

Article

Variation and QTL Analysis of Dynamic Tillering in Rice Under Nitrogen and Straw Return Treatments

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Abstract: Rice tillering is an important trait that is genetically and environmentally co-regulated. Nitrogen is one of the key nutrients affecting tillering, and straw return further affects tiller development by altering soil heterogeneity. In order to analyze the genetic regulation mechanism of rice tillering and its interactions with the environment, 124 recombinant inbred line (RIL) populations derived from two superior Peijiu lines, 9311 and PA64s, were used as materials in this study, and the dynamic tillering phenotypes were measured under three treatments (no nitrogen application, nitrogen application, and nitrogen + straw return) for two consecutive years. Using an existing genetic map, we conducted single-environment, multi-environment, and meta-QTL analyses to systematically identify tiller-related genetic loci and their environmental interactions. The main findings were as follows: (1) A total of 57 QTLs were identified in the single-environment QTL analysis, of which 44 were unreported new QTLs. Four QTLs showed temporal pleiotropy, ten QTLs contributed more than 10% to the phenotypes under the no-N treatment, and five QTLs contributed more than 10% under the straw return treatment. Among them, the phenotypic contribution of mks1-355 (*qD1tn1-3*) and mks1-352 (*qD2TN1-2*) both exceeded 40%. (2) Multi-environmental QTL analysis detected 15 QTLs. Of these, *qmD1TN1* (mks1-356) showed no environmental interaction effect, while *qmD1TN12* (mks12-267), *qmD2TN1* (mks1-334), *qmD2TN3-1* (mks3-105), and *qmD5TN6* (mks6-71) exhibited antagonistic pleiotropy, suggesting that these QTL need to be considered for environmental specificity in breeding. (3) Meta-QTL analysis localized 52 MQTLs, of which MQTL3.1 and MQTL6.8 contained 82 and 59 candidate genes, respectively, and no reported tiller-related genes were found. (4) mks1-355 (*qD1tn1-3*), mks1-352 (*qD2TN1-2*), and mks1-356 (*qmD1TN1*) may be located in the same genetic locus, and their phenotypic contributions were more than 40%. These QTLs were detected stably for two consecutive years, and they may be the main effector QTLs in tillering that are less affected by the environment. Further analysis revealed that these QTLs corresponded to MQTL1.6, which contains 56 candidate genes. Of these, the expression level of *OsSPL2* gene in the parental line 9311 was significantly higher than that of PA64s, and there were polymorphic differences in the coding region. It was hypothesized that *OsSPL2* was the main effector gene of this QTL. This study provides important genetic resources for mining candidate genes related to tillering and nitrogen efficiency in rice and lays a theoretical foundation for directional breeding and molecular marker development in specific environments.

Keywords: rice; dynamic tillering; single-environment QTL analysis; multi-environment QTL analysis; meta-analysis; high nitrogen efficiency



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1. Introduction

Tiller number in rice is a quantitative trait controlled by multiple genes, and its development is regulated by both genetic and environmental factors. Genetically, a series of genes have been reported to be directly involved in tillering regulation, such as *MOC1* [1], *FON1* [2], *IPA1* [3], *OsTB1* [4], and *D3* [5]. In terms of environment, fertilizer application, planting density, cultivation conditions, and climatic factors significantly affected tiller development [6,7]. Among them, nitrogen is one of the key nutrients affecting tillering in rice, and moderate nitrogen application can significantly promote tillering [8,9]. However, there is a threshold effect on the uptake of nitrogen fertilizer in rice. Over-application of nitrogen not only makes it difficult to further increase the number of tillers, but also reduces nitrogen fertilizer utilization and leads to an increase in the number of ineffective tillers [10,11]. Therefore, optimizing nitrogen fertilizer management is of great significance to improve rice yield and resource utilization efficiency. As an important agricultural resource, crop straw can provide an organic nitrogen source, carbon source, and nutrients such as phosphorus and potassium after decomposition by soil microorganisms, partially replacing chemical nitrogen fertilizers [12–14]. Returning straw to the field not only improves the decline in soil fertility and the degradation of physicochemical properties caused by long-term cultivation, but it also reduces the environmental pollution caused by straw burning and promotes the nutrient cycling of the ecosystem in the field [12–14]. This measure not only helps to reduce the cost of nitrogen fertilizer application, but also responds to the strategic needs of national rural revitalization and sustainable development.

Quantitative trait loci (QTL) localization is an important tool for mining yield-related genes in rice. Currently, more than 200 QTLs related to tiller number in rice have been reported [15]. However, studies on dynamic tillering QTLs and their interactions with the environment are still relatively limited. Previously, QTL analyses of dynamic tillering in rice have been conducted mainly using single segment substitution lines (SSSL) and double haploid (DH) populations [16–18], but the dynamic characteristics of tillering QTLs and their environmental interactions (QEI) have not yet been systematically analyzed under straw-returned and nitrogen-stressed conditions. Xing et al. [19] pointed out that the interactions between genotypes and the environment have a significant effect on developmental and agronomic traits, and this interactions may lead to a significant increase in the number of tillers in rice. In addition, such interactions may lead to genetic variation in the same QTLs in different environments [20]. Therefore, it is important to analyze the environmental response mechanism of tillering QTLs for rice breeding.

Molecular marker-assisted selection (MAS) is an efficient and precise breeding strategy. In recent years, a series of nitrogen-efficient genes (e.g., *OsNR2* [21], *NRT1.1B* [22], *OsTCP19* [23], and *OsLHT1* [24]) have been cloned, and related molecular markers have been developed for application in breeding practice [25]. Among them, *OsTCP19* was shown to be a key gene for efficient response to tiller nitrogen [23]. In addition, meta-QTL analysis can significantly improve the accuracy of QTL localization and provide important clues for candidate gene mining by integrating QTL information from multiple studies [26]. Therefore, systematic analysis of the dynamic characteristics of tillering QTLs and their environmental interaction effects under straw return and N stress conditions not only helps to mine N-efficient related genes, but also provides a theoretical basis for molecular design breeding.

In this study, the phenotypic identification of dynamic tiller number in rice was systematically carried out using the parental lines 9311 and PA64s and 124 recombinant inbred lines (RIL) populations derived from them, and three treatments, namely, straw return + normal nitrogen (GNSB), normal nitrogen (GN), and nitrogen stress (NN), were set in 2021 and 2022. Combined with single-environment QTL analysis, multi-environment

QTL analysis, and meta-QTL integration analysis, we aimed to explore tillering-related genetic loci and their environmental interaction effects and to provide theoretical support for rice N-efficient breeding and molecular marker development.

The specific objectives were as follows: 1. Identify dynamic tillering QTLs under three treatments (no N, N, N + straw return) across two years; 2. Detect QTL-by-environment interactions (QEI) and pleiotropic effects; 3. Validate stable QTLs using meta-analysis and identify candidate genes for nitrogen-efficient tillering. We hypothesized that nitrogen and straw return treatments would modulate the expression of tillering QTLs, with specific loci exhibiting stable effects across environments or environment-specific effects.

2. Materials and Methods

2.1. Test Materials and Field Trials

A population of 124 recombinant inbred lines derived from a cross between indica PA64s (female parent) and indica 9311 (male parent) was used as the material in this experiment. These materials were planted in Qinglian Town, Mianyang City, Sichuan Province. The seeds of the populations were arranged in sequence and transplanted in single rows of 10 plants each, with a plant spacing of 15 cm and a row spacing of 33 cm, and the plot area was designed to be 1.5 m². The seeds were sown in the middle of April, transplanted in the second half of May, and subjected to conventional field management.

2.2. Investigation of Dynamic Tiller Number in Rice Under Three Treatments and Data Analysis

The 124 lines and their parental materials were grown under three treatments, namely, straw return + normal N, normal N, and no N application with two-year replications (2021 and 2022). The treatments under 2021 and 2022 are denoted by E1 (straw return + normal N in 2021), E2 (normal N in 2021), E3 (no N application in 2021), E4 (straw return + normal N in 2022), E5 (normal N in 2022), and E6 (no N application in 2022). The detailed data of soil treatment can be found in Table S4. From two weeks after transplanting, the number of tillers per line was counted randomly in five replicates for every 7 days until the highest number of tillers appeared in each plot. Tiller numbers were recorded continuously for six weeks (expressed as D1–D6). Individual tiller counts of plants in each plot at different measurement stages were used as raw data in the analyses. All tiller raw data were analyzed using ANOVA using SPSS 22.0 and QTL IciMapping v4.1.

2.3. Genetic Mapping

Genetic linkage maps have been constructed in a previous study [24]. Having two parents (9311 and PA64s) that had been sequenced completely, 124 recombinant autogamous singletons (F18 generation) were then sequenced with low coverage and analyzed with recombinant bin. Only one genotype of 9311 or PA64s was present in each recombinant segment. Then, segments with the same genotype were combined into one recombinant bin, resulting in a total of 3524 bins for molecular markers. Finally, a high-density map was then obtained by constructing a genetic map in Mapmaker/EXP3.0 with a total length of 1381.9 cM and an average marker spacing of 0.392 cM. A high-density genotype map was successfully constructed by identifying SNPs and recombination sites using high-throughput resequencing (Figure 1).

2.4. Single- and Multi-Environment QTL Analyses for Dynamic Tillering in Rice

Single-environment QTLs were detected using composite interval mapping (CIM) with a LOD threshold of 3.0 (corresponding to $p < 0.01$, validated via 1000 permutations). Multi-environment analysis used genome-wide significance ($p < 0.05$), and FDR correction (Benjamini–Hochberg, $q < 0.10$) was used to control the false discovery rate.

