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QTL Analysis of Morpho-Agronomic Traits in Garden Asparagus (*Asparagus officinalis* L.)

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Abstract: In order to understand the genetic control of quantitative agronomic traits in garden asparagus, we performed a quantitative trait loci (QTL) analysis. A population (n = 167) derived from a cross between a female and male plants was evaluated for morpho-agronomic traits over three years. Interval mapping (IM) and restricted multiple QTL mapping (rMQM) analysis was applied, and 18 QTLs were detected. QTLs were located in two linkage groups (LG): 5 in LG5 and 13 in LG6. The physical position of markers of both groups was mapped onto the reference genome through BLAST analysis. LG5 and LG6 match with chromosome 1 (sex-determining chromosome) and chromosome 5, respectively. Haplotypes of both chromosomes of the heterozygous parent and their progeny were obtained, and a bin map was developed. Bins were used to map the QTLs on the reference genome and to perform the association analysis with the morpho-agronomic traits. Two major and stable QTLs over the years (R2 > 10%) for number of stalk and earliness were mapped in the end of chromosome 1 into a bin that spans 3.25 Mb and includes the sex-determination locus. In chromosome 5, some QTLs were located in the center of chromosome for the year 2016. Branching is tightly regulated by both internal factors (such as plant hormones) and external factors (such as light conditions). QTLs for branching height and earliness were detected in a bin that spans 4.96 Mb. Functional annotation of genes within the two bins revealed candidate genes with potential roles in SA and light signaling and photomorphogenesis pathways that may be involved in branching and/or tillering. This is the first study providing the identification of genomic regions associated with yield-related morpho-agronomic traits in asparagus.

Keywords: yield; genetic map; physical map; bins; candidate genes; QTLs analysis

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1. Introduction

The *Asparagus* genus is a large genus composed of about two hundred species [1]. Garden asparagus (*Asparagus officinalis* L.) is a monocotyledonous, perennial, dioecious, and diploid (2n = 2x = 20) species. The genome size is 1.187 Gb, with 93.6% of sequences assigned to 10 chromosomes [2]. *A. officinalis* is the most economically important species within the genus, with a cultivated area similar to other important vegetable crops such as pumpkins, garlic, green beans, or eggplant [3]. Beyond the use of the species for human food, asparagus has also been cultivated for medicinal purposes since ancient times. Asparagus contains higher profiles for different bioactive compounds that may provide pharmacological and healthy properties [4–6].

Sex is an important morpho-agronomic trait in garden asparagus because male plants produce higher yield, give earlier productions than females, and they do not produce seeds, avoiding their further development into seed [7]. A single-gene (M/m) model system controlling the sex trait (males, Mm; females, mm) proposed by Rick and Hanna [8] is commonly accepted. Nowadays, the availability of the sequence of the reference genome has provided a deeper understanding of the genetic control for sex. Two genes, namely *SOFF* and *MSE1/AoMYB35/AspTDF1*, located in a male-specific region (~1 Mb) and involved, respectively, in the pistil and stamen development, have been recently proposed

as responsible for the genetic control of the sex trait [2,9,10]. Due to the superior agronomic performance of male plants in crop conditions, the development of male hybrids composed solely by male plants is a main objective in asparagus-breeding programs.

Therefore, in asparagus, the production of male plants has a clear effect in yield. However, yield can be also determined by quantitative traits such as number and diameter of the spears or spears quality, the genetic control of which remains poorly understood. Research based on quantitative traits is conducted by estimating correlations between agronomic traits and yield in order to identify those traits most reliable to be used as selection criteria for elite plants. Several studies have established that total number of spears per plant and spear diameter have a strong positive correlation with yield while maintaining a negative correlation between them [11–13]. Other studies using a half-sib family population have found positive correlation between marketable yield and both number of market spears and spear diameter although the number of marketable spears shows a moderate positive correlation with spear diameter [14]. Spear tight heads is another important agronomic trait for the market because it is related to spear quality. Spear head tightness has a strong positive correlation with the branching height of the fern during summer and autumn [12]. Earliness in production has also been reported to be positively correlated with yield [7,13,15]. There are scarce reports about the gene action involved in the expression of yield, and additive and non-additive gene effects in equal importance have been reported [16].

The development of a saturated genetic map in garden asparagus remains far behind the advancements in other important vegetable crops. Significant achievements with the development of incomplete genetic maps were made from the 1990s to the first decade of the current century (reviewed in [17]). Recently, our own laboratory has developed a high-density SNP genetic linkage map through genotyping by sequencing. The map contains 10 linkage groups, each one corresponding to one chromosome [18]. Mapping quantitative trait loci (QTL) is a fundamental tool for understanding the genetic architecture controlling quantitative agronomic traits. Identification of QTLs allows the development of molecular markers linked to those traits that can be eventually used by the breeding programs via marker-assisted selection. To our knowledge, only one study on QTL has been developed in the species [19]. The authors attempted to develop AFLP markers associated with several traits (green spear quality, mean spear weight, and marketable yield), but the analysis did not find significant association in the populations. Following that pioneer study, our own laboratory released a high-density SNP linkage map [18], and now, we aim to generate QTL mapping in garden asparagus.

Here, we focus on important economic traits that certainly require a better understanding in asparagus research, such as diameter and number of spears, height of first branching, or earliness in spear production. The identification of genomic regions of interest allowed us to search for candidate genes that may play a significant role in the phenotypic expression of those major morpho-agronomic traits in asparagus.

2. Materials and Methods

2.1. Plant Material

A diploid F_1 population of 167 individuals derived from the controlled cross (hand pollinated) of PS010 × WN124 was used in this study. PS010 is a female plant from unknown origin. WN124 is a male plant belonging to a naturalized accession from Asmundtorp (Skåne County, Sweden), provided by the Nordic GenBank. Both accessions were characterized for morpho-agronomic traits in a field plot at the University of Córdoba (Córdoba, Spain). PS010 showed higher branching height, greater stalk thickness, lower number of stalks, and inferior earliness compared to the parent WN124. In late spring 2012, the population was planted in a field experiment at the Research Center of the Regional Government of Andalusia (IFAPA, Córdoba). The field plot was four rows of 25 m length with 1.5 m between rows and 0.5 m between plants within a row. Standard cultural agronomic practices were applied.

