, there were significant differences in phenotypic and environmental interactions. Correlation analysis showed that the PH of broccoli was significantly positively correlated with PL and LW in three environments between 2017 and 2018 (Table 2), indicating that the apical branch angle and the base branch angle of broccoli were strongly correlated and associated. From Table 2, we found that the traits of PH, PL and LW were stable and that there was a minor environmental effect. The frequency distributions of PH, PL and LW in the broccoli DH population largely conformed to the normal frequency (Fig. 1). Three traits had a wide range of variation in DH populations in different environments, which were expected to be used for further QTL mapping.

Table 1
Analysis of variance (ANOVA) for PH, PL and LW in three environments.

Model /Source	Sum of Squares	df	Mean Square	F	Sig.
PH/environment	390.03	3	130.01	0.05	0.99b
PL/environment	18145.95	3	6048.65	2.39	0.07b
LW/environment	12701.07	3	4233.69	1.65	0.18b

Table 2
Correlation coefficients of PH, PL and LW in the three environments during the two years.

	PL-1	LW-1	PH-2	PL-2	LW-2	PH-3	PL-3	LW-3
PH1	.676**	.506**	.929**	.656**	.554**	.906**	.649**	.535**
PL1		.651**	.734**	.924**	.673**	.711**	.909**	.623**
LW1			.542**	.657**	.925**	.526**	.606**	.918**
PH2				.734**	.609**	.930**	.713**	.584**
PL2					.711**	.716**	.911**	.650**
LW2						.580**	.665**	.944**
PH3							.710**	.576**
PL3								.656**
Note: ** Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively								

Construction Of The Genetic Linkage Map

QTL IciMapping 4.0 software was used to screen the polymorphic SSR markers for genetic maps, excluding unclear bands and partial separation markers. Finally, a total of 438 SSR markers were used for mapping. The map contained 9 linkage groups, covering a length of 1168.26 cM with an average distance between markers of 2.67 cM and ranging from 48.35 to 430.52 cM. The longest linkage group in the map was linkage group 3 with a length of 430.52 cM, containing 169 SSR markers, and the shortest linkage group was linkage group 1 with a length of 48.35 cM, containing 29 SSR markers, as shown in Table 3.

Table 3
Parameters of the genetic linkage map of broccoli.

Linkage group	No. of marker	Genetic distance (cM)	Average distance (cM)
1	29	48.35	1.67
2	35	84.45	2.41
3	169	430.52	2.55
4	74	127.44	1.73
5	23	90.40	3.93
6	30	49.76	1.66
7	30	133.51	4.45
8	23	93.31	4.06
9	25	110.52	4.42

QTL Mapping of Plant Height (PH)

QTL mapping was performed on PH, and 31 QTL loci were detected, of which 10 had positive additive effect values, as shown in Table 4. The interpretable contribution rate was 4.46%-20.05%, and the additive effect value was 1.86–3.92. Among the loci, PH1-A05 had the lowest contribution rate of 4.46% and LOD value of 3.01. Four QTLs were repeatedly detected in three different environments. One locus was QTL-*phc1* on chromosome 1, the genetic distance was 7.20 cM, the left marker was 8C024, and the right marker was sf4482 with a highest contribution rate of 20.05% and an LOD value of 13.23. The second was QTL-*phc2* on chromosome 2, the genetic distance was 56.50 cM, the left marker was ol12-F11, and the right marker was sc39144 with a contribution rate of 4.55% and LOD value of 1.93. There were 2 QTLs (*phc4-1*, *phc4-2*) on chromosome 4, and the first genetic distance was 49.80 cM. Between the markers 8C0478 and Sc32130, the contribution rate was 9.25%, and the LOD value was 3.58. Another QTL, *phc4-2*, had a genetic distance of 51.40 cM between the markers Sc32130 and ol10-F07 with a contribution rate of 6.98% and a LOD value of 3.41. These four QTLs were the main functional position with positive additive effects shown in Table 4 and Fig. 2.

Table 4
QTLs associated with PH detected in individual environments during 2017–2018.