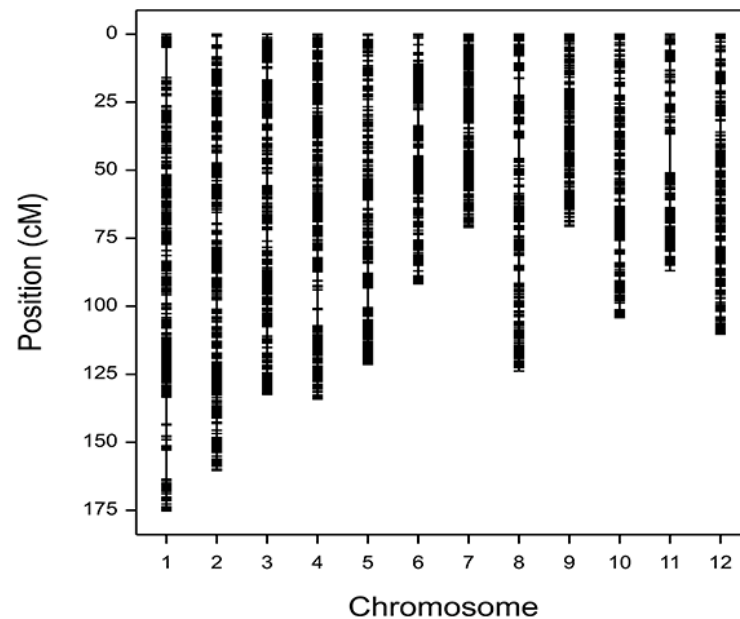


Figure 1. Genetic mapping of recombinant inbred line (RIL) populations.

2.5. Tiller Number-Related QTL Collection and Meta-QTL Analysis

Thirty QTL articles related to tiller number from 2002 to 2022 were collected mainly through the Chinese National Knowledge Base, NBCI PubMed, and Web of Science. The collected QTL information included QTL name, population type, QTL chromosome, R^2 value (phenotypic variation explained, PVE), LOD value, confidence interval, and map position. A QTL consensus map for tiller number trait in rice was constructed by integrating the collected markers and QTLs using BioMercator 4.2 software. The collected QTLs were then clustered and distributed using the mapping function of BioMercator 4.2 software. Finally, meta-analysis was performed on the entire dataset to determine the effect size and confidence level of each QTL. By comparing areas of overlap and effect sizes between studies, it was possible to determine which QTLs were reliable. The locations of reliable QTLs are compared to known genome sequences to identify potential candidate genes in the genome. Candidate genes are further subjected to functional annotation, expression analysis, and genetic validation to determine whether they are associated with the target complex trait.

2.6. Candidate Gene Analysis

Firstly, the genes were identified by <https://archive.gramene.org/markers> (accessed on 10 August 2022) to determine the physical location of the markers at both ends of the MQTL on the chromosome. All the genes with physical location within the two side markers were considered as candidate genes. The candidate genes within the interval were retrieved from the Rice Annotated Gene Database (RAPDB, <http://RAPDB.dna.affrc.go.jp> (accessed on 11 August 2022)), and the candidate genes within the interval were retrieved from the Chinese National Rice Gene Database (<https://www.ricedata.cn/gene/> (accessed on 12 August 2022)) and <https://funricegenes.github.io> (accessed on 15 August 2022) to search for candidate genes that have been reported to be associated with tiller number.

3. Results

3.1. Phenotypic Variation of Dynamic Tillering in Rice Under Three Treatments

The number of tillers of parents 9311 and PA64s differed in different periods and treatments, except that the number of tillers of PA64s was slightly lower than that of 9311

in E1 and E3 treatments in D1 period, and the number of tillers of PA64s was significantly higher than that of 9311 in the rest of the periods (Table 1). The fastest tiller growth was observed from D1 to D3, and the number of tillers showed a decreasing trend from D5 to D6, which could be attributed to the production of ineffective tillers at the late tillering stage (Figure 2). The most serious stress on tiller number in the three periods of D2, D3, and D4 was caused by the no-N treatment, and the degree of stress gradually decreased. The number of tillers in this environment decreased by 31.5%, 17.2%, and 8% and 30%, 11.3%, and 9.9% compared with that in the same period of time under the straw-returned field and the normal N treatments, respectively, and there was no significant difference between the number of tillers under the straw-returned field and the normal N treatments in the respective periods (Table 1). Meanwhile, the violin plots of tiller numbers in the RIL population at different periods showed a normal distribution under all three treatments, and all showed superparental segregation (Figure 2), which is suitable for QTL analysis.

Table 1. Statistical analysis of dynamic tiller number traits in the RIL population under three treatments.

Stage	Year	Environment	Parent		RIL Population			ANOVA		
			PA64s	9311	Mean	Range	CV (%)	RILs	Treatment	G × E
D1	2021	E1	4.5	5	4.2 ± 0.7	2.3~6.8	16.3	**	NS	**
		E2	5.0 *	3.3	4.3 ± 0.4	3.0~7.0	9.5			
		E3	3.8	4	4.2 ± 0.6	3.0~6.8	14.1			
	2022	E4	13.0 *	8	7.9 ± 0.6	4.3~11.7	7.3			
		E5	9.3	9.3	9.3 ± 1.0	4.0~14.3	10.2			
		E6	9.3 *	5.7	7.3 ± 0.5	3.7~12.3	7.5			
D2	2021	E1	10.3 *	8.3	7.3 ± 1.0	4.5~13.5	13.8	**	**	**
		E2	9.3 *	6.3	6.9 ± 1.1	4.0~11.8	15.7			
		E3	6.5 *	5	4.9 ± 1	2.8~8.3	20.9			
	2022	E4	14.7 *	10	13.8 ± 1.1	7.0~22.3	7.9			
		E5	14.0 *	11.3	13.9 ± 1.2	7.3~23.3	8.5			
		E6	11.3 *	9	9.6 ± 0.7	5.0~14.7	7.5			
D3	2021	E1	16.0 *	12.8	12.6 ± 1.1	8.3~21.0	8.9	**	**	**
		E2	14.3 *	9.3	11.5 ± 1.7	6.3~21.3	14.3			
		E3	14.3 *	8	9.8 ± 1.3	5.3~15.5	13.6			
	2022	E4	17.7 *	13	14.9 ± 1.6	9.3~25.0	10.8			
		E5	18.0 *	12	14.2 ± 1.0	8.0~25.7	7			
		E6	13.5 *	11	13.1 ± 0.8	7.7~23.0	5.9			
D4	2021	E1	19.3 *	12.8	12.7 ± 1.7	7.8~24.5	13.2	**	**	**
		E2	24.3 *	13.5	13.7 ± 2.2	7.5~21.3	16			
		E3	14.0 *	8.5	11.9 ± 2.1	6.8~17.8	17.7			
	2022	E4	18.0 *	15.3	15.5 ± 1.2	9.7~23.3	7.7			
		E5	18.5 *	13.7	15.0 ± 1.3	9.3~23.3	8.8			
		E6	15.7 *	12.7	14.0 ± 1.0	8.3~22.7	7.2			
D5	2021	E1	18.3 *	11.3	13.0 ± 1.9	7.3~24.3	14.6	**	**	**
		E2	26.7 *	13.7	12.2 ± 2.4	6.3~22.5	19.6			
		E3	23.0 *	12.5	12.8 ± 2.0	7.8~19.0	15.5			
	2022	E4	21.3 *	15.7	14.4 ± 1.3	9.3~24.0	8.9			
		E5	19.3 *	14	15.7 ± 1.2	9.0~22.3	7.6			
		E6	18.7 *	13.5	14.3 ± 1.1	7.3~23.0	7.6			
D6	2021	E1	17.5 *	11.5	11.4 ± 1.8	7.0~17.8	16.3	**	NS	**
		E2	22.5 *	13.5	11.3 ± 2.4	6.5~15.3	21.2			
		E3	17.3 *	11	11.7 ± 1.9	7.3~22.0	16			
	2022	E4	19.7 *	14.7	16.6 ± 0.8	9.0~25.3	5.1			
		E5	19.8 *	13.7	16.4 ± 1.4	9.0~26.0	8.3			
		E6	19.3 *	13.7	15.8 ± 1.4	9.0~23.3	8.9			

* indicates that there is a significant difference. ** indicates that there is a highly significant difference. NS indicates that there is no significant difference.

