agronomy

MDPI

Article

Fine Mapping of *Bush* Gene and Development of Molecular Marker for Bush Type in Pumpkin (*Cucurbita maxima* Duch.)

Chun Li [1,2,3], Zhen He [1,4], Yifan Li [1,2,3], Yanhong Lan [1,2,3], Fangyi Gong [1,2,3], Peng Cai [1,2,3], Nan Yang [1,2,3], Yan Liu [1,2,3], Feng Xia [1,2,3], Genyun Liang [1,2,3] and Xiaojun Liu [1,2,3,*]

[1] Horticulutre Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China; lcharry99@scsaas.cn (C.L.); 2022211031@stu.sicau.edu.cn (Z.H.); liyifan@scsaas.cn (Y.L.); yanhong_lan24@scsaas.cn (Y.L.); gfy2024@scsaas.cn (F.G.); cp215@sasaas.cn (P.C.); 2018111006@stu.sicau.edu.cn (N.Y.); 2018305018@stu.sicau.edu.cn (Y.L.); xiafeng@scsaas.cn (F.X.); liang_gyrb@scsaas.cn (G.L.)
[2] Vegetable Germplasm Innovation and Variety Improvement Key Laboratory of Sichuan Province, Chengdu 610066, China
[3] Sichuan Province Engineering Technology Research Center of Vegetables, Chengdu 611934, China
[4] Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, China
* Correspondence: lxjsc@scsaas.cn

Abstract: The bush trait is an important agronomic trait with great value for pumpkin crops. In this study, a bush-type line, CS82, identified in pumpkin (*Cucurbita maxima*) showed no obvious main vine, and all petioles clustered at the extremely shortened stem with limited internodes. The microscopy analysis revealed that the bush-type phenotype may be due to the degeneration of the shoot apex. Genetic analysis showed that the bush-type phenotype is controlled by a single dominant nuclear gene. Exogenous gibberellin treatment could not recover the bush-type phenotype to the wild type, indicating that the bush-type phenotype is not due to the mutation of gibberellin biosynthesis genes. The BSA-seq analysis preliminarily mapped the *Bush* gene to Chr.15 of the pumpkin genome. Further fine mapping limited the *Bush* gene to a physical distance of 95.65 kb with 19 genes. Based on the gene function and the 63 bp deletion, *CmaCh15G011490*, encoding an axial regulator YABBY 5-like protein, was selected as the candidate gene for the *Bush* gene. A quick and efficient method was developed for bush-type phenotype identification, which is useful for bush-type variety breeding in pumpkin.

Keywords: BSA-seq; bush trait; *Cucurbita maxima*; plant architecture

Citation: Li, C.; He, Z.; Li, Y.; Lan, Y.; Gong, F.; Cai, P.; Yang, N.; Liu, Y.; Xia, F.; Liang, G.; et al. Fine Mapping of *Bush* Gene and Development of Molecular Marker for Bush Type in Pumpkin (*Cucurbita maxima* Duch.). *Agronomy* **2024**, *14*, 2967. https://doi.org/10.3390/agronomy14122967

Academic Editor: John Stephen C. Smith

Received: 8 November 2024
Revised: 8 December 2024
Accepted: 9 December 2024
Published: 13 December 2024

1. Introduction

Plant architecture is an important agronomic trait determining planting mode and field management, which impacts labor costs and crop yield [1,2]. A crucial morphological change in cultivated rice domestication was the transition of the plant architecture from a prostrate growth habit with a wide tiller angle to an erect growth habit with a narrow tiller angle [3]. And the first Green Revolution of cereals enabled by semidwarf breeding greatly improved lodging resistance and grain yield [4,5]. Pumpkin is an economically important vegetable crop worldwide [6]. Its prostrate growth habit and long vines result in a large area occupied by a pumpkin plant during growth. Dwarf and bush breeding would effectively increase planting densities to improve the per-unit yield, which will be a main aspect in pumpkin breeding in the future [7,8].

Bush and dwarf breeding could increase the yield and improve the quality of cucurbit crops; breeders have paid much attention to dwarf or bush plants in watermelon, pumpkin, and melon [9–11]. To accelerate the breeding process, many dwarf or bush genes have been identified in cucumber, watermelon, and pumpkin [6,7,12]. In cucumber, *cp* was the first dwarf mutant identified, the *cp* gene encodes the cytokinin oxidase (CKX), and a 3 bp

deletion in its first exon of PI 308,915 resulted in an extremely reduced internode length [13]. *CsCLAVATA1*, encoding CLAVATA1-type receptor-like kinase, was the candidate gene for the dwarf phenotype of the *Csdw* mutant [12]. Five more genes have been identified for dwarf phenotypes in watermelon. *Cldf* encodes a gibberellin 3β-hydroxylase involved in the GA biosynthesis pathway. A 13 bp deletion in *Cldf* resulted in GA deficiency and the dwarf phenotype in line N21, and exogenous GA3 application rescued its dwarf phenotype [7]. Relatively few QTLs and genes have been identified in pumpkin. Three QTLs were recovered from pumpkin (*C. maxima*) with a dwarf vine using a high-density genetic map, and *Cma_004516* was found within the *qCmB2*-encoded gibberellin (GA)20-oxidase in the GA biosynthesis pathway [6]. Three QTLs of the gibberellin-sensitive dwarf trait were located in *Cucurbita pepo*, and the major QTL harbored a gene encoding a predicted gibberellin 2-β-oxidase [14]. *Cp4.1LG10g05910.1* in the *CpDw* locus is involved in the GA biosynthesis pathway in *C. pepo*, and two nonsynonymous SNPs in the exons and several SNPs and InDels in the promoter may cause the shortened vine [15]. The *Bu* gene of the bush trait in *C. moschata* encodes a YABBY1 protein, and a 76 bp deletion was found in the 5′ untranslated region, which enhanced the translation of YABBY1, suppressing the stem elongation in a dose-dependent manner [8].

The YABBY gene family is a plant-specific transcription factor family playing important roles in plant growth, development, and morphogenesis [16–18]. Two highly conserved domains are shared by all *YABBY* genes: an N-terminal C2C2 zinc finger domain and a helix–loop–helix motif [16,18]. Based on the phylogenetic relationship, the YABBY gene family includes six subfamilies among the extant angiosperms: *FIL*, *YAB3*, *YAB2*, *YAB5*, *CRC*, and *INO* [19–21]. *FIL*, *YAB2*, *YAB3*, and *YAB5* are expressed both in the leaf primordia and floral organs and are involved in lateral organ polarity development, the formation of margins, the maturation of the leaf, and the development and phyllotaxis of the shoot apical meristem [22–24]. *CRC* is mostly limited to carpel and nectary development [25], and *INO* is restricted to the gynobasal side of developing ovules, promoting the formation and asymmetric growth of ovule outer integument [26].

