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Genetic Dissection of Stem Branch Trait and Envisioning of Fixing Heterosis by Vegetative Reproduction in *Oryza rufipogon*

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Abstract: The stem branch trait of the stolon enables the common wild rice to produce new individuals through vegetative reproduction habit. In order to understand the genetic mechanism of stem branch character, we developed introgression lines (ILs) with the irrigated rice variety Yundao1 (YD1, *Oryza sativa*) as the recipient parent and Yuanjiang common wild rice (YJCWR, *O. rufipogon*) as the donor parent for subsequent identification of the relevant genes. An IL named IL-J85 was selected, which can originate new individuals from stem branches on stem nodes. Furthermore, the newly formed individuals can survive cutting to bear normally and produce the same yield per plant as IL-J85, which saved the growth time and production cost. Two QTLs (quantitative traits loci) related to the stem branch trait, *qSBR1* and *qSBR5*, were first mapped on chromosomes 1 and 5. The near isogenic lines NIL-Y37 and NIL-D1 in the background of Yunjing 37 and Dianjingyou 1 were cultivated, showing the same characteristics as IL-J85. Our results provide new insights into the underlying genetic mechanism of the stem branch trait in the common wild rice and have the value of breeding utilization using vegetative reproduction to fix heterosis and breed new rice varieties with the cutting characteristic.

Keywords: common wild rice; *Oryza rufipogon*; stem branch; vegetative reproduction

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1. Introduction

As an annual crop and one of the three major food crops, rice (*Oryza sativa*) provides staple food for 3 billion people worldwide and plays an essential role in world food security [1,2]. Its importance is even more evident in China due to its long history of cultivation, given that it is the most dominant staple food for over 60% of the Chinese [3]. The traditional cultivation modes require sufficient irrigation water and have caused the serious nutrient loss. Moreover, labor costs, seeds, and other production costs keep rising, which affect people's enthusiasm and motivation to cultivate perennial rice [4]. Recently, researchers placed efforts into using the perennial habit of wild rice varieties to breed perennial rice/upland rice and fixing heterosis through vegetative reproduction to increase harvest area and reduce production costs and soil erosion, thereby protecting the environment [4,5].

Different perennial characteristics were performed in the species of *Oryza*. The common wild rice (*O. rufipogon*) grows perennially with stolons. However, *O. longistaminata*, *O. rhizomatis*, and *O. australiensis* grow permanently with rhizomes. Both *O. longistaminata* and *O. rufipogon* with the typical perenniality have the same AA genome as *O. sativa*, which

are considered ideal donors for transplanting the perennial habit [6]. Rhizomes determine the perennial growth of *O. longistaminata* and are controlled by one pair of dominant complementary genes, *Rhz2* and *Rhz3*, located on chromosomes 3 and 4, respectively [5]. Further studies have showed that a very complex gene regulatory network mediated the growth and development of rhizomes in *O. longistaminata* [7]. *O. rufipogon* is the ancestor of *O. sativa* [8], and there are almost no reproductive disorders between them. It is sensitive to photoperiod and has a high outcrossing rate, preferring to live near swamps, lakes, and ditches with perennial habit [9]. *O. rufipogon* has a prostrate growth for many years by branching stolons and functional roots to form new individuals and finally achieving perennial survival through vegetative reproduction [10].

Various important agronomical traits were selected during the domestication of cultivated rice, including seed shattering, erect plant architecture, panicle shape, awn length, grain size and quality, and hull color [11]. Some key genes controlling rice domestication are well-understood, including the shattering gene *Sb1* and *qSH1* [12,13]; awn length gene *An-1*, *An-2*, and *RAE2* [14–17]; hull color gene *Bh4* [18]; pericarp color gene *Rc* [19]; panicle shape and ligule development gene *OsLG1* [20]; grain width gene *GW5* [21,22]; and tiller angle gene *PROG1* [23,24]. The selection and domestication of these genes resulted in dramatic morphological changes in cultivated rice in Asia, from wild varieties adapted to natural environments to cultivated varieties adapted to agricultural environments. In addition, about 40% of wild rice alleles, including perennial alleles in the process of domestication from the wild rice to the cultivated rice, were lost [25], which resulted in narrowing of the genetic basis of cultivated varieties and further restricted improvement and breakthrough of yield potential of rice varieties. Genetic and molecular analyses of the perennial habit of *O. rufipogon* are the basis for breeding utilization and also help reveal the origin and domestication process of cultivated rice. Creeping growth gene *PROG1* and photoperiod sensitive gene *Hd1* and *Ehd1* at the heading stage were associated with the perennial habit of *O. rufipogon*. However, the formation of new individuals by stem branches and adventitious roots on stolons and the critical part of the perennial habit, is little known.

Tiller, the shoot branch in rice which is closely related to rice yield, has been extensively studied, and the formation process of tiller has been elucidated [26,27], including *MOC1* [28], *MOC3/TABLE1* [29,30], *TAD1/TE* [31,32], *OSH1* [30], *LAX1,* and *LAX2* [33,34], which influence tiller bud initiation. Furthermore, *D27* [35], *OsMAX1* [36], *OsMADS57* [37], *D53* [38], *OsOTUB1* [39], *IPI1* [40], *IPA1* [41], *OsTB1* [42,43], and *DLT* [44] have been demonstrated to participate in tiller bud outgrowth. Phytohormones also regulate tiller bud initiation and outgrowth. In rice, strigolactones (SLs), auxin, gibberellins (GAs), and abscisic acid (ABA) can repress bud outgrowth, whereas brassinosteroids (BRs) and cytokinins (CKs) promote bud outgrowth [45].