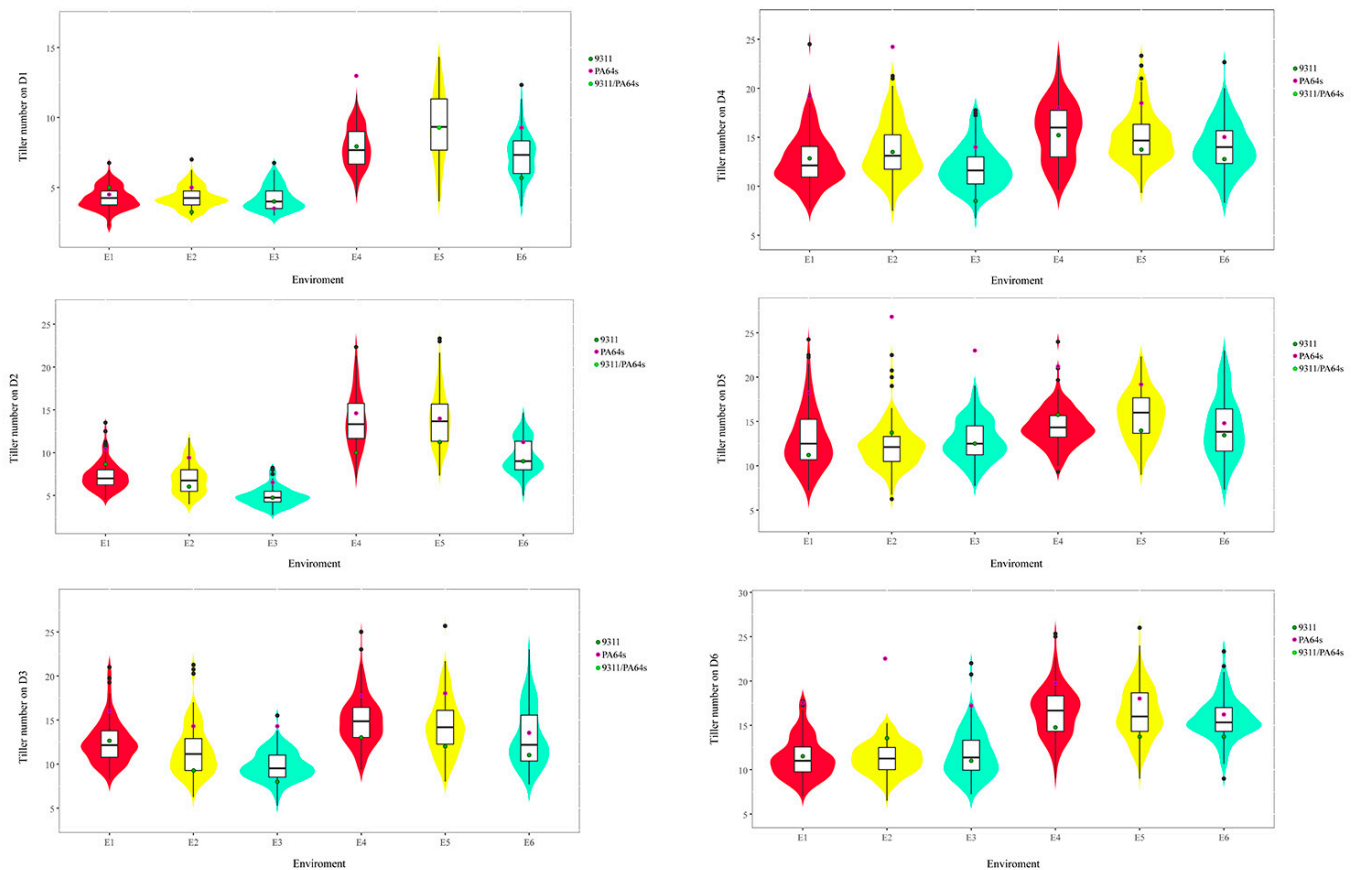


Figure 2. Violin plots showing the distribution of tiller numbers in the RIL population and parents (9311: dark green; PA64s: purple) across six developmental stages (D1–D6) under three treatments: E1/E4 (straw + N), E2/E5 (N), and E3/E6 (no N). Superparental segregation indicates transgressive variation suitable for QTL analysis. Note: Dark green and purple colors indicate the parents 9311 and PA64s, respectively, and green colors indicate the same number of tillers. D1–D6 represent the tillering traits at each of the six periods.

3.2. QTL Correlation Analysis of Tiller Number Across Six Periods in Recombinant Inbred Lines of Rice

3.2.1. Single-Environment QTL Analysis of Tiller Number Traits in RIL Populations in Six Periods

Through the constructed genetic linkage map, a total of 57 QTLs were detected in tiller number across six periods of the population in mono-environment. The QTLs were distributed on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12, of which 25 phenotypic incremental alleles were from PA64s and 32 phenotypic incremental alleles were from 9311 (Table 2). The LOD values of these 57 QTLs ranged from 3.05 to 6.01, and the phenotypic contribution ranged from 6.49% to 47.95%. Of the 57 QTLs, 44 were novel, including two major-effect QTL, namely, *qD1tn1-3* (PVE = 41.61%, no N 2022) and *qD2TN1-2* (PVE = 47.95%, N 2021), located 0.57 cM apart on chromosome 1, suggesting a shared genetic locus. (Table 2). Therefore, it was hypothesized that there was a primary QTL or primary gene near these two markers controlling tillering traits in this period and that this primary QTL or primary gene was stable and temporally multiplexed and could be detected in different environments and times. This QTL could be detected under normal nitrogen and no nitrogen treatments, suggesting that this QTL may be less affected by nitrogen and also have high contribution to population tillering under low nitrogen environments.

Table 2. QTLs for number of tillers in the dynamic phase in a single environment detected in the RIL population of Pa64s × 9311.

Stage	QTL							
	Name	Locus	Environment	Linkage Group	Position of Highest Peak (cM)	−log10(P)	Effect ^a	PVE
D1	<i>qD1tn1-1</i> ^b	mks1-66	E4	1	36.57	3.12	−0.40	7.39
	<i>qD1tn1-2</i>	mks1-305	E4	1	123.05	3.23	−0.40	7.38
	<i>qD1tn1-3</i>	mks1-355	E6	1	152.44	4.81	1.02	41.61
	<i>qD1tn2</i>	mks2-228	E6	2	90.69	4.09	−0.52	10.70
	<i>qD1tn4</i>	mks4-278	E6	4	90.75	4.70	−0.57	12.82
	<i>qD1TN6-2</i>	mks6-69	E1	6	24.44	4.03	0.28	11.09
	<i>qD1TN6-1</i>	mks6-222	E1	6	82.85	3.63	0.25	8.86
	<i>qD1tn9</i>	mks9-131	E4	9	42.55	4.52	0.50	11.85
D2	<i>qD2TN1-4</i>	mks1-61	E3	1	34.43	3.65	0.33	9.19
	<i>qD2tn1</i>	mks1-305	E4	1	123.05	3.19	−1.00	9.05
	<i>qD2TN1-2</i>	mks1-352	E2	1	151.87	5.18	1.21	47.95
	<i>qD2tn2</i>	mks2-17	E6	2	10.29	4.84	−0.67	11.36
	<i>qD2TN2-1</i>	mks2-107	E1	2	40.9	3.11	0.49	8.33
	<i>qD2tn3</i>	mks3-165	E5	3	65.05	5.70	1.19	13.93
	<i>qD2tn4-1</i>	mks4-30	E6	4	8.49	3.08	−0.53	7.22
	<i>qD2tn4-2</i>	mks4-340	E5	4	122.16	3.69	0.94	8.56
	<i>qD2tn5-1</i>	mks5-196	E5	5	89.99	3.25	0.83	6.72
	<i>qD2tn5-2</i>	mks5-286	E6	5	118.71	5.05	0.68	11.65
	<i>qD2tn6-1</i>	mks6-81	E5	6	34.84	3.16	−0.89	7.79
	<i>qD2tn6-2</i>	mks6-135	E6	6	51.64	3.08	−0.53	7.09
	<i>qD2TN8</i>	mks8-91	E2	8	48.49	3.75	−0.54	9.52
D3	<i>qD3tn1</i>	mks1-71	E6	1	38.69	4.02	1.07	9.95
	<i>qD3TN3-1</i>	mks3-139	E2	3	50.82	3.68	0.88	9.24
	<i>qD3TN3-2</i>	mks3-213	E1	3	89.43	3.26	−0.65	6.49
	<i>qD3tn4-1</i>	mks4-355	E6	4	125.8	3.68	1.07	9.98
	<i>qD3tn4-2</i>	mks4-374	E4	4	132.58	3.20	0.79	8.02
	<i>qD3TN6</i>	mks6-235	E1	6	89.03	3.22	0.67	6.87
	<i>qD3TN7</i>	mks7-139	E1	7	39.72	3.50	−0.69	7.17
	<i>qD3TN8-1</i>	mks8-27	E1	8	10.65	4.83	−0.83	10.61
	<i>qD3TN12-1</i>	mks12-8	E2	12	1.53	5.02	−1.19	16.80
D4	<i>qD4tn1</i>	mks1-382	E5	1	171.08	3.22	0.71	7.43
	<i>qD4TN2-1</i>	mks2-344	E3	2	135.73	3.53	0.61	7.83
	<i>qD4TN3</i>	mks3-192	E3	3	81.08	4.66	−0.71	10.37
	<i>qD4TN4</i>	mks4-18	E2	4	4.81	4.49	−0.95	11.13
	<i>qD4tn4</i>	mks4-62	E5	4	19.71	4.73	−0.93	12.44
	<i>qD4TN5-1</i>	mks5-29	E2	5	11.63	3.89	0.91	10.30
	<i>qD4TN5-2</i>	mks5-37	E3	5	14.92	3.05	0.56	6.60
	<i>qD4TN6</i>	mks6-212	E3	6	79.37	4.65	0.74	11.33
	<i>qD4TN8-1</i>	mks8-1	E1	8	0	3.76	−0.83	9.68
	<i>qD4TN8-2</i>	mks8-36	E2	8	12.95	4.30	−0.96	11.26
	<i>qD4tn9</i>	mks9-144	E5	9	47.38	5.64	1.00	14.59
	<i>qD4tn9</i>	mks9-144	E6	9	47.38	3.77	0.86	11.27
	<i>qD4TN10</i>	mks10-197	E2	10	75.21	3.23	0.73	6.63
	<i>qD4TN12</i>	mks12-197	E1	12	75.31	3.79	0.95	12.78
D5	<i>qD5TN2-1</i>	mks2-199	E3	2	82.35	5.39	0.85	13.74
	<i>qD5tn3</i>	mks3-165	E5	3	65.05	6.01	1.19	18.71
	<i>qD5TN6</i>	mks6-235	E1	6	89.03	3.31	1.03	9.20
	<i>qD5tn7</i>	mks7-105	E5	7	27.85	4.66	1.00	13.15
D6	<i>qD6TN1</i>	mks1-216	E2	1	90.37	3.44	0.54	9.27
	<i>qD6TN4</i>	mks4-265	E2	4	83.48	5.22	0.69	15.51
	<i>qD6tn5</i>	mks5-179	E4	5	80.67	3.97	−0.94	10.69
	<i>qD6TN6-2</i>	mks6-195	E3	6	70.51	3.98	0.86	10.61
	<i>qD6TN7</i>	mks7-139	E3	7	39.72	4.08	−0.83	9.89
	<i>qD6tn7</i>	mks7-146	E4	7	41.25	3.44	−0.82	8.14
	<i>qD6tn10-1</i>	mks10-64	E4	10	30.1	3.12	−0.79	7.49
	<i>qD6tn10-2</i>	mks10-68	E6	10	31.25	3.59	−0.76	9.83
	<i>qD6tn12</i>	mks12-294	E6	12	110.04	3.29	−0.72	8.71

Note: ^a Positive values indicate that 9311 carries an allele that positively affects the trait, whereas negative values indicate that PA64s carry an allele that favorably affects the trait; ^b “TN” indicates detected in 2021; “tn” indicates detected in detected in 2022.