In this study, we identified a candidate *Bush* gene determining the bush-type phenotype through BSA-seq and map-based cloning. A quick and efficient method for the identification of bush-type individuals was developed based on the 63 bp deletion in the promoter sequence of *CmaCh15G011490*. Our work provides a candidate gene and a method for identifying bush-type individuals quickly and efficiently, which is useful for accelerating bush-type variety breeding in pumpkin.

2. Materials and Methods

2.1. Plant Materials

A bush-type inbred line, CS82, and a wild-type inbred line, CM82, of pumpkin (*C. maxima*) were obtained from the high generations of self-crossing in our laboratory. The bush trait of CS82 (*C. maxima*) was transferred from the spontaneous bush-type mutant of pumpkin (*C. moschata*) found in Shanxi, China [27]. To analyze the inheritance of the bush trait in pumpkin (*C. maxima*), bush-type line CS82 and wild-type line CM82 were crossed to construct F_1 generation. Then, F_1 generation were self-crossed to construct the F_2 segregating population and backcrossed with the two parents CS82 (P_1) and CM82 (P_2) to construct BC_1P_1 and BC_1P_2. All plant materials were grown in the paddy field during the natural planting season of pumpkin (*C. maxima*) in Xindu, Chengdu, China.

2.2. Phenotype Identification

The phenotypes were obviously different between the bush-type and wild-type pumpkins (*C. maxima*). Wild-type CM82 showed an apparent main vine with a stem apex and more than twenty nodes at the mature stage. Bush-type CS82 did not have any obvious main vine, and the petioles were clustered at the extremely shortened stem with limited internodes. Some individuals of bush-type pumpkin (*C. maxima*) had stretched stems with

no stem apex and no more than five nodes. At the flowering stage, the phenotypes of the P_1, P_2, F_1, BC_1P_1, BC_1P_2, and F_2 population were identified.

2.3. Microscopy Analysis of the Shoot Apex

The bush phenotype of pumpkin (*C. maxima*) may be due to the abortion of the shoot apical meristem. Then, the microscopy structures of the shoot apices of bush-type and wild-type pumpkins (*C. maxima*) were observed using photomicrographic techniques. The shoot apices of bush-type and wild-type pumpkins (*C. maxima*) at the flowering stage were collected and observed with light microscopy (AXIO Scope A1, Carl Zeiss AG, Oberkochen, Germany).

2.4. Exogenous Gibberellin Treatment

To investigate whether the bush-type pumpkins will recover to the wild phenotype after treating with gibberellin, we conducted an exogenous gibberellin treatment with two bush-type pumpkins (CS01 and CS02). At the two-leaf stage, both bush-type and wild-type pumpkins were treated with 0.6 mM GA_3 five times over a 4-day interval [28]. A control group was treated with ddH_2O for the same number of times and over the same interval. After treating with gibberellin and ddH_2O, we observed the responses of bush-type pumpkins (*C. maxima*) to gibberellin.

2.5. Preliminary Position with BSA Method

According to Takagi's method [29], thirty bush-type plants and thirty wild-type plants were randomly selected from the F_2 segregating population to construct a bush-type pool (Bu) and a wild-type pool (WT), respectively, with an equal weight of fresh leaf. The genomic DNAs were extracted from the two parents and the two pools using the CTAB method and then sent to Gene Denovo Biotechnology Co. Ltd. (Guangzhou, China) for high-throughput sequencing with the Illumina Hiseq PE150 platform (Illumina, Inc. San Diego, CA, USA). The FastQC (0.12.0) software was used to assess the quality of the data from the two parents and the two pools (http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 30 August 2022)). High-quality short-sequence reads after filtering were mapped to the pumpkin (*C. maxima*) reference genome sequence (Cmaxima_genome_v1.1) from the Cucubit Genomic Database (http://www.cucurbitgenomics.org/ (accessed on 30 August 2022)). The GATK (4.1) software was used to call SNPs in the alignment files, and the SNP index was calculated for each SNP locus in the two pools. The preliminary region of the *Bush* gene was identified based on the SNPs and InDels according to Fekih's method.

2.6. Fine Mapping and Identification of Candidate Gene

For the fine mapping, 115 wild-type individuals with normal vine were identified from a larger F_2 segregating population derived from a cross between two parents, CM82 and CS82. New polymorphic indel markers were developed based on the genomic differences between the two parents to further narrow down the candidate region. All of the newly developed primers for the fine mapping are listed in Table S1. After fine mapping, the information including gene IDs and gene functions of all genes within the narrowed region were extracted from the Cucurbit Genomics Database.

2.7. Sequence Variation Detection and Development of Indel Marker

According to the genomic data, a 63 bp indel was detected in the promoter region of the candidate gene between the two parents. The specific primers (Bu-indel, Table S1) were designed on the flanking regions of the 63 bp indel based on the pumpkin reference genome sequence (Cmaxima_genome_v1.1). PCR was performed to amplify the fragment containing the 63 -bp indel from the two parents using Phanta Max Super-Fidelity DNA Polymerase (R223-01, Vazyme Biotech Co., Ltd., Nanjing, China). The PCR products of the two parents were sent to Sangon Biotech Co., Ltd. (Shanghai, China) for sequencing.

2.8. Molecular Marker-Assisted Breeding of Bush-Type Pumpkin

The seeds of pumpkin materials to be identified were sowed in 32-hole plugs. Three 32-hole plugs constituted an 8 × 12 plug tray, corresponding to a 96-well PCR plate. Flesh leaf tissue about 2 mm ×5 mm was collected from the cotyledon and transferred from the 8 × 12 plug tray to the 96-well PCR plate. Genomic DNAs were extracted using a simple method: (1) 20 μL of 0.25 M NaOH was added to the wells of the PCR plate using the 8 channel pipette, followed by incubation at 95 °C for 1 min; (2) 50 μL of 0.1 M Tris-HCl was then added, and the solution in the wells was used as a DNA template to perform the PCR. The PCR reactions were conducted using the reaction system (10 μL), 5.0 μL of 2 × ChamQ SYBR Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China), 1.0 μL of DNA template, 0.5 μL of forward and reverse primers (10 μmol/μL), and 3.0 μL of ddH_2O, with the following procedure conditions: an initial pre-denaturation at 95 °C for 3 min, followed by 32 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 20 s, with a final extension step at 72 °C for 5 min.