The above studies mainly focus on the tiller, a lateral shoot arising from an axillary bud at basal nodes in rice. However, the genetic and molecular mechanism of the stem branch, a lateral shoot arising from an axillary bud at high nodes aboveground, remains elusive. Herein, we investigated the stem branch trait of an introgression line, a cross between cultivated rice (*O. sativa*) and its wild relative, *O. rufipogon*. The introgression line formed new individuals from the branches on stem nodes with adventitious roots, which was the same as the *O. rufipogon*. Furthermore, these individuals can survive by cutting, bear normally, and produce the same yield per plant with the introgression line. This study will provide theoretical basis and practical value for rice breeding using the stem branch trait to fix heterosis and cultivate new rice varieties of vegetative reproduction.

2. Materials and Methods

2.1. Plant Materials and Natural Field Experiment Conditions

The *japonica* paddy rice varieties, including Yundao1 (YD1), Yunjing37 (YJ37), and Dianjingyou1 (DJY1), were used as the recurrent parents. The common wild rice (YJCWR) collected from Yuanjiang county, Yunnan Province, China was used as the donor parent.

IL-J85 was an introgression line from the cross between YD1 and YJCWR. NIL-Y37 and NIL-D1 (BC_3F_5) were the near-isogenic lines by crossing/backcrossing between IL-J85 and YJ37, respectively. C-J85, C-Y37, and C-D1 were the cuttings from the stem branches of IL-J85, NIL-Y37, and NIL-D1, respectively. S-J85, S-Y37, and S-D1 were the seed seedlings of IL-J85, NIL-Y37, and NIL-D1, respectively. BC_3F_2 and BC_3F_3, derived from the cross between YD1 and IL-J85 were cultivated to map the QTLs responsible for the stem branch trait.

All rice materials used for agronomic analysis were cultivated at the breeding base of the Xishuangbanna Botanical Garden, Chinese Academy of Sciences (21°56′ N, 101°15′ E) under natural field conditions twice a year in the spring and fall of 2021. The seeds were soaked for 48 h, and then germinated in a growth chamber at 28 °C for 24 h, subsequently sown on seedbeds. The seed seedlings were transplanted to paddy field at the four-leaf stage with 6 rows per plot and 12 plants per row. The density was 25 cm × 20 cm between each individual. For the breeding utilization of the stem branch trait, about 10 days before harvest of the NILs in the previous season, the seeds of the NILs were sowed normally on the seedling bed. Furthermore, the stem branches were cut and collected in 15 days later after harvest of the NILs, and then the cuttings (C-Y37 and C-D1) were placed in water for 3 days to enable the growth of the adventitious root. The seed seedlings and cuttings of NILs were transplanted to the paddy field simultaneously. The seed seedlings and cuttings of IL-J85 were treated and planted as mentioned above. To better measure phenotypes, all rice materials were planted in random three repetitions. The water in the paddy field was covered 2–5 cm above the soil before the rice seeds matured and supplemented with the compound NPK fertilizer (2 kg/100 m^2).

2.2. Phenotypes Analysis

The materials were harvested in the center of each plot in each line to investigate the phenotypes. For the parent YD1 and IL-J85, the agronomic traits, including plant height, primary panicle length, flag leaf length, flag leaf width, flag leaf length–width ratio, the number of panicles, primary branches per panicle, secondary branches per panicle, 1000-grain weight, grain yield per plant, and internode length were investigated for 30 individuals. The number of stem branches and tillers per plant were conducted in 15 days later after harvest. For the analysis in the breeding utilization of the stem branch trait, the agronomic traits, including heading date, plant height, primary panicle length, flag leaf length, flag leaf width, the number of panicles, primary branches per panicle, secondary branches per panicle, 1000-grain weight, and grain yield per plant, were investigated for 30 individuals of the seed seedling and cutting of corresponding ILs/NILs respectively. Each experiment was performed for three biological repetitions. All rice seeds for phenotypes analyses were air-dried. Grain weight was measured with a Yield-Traits Scorer (YTS-5DS).

2.3. DNA Extraction

Rice genomic DNA from leaves was obtained according to the method described by Abdel-Latif et al. [46].

2.4. Mapping of qSBR1 and qSBR2

We hybridized IL-J85 with YD1 to obtain the BC_3F_1. The BC_3F_1 generation all performed the stem branch trait. BC_3F_2 was obtained from the selfing of BC_3F_1. We used 210 individuals of the BC_3F_2 segregating population to map *qSBR1* and *qSBR5* regions based on the number of stem branches per plant. A total of 100 recombinant individuals from BC_3F_3 segregating populations were used to fine map the locus of the stem branch trait through the targeted sequencing genotype detection technology of 10 K SNP chip (Shijiazhuang Molbreeding Biotechnology Co., Ltd., Shijiazhuang, China).

2.5. Statistical Analysis

QTL IciMapping 4.1 software was used to construct a genetic linkage map based on the genotypes and phenotypes, and Excel 2007 and SPSS 22 were used for data analysis. All samples were subjected to three replicate measurements. The data were presented as the mean ± standard deviation (SD). Student's *t*-test or one/two-way ANOVA was performed to determine the significant difference, as indicated by * and ** at $p < 0.05$ and $p < 0.01$, respectively.

3. Results

3.1. Phenotypic Analysis of YD1 and IL-J85

The common wild rice (*O. rufipogon*; YJCWR) was collected from Yuanjiang County, Yunnan Province, China (Figure 1a,b). Yundao1 (*O. sativa*; YD1) a paddy *japonica* rice variety in Yunnan, was bred by Yunnan Academy of Agricultural Sciences (and Figure 1c). In order to explore the excellent traits from YJCWR, we constructed a set of introgression lines (ILs, BC_2F_8) with YJCWR as the donor parent and YD1 as the recurrent parent. Among these ILs, one introgression line IL-J85, showed stem branches at high nodes aboveground 15 days later after harvest, but the stem branch trait did not occur before maturity compared with YD1 (Figure 1c,d). We found that the stem could form more branches after crushing artificially or submerged naturally, which was similar to the trait of YJCWR (Figure 1e). These results suggest that the stem branch trait of introgression line IL-J85 was inherited from YJCWR.