Two QTLs, *qD2tn1* and *qD1tn1-2*, were detected in the D1 and D2 periods, respectively. Their central markers were all located in mks1-305 and were detected under the E4 (straw return in 2022) treatment, and its LOD values were 3.19 and 3.23, with a phenotypic contribution of 9.05% and 7.38%. Two QTLs, *qD2tn3* and *qD5tn3*, were detected in D2 and D5 periods. The LOD values of these two QTLs were 5.7 and 6.01, respectively, with phenotypic contributions of 13.93% and 18.71%, respectively, and their central markers were all located in mks3-165 detected under E5 (2022 normal nitrogen) treatment. *qD3TN6* and *qD5TN6*, the central markers of these two QTLs, were detected in the D3 and D5 periods,

respectively, and their central markers were all located in mks6-235, both detected under E1 (2021 straw return + normal N) treatment, with LODs of 3.22 and 3.31 and phenotypic contributions of 6.87% and 9.2%, respectively (Table 2). mks1-305 and mks3-165 both may have a QTL with temporal pleiotropy. *qD3TN7* and *qD6TN7* are two QTLs whose central markers are located in mks7-139. mks7-139 was detected under the E1 environment at the D3 period and the E3 environment at the D6 period with LOD values of 3.5 and 4.08, respectively, and contributions of 7.17% and 9.89%, respectively. This QTL may contain temporally and spatially pleiotropic genes controlling the phenotypes in these two periods. At the same time, this QTL may contain tiller nitrogen efficiency response genes. The QTL *qD4tn9* was detected in E5 and E6, with LOD values of 5.64 and 3.77, respectively. The contributions to the phenotypes in the corresponding environments were 14.59% and 11.27%, respectively. Therefore, this QTL may be present in tiller nitrogen efficiency response-related genes.

3.2.2. Multi-Environmental QTL Analysis of Tiller Number Traits in RIL Populations Across Six Periods

To determine the genetic basis of tillering plasticity in rice under straw return and no N application treatments, the population was subjected to a multi-environmental QTL analysis, and a total of 15 QTLs were localized under the three treatments (Figure 3). There were 14 QTLs with $Q \times E$ interactions and only one QTL without $Q \times E$ interactions (Table 3). *qmD1TN1* with its centromeric marker mks1-356 locus located at position 152.63 cM on chromosome 1 was detected at the D1 period. This QTL was separated from the *qD2TN1-2* (mks1-352) and *qD1tn1-3* (mks1-355) loci by 0.76 cM and 0.19 cM, respectively. This locus was detected with *p*-values less than 0.01 under all three treatments, and alleles with an additive effect value of 0.33 phenotypic increment were all from 9311. All of the phenotypic contributions exceeded 14% in 2021 and contributed slightly less to the locus in 2022. The absence of QTL–environment interaction effects for *qmD1TN1* suggests that this QTL may not be affected by the environment and possesses a role in tillering regulation under no-N application treatment.

Table 3. QTLs detected in the RIL population of A64s $\times$ 9311 for dynamic tillering in multiple environments.

Stage	Name	Locus	Linkage Group	Position of Highest (cM)	$-\log_{10}(P)$	$Q \times E$	Treatment	Effect ^a	<i>p</i> ^b	R ²
D1	<i>qmD1TN1</i>	mks1-356	1	152.63	2.66	no	E1	0.33	0.00	15.70
							E2	0.33	0.00	18.00
							E3	0.33	0.00	14.30
							E4	0.33	0.00	5.10
							E5	0.33	0.00	1.90
							E6	0.33	0.00	4.40
	<i>qmD1TN2</i>	mks2-106	2	38.85	3.67	yes	E1	0.15	0.24	3.20
							E2	0.02	0.90	0.00
							E3	0.04	0.77	0.20
							E4	−0.03	0.81	0.00
							E5	0.61	0.00	6.30
							E6	−0.11	0.40	0.40
	<i>qmD1TN6</i>	mks6-71	6	24.82	6.60	yes	E1	0.21	0.10	6.30
							E2	−0.22	0.09	7.70
							E3	−0.05	0.72	0.30
							E4	0.23	0.07	2.40
							E5	−0.70	0.00	8.40
							E6	−0.05	0.71	0.10
	<i>qmD1TN12</i>	mks12-267	12	100.89	3.50	yes	E1	−0.12	0.38	2.00
							E2	0.00	1.00	0.00
							E3	0.09	0.48	1.10
							E4	−0.03	0.83	0.00
							E5	0.56	0.00	5.40
							E6	−0.26	0.04	2.80

Table 3. Cont.

Stage	Name	Locus	Linkage Group	Position of Highest (cM)	$-\log_{10}(P)$	$Q \times E$	Treatment	Effect ^a	p^b	R^2
D2	<i>qmD2TN1</i>	mks1-334	1	131.32	5.81	yes	E1	−0.01	0.95	0.00
							E2	−0.03	0.90	0.00
							E3	−0.04	0.84	0.10
							E4	−1.05	0.00	9.90
							E5	0.48	0.02	2.30
							E6	−0.15	0.46	0.60
	<i>qmD2TN3-1</i>	mks3-105	3	34.84	4.66	yes	E1	0.18	0.44	1.10
							E2	0.31	0.17	3.10
							E3	0.16	0.49	2.00
							E4	−1.01	0.00	9.20
							E5	0.44	0.05	1.90
							E6	0.08	0.74	0.10
	<i>qmD2TN3-2</i>	mks3-164	3	64.86	2.95	yes	E1	−0.09	0.68	0.30
							E2	0.39	0.08	4.90
							E3	−0.07	0.75	0.40
							E4	0.55	0.01	2.70
							E5	0.83	0.00	6.80
							E6	0.18	0.41	0.90
	<i>qmD2TN6</i>	mks6-81	6	34.84	4.31	yes	E1	−0.12	0.60	0.50
							E2	−0.31	0.17	3.00
							E3	−0.09	0.69	0.60
							E4	0.13	0.56	0.10
							E5	−1.13	0.00	12.40
							E6	−0.33	0.14	2.70
D3	<i>qmD3TN3</i>	mks3-108	3	37.97	2.62	yes	E1	−0.30	0.26	1.30
							E2	0.79	0.00	7.50
							E3	0.14	0.60	0.70
							E4	0.23	0.38	0.70
							E5	0.76	0.00	5.70
							E6	0.36	0.17	1.20
D4	<i>qmD4TN1</i>	mks1-382	1	171.08	3.36	yes	E1	0.08	0.72	0.10
							E2	−0.35	0.15	1.50
							E3	0.04	0.87	0.00
							E4	0.47	0.05	2.30
							E5	0.86	0.00	10.70
							E6	−0.18	0.46	0.50
	<i>qmD4TN2</i>	mks2-344	2	135.73	2.91	yes	E1	0.43	0.07	2.60
							E2	0.78	0.00	7.50
							E3	0.61	0.01	7.90
							E4	−0.07	0.77	0.10
							E5	0.22	0.35	0.70
							E6	−0.17	0.47	0.40
	<i>qmD4TN4</i>	mks4-14	4	3.27	3.29	yes	E1	−0.05	0.85	0.00
							E2	−0.78	0.00	7.60
							E3	−0.02	0.93	0.00
							E4	−0.81	0.00	7.00
							E5	−0.70	0.01	7.10
							E6	−0.09	0.73	0.10
	<i>qmD4TN9</i>	mks9-143	9	47.19	3.28	yes	E1	−0.32	0.18	1.40
							E2	−0.01	0.96	0.00
							E3	0.20	0.40	0.80
							E4	0.48	0.04	2.40
							E5	0.77	0.00	8.60
							E6	0.59	0.01	5.20
D5	<i>qmD5TN6</i>	mks6-71	6	24.82	2.93	yes	E1	0.65	0.01	3.70
							E2	−0.10	0.70	0.10
							E3	−0.24	0.34	1.10
							E4	−0.23	0.37	1.00
							E5	−0.78	0.00	7.90
							E6	0.17	0.52	0.20
D6	<i>qmD6TN7</i>	mks7-145	7	41.06	4.31	yes	E1	−0.36	0.09	2.50
							E2	0.05	0.83	0.10
							E3	−0.79	0.00	8.90
							E4	−0.84	0.00	8.40
							E5	−0.07	0.75	0.10
							E6	−0.07	0.75	0.10

Note: ^a Positive values indicate that 9311 carries an allele that positively affects the trait, whereas negative values indicate that PA64s carries an allele that favorably affects the trait; ^b Whether or not the QTL has a significant QTL $\times$ environment effect.