Trait ID	Trait Name	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add
1	PH1	C01	7.20	8C024	sf4482	13.22	20.05	3.92
1	PH1	C02	56.50	ol12-F11	sc39144	3.58	4.55	1.93
1	PH1	C03	172.10	8C0194	8C01000	10.68	16.45	-3.64
1	PH1	C04	31.20	sf14184	ol11-H09	5.28	7.07	2.33
1	PH1	C04	49.80	8C0478	Sc32130	4.74	9.25	2.74
1	PH1	C04	50.70	Sc32130	ol10-F07	8.02	21.06	-4.05
1	PH1	C04	51.40	Sc32130	ol10-F07	3.41	6.80	2.31
1	PH1	C05	46.00	Sc53056	sc22567	3.01	4.46	1.86
2	PH2	C05	81.60	sc3314	8C0435	2.60	5.72	3.50
2	PH2	C01	6.90	8C024	sf4482	8.02	11.13	3.05
2	PH2	C01	9.90	8C0416	sc17086	4.53	4.82	-2.01
2	PH2	C02	56.50	ol12-F11	sc39144	4.72	4.92	2.10
2	PH2	C03	172.70	8C01000	BR-S008	13.06	15.32	-3.65
2	PH2	C03	240.00	8C0828	8C039	4.56	5.39	-2.89
2	PH2	C04	31.20	sf14184	ol11-H09	5.28	7.07	2.33
2	PH2	C04	49.80	8C0478	Sc32130	4.74	9.25	2.74
2	PH2	C04	50.70	Sc32130	ol10-F07	12.56	25.06	-4.63
2	PH2	C04	51.40	Sc32130	ol10-F07	6.36	11.11	3.09
2	PH2	C05	83.70	Sc33948	Sc24249	4.26	6.30	-4.21
3	PH3	C01	7.10	8C024	sf4482	9.15	10.79	2.90
3	PH3	C01	9.40	Sc7918	8C0416	5.61	8.16	-2.52
3	PH3	C02	56.50	ol12-F11	sc39144	6.05	6.10	2.26
3	PH3	C02	57.20	ol12-F11	sc39144	3.21	4.04	-1.80
3	PH3	C03	76.00	ol10-G08	sc46974	3.98	4.70	1.94
3	PH3	C03	166.80	8C0790	sc28408	3.55	5.68	-2.24
3	PH3	C03	173.00	8C01000	BR-S008	9.39	11.39	-3.04
3	PH3	C03	238.90	Sc2207	Sc4534	4.52	10.63	-3.30
3	PH3	C04	31.30	ol11-H09	8C0167	7.46	7.66	2.44
3	PH3	C04	49.80	8C0478	Sc32130	6.57	9.70	2.82
3	PH3	C04	50.60	Sc32130	ol10-F07	9.34	13.02	-3.23
3	PH3	C04	51.40	Sc32130	ol10-F07	5.24	7.90	2.51

Qtl Mapping Of Maximum Petiole Length (pl)

As shown in Table 5, a total of 25 QTL loci were detected by the broccoli DH population, distributed on chromosomes 1, 3, 4 and 6. There were 5 positive QTLs for additive effects, and the additive effect values were 0.62–1.39, which could explain the contribution of 3.93%-20.02%. There was one QTL repeatedly detected in three different environments. QTL-*plc6* was on chromosome 6 with a genetic distance of 11.10 cM, the left marker was sc2170, and the right marker was sf43960. At the same time, the contribution rate of this locus was the largest, 20.02%, and the

LOD value was also high, 13.19. In the first environment, two QTLs (*plc4-1*, *plc4-2*) were both located adjacent to chromosome 4, the left marker was sf53389 and the right marker was 8C0474, but QTL-*plc4-1* and QTL-*plc4-2* had opposite additive effect values of -0.99 and 1.07, respectively. Similarly, in the third environment, it happened again with similar position and additive effect, the left marker was labeled Sc32130 and the right marker was labeled ol10-F07 with opposite additive effect of -1.02 and 0.89 (Table 5 and Fig. 3).

Table 5
QTL associated with PL of broccoli detected in individual environments during 2017–2018.