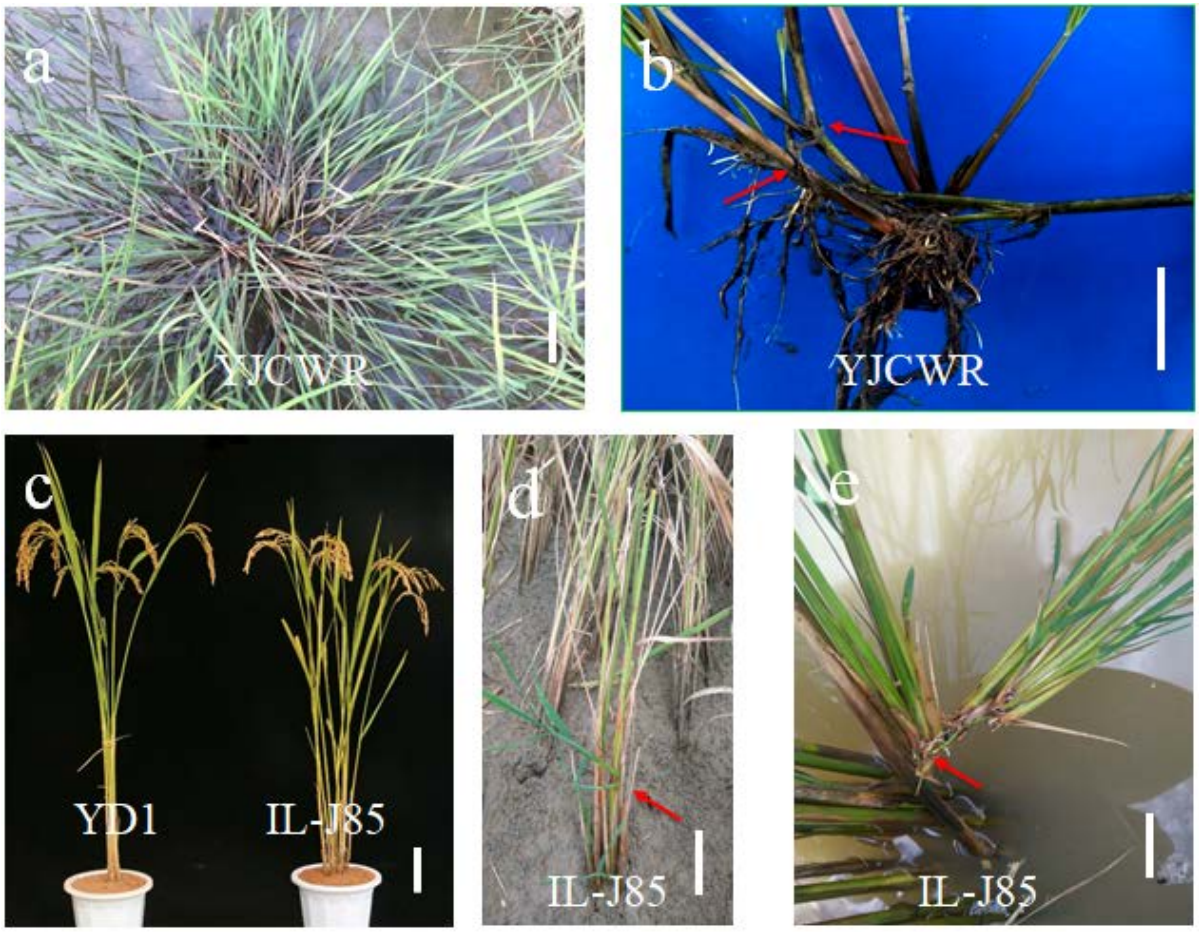

Figure 1. The phenotype of YJCWR, YD1, and IL-J85. (**a**,**b**) The phenotype of YJCWR. (**c**) YD1 and IL-J85 in the mature period. (**d**) IL-J85 15 days later after harvest. (**e**) IL-85 after crushing artificially or submerged naturally; the red arrow indicated the stem branches. Bar = 10 cm.

We conducted a comparative test on the main agronomic traits between IL-J85 and its recurrent parent, YD1. The results showed that plant height (PH), primary panicle length (PL), flag leaf length (FL), flag leaf width (FW), primary branches per panicle (PP), and secondary branches per panicle (SP) of IL-J85 decreased, but the number of panicles (NP) increased compared with those of YD1; whereas, there was no difference between YD1 and IL-J85 in flag leaf length–width ratio (FL/FW) (Table 1). We further analyzed (1st–5th) the internode length (INL) from top to bottom, and found that INL2, INL3, and INL4 of IL-J85 were significantly shorter than YD1, while INL1 and INL5 of IL-J85 had no significant difference from that of YD1 (Table 1). In addition, the number of stem branches per plant (TB) and tillers number per plant (TP) of two parents after harvest were investigated, and it was found that both TB and TP of IL-J85 were significantly higher than YD1 (Table 1). YD1 could produce a small amount of tillers after harvest, but did not produce stem branches which was different from IL-J85. The 1000-grain weight and grain yield per plant (GY) had no significant difference between YD1 and IL-J85, indicating that the stem branch trait after harvest did not affect the yield in IL-J85 compared with that in YD1 (Table 1). These results suggest that the stem branch trait of IL-J85 have the potential for breeding practice.

Table 1. Investigation of agronomic traits in YD1 and IL-J85.