2.2. Assessment of Morpho-Agronomic Traits

The population was evaluated during 2016, 2017, and 2018. The statistical analysis was performed using the software IBM SPSS Statistics for Windows, v. 25.0. Armonk, NY: IBM Corp and RStudio (v. 1.3.1056). The morpho-agronomic traits addressed were:

- Branching height, evaluated in the autumn of 2016 and 2017 as follows:
  - Mean branching height (BH), obtained as the mean height of the first branching of the fern stalks;
  - Maximum branching height (MBH), measured on the fern stalk with the greatest distance from ground level to the first branch.
- Earliness in yield was evaluated in the spring as follows:
  - Phenological stage reached by the spears of each plant (PS) evaluated in a single day, i.e., 8 March 2017 and 28 March 2018, and scored on a 0 to 4 numeric rating scale (0 = no spear; 1 = spear under 20 cm high; 2 = spear higher than 20 cm; 3 = spear with primary branches; 4 = spear with secondary branches);
  - Spear area under the curve of progress of the number of spears (SPA) recorded along different dates, namely 1, 8, and 15 April in 2016, and 27 February and 7 and 28 March in 2018.
- Number of stalks per plant (NS) and stalk thickness (ST) (mm), measured on the stalk with the largest diameter of the plant at 10 cm from soil level by digital caliper, were evaluated in the autumn of 2016 and 2017.

2.3. QTL Analysis

To perform the quantitative analysis of the traits, we used the parental genetic maps developed by Moreno et al. [18], which include a total of 1403 markers (1376 SNPs and 27 EST-SSRs) distributed in the female and male parental maps, with 10 linkage groups each. QTL analysis was performed using MapQTL5 [20]. A two-way pseudo-testcross strategy was carried out to identify QTLs [21]. QTLs were detected using interval mapping (IM) and restricted multiple QTL mapping (rMQM). The detection of the QTL interval was tested by a likelihood-ratio statistic (LOD). LOD threshold values for QTL significance were determined using a permutation test with 1000 iterations for each trait. QTL with LOD values higher than the genome-wide threshold at $p < 0.05$ were considered significant. A step size of 5 cm and a maximum of 5 neighboring markers were used in QTL detection. The QTL maps were drawn by MapChart 2.32 [22].

There were 12.7% of the markers from the genetic linkage maps that were heterozygous in both parents (*hk* genotypes). These markers cannot be analyzed following the above strategy because the allelic contribution of each parent cannot be identified. Therefore, we used a single-marker analysis of variance (ANOVA) to determine the association between these markers and the phenotypic traits (QTLs). A significance threshold of LOD = $-\mathrm{Log}_{10}$ (p) values ≥ 3.0, corresponding to p-value < 0.001, was used to minimize possible false-positive QTLs and define significant marker–QTL association. A one-way ANOVA test was performed in RStudio.

In order to overlap QTLs with physical position and narrow down the genomic region that spans the QTLs, a collinearity analysis between the genetic map and the physical location of each marker was performed as a first step. For that, SNPs markers were physically localized by aligning their sequences against the *A. officinalis* genome Aspof.V1 (GenBank assembly accessionGCF_001876935.1) using BLAST [23]. Similarly, the physical location of the EST-SSR markers was obtained by mapping the forward and reverse primer sequences against the asparagus genome. Next, we selected those chromosomes with QTLs for further analysis. Then, haplotypes of both chromosomes of the heterozygous parent were developed and compared with progeny's haplotypes to identify recombination breakpoints in the mapping population. The haplotypes of both chromosomes of the heterozygous parent were obtained by analyzing the coupling or repulsion phases between alleles mapped physically through its descendance. For two linked, heterozygous loci *A/a*

y *B/b* in a cross test (*AaBb* × *aabb*), if the alleles are in coupling phase, then the haplotype of a chromosome of the heterozygous parent will be *AB* and the other *ab*. On the other hand, if the alleles are in repulsion, then the haplotype will be *Ab* for one chromosome and *aB* for the other one. Using the two haplotypes, the recombination breakpoint for overall progeny was determined considering the transition from one to another haplotype. Non-recombinant regions between recombination breakpoints were considered as a bin. Bins were employed to map physically the QTLs on the reference genome, and then, they were used to perform the association with the morpho-agronomic traits using a one-way ANOVA. A significant threshold was established at $LOD = -Log_{10}(P)$ values ≥ 3, and the statistical analysis was performed in R.

2.4. Selection of Candidate Genes

Candidate genes were selected based on the functional annotation of the genes in the bin regions showing the highest significance for each QTL. Sequences contained by the bin regions were downloaded from the genome browser at NCBI (Genome Data Viewer) [24]. Next, sequences were submitted to the functional annotation pipeline using the Blast2GO methodology [25] implemented in the OmicsBox platform (BioBam Bioinformatics, Valencia, Spain, v. 2.1.10). For the mapping and annotation, the following configuration settings were used: BLASTP against NCBI non-redundant (nr) protein database, E-value filter $\leq 10-6$, length cutoff of 33, maximum 5 BLAST hits per sequence, and annotation cutoff of 50. Furthermore, to improve annotation, InterProScan was performed, and results were merged to GO annotation.

3. Results and Discussion

3.1. Assessment of Morpho-Agronomic Traits

The evaluation of the morpho-agronomic traits was conducted in spring and autumn for three years (2016–2018). Descriptive statistics of the morpho-agronomics traits studied in the offspring are summarized in Table 1. Kolmogorov–Smirnov test with Lilliefors correction was performed to assess the normality distribution of the morpho-agronomic traits. Solely mean branching height (BH) in 2016 and stalk thickness (ST) in 2017 showed a normal distribution (Table 1). Therefore, the non-parametric Spearman correlation coefficients between traits were calculated (Table 2). For the two years in which the branching height was evaluated (2016 and 2017), a high, positive, and significant correlation (0.74 and 0.82, respectively) was found between the two characters (BH and MBH) used to evaluate the trait. Accordingly, either of both characters could be used to estimate branching height. This trait has been reported to be highly related to the tightly close head of spears [12,26]. Stalk thickness (ST) was moderate, positive, and significantly correlated (0.52–0.60) with branching height (MBH and BH) for the two years, suggesting some genetic linkage between both characters. Similar correlation values (0.52 and 0.68) and statistical significance were also found between number of stalks (NS) and earliness (PS or SPA). Lower correlation values (0.21–0.25) were found between stalk thickness (ST) with both phenological stage (PS) and number of stalks (NS). A high, positive, and significant correlation (0.75) was also found between the traits employed to evaluate earliness in yield (PS and SPA).