Trait ID	Trait Name	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add
1	PL1	C01	10.70	sc17086	B2	14.47	22.81	-1.48
1	PL1	C03	170.90	8C0194	8C01000	2.70	3.25	-0.57
1	PL1	C03	232.00	Sc40920	Sc14212	4.37	6.14	-0.93
1	PL1	C04	43.60	sf53389	8C0474	6.83	9.88	-0.99
1	PL1	C04	44.70	sf53389	8C0474	8.76	11.67	1.07
1	PL1	C04	46.10	8C0474	sc27943	2.57	3.73	-0.60
1	PL1	C06	10.70	sc23682B	sc2170	14.84	20.51	-1.41
1	PL1	C06	11.10	sc2170	sf43960	13.19	20.02	1.39
2	PL2	C01	10.00	8C0416	sc17086	25.56	27.63	-1.63
2	PL2	C02	22.10	sc1745A	8C0462	6.55	5.44	-0.91
2	PL2	C02	53.20	8C0413	sc47877	4.07	4.05	-0.63
2	PL2	C03	171.80	8C0194	8C01000	4.59	4.30	-0.65
2	PL2	C04	31.30	ol11-H09	8C0167	4.90	3.98	0.62
2	PL2	C04	49.80	8C0478	Sc32130	10.97	13.62	1.18
2	PL2	C04	50.50	8C0478	Sc32130	7.82	8.38	-0.91
2	PL2	CC06	3.30	Sc47235	8C0815	4.36	4.44	-0.66
2	PL2	C06	10.70	sc23682B	sc2170	17.62	16.92	-1.28
2	PL2	C06	11.10	sc2170	sf43960	13.24	13.94	1.16
2	PL2	C06	28.00	Sc40045	Sc1090-14681	2.90	7.58	-0.88
3	PL3	C01	10.40	sc17086	B2	15.36	26.53	-1.65
3	PL3	C04	49.80	8C0478	Sc32130	5.20	9.83	1.03
3	PL3	C04	50.60	Sc32130	ol10-F07	5.50	9.84	-1.02
3	PL3	C04	51.40	Sc32130	ol10-F07	3.87	7.56	0.89
3	PL3	C06	10.70	sc23682B	sc2170	12.81	19.53	-1.42
3	PL3	C06	11.10	sc2170	sf43960	10.09	16.80	1.31

Qtl Mapping Of Maximum Outer Leaf Width (lw)

As shown in Table 6, a total of 19 QTL loci were detected in the DH population, distributed on chromosomes 1, 3, 4 and 6. There were 9 QTLs with positive additive effect values; the interpretable contribution rate was 4.01%–19.97%, and the additive effect values were 0.72–3.09. Two QTLs (*lwc1* and *lwc3*) were repeatedly detected in three different environments during 2017–2018. QTL-*lwc1* was on chromosome 1, the genetic distance was 7.40 cM, the left marker was sf4482, and the right marker was Sc7918 with a contribution rate of 16.16% and LOD value of 8.25. The other QTL-*lwc3* was on chromosome 3, the genetic distance was 14.70 cM, the left marker was Sc52751, and the right marker was RA2-E12, with the largest contribution rate of 19.97% and an LOD value of 7.16. In the second environment, two QTLs (*lwc4-1*, *lwc4-2*) were located adjacent to chromosome 4, the left marker was 8C0478 and the right marker was Sc3213 with opposite additive effect values of 1.64 and -1.26. The two QTLs might be functional in regulating LW of broccoli (Table 6 and Fig. 4).

Table 6
QTL associated with LW of broccoli detected in individual environments during 2017–2018.

