



Development of the PARMS marker of the *TAC1* gene and its utilization in rice plant architecture breeding

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Abstract The ideal plant architecture is a new strategy for super high yield breeding of rice. Tiller angle is an important plant architecture character of rice. A reasonable tiller angle is a key factor for the ideal plant architecture and achieving high-yield breeding. Molecular design breeding is the most potential new direction of crop breeding in the future. The development of accurate and efficient functional

molecular markers of target trait genes is crucial for molecular design breeding. The *TAC1* (*Tiller Angle Controlling*) gene is the primary gene that regulates tiller angle in rice. This gene can be used to improve the compact plant architecture of indica and japonica rice varieties. The SNP variation from A to G at the fourth intron 3' splicing point in *TAC1* changes plant architecture. Based on the SNP variation, PM-TAC1 was successfully developed as a fluorescent functional molecular marker, via the penta-primer amplification refractory mutation system. Ninety-three rice materials were genotyped using this marker, and the marker was effectively used in rice plant architecture breeding. The

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successful development of this marker will contribute to the molecular breeding of rice plant architecture.

Keywords *TAC1* · Plant architecture · PARMS marker · Rice · MAS breeding

Introduction

New plant architecture breeding (or super rice breeding) of rice was proposed in the 1990s (Khush 1996). Super hybrid rice varieties with the ideal plant architectures are essential to increase global food supply and ensure food security. Tillering angle is one of the indexes used in measuring the degree of plant tightness. It is an important plant architecture character of rice. It has a considerable influence on canopy structure, photosynthetic efficiency, material production, and disease resistance of rice. (Wang and Li 2005; Sun et al. 2019).

Although tillering angle is affected by the environment, it is primarily determined by genetic factors (Dong et al. 2016). The *TAC1* (*Tiller Angle Controlling 1*) is a major quantitative trait locus of tiller angle in rice (Yu et al. 2007). *TAC3*, together with *-TAC1* and *D2*, greatly controls tiller angle in rice cultivars (Dong et al. 2016). The gene *PROG1* controls the creeping growth habit of wild rice, which is essential to reveal the molecular mechanism of rice evolution (Jin et al. 2008; Tan et al. 2008). *LPA1* is a loose plant architecture gene that controls tillering angle and leaf angle by controlling the growth of the paraxial surface between the tiller and the leaf angle (Wu et al. 2013; Liu et al. 2016). *Spk(t)* controls the tillering angle of rice, and the dominant allele from Kasalath increases tillering angle that makes rice present scattered characteristics (Yamamoto T 1997; Miyata et al. 2005). *TIG1* positively regulates *EXPA3*, *EXPB5*, and *SAUR39* expression to promote cell elongation and increase tiller angle (Zhang et al. 2019a, b). The rice phytochrome-interacting factor-like protein OsPIL15 regulates tiller angle through light and gravity signals in rice (Xie et al. 2019). Numerous genes have been demonstrated to affect tillering angle through auxin regulation. *LAZY1* affects tillering angle by negatively regulating the polar transport of auxin. After the loss of *LAZY1* function, the polar transport capacity of auxin is greatly enhanced, thereby enhancing stem geotropism and making the whole plant loose

(Li et al. 2007). *OsBRXL4* regulates the centripetal and tillering angle of rice by affecting the nuclear localization of *LAZY1* (Li et al. 2019). *OsPIN2* depends on the auxin regulatory pathway to participate in the regulation of rice plant architecture. *OsPIN2* overexpression in rice inhibits *OsLAZY1* expression, thereby increasing tiller and tiller angle and decreasing plant height (Chen et al. 2012). *OsLIC1* regulates the plant architecture characteristics of rice, such as leaf angle, plant height, tiller angle, and grain number per ear, through the BR signaling pathway (Wang et al. 2008). *OsLIC1* is a direct target of *OsBZR1*, which can be phosphorylated by GSK3 kinase. As a zinc finger transcription factor antagonistic to *BZR1*, it weakens the BR signal (Zhang et al. 2012). *OsmiR167a* represses its targets, namely, *OsARF12*, *OsARF17* and *OsARF25*, to control rice tiller angle by fine-tuning auxin asymmetric distribution in shoots (Li et al. 2020).

