



Article Genetic Diversity and Genome-Wide Association Study of Architectural Traits of Spray Cut Chrysanthemum Varieties

Daojin Sun, Luyao Zhang, Jiangshuo Su, Qi Yu, Jiali Zhang, Weimin Fang, Haibin Wang, Zhiyong Guan, Fadi Chen and Aiping Song *[®]

State Key Laboratory of Crop Genetics and Germplasm Enhancement, Key Laboratory of Landscaping, Ministry of Agriculture and Rural Affairs, Key Laboratory of Biology of Ornamental Plants in East China, National Forestry and Grassland Administration, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China; 2020104106@njau.edu.cn (D.S.); 2019804185@njau.edu.cn (L.Z.); sujiangshuo@njau.edu.cn (J.S.); 2019104103@njau.edu.cn (Q.Y.); 2020804199@stu.njau.edu.cn (J.Z.); fangwm@njau.edu.cn (W.F.); hb@njau.edu.cn (H.W.); guanzhy@njau.edu.cn (Z.G.); chenfd@njau.edu.cn (F.C.) * Correspondence: aiping_song@njau.edu.cn

Abstract: The architecture of spray cut chrysanthemum is crucial for the quality and quantity of cut flower production. However, the mechanism underlying plant architecture still needs to be clarified. In this study, we measured nine architecture-related traits of 195 spray cut chrysanthemum varieties during a two-year period. The results showed that the number of upper primary branches, number of lateral flower buds and primary branch length widely varied. Additionally, plant height had a significant positive correlation with number of leaf nodes and total number of lateral buds. Number of upper primary branches had a significant negative correlation with primary branch diameter, primary branch angle and primary branch length. Plant height, total number of lateral buds, number of upper primary branches, stem diameter, primary branch diameter and primary branch length were vulnerable to environmental impacts. All varieties could be divided into five categories according to cluster analysis, and the typical plant architecture of the varieties was summarized. Finally, a genome-wide association study (GWAS) was performed to find potential functional genes.

Keywords: spray cut chrysanthemum; GWAS; plant architecture; statistical analysis

1. Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the four most popular cut flowers worldwide and is an important component in the floral industry [1]. Branching is one of the most important agricultural traits of chrysanthemum, playing an important role in morphological formation, and affecting ornamental quality and economic value. Operations involving decapitation and/or removal of lateral buds constitute nearly 1/3 of production costs [2]. The growth and development of chrysanthemum are largely affected by various environmental factors. As a quantitative trait controlled by multiple genes, branching is affected by both the environment and the genetic background [3,4], and the underlying molecular mechanism that governs branching still needs to be elucidated.

Shoot branching is controlled by various hormone signaling pathways, including auxin, strigolactones (SLs), cytokinins (CKs) and brassinosteroids (BRs) [5]. Apical dominance is a universal phenomenon in plants and is mainly maintained by auxin. According to the auxin canalization model, auxin can act as a second messenger to regulate downstream signals or functions [6]. Moreover, auxin acts upstream of SLs and CKs, which promote and inhibit shoot branching, respectively. The biosynthesis of CKs is repressed by auxin, as the key CK biosynthesis-related enzyme Isopentenyltransferase (IPT) is downregulated by auxin [7,8]. The biosynthesis of SLs is activated by auxin, as the key SL biosynthesis-related genes *carotenoid cleavage dioxygenase*7 (*CCD7*) and *carotenoid cleavage dioxygenase*8 (*CCD8*) are upregulated by auxin [9]. Through the BR signaling component



Citation: Sun, D.; Zhang, L.; Su, J.; Yu, Q.; Zhang, J.; Fang, W.; Wang, H.; Guan, Z.; Chen, F.; Song, A. Genetic Diversity and Genome-Wide Association Study of Architectural Traits of Spray Cut Chrysanthemum Varieties. *Horticulturae* 2022, *8*, 458. https://doi.org/10.3390/ horticulturae8050458

Academic Editor: Luigi De Bellis

Received: 26 April 2022 Accepted: 18 May 2022 Published: 19 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). brassinazole-resistant1 (BZR1), BRs can promote increased tillering in rice [10] and bud outgrowth of tomato [11]. Sugars are a major source of carbon and energy in plants. A recent study indicated that sugars can promote initial bud outgrowth and downregulate the expression levels of *BRANCHED1* (*BRC1*) [12]. However, the gene regulatory network governing plant architecture still needs to be elucidated, and new key genes and pathways need to be identified for continued research.

The environment also plays an important role in determining plant architecture [13]. Treatments involving drought, heat and drought plus heat were shown to reduce the shoot outgrowth of *Pinus edulis* [14]. Leaf distribution, branch distribution and canopy photosynthetic rate were also influenced by temperature in potato [15]. Light is a pivotal environmental factor that influences the growth of shoots, and increasing light intensity can promote the growth of branches in herbaceous and tree species [16–18]. Nitrogen is an important nutrient element in the soil and can alter the amino acid content and influence the branch growth of plants [19]. *TaNAC2-5A* is a nitrate-inducible gene and can increase tiller numbers and spikelet numbers of wheat [20]. Various environmental factors influence the architecture of plants, which reflects their adaptation and evolution.

Genome-wide association studies (GWASs) are efficient tools to exploit complex genetic mechanisms through associations of agronomic traits with single-nucleotide polymorphisms (SNPs) within a group of individuals or natural inbred lines [21,22]. For chrysanthemum, GWASs have been used to identify genes related to waterlogging resistance and flower color [23–25], providing a reference for transgenic breeding. GWASs have also been used to identify key regulatory genes controlling plant architecture. In *Brassica napus*, plant height, branch initiation height and branch number have been used to identify functional loci [26–29]. In rice, plant height, tillering, and panicle morphology were examined, and the gibberellic acid (GA) signaling-related gene *OsSPY* was found to be associated with semidwarfism and small panicles [30]. However, no architecture-related research has been conducted in spray cut chrysanthemum, and the molecular mechanism controlling architecture still needs to be elucidated.