The relationships between the characters evaluated in the present study and important morpho-agronomic traits, such as plant yield or spear quality, have been pointed out in previous studies. High stalk numbers that display medium-high diameter size have been described as important plant yield components [27–29]. Branching height is highly related with the spear head tightness [12,26]. This last morphological trait is important for green spear production under warm climates because plants with large branching height tend to avoid high percentages of non-marketable spears [17]. According to the "standard FFV-04" rules, concerning the marketing and commercial quality control of asparagus, a compact tip of the spears is a requirement to be classified as "extra" class quality [30]. During harvest season, earliness in spear production has been found highly correlated with full-season yield [7,13,15]. Earliness in spear production is also interesting from a commercial point

of view, as prices paid for the spears at the beginning of the harvest season are usually noteworthily higher. In the present study, significant correlations were also found between earliness in spear production and total stalk number per plant.

Table 1. Descriptive statistics and normality analysis of morpho-agronomic traits in a F_1 population of garden asparagus.

Characteristics [a]						Kolmogorov–Smirnov Test [b]	
	Years	N	Mean ± SE	Minimum	Maximum	Test Statistic	P (2-Tailed)
BH (cm)	2016	163	45.08 ± 0.682	20	67.50	0.056	0.200
	2017	155	43.00 ± 0.696	12	62	0.113	0.000
MBH (cm)	2016	163	55.57 ± 0.807	22	81	0.07	0.048
	2017	157	51.54 ± 0.923	17	81	0.098	0.001
NS	2016	163	10.96 ± 0.492	1	32	0.126	0.000
	2017	157	14.09 ± 0.726	1	52	0.101	0.001
PS	2017	166	1.12 ± 0.090	0	4	0.232	0.000
	2018	130	3.23 ± 0.095	0	4	0.292	0.000
SPA	2016	164	55.89 ± 3.622	0	252	0.114	0.000
	2018	134	142.84 ± 11.891	0	658	0.175	0.000
ST (mm)	2016	163	10.41 ± 0.182	4.30	18.50	0.061	0.200
	2017	157	10.66 ± 0.218	3.80	18	0.048	0.200

[a] BH, mean branching height; MBH, maximum branching height; NS, stalk number per plant; PS, phenological stage; SPA, spear area under the curve of progress of the number of spears; ST, stalk thickness. [b] Lilliefors significance correction.

Table 2. Spearman correlation coefficients between morpho-agronomic traits in a F_1 population of garden asparagus.

Traits	Year	BH	MBH	NS	PS	SPA
MBH	2016	0.74 **				
	2017	0.82 **				
	2018	nd				
NS	2016	−0.11	0.14			
	2017	0.17 *	0.23 **			
	2018	nd	nd			
PS	2016	nd	nd	nd		
	2017	0.22 **	0.25 **	0.52 **		
	2018	nd	nd	nd		
SPA	2016	0.23 **	0.36 **	0.68 **	nd	
	2017	nd	nd	nd	nd	
	2018	nd	nd	nd	0.75 **	
ST	2016	0.52 **	0.60 **	0.25 **	nd	0.41 **
	2017	0.59 **	0.52 **	0.25 **	0.21 **	nd
	2018	nd	nd	nd	nd	nd

* and ** are significant correlations at the 0.05 and 0.01 levels (2-tailed), respectively. BH, mean branching height; MBH, maximum branching height; NS, stalk number per plant; PS, phenological stage; SPA, spear area; ST, stalk thickness; nd, no data.

3.2. QTL Mapping

QTL analysis performed by rMQM resulted in identification of 72 markers (70 SNPs and 2 sex markers) significatively associated with the quantitative traits (Table S1). All significant markers were located on the paternal genetic map (<*nn* × *np*>), with the male WN124 being the heterozygous parent. A total of 18 QTLs (LOD score threshold > 2.5) distributed on LG5 and LG6 were identified for the studied traits along different years (Figure 1, Table S1). QTL regions for different morpho-agronomic traits co-located on LG5 and LG6. QTLs on LG5 were sited on one extreme of the linkage group, while on LG6, they were distributed in a broad genomic window (Figure 1). The percentage of the total phenotypic variation (PV) explained by the QTLs ranged from 7% for SPA in 2016 (*SPA16.b*)

to 33.4% for NS in 2017 (*NS17*), and the LOD values were between 2.86 (*SPA16.b*) and 13.85 (*NS17*) (Table S1).