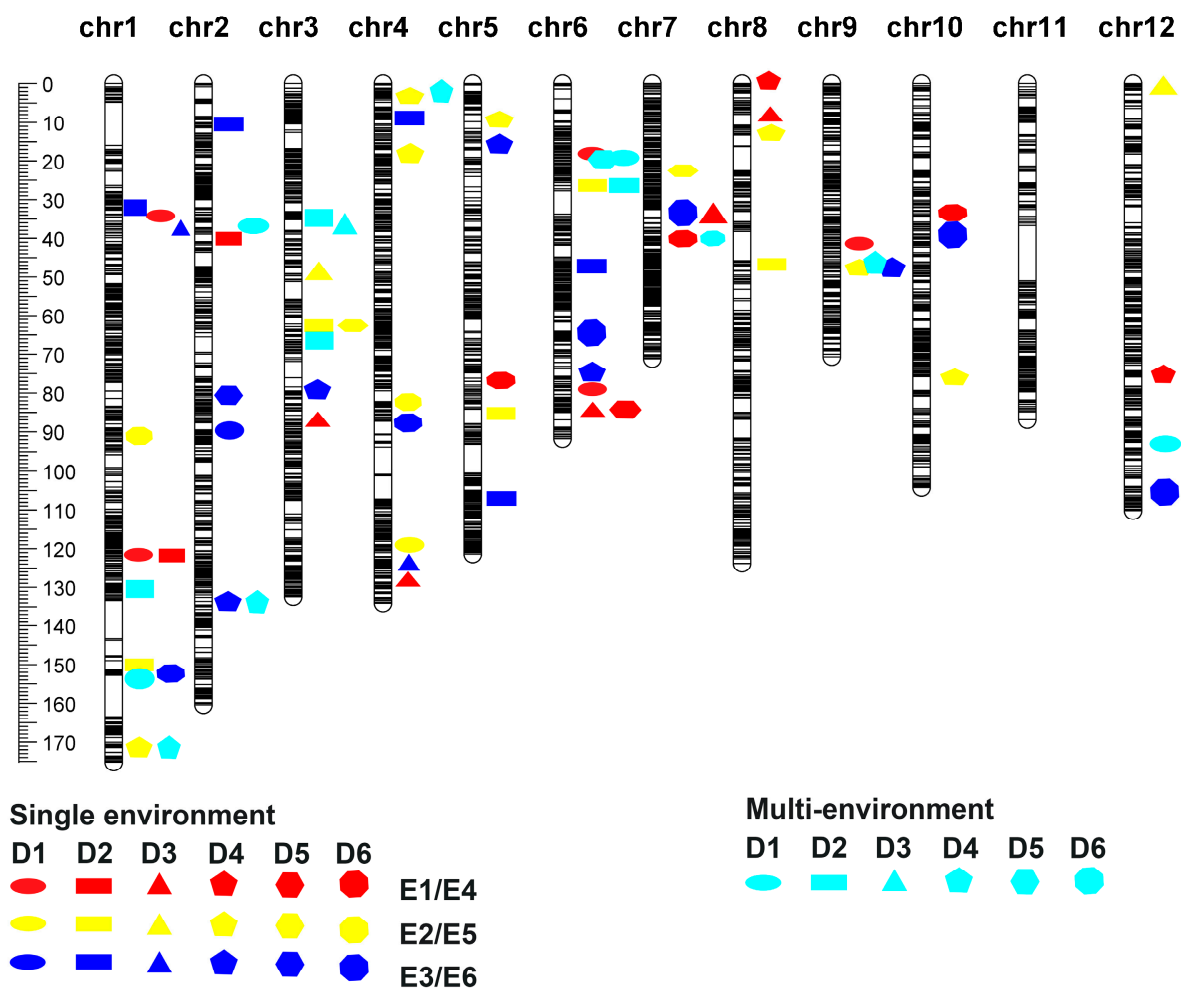


Figure 3. Genetic map of QTLs associated with dynamic tiller number across six periods in the RIL population.

The QTL *qmD1TN12* (mks12-267) was detected under normal nitrogen and no nitrogen application treatments in the D1 period ($p < 0.05$). Three QTLs, *qmD2TN1* (mks1-334), *qmD2TN3-1* (mks3-105), and *qmD5TN6* (mks6-71), were detected in the straw-returned field and normal nitrogen environment ($p < 0.05$), and antagonistic pleiotropy was present in all four QTLs (Table 3). *qmD1TN12* (mks12-267) derived its phenotypic increment allele from PA64s under no nitrogen treatment and 9311 under normal nitrogen environments. *qmD2TN1* (mks1-334) and *qmD2TN3-1* (mks3-105) were detected in straw-returned environments. Their phenotypic increment alleles were from PA64s in the straw-returned environment and 9311 in the normal nitrogen environment. The *qmD5TN6* (mks6-71) locus was detected in the E1 and E5 environments, and its phenotypic increment alleles were from 9311 in the straw-returned environment and PA64s in the normal nitrogen environment. *qmD1TN2* (mks2-106) and *qmD2TN6* (mks6-81) are two QTLs that were condition neutral. Both were detected under E5 (2022 normal nitrogen) treatment, suggesting that these two QTLs may only play a role in regulating tillering under normal nitrogen environments. The remaining QTLs in different environments all showed differential sensitivity, with *qmD4TN4* (mks4-14) and *qmD6TN7* (mks7-145) phenotypically potentiating alleles from PA64s in multiple environments. The QTL *qmD4TN4* (mks4-14) was detected under the E2, E4, and E5 treatments, and the additive effect in the straw-returned treatment was higher than the effect value under the normal nitrogen treatment. The values were higher than those under normal nitrogen treatment, suggesting that this QTL may have a higher regulatory effect on tillering under straw return treatment than under normal nitrogen

environment at the tillering bloom. *qmD6TN7* (mks7-145) was detected under E3 and E4 treatments, with effect values of -0.79 and -0.84 under straw return and no nitrogen treatment, respectively, and a smaller difference in effect values was noted between the no-nitrogen treatment and normal nitrogen treatment. The difference between the effect values of -0.05 and -0.84 under the no-N treatment and the normal-N treatment was small, so this QTL may possess genes that act as potentiators for tillering under the no-N treatment. In addition, *qmD1TN6* (mks6-71) was also detected in the D1 period, but showed conditionally neutral only under normal nitrogen environment. The phenotypic potentiation allele was from PA64s, and the LOD of this QTL reached 6.6 in multi-environmental analysis. The QTL was expressed under normal nitrogen treatment, and the results also indicated that there might be a gene that not only exhibited antagonistic pleiotropy but also was expressed under normal nitrogen treatment in the vicinity of the mks6-71 marker.

3.3. Meta-QTL Analysis of Tillering Traits in Rice

3.3.1. Collection of Information Related to QTLs for Tiller Number in Rice

A total of 222 QTLs related to tiller number were collected from 30 reported research populations between 2002 and 2021 (Table 4), and a pre-consensus map covering 12 chromosomes suitable for meta-QTL analyses was constructed by integrating the genetic maps of the studied tiller numbers (Figure 4). The map also had 1865 highly saturated markers with a full length of 2523.2 cM and an average genetic distance of 0.93 to 3.32 cM per marker (Table 5). The highest number of QTLs were localised on chromosomes 1 and 6, with 36 and 37, respectively, and the lowest number of QTLs were localised on chromosome 12, with eight.

Table 4. QTL information reported in previous studies.

Map Name	Traits	Parental	Population Type	Population Size	Number of QTL	Reference
2021-Wang	TN	Changhui 121/Koshihikari	BC	208	8	(Wang et al., 2021) [27]
2021-Luo	TN	Oryza rufipogon Griff./Oryza sativa L.	BC	104	2	(Luo et al., 2021) [28]
2021-B. Rajurkar	TN	IR62266/Norungan	RIL	132	35	(B. Rajurkar et al., 2021) [29]
2021-Kwon	TN	9311/Milyang352	DH	117	3	(Kwon et al., 2021) [30]
2021-Suman	TN	PR116/Ranbir Basmati	RIL	44	1	(Suman et al., 2021) [31]
2022-Kavitha Beerelli	TN	IET27223/IET26772	RIL	174	4	(Kavitha Beerelli et al., 2022) [32]
2017-Zhang	TN	XieqingzaoB/Zhonghui9308	RIL	195	3	(Zhang et al., 2017) [33]
2018-Lei	TN	Dongnong422/Kongyu131	RIL	190	7	(Lei et al., 2018) [15]
2018-Xu	TN	Shennong265/Haogelao	BC	178	3	(Xu et al., 2018) [34]
2019-Li	TN	Liaoyou5218/5216A	BC	167	2	(Li et al., 2019) [35]
2019-Zhou	TN	Mowanggu/CO39	BC	280	4	(Zhou et al., 2019) [36]
2020-Mona	TN	Hashemi/Nemat	RIL	140	3	(Emami et al., 2020) [37]
2007-Liang	TN	Nipponbare/Guangluai4hao	RIL	90	6	(Liang et al., 2007) [38]
2007-Ye	TN	PA64s/Nipponbare	RIL	180	2	(Ye et al., 2007) [39]
2013-Vennu	TN	Nipponbare/9311	RIL	254	9	(Vennu et al., 2013) [40]
2009-Xu	TN	Nipponbare/Kasalath	BC	98	4	(Xu et al., 2009) [41]
2012-Liang	TN	XieqingzaoB/Zhonghui9308	RIL	226	4	(Liang et al., 2012) [42]
2013-Zhou	TN	Guanghui 116/LaGrue	RIL	307	4	(Zhou et al., 2013) [43]
2014-Sun	TN	Ch5-10/Ch6-11	RIL	113	9	(Sun et al., 2014) [44]
2014-Lim	TN	Milyang23/SNUSG1	RIL	178	5	(Lim et al., 2014) [45]
2015-Sun	TN	Dongnong425/Changbai10	RIL	180	7	(Sun et al., 2015) [46]
2015-Xu	TN	JY293/M201	RIL	234	2	(Xu et al., 2015) [47]
2017-Malathi	TN	MTU7029/IRGC81848	BC	94	2	(Surapaneni et al., 2017) [48]
2008-Jiang (1)	TN	Zhaiyeqing8hao/Jingxi17	DH	127	30	(Jiang (1) et al., 2008) [49]
2008-Jiang (2)	TN	Taizhong1/Chunjiang06	DH	120	45	(Jiang (2) et al., 2008) [50]
2002-W. C. Kennard	TN	Johnson/Dora Lake	RIL	172	3	(W. C. Kennard et al., 2002) [51]
2003-Kobayashi	TN	Milyang 23/Akihikari	RIL	191	2	(Kobayashi et al., 2003) [52]
2003-Ren	TN	MH63/B5	RIL	187	4	(Ren et al., 2003) [53]
2004-N. Miyamoto	TN	IR36/Genjah Wangkal	RIL	100	4	(N. Miyamoto et al., 2004) [54]
2005-Liang	TN	Nipponbare/Guangluai4	RIL	100	5	(Liang et al., 2005) [55]