In this study, we performed phenotypic measurements and a statistical analysis on nine architectural traits of 195 spray cut chrysanthemum varieties in two continuous years (2019 and 2020). Because the environments of these two years were different, we defined the environment in 2019 as EN2019 and the environment in 2020 as EN2020. The effect of different environmental factors, variation and relationships of different traits and cluster analysis of all spray cut chrysanthemum were analyzed. GWAS was also performed to find latent functional genes controlling architectural traits in chrysanthemums.

2. Materials and Methods

2.1. Plant Materials

A total of 195 spray cut chrysanthemum varieties were used in this study, including varieties developed by Nanjing Agricultural University and those collected from around the world. These varieties were maintained at the Chrysanthemum Germplasm Resource Preserving Centre of Nanjing Agricultural University, China (E118°85', N31°95'). The 195 varieties evaluated during the two years are listed in Table S1.

2.2. Phenotypic Evaluation of Architectural Traits

Seedlings were planted in seedbeds in June 2019 and June 2020. Vigorously growing and similarly appearing rooted seedlings were selected and transplanted into a greenhouse in July of the same year. Fifty seedlings of each variety were planted in accordance with a row spacing of 10 cm \times 10 cm, and conventional field management practices were performed. Flowering occurred from late October to late November. The monthly average temperature, monthly precipitation and monthly average relative humidity of EN2019 and EN2020 in planting location, Jiangning District, Nanjing, China (E118°85', N31°95') were shown in Table S2.

Nine phenotypic traits were measured. 1. For plant height, the height of the aboveground part of the plant was measured with a ruler, with a precision of 0.1 cm; 2. for number of leaf nodes, the number of leaf nodes on the trunk of the aboveground part of the plant was counted visually; 3. for total number of lateral buds, the number of nodes of all germinating buds or sprouted branches on the stem of the plant was counted visually; 4. for number of upper lateral branches, the number of all primary branches within 15 cm from the top of the plant was counted visually; 5. for number of lateral flower buds, the total number of flower buds on all primary branches was counted visually; 6. for stem diameter, the diameter at 40 cm below the top of the plant was measured with a digital Vernier caliper with precision of 0.01 mm; 7. for branch diameter, the diameter at 1/2 of the three nearest primary branches around the main bud was measured with a digital Vernier caliper with a precision of 0.01 mm; 8. for branch angle, the angle of the three nearest primary branches around the main bud was measured with a protractor; 9. for branch length, the length of all primary branches was measured with a ruler, with a precision of 0.1 cm. At the full-flowering stage, the measurement was performed on six plants for each variety, and the mean values were taken.

2.3. Phenotypic Data Analysis

Microsoft Excel 2019 was used for basic descriptive statistical analysis of the 9 architecturerelated phenotypic traits of the 195 cut chrysanthemum varieties in EN2019 and EN2020 environments, and IBM SPSS 25.0 statistical software was used for correlation analysis of the EN2019 and EN2020 data. Significant differences (paired-sample t tests) were assessed and violin mapping and cluster analysis of two environmental data were conducted by R 4.0.4 (https://www.r-project.org/, accessed on 7 December 2021).

2.4. GWAS and Candidate Gene Annotation

In our previous study [31], 199 chrysanthemum accessions were sequenced, of which forty-four spray cut chrysanthemum varieties were also measured in our study and the list is shown in Table S3. In order to obtain more meaningful information, we performed GWAS using these raw sequencing data. SLAF-seq raw reads whose quality scores were <30 and separated by barcodes were discarded. The highest depth tag in each SLAF was chosen as a reference due to the lack of a reference genome sequence. The qualified sequencing data of the samples were aligned to the genome reference sequence of chrysanthemum using Burrow–Wheeler Aligner (BWA) V0.7 (http://bio-bwa.sourceforge.net/, accessed on 7 December 2021) [32], and then SNP sites were detected by SAMtools V1.4 (http://samtools.sourceforge.net/, accessed on 7 December 2021) [33]. After removing the SNPs with a sequencing depth less than 3, a data loss percentage greater than 20%, and a minor allele frequency (MAF) less than 5%, 191,417 high-quality SNPs were ultimately identified for further analysis.

GCTA software V1.93 (https://yanglab.westlake.edu.cn/software/gcta/#Overview, accessed on 7 December 2021) [34] was used for principal component analysis (PCA) and construction of a kinship matrix, yielding an eigenvector principal component (PC) matrix of all the individuals and a kinship matrix comprising data between every pair of individuals. Combining the data of the nine phenotypic traits and the SNP sequencing data, a GWAS was conducted via the compressed mixed linear model (cMLM) of GAPIT software V3 (https://www.zzlab.net/GAPIT/, accessed on 7 December 2021) [35] and via the cMLM and mixed linear model (MLM) of TASSEL software V5.0 (https://www.maizegenetics.net/tassel, accessed on 7 December 2021) [36]. The mean values were used for the GWAS, and the significance threshold was set at $p \leq 0.001$. As a result, the SNPs found to be significantly associated with the phenotypic data and the phenotypic variance explained (PVE) were identified for gene mining.

According to the significant SNP sites detected by cMLM model of TASSEL, candidate genes within 300 k of SNP sites were found. The function of genes was annotated via The Arabidopsis Information Resource (TAIR) website (https://www.arabidopsis.org/,

accessed on 7 May 2022) by BLASTX [37]. Through the functional annotations of Arabidopsis and other function reported in other plants, the genes related to plant architecture, hormone signaling pathways or plant development regulation were further selected as final candidate functional genes.

3. Results

3.1. Analysis of Significantly Different Architectural Traits of Spray Cut Chrysanthemum between EN2019 and EN2020

To explore the effects of different years on architectural traits, nine architectural traits in EN2019 and EN2020 were compared, and their significance was assessed (paired-sample t tests). As shown in Figure 1, there were significant differences in plant height, total number of lateral buds, number of upper primary branches, stem diameter, primary branch diameter and primary branch length between EN2019 and EN2020, while there were no significant differences in number of leaf nodes, number of lateral flower buds or primary branch angle. Among these traits, the median plant height, stem diameter, primary branch diameter and primary branch length in EN2019 were larger than those in EN2020. The median total number of lateral buds in EN2019 was similar to that in EN2020, while the median number of upper primary branches in EN2019 was smaller than that in EN2020.