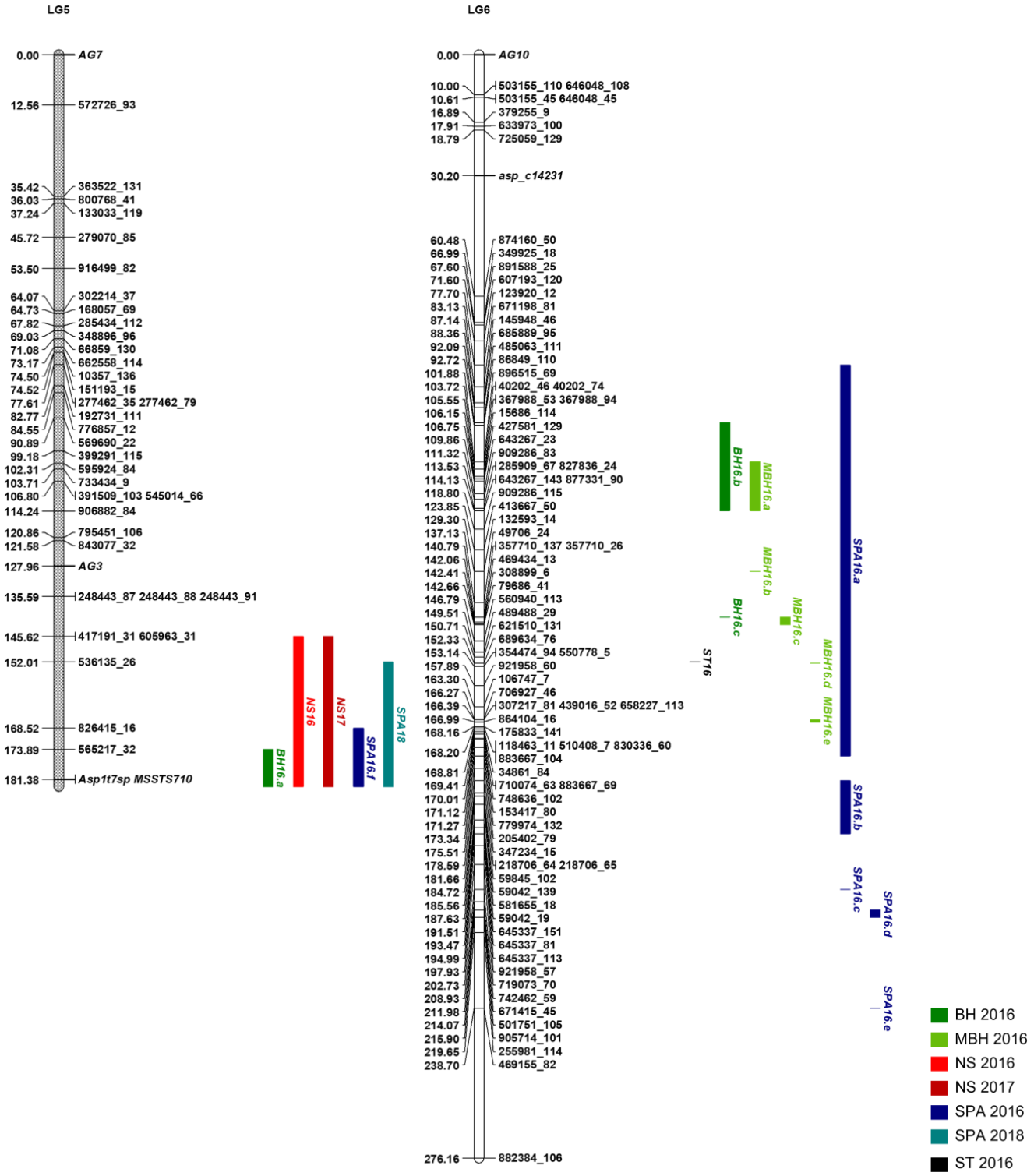

Figure 1. QTLs for morpho-agronomic traits identified in the asparagus F_1 population derived from the cross PS010 × WN124. The genetic distance is expressed in cM for each SNP. Stalk thickness (ST); branching height (BH); maximum first branching height (MBH); number of stems (NS); spears area (SPA). Only LGs with QTLs are shown.

Comparisons between physical and parental genetic maps showed a good collinearity (Figure 2). The physical location of the markers on the reference genome allowed us to assign the genetic linkage groups reported by Moreno et al. [18] to the chromosome nomenclature proposed for the reference genome by Harkerss et al. [2]. Accordingly, LG5 and LG6 containing the 18 QTLs identified correspond to chromosome 1 (sex-determining chromosome) and chromosome 5, respectively. The physical location of the markers on both chromosomes allowed us to detect recombination breakpoints comparing the progeny haplotypes with those of the heterozygous parent. The average of breakpoints per individual was 1.6 and 0.77 for chromosome 1 and 5, respectively (Figures 3 and 4). The difference in breakpoints could be explained by the high recombination frequency observed in the left end of chromosome 1 (Figure 3). The results agree with those obtained in other species, such as *Brassica napus* with 1.51 and 0.73 crossover events per individual and per chromosome [31,32]. Identification of recombination breakpoints allowed us to construct a bin map providing a confident and accurate physical location of the QTLs in both chromosomes. The bin map contained 17 and 25 bins in chromosome 1 and 5, respectively. In both chromosomes there is a wide bin (*m* in chromosome 1 and *c* in chromosome 5) with a low recombination rate compared to the respective flanking bins that could be related with the centromeric region of the chromosomes (Figure 3B,C and Figure 4B,C).

Using the bin map, six and four QTLs were detected in chromosomes 1 and 5, respectively (Figures 5 and 6). In chromosome 1, all QTLs detected were located in the left end of chromosome, while the distribution of QTLs in chromosome 5 was close to the center. The large difference in the number of QTLs obtained in the bin map compared to the genetic map, namely 5 (LG5) and 13 (LG6), may be explained by misplacement of the markers on the genetic map. Once the markers mapped onto the reference genome, they clustered together to form unique QTL regions. The result may be explained because of the relative order of the markers on the genetic maps, which in turn is also influenced by missing values, population size, or segregation distortion, making it worse as the distance between loci decreases [33]. The wider genomic window for QTLs observed in chromosome 5 may be a consequence of the lowest recombination frequency in the area that seems to be located in a pericentromeric region (Figure 4). Regions with lower gene density undergo fewer recombination events, as has been observed in the pericentromeric region of different crops such as tomato, potato, and wheat, among others [34].

Number of stalks per plant is also an important yield component in asparagus [11,35,36]. A strong QTL was detected in chromosome 1 in two years of assessment. This major and stable QTL (i.e., with a PV higher than 10% and present for more than one year) was located in the extreme of chromosome 1, and bin *d* explained the highest phenotypic variation with values of 22.3 and 33.4% in 2016 and 2017, respectively (Figure 5, Table 3). Bin *d* spans 3.25 Mb and includes the sex-determining region. Stalk number has been highly correlated with yield [13,26,29], and it is well-known that male plants produce higher yield than female ones [13,36,37]. Our results agree with this association between sex and yield that may be explained by genetic linkage or pleiotropic genetic effects.

The QTL for branching height (BH or MBH) located on chromosome 5 in 2016 had a high and significant association with a large genomic region of 21.42 Mb that contains six bins (*h*, *i*, *j*, *k*, *l*, and *m*; Figure 6, Table 4). The significance for MBH was stronger than BH, and bin *l* (4.96 Mb length) explained the highest value of the phenotypic variation for MBH (R^2 = 10.78%). A small decrease in the significant association values was observed in the contiguous bins on the left end of bin *l*. This result could be due to the low recombination present in the area near the centromeric region. However, the same effect does not occur on the right end of the bin *l*, where the recombination frequency increases (Figure 4). In chromosome 1, another QTL for BH was also detected in 2016 (Figure 5, Table 3). Here, two bins (*c* and *d*) were significantly associated with the trait, explaining up to 9.84% of the variation and delimiting a window of 4.62 Mb, which includes the sex-determining region.

