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

CURRENT STATUS: Posted

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Subject Areas

Medical Genetics  Molecular Genetics

Keywords

broccoli, DH population, QTL mapping, planting height, leaf characteristics
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 $F_1$ obtained by crossing the broccoli inbred lines 86101 ($P_1$) and 90196 ($P_2$).

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 antivirus 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.

| 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 1**

Analysis of variance (ANOVA) for PH, PL and LW in three environments.
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 |
|   | PH1  | C03        |   |   |   |   |   |   |   |   |
|---|------|------------|---|---|---|---|---|---|---|---|
| 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 |
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 | C06 | 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 QLT-Iwc3 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|
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.
|   | 2     | 56.50  | 112-F11 | sc3914 4 | 14.35 | 14.03 | 0.33 | 5.17 | 5.15 | 0.02 | 2.09 | -0.16 | 0.00 | 0.16 |
|---|-------|--------|---------|----------|-------|-------|------|------|------|------|------|--------|------|------|
| 2 | 57.20 | 56.50  | 112-F11 | sc3914 4 | 7.11  | 6.87  | 0.24 | 2.82 | 2.80 | 0.02 | -1.51 | 0.17  | -0.03 | -0.14 |
| 3 | 11.30 | sc387  | sc3393 9 | 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| sc4697 4| 4.17    | 2.30  | 1.87  | 1.36 | 0.90 | 0.45 | 0.86  | -0.45 | -0.41 | 0.86  |
| 3 | 154.60| Sc2527 7| 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 | sc2840 8| 5.94    | 5.59  | 0.36  | 2.25 | 2.20 | 0.06 | -1.40 | 0.05  | 0.25  | -0.30 |
| 3 | 172.90| 8C0100 | 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 | sc908   | 3.33    | 3.25  | 0.07  | 1.42 | 1.42 | 0.00 | 1.11  | -0.01 | -0.04 | 0.05  |
| 3 | 263.50| sc1602 | 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  | sf1418 4| 2.84    | 1.92  | 0.92  | 0.89 | 0.89 | 0.66 | -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 | sc3213 0| 20.41   | 20.01 | 0.41  | 8.90 | 8.79 | 0.11 | 2.72  | -0.24 | 0.43  | -0.19 |
| 4   | 50.60 | Sc3213 0 | 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 | Sc3213 0 | 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 | Sc5305 6 | sc2256 7 | 5.55  | 4.87  | 0.68 | 2.10  | 1.72  | 0.38 | 1.18  | 0.52  | 0.24  | -0.76 |
| 5   | 48.40 | sc2256 7 | sc5240 6 | 2.87  | 1.91  | 0.96 | 1.08  | 0.71  | 0.37 | -0.76 | -0.44 | -0.34 | 0.78 |
| 5   | 83.70 | Sc3394 8 | Sc2424 9 | 8.31  | 8.16  | 0.15 | 3.54  | 3.45  | 0.09 | -3.01 | 0.01  | -0.60 | 0.59 |
| 7   | 112.60| Sc1423 2 | 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 sepal 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 (BC0478) 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 (P1) and 90196 (P2), F1 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 P1, P2, F1, 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. P1, P2, and F1 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**

**Acknowledgments**

We thank the academic English editors (AJE) (available online: https://www.aje.com/services/editing/) for editing the English text of a draft of this manuscript.

**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.

**Funding**

This work was supported by grants from the National Key Research and Development Program of China (Grant No. 2017YFD0101805), the National Natural Science Foundation of China (Grant No. 31501761), the China Agriculture Research System (Grant No. CARS-23-08A), the Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (Grant No. CAAS-ASTIP-IVFCAAS), and the State Key Laboratory of Vegetable Germplasm Innovation (Grant No. SKL-VGI).