Figure 1. Violin plots indicating variation in architectural traits of 195 spray cut chrysanthemum varieties in EN2019 and EN2020. (a) Plant height; (b) Number of leaf nodes; (c) Total number of lateral buds; (d) Number of upper primary branches; (e) Number of lateral flower buds; (f) Stem diameter; (g) Primary branch diameter; (h) Primary branch angle; (i) Primary branch length. Note: ***, ** and * indicate significant differences at the 0.001, 0.01 and 0.05 probability levels, respectively.

3.2. Descriptive Statistics of the Architectural Characteristics of Spray Cut Chrysanthemum in EN2019 and EN2020

The basic statistical analysis results of the data of the nine phenotypic traits of the 195 spray cut chrysanthemum species in EN2019 and EN2020 are shown in Table 1. Although there were different coefficients of variation (CVs) for all the traits, the same traits

in both years showed a significant positive correlation. In EN2019, the CV varied from 12.67% to 85.84%, among which the CV of stem diameter was the smallest and the CV of number of lateral flower buds was the largest. In EN2020, the CV varied from 13.30% to 73.81%, and traits with extreme values were the same as those in EN2019. These results showed that stem diameter trait was rather stable, while number of lateral flower buds varied among the different spray cut chrysanthemum varieties. In addition, compared with the other traits, primary branch length and number of upper primary branches also was associated with larger CVs, which were more than 30%.

 Table 1. Phenotypic characteristics of the architectural traits of 195 spray cut chrysanthemum varieties.

Trait	Environment	Max	Min	Rang	Mean	SD	<i>CV</i> /%	Skewness	Kurtosis	r
Plant height/cm	EN2019	153.67	36.10	117.57	101.84	21.20	20.82	-0.22	-0.16	0.794 **
	EN2020	141.42	42.98	98.43	94.67	19.89	21.01	-0.15	-0.55	
Number of leaf nodes	EN2019	73.33	19.17	54.17	46.51	8.62	18.52	0.24	0.93	0.701 **
	EN2020	79.50	27.17	52.33	47.32	9.82	20.75	0.63	0.41	
Total number of lateral buds	EN2019	73.33	19.17	54.17	45.40	8.94	19.68	0.20	0.81	0.649 **
	EN2020	79.17	26.33	52.83	46.60	9.77	20.96	0.57	0.43	
Number of upper primary	EN2019	13.00	2.00	11.00	5.04	1.60	31.75	1.56	4.16	0 502 **
branches	EN2020	15.33	2.67	12.67	5.95	2.13	35.82	1.26	2.16	0.593 **
Number of lateral flower	EN2019	52.67	2.83	49.83	10.83	9.30	85.84	2.45	6.69	0 700 **
buds	EN2020	56.50	2.67	53.83	11.25	8.30	73.81	2.71	9.85	0.738 **
Stem diameter/mm	EN2019	7.87	3.62	4.24	5.54	0.70	12.67	0.25	0.58	0.618 **
	EN2020	7.21	2.77	4.44	5.07	0.67	13.30	-0.07	0.86	
Primary branch	EN2019	4.40	1.24	3.16	2.18	0.46	20.90	1.34	3.35	0 710 **
diameter/mm	EN2020	3.88	1.25	2.62	2.09	0.45	21.40	1.14	2.26	0.712 **
Primary branch angle/°	EN2019	45.00	16.22	28.78	31.49	4.72	14.99	0.22	0.28	0.718 **
	EN2020	53.38	20.11	33.17	31.03	4.30	13.85	0.80	3.11	
Primary branch length/cm	EN2019	78.08	2.22	75.86	16.47	8.75	53.11	3.15	15.82	0.521 **
	EN2020	54.96	1.63	53.33	13.39	7.77	58.06	3.04	11.42	

Note: *r* refers to the Pearson correlation coefficient between the two environments (EN2019 and EN2020). ** indicates a significant difference at the 0.01 probability level.

3.3. Correlation Analysis of Architectural Traits in EN2019 and EN2020

Table 2 shows the Pearson correlations between the nine phenotypic traits in EN2019 and EN2020. In total, there were 72 pairs of architecture correlations in EN2019 and EN2020. Among all the architectural traits, the positive correlation between the number of leaf nodes and total number of lateral buds was the strongest, whose correlation coefficients in EN2019 and EN2020 were 0.913 and 0.986, respectively, followed by the positive correlation between primary branch length and primary branch diameter, whose correlation coefficients were 0.639 and 0.661, respectively. Similarly, the negative correlation between primary branch length and number of upper primary branches in EN2019 was the strongest, with a correlation coefficient of -0.317; in EN2020, the negative correlation between primary branch diameter and number of upper primary branches was the strongest, with a correlation coefficient of -0.466.

3.4. Cluster Analysis of the Architectural Traits of 195 Spray Cut Chrysanthemum Species

Cluster analysis was performed using both the K-means clustering algorithm and the Pedigree clustering algorithm based on the nine architectural traits during the twoyear period (Figure 2). The best preset number of K calculated by K-means in EN2019 and EN2020 was 5 (Figure 2a,b), which was verified by pedigree clustering (Figure 2c,d). However, in EN2019, the 195 spray cut chrysanthemum varieties were divided into six categories according to the pedigree clustering diagram, which was inconsistent with the results of the K-means clustering; in EN2020, the distribution of varieties in the pedigree clustering diagram.