2.9. Statistical Analysis

Microsoft Excel (Office16) was used to analyze all the phenotype data of each population using the chi-square test. According to the chi-square test, the chi-square value for the bush-type phenotype was less than 3.84 (the chi-square critical value for a significance level of 0.05), indicating that the heredity of the bush-type phenotype fit a 3:1 Mendelian ratio.

3. Results

3.1. Bush-Type Pumpkin (C. maxima) Shows Compact Plant with Degraded Shoot Apex and Limited Nodes

Compared with the wild-type line CM82, the bush-type line CS82 exhibited a compact plant architecture (Figure 1A,B). CM82 had a prominent main vine with a shoot apex at the mature stage. In contrast, CS82 lacked an obvious main vine, and its petioles were clustered at the extremely shortened stem with limited internodes (Figure 1A,B). To further analyze the cause of the bush-type phenotype, we observed the shoot apex using photomicrography. As shown in Figure 1C,D, an obvious shoot apex with a flower bud and foliar buds was observed on the stem of wild-type pumpkin (*C. maxima*), whereas no shoot apex was found in the bush-type pumpkin (*C. maxima*). The results above suggested that the shoot apex of bush-type pumpkin (*C. maxima*) may degenerate, leading to its bush-type phenotype.

To investigate the inheritance of the bush trait in pumpkin (*C. maxima*), we identified and analyzed the phenotypes of P_1, P_2, F_1, BC_1P_1, BC_1P_2, and F_2 populations at the flowering stage. CS82 (P_1) showed the bush phenotype, while CM82 (P_2) exhibited the wild phenotype. A total of 48 individuals in the F_1 population (CS82 × CM82) displayed the bush phenotype. All plants from the F_1 × CS82 backcross showed the bush phenotype, consistent with P_1 and F_1, while in the F_1 × CM82 backcross population, 36 bush-type and 42 wild-type plants were identified, demonstrating a good fit in a 1:1 segregation ratio ($\chi^2 = 0.46 < \chi^2_{0.05} = 3.84$). In the F_2 segregating population, 104 bush-type plants and 33 wild-type plants were found, which fit well to the Mendelian ratio of 3:1 ($\chi^2 = 0.06 < \chi^2_{0.05} = 3.84$). The results above indicated that the bush phenotype in pumpkin (*C. maxima*) is controlled by a single dominant nuclear gene.

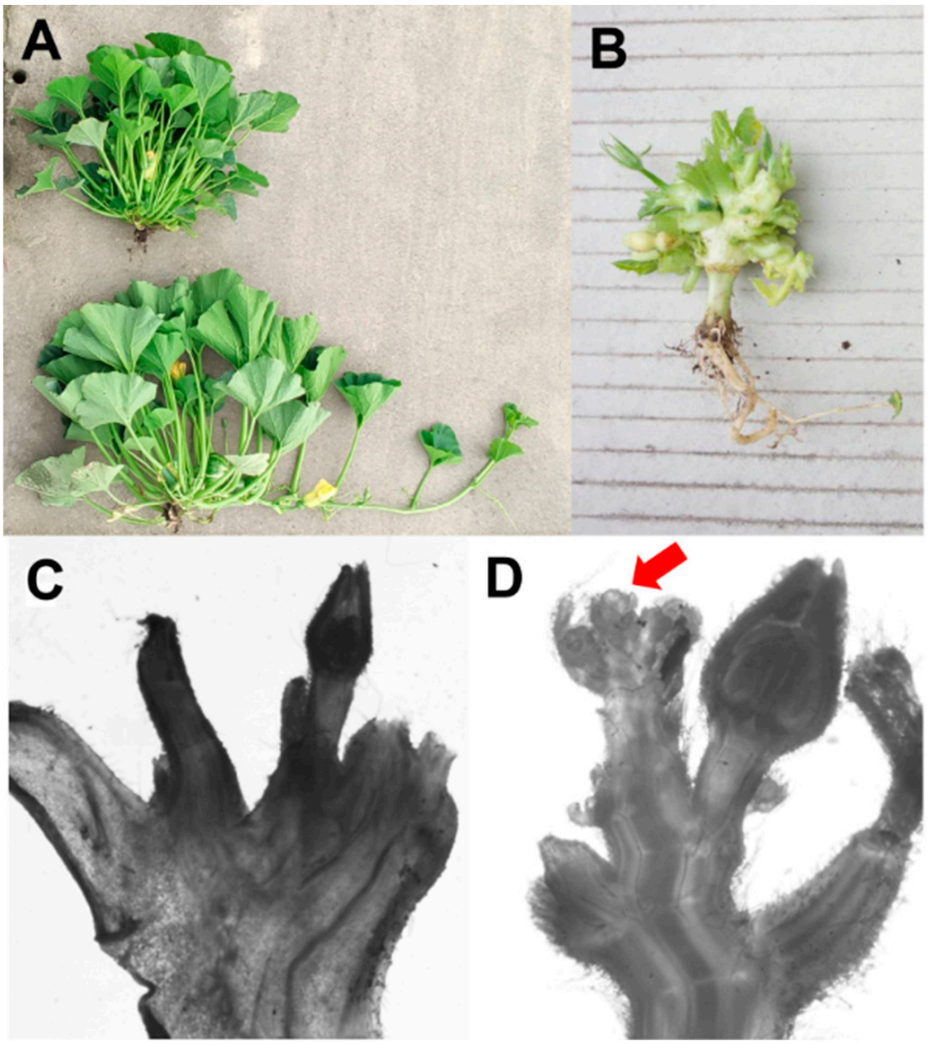

Figure 1. Phenotypic comparison between CS82 (bush-type, top) and CM82 (wild-type, bottom). (**A**) The plant architecture of CS82 and CM82 at the mature stage. (**B**) The clustered stem without petioles and blades of bush-type plant with limited and short internodes. (**C**) The shoot apex of bush-type plant. (**D**) The shoot apex of wild-type plant. The red arrow indicates the shoot apex with flower buds and foliar buds.

3.2. Bush-Type Phenotype Cannot Be Recovered to Wild-Type by Exogenous Gibberellin

As is well known, gibberellins (GAs) are important plant hormones that play a crucial role in stem elongation, and plants deficient in GAs show a dwarf phenotype [5,30,31]. To further investigate the causation of the bush phenotype, bush-type pumpkins were treated with gibberellin. After gibberellin treatment, the wild-type pumpkin and a bush-type pumpkin (CS02) displayed obviously elongated stems, while another bush-type pumpkin (CS01) showed unchanged stem length (Figure 2). However, both types of bush-type pumpkins (CS01 and CS02) did not develop any new shoot apices after gibberellin treatment (Figure 2). The results above indicated that the bush phenotype cannot be recovered to the wild-type by exogenous gibberellin and is not due to the mutation in gibberellin biosynthesis genes.

