Identification of QTL underlying the leaf length and area of different leaves in barley

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Leaf is the main organ of photosynthesis, which significantly impacts crop yield. A high-density linkage map containing 1894 single nucleotide polymorphism (SNP) and 68 simple sequence repeats (SSR) markers was used to identify quantitative trait locus (QTL) for flag leaf length (FLL), second leaf length (SLL), third leaf length (TLL), fourth leaf length (FOLL), flag leaf area (FLA), second leaf area (SLA), third leaf area (TLA) and fourth leaf area (FOLA). In total, 57 QTLs underlying the top four leaf length and area traits were identified and mapped on chromosome 2H, 3H, 4H and 7H. Individual QTL accounted for 5.17% to 37.11% of the phenotypic variation in 2015 and 2016. A major stable QTL $qFLL_{2\text{-}2}\text{H}$ close to the marker 2HL_25536047 was identified on the long arm of chromosome 2H. The most important QTL clustered region at M_256210_824 - 2HL_23335246 on chromosome 2H was associated with FLL, SLL, FLA and SLA and explained high phenotypic variation. These findings provide genetic basis for improving the leaf morphology of barley. In addition, our results suggested that the top four leaves were significantly positively correlated with plant height and some yield-related traits.

Barley (*Hordeum vulgare* L.), as one of the earliest domesticated and most important cereal crops for humanity, is widely used for food, feed and malting. Leaf is the main organ of photosynthesis and plays a crucial role in determining yield of crops1. In cereals, the upmost three leaves, particularly the flag leaf, assimilate the most light energy and are main source of generation for carbohydrate production2–4. The top two leaves produce over 80% photosynthesis of the whole plant during grain filling4. Moreover, leaf size and shape are regarded as important traits determining photosynthesis capability and grain yield5–7. Therefore, it is necessary to disclose the genetic mechanism of leaf characteristics.

QTL underlying leaf morphological traits has been identified in some crops8–11, such as QTL controlling flag leaf length in rice8, QTL for flag leaf size and morphology in wheat10.

To date, the morphological traits of flag leaf have been widely studied in barley and were determined by polygenes with vulnerability to influence from environment12–18. Digel et al.19 identified PHOTOPERIOD-H1 (Ppd-H1) as a candidate gene controlling leaf size in barley. Xue et al.17 reported QTLs for FLL and FLW on chromosome 5H and 7H using a comprehensive DArT and SSR genetic map. Liu et al.18 identified two pleiotropic genomic regions on chromosome 2H and 7H controlling FLL, FLW, FLA and some physiological traits. The limitation of previous studies was the limited number of markers used. High-density map can improve the accuracy of QTL and precise location of important traits20.

Our group has constructed a high density of linkage map containing 1962 SNP and SSR markers using a barley DH population21, which has previously been used for identifying QTLs underlying yield-related traits22, physiological traits of flag leaf including net photosynthesis rate23 and mapping a new dwarf gene *btwd*123. The objectives of this study were to use this high density of linkage map to identify QTL associated with the topmost four leaf length and area traits; and to reveal the relationships among the topmost four leaves, plant height and yield-related traits. The detected QTLs and their closely linked markers can be used for marker-assisted selection (MAS) in barley breeding.
Methods
Experimental materials and field trial. The 122 doubled haploid (DH) lines from a cross between dwarf-barley cultivar Huaai11 and feed-barley cultivar Huaadama6 were obtained\(^2^)\). Huaai11 is a six-rowed, dwarf-barley cultivar derived from barley landrace, Daozifu Baoqingke. Huaadama6 is a two-rowed, feed-barley cultivar. In addition to the differences in plant height between two parents, they also showed distinct difference in leaf length and area traits. The DH lines and parents were sown in a plot with line length of 1.5 m and line interval of 0.2 m at the experimental farm of Huazhong Agricultural University, Wuhan, China in 2015 and 2016. The field trial followed a completely randomized block design, with 3 replications each year. Each DH and parental line was grown in two rows with eight seedlings in each row.