EN2020 EN2019	Plant Height	Number of Leaf Nodes	Total Number of Lateral Buds	Number of Upper Primary Branches	Number of Lateral Flower Buds	Stem Diameter	Primary Branch Diameter	Primary Branch Angle	Primary Branch Length
Plant height	1	0.196 **	0.188 **	-0.298 **	-0.195 **	0.153 *	0.027	0.112	0.142 *
Number of leaf nodes	0.250 **	1	0.913 **	0.197 **	0.079	0.388 **	0.012	-0.154 *	-0.059
Total number of lateral buds	0.253 **	0.986 **	1	0.133	0.041	0.334 **	-0.016	-0.160*	-0.138
Number of upper primary branches	-0.369 **	0.134	0.126	1	0.231 **	0.110	-0.200 **	-0.293 **	-0.317 **
Number of lateral flower buds	-0.232 **	0.351 **	0.341 **	0.240 **	1	0.109	0.579 **	-0.074	0.534 **
Stem diameter	0.322 **	0.250 **	0.257 **	-0.019	-0.191 **	1	0.394 **	-0.041	0.044
Primary branch diameter	0.096	0.028	0.026	-0.466 **	0.368 **	0.147 *	1	0.147 *	0.639 **
Primary branch angle	0.063	-0.215 **	-0.242 **	-0.253 **	-0.108	-0.031	0.264 **	1	0.148 *
Primary branch length	0.113	0.063	0.057	-0.402 **	0.513 **	-0.279 **	0.661 **	0.160 *	1

Table 2. Pearson correlations between the architectural traits of 195 spray cut chrysanthemum varieties.

Note: ** and * indicate significant differences at the 0.01 and 0.05 probability levels, respectively.



Figure 2. Cluster analysis map of 195 spray cut chrysanthemum varieties in EN2019 and EN2020. (a) K-means cluster of 195 spray cut chrysanthemum varieties in EN2019; (b) K-means cluster of 195 spray cut chrysanthemum varieties in EN2020; (c) Pedigree cluster of 195 spray cut chrysanthemum varieties in EN2019; (d) Pedigree cluster of 195 spray cut chrysanthemum varieties in EN2020. Different colors in every figure refer to different clusters.

Therefore, the categories in EN2020 were more suitable for summarizing the 195 spray cut chrysanthemum varieties. As shown in Figure 3, the 195 were divided into five categories, accounting for 3.08%, 12.31%, 29.74%, 13.85% and 41.45% of all the varieties, of which the typical architecture types were summarized. In the first category, there were only six varieties, represented by Nannong Taoliu (Figure 3a), which had a low startingbranch height, long primary branches, a semispreading plant growth habit and a large number of secondary branches. There were tertiary branches on the plants only in the first category. The second category, represented by Nannong Meifengche (Figure 3b), included 24 varieties, which had loosely distributed branches and longer primary branches. There were fewer leaf nodes and secondary branches on the plants in the second category. The third category, represented by Nannong Cuilongzhao (Figure 3c), included 58 varieties whose starting-branch height was lower than 1/3 of the total plant height. The plants in this category had a relatively large distribution of flowering branches and secondary branches. The fourth category, represented by Nannong Songmang (Figure 3d), included 27 varieties, which had a higher starting-branch height and shorter flowering branches. These compact flowering branches displayed a nearly spherical appearance. The fifth category, represented by Nannong Bingqing (Figure 3e), included 80 varieties, the plants of



which had a relatively large amount of leaf nodes. The flowering branches of the plants in this category displayed a tower-like shape.

Figure 3. Five representative varieties with different architectural traits among 195 spray cut chrysanthemum. (a) Nannong Taoliu; (b) Nannong Meifengche; (c) Nannong Cuilongzhua; (d) Nannong Songmang; (e) Nannong Bingqing. Bars = 10 cm. In each sub figure, the left one refers to an intact plant, middle one refers to an intact plant without leaves and the right one refers to an intact plant without leaves and flowers.

3.5. GWAS and Mining of Genes Controlling Plant Architecture

Combining the data concerning 191,417 high-quality SNPs and the data of the nine phenotypic traits in EN2019 and EN2020, we performed a GWAS via the cMLM method of GAPIT software and the cMLM and MLM methods of TASSEL software, with the PC matrix and kinship matrix serving as covariates. When the significance threshold was 1×10^{-3} , 281 SNPs associated with each trait and corresponding PVE values were obtained. According to the data in Tables S4–S16, GAPIT software revealed 113 SNPs associated with plant architecture in EN2019 and 93 SNPs in EN2020, and the PVE values ranged from 24.06% to 44.46% in EN2019 and from 25.20% to 44.48% in EN2020. The cMLM model of TASSEL software revealed 35 SNPs associated with plant architecture in EN2019 and from 25.20% to 44.68% in EN2020, and the PVE values ranged from 24.06% to 59.73% in EN2020.

Among the identified SNPs, 18 were detected by GAPIT. 5 $_55325230$, 5 $_55325289$ and 10 $_268875261$ were associated with plant height in EN2019 and EN2020; 27 $_13304666$ was associated with stem diameter in EN2019 and EN2020; 9 $_193339518$ was associated with primary branch diameter in EN2019 and EN2020. Eight SNPs (17 $_232431589$, 27 $_194241646$, 27 $_194241707$, 17 $_46614191$, 8 $_66464970$, 27 $_55624912$, 27 $_112009133$ and 14 $_58564373$) were associated with number of leaf nodes and total number of lateral buds in EN2019. Five SNPs (3 $_163391415$, 25 $_13632558$, 25 $_13632658$, 22 $_108888654$

and 7_185418131) were associated with number of leaf nodes and total number of lateral buds in EN2020. According to the cMLM model of TASSEL, the same 10 SNPs were detected: 23_171200599 was associated with plant height in EN2019 and EN2020, 8 SNPs (11_125802422, 17_280870788, 21_242369101, 24_15091361, 23_293787130, 23_293967285, 23_294075102 and 23_308947968) were associated with number of leaf nodes and total number of lateral buds in EN2019, and 9_215337463 was associated with number of leaf nodes and total number of lateral buds in EN2020. Additionally, 19_104723464 was associated with number of lateral flower buds in EN2020. According to the MLM method of TASSEL, 23_171200599 was also associated with plant height in EN2020; 11_125802422 was associated with total number of lateral buds in EN2019; 17_280870788 was associated with number of leaf nodes in EN2019; 21_242369101, 24_15091361, 23_293787130, 23_293967285, 23_294075102 and 23_308947968 were associated with the number of leaf nodes and total number of lateral buds in EN2019; 9_215337463 was associated with number of leaf nodes in EN2019; 9_215337463 was associated with number of leaf nodes in EN2019; 9_215337463 was associated with number of leaf nodes in EN2020.