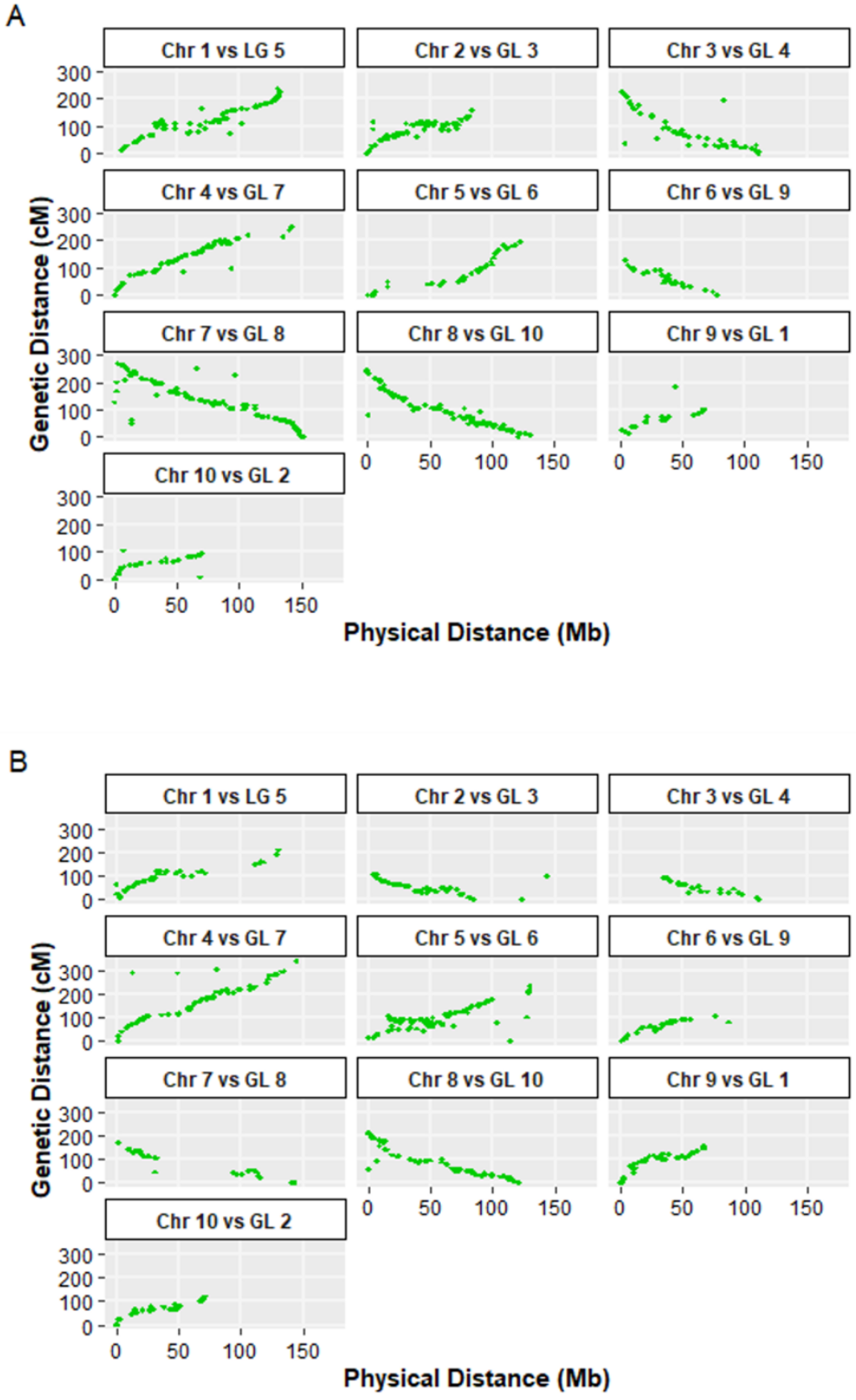

Figure 2. Distribution of markers according to their position in the genetic and physical map obtained in a mapping population (PS010 × WN 124) of *A. officinalis*. (**A**) Maternal map. (**B**) Paternal map.

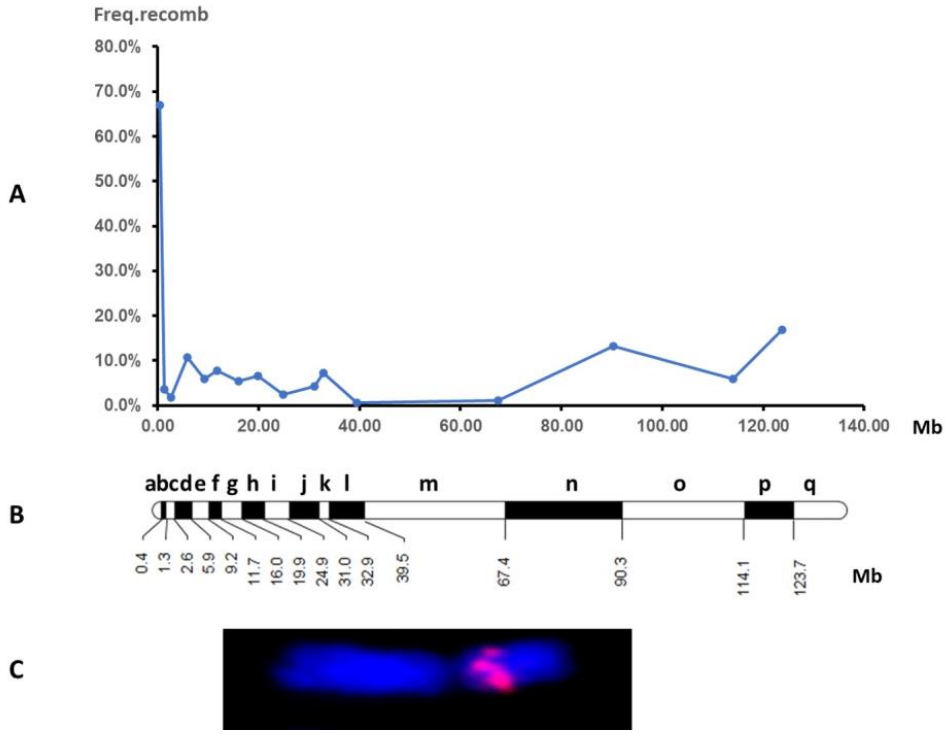

Figure 3. (**A**) Distribution of recombination frequency between bins along chromosome 1 of *A. officinalis*. (**B**) Map bins. (**C**) Fluorescence in situ hybridization (FISH) with rDNA 5s probe on chromosome 1 [18].