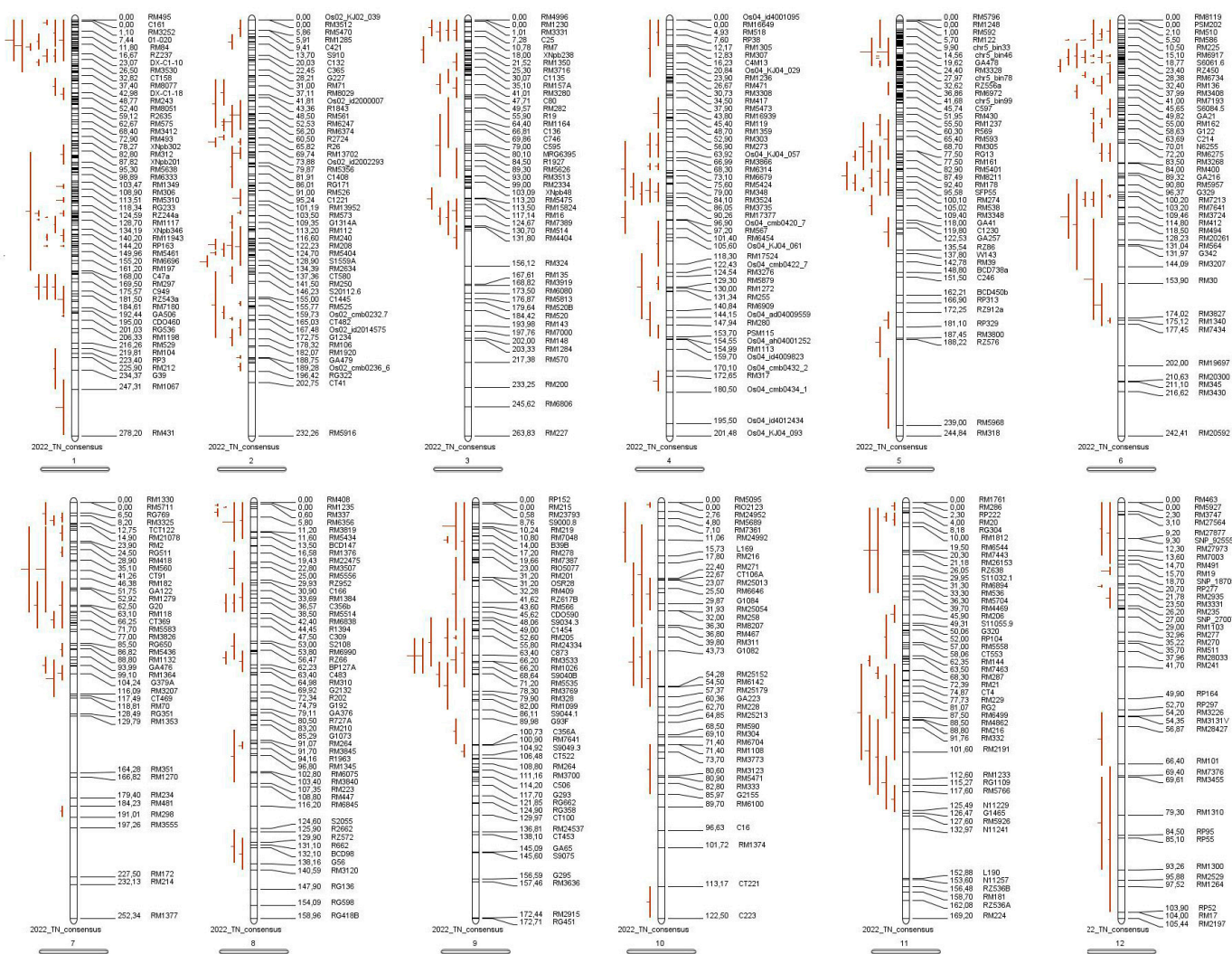


Figure 4. Consensus mapping of tiller number traits.

Table 5. Integration of information from mapping.

Chr ^a	No. of Markers ^b	No. of QTLs ^b	Length (cM)	Average (cM)
1	299	36	278.2	0.93
2	266	30	256.4	0.96
3	164	17	285.1	1.74
4	130	20	201.5	1.55
5	266	25	244.8	0.92
6	201	37	258.2	1.28
7	76	15	252.3	3.32
8	99	16	159.0	1.61
9	124	15	172.7	1.39
10	57	10	122.5	2.15
11	111	16	169.2	1.52
12	72	8	123.2	1.71
Total	1865	245	2523.2	

^a chromosome^b number^a represents chromosome and ^b represents the number of markers.

3.3.2. Meta-QTL Analysis of Tiller Number Traits in Rice

Meta-analysis of the 222 QTLs collected was performed using BioMercator V4.2 software. A total of 52 MQTLs with 95% confidence intervals associated with tiller number

were calculated. The distribution of MQTLs on each staining ranged from 0 to 8. No MQTLs were detected on chromosomes 10 and 12, and the highest number of MQTLs detected on chromosome 6 was 8 (Table 6). The PVE values of the MQTL ranged from 4 to 43%, with 23 MQTLs with PVE values exceeded 20%. MQTL 11.2 had the highest PVE value of 43%.

Table 6. Meta-QTL analysis of tiller number traits in rice.

MQTL	QTL Model	Position (cM)	PVE (%)	No. of Initial QTLs	MQTL CI (95%)	Interval	Physical Length of MQTLs (Mb)	Partial Initial QTL	Candidate Genes
MQTL1.1	7	2.78	7	2	3.94	RM3148–RM5423	1.42	mks1-334 qD1tn1-3, qD2tn1-2, mks1-356	SPL33; MHZ4
MQTL1.2		23.29	23	8	2.94	R1944–RM8111	1.73		OsNPR1; OsSCAR1; DPF; OsBZR2
MQTL1.3		48.21	6	2	7.06	RM259–RM576	0.69		OsGRX3
MQTL1.4		114.22	13	4	2.44	GA330–RG345	3.79		OsRH2; OsATG7; Osa-miR319a;
MQTL1.5		132.81	16	6	1.00	RM1117–RM3324	2.43		OsFBK1
MQTL1.6		151.31	15	5	1.02	RM3520–RM414	0.50		THIS1; OsIAA6; D10
MQTL1.7		197.75	21	7	0.29	RM5362–RM1067	1.84		OsRLCK57
MQTL2.1	6	9.21	7	2	6.59	R2510–CT16	1.57	qD2tn2	OsSPL3; OsmiR168a; OsTEF1
MQTL2.2		66.43	33	10	2.04	RM71–G243A	2.99		EP3
MQTL2.3		108.02	10	3	2.45	R26–RM318	3.30		OsmiR156i; OsPRR1; OsPLIM2a
MQTL2.4		130.83	23	7	2.91	R3393–CT388	3.15		OsPIN1; OsMADS57; OsNPF7.2;
MQTL2.5		164.77	17	5	2.19	CT482–CT87	1.88		OsDOF11
MQTL2.6		189.23	10	3	0.37	CT41–RM535	0.41		OsNR2; DES4
MQTL3.1	4	8.03	29	5	5.97	C25–RM489	0.37	mks3-105, mks3-108 qD4TN3	OsIAA10; OsDHHOC6
MQTL3.2		36.38	24	4	5.86	RM232–RM3280	1.15		OsTB1; OsSLR1
MQTL3.3		84.02	17	3	15.54	MRG6395– RM5626	2.61		
MQTL3.4		124.4	29	5	2.87	RM426–RM504	0.54		
MQTL4.1	4	7.73	11	2	11.67	RM518–RM252	3.62	qD2tn4-1, mks4-14 qD6TN4	OsETR2; OsPT4
MQTL4.2		69.98	31	6	2.50	RM3308–RM6679	0.73		OsAFB2; LAX2
MQTL4.3		85.88	32	6	5.30	RM6997–RM1165	2.55		OsNAC2
MQTL4.4		145.47	26	5	1.17	RM255–RM5968	3.31		OsNPF7.3; RFL; OsGS2; MOC3
MQTL5.1	6	21.57	12	2	0.08	RM13–RM437	1.87	qD2tn5-2	OsTIR1
MQTL5.2		22.96	14	4	0.11	RM437–RM7118	2.21		OsBC1L4
MQTL5.3		40.82	4	1	7.93	RM289–RM249	2.97		OsZIP9; OsZIP5; PILS6b
MQTL5.4		77.11	23	5	4.98	RM164–RM3295	3.07		OsmiSSB1; OsPUP7
MQTL5.5		99.32	25	6	3.17	RM3476–G81	0.48		
MQTL5.6		114.77	9	2	10.10	RM3348–GA257	3.52		
MQTL6.1	8	11.99	17	6	0.61	RM510–RM6587	0.54	qD1TN6-2, mks6-71 qD2tn6-1, mks6-81 qD4TN6	D3; Hd3a
MQTL6.2		21.45	14	6	1.37	CT201–CT115	0.82		AID1; OsNPY2
MQTL6.3		25.3	11	3	0.84	RM314–RM253	0.58		OsKASI
MQTL6.4		35.12	14	5	2.39	RM2615–R2147	0.56		OsTCP19
MQTL6.5		58.33	11	4	4.10	RM5745–RM1161	1.26		Osa-miR1871; OsMFAP1
MQTL6.6		77.8	6	2	7.97	RM541–RM5957	5.01		OsUBR7; OsAAP3; OsSPX1; OsBZR3;
MQTL6.7		103.9	19	7	1.99	RM7641–RM340	1.34		MOC1
MQTL6.8		175.44	8	3	0.75	G342–RM494	0.27		OsPIN2; OsARF18
MQTL7.1	4	11.69	29	4	8.87	RM3325– RM21103	2.34	qD5tn7	FOI1
MQTL7.2		30.81	16	3	10.13	RG477–CT91	6.00		OsTCP21; POW1
MQTL7.3		69.48	22	3	12.12	G20–RM5583	1.83		CHR729
MQTL7.4		97.39	27	4	6.29	RM1132–RM429	2.82		OsBZR1; OsLBD39; OsGH3.8;
MQTL8.1	6	1.22	9	1	1.20	S2104–RM1381	0.27	qD4TN8-1	OsNPF7.1
MQTL8.2		2.24	22	4	0.68	RM6925–RM6356	0.91		OsPMEI28
MQTL8.3		37	25	4	4.38	RM5428–C309	5.45		OsCCA1; OsLIS-L1; DTH8; OsGPT1
MQTL8.4		59.2	19	3	4.05	RM6990–RM5767	4.46		PAY1
MQTL8.5		93.24	12	2	3.06	G1073–CT56	1.81		SAD1; OsmiR156f; Osmtd1
MQTL8.6		133.15	13	2	5.15	RZ66–RG136	1.52		OsSPL14; OsPIN5b
MQTL9.1	4	15.61	21	3	2.95	RM219–RM7387	2.25		OsWRKY74
MQTL9.2		56.44	29	4	1.88	RM566–RM434	0.96		OsTCP17; OsmiR156g
MQTL9.3		67.76	37	6	6.00	RM3533–RM5519	1.34		LGDI; OsZHD1; Oshox4; OsmiR156k;
MQTL9.4		101.77	13	2	1.67	RM328–RM205	3.00		OsSPL17
MQTL11.1	3	4.71	31	5	2.33	RM286–RM26153	3.25		OsSPL18; OsFTL4; OsSHI1; OsNLP4;
MQTL11.2		63.56	43	7	3.08	RM6272–CT4	3.11		OsNRPD1b; OsAHP2; OsDRP1E
MQTL11.3		116.17	26	4	5.25	RM1233–RM5766	1.81		D53; OsGalLDH; OsTOM2; NAL2
									OsTOM3; miR535
									DHD1

Of the 57 QTLs detected in the RIL population by crossing 9311 to PA64s, 13 QTLs were covered by MQTLs. Among them, on chromosome 1, two QTLs were covered by the same MQTL. Specifically, *qD1tn1-3* and *qD2tn1-2* were covered by MQTL1.6. On chromosome 2 two QTLs were covered, including *qD2tn2* by MQTL2.1 and *qD4TN2-1* by MQTL2.4. On chromosome 3, *qD4TN3* was covered by MQTL3.3. On chromosome 4, two QTLs were covered by MQTLs, including *qD2tn4-1* by MQTL4.1 and *qD6TN4* by MQTL4.3. On chromosome 5, *qD2tn5-2* was covered by MQTL5.6. On chromosome 6, three QTLs were covered by MQTL, including *qD1TN6-2* covered by MQTL6.3, *qD2tn6-1* covered by MQTL6.4, and *qD4TN6* covered by MQTL6.6. On chromosome 7, *qD5tn7* was covered by MQTL7.2. On chromosome 8, *qD4TN8-1* was covered by MQTL8.1.