After comparing genes related to SNP loci of three models, the cMLM model of TASSEL software was chosen finally. Combining the annotation of TAIR, candidate genes are shown in Table 3, and the Manhattan plots of cMLM model of TASSEL can be found in supplementary file S1. We identified four candidate genes: *phyB*, *BRH1*, *CPC* and *bZIP16*.

Table 3. List of SNP sites, candidate genes, and functional annotation for selected architectural traits in spray cut chrysanthemums identified with cMLM model of TASSEL.

SNP Site	Significantly Associated Traits	Candidate Genes	Homologs in Arabidopsis
19_104723464	Number of lateral flower buds/Number of upper primary branches	evm.model.scaffold_940.421	AT2G18790.1 (phytochrome B, phyB)
9_215337463	Number of leaf nodes/Total number of lateral buds	evm.model.scaffold_11169.22	AT3G61460.1 (brassinosteroid-responsive RING-H2, BRH1)
17280870788	Total number of lateral buds	evm.model.scaffold_3682.68	AT2G46410.1 (Homeodomain-like superfamily protein, CPC)
21242369101	Total number of lateral buds	evm.model.scaffold_881.80	AT2G35530.1 (basic region/leucine zipper transcription factor 16, bZIP16)

4. Discussion

In the two consecutive years of EN2019 and EN2020, the number of upper primary branches, number of lateral flower buds and primary branch length presented the highest CV values; these traits were also the key traits used to determine the output of spray cut chrysanthemum. These results indicated that the architectural traits of the 195 spray cut chrysanthemum varieties selected by artificial breeding were diverse and controlled by complex gene pathways. As such, when selecting the appropriate spray cut chrysanthemum, breeders should consider these three traits to have high priority.

During their growth, plants have limited resources. They can allocate resources reasonably through different gene pathways to complete their life cycle. In the present study, plant height had significant slightly large positive Pearson coefficients with number of leaf nodes and total number of lateral buds, but had significant large negative Pearson coefficients with number of upper flower branches and number of lateral flower buds. Plant height was also found to positively correlate with tiller number in sorghum [38], and negatively correlated with fruit branch length in Chinese upland cotton [39]. The correlation relationships in chrysanthemums reflect the mutual negative relationship between vegetative growth and reproductive growth. The number of upper primary branches had very significant negative correlations with primary branch diameter, primary branch angle and primary branch length, which are key traits determining the quality of spray cut chrysanthemum. Therefore, balancing the number of upper primary branches and their quality is highly important.

Plant architecture is species specific, and influenced by environmental conditions such as light, temperature, humidity and nutrient status [40]. Low temperature can lead to the dwarfed rosette and leaves with increased thickness in Arabidopsis [41]. Main differences between EN2019 and EN2020 were monthly precipitation and monthly average relative humidity according to Table S2. Monthly precipitation and monthly average relative humidity of EN2020 in nearly each month were larger than that of EN2019. The mean values of plant height, stem diameter, primary branch diameter, primary branch angle and primary branch length decreased in EN2020 compared with EN2019, indicating a weaker growth state in EN2020. These differences in growth might be attributed to three main factors. First, the growth of chrysanthemum is sensitive to continuously cropped soils, which is related to changes in physicochemical properties, soil microorganisms and allelopathy of plants [42]. Second, the plum rain season in Nanjing was longer in 2020 than in 2019, as mentioned above, which increased the air humidity during the rooting period of the seedlings and the initial root growth stages. Changes in vapor pressure deficit (VPD) and relative humidity (RH) affect the height and flowering time of chrysanthemum plants [43,44]. This explained why the growth of seedlings in EN2020 was rather poor to some extent. Lastly, the seedlings of EN2020 were collected from mother plants overwintering in EN2019, whose growth state might be worse than that in EN2019. Number of leaf nodes, number of lateral flower buds and primary branch angle showed no significant difference in two years, which means that they might not be significantly influenced by a changed environment.

In this experiment, due to the differences in each trait in the two years, two cluster methods were used to cluster the data from the two years. After the trials, the best K value was set as 5. According to the clustering results, we divided the spray cut chrysanthemum varieties into five categories; these categories could be used as typical architecture types for summarize the architectural traits of spray cut chrysanthemum.

A low red light:far-red light ratio (R:FR) can lead to shade avoidance syndrome of plants, resulting in enhanced shoot elongation and reduced branching, and phyB is a major sensor of R:FR signal [45]. phyB has been found to control shooting branching together with photosynthetic photon flux density (PPFD) and PIF4/PIF5 in Arabidopsis, regulating related hormone pathway and expression levels of downstream genes such as BRC1 [46,47]. The *phyB* mutant in sorghum also showed enhanced apical dominance and shortened bud length and the expression levels of TEOSINTE BRANCHED1 (TB1), Dormancy-associated gene-1 (DRM1) and MORE AXILLARY BRANCHES2 (MAX2) were found to increase in the axillary buds [48,49]. The number of upper primary branches in chrysanthemums was found to correlate with *phyB* in our study, indicating the functional role of controlling plant architecture. Three other genes were also found to participate in plant development regulation and hormone signal pathway. BRH1 is a BR-responsive gene, and overexpression of BRH1 results in the production of rounded leaves and may result in the growth and development of rosette leaves [50]. By promoting the conversion of nonhair cells to root hair cells, the R3-type MYB transcription factor protein CAPRICE (CPC) was shown to induce root hair formation in the root epidermis [51]. bZIP16 can promote seed germination and hypocotyl elongation in the initial stages of seedling development [52]. These three genes also play a role in plant development in other plants, which might control plant architecture in chrysanthemums.