Trait measurement. At the stages of pre-filling and with fully unfolded flag leaves on the main stem, 6 plants in the middle of rows from each replication were randomly chosen to measure 8 morphological traits including flag leaf length (FLL, cm), second leaf length (SLL, cm), third leaf length (TLL, cm) and fourth leaf length (FOLL, cm), as well as flag leaf area (FLA, cm\(^2\)), second leaf area (SLA, cm\(^2\)), third leaf area (TLA, cm\(^2\)) and fourth leaf area (FOLA, cm\(^2\)). The leaf length was examined from leaf base to tip. Leaf area was calculated using width × length × 0.75\(^2^)\), in which the length and width were measured on the widest and longest part of the blade, respectively. The measurement of plant height (PH) and yield-related traits of all lines including main spike length (MGL), grain number per plant (GP), spikelet number per plant (SLP), grain weight per spike (GWS), grain weight per plant (GWP) and thousand grain weight (TWG) was described by Wang et al\(^2^)\).

Phenotypic data analysis. The mean values of each year were used for statistics, correlation analysis and QTL mapping. Descriptive statistics of all phenotypic data were performed using IBM SPSS Statistics 22 software. Correlation analysis between traits and their significance levels were calculated using the Pearson's correlation coefficients analysis method and two-tailed T test. The broad-sense heritability value was calculated using 

\[ h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/n + \sigma_r^2/n} \]

where the genotype variance \(\sigma_g^2 = (MS_g - MS_e)/rn\), genotype and environment interaction variance \(\sigma_{ge}^2 = (MS_{ge} - MS_{g+e})/rn\), error variance \(\sigma_r^2 = MS_{error}\), \(MS_g\), \(MS_e\) and \(MS_{error}\) represent genotype mean square, genotype and environment interaction mean square and error mean square, respectively, \(r\) and \(n\) represent the number of replicates of each genotype and environments, respectively. The variance of the components was estimated using a general linear model (GLM).

QTL analysis. The genetic map containing 1894 SNP and 68 SSR markers\(^2^)\) was used to identify QTL using WinQTLCart v2.5 with composite interval mapping\(^2^)\). The window size and walking speed were set to 10 CM, and 1 CM, respectively. After performing 1000 permutations, a LOD (Logarithm of the odds) threshold was obtained\(^2^)\). The QTL with LOD score greater than LOD threshold was considered to be an important QTL using the significance level (\(P = 0.05\)) as the LOD threshold. QTL was considered as minor or major, depending on the phenotypic variance in a primary genetic analysis, if the phenotypic variance was more than 15% which was considered as major QTL\(^2^)\). QTL nomenclature followed the principle of McCouch\(^2^)\). The QTL location on the map was drawn using MapChart ver. 2.2 software\(^2^)\).

Results
Phenotypic data evaluation. Phenotypes of the parents and the distribution among DH lines for FLL, SLL, TLL, FOLL, FLA, SLA, TLA, and FOLA in two consecutive years (year 2015 and 2016) were shown in Table 1. The values of all traits in Huaadama6 were higher than those in Huai11, and the two parents showed significant differences in all traits (\(p < 0.01\)). The frequency distributions of those traits in two years were consecutive (Supplementary Fig. S1), and the values of skewness and kurtosis for all traits were among 1 and 1, indicating all traits controlled by multiple genes (Table 1). ANOVA showed that genotypic effects of 122 DH lines and parents were significant for all traits (\(p < 0.01\)). Year effects were significant (\(p < 0.05\)) for all traits except SLL, FOLL and SLA. Genotype × year interactions were also significant (\(p < 0.05\)) for all traits except SLA. (Table 2). The variable coefficients varied from 13.57% to 33.94% in 2015, and 11.12% to 30.89% in 2016. The broad-sense heritability \((h^2)\) of all traits ranged from 88.54% to 91.11%, indicating that genetic factors played an important role in determining these traits.