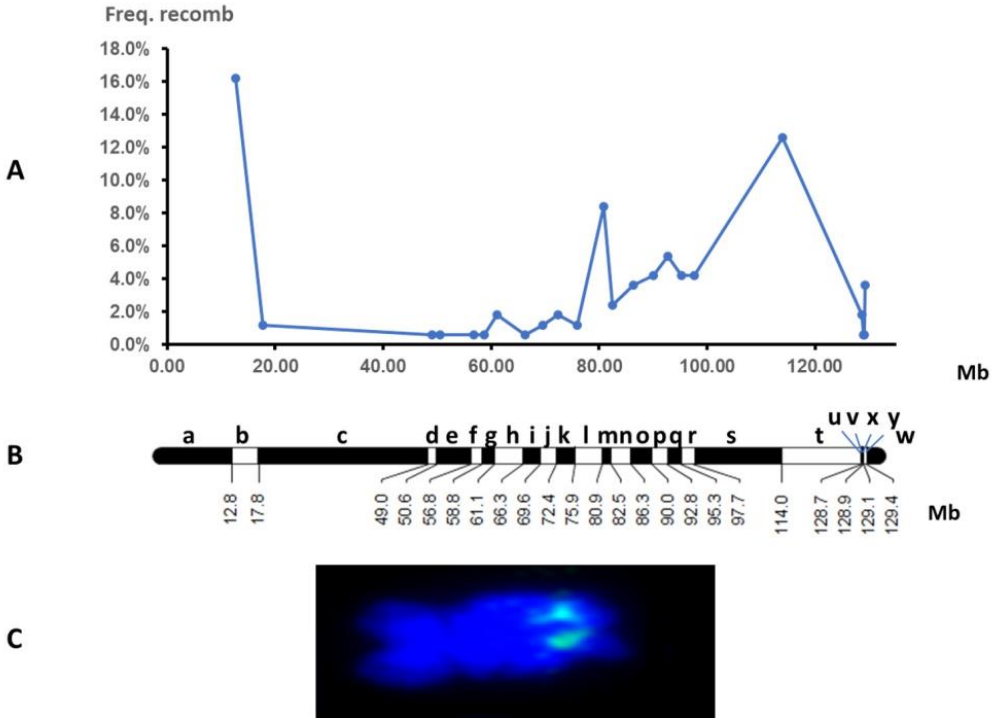

Figure 4. (**A**) Distribution of recombination frequency between bins along chromosome 5 of *A. officinalis*. (**B**) Map bins. (**C**) Fluorescence in situ hybridization (FISH) with rDNA 45s probe on chromosome 5 [18].

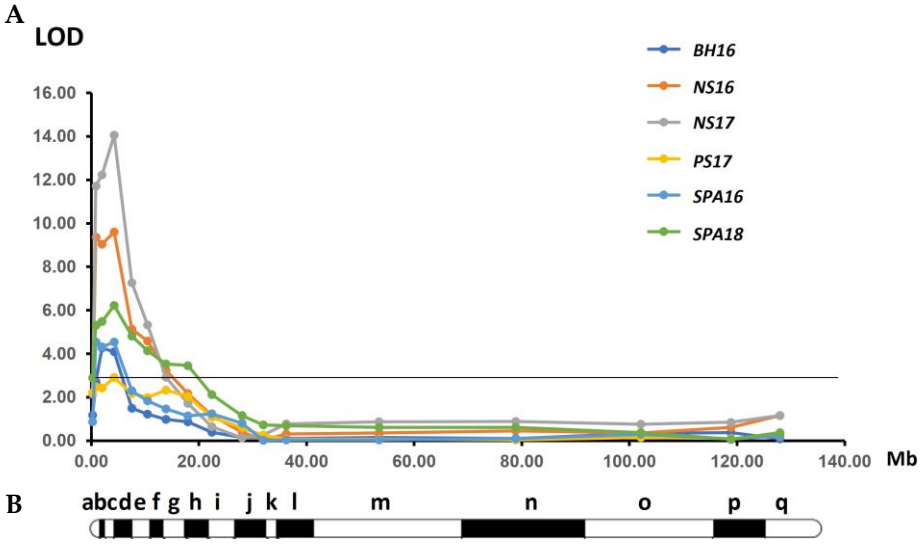

Figure 5. (**A**) QTLs detected for several morpho-agronomic traits on chromosome 1 of *A. officinalis*. The traits are mean branching height (BH), number of stalks (NS), and earliness measured as phenological stage (PS) and spear area (SPA). The LOD is calculated as $-\mathrm{Log}_{10}(P)$ values. The horizontal black line is the established threshold of LOD $\geq$ 3. (**B**) Bins chromosome 1.

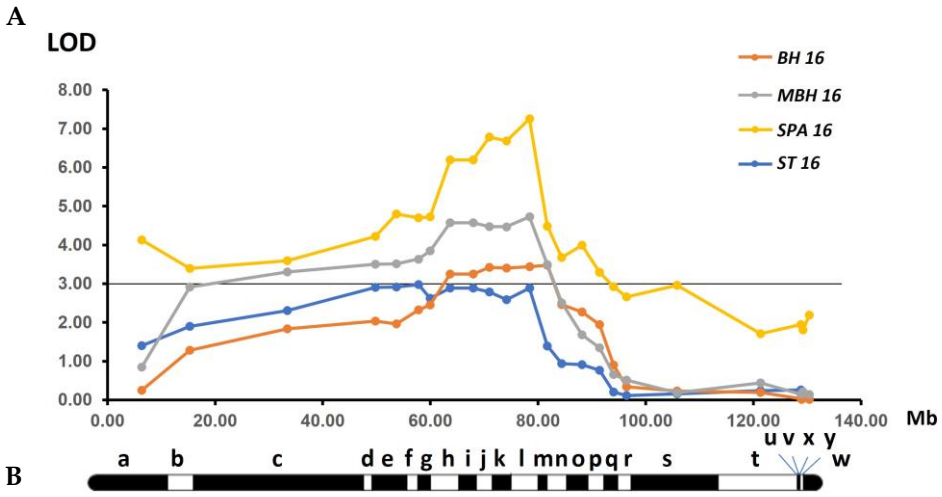

Figure 6. (**A**) QTLs detected for several morpho-agronomic traits on chromosome 5 of *A. officinalis*. The traits are mean branching height (BH), maximum branching height (MBH), and earliness measured as spear area (SPA) and stalk thickness (ST). The LOD is calculated as $-\mathrm{Log}_{10}(P)$ values. The horizontal black line is the established threshold of LOD $\geq$ 3. (**B**) Bin map of chromosome 5.