5. Conclusions

In this study, we found that the number of leaf nodes, number of lateral flower buds and primary branch angle were less influenced by environmental factors, while plant height, stem diameter, total number of lateral buds, number of upper primary branches, primary branch diameter and primary branch length were significantly influenced by environmental factors. The number of upper primary branches, number of lateral flower buds and primary branch length presented larger variation degree in 195 species. The number of upper primary branches had very significant negative correlations with primary

11 of 13

branch diameter, primary branch angle and primary branch length. We also summarized five clusters with typical architecture and predicted four candidate functional genes (*phyB*, *BRH1*, *CPC* and *bZIP16*) which might control plant architecture in chrysanthemums.

Supplementary Materials: The following supporting information can be downloaded at: https://www.action.com/actionals //www.mdpi.com/article/10.3390/horticulturae8050458/s1, Table S1: 195 spray cut chrysanthemum varieties tested; Table S2. The monthly average temperature, monthly precipitation and monthly average relative humidity of EN2019 and EN2020 in planting location, Jiangning District, Nanjing, China (E118°85', N31°95') Table S3: 44 spray cut chrysanthemum varieties sequencing completed; Table S4: SNPs (GAPIT-cMLM) for the architecture traits of plant height and number of leaf nodes; Table S5: SNPs (GAPIT-cMLM) for the architecture traits of number of upper primary branches, number of lateral flower buds and stem diameter; Table S6: SNPs (GAPIT-cMLM) for the architecture traits of stem diameter, primary branch diameter and primary branch angle; Table S7: SNPs (GAPITcMLM) for the architecture traits of primary branch length; Table S8: SNPs (GAPIT-cMLM) for the architecture traits of primary branch length; Table S9: SNPs (Tassel-cMLM) for the architecture traits of plant height, number of leaf nodes, total number of lateral buds, number of upper primary branches and number of lateral flower buds; Table S10: SNPs (Tassel-cMLM) for the architecture traits of number of lateral flower buds, stem diameter, primary branch diameter and primary branch length; Table S11: SNPs (Tassel-MLM) for the architecture traits of plant height, number of leaf nodes and total number of lateral buds; Table S12: SNPs (Tassel-MLM) for the architecture traits of number of upper primary branches number of lateral flower buds and stem diameter; Table S13: SNPs (Tassel-MLM) for the architecture traits of stem diameter, primary branch diameter, primary branch angle and primary branch length; Table S14: SNPs (Tassel-MLM) for the architecture traits of primary branch length; Table S15: SNPs (Tassel-MLM) for the architecture traits of primary branch length; Table S16: SNPs (Tassel-MLM) for the architecture traits of primary branch length. Supplementary Figures S1–S18: The Manhattan plots of SNPs detected by the cMLM model of TASSEL software.

Author Contributions: Conceptualization, A.S.; methodology, A.S. and J.S.; software, A.S., J.S. and D.S.; validation, D.S., L.Z. and Q.Y.; formal analysis, D.S. and L.Z.; writing—original draft preparation, D.S. and L.Z.; writing—review and editing, D.S., L.Z., J.S., Q.Y., J.Z., W.F., H.W., Z.G., F.C. and A.S.; funding acquisition, A.S., F.C., W.F. and H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by China Agriculture Research System (CARS-23-A18), National Natural Science Foundation of China (32172609, 31870694, 31872149), the earmarked fund for Jiangsu Agricultural Industry Technology System (JATS [2021]454), and a project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institution.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Data analysis was supported by the high-performance computing platform of Bioinformatics Center, Nanjing.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Su, J.; Jiang, J.; Zhang, F.; Liu, Y.; Ding, L.; Chen, S.; Chen, F. Current achievements and future prospects in the genetic breeding of chrysanthemum: A review. *Hortic. Res.* **2019**, *6*, 109. [CrossRef] [PubMed]
- Chen, X.; Zhou, X.; Xi, L.; Li, J.; Zhao, R.; Ma, N.; Zhao, L. Roles of *DgBRC1* in regulation of lateral branching in chrysanthemum (*Dendranthema* × grandiflora cv. Jinba). *PLoS ONE* 2013, 8, e61717. [CrossRef] [PubMed]
- 3. Yang, J.; Jeong, B.R. Side lighting enhances morphophysiology by inducing more branching and flowering in chrysanthemum grown in controlled environment. *Int. J. Mol. Sci.* **2021**, *22*, 12019. [CrossRef] [PubMed]
- Sun, D.; Zhang, L.; Yu, Q.; Zhang, J.; Li, P.; Zhang, Y.; Xing, X.; Ding, L.; Fang, W.; Chen, F.; et al. Integrated Signals of Jasmonates, Sugars, Cytokinins and Auxin Influence the Initial Growth of the Second Buds of Chrysanthemum after Decapitation. *Biology* 2021, 10, 440. [CrossRef]
- 5. Barbier, F.F.; Dun, E.A.; Kerr, S.C.; Chabikwa, T.G.; Beveridge, C.A. An update on the signals controlling shoot branching. *Trends Plant Sci.* **2019**, *24*, 220–236. [CrossRef]