Correlation analysis. The Pearson correlation coefficients \((r)\) between topmost four leaves traits, plant height and yield-related traits were calculated based on the mean value of each year (Table 3). The top four leaf traits showed significant positive correlation with MSL, PH and TWG (\(p < 0.01\)). GP was significantly and negatively correlated with FLL \((r = -0.214, P < 0.05\) in 2015, \(r = -0.345, P < 0.01\) in 2016) and FLA \((p < 0.01)\). Significant negative correlations were also detected between SLP and FLL \((p < 0.01)\), SLL \((r = -0.216, P < 0.05\) in 2015, \(r = -0.249, P < 0.01\) in 2016), FLL \((p < 0.01)\), SLL \((r = -0.218, P < 0.05\) in 2015, \(r = -0.235, P < 0.01\) and TLA \((r = -0.189, P < 0.05\) in 2015). GWS was significantly positively correlated with TLL, FOLL, SLA, TLA and FOLA in 2015, and all leaf traits in 2016 (\(P < 0.01\)). Significant positive correlations between GWP and top four leaves traits except FLL, FLA, TLA and FOLA in 2016 were observed (Table 3).

QTL analysis. In total, 57 QTLs for top four leaves length and area were identified on chromosome 2H (22 QTLs), 3H (8 QTLs), 4H (12 QTLs) and 7H (15 QTLs). Thirty-one and 26 QTLs were detected in 2015 and 2016, respectively. The individual QTL accounted for 5.17–37.11% and 5.28–35.64% phenotypic variation in 2015 and 2016, respectively (Supplementary Table S1). Among the 57 QTLs, 26 QTLs (46%) were detected in the both years.

Flag-leaf length. A total of 29 QTLs for the leaf length traits were detected. All positive alleles were contributed by Huaadama6. Six QTLs for FLL trait over two years were identified on 2H (5 QTLs) and 7H (1 QTL) with
qFOLL3-1 was on the verge of the SNP marker 3_511749149. The rest of QTLs were only identified in major QTL qFOLL3-1; 7H (2 QTLs), contributing 5.28–31.26% to phenotypic variation (Supplementary Table S1). Only phenotypic variation in 2015.

qTLL4-2 and qTLL7-1 were identified in one year and accounted for 5.50–28.25% phenotypic variations (Supplementary Table S1). Nine QTLs for SLL trait were identified on 2H (3 QTLs), 3H (2 QTLs), 4H (1 QTL) and 7H (3 QTLs) with individual QTL accounting for 5.30–37.11% phenotypic variation, respectively. However, four QTLs, qFLL2-2, qFLL2-5, qFLL7-2 and qFLL7-3 were detected in one year and accounted for 5.30%, 12.39%, 6.62% and 9.02% of phenotypic variations ranging from 5.30% to 37.11% (Supplementary Table S1). However, four QTLs, qFLL2-4, qFLL2-5, qFLL2-8 and qFLL7-2 were detected in one year and accounted for 5.30%, 12.39%, 6.62% and 9.02% of phenotypic variation, respectively. Only one common QTL qFLL2-2 was identified on chromosome 2H across two years and explained 37.11% and 30.71% of phenotypic variations in 2015 and 2016, respectively. The major QTL qFLL2-2 was near to the SNP marker 2HL_25536047.

**Second-leaf length.** Nine QTLs for SLL trait were identified on 2H (3 QTLs), 3H (2 QTLs), 4H (1 QTL) and 7H (3 QTLs) with individual QTL accounting for 5.39–34.64% of phenotypic variations (Supplementary Table S1). QTLs qSLL2-2, qSLL3-1 and qSLL7-1 were identified in both years. qSLL2-2 accounted for 15.22% (year 2015) and 34.64% (year 2016) of phenotypic variation, qSLL3-1 accounted for 12.04% (year 2015) and 28.25% (year 2016) of phenotypic variation.