Earliness, evaluated as PS and SPA, was related to chromosomes 1 and 5, each containing one QTL. In 2016, a strong association for SPA was detected on chromosome 5, and bin *l* explained a total of 16.7% of the phenotypic variation (Table 4, Figure 6). The other QTL for SPA was detected at the end of chromosome 1 in both 2016 and 2018. The highest percentage of the total variation (17.6%) was explained by bin *d* in 2018 (Figure 5, Table 3). Concerning PS, in 2017, a light significant association with bin *d* was also found although it had a lower LOD value (LOD = 2.9) and explained a lower percentage of the phenotypic variation (R^2 = 6.3%) than for SPA. Therefore, a stable QTL for earliness yield could be considered within this genomic region of chromosome 1 that may explain the moderate positive correlation found with stalk number in this study (Table 2). In regard to stalk thickness (ST), a weak QTL was located on chromosome 5 (in 2016), with several bins (*d* to *l*) spanning a large genomic region (31.86 Mb; Table 4), which showed an upward trend but without exceeding the established threshold of LOD = 3. Bin *f* was the most significatively

associated bin, explaining 6.5% of the phenotypic variation. Noticeably, QTLs for branching height, earliness, and stalk thickness in chromosome 5 were detected only in 2016. It is accepted that spear emergence depends on soil temperature, and high temperature affects the spear head tightness, which in turn is highly correlated with branching height [12,26]. Our findings may be the result of different environmental conditions during the harvesting period in the years that we assessed the phenotypic data. The first months of the harvesting time in 2016 were, actually, colder than those in 2017 and 2018 (Figure S1).

Table 3. Bins detected in chromosome 1 and QTLs identified. BH, mean branching height; NS, number of stalks; PS, phenological stage; SPA, spear area. The abbreviations of the trait names are followed by the year in which the trait was assessed (e.g., 16, 17, and 18 refer to the years 2016, 2017, and 2018, respectively).

		Branching Height		**Stalk Number**				**Earliness**					
		BH16		**NS16**		**NS17**		**PS17**		**SPA16**		**SPA18**	
Bin	**Length (Mb)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**
a	0.43	1.17	2.16	2.14	4.58	2.15	4.79	2.22	4.71	0.86	1.42	2.88	7.90
b	0.84	2.69	5.87	9.35	21.89	11.72	27.90	2.47	5.20	4.53	10.43	5.30	14.98
c	1.37	4.27	9.84	9.04	21.20	12.23	28.99	2.43	5.10	4.31	9.88	5.48	15.49
d	3.25	4.09	9.39	9.60	22.44	14.06	32.77	2.91	6.29	4.54	10.44	6.22	17.63
e	3.31	1.49	2.86	5.12	11.96	7.27	17.73	2.17	4.46	2.29	4.82	4.82	13.55
f	2.50	1.22	2.22	4.58	10.62	5.33	12.92	1.98	4.01	1.84	3.70	4.14	11.54
g	4.33	0.98	1.66	3.23	7.23	2.92	6.68	2.33	4.86	1.47	2.80	3.53	9.71
h	3.82	0.88	1.42	2.16	4.54	1.72	3.55	2.04	4.14	1.14	2.01	3.46	9.47
i	5.09	0.39	0.43	1.15	2.06	0.65	0.97	1.08	1.85	1.23	2.24	2.12	5.41
j	6.08	0.15	0.09	0.40	0.46	0.14	0.08	0.60	0.81	0.81	1.27	1.16	2.55
k	1.87	0.17	0.11	0.01	0.00	0.25	0.23	0.24	0.20	0.03	0.00	0.73	1.37
l	6.60	0.10	0.04	0.30	0.29	0.77	1.24	0.07	0.02	0.03	0.01	0.69	1.25
m	27.94	0.15	0.09	0.36	0.38	0.87	1.47	0.06	0.02	0.04	0.01	0.61	1.05
n	22.81	0.10	0.04	0.45	0.55	0.89	1.51	0.04	0.01	0.10	0.05	0.61	1.05
ñ	23.81	0.35	0.36	0.36	0.38	0.76	1.22	0.15	0.09	0.29	0.26	0.37	0.50
o	9.61	0.38	0.42	0.61	0.85	0.85	1.41	0.10	0.04	0.06	0.02	0.07	0.03
p	8.73	0.08	0.03	1.16	2.09	1.16	2.17	0.21	0.16	0.23	0.19	0.37	0.49

[a] LOD, $-\log_{10}(P)$ values. R^2, percentage of the total phenotypic variation explained by the bin.

The analysis of heterozygous markers in both parents (<*hk* × *hk*>) (a total of 178 SNPs/12.69% of the total SNPs) reported a significant association for 13 markers, which had LOD values ranging from 3 to 6.65 and explained from 6.90% to 16.72% of phenotypic variation (Table S2). The physical location of the markers showed their location in significant bins for all traits considered. The marker associated with stalk thickness was located in bin *l* on chromosome 5, which is one of the bins that showed an upward trend but did not exceed the previously established threshold (Table 4).

3.3. Candidate Genes

In a first attempt to narrow the list of candidate genes governing important agronomic traits in asparagus, we performed a functional annotation of the loci based on assignment of GO identity. The analysis was performed for bins on chromosome 1 and chromosome 5, which showed the highest percentage of explained PVs and reached a minimum of 10 % (bin *d* and bin *l*). Bin *d* on chromosome 1 showed the highest significance for all the QTLs detected on that chromosome. The highest value of phenotypic variation was shown by NS. The bin spans 3.25 Mb and contains 112 loci (36.7 loci per Mb; avg. chr.1: 32 loci/Mb; Figure S2). After removing 18 loci non-coding RNAs, a set of 94 sequences were run into the functional annotation pipeline, with a final result of 83 sequences fully annotated. The distribution of GO terms and sequences over the ontologies of "molecular function", "biological process", and "cellular component" is shown in Figure S3. The LOC109846629 encodes a ras-related protein RGP1 with nucleotide and hydrolase molecular function activities (GO:0000166 and GO:0016787, respectively). Previous studies have demonstrated the correlation of *RGP1* with effects on morphological traits such as the induction of