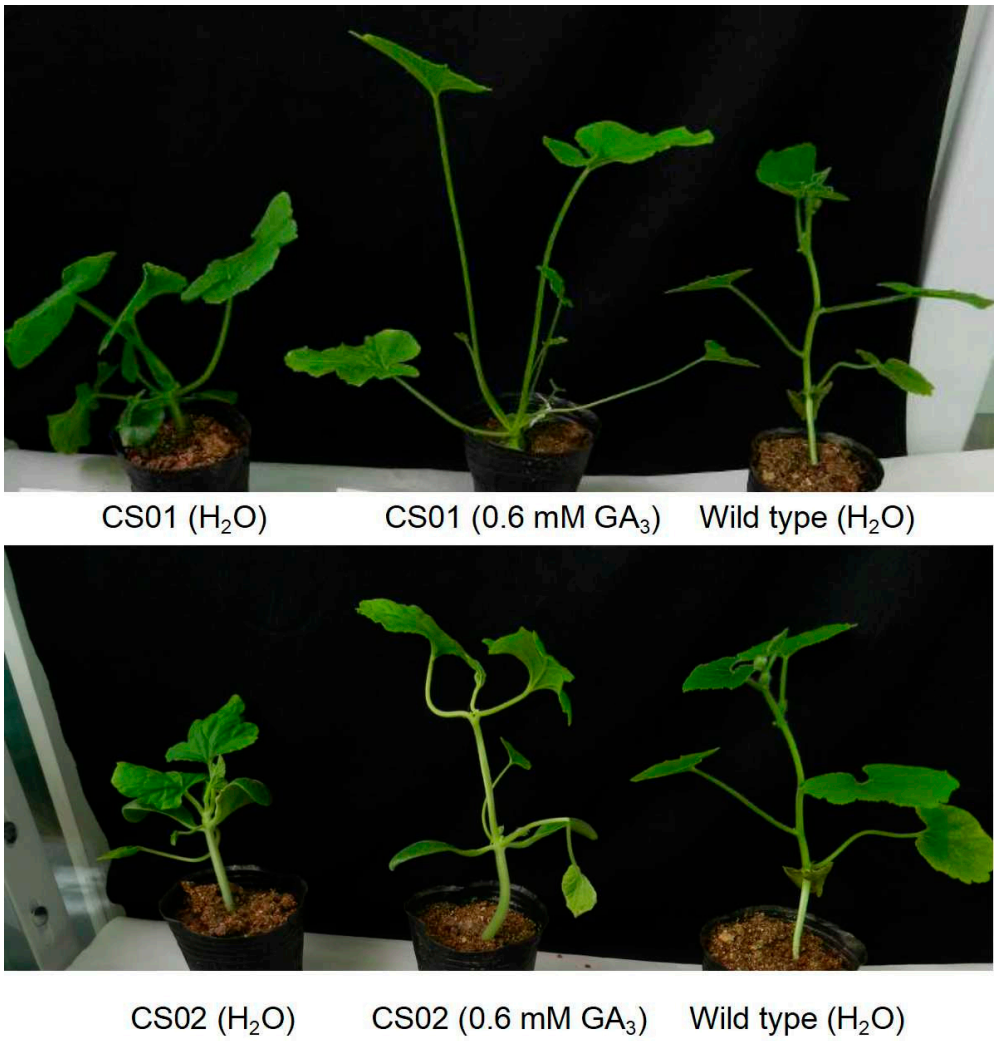

Figure 2. Morphological phenotypes of bush-type (CS01 and CS02) and wild-type pumpkins after exogenous GA_3 treatment. Bush-type pumpkins (CS01 and CS02) were treated with 0.6 mM of GA_3 and ddH_2O. A control group was treated with ddH_2O with the same times and interval.

3.3. BSA-Seq Analysis Preliminarily-Mapped Bush Gene on Chr.15

BSA-seq analysis was conducted to preliminarily map the *Bush* gene using the F_2 segregating population. Thirty bush-type individuals and thirty wild-type individuals were selected for high-throughput sequencing. After quality control, 113,698,634 (98.74%) and 93,198,628 (98.74%) high-quality clean reads were identified in the Bu pool and WT pool, respectively. Of these, 112,603,590 (99.04%) reads in the Bu pool and 92,457,164 (99.20%) reads in the WT pool were in alignment with the reference genome of pumpkin (*C. maxima*). In total, 992,432 SNPs and 191,632 InDels were identified between the two parents. To detect the genomic regions associated with the bush trait, the SNP index of each SNP locus was calculated between the Bu pool and the WT pool using filtered SNPs. As shown in Figure 3, a confidence interval of 95% based on Δ(SNP index) was identified in the region of 2.80 Mb to 9.17 Mb of Chr. 15 between the Bu pool and WT pool.