The tiller angle genes that control the two extreme types of rice, namely, dispersed or erect, such as *PROG1*, *LPA1*, *Spk(t)* and *TIG1*, have little practical breeding value. By contrast, the major QTL *TAC1* increases the tillering angle of rice lines appropriately (Yu et al. 2007; Jiang et al. 2012), and thus plays an important role in breeding. *TAC1* was isolated via map-based cloning by using the loose plant architecture indica rice variety ‘IR24’ as the genetic background and the compact infiltrating line ‘IL55’ that infiltrates a few chromosome segments of the japonica rice variety ‘asominori’ as the material. (Yu et al. 2007; Jiang et al. 2012). A comparison of the gene sequences of *TAC1* from IR24 and IL55 revealed an important SNP variation from A to G at the 3’ splicing site of the fourth intron. The genome sequence changed from “AGGA” in *TAC1* to “GGGA” in *tac1*. As a result, the intron could not be cut normally during mRNA processing, and poly (A) was added ahead of time, resulting in the cDNA of *TAC1* and *tac1* to encode the same amino acid sequence but with completely different 3’ untranslated regions. *TAC1* with “AGGA” is a loose plant architecture, whereas *tac1* with “GGGA” is a compact plant architecture (Yu et al. 2007).

The penta-primer amplification refractory mutation system (PARMS) is an SNP genotyping technology based on the tetra-primer amplification refractory mutation system (Ye et al. 2001). In this technique, five primers (a pair of universal fluorescent primers, a pair of allele-specific primers, and a pair of reverse shared primers) are used to amplify SNP or short indel

loci with allele specificity. Genotyping is then conducted by fluorescence scanning (Zhang et al. 2019a, b; Lu et al. 2020).

Molecular marker-assisted selection (MAS) is a highly efficient breeding method because it offers a rapid and precise selection of the desired genes. These markers are based on PCR and gel electrophoresis. Herein, on the basis of SNP variations in the *TAC1* gene from A to G at the 3' splicing site of the fourth intron, we developed the functional molecular marker PM-TAC1 via PARMS and utilized it in MAS breeding to improve the plant architecture of rice.

Materials and methods

Experimental materials

Ninety-three rice experimental materials were planted in the experimental field of Guangxi Academy of Agricultural Sciences. These materials included 31 maintainer lines of CMS lines, 6 temperature-/photoperiod-sensitive genic male sterile (TGMS) lines, 24 restorer lines of CMS lines, and 32 varieties (Table S1). Plant architecture was investigated at the heading stage.

Primer design of the PARMS markers of *TAC1*

BLAST was used for sequence alignment, and the online primer design tool Primer3Plus (<https://primer3plus.com>) was utilized to design the primer sets for all markers. The PARMS molecular marker PM-TAC1 was designed based on the SNP variations from A to G at the 3' splicing site of *TAC1* intron 4. These variations cause the plant architecture to change from loose to compact. The marker consists of three specific primers of *TAC1*. Two different bases of the SNP variations were introduced into the designed forward primers. In addition, two common primers that are consistent with the underlined parts of the two forward primers had different fluorescent marks at the tail. The underlined sequence was annealed to the oligonucleotides labeled with the FAM/HEX fluorophore (Table 1.). Using this technique, three primers were designed according to the sequence of *TAC1*, and two universal fluorescent primers were added to the PCR reaction system for amplification (Fig. S1). According to the SNP variations, the allele sequence

of *TAC1* was amplified to obtain the FAM fluorescence signal value by matching with the forward primer TAC1-Fg and the HEX fluorescence signal value and by matching with the forward primer TAC1-Fa. When the rice sample was heterozygous at this site, two forward primers were simultaneously amplified.

Sample collection and DNA extraction

The leaves of the 93 rice materials were used for DNA extraction via the CTAB method (Murray and Thompson 1980). The extracted DNA was dissolved in 1 × TE buffer for subsequent PCR amplification.

PCR amplification and genotyping assays

The *TAC1* gene of these rice varieties was amplified via PCR. The master mix for the PARMS markers was purchased from Gentides Biotech Co., Ltd. (Wuhan, China). The PCR reaction system (10 μL) included 5 μL 2 × PARMS master mix (containing two common fluorescent primers, PCR buffer, dNTP and Taq enzymes, and internal standard ROX), 0.15 μL 10 mM TAC1-Fg primer, 0.15 μL 10 mM TAC1-Fa primer, 0.4 μL 10 mM TAC1-R primer, 1 μL 50 ng DNA template, and 3.3 μL of ddH₂O. Touchdown PCR was performed as follows: 95 °C for 5 min, followed by 10 cycles of 95 °C for 20 s and 65 °C (–0.8 °C per cycle) for 1 min, and 32 cycles of 95 °C for 20 s and 57 °C for 1 min and 72 °C for 7 min.