Trait ID	Trait Name	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add
1	PL1	C01	10.70	sc17086	B2	14.47	22.81	-1.48
1	PL1	C03	170.90	8C0194	8C01000	2.70	3.25	-0.57
1	PL1	C03	232.00	Sc40920	Sc14212	4.37	6.14	-0.93
1	PL1	C04	43.60	sf53389	8C0474	6.83	9.88	-0.99
1	PL1	C04	44.70	sf53389	8C0474	8.76	11.67	1.07
1	PL1	C04	46.10	8C0474	sc27943	2.57	3.73	-0.60
1	PL1	C06	10.70	sc23682B	sc2170	14.84	20.51	-1.41
1	PL1	C06	11.10	sc2170	sf43960	13.19	20.02	1.39
2	PL2	C01	10.00	8C0416	sc17086	25.56	27.63	-1.63
2	PL2	C02	22.10	sc1745A	8C0462	6.55	5.44	-0.91
2	PL2	C02	53.20	8C0413	sc47877	4.07	4.05	-0.63
2	PL2	C03	171.80	8C0194	8C01000	4.59	4.30	-0.65
2	PL2	C04	31.30	ol11-H09	8C0167	4.90	3.98	0.62
2	PL2	C04	49.80	8C0478	Sc32130	10.97	13.62	1.18
2	PL2	C04	50.50	8C0478	Sc32130	7.82	8.38	-0.91
2	PL2	CC06	3.30	Sc47235	8C0815	4.36	4.44	-0.66
2	PL2	C06	10.70	sc23682B	sc2170	17.62	16.92	-1.28
2	PL2	C06	11.10	sc2170	sf43960	13.24	13.94	1.16
2	PL2	C06	28.00	Sc40045	Sc1090-14681	2.90	7.58	-0.88
3	PL3	C01	10.40	sc17086	B2	15.36	26.53	-1.65
3	PL3	C04	49.80	8C0478	Sc32130	5.20	9.83	1.03
3	PL3	C04	50.60	Sc32130	ol10-F07	5.50	9.84	-1.02
3	PL3	C04	51.40	Sc32130	ol10-F07	3.87	7.56	0.89
3	PL3	C06	10.70	sc23682B	sc2170	12.81	19.53	-1.42
3	PL3	C06	11.10	sc2170	sf43960	10.09	16.80	1.31

Analysis of Epistatic and Interaction Effects of QTLs for Broccoli Planting Density

Using the MET module of QTL IciMapping V4.0 software, the interaction between EPH-QTL and the environment of PH, PL and LW was analyzed by the ICIM-EPI mapping method. As shown in Table 7, there were 31 upper QTLs for PH detected through 1000 repeated alignment tests and 5.0 cM scanning steps, covering 27 of the 7 linkage groups, except for linkage groups 6 and 8. Twenty-seven QTLs showed epistatic interactions among different linkage groups, while the other 3 QTLs showed epistatic interactions within a single linkage group. The different positions of 8C024-sf4482 in linkage group 1 had different epistatic effects (Fig. 5), thereby indicating that one locus had different effects on other loci involved, only one of the 39 loci interacted with QTL, and the other interacted with more than one QTL (Fig. 6). The interaction between these QTLs and the environment was also very different. Similarly, 53 upper QTLs for LW were detected, covering 42 QTLs in 9 linkage groups, showing superior interactions between different linkage groups. The different positions of Sc47235-8C0815 in linkage group 6 had different epistatic effects (Fig. 5), indicating that the locus of one locus had different effects on other loci involved; only one of the 30 loci interacted with QTLs, and the other interacted with more than one QTL (Fig. 6). Thirty-two upper QTLs were detected in PL, covering 32 of the eight linkage groups, except for linkage group 7 of the 43 loci involved; only one of the 38 loci interacted with QTL, and the other interacted with more than one QTL (Fig. 5, 6). The phenotypic contribution in three QTL × environment interactions was lower than the additive effect, which suggested

that the additive effects of these QTLs had a significant effect on phenotypic variation of multiple traits. We also detected two environment-specific QTLs with low LOD values, which showed that the environment also had an important impact on phenotypic variation.

Table 7
Profiles of ICIM-ADD testing of the main QTL and environment interactions for PH, PL and LW during 2017–2018.