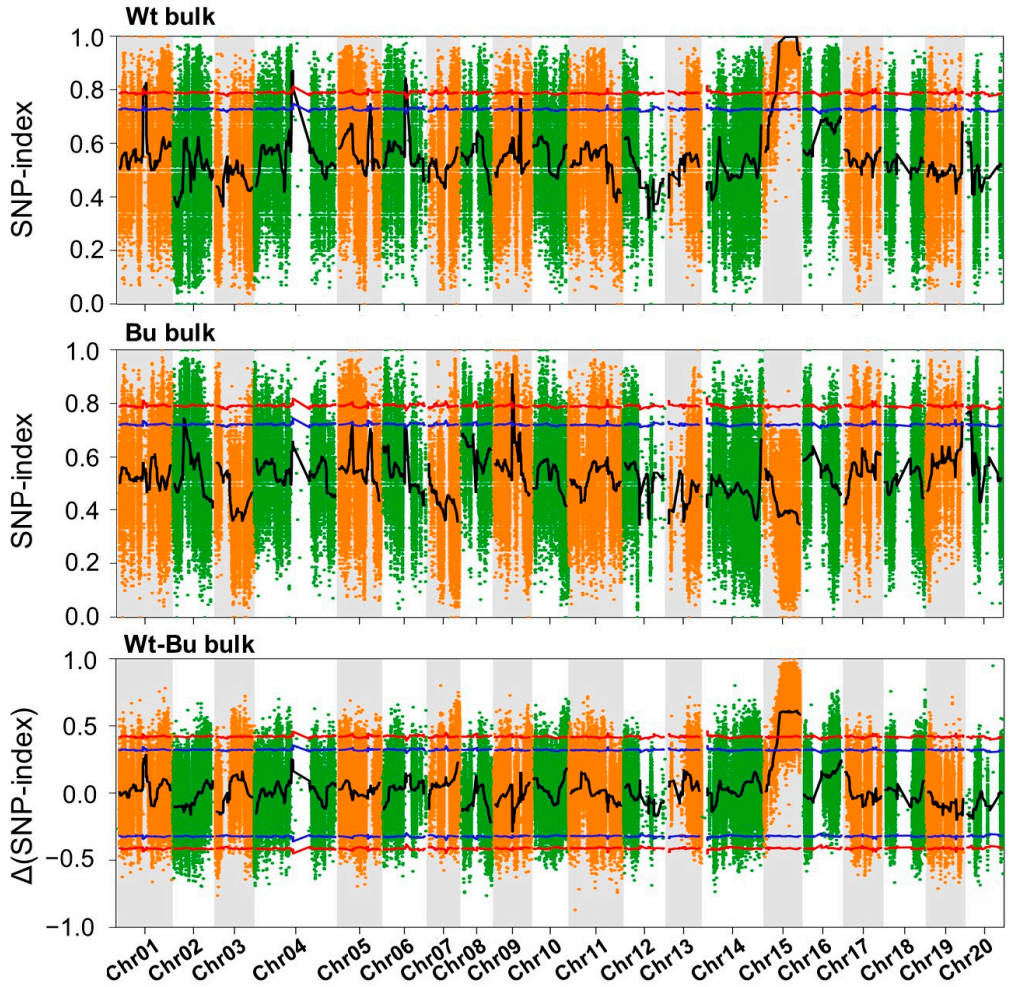

Figure 3. Preliminarily mapping of *Bush* gene with BSA-seq analysis. SNP index plots of WT bulk (**top**) and Bu bulk (**middle**); Δ(SNP index) plot (**bottom**) of 20 chromosomes of pumpkin (*C. maxima*). Confidence intervals of 99% (blue line) and 95% (red line). Based on the confidence level of 99%, the *Bush* gene was preliminarily mapped to the region of 2.80 Mb to 9.17 Mb on Chr. 15. The green and orange in the figure were used to distinguish the adjacent chromosomes.

3.4. A YABBY Gene Was Selected as the Candidate Gene of Bush Gene

A very large region of 6.37 Mb was preliminarily identified through BSA-seq analysis. To further narrow down the region of the *Bush* gene, a larger F_2 segregating population consisting of 445 plants was constructed, and 54 polymorphic InDel markers between the two parents were developed for fine mapping of the *Bush* gene. Finally, five polymorphic InDel markers were used to screen the 445 F_2 plants, and the *Bush* gene was mapped to an interval between InDel markers D28 and D32 with genetic distances of 1.33 cM and 0.44 cM, respectively, and a physical distance of 95.65 kb (Figure 4).

According to the reference genome of pumpkin (*C. maxima*), 19 genes were identified in the 95.65 kb region (Table 1). Based on annotations in the Cucurbit Genomics Database (http://cucurbitgenomics.org/v2/ (accessed on 14 December 2022)), the functions of 18 genes, excluding *CmaCh15G011490*, are not related to the ontogeny and development of the shoot apex. However, *CmaCh15G011490* encodes an axial regulator, YABBY 5-like, which is a transcription factor playing a crucial role in the shoot meristem identity in *Arabidopsis* [20]. Since the bush phenotype is caused by the degeneration of the shoot apical meristem, we selected *CmaCh15G011490* as the candidate gene for the bush trait. The results of the high-throughput sequencing revealed no SNPs or InDels in the gene structure (exons and introns) of *CmaCh15G011490* between the two parents, but an InDel was found

at the promoter region of *CmaCh15G011490*. Then, we designed a pair of primers based on the promoter sequence of *CmaCh15G011490* to amplify the InDel locus in both parents. Sequencing revealed a 63 bp deletion in the promoter sequence of *CmaCh15G011490* in bush-type line CS82 compared to the wild-type line. Based on the gene function and the variable locus, *CmaCh15G011490* was selected as the candidate gene for the bush trait.

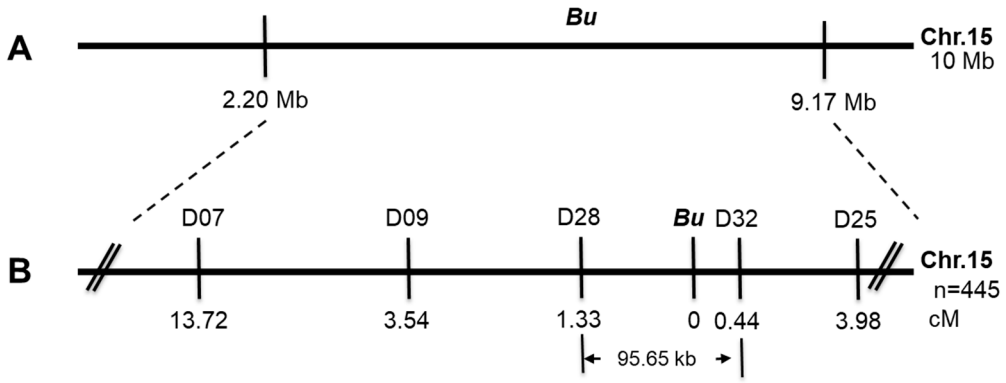

Figure 4. Fine mapping the *Bush* gene. (**A**) The *Bush* gene was preliminarily mapped to the region of 2.80 Mb to 9.17 Mb on Chr. 15. (**B**) The *Bush* gene was narrowed down to a region between InDel markers D28 and D32 with a physical distance of 95.65 kb.

Table 1. The 19 candidate genes identified within the 95.65 kb region according to the reference genome of pumpkin (C. maxima).