Traits	YD1	IL-J85
PH (cm)	108.51 ± 6.27	91.52 ± 5.29 **
PL (cm)	23.28 ± 1.92	19.15 ± 1.84 **
FL (cm)	29.12 ± 5.48	22.04 ± 3.19 **
FW (cm)	1.63 ± 0.12	1.29 ± 0.11 **
FL/FW	17.78 ± 2.73	17.02 ± 1.64
NP	7.43 ± 2.37	10.07 ± 2.85 **
PP	14.97 ± 1.4	8.47 ± 0.94 **
SP	38.00 ± 5.72	11.57 ± 3.11 **
1000-grain weight (g)	29.05 ± 0.75	28.62 ± 1.57
GY (g)	22.03 ± 7.31	21.34 ± 3.51
INL1 (cm)	21.74 ± 1.62	20.89 ± 1.17
INL2 (cm)	38.48 ± 2.67	33.03 ± 1.21 **
INL3 (cm)	22.31 ± 1.01	18.91 ± 1.37 **
INL4 (cm)	15.44 ± 1.93	13.4 ± 1.3 *
INL5 (cm)	6.36 ± 2.55	6.43 ± 2.44
TP	1.9 ± 3.00	7.13 ± 3.84 **
TB	0.00 ± 0.00	5.06 ± 2.81 **

PH: plant height; PL: primary panicle length; FL: flag leaf length; FW: flag leaf width; FL/FW: flag leaf length–width ratio; NP: the number of panicles; PP: primary branches per panicle; SP: secondary branches per panicle; GY: grain yield per plant; INL: internode length; TB: the number of stem branch per plant after harvest; TP: the tiller number of per plant after harvest; mean ± standard deviations (SD). (n = 30); * indicated significantly different values (student's *t*-test, $p < 0.05$), and ** indicated highly significantly different values (student's *t*-test, $p < 0.01$).

The number of stem branches (TB) was significantly correlated with the number of panicles (NP); grain yield per plant (GY) were significantly correlated with NP and primary panicle length (PL), but not correlated with flag leaf related traits (Table 2); TB also affected plant architecture traits, including PH, PL, and flag leaf related traits, indicating that there may be one pleiotropic gene or linkage inheritance.

Table 2. Correlative coefficients among different agronomic traits of IL-J85.

	PH (cm)	NP	FL (cm)	FW (cm)	FL/FW	PL (cm)	GY (g)	TP	TB
PH (cm)	1								
NP	−0.70 **	1							
FL (cm)	0.73 **	−0.69 **	1						
FW (cm)	0.91 **	−0.74 **	0.88 **	1					
FL/FW	0.25	−0.43	0.81 **	0.45 *	1				
PL (cm)	0.89 **	−0.71	0.89 **	0.96 **	0.52 *	1			
GY (g)	0.45	−0.41 *	0.18	0.11	0.25	0.63 **	1		
TP	0.09	0.00	−0.28	−0.04	−0.48 *	−0.19	−0.18	1	
TB	−0.57 *	0.66 **	−0.73 **	−0.84 **	−0.38	−0.58 *	−0.34	−0.01	1

PH: plant height; PL: primary panicle length; FL: flag leaf length; FW: flag leaf width; FL/FW: flag leaf length–width ratio; NP: the number of panicles; GY: grain yield per plant; TB: the number of stem branches per plant after harvest; TP: the tillers number of per plant after harvest; * represent significant correlation ($p < 0.05$), and ** represent highly significant correlation ($p < 0.01$).

3.2. Genetics Analysis of Stem Branch Trait in IL-J85

To carry out a genetic analysis of the stem branch trait in IL-J85, we hybridized IL-J85 with the recurrent parent YD1 to obtain the BC_3F_1 population (Figure 2a). All of the BC_3F_1 generation showed a similar stem branch trait to IL-J85. Thus, we speculated that the stem branch trait in IL-J85 was effectively dominant. To further assess the characteristics of phenotypical variation in the stem branch trait of IL-J85, the BC_3F_2 population were obtained from the selfing of BC_3F_1. We found the distribution in the BC_3F_2 population based on the number of stem branches per plant presented a multimodal distribution (Figure 2b). These findings indicated that major QTLs controlled the stem branch trait.

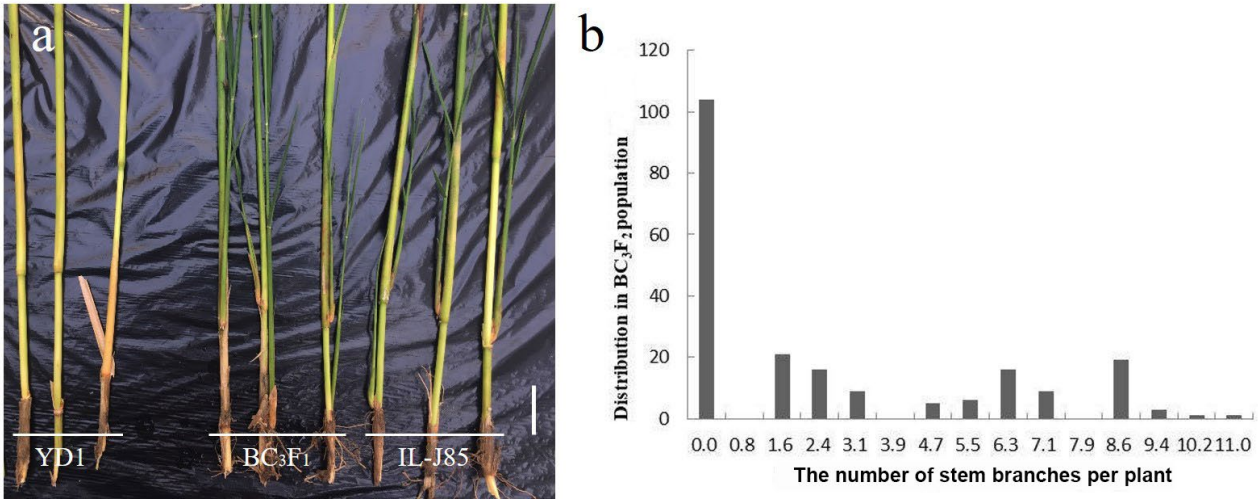

Figure 2. The phenotype of BC_3F_1 in the mature period and the distribution in BC_3F_2 population. (**a**) YD1, BC_3F_1, and IL-J85 15 days later after harvest (bar = 10 cm). (**b**) The distribution in BC3F2 population based on the number of stem branches per plant (N = 210).