Chromosome	Position	Left Marker	Right Marker	LOD	LOD (A)	LOD (AbyE)	PVE	PVE (A)	PVE (AbyE)	Add	Aby E_01	Aby E_02	Aby E_03
1	2.90	8C024	sf4482	2.97	2.84	0.14	1.40	1.27	0.12	-1.01	-0.40	0.36	0.04
1	7.20	8C024	sf4482	30.30	30.21	0.09	12.47	12.13	0.34	3.10	0.73	-0.38	-0.35
1	9.70	8C0416	sc17086	10.80	9.63	1.18	3.75	3.44	0.31	-1.65	0.70	-0.30	-0.39
2	34.90	17–69	Sc19852	3.00	2.89	0.11	1.20	1.13	0.07	-0.96	-0.29	-0.01	0.29
2	56.50	ol12-F11	sc39144	14.35	14.03	0.33	5.17	5.15	0.02	2.09	-0.16	0.00	0.16
2	57.20	ol12-F11	sc39144	7.11	6.87	0.24	2.82	2.80	0.02	-1.51	0.17	-0.03	-0.14
3	11.30	Sc387	Sc33939	2.52	2.35	0.17	1.01	0.94	0.08	-0.90	-0.33	0.02	0.31
3	32.30	915	01-137	3.30	3.04	0.27	1.10	1.04	0.06	-1.07	-0.10	0.35	-0.25
3	76.00	ol10-G08	sc46974	4.17	2.30	1.87	1.36	0.90	0.45	0.86	-0.45	-0.41	0.86
3	154.60	Sc25277	BG305	4.01	3.90	0.11	1.34	1.33	0.02	1.58	-0.15	0.24	-0.09
3	163.20	8C0106	8C0643	4.66	4.25	0.41	1.81	1.66	0.15	-1.53	-0.40	-0.25	0.65
3	166.80	8C0790	sc28408	5.94	5.59	0.36	2.25	2.20	0.06	-1.40	0.05	0.25	-0.30
3	172.90	8C01000	BR-S008	32.25	32.05	0.20	13.29	13.15	0.14	-3.29	-0.08	-0.38	0.45
3	187.40	Sc1481	8C0202	3.84	3.68	0.16	1.73	1.70	0.03	-1.65	0.11	-0.33	0.22
3	238.80	sc5427	Sc2207	5.53	3.34	2.19	1.86	1.17	0.68	-1.30	-0.16	1.29	-1.13
3	240.00	8C0828	8C039	6.26	4.73	1.53	2.23	1.63	0.60	-1.53	-0.15	-1.05	1.20
3	243.40	8C039	8C076	3.42	3.39	0.04	1.36	1.33	0.03	-1.28	-0.27	0.11	0.16
3	251.60	Sc1031-14182	Sc908	3.33	3.25	0.07	1.42	1.42	0.00	1.11	-0.01	-0.04	0.05
3	263.50	Sc16020	Sc9869	3.01	2.82	0.18	1.33	1.31	0.0	1.02	-0.16	0.17	-0.01
3	400.00	BG353	112	2.67	2.60	0.06	1.08	1.04	0.04	-0.96	-0.27	0.13	0.14
4	25.60	BG380	sf14184	2.84	1.92	0.92	0.89	0.66	0.23	-0.74	0.62	-0.25	-0.36
4	31.30	ol11-H09	8C0167	20.77	20.42	0.35	7.76	7.72	0.04	2.48	-0.20	0.24	-0.03
4	49.80	8C0478	Sc32130	20.41	20.01	0.41	8.90	8.79	0.11	2.72	-0.24	0.43	-0.19
4	50.60	Sc32130	ol10-F07	28.68	28.34	0.34	11.96	11.88	0.08	-3.12	0.15	-0.36	0.22
4	51.40	Sc32130	ol10-F07	14.51	14.23	0.28	6.76	6.70	0.06	2.33	-0.21	0.29	-0.08
5	46.40	Sc53056	sc22567	5.55	4.87	0.68	2.10	1.72	0.38	1.18	0.52	0.24	-0.76
5	48.40	sc22567	sc52406	2.87	1.91	0.96	1.08	0.71	0.37	-0.76	-0.44	-0.34	0.78
5	83.70	Sc33948	Sc24249	8.31	8.16	0.15	3.54	3.45	0.09	-3.01	0.01	-0.60	0.59
7	112.60	Sc14232	1739–17431	4.56	4.46	0.10	1.89	1.88	0.01	2.01	-0.1	0.14	-0.14
9	108.30	15164	11759	4.49	4.05	0.44	1.63	1.44	0.19	1.34	0.35	0.34	-0.69

Discussion

The DH population was stable and could be reused in the population, which was especially suitable for analyzing traits that are easily affected by the environment. This population could be planted in different environmental conditions at the same time, and the QTL mapping of the target

traits under multiple environmental conditions was helpful for finding genes that were stably expressed in different environments [14]. Therefore, mapping QTLs for agronomic traits in *Brassica* plants by DH population is a useful method. Zhao et al. [15] used two genetic linkage maps constructed from the same parental DH and RIL populations to identify and compare QTLs controlling rice crop traits in different years. He et al. [16] identified 102 additive quantitative characteristic loci (QTLs) in six environments based on high-density SNP profiles by a DH population of *Brassica napus*.