No.	Gene	Function
1	*CmaCh15G011480*	Monocopper oxidase-like protein SKU5
2	*CmaCh15G011490*	Axial regulator YABBY 5-like
3	*CmaCh15G011500*	Thylakoid lumenal protein TL20.3
4	*CmaCh15G011510*	Histone-lysine N-methyltransferase
5	*CmaCh15G011520*	Vacuolar-processing enzyme
6	*CmaCh15G011530*	Elongation factor G
7	*CmaCh15G011540*	Plant invertase/pectin methylesterase inhibitor superfamily protein
8	*CmaCh15G011550*	Protein ODORANT1-like
9	*CmaCh15G011560*	Retrovirus-related Pol polyprotein from transposon TNT 1-94
10	*CmaCh15G011570*	Retrovirus-related Pol polyprotein from transposon TNT 1-94
11	*CmaCh15G011580*	Geraniol 8-hydroxylase-like
12	*CmaCh15G011590*	Cytochrome P450
13	*CmaCh15G011600*	Transposable element protein
14	*CmaCh15G011610*	Cytochrome P450 84A1-like
15	*CmaCh15G011620*	Maspardin
16	*CmaCh15G011630*	40S ribosomal protein S13
17	*CmaCh15G011640*	Protein of unknown function (DUF1442)
18	*CmaCh15G011650*	IRK-interacting protein-like
19	*CmaCh15G011660*	DNA photolyase

3.5. A Quick and Efficient Method Was Developed for Bush-Type Individual Identification at Seedling Stage

Molecular marker-assisted breeding can identify the individuals with specific phenotypes using tissues from all development stages of plants, regardless of season and environment impact, which is crucial for breeders. A high-throughput genotyping system could quickly and efficiently identify individuals with specific phenotypes. However, the high cost of this system hinders most breeders from using it. In this study, we developed a quick and efficient method for bush-type individuals' identification at the seedling stage using conventional experimental equipment. The tissues were collected from the unfolding cotyledon of seven-day-old seedlings. In the laboratory, two laboratorians could process more than two thousand samples in one day (8 h) using several common PCR thermocyclers

(T100™ Thermal Cycler, Bio-Rad Laboratories, Inc. Hercules, USA) and polyacrylamide gel electrophoresis apparatus (DYCZ-20H, Beijing Liuyi Biotechnology Co., Ltd., Beijing, China). For most laboratories, our identification method is suitable for quick and efficient bush-type individuals' identification at the seedling stage.

4. Discussion

Plant architecture is an important agronomic trait that determines labor costs and crop yield [1,2]. CS82 is a bush-type inbred line with all petioles clustered at the extremely shortened vine, showing a compact plant architecture suitable for dense planting. Compared to the wild-type inbred line CM82, CS82 has shorter and limited internodes, resulting in an extremely shortened vine. Paraffin sections of the shoot apex revealed that the bush phenotype of pumpkin (*C. maxima*) may be caused by the degeneration of the shoot apical meristem.

Plant hormones are the primary regulators of plant architecture [32]. Gibberellins, brassinosteroids, cytokinins, auxin, and strigolactones are the most commonly studied hormones that regulate plant architecture [33–35]. In cucurbit crops, most dwarf mutants were caused by a deficiency of gibberellins, such as *cp* of cucumber [13], *Cldf* of watermelon [7], X10 in squash [15], and WJ209 in sponge gourd [36]. However, the bush phenotype of pumpkin (*C. maxima*) is not related to gibberellins. Exogenous gibberellin treatment does not elongate the internode length and increase the internode number of bush-type individuals, failing to recover the phenotype to wild-type (Figure 2).

A spontaneous bush-type mutant of *C. moschata* was discovered by a retired teacher in Shanxi Province, China, in 1982 [37]. The bush-type phenotype of *C. moschata* is an exciting finding which could revolutionize the planting mode and improve the per-unit yield [7,8]. To accelerate the breeding process of bush-type varieties, several molecular markers were developed using different methods [38,39]. Li et al. developed an RAPD molecular marker, S1225-548, linked to the *Bush* gene with a genetic distance of 2.29 cM [39]. Wang et al. comparatively mapped the *Bush* gene of *C. moschata* onto the chromosome 5 of the cucumber genome and developed a molecular marker, IF3629, linked to the *Bush* gene with a genetic distance of 1.0 cM [38]. Finally, Wang et al. cloned the *Bush* gene encoding a transcription factor, YABBY1. A 76 bp deletion in the promoter region was found to cause the bush phenotype in *C. moschata* [8]. In our study, we identified a bush-type inbred line of pumpkin (*C. maxima*). The BSA-seq analysis and fine mapping identified *CmaCh15G011490*, encoding an axial regulator YABBY 5-like, as the candidate gene for the *Bush* gene. A 63 bp deletion was found in the promoter region of *CmaCh15G011490* in the bush-type inbred line CS82 compared to the wild-type inbred line CM82. Next, more experiments, including gene expression analysis, complementation tests, and downstream gene identification, will be conducted to explore the link between the 63 bp deletion in the promoter and its functional impact on the *Bush* gene. Interestingly, we found that the promoter sequence of *CmaCh15G011490* in CS82 is identical to the corresponding sequence from the reference genome of *C. moschata* [40], while a 76 bp deletion was found in CS82 compared to *C. moschata*, which is in agreement with the result of Wang et al. [8]. The results above and the interspecific transfer of bush traits in our lab previously [27] indicate that the bush trait of *C. maxima* was caused by the *Bush* gene spontaneously mutated and found in *C. moschata*.

Marker-assisted selection (MAS) is a quick and effective tool for selecting individuals with the target characters, especially for dominant traits, improving the productivity and accuracy of classical plant breeding [41]. In classical plant breeding, two seasons are required to identify homozygous individuals with dominant traits. The bush-type is an advantage phenotype for close planting in the artificial cultivation of pumpkin (*C. maxima*). Based on the deletion in the promoter region of *CmaCh15G011490*, an InDel marker was developed for identifying bush-type plants. Additionally, a quick and efficient method was developed for bush-type plants' identification at the seedling stage. Currently, two identification systems are commonly used in MAS: the SSR-based gel electrophoresis system

and the KASP-based high-throughput system. For the SSR-based system, most laboratories equipped with simple instruments (PCR amplifiers and electrophoresis apparatus) could carry out MAS, though its efficiency is low. In contrast, the KASP-based system requires more expensive equipment but offers high efficiency. In our method, we used PCR amplifiers and electrophoresis apparatus with three 32-hole plugs and a simple DNA extraction method, which significantly increased the identification efficiency. The method developed in this study could accelerate the breeding progress of the bush-type variety in pumpkin (*C. maxima*).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14122967/s1, Table S1: The primers newly developed in our study.