3.3. QTL Mapping for Stem Branch Trait

In order to map the stem branch trait, a total of 452 SSR markers, relatively uniformly distributed throughout the rice genomes, were used to assess polymorphism between IL-J85 and YD1. Sixteen SSR markers were polymorphic and distributed on nine chromosomes as nine segments except for chromosomes 2, 4, and 10 in IL-J85, indicating that they were introgressed from YJCWR. To evaluate whether a QTL locus of stem branch was harbored in these nine chromosome regions, the genotypes of 210 individuals from the BC_3F_2 population were investigated using the 16 polymorphic SSR markers. The loci on chromosomes 1 and 5 had significant effects, whereas the effects of other introgressed segments were barely significant (Table 3). These results suggest that these two introgressed regions were responsible for the stem branch trait.

A two-way ANOVA was performed with the markers showing the most effect from these two regions (RM259 and RM26) (Figure 3). The results showed that the effect of these two regions alone and their interaction on the stem branch trait were significant. A QTL analysis showed that two QTLs on chromosomes 1 (in the interval between RM6521 and RM259) and 5 (in the interval between RM26 and RM19225) explained 15.65% and 13.45%, respectively (Tables 4 and 5). We designated the locus on chromosome 1 as *qSBR1* (*Stem branch of Rice 1*) and the locus on chromosome 5 as *qSBR5* (*Stem branch of Rice 5*).

Table 3. One-way ANOVA of stem branch presence or absence using marker genotypes as groups.

Chromosome	Markers	MS	df Error	MS Error	F	*p*
1	RM259	16.11	238	0.58	27.57	0.00
3	RM6147	0.24	238	0.21	1.15	0.28
5	RM274	6.63	237	0.60	11.00	0.00
5	RM26	11.98	238	0.67	17.97	0.00
5	RM5162	7.43	236	0.67	11.05	0.00
5	RM19151	7.37	238	0.86	8.54	0.00
5	RM5261	6.23	238	0.60	10.41	0.00
6	RM3330	1.13	236	0.42	2.67	0.10
7	RM542	0.41	238	0.57	0.72	0.40
8	RM6976	1.43	237	0.74	1.95	0.16
9	RM5526	0.55	238	0.64	0.87	0.35
11	RM3605	0.19	238	0.67	0.28	0.60
12	RM1246	0.01	238	0.27	0.02	0.88

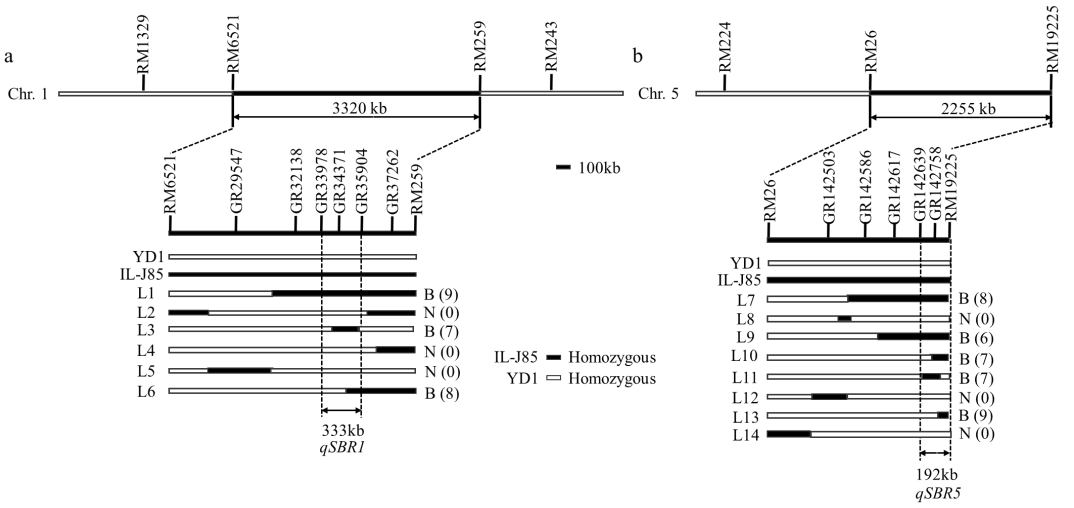

Figure 3. Fine mapping of the *qSBR1* and *qSBR5* locus. (**a**) *qSBR1* was initially mapped to the interval between the markers RM6521 and RM259, and then narrowed to a 333 kb region between the markers GR33978 and GR35904. (**b**) *qSBR5* was initially mapped to the interval between the markers RM26 and RM19225, and then narrowed to a 192 kb region between the markers GR142639 and RM19225. B: stem branch; N: no-stem branch; the number of individuals is in parentheses; L1–L6: individuals that exchanged on chromosome 1; L7–L14: individuals that exchanged on chromosome 5.

Table 4. Two-way ANOVA using one marker locus from each of the two putative regions related to stem branch trait.

Markers	Chromosome	MS Error	df Error	F	*p*
RM259	1	7.509	2	14.631	0.000
RM26	5	3.976	2	7.746	0.000
RM259* RM26		3.716	3	5.430	0.000

Table 5. QTLs controlling stem branch in rice.