- 6. Rameau, C.; Bertheloot, J.; Leduc, N.; Andrieu, B.; Foucher, F.; Sakr, S. Multiple pathways regulate shoot branching. *Front. Plant Sci.* **2015**, *5*, 741. [CrossRef]
- Nordström, A.; Tarkowski, P.; Tarkowska, D.; Norbaek, R.; Åstot, C.; Dolezal, K.; Sandberg, G. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: A factor of potential importance for auxin–cytokinin-regulated development. *Proc. Natl. Acad. Sci. USA* 2004, 101, 8039–8044. [CrossRef]
- Tanaka, M.; Takei, K.; Kojima, M.; Sakakibara, H.; Mori, H. Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. *Plant J.* 2006, 45, 1028–1036. [CrossRef]
- Alder, A.; Jamil, M.; Marzorati, M.; Bruno, M.; Vermathen, M.; Bigler, P.; Ghisla, S.; Bouwmeester, H.; Beyer, P.; Al-Babili, S. The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science* 2012, 335, 1348–1351. [CrossRef]
- 10. Wu, C.; Trieu, A.; Radhakrishnan, P.; Kwok, S.F.; Harris, S.; Zhang, K.; Wang, J.; Wan, J.; Zhai, H.; Takatsuto, S.; et al. Brassinosteroids regulate grain filling in rice. *Plant Cell* **2008**, *20*, 2130–2145. [CrossRef]
- 11. Xia, X.; Dong, H.; Yin, Y.; Song, X.; Gu, X.; Sang, K.; Zhou, J.; Shi, K.; Zhou, Y.; Foyer, C.H.; et al. Brassinosteroid signaling integrates multiple pathways to release apical dominance in tomato. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2004384118. [CrossRef]
- 12. Mason, M.G.; Ross, J.J.; Babst, B.A.; Wienclaw, B.N.; Beveridge, C.A. Sugar demand, not auxin, is the initial regulator of apical dominance. *Proc. Natl. Acad. Sci. USA* 2014, 111, 6092–6097. [CrossRef]
- 13. Wang, B.; Smith, S.M.; Li, J. Genetic regulation of shoot architecture. Annu. Rev. Plant Biol. 2018, 69, 437–468. [CrossRef]
- Adams, H.D.; Collins, A.D.; Briggs, S.P.; Vennetier, M.; Dickman, L.T.; Sevanto, S.A.; Garcia-Forner, N.; Powers, H.H.; McDowell, N.G. Experimental drought and heat can delay phenological development and reduce foliar and shoot growth in semiarid trees. *Glob. Chang. Biol.* 2015, *21*, 4210–4220. [CrossRef]
- 15. Fleisher, D.H.; Timlin, D.J.; Reddy, V.R. Temperature influence on potato leaf and branch distribution and on canopy photosynthetic rate. *Agron. J.* **2006**, *98*, 1442–1452. [CrossRef]
- 16. Leduc, N.; Roman, H.; Barbier, F.; Péron, T.; Huché-Thélier, L.; Lothier, J.; Demotes-Mainard, S.; Sakr, S. Light signaling in bud outgrowth and branching in plants. *Plants* **2014**, *3*, 223–250. [CrossRef]
- 17. Bahmani, I.; Hazard, L.; Varlet-Grancher, C.; Betin, M.; Lemaire, G.; Matthew, C.; Thom, E. Differences in tillering of long- and short-leaved perennial ryegrass genetic lines under full light and shade treatments. *Crop Sci.* 2000, 40, 1095–1102. [CrossRef]
- 18. Evers, J.B.; Vos, J.; Andrieu, B.; Struik, P.C. Cessation of tillering in spring wheat in relation to light interception and red: Far-red ratio. *Ann. Bot.* **2006**, *97*, 649–658. [CrossRef]
- 19. Luo, L.; Zhang, Y.; Xu, G. How does nitrogen shape plant architecture? J. Exp. Bot. 2020, 71, 4415–4427. [CrossRef]
- He, X.; Qu, B.; Li, W.; Zhao, X.; Teng, W.; Ma, W.; Ren, Y.; Li, B.; Li, Z.; Tong, Y. The nitrate-inducible NAC transcription factor TaNAC2-5A controls nitrate response and increases wheat yield. *Plant Physiol.* 2015, 169, 1991–2005. [CrossRef]
- 21. Tian, D.; Wang, P.; Tang, B.; Teng, X.; Li, C.; Liu, X.; Zou, D.; Song, S.; Zhang, Z. GWAS Atlas: A curated resource of genome-wide variant-trait associations in plants and animals. *Nucleic Acids Res.* **2020**, *48*, D927–D932. [CrossRef] [PubMed]
- 22. Cano-Gamez, E.; Trynka, G. From GWAS to function: Using functional genomics to identify the mechanisms underlying complex diseases. *Front. Genet.* 2020, *11*, 424. [CrossRef]
- 23. Su, J.; Zhang, F.; Li, P.; Guan, Z.; Fang, W.; Chen, F. Genetic variation and association mapping of waterlogging tolerance in chrysanthemum. *Planta* **2016**, *244*, 1241–1252. [CrossRef]
- 24. Su, J.; Zhang, F.; Chong, X.; Song, A.; Guan, Z.; Fang, W.; Chen, F. Genome-wide association study identifies favorable SNP alleles and candidate genes for waterlogging tolerance in chrysanthemums. *Hortic. Res.* **2019**, *6*, 21. [CrossRef]
- 25. Sumitomo, K.; Shirasawa, K.; Isobe, S.; Hirakawa, H.; Hisamatsu, T.; Nakano, Y.; Yagi, M.; Ohmiya, A. Genome-wide association study overcomes the genome complexity in autohexaploid chrysanthemum and tags SNP markers onto the flower color genes. *Sci. Rep.* **2019**, *9*, 13947. [CrossRef]
- He, Y.; Wu, D.; Wei, D.; Fu, Y.; Cui, Y.; Dong, H.; Tan, C.; Qian, W. GWAS, QTL mapping and gene expression analyses in *Brassica* napus reveal genetic control of branching morphogenesis. Sci. Rep. 2017, 7, 15971. [CrossRef]
- Li, F.; Chen, B.; Xu, K.; Gao, G.; Yan, G.; Qiao, J.; Li, J.; Li, H.; Li, L.; Xiao, X.; et al. A genome-wide association study of plant height and primary branch number in rapeseed (*Brassica napus*). *Plant Sci.* 2016, 242, 169–177. [CrossRef]
- Liu, H.; Wang, J.; Zhang, B.; Yang, X.; Hammond, J.P.; Ding, G.; Wang, S.; Cai, H.; Wang, C.; Xu, F. Genome-wide association study dissects the genetic control of plant height and branch number in response to low-phosphorus stress in *Brassica napus*. *Ann. Bot.* 2021, *128*, 919–930. [CrossRef]
- Zheng, M.; Peng, C.; Liu, H.; Tang, M.; Yang, H.; Li, X.; Liu, J.; Sun, X.; Wang, X.; Xu, J.; et al. Genome-wide association study reveals candidate genes for control of plant height, branch initiation height and branch number in rapeseed (*Brassica napus* L.). *Front. Plant Sci.* 2017, *8*, 1246. [CrossRef] [PubMed]
- Yano, K.; Morinaka, Y.; Wang, F.; Huang, P.; Takehara, S.; Hirai, T.; Ito, A.; Koketsu, E.; Kawamura, M.; Kotake, K.; et al. GWAS with principal component analysis identifies a gene comprehensively controlling rice architecture. *Proc. Natl. Acad. Sci. USA* 2019, 116, 21262–21267. [CrossRef] [PubMed]
- Chong, X.; Zhang, F.; Wu, Y.; Yang, X.; Zhao, N.; Wang, H.; Guan, Z.; Fang, W.; Chen, F. A SNP-enabled assessment of genetic diversity, evolutionary relationships and the identification of candidate genes in chrysanthemum. *Genome Biol. Evol.* 2016, *8*, 3661–3671. [CrossRef]
- 32. Li, H.; Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **2009**, *25*, 1754–1760. [CrossRef]

- Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009, 25, 2078–2079. [CrossRef]
- 34. Yang, J.; Lee, S.H.; Goddard, M.E.; Visscher, P.M. GCTA: A tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* 2011, 88, 76–82. [CrossRef]
- Lipka, A.E.; Tian, F.; Wang, Q.; Peiffer, J.; Li, M.; Bradbury, P.J.; Gore, M.A.; Buckler, E.S.; Zhang, Z. GAPIT: Genome association and prediction integrated tool. *Bioinformatics* 2012, 28, 2397–2399. [CrossRef]
- 36. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* 2007, 23, 2633–2635. [CrossRef]
- Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. J. Mol. Biol. 1990, 215, 403–410. [CrossRef]
- Luo, F.; Pei, Z.; Zhao, X.; Liu, H.; Jiang, Y.; Sun, S. Genome-wide association study for plant architecture and bioenergy traits in diverse sorghum and sudangrass germplasm. *Agronomy* 2020, *10*, 1602. [CrossRef]
- Su, J.; Li, L.; Zhang, C.; Wang, C.; Gu, L.; Wang, H.; Wei, H.; Liu, Q.; Huang, L.; Yu, S. Genome-wide association study identified genetic variations and candidate genes for plant architecture component traits in Chinese upland cotton. *Theor. Appl. Genet.* 2018, 131, 1299–1314. [CrossRef]
- 40. Reinhardt, D.; Kuhlemeier, C. Plant architecture. EMBO Rep. 2002, 3, 846–851. [CrossRef]
- Atkin, O.K.; Loveys, B.; Atkinson, L.J.; Pons, T.J. Phenotypic plasticity and growth temperature: Understanding interspecific variability. J. Exp. Bot. 2006, 57, 267–281. [CrossRef]
- 42. Li, P.; Chen, J.; Li, Y.; Zhang, K.; Wang, H. Possible mechanisms of control of Fusarium wilt of cut chrysanthemum by *Phanerochaete chrysosporium* in continuous cropping fields: A case study. *Sci. Rep.* **2017**, *7*, 15994. [CrossRef]
- 43. Mortensen, L.M. Effects of air humidity on growth, flowering, keeping quality and water relations of four short-day greenhouse species. *Sci. Hortic.* **2000**, *86*, 299–310. [CrossRef]
- Körner, O.; Challa, H. Temperature integration and process-based humidity control in chrysanthemum. *Comput. Electron. Agric.* 2004, 43, 1–21. [CrossRef]
- 45. Casal, J.J. Shade avoidance. Arab. Book/Am. Soc. Plant Biol. 2012, 10, e0157. [CrossRef] [PubMed]
- 46. Su, H.; Abernathy, S.D.; White, R.H.; Finlayson, S.A. Photosynthetic photon flux density and phytochrome B interact to regulate branching in *Arabidopsis*. *Plant Cell Environ*. **2011**, *34*, 1986–1998. [CrossRef]
- 47. Holalu, S.V.; Reddy, S.K.; Blackman, B.K.; Finlayson, S.A. Phytochrome interacting factors 4 and 5 regulate axillary branching via bud abscisic acid and stem auxin signalling. *Plant Cell Environ.* **2020**, *43*, 2224–2238. [CrossRef]
- Kebrom, T.H.; Burson, B.L.; Finlayson, S.A. Phytochrome B represses *Teosinte Branched1* expression and induces sorghum axillary bud outgrowth in response to light signals. *Plant Physiol.* 2006, 140, 1109–1117. [CrossRef]
- 49. Kebrom, T.H.; Mullet, J.E. Transcriptome profiling of tiller buds provides new insights into PhyB regulation of tillering and indeterminate growth in sorghum. *Plant Physiol.* **2016**, *170*, 2232–2250. [CrossRef]
- 50. Wang, X.; Chen, E.; Ge, X.; Gong, Q.; Butt, H.; Zhang, C.; Yang, Z.; Li, F.; Zhang, X. Overexpressed *BRH1*, a RING finger gene, alters rosette leaf shape in *Arabidopsis thaliana*. *Sci. China Life Sci.* **2018**, *61*, 79–87. [CrossRef]
- 51. Tominaga-Wada, R.; Wada, T. CPC-ETC1 chimeric protein localization data in *Arabidopsis* root epidermis. *Data Brief* **2018**, *18*, 1773–1776. [CrossRef]
- 52. Hsieh, W.-P.; Hsieh, H.-L.; Wu, S.-H. *Arabidopsis* bZIP16 transcription factor integrates light and hormone signaling pathways to regulate early seedling development. *Plant Cell* **2012**, *24*, 3997–4011. [CrossRef]