**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.

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**References**

1. Arnison PG, Donaldson P, Ho LCC, Keller WA. The influence of various physical parameters on anther culture of broccoli (*Brassica oleracea var. italica*). Plant Cell Tissue Organ Cult. 1990;20:147–55.
2. Li ZS, Liu YM, Fang ZY, Yang LM, Zhuang M, Zhang YY, Lv HH. Natural sulforaphane from broccoli seeds against influenza A virus replication in MDCK cells. Natural Product Communications. 2019;14.
3. Hale AL, Farnham MW, Nzaramba MN, Kimbeng CA. Heterosis for horticultural traits in broccoli. Theoretical And Applied Genetics. 2007;115.
4. Guo J, Zu YX, Gu MF, Wu YC, Zheng JQ. Effects of sowing date and density on the yield and agronomic characters of lucky broccoli. Jiangsu Agricultural Sciences. 2010:214–215.
5. Zheng HZ. Comparative experiments of different planting density of ‘Yanxiu' broccoli. Chinese Horticulture Abstracts. 2018;34:56.
6. Zalapa JE, Staub JE, Mccreight JD, Chung SM, Cuevas H. Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. Theoretical Applied Genetics. 2007;114:1185–201.
7. Gardner AM, Brown AF, Juvik JA. QTL analysis for the identification of candidate genes controlling phenolic compound accumulation in broccoli (*Brassica oleracea L. var. italica*). Molecular Breeding. 2016;36.
8. Wang H, Huang WH, Wang J, Liu J, Wang WX, Fu L, Zaman QU, Hu Q, Mei DS. QTL mapping for yield components of *Brassica napus* L. using double haploid and immortalized F2 populations. Oil Crop Science. 2018;3:203–14.
9. Lv HH, Wang QB, Zhang YY, Yang LM, Fang ZY, Wang XW, Liu YM, Zhuang M, Yu HL, Liu B. Linkage map construction using InDel and SSR markers and QTL analysis of heading traits in *Brassica oleracea* var. *capitata* L.. Molecular Breeding. 2014;34:87–98.
10. Fan YY, Liu YM, Li ZS, Fang ZY, Yang LM, Zhuang M, Zhang YY, Sun PT. Expression Analysis of Chlorophyll Degradation Related Genes in the Process of Senescence of Broccoli. Acta Horticulture Sinica. 2015;42:1338–46.
11. Brown AF, Yousef GG, Chebrolu KK, Byrd RW, Everhart KW, Thomas A, Reid RW, Parkin IA, Sharpe AG, Oliver R. High-density single nucleotide polymorphism (SNP) array mapping in *Brassica oleracea*: identification of QTL associated with carotenoid variation in broccoli florets. Theor Appl Genet. 2014;127:2051–64.
12. Walley PG, Carder J, Skipper E, Mathas E, Lynn J, Pink D. A new broccoli × broccoli immortal mapping population and framework genetic map: tools for breeders and complex trait analysis. Theor Appl Genet.
13. Yu HF, Wang JS, Zhao ZQ, Sheng XG, Shen YS, Branca F, Gu HH. Construction of a high-density genetic map and identification of loci related to hollow stem trait in broccoli (Brassica oleracea L. italica). Frontiers in plant science 2019;10.

14. Miao TY, Liu YM, Fang ZY, Yang LM, Zhuang M, Zhang YY, Yuan SX, Sun PT. Genetic analysis of the main agronomic traits of DH population in Brassica oleracea var. capitata. Acta Horticulturae Sinica. 2008;35:59–64.

15. Zhao XH, Yang Q, Jia BY, Kim SM, Lee HS, Eun MY, Kim KM, Sohn JK. Comparison and Analysis of QTLs, Epistatic Effects and QTL × Environment Interactions for Yield Traits Using DH and RILs Populations in Rice. Journal of Integrative Agriculture. 2013;12.

16. He YY, Wu DM, Fu Y, Qian W. Detection of QTLs of Brassica napus plant height related traits using DH and IF2 Populations. Acta Agronomica Sinica. 2018;44:533–41.

17. Qi P. QTL Analysis of Wheat Plant Height and Leaf Area and Preliminary Study on Fertilizer Effect of Oil Sunflower XN1616. Northwest A & F University, 2015.

18. Shen YS, Xiang Y, Xu ES, Ge XH, Li ZY. Major Co-localized QTL for Plant Height, Branch Initiation Height, Stem Diameter, and Flowering Time in an Alien Introgression Derived Brassica napus DH Population. Frontiers in Plant Science. 2018;9.

19. Luo QH, Long SF, Zhang XC, Liu XW, Peng Q, Jiang X, Wu X, Zhang ZB, Wang ZN, Zhu SS. QTL Analysis of Rice Appearance Quality Traits. Molecular Plant Breeding. 2020:1–23.

20. Li DX, Yang J, Sun K, Li DD, Yang GL, Guo T, Wang H, Chen ZQ. Locating new QTLs for heading of rice based on high density genetic map. Journal of Northwest A & F University (Natural Science Edition). 2020:1–7.

21. Zhang Y, Sun JM, Han RQ, Chen JS, Liu B, Deng ZY, Li YY, Tian JC. QTL analysis of yield conditions based on three factors of wheat yield. Journal of Triticeae Crops. 2019;39:42–9.