Trait	QTL	Chromosome	Markers	Range (cm)	LOD	PVE (%)
Stem branch	*qSBR1*	1	RM6521-RM259	16.50–29.78	15.65	15.65
	qSBR5	5	RM26-RM19225	109.37–118.39	7.53	13.45

In order to further fine map the two QTLs, we selected six individuals with heterozygous QTLs segments from the BC_3F_2 population to obtain the BC_3F_3 populations. Through the genotype analysis of 1461 individual plants, 50 recombinations of distinct stem branch individuals similar to IL-J85, and 50 non-stem branch individuals similar to YD1 were selected from the BC_3F_3 segregation population. The targeted sequencing genotype detection technology of the 10k SNP chip was used to analyze the genotype of these recombinant individuals. Through parental polymorphism analysis, a total of 67 and 132 polymorphic SNP markers were screened in the *qSBR1* and *qSBR5* intervals. The total length of *qSBR1* interval was 18 cm with an average of 0.28 cm, and *qSBR5* interval was 18.39 cm with an average of 0.14 cm. Compared with the phenotype and genotype of recombinants, we narrowed the *qSBR1* and *qSBR5* to chromosome 1 (18.64–19.94 cm, 333 kb) and chromosome 5 (117.54–118.30 cm, 192 kb) (Figure 3).

3.4. Effects of Different Genotype Combinations of Two QTLs on Stem Branch and Related Trait

The investigation of the BC_3F_3 population found the basal regenerative tiller and stem branch trait 15 days later after harvest. We further analyzed the effects of different genotype combinations of two QTLs on the tiller and stem branch trait by QTL closely linked markers (*qSBR1* Marker ID: GR34371; *qSBR5* Marker ID: GR142758). The results showed that there was no significant difference in the number of regenerative tillers when two QTLs showed homozygous YD1 and one QTL showed heterozygous or homozygous introgression. However, with the combined introgression of two QTLs, whether heterozygous or homozygous, the number of regenerative tillers was significantly more than the YD1 genotype (Figure 4a). The same situation appeared on stem branch trait (Figure 4b). When the two QTLs were YD1 genotype, the stem branch trait did not occur, and one QTL introgression significantly increased the number of stem branches, and the simultaneous introgression of two QTLs significantly increased the number of stem branches compared with the YD1 genotype (Figure 4b). Interestingly, there was no significant difference between heterozygous and homozygous genotypes introgression of two QTLs. These results indicate that two QTLs simultaneously affected the growth of regenerated tiller and stem branch.

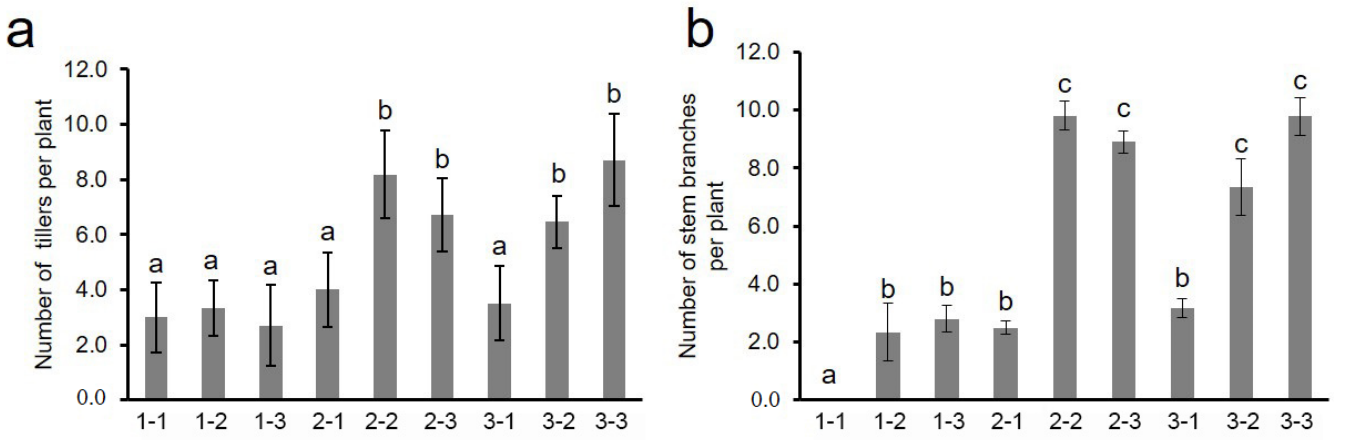

Figure 4. Effects of different genotype combinations of two QTLs on branch trait. (**a**) Number of tillers per plant. (**b**) Number of stem branches per plant. 1, 2, 3 indicated homozygotes of YD1, and homozygotes and heterozygotes of IL-J85, respectively; the number in front and behind represent *qSBR1* and *qSBR5*, respectively. The different letters above each bar indicated statistically significant differences as determined by one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

3.5. Breeding Utilization of Stem Branch Trait

In order to evaluate the breeding value of the stem branch trait, near-isogenic lines (NILs; NIL-Y37, NIL-D1) were cultivated using two *japonica* rice varieties Yunjing 37 (YJ37) and Dianjingyou 1 (DJY1) to cross and backcross IL-J85, respectively, through molecular markers assisted selection.

We further investigated the growth period of cuttings of ILs/NILs from transplanting to heading and found that the heading date (HD) of cuttings were shorter than that of seed seedlings (Table 6 and Figure 5). Other agronomic traits were investigated in the mature period. S-J85 was higher than C-J85 in plant height (PH), but the difference was not significant. However, S-Y37 was significantly higher than C-Y37 and S-D1 was significantly higher than C-D1; there was no significant difference of the primary panicle length (PL) between S-J85 and C-J85, but PL of S-Y37 was significantly shorter than that of C-Y37. Similarly, PL of S-D1 was shorter than that of C-D1; the flag leaf length (FL) of S-Y37 was significantly higher than that of C-Y37, while there was no significant difference between S-J85 and C-J85, and S-D1 and C-D1, respectively; in addition, there was no significant difference between S-J85 and C-J85, S-Y37 and C-Y37, and S-D1 and C-D1 for their flag leaf width (FW) and number of panicles (NP); both the primary branches per panicle (PP) and the secondary branches per panicle (SP) of S-Y37 were significantly higher than that of C-Y37; however, there was no significant difference between S-J85 and C-J85, S-D1 and C-D1, respectively; the 1000-grain weight of S-D1 was significantly higher than that of C-D1. Likewise, there was no significant difference between S-J85 and C-J85, and S-Y37 and C-Y37, respectively; the grain yield per plant (GY) of S-Y37 was significantly higher than that of C-Y37, but there was no significant difference between S-J85 and C-J85, and S-D1 and C-D1, respectively. These results showed that the cuttings can reduce the heading date compared with the seed seedlings in different backgrounds. Interestingly, there was no significant difference between the cuttings and the seed seedlings of S-J85 for their 1000-grain weight and GY. However, there was a partial difference between S-Y37 and C-Y37, and S-D1 and C-D1, in different backgrounds. These results suggest that the stem branch character can be used as vegetative reproduction in rice breeding utilization.