tillering. The formation of tillers from axillary meristem is regulated by phytohormones such as cytokinin, which acts as positive regulator of tillering, and auxin, which act as an inhibitor of tillering [38]. Rice lines overexpressing the *RGP1* gene have shown increased tillering [39], whereas the induction of tillering through overexpression of *RGP1* in tobacco has been shown to be associated with increases in the levels of both cytokinin and salicylic acid (SA) [40]. In a recent study, exogenous application of SA in wheat plants induced the production of cytokinin leading to tiller initiation [41]. At least, a second ras-related protein encoded by LOC109847064 (sharing 75% identity at the amino acid level) is also present in bin *d* with the same molecular activities (GO:0000166, GO:0016787). Another interesting molecular function in bin *d* is associated with LOC109846575, which encodes a bZIP transcription factor TGA10-like protein (GO:0003700, transcription factor activity). It is known that signal transduction pathways, such as the SA, are involved in the regulation of defense responses. The transcriptome profile of asparagus plants inoculated with the biocontrol agent *Yarrowia lipolytica* has resulted in induction of the SA signaling pathway, revealed by the up-regulation of the *TGA10* transcription factor [42]. We speculate that *RGP1* and *TGA10* may have a concomitant role in tilling formation through the SA signal transduction. As it is mentioned above, male plants produce higher yield than female ones. The physiological aspect has been underlined to explain this fact because male plants do not have to divert photosynthates to seed production [43]. According to our results, the presence of the candidate genes *RGP1* and *TGA10*, which are tightly linked to the sex-determining region, may also explain the different agronomic performance of asparagus plants related to yield.

Table 4. Bins detected in chromosome 5 and QTLs identified. BH, mean branching height; MBH, maximum branching height; SPA, spear area; ST, stalk thickness. Termination 16 refers to the year 2016, when the QTLs were detected.

		Branching Height				**Earliness**		**Stalk Thickness**	
		BH16		**MHB16**		**SPA16**		**ST16**	
Bin	**Length (Mb)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**	**LOD** [a]	**R^2 (%)**
a	12.70	0.24	0.20	0.85	1.33	4.13	9.26	1.40	2.59
b	5.02	1.28	2.32	2.91	6.30	3.39	7.45	1.89	3.79
c	31.24	1.83	3.64	3.31	7.28	3.60	7.95	2.30	4.80
d	1.53	2.03	4.13	3.50	7.77	4.22	9.48	2.90	6.28
e	6.24	1.96	3.96	3.51	7.79	4.80	10.90	2.91	6.31
f	1.98	2.32	4.84	3.63	8.09	4.70	10.66	3.00	6.47
g	2.33	2.45	5.16	3.84	8.62	4.72	10.71	2.62	5.59
h	5.14	3.25	7.18	4.57	10.47	6.20	14.31	2.88	6.27
i	3.34	3.25	7.18	4.57	10.47	6.20	14.31	2.88	6.27
j	2.75	3.42	7.57	4.47	10.15	6.78	15.60	2.79	6.00
k	3.59	3.41	7.53	4.47	10.15	6.68	15.37	2.58	5.49
l	4.96	3.44	7.62	4.72	10.78	7.25	16.69	2.88	6.23
m	1.63	3.47	7.69	3.49	7.74	4.49	10.13	1.39	2.59
n	3.75	2.46	5.19	2.51	5.31	3.67	8.15	0.94	1.53
o	3.72	2.27	4.74	1.68	3.30	3.99	8.98	0.91	1.48
p	2.79	1.95	3.92	1.35	2.47	3.30	7.22	0.76	1.15
q	2.46	0.90	1.45	0.66	0.93	2.92	6.28	0.20	0.15
r	2.44	0.34	0.35	0.51	0.64	2.66	5.65	0.11	0.05
s	16.25	0.23	0.19	0.18	0.13	2.96	6.37	0.16	0.10
t	14.76	0.19	0.15	0.44	0.55	1.71	3.55	0.24	0.21
u	0.20	0.03	0.00	0.15	0.08	1.94	3.88	0.26	0.22
v	0.15	0.02	0.00	0.16	0.10	1.93	3.85	0.20	0.15
x	0.29	0.11	0.05	0.22	0.16	1.81	3.57	0.14	0.08
w	2.65	0.02	0.00	0.14	0.08	2.19	4.49	0.02	0.00

[a] LOD, $-\log10(P)$ values. R^2, percentage of the total phenotypic variation explained by the bin.

Related to chromosome 5, bin *l* spans 4.96 Mb and contains 133 loci (22.4 loci per Mb; avg. chr.5: 33 loci/Mb; Figure S1). After removing 20 sequences described as non-coding RNA or pseudogenes, a set of 113 loci was further analyzed, with a final result of 70 sequences fully annotated (Figure S4). Three copies of a FAR1-related sequence protein (LOC109842041, LOC109842030, and LOC109842068) were annotated under biosynthetic process (GO:0009058). FAR1 protein is a transcription factor essential for phytochrome A-mediated light signaling. Recent studies have started to elucidate the complex regulatory network regulating branching through integration of the light and plant hormone signaling pathways. The *Arabidopsis FAR1* has been suggested to act as the nexus in the signaling cross-talk mechanisms [44]. Another interesting annotation (anatomical structure development; GO:0048856) was associated with LOC109842035, which encodes a protein MAIN 1-like protein. MAIN protein has a crucial role in dividing cells to maintain genome stability in the meristems of *Arabidopsis* [45]. The loss-of-function mutant shows cell differentiation defects in root and shoot tissues [46]. Another potential candidate based on the interest of the GO term identity is LOC109844046 (TCP4 protein; GO:0003700, DNA-binding transcription factor activity), which encodes a *TCP*-family transcription factor that was initially identified as a key repressor of lateral branching in maize [47]. Recent studies have found TCPs to promote plant adaptation in the control of several processes such as leaf and flower development, photomorphogenesis, and thermomorphogenesis [48–50]. Lately, the *Arabidopsis TCP4* has been shown to have a role in the suppression of leaf area under high temperature by a reduction in cell number [51]

4. Concluding Remarks

This is the first study reporting association between morpho-agronomic traits and specific regions in the asparagus genome. Through association analysis and bin mapping, morpho-agronomic-trait QTLs related to yield were identified and located on chromosomes 1 and 5 in the reference genome sequence. In addition, two major QTLs for number of stalks and earliness in yield were also identified. The construction of a bin map allowed us to obtain an accurate location of the QTLs in the relevant chromosomes. On the other hand, the availability of the genome sequence allowed us to carry out the functional annotation of genes contained by significant bins. The genomic regions of several candidate genes related to branching/tillering, which is a major component of plant architecture and asparagus productivity, were identified. The scientific literature from plant species without floral dimorphism provides support for the association of these genes with significant traits related to yield. Our results will contribute to a deeper understanding of the genes underlying those traits, which may eventually facilitate the work of plant breeders.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010041/s1.

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