**Third-leaf length.** For TLL, seven QTLs were detected on 2H (2 QTLs), 3H (2 QTLs), 4H (2 QTLs) and 7H (1 QTL) with individual QTL accounting for 5.50–28.25% phenotypic variations (Supplementary Table S1). The QTLs qTLL2-2, qTLL3-1 and qTLL4-2 were identified in the both years. The qTLL2-2 explained 27.43% of phenotypic variation in 2015, and 19.74% in 2016. The major QTL qTLL2-2 was adjacent to the SNP marker 2HL_25536047. The rest of QTLs were identified in one year and accounted for 5.39–10.23% of phenotypic variations.

**Fourth-leaf length.** For FOLL, seven QTLs were identified on 2H (2 QTLs), 3H (2 QTLs), 4H (1 QTL) and 7H (2 QTLs), contributing 5.28–31.26% to phenotypic variation (Supplementary Table S1). Only qFOLL3-1 was identified in both years, and explained 16.22% (year 2015) and 31.26% (year 2016) of phenotypic variation. The major QTL qFOLL3-1 was on the verge of the SNP marker 3_511749149. The rest of QTLs were only identified in one year and explained 5.28–21.49% of phenotypic variations.
Flag-leaf area. In total, 28 QTLs for leaf area traits were identified. Ten QTLs for FLA trait across two years were detected with phenotypic variations explained by each QTL varying from 5.17% to 23.46% (Supplementary Table S1). These QTLs were mapped on 2H (3 QTLs), 3H (2 QTLs), 4H (3 QTLs) and 7H (2 QTLs). Six positive alleles on 2H and 7H were contributed by Huadamai6, and the positive alleles from Huadamai6 increased the FLL, SLL, SLA, TLL, FOLL, TLA and FOLA. This QTL cluster qTLL4-2 and the favorite alleles from Huadamai6 increased these traits simultaneously. The C4 on chromosome 4H was identified in both years and contributed 10.31% (2015) and 23.46% (2016). The remaining QTLs were detected in one year and accounted for 6.99–35.64% of phenotypic variations, and all positive alleles were contributed to the QTL cluster associated with top four leaf length and area traits were found on chromosome 2H (two clusters), 3H (one cluster), 4H (one cluster) and 7H (one cluster) (Fig. 1, Table 4). These QTLs increased top four leaf length and area were contributed by Huadamai6 alleles in these QTL cluster regions. Six QTLs were co-localized in C1 on chromosome 2H underlying TLL, FOLL, SLL, TLA and FOLA. The major QTL qFLL2-2 in C2 was identified in two years and co-localized with qSL2-2 and qFLL2-2, the positive alleles from Huadamai6 increased the FLL, SLL and FLLA. A major QTL for FOLL (qFOLL3-1) in C3 clustered with two stable QTL qSL3-1 and qFOLL3-1, and the favorite alleles from Huadamai6 increased these traits simultaneously. The C4 on chromosome 4H contained stable QTLs (qTLA4-2, qFOLL4-2 and qFOL4-2) controlling TLL, TLA and FOLA. Nine QTLs were co-localized in C5 on chromosome 7H underlying FLL, SLL, SLLA, TLL, FOLL, TLA and FOLA. This QTL cluster contained two stable QTLs (qSL7-1 and qSL7-2) controlling SLL, SLA.

| Trait | 2015 | 2016 |
|-------|------|------|
| PH    | 0.275 | 0.282 |
| TLL   | 0.287 | 0.315 |
| FLL   | 0.288 | 0.315 |
| SLL   | 0.308 | 0.315 |
| SLA   | 0.311 | 0.315 |
| FOLL  | 0.317 | 0.315 |
| TLA   | 0.318 | 0.315 |
| FOLA  | 0.333 | 0.315 |