Table 6. Investigating the agronomic traits of seed seedlings and cuttings from ILs/NILs in different backgrounds.

Traits	YD1 Background		YG37 Background		DJY1 Background	
	S-J85	C-J85	S-Y37	C-Y37	S-D1	C-D1
HD (d)	65.30 ± 1.11	60.52 ± 0.50 *	65.50 ± 1.00	60.60 ± 0.60 *	64.90 ± 0.99	59.42 ± 0.36 *
PH (cm)	93.35 ± 5.85	90.76 ± 4.01	102.47 ± 4.39	95.87 ± 3.29 **	108.10 ± 3.42	104.85 ± 4.95 *
PL (cm)	19.87 ± 1.65	19.74 ± 1.31	21.27 ± 1.22	22.36 ± 1.26 *	20.95 ± 1.69	22.92 ± 1.45 **
FL (cm)	30.73 ± 3.32	29.31 ± 3.27	41.68 ± 4.50	33.00 ± 5.60 **	39.90 ± 5.10	39.88 ± 3.34
FW (cm)	1.27 ± 0.09	1.21 ± 0.10	1.67 ± 0.09	1.65 ± 0.11	1.78 ± 0.09	1.73 ± 0.08
NP	6.27 ± 1.56	7.44 ± 2.58	11.337 ± 3.68	9.27 ± 1.83	6.40 ± 1.40	7.27 ± 1.49
PP	8.82 ± 1.25	9.63 ± 1.63	12.20 ± 1.57	8.73 ± 0.96 **	13.40 ± 1.50	12.73 ± 2.15
SP	18.45 ± 5.73	14.81 ± 4.55	39.20 ± 6.93	21.73 ± 4.11 **	40.20 ± 9.17	39.53 ± 8.06
1000-grain weight (g)	31.55 ± 0.47	30.79 ± 1.40	27.58 ± 0.69	27.26 ± 0.21	29.99 ± 0.16	28.95 ± 1.31 **
GY (g)	12.73 ± 4.05	12.65 ± 3.61	29.37 ± 6.32	24.33 ± 4.74 *	22.34 ± 5.28	23.10 ± 5.93

C-: cutting of ILs/NILs; S-: seed seedling of ILs/NILs; HD: heading date; PH: plant height; PL: primary panicle length; FL: flag leaf length; FW: flag leaf width; NP: the number of panicles; PP: primary branches per panicle; SP: secondary branches per panicle; GY: grain yield per plant; mean ± standard deviations (SD). (n = 15); * represented significantly different values (student's *t*-test, $p < 0.05$), and ** represented highly significantly different values (student's *t*-test, $p < 0.01$).