22. Lu N, Zhang MM, Xiao Y, Han DH, Liu Y, Zhang Y, Yi F, Zhu TQ, Ma WJ, Fan EQ, Gu GZ, Wang JH. Construction of a high-density genetic map and QTL mapping of leaf traits and plant growth in an interspecific F1 population of Catalpa bungei × Catalpa duclouxii Dode. BMC plant biology. 2019;19:596.

23. Yu HF, Qi ZR, Chen JS, Wang JS, Sheng XG, Zhao ZQ, Shen YS, Gu HH. Genetic and QTL mapping of stem traits in broccoli. Molecular plant breeding. 2019;17:5037–44.

24. Jia B, Cui M, Yin ZT, Yan WG, Xie QC. QTL mapping of main traits in maize tassels. Zhejiang Agricultural Sciences. 2018;59:228–61.

25. Gerardo NL, Cristóbal B, Catalina P, Claudio U, Dayan S, Elisa V, Maria TD, Pere A, Ignazio V, Francisca BH, Reinaldo CV, Claudio M. High-density genetic map and QTL analysis of soluble solid content, maturity date, and mealiness in peach using genotyping by sequencing. Scientia Horticulturae. 2019;257.

26. Sebastian RL, Kearsey MJ, King GJ. Identification of quantitative trait loci controlling developmental characteristics of Brassica oleracea L. Theor Appl Genet. 2002;104:60 l–609.

27. Wang WW, Wang HY, Liu J, Liang JS, Li CH, Tang W. QTL mapping of potato importance and molecular marker-assisted breeding of three disease resistance traits. Crops. 2018:10–16.

28. Shen YS, Yang Y, Xu ES, Ge XH, Xiang Y, Li ZY. Novel and major QTL for branch angle detected by using DH population from an exotic introgression in rapeseed (Brassica napus L.). Theor Appl Genet. 2018;131:67–78.

29. Holme IB, Torp AM, Hansen LN, Andersen SB. Quantitative trait loci affecting plant regeneration from protoplasts of Brassica oleracea. Theor Appl Genet. 2004;108:1513–20.

30. Yu HF, Wang JS, Sheng XG, Zhao ZQ, Shen YS, Branca F, Gu HH. Construction of a high-density genetic map and identification of loci controlling purple sepal trait of flower head in Brassica oleracea L. italica. BMC plant biology. 2019;19.

31. Liu ZF, Xie B, Sun B, Sun XW. Genetic map construction of Chinese pumpkin (Cucurbita moschata D.) and QTL mapping of fruit flavonoids. China Cucurbits Vegetables. 2018;31:5–10.

32. Wang BW, Zhai JM, Shi CQ, Zheng JX, Yu YZ, Huang AX. QTL Mapping and Genetic Analysis of a High Resistance to Maize Southern Rust Gene. Scientia Agricultura Sinica. 2019;52:2033–41.

33. Liu JF, Zhang B, Li M, Liu L, Wen FY. The molecular genetic map of Chinese cabbage was constructed by using DH population. Acta Agriculturae Boreali-Sinica. 2015;30:156–60.

34. Gai Y, Zhang YM, Wang JK. Genetic system of quantitative traits in plants. Beijing: Science Press; 2003.
35. Li XX, Fang ZY. Descriptors and data standard for cauliflower (*Brassica oleracea* L. var. *botrytis* L. and *Brassica oleracea* L. var. *italica* Planck). Beijing: China Agriculture Press; 2008. (in Chinese).
36. Li ZS, Li L, Liu YM, Fang ZY, Yang LM, Zhuang M, Zhang YY, Lv HH. Transcriptome reveals the gene expression patterns of sulforaphane metabolism in broccoli florets. PLoS One. 2019;14:e0213902.
37. Su CF, Zhao TJ, Gai JY. Simulation comparison of application effects of QTL mapping methods in different statistical genetic models. Acta Agronomica Sinica. 2010;36:1100-7.
38. Tang YZ. The regulation of tiller panicle formation and the QTL mapping of control gene in coordinated wheat. Sichuan Agricultural University. 2016.
39. Tan RJ, Wen ZX, Gu CH, Wang DC, Song QJ, Reppy C, Xing XP, Li HL. Comparison of construction methods of high-density snp-labeled genetic map of soybean. Journal of Henan Agricultural University. 2013;47:671-6.
40. Rong FX. QTL mapping and analysis of chlorophyll fluorescence parameters in floc phase and sea land infiltration system cotton fiber quality related characters. Shihezi University. 2015.

**Figures**

Figure 1
Frequency distributions of PH, PL and LW in the broccoli DH population.
Figure 2
Mapping of QTLs for PH of broccoli in three environments.
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.
Cyclic representation of significant epistatic QTL and LOD profiles of ICIM-EPI testing for PH, PL and LW of broccoli