Flag-leaf area. In total, 28 QTLs for leaf area traits were identified. Ten QTLs for FLA trait across two years were detected with phenotypic variations explained by each QTL varying from 5.17% to 23.46% (Supplementary Table S1). These QTLs were mapped on 2H (3 QTLs), 3H (2 QTLs), 4H (3 QTLs) and 7H (2 QTLs). Six positive alleles on 2H and 7H were contributed by Huadamai6, and the positive alleles from Huadamai6 increased the FLL, SLL, SLA, TLL, FOLL, TLA and FOLA. This QTL cluster qTLL4-2 and the favorite alleles from Huadamai6 increased these traits simultaneously. The C4 on chromosome 4H was identified in both years and contributed 10.31% (2015) and 23.46% (2016). The remaining QTLs were detected in one year and accounted for 6.99–35.64% of phenotypic variations, and all positive alleles were contributed to the QTL cluster associated with top four leaf length and area traits were found on chromosome 2H (two clusters), 3H (one cluster), 4H (one cluster) and 7H (one cluster) (Fig. 1, Table 4). These QTLs increased top four leaf length and area were contributed by Huadamai6 alleles in these QTL cluster regions. Six QTLs were co-localized in C1 on chromosome 2H underlying TLL, FOLL, SLL, TLA and FOLA. The major QTL qFLL2-2 in C2 was identified in two years and co-localized with qSL2-2 and qFLL2-2, the positive alleles from Huadamai6 increased the FLL, SLL and FLLA. A major QTL for FOLL (qFOLL3-1) in C3 clustered with two stable QTL qSL3-1 and qFOLL3-1, and the favorite alleles from Huadamai6 increased these traits simultaneously. The C4 on chromosome 4H contained stable QTLs (qTLA4-2, qFOLL4-2 and qFOL4-2) controlling TLL, TLA and FOLA. Nine QTLs were co-localized in C5 on chromosome 7H underlying FLL, SLL, SLLA, TLL, FOLL, TLA and FOLA. This QTL cluster contained two stable QTLs (qSL7-1 and qSL7-2) controlling SLL, SLA.

Table 3. Correlation analysis between top four leaves traits and yield-related traits based on data from each year. * Significant at 0.05, ** Significant at 0.01 level, respectively. Values above the diagonal are correlation coefficients in 2016; values below the diagonal are correlation coefficients in 2015. MSL, main spike length; GP, grain number per plant; SLP, spikelet number per plant; GWS, grain weight per spike; GWP, grain weight per plant; TGW, thousand grain weight; PH, Plant height; FLL, flag leaf length; SLL, second leaf length; TLL, third leaf length; FOLL, fourth leaf length; FLA, flag leaf area; SLA, second leaf area; TLA, third leaf area; FOLA, fourth leaf area.
Discussion

Leaf is the main organ of photosynthesis, and the top two leaves produce over 80% of the net photosynthesis product during grain filling, particularly the flag leaf, which assimilates most of the light energy and converts it to 41–43% of the carbohydrates for kernel filling\(^3,30\). Thus, leaf morphological traits, such as length, width and area,
are considered as important components in determining grain yield potential. Liu et al. have reported the correlation and QTL of physiological and morphological traits of flag leaf in barley. However, QTL analysis related to the uppermost four leaf length and area traits has not been reported.

In our study, we detected 57 QTLs for eight leaf morphological traits: FLL, SLL, TLL, FOLL, FLA, SLA, TLA and FOLA. Six QTLs for FLL in two years were located on chromosome 2H and 7H (Fig. 1, Supplementary Table S1). A major QTL qFLL2-2 that was stable and not influenced by environment was close to the SNP marker 2HL_25536047 on the chromosome 2H long arm. QTL for flag-leaf length was previously reported on chromosome 2H, 3H, 5H and 7H. The QTL qFLL2-2 was different from those QTL reported by Elberse et al. on 2HS, and is likely a new QTL. The QTL qFLL2-4 on 2H and nearby the SNP marker 2HL_13648618 was associated with row number (Vrs1). The QTL qFLL7-2 on 7H is different from the QTL on 7HS reported by Xue et al.