Author Contributions: Conceptualization, X.L. and G.L.; methodology, C.L. and G.L.; software, C.L.; validation, C.L. and Z.H.; investigation, C.L., G.L., Z.H., Y.L. (Yifan Li), Y.L. (Yanhong Lan), F.G., P.C., N.Y., Y.L. (Yan Liu) and F.X.; resources, X.L.; data curation, C.L., G.L., Z.H., Y.L. (Yifan Li), Y.L. (Yanhong Lan), F.G., P.C., N.Y., Y.L. (Yan Liu) and F.X.; writing—original draft preparation, C.L.; writing—review and editing, C.L.; supervision, X.L. and G.L.; project administration, C.L.; funding acquisition, X.L. and G.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China (2022YFD1601504), the China Agriculture Research System (CARS-23-G38), the Key Research and Development Program of Sichuan Province (2021YFYZ0022), the Sichuan Innovation Team of the National Modern Agricultural Industry Technology System (SCCXTD-2024-5), and the 1 + 9 open competition projects of the Sichuan Academy of Agricultural Sciences (1 + 9KJGG001).

Data Availability Statement: Data are contained within the article and Supplementary Materials.

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

References

1. Liu, X.; Chen, J.; Zhang, X. Genetic regulation of shoot architecture in cucumber. *Hortic. Res.* **2021**, *8*, 143. [CrossRef] [PubMed]
2. Wang, B.; Smith, S.M.; Li, J. Genetic regulation of shoot architecture. *Annu. Rev. Plant Biol.* **2018**, *69*, 437–468. [CrossRef]
3. Chen, Q.; Li, W.; Tan, L.; Tian, F. Harnessing knowledge from maize and rice domestication for new crop breeding. *Mol. Plant* **2021**, *14*, 9–26. [CrossRef] [PubMed]
4. Chen, E.; Huang, X.; Tian, Z.; Wing, R.A.; Han, B. The genomics of *Oryza* species provides insights into rice domestication and heterosis. *Annu. Rev. Plant Biol.* **2019**, *70*, 639–665. [CrossRef] [PubMed]
5. Sasaki, A.; Ashikari, M.; Ueguchi-Tanaka, M.; Itoh, H.; Nishimura, A.; Swapan, D.; Ishiyama, K.; Saito, T.; Kobayashi, M.; Khush, G.S.; et al. A mutant gibberellin-synthesis gene in rice. *Nature* **2002**, *416*, 701–702. [CrossRef]
6. Zhang, G.; Ren, Y.; Sun, H.; Guo, S.; Zhang, F.; Zhang, J.; Zhang, H.; Jia, Z.; Fei, Z.; Xu, Y.; et al. A high-density genetic map for anchoring genome sequences and identifying QTLs associated with dwarf vine in pumpkin (*Cucurbita maxima* Duch.). *BMC Genom.* **2015**, *16*, 1101. [CrossRef]
7. Wei, C.; Zhu, C.; Yang, L.; Zhao, W.; Ma, R.; Li, H.; Zhang, Y.; Ma, J.; Yang, J.; Zhang, X. A point mutation resulting in a 13 bp deletion in the coding sequence of *Cldf* leads to a GA-deficient dwarf phenotype in watermelon. *Hortic. Res.* **2019**, *6*, 132. [CrossRef]
8. Wang, S.; Wang, K.; Li, Z.; Li, Y.; He, J.; Li, H.; Wang, B.; Xin, T.; Tian, H.; Tian, J.; et al. Architecture design of cucurbit crops for enhanced productivity by a natural allele. *Nat. Plants* **2022**, *8*, 1394–1407. [CrossRef] [PubMed]
9. Denna, D.W.; Munger, H.M. Morphology of the bush and vine habits and the allelism of the bush genes in *Cucurbita maxima* and *C. pepo* squash. *Proc. Am. Soc. Hort. Sci.* **1963**, *82*, 370–377.
10. Lester, D.R.; Ross, J.J.; Davies, P.J.; Reid, J.B. Mendel's stem length gene (*Le*) encodes a gibberellin 3 beta-hydroxylase. *Plant Cell* **1997**, *9*, 1435–1443. [CrossRef] [PubMed]
11. Huang, H.; Zhang, X.; Wei, Z.; Li, Q.; Li, X. Inheritance of male-sterility and dwarfism in watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai]. *Sci. Hortic.* **1998**, *74*, 175–181. [CrossRef]
12. Xu, L.; Wang, C.; Cao, W.; Zhou, S.; Wu, T. CLAVATA1-type receptor-like kinase CsCLAVATA1 is a putative candidate gene for dwarf mutation in cucumber. *Mol. Genet. Genom.* **2018**, *293*, 1393–1405. [CrossRef] [PubMed]
13. Li, Y.; Yang, L.; Pathak, M.; Li, D.; He, X.; Weng, Y. Fine genetic mapping of *cp*: A recessive gene for compact (dwarf) plant architecture in cucumber, *Cucumis sativus* L. *Theor. Appl. Genet.* **2011**, *123*, 973–983. [CrossRef] [PubMed]
14. Xiang, C.; Duan, Y.; Li, H.; Ma, W.; Huang, S.; Sui, X.; Zhang, Z.; Wang, C. A high-density EST-SSR-based genetic map and QTL analysis of dwarf trait in *Cucurbita pepo* L. *Int. J. Mol. Sci.* **2018**, *19*, 3140. [CrossRef] [PubMed]