QTL IciMapping 4.0 software has been widely used for mapping QTLs in crops. Qi [17] used QTL IciMapping 4.0 software to locate 154 marker sites on chromosome 21 of wheat. QTL mapping analysis was performed on three characteristics of wheat flag leaf length, flag leaf width and plant height traits, and six additive QTLs were detected and distributed on chromosome 6 of wheat. QTL analysis of appearance quality traits, such as rice grain length and chalkiness, in two different ecological environments was reported based on this software, and the QTL results showed that 16 appearance-quality QTLs were located in two environments, distributed on chromosomes 1, 2, 3, 5, 9, and 10, and the LOD value was between 2.50-10.98 with a contribution rate of 7.64%-19.89% [18]. Using this method, a high-density genetic map constructed by resequencing parents and simplified genome sequencing of rice RIL populations and QTL mapping of heading stage under two environments, a total of 4 QTLs affecting heading date were located on chromosomes 3, 6, and 8 [19].

QTL mapping has become one of the common methods for quantitative trait genetic research [20–22]. Most agronomic traits of broccoli are quantitative traits that are controlled by multiple major and minor genes [23, 24]. To date, based on different molecular markers, researchers have constructed multiple genetic linkage maps and successfully mapped them to multiple QTL sites of *Brassica* crops [25–27]. Shen et al. [28] used 208 DH populations to locate 17 rape branch angle QTLs. Each of the three main QTLs could explain approximately 10% of the phenotypic variation, and 27 candidate genes were obtained in the QTL interval. Holme et al. [29] obtained 128 QTLs controlling the regeneration of cabbage protoplasts using 128 combination materials and an F₂ population and used two QTLs to assist in the selection of broccoli lines with high protoplast regeneration capacity. Yu et al. [30] constructed a high-density genetic map of 127 broccoli DH populations based on the phenotypic data of three seasons, and the traits of purple sepals of flower heads and hollow stems were identified.

In this study, the longest linkage map of broccoli derived from broccoli varieties was constructed, covering the genome length of 1168.26 cM, which would be helpful for mapping QTLs for important agronomic traits in broccoli and other *Brassica oleracea* plants. By using a high-density genetic linkage map and combining phenotypic test data, genetic correlation analysis and QTL initial mapping of the 3 traits PH, PL and LW of broccoli were performed. Finally, four main QTLs (*phc1*, *phc2*, *phc4-1*, *phc4-2*), one main QTL (*plc6*), and two main QTLs (*lwc1*, *lwc3*) were detected and found during the two years. In addition, in scanning QTLs for PL, two QTLs (*plc4-1* and *plc4-2*) were both located adjacent to chromosome 4, and there were similar genetic distances and additive effects. At the same time, additive effects were noted in this position, which indicated that there might be the same candidate genes. In QTLs for LW of broccoli, two QTLs (*lwc4-1*, *lwc4-2*) were also located adjacent to chromosome 4, and there were both the same left marker (8C0478) and right marker (Sc3213) with opposite additive effect values of 1.64 and – 1.26. This result also suggested that the two QTLs might be functional in regulating LW of broccoli. This research provides an experimental basis for molecular marker-assisted breeding in the planting density of broccoli [31, 32].

In the localization analysis on planting density in broccoli, 7 main QTLs were repeatedly found in both years, and there were stable traits in three environmental tests, indicating that there might be major genes regulating PH, PL and LW of broccoli. According to the correlation coefficients of PH, PL and LW of broccoli in the three environments during the two years, PL could promote PH more than LW, PL might be promoted by PH more than LW, and LW was mainly dependent on PL. This research focused on factors affecting the planting density of broccoli by mapping QTLs for PH, PL and LW, and the results may be helpful for further research on the development and regulation mechanism of broccoli regarding plant leaves and height..