As expected, three coincident genetic regions were detected between FLL and FLL. For example, qFLLA-2, qFLLA-4 and qFLLA-7-2 were located at the same regions as qFLLA-2, qFLLA-5 and qFLLA-7-2. In this study, we detected 10 QTLs associated with FLL on 2H, 3H, 4H and 7H over two years (Fig. 1, Supplementary Table S1). A stable QTL qFLLA-2 was detected on 2H. The QTL qFLLA-2 was close to the SNP marker 2HL_25536047 and SSR marker GBM1218. Li et al. detected Qla1.2 for FLL close to the marker HVHOTR1 on 2H in a B.C2 population. The genetic-linkage map of Varshney et al. illustrated the marker HVHOTR1 was near to the marker GBM1218, indicating that qFLLA-2 was likely the same locus to the QTL Qla2.1.

QTL for leaf morphological traits from the second to the fourth leaf of barley has not been studied in detail. In our study, 41 QTLs associated with SL, TLL, FOLL, SLA, TLA and FOLA were detected over two years with phenotypic variation ranging from 5.28% to 35.64%. The six traits were controlled by genetic regions on 2H, 3H, 4H and 7H (Fig. 1, Supplementary Table S1). Wherein, ten QTLs were detected in both years and included three major QTLs. These major QTLs can be used in MAS to improve the leaf morphological traits from the second leaf to the fourth leaf.

In the present study, we detected 57 QTLs associated with top four leaf length and area traits, including five QTL clusters on 2H, 3H, 4H and 7H. Our previously reported QTL for FLL and FLA in C2 on chromosome 2H also influenced those traits in other populations. The most important QTL cluster region at the M_256210_824 - 2HL_23352546 was close to the marker GMS3 on 2H, associated with QTLs for FLL, FLA and SLA (Fig. 1, Table 4, Supplementary Table S1). QTLs affecting the chlorophyll, thousand grain weight and plant height were previously located on 2H close to the SSR marker GMS3 inferred from GrainGenes (http://wheat.pw.usda.gov/GG3/), which was in the same region detected here. This region also has QTL associated with grain number per spike and net photosynthetic rate, stomatal conductance and chlorophyll content of the leaf flag physiological traits previously detected in the same population, indicating this QTL cluster region not only affected the morphology of leaf, but also had positive effect on the physiological traits of flag leaf.

The second noticeable coincident QTL region was Bmag13 - 3_504106156 on 3H containing qSLA1-1, qTLA1-1 and qFOLL1-1 underlying SL, TLL and FOLL (Fig. 1, Table 4). Ren et al. detected QTL for heading date on 3H, which was linked with the marker Bmag13 in the same population, and near to this QTL cluster region. The QTLs underlying rachis internode length, plant height, grain yield, number of tillers and days until heading were near to the marker Bmag13 in this clustered QTL region.

The third QTL cluster was in the region 4_42378533 - 4_8073993 on 4H determined SLL, TLL, FOLL, SLA, TLA and FOLA (Fig. 1, Table 4). According to GrainGenes (http://wheat.pw.usda.gov/GG3/), this region affecting ears per m2, heading date, thousand kernel weight, plant height and yield were located on 4H, and near to the SSR marker HVM40 in this clustered QTL region. The fourth QTL cluster in the region 7_542140217 - 7HL_3360534 was close to the SSR marker GBM1102 on 7H, which was associated with FLA, SLL, TLA, FOLL, TLA and FOLA (Fig. 1, Table 4). Search against GrainGenes (http://wheat.pw.usda.gov/GG3/) found that QTLs affecting plant height, heading date and single plant yield were located on 7H adjacent to the marker GBM1102, which was similar to this region detected here. Considerable to note was the above-mentioned four QTL cluster regions, each containing QTL controlling plant height and heading date. Previous studies reported that plant-height and heading-date traits had significant effect on grain yield. The co-localization of these QTLs likely resulted from closely linked QTL or pleiotropic QTL.