15. Ding, W.; Wang, Y.; Qi, C.; Luo, Y.; Wang, C.; Xu, W.; Qu, S. Fine mapping identified the gibberellin 2-oxidase gene *CpDw* leading to a dwarf phenotype in squash (*Cucurbita pepo* L.). *Plant Sci.* **2021**, *306*, 110857. [CrossRef]
16. Zhang, T.; Li, C.; Li, D.; Liu, Y.; Yang, X. Roles of YABBY transcription factors in the modulation of morphogenesis, development, and phytohormone and stress responses in plants. *J. Plant Res.* **2020**, *133*, 751–763. [CrossRef]
17. Romanova, M.A.; Maksimova, A.I.; Pawlowski, K.; Voitsekhovskaja, O.V. YABBY genes in the development and evolution of land plants. *Int. J. Mol. Sci.* **2021**, *22*, 4139. [CrossRef] [PubMed]
18. Yin, S.; Li, S.; Gao, Y.; Bartholomew, E.S.; Wang, R.; Yang, H.; Liu, C.; Chen, X.; Wang, Y.; Liu, X.; et al. Genome-wide identification of YABBY gene family in Cucurbitaceae and expression analysis in cucumber (*Cucumis sativus* L.). *Genes* **2022**, *13*, 467. [CrossRef]
19. Meister, R.J.; Oldenhof, H.; Bowman, J.L.; Gasser, C.S. Multiple protein regions contribute to differential activities of YABBY proteins in reproductive development. *Plant Physiol.* **2005**, *137*, 651–662. [CrossRef]
20. Sarojam, R.; Sappl, P.G.; Goldshmidt, A.; Efroni, I.; Floyd, S.K.; Eshed, Y.; Bowman, J.L. Differentiating Arabidopsis shoots from leaves by combined YABBY activities. *Plant Cell* **2010**, *22*, 2113–2130. [CrossRef] [PubMed]
21. Yang, Z.; Gong, Q.; Wang, L.; Jin, Y.; Xi, J.; Li, Z.; Qin, W.; Yang, Z.; Lu, L.; Chen, Q.; et al. Genome-wide study of YABBY genes in upland cotton and their expression patterns under different stresses. *Front. Genet.* **2018**, *9*, 33. [CrossRef] [PubMed]
22. Siegfried, K.R.; Eshed, Y.; Baum, S.F.; Otsuga, D.; Drews, G.N.; Bowman, J.L. Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. *Development* **1999**, *126*, 4117–4128. [CrossRef] [PubMed]
23. Pfannebecker, K.C.; Lange, M.; Rupp, O.; Becker, A. Seed plant-specific gene lineages involved in carpel development. *Mol. Biol. Evol.* **2017**, *34*, 925–942. [CrossRef] [PubMed]
24. Filyushin, M.A.; Slugin, M.A.; Dzhos, E.A.; Kochieva, E.Z.; Shchennikova, A.V. Coexpression of *YABBY1* and *YABBY3* genes in lateral organs of tomato species (*Solanum*, Section Lycopersicon). *Dokl. Biochem. Biophys.* **2018**, *478*, 50–54. [CrossRef]
25. Bowman, J.L.; Smyth, D.R. *CRABS CLAW*, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **1999**, *126*, 2387–2396. [CrossRef]
26. Gallagher, T.L.; Gasser, C.S. Independence and interaction of regions of the INNER NO OUTER protein in growth control during ovule development. *Plant Physiol.* **2008**, *147*, 306–315. [CrossRef]
27. Liu, X.J.; Li, Y.J.; Zhang, Z.Y.; Liu, D.C.; Fang, C.; Liang, G.Y. Preliminary study on interspecific transfer of bush trait through *Cucurbita moschata* by *Cucurbita maxia*. *Southwest China J. Agr. Sci.* **2009**, *22*, 130–132. (In Chinese with English abstract). [CrossRef]
28. Yu, H.F. Studies on the Physiological and Molecular Mechanism of Vine-Elongation Induced by GA in *Cucurbita moschata*. Ph.D. Thesis, Zhejiang University, Hangzhou, China, 2005.
29. Takagi, H.; Abe, A.; Yoshida, K.; Kosugi, S.; Natsume, S.; Mitsuoka, C.; Uemura, A.; Utsushi, H.; Tamiru, M.; Takuno, S.; et al. QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *Plant J.* **2013**, *74*, 174–183. [CrossRef]
30. Ayele, B.T.; Magome, H.; Lee, S.; Shin, K.; Kamiya, Y.; Soh, M.-S.; Yamaguchi, S. *GA-sensitive dwarf1-1D* (*gsd1-1D*) defines a new mutation that controls endogenous GA levels in *Arabidopsis*. *J. Plant Growth Regul.* **2014**, *33*, 340–354. [CrossRef]
31. Zhang, J.; Zhang, X.; Chen, R.; Yang, L.; Fan, K.; Liu, Y.; Wang, G.; Ren, Z.; Liu, Y. Generation of transgene-free semidwarf maize plants by gene editing of gibberellin-oxidase20-3 using CRISPR/Cas9. *Front. Plant Sci.* **2020**, *11*, 1048. [CrossRef]
32. Guo, W.; Chen, L.; Herrera-Estrella, L.; Cao, D.; Tran, L.-S.P. Altering plant architecture to improve performance and resistance. *Trends Plant Sci.* **2020**, *25*, 1154–1170. [CrossRef]
33. Tong, H.; Chu, C. Functional s pecificities of brassinosteroid and potential utilization for crop improvement. *Trends Plant Sci.* **2018**, *23*, 1016–1028. [CrossRef] [PubMed]
34. Waldie, T.; Leyser, O. Cytokinin targets auxin transport to promote shoot branching. *Plant Physiol.* **2018**, *177*, 803–818. [CrossRef]
35. Liao, Z.; Yu, H.; Duan, J.; Yuan, K.; Yu, C.; Meng, X.; Kou, L.; Chen, M.; Jing, Y.; Liu, G.; et al. SLR1 inhibits MOC1 degradation to coordinate tiller number and plant height in rice. *Nat. Commun.* **2019**, *10*, 2738. [CrossRef] [PubMed]
36. Zhao, G.; Luo, C.; Luo, J.; Li, J.; Gong, H.; Zheng, X.; Liu, X.; Guo, J.; Zhou, L.; Wu, H. A mutation in *LacDWARF1* results in a GA-deficient dwarf phenotype in sponge gourd (*Luffa acutangula*). *Theor. Appl. Genet.* **2021**, *134*, 3443–3457. [CrossRef] [PubMed]
37. Wang, J.S. Brief report on the discovery and breeding of dwarf pumpkin (*Cucurbita moschata*). *China Veg.* **1986**, *50*. (In Chinese) [CrossRef]
38. Wang, S.H.; Li, H.Z.; Zhang, Z.H.; He, J.; Jia, C.C.; Zhang, F.; Huang, S.W. Comparative mapping of the dwarf gene Bu from tropical pumpkin (Cucurbita moschata Duchesne). *Acta Hortic. Sinica* **2011**, *38*, 95–100. (In Chinese with English abstract). [CrossRef]
39. Li, Y.L.; Li, H.Z.; Cui, C.S.; Zhang, H.Y.; Gong, G.Y. Molecular markers linked to the dwarf gene in squash. *J. Agr. Biotechnol.* **2007**, *15*, 279–282. (In Chinese with English abstract).
40. Sun, H.; Wu, S.; Zhang, G.; Jiao, C.; Guo, S.; Ren, Y.; Zhang, J.; Zhang, H.; Gong, G.; Jia, Z.; et al. Karyotype stability and unbiased fractionation in the paleo-allotetraploid Cucurbita genomes. *Mol. Plant* **2017**, *10*, 1293–1306. [CrossRef]
41. Hasan, N.; Choudhary, S.; Naaz, N.; Sharma, N.; Laskar, R.A. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *J. Genet. Eng. Biotechnol.* **2021**, *19*, 128. [CrossRef] [PubMed]