Conclusions

In this study, according to QTL IciMapping 4.0 software, a genetic linkage map with the longest length of 1168.26 cM in broccoli was constructed by a permanent DH population. Finally, there were a total of 7 major QTLs repeatedly detected for the planting density of broccoli during two years, that is, four major QTLs (*phc1*, *phc2*, *phc4-1*, *phc4-2*), one major QTL (*plc6*), and two major QTLs (*lwc1*, *lwc3*), repeatability for PH, PL and LW of broccoli was observed, and all the QTLs had a higher contribution rate with positive results. Meanwhile, four adjacent QTLs, that is, two QTLs (*plc4-1*, *plc4-2*) responsible for PL and two QTLs (*lwc4-1*, *lwc4-2*) responsible for LW, were also identified as possible positions of candidate genes in regulating plant leaves. This study may establish a foundation for further research on the growth mechanisms of broccoli, especially plant leaves and height.

Materials And Methods

Plant materials

The high-generation inbred lines 86101 (P_1) and 90196 (P_2), F_1 hybrid of two parents selected by the Chinese Academy of Agricultural Sciences, Institute of Vegetables and Flowers (CAAS-IVF), were used to construct a DH population containing 176 genotypes by microspore culture [33].

Field Trial Design

The materials of the P_1 , P_2 , F_1 , and DH groups were planted in early and late sowing in 2017 and early sowing in 2018 at the North Farm Experimental Farm of Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences and were generally managed in the field. P_1 , P_2 , and F_1 were planted with 50 plants individually with three repeats ($n = 3$). The DH population was arranged using repeated internal grouping and random block design methods [34]. The 176 families of the DH population were divided into 11 groups, and each group contained 16 families. Three replicates were set randomly, and 3 replicates were designed per repeat block. The random block consisted of 2 replicates with 3 rows in each plot, 18 plants in each row, a row spacing of 0.33 m, and a plant spacing of 0.10 m. Field management was the same as conventional production, ensuring that the external growth environment of all samples was consistent, and statistics would be obtained after maturity.

Characteristics Investigation And Statistical Methods

When the florets matured, the PH, PL and LW of the broccoli were investigated on a case-by-case basis in different generations and DH populations, and the DH population was investigated 5 times per line. The survey criteria refer to the “Standards and Data Standards for Germplasm Resources of Cauliflower and Broccoli” [35]. Statistics were performed using DPS and Excel, and the normality test, correlation analysis of phenotypic data, and analysis of variance (ANOVA) were carried out using SPSS 17.0 software [36].

Construction of Genetic Maps, Epistatic QTLs for QTL Initial Mapping and Multienvironmental Interaction

A complete set of DH permanent populations of broccoli was constructed containing 176 plants. The genetic linkage map was constructed by ICIM-ADD (ICIM additive mapping) using IciMapping 4.0 software, and the QTL of the DH population polymorphism shape was initially identified for QTL analysis [37, 38]. The scan step was set to 0.10 cM, and the LOD value was set to 3.00. At the same time, the epistatic QTL for the multienvironmental test environment occurred in the three environments of early sowing and late sowing in 2017 and early sowing in 2018 [39, 40].

Abbreviations

QTL

Quantitative trait locus; DH:Doubled haploid; PH:Plant height; PL:Maximum outer petiole length; LW:Leaf width; ICIM:Inclusive composite interval mapping; SSR:Simple sequence repeats.

Declarations

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Author Contributions

Data curation, Z.L., J.S.; Formal analysis, Z.L.; Funding acquisition, Z.L.; Project administration, Z.L.; Resources, Y.L.; Supervision, Z.L. and Y.L.; Validation, Z.F., L.Y., M.Z., Y.Z., H.L., Y.W., F.H. and J.J.; Writing-original draft, J.H.; Writing, review and editing, J.H. and Z.L. All authors have reviewed and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests Conflict of Interest

The authors declared that they have no competing interests.

Author details

¹Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture, Beijing 100081, China. ²Key Laboratory of Genetic Improvement of Horticultural Crops, Shandong Province, Weifang Academy of Agricultural Sciences, Weifang, Shandong, China

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Figures

Figure 3

Mapping of QTLs for PL by DH population in three environments.

Figure 4

Mapping of QTLs for LW by DH population in three environments.

Figure 5

Distribution and positions of QTLs for PH, PL and LW in the chromosome.

Figure 6

Cyclic representation of significant epistatic QTL and LOD profiles of ICIM-EPI testing for PH, PL and LW of broccoli