The raw QTLs on each MQTL ranged from 1 to 10, with a maximum of 10 raw QTLs for MQTL2.2 and a minimum of 1 for MQTL8.1. The 95% confidence intervals for the MQTLs ranged from 0.29 to 12.12 cM, and the physical lengths ranged from 0.27 to 6.0 Mb. There were only three MQTLs, MQTL6.6, MQTL7.2, and MQTL8.3, with physical lengths greater than 5 Mb, and the rest of the MQTLs were less than 5 Mb. The physical lengths of five MQTLs, MQTL1.6, MQTL2.6, MQTL5.5, MQTL6.8, and MQTL8.1, were all less than 0.5 Mb, and the physical lengths of MQTL6.8 and MQTL8.1 were the shortest at 0.27 Mb. These five MQTLs can be further studied and analyzed to mine tillering-related genes.

3.4. Analysis of Candidate Genes Related to Tillering in Rice

3.4.1. MQTL Candidate Gene Analysis

The obtained MQTLs were screened for candidate genes. The physical locations of the MQTLs were obtained using the Gramene database (<https://archive.gramene.org/markers/> (accessed on 10 August 2022)), and the 52 MQTLs included a total of 19,274 candidate genes (Table S2). MQTL6.8 encompassed a minimum of 59 genes with a physical length of about 0.27 Mb. MQTL7.2 contains the most candidate genes with 936 genes and a physical length of about 6 Mb. Five MQTLs, MQTL1.6, MQTL2.6, MQTL3.1, MQTL6.8, and MQTL8.1, contain less than 100 candidate genes, which allows for the mining relevant genes related to tiller number. MQTL3.1, MQTL3.2, MQTL3.3, MQTL5.2, MQTL5.3, MQTL7.2, and MQTL8.2 do not have genes that have been reported to be related to tillering and can be further mined for tiller-related candidate genes. MQTL3.1 has a physical length of 0.37 Mb and a phenotypic contribution rate of 29%, and the interval is between 3,964,690 and 4,333,950. This MQTL can be screened for tillering-related candidate genes. MQTL1.6 (56 candidate genes) contains *OsSPL2*, showing higher expression in 9311, and a coding SNP (T441G) that is hypothesized to be the main effector gene for the stable QTL cluster (*qmD1TN1*, *qD1tn1-3*, *qD2TN1-2*).

Candidate gene screening of 52 MQTLs using the Rice Data Centre library (<https://www.ricedata.cn/gene/> (accessed on 12 August 2022)) yielded 104 candidate genes related to tillering (Table 5). Some played important roles in rice tiller growth, e.g., *MOC1* [17], *FON1* [1], *OsTB1* [3], and *D3* [5]. Some genes regulate tiller response to nitrogen, e.g., *OsTCP19* [20], *OsNPF7.1* [25], *OsNPF7.2* [26], and *OsNR2* [18].

At a tiller number of 6 in 9311 and 11 in PA64s, RNA from parental tiller shoots was extracted (Figure 5A), and the relative expression of some genes related to positive or negative tiller regulation reported by Wang et al. [56] was determined (Table S1). *HTD1* [57] and *D3* [5] negatively regulated the growth of tiller shoots, and *DLT* [58] positively regulated the growth of tillers. The results determined in this study showed that the relative expression levels of *D3* and *HTD1* genes were significantly higher in the parental line 9311 than in PA64s. The relative expression levels of *DLT* genes were significantly higher in the parental line PA64s than in 9311, and the expression of genes related to the negative regulation of tillering was relatively higher in 9311 than in PA64s (Figure 5B).

3.4.2. Analysis of MQTL1.6-Related Candidate Genes

By analyzing the QTLs detected in a single environment, it was found that the two QTLs, *qD1tn1-3* and *qD2TN1-2*, both of which provided extremely high contributions to the tiller number phenotype in the pre-tiller stage, reached more than 40%, and the positional distance between the highest points of the two QTLs differed by 0.57 cM. One QTL (mks1-356) was detected in the pre-tiller stage in multi-environmental QTL analyses, and it was identified that mks1-356 did not exist in the pre-tiller stage. It was located 0.76 cM and 0.19 cM away from the *qD2TN1-2* and *qD1tn1-3* loci, and no environmental interactions were identified for mks1-356 using multi-environmental QTL analysis. MQTL1.6

covers this segment, which contains 56 candidate genes (Table S3), and the analysis of related genes that have been reported for tiller number by the National Rice Data Centre (<https://www.ricedata.cn/gene/> (accessed on 12 August 2022)) revealed that this segment contains an *OsSPL2* gene that has been reported as a Squamosa promoter-binding protein. This gene has not been studied in depth, and information is limited [59]. Quantitative gene analysis of *OsSPL2* in 9311 and PA64s (Table S1) revealed that the relative expression level of the *OsSPL2* gene in 9311 was significantly higher than that in PA64s during the pre-tillering stage. In addition, there was a single nucleotide mutation (SNP) (T → G) in the third exon at position 441, where the amino acid was mutated from phenylalanine to valine (Figure 6). Thus, we hypothesized that this locus caused the expression difference between the parents. The candidate gene for *qD1tn1-3* and *qD2TN1-2* is *OsSPL2*, and this gene may not be affected by the environment as a tiller nitrogen efficiency response gene.

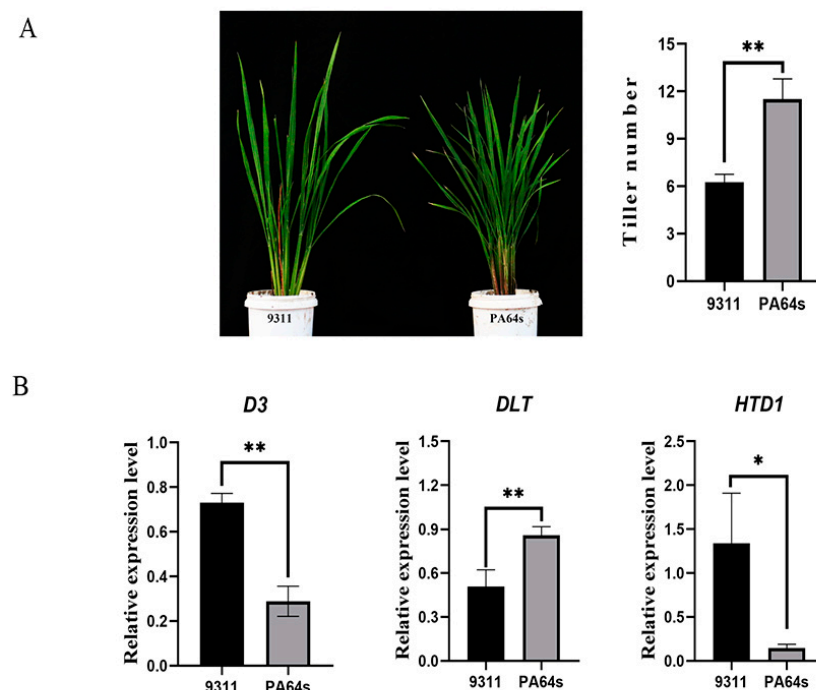


Figure 5. Expression levels of genes related to regulating tiller number in rice. (A): Graph of parental monocots at the tillering stage; (B): number of parental tillers at the time of taking tiller buds; (B-D3): expression level of the *D3* gene in parents; (B-DLT): expression level of the *DLT* gene in parents; (B-HTD1): expression level of the *HTD1* gene in parents. *, $p < 0.05$; **, $p < 0.01$.

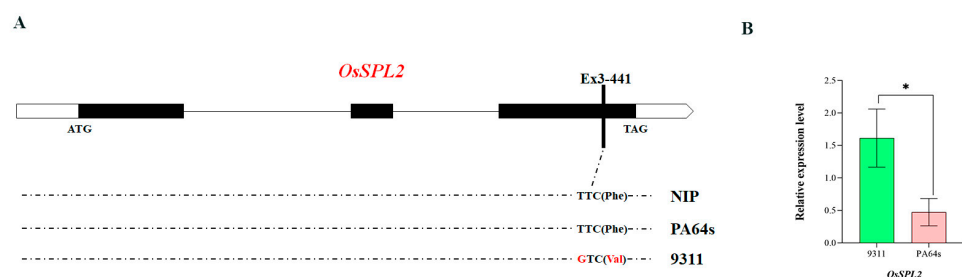


Figure 6. SNPs and relative expression of the *OsSPL2* gene in the parental strains. (A): Structure and comparison of the *OsSPL2* gene in parental strains PA64s and 9311 and the reference Nipponbare; (B): indicates the expression level of the *OsSPL2* gene in different parental strains. *, indicates the significance of the difference in expression levels between PA64s and 9311 at $p < 0.05$.