The leaf is the main organ of photosynthesis, in which the top three leaves, especially the flag leaf, absorb most of the plant’s light energy and is the main source of carbohydrate production. In this study, the top four leaf traits were significantly positively correlated with yield-related traits, such as MSL and TGW, with the highest correlation coefficient between flag leaf and TGW. Significant positive correlation was also detected between flag leaf and GWS and GWP (Table 3). Previous studies have also reported the importance of leaf traits such as leaf length, leaf angle and leaf area on yield. We detected co-located QTL between the top four leaf traits and yield-related traits, such as MSL, SLP, GWP and TGW, on 2H, 4H and 7H. Therefore, QTLs for different leaf traits co-localized with yield-related traits can be effectively utilized in MAS. In addition, significant positive correlations between the top four leaves and PH, and QTL for FLL (qFLL2-2) near the dwarf gene btwd1 on chromosome 7H suggested that leaf size might be affected by plant height.

A major QTL was detected for FLL, SLL, FLA and SLA on 2HL. Blast SNP-tag sequence searched against http://floresta.eead.csic.es/barleymap using the Barleymap program to anchor the QTL marker (2HL_25536047) in the Barke × Morex POPSEQ population. The QTL annotation by Barleymap program found three potentially relevant functional genes, FHY3/FAR1, gibberellin-regulated gene and SAUR gene. Tang et al. reported FHY3 (FAR-RED ELONGATED HYOCOTYL3) and FAR1 (FAR-RED IMPAIRED RESPONSE1) were transcriptional factors derived from ancient transposases in evolutionary process. They were involved in chlorophyll biosynthesis via the activation of HEM1 gene expression in Arabidopsis thaliana, and may have a wide range of functions in plant growth and development. Aubert et al. described that gibberellin-regulated, gene-encoded and gibberellin-regulated family proteins have some role in plant development. Gil and Green found that SAUR gene expressed in growing hypocotyls or other extended tissues had a certain function in regulating cell elongation.
The above-mentioned genes might be considered as candidates in determining these traits. Of course, we cannot rule out the role of other genes in this QTL region. At present, the function of these genes on barley is unknown and will be characterized in future studies.

In this research, a total of 57 QTLs associated with top four leaf length and area traits were identified with individual QTL explaining 5.17% and 37.11% of the phenotypic variation. Five clustered QTL regions were detected on chromosome 2H, 3H, 4H, and 7H. Ten QTLs were co-localized in the C2 cluster, such as qFLL2-2, qSLL2-2, qFLA2-2, and qSLA2-3. Two major QTLs qFLL2-2, qSLL2-2 and one stable qFLA2-2 explained high phenotypic variation in this QTL cluster. Two stable QTLs qSLL3-1, qTLL3-1 and one major qFOLL3-1 on 3H detected in two years were associated with SLL, TLL and FOLL and co-localized in the C3. The C4 on 4H contained stable QTLs (qTLL4-2, qTLA4-2 and qFOLA4-1) controlling TLL, TLA and FOL. Five QTLs were co-localized in the C5 on 7H associated with TLL, FOLL, FLA, TLA and FOL. These QTL clusters could be used as target regions for improving leaf morphology of barley.

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**Author Contributions**

B.D. performed this study, statistical analysis and manuscript writing. Conceived and designed the experiments: D.S., G.S. Performed the experiments: L.L., Q.W., X.R. Analyzed the data: Q.W., X.R. Contributed reagents/materials/analysis tools: X.R., D.S., G.S. Wrote the paper: D.S., G.S. C.L. produced the Huaai 11 and Huadamai 6 DH population. All authors have read and approved the manuscript.

**Additional Information**

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