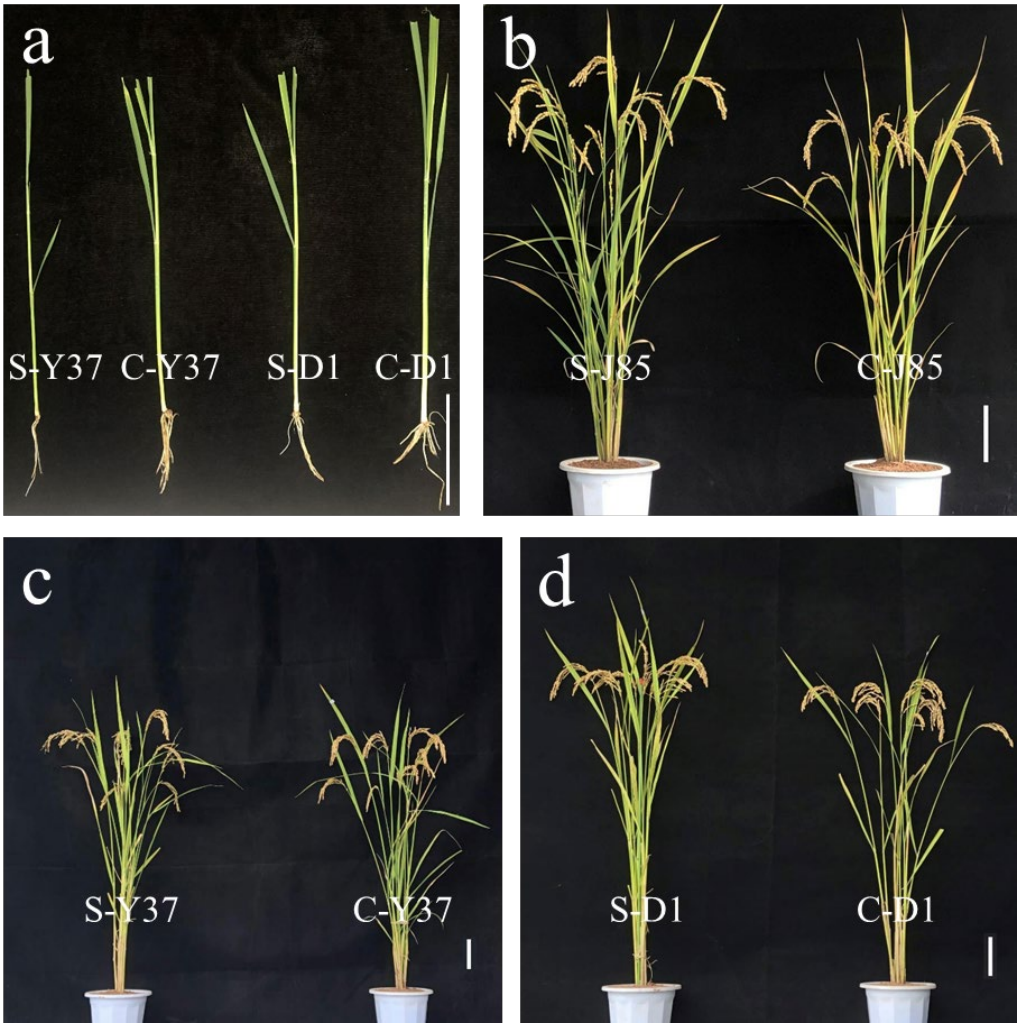

Figure 5. Investigating plant architecture of seed seedling and cutting of ILs/NILs at the seedling and mature period. (**a**) S-Y37 and S-D1 are seed seedlings, C-Y37 and C-D1 are cuttings from the stem branches (bar = 5 cm). (**b**–**d**) S-J85 and C-J85, S-Y37 and C-Y37, S-D1 and C-D1 are at mature period; (bar = 10 cm).

4. Discussion

Rice is the staple food for half of the population worldwide. Breeders have placed efforts into improving output per unit area to fulfill the increasing demand of food. Double-cropping rice can be developed in tropical areas to increase rice production. Seed is the main expense of the cost of rice production, especially for hybrid rice. South China and the middle and lower reaches of the Yangtze River are the main areas for rice production. In most rice plant regions, the whole growth duration for a single cropping rice season was surplus but insufficient for double cropping rice. IL-J85 with the stem branch trait discovered in this research can be transplanted to cuttings after harvest and performed with a similar yield compared with YD1, which saved the growth time and production cost. A correlation analysis found that grain yield per plant was negatively correlated with the number of panicles; however, the number of stem branches was significantly correlated with the number of panicles. Furthermore, the number of stem branches affected plant agronomic traits including plant height, flag leaf length, flag leaf width, the number of panicles, and primary panicle length, indicating that there may be one pleiotropic gene or linkage inheritance. A genotypic analysis showed that the phenotype of the stem branch in the heterozygous state was consistent with that in the homozygous state, indicating that it can be broadly used in hybrid rice production. These results suggest that common wild rice has practical value for rice production by using the stem branch trait to fix heterosis in breeding rice varieties of vegetative reproduction.

The novel beneficial alleles and potential genetic diversity have been lost during wild rice domestication in cultivated rice. The wild rice species can better adapt to different environments and various abiotic and biotic stresses owing to the reservoir of novel genes/QTLs. Some agronomically important genes/QTLs for improving abiotic and biotic stresses, resistance, productivity, and grain quality characteristics were identified from AA genome donor wild species and labelled with breeder-friendly molecular markers to elite genetic backgrounds [47]. *O. rufipogon* (2n = 24, AA) was considered as the progenitor of *O. sativa*. There were several reports of using chromosomal segment substitution lines (CSSLs), backcross inbred lines (BILs), ILs, and NILs to introgress QTLs related to yield and grain quality from different *O. rufipogon* accessions into elite *indica* and *japonica* genetic backgrounds [48–52]. In this research, the introgression line IL-J85, constructed with YD1 and Yuanjiang common wild rice (*O. rufipogon*), had the stem branch trait and could be cut to form new individuals to be achieved a with similar yield of seed seedlings. The *qSBR1* and *qSBR5* responsible for the stem branch trait were first mapped on chromosomes 1 and 5. However, rice tiller is regulated by complex genes, hormones, and environmental factors. The molecular mechanism and regulatory network of the stem branch trait still need further elucidation.

Although there has been a doubling of major grain crop yields since the 1950s, people subjected to malnutrition prevail worldwide [53]. However, the crop yield increase cannot fulfill the demand for food with an ever-growing population [54]. Global food security depends on annual grains:cereals, oilseeds, and legumes, which damage essential ecosystem services, making some beyond sustainable boundaries [55,56]. The development of perennial varieties of important grain crops can expand choices of farmers to produce grains under less favorable circumstances, which can ensure food and ecosystem security [4]. Perennial crops are superior to annuals in sustaining important ecosystem functions, specifically on marginal landscapes or where resources are limited [57]. Perennial crops tend to have longer growing seasons and deeper rooting depths than annual counterparts. In addition, they intercept, retain, and utilize more precipitation [4,58,59]. Breeders are devoted to pursuing of high yield, high quality, high resistance, and other valuable characteristics of rice varieties. In this research, IL-J85 in YD1 background performed the stem branch trait and can be cut to form new individuals with similar yield of seed seedlings, which save rice seed cost, reduce seedling raising time and water usage. In addition, the cultivated near isogenic lines NIL-Y37 and NIL-D1 in the two backgrounds Yunjing 37 and Dianjingyou

1 perform the same characteristics as IL-J85, indicating the stem branch trait can be used in rice production and breeding utilization of rice varieties of vegetative reproduction.

5. Conclusions

This study found that IL-J85 can form new individuals by branches and adventitious roots on stem nodes, which can survive by cutting and bear the same yield per plant as IL-J85, thereby saving the growth time and production cost. The stem branch trait QTLs, namely *qSBR1* and *qSBR5*, were first mapped on chromosomes 1 and 5 by mapping, MBS, and the targeted sequencing genotype detection technology of 10k SNP chip. The NIL-Y37 and NIL-D1 in the background YJ37 and DJY1 performed the same characteristics as IL-J85, which has the stem branch trait and could be cut to form new individuals. Our results provide new insights into the genetic mechanism of the stem branch characteristic in common wild rice, and have practical value using the stem branch trait to fix heterosis in breeding rice varieties of vegetative reproduction.

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