4. Discussion

4.1. Comparison of the QTLs Identified in This Study with Reported QTLs

Some of the QTLs detected in this study were located in the same or similar genomic regions as those reported by previous authors. For example, *qD2TN1-2*, *qD1tn1-3*, *qmD1TN1*, *qD4TN8-1*, and *qmD6TN7* were located in similar positions as *qNPT1-2*, *qNTT8-1*, and *qNTT7-2*, respectively, as reported by B. Rajurkar [29]. *qD2tn2* and *qD6TN4* were located in similar positions as *qTN-2* and *qTN-4* as reported by Liang [55]. *qD4TN2-1* and *qmD4TN2* are close to *qTNL2-1* as reported by Kwon [30]. In addition, *qD4TN3* and *qD5tn7* are similar in positions as *qTN3* and *qTN7* as reported by Liang [42]. *qD2tn4-1* is similar in position to *qPTN4.1* as reported by Beerelli [32]. *qD2tn5-2* is similar in position to *qMTN5-2* as reported by Sun [46]. *qD1TN6-2*, *qD2tn6-1*, *qmD2TN3-1*, *qmD3TN3*, *qmD2TN6*, and *qmD4TN4* were similar in positions to *qCTN6-5*, *qCTN6-6*, *qCTN3-1*, and *qCTN4-3* as reported by Jiang [16], and *qD4TN6* was similar in position to *qTN-6* as reported by Wang [27]. These results further verified the reliability of the QTL localization in this study and also indicated that some of the QTLs were conserved under different genetic backgrounds and environmental conditions.

4.2. Adaptive QTLs Associated with Dynamic Tillering in Rice RIL Populations Under Three Treatments

Phenotypic plasticity is the ability of the same genotype to exhibit different phenotypes in different environments [60,61]. Studies have shown that the plasticity of tillering traits to soil heterogeneity is universal, and this plasticity helps rice adapt to stress related to nitrogen deficits [61]. In this study, single-environment QTL analysis identified 10 QTLs (e.g., *qD2tn2*, *qD5TN2-1*, *qD1tn2*, *qD4TN3*, etc.) that contributed more than 10% under no-N treatment, and these QTLs may contain the main effector genes for the efficient response of tillering to nitrogen. Among them, the efficiency alleles of *qD1tn2*, *qD1tn4*, *qD2tn2*, and *qD4TN3* were derived from PA64s, suggesting that these QTLs positively regulate tiller number under low nitrogen conditions. In addition, *qD1TN6-2*, *qD3TN8-1*, *qD4TN12*, and *qD6tn5* contributed more than 10% under straw return treatment, suggesting that these QTLs may be involved in regulating rice adaptation to soil heterogeneity.

QTL–environment interaction (QEI) analyses reveal differences in phenotypic plasticity of different genotypes in different environments [62,63]. In this study, *qmD1TN1* (mks1-356) did not have environmental interactions effects, and its contribution was higher in the normal nitrogen environment than under the no-nitrogen treatment condition, suggesting that this QTL may attenuate the repressive effect of 9311-related tiller genes under low nitrogen conditions. In addition, four QTLs with antagonistic pleiotropy (e.g., *qmD1TN12*, *qmD2TN1*, *qmD2TN3-1*, and *qmD5TN6*) were identified in this study, and these QTLs showed opposite effect values under different environments. For example, *qmD1TN12* (mks12-267) increased the number of tillers under no-N treatment, whereas it decreased the number of tillers under the normal N environment. *qmD2TN1* (mks1-334) and *qmD2TN3-1* (mks3-105) increased the number of tillers under straw return condition, whereas they showed opposite effects under the normal N environment. These results suggest that the environmental specificity of QTLs needs to be considered in breeding for optimal trait selection in specific environments [64].

4.3. Dynamic Tillering in Rice RIL Populations Under Three Treatments Associated with Pleiotropic QTLs and Primary QTLs

QTL pleiotropy refers to the phenomenon that a single QTL locus can regulate multiple phenotypic traits. QTLs related to rice quality and grain traits have been shown to be pleiotropic [65,66]. Liu et al. [17] reported dynamic QTLs for tiller number over time,

revealing differences in tiller QTLs at different developmental stages [16,67]. In this study, four QTLs with temporal pleiotropy were identified, of which mks3-165 (*qD2tn3*, *qD5tn3*) and mks6-235 (*qD3TN6*, *qD5TN6*) were detected in the rapid tiller growth and later stages of tiller development, respectively, suggesting that these QTLs may regulate rapid tiller growth and the formation of the highest tiller number. In addition, mks1-305 (*qD1tn1-2*, *qD2tn1*) was detected in the middle and early stages of tillering and might be related to the formation of tiller buds. In addition, mks7-139 (*qD6TN7*, *qD3TN7*) was detected in the rapid growth and late stages of tillering and showed significant effects under different treatments. These results indicate that this QTL is spatio-temporally multiplexed and might regulate the final effective spike formation.

In addition, two main effector QTLs (*qD1tn1-3* and *qD2TN1-2*) with more than 40% phenotypic contribution were identified in this study, and both of their potentiated alleles were derived from 9311. In the multi-environmental analysis, *qmD1TN1* (mks1-356) did not have environmental reciprocal effects and was located in close proximity to *qD1tn1-3* and *qD2TN1-2*, suggesting that these three QTLs may be located at the same genetic locus. This main effect QTL may play a key role in tiller shoot formation and rapid growth period and is not affected by the environment, suggesting that it may be a main effect gene for efficient response to tiller nitrogen.

4.4. Meta-QTLs and Candidate Genes Associated with Tiller Traits in Rice

Meta-QTL analysis can significantly improve QTL localization accuracy and provide important clues for candidate gene mining by integrating QTL information from multiple studies [22,68]. In this study, 52 MQTLs were identified, of which MQTL3.1 and MQTL6.8 contained 82 and 59 candidate genes, respectively, and no reported tillering-related genes were found, suggesting that these intervals may contain new tillering-regulated genes. In addition, this study covered several nitrogen-efficient related genes (e.g., *OsTCP19* [23], *NPF7.1* [69], *OsNPF7.2* [70], and *OsNR2* [21]). Of these, *OsTCP19* was confirmed to be a key gene in the nitrogen-efficient response of tillering, and a 29 bp deletion in its promoter region was associated with a nitrogen-efficient phenotype. This gene is located within the *qD2tn6-1* interval and could be a priority target for the development of molecular markers for nitrogen efficiency in tillers.

The primary QTLs identified in this study, *qD1tn1-3* and *qD2TN1-2*, were covered by MQTL1.6, an interval containing 56 candidate genes. Of these, *OsSPL2* is the only reported tillering-related gene, and its function has not been analyzed in depth. *OsSPL2* belongs to the Squamosa promoter binding protein-like (SPL) family of transcription factors, members of which play key regulatory roles in plant growth and development and stress response [59,71,72]. In this study, we found that the expression level of *OsSPL2* in 9311 was significantly higher than that of PA64s, and its coding region was polymorphically different among the parents (site 441 of the third exon was mutated from T to G). This was hypothesized to be a possible cause of the functional difference. Therefore, *OsSPL2* may be a key gene for this primary QTL, and its independence from environmental influences has the potential to be developed as a molecular marker. In addition, other candidate genes within the MQTL1.6 interval (e.g., *LOC_Os01g69980* and *LOC_Os01g70050*) may also be involved in tillering regulation, which is worthy of further investigation.

4.5. Breeding Implications

The stable QTL *qmD1TN1* (mks1-356) and its candidate gene *OsSPL2*, unaffected by environmental interactions, represent ideal targets for MAS to enhance tiller establishment under low N or straw return. Molecular markers linked to mks1-355/mks1-352 could efficiently introgress high-tillering alleles from 9311 into breeding lines. Pyramiding

environment-specific QTLs with stable major-effect QTLs may optimize tiller architecture for sustainable systems. Candidate lines carrying the MQTL1.6 haplotype from 9311, combined with PA64s-derived alleles for *qD2tn2* (low N tolerance), could balance tiller number and nitrogen efficiency.

4.6. Limitations of Year-to-Year Reproducibility and Statistical Stringency

In this study, although 57 tiller-related QTLs were identified, limitations still exist in their year-to-year reproducibility and statistical rigor. Inter-annual environmental variations (such as soil nitrogen and temperature) led to unstable detection of some key QTLs. Genotype–environment interaction ($G \times E$) analysis indicates that their expression is regulated by specific environmental conditions, suggesting that QTL effects are context-dependent rather than universally stable. In terms of statistical methods, although the FDR correction ($q < 0.10$) was used to control false positives, the population of 124 recombinant inbred lines (RILs) had insufficient power to detect small-effect QTLs ($PVE < 10\%$), and some minor-effect loci might have been missed. Future research can improve the reliability and universality of QTL detection by expanding the population size, conducting controlled-environment experiments, or integrating multi-model analysis, providing more robust genetic targets for precision breeding.

5. Conclusions

This study systematically analyzed the molecular basis of genetic regulation of tillering and environmental interactions in rice and revealed the dynamic characteristics of tillering-related QTLs and key candidate genes in multiple environments. Using single-environment, multi-environment, and meta-QTL integration analyses, 44 new unreported QTLs were identified, among which three main effective QTLs (*mks1-355*, *mks1-352*, and *qmD1TN1*) contributed more than 40% in two consecutive years of experiments and were unaffected by environmental interactions. These QTLs were further locked by the MQTL1.6 interval and might be the same genetic locus. It is worth noting that there are significant expression differences and polymorphisms of the candidate gene *OsSPL2* among the parents, suggesting that it may be the main effector gene of tillering, and *OsSPL2* may be a priority target for molecular marker development. In addition, four QTLs with antagonistic pleiotropy (e.g., *qmD1TN12*) were identified, which provides a theoretical basis for the targeted selection of favorable traits under specific environments. These QTLs exhibit stage- and environment-specific stability, with consistent effects during early tillering under defined nitrogen/straw treatments, rather than universal stability across all conditions. In the future, independent validation in a larger RIL population ($n > 200$) and transgenic analysis of *OsSPL2* will solidify the functional relevance of the MQTL1.6 cluster. These steps are crucial for translating stage-specific QTLs into widely applicable breeding tools.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15111115/s1>, Table S1: Primers for gene wxpression analysis and sequencing; Table S2: Analysis of Rice Tiller Nunber Rleated MQTL candidate Genes; Table S3: 56.MQTL 1.6 candidate genes; Table S4: Soil treatment information form.

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