The PCR products were analyzed on a Multiscan Spectrum (Tecan Infinite 200-TWT, Grodig, Austria), which can detect the FAM, HEX, and ROX fluorescent signals. The fluorescence signal value was then filed by the SNP decoder software (<http://www.snpway.com/snpdecoder01/>) to obtain each sample amplification of the FAM and HEX fluorescence signal strengths. Each point with the graphical output signal was acquired according to the fluorescence signal intensity. Automated genotyping was performed to generate genotype results. According to the analysis of fluorescence signal values, the fluorescence signal of FAM (blue) obtained via fluorescence scanning was the *tac1* allele (G) type material of compact plant architecture, whereas the fluorescence signal of HEX (green) was the *TAC1* allele (A) type material of loose plant architecture. The fluorescence scanning result was red, indicating that the sample was heterozygous.

Table 1 PARMs primer sets for genotyping

PARMS primer	Sequences	Remarks
TAC1-Fg	<u>GAAGGTGACCAAGTTCATGCTATTCCTTTCACCTTTTGCg</u>	Forward primer
TAC1-Fa	<u>GAAGGTGCGGAGTCAACGGATTATTCCTTTCACCTTTTGCa</u>	Forward primer
TAC1-R	<u>TGCATGGATGTTTCTCTTGAGC</u>	Reverse primers
#1	<u>GAAGGTGACCAAGTTCATGCT-FAM</u>	Common primer labeled with the FAM fluorophore
#2	<u>GAAGGTGCGGAGTCAACGGATT-HEX</u>	Common primer labeled with the HEX fluorophore

Sequence verification of the SNP variations

The online primer design tool Primer3Plus (<https://primer3plus.com>) was utilized to design the primer sets, the forward primer TAC1F1 (5'-GGCTGGAGCTATGATGGACC-3') and the reverse primer TAC1R1 (5'-ATCACTGGCGAGCGAA-TAGG-3'). PCR products were Sanger-sequenced and the CLUSTAL W soft (<https://myhits.sib.swiss/cgi-bin/clustalw>) was used for sequence alignment. The PCR reaction system (10 μ L) included 1 μ L 10 \times PCR mix (containing PCR buffer, dNTP and Taq enzymes), 0.15 μ L 10 mM TAC1F1 primer, 0.15 μ L 10 mM TAC1R1 primer, 1 μ L 50 ng DNA template, and 7.7 μ L of ddH₂O. PCR was performed as follows: 95 $^{\circ}$ C for 5 min, followed by 30 cycles of 95 $^{\circ}$ C for 30 s and 57 $^{\circ}$ C for 30 s and 72 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 5 min.

Utilization of *tacl* in breeding

The L204 of compact plant architecture was hybridized with the R998 of loose plant architecture. R998 was backcrossed as a recurrent parent with F₁ generation, and the BC₁F₁ population was obtained. From BC₁F₁ generation, the PM-TAC1 fluorescent molecular marker was used to track and detect each generation. The lines of the *tacl* allele (G) type and compact plant architecture were selected to backcross with R998, the BC₂F₁ population was obtained. After two generations of backcross and one generation of selfcross, the BC₂F₂ population was obtained. The breeding material of the *tacl* allele (G) type and compact plant architecture was selected. Finally,

BC₂F₃ was selected as the breeding material of stable genetic compact plant architecture.

Results

Development of *TAC1* PARMs marker

Ninety-three rice materials were genotyped using the *TAC1* functional molecular fluorescence marker PM-TAC1. PM-TAC1 matched well with the corresponding SNP to generate a fluorescence signal and achieve the purpose of genotyping (Table S2). Results showed that 22 materials were *tacl* genotypes, 70 materials were *TAC1* genotypes, and 1 materials was a *TAC1//tacl* hybrid genotype (Fig. 1).

The detected FAM fluorescence signal (blue dot) is a compact plant architecture rice variety with *tacl* genotype. The detected HEX fluorescence signal

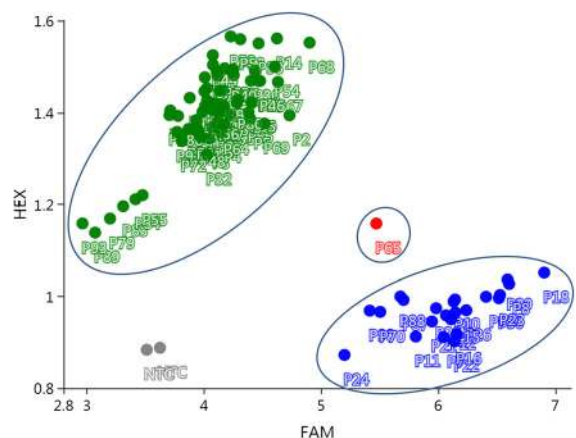


Fig. 1 Genotyping of 93 rice varieties using the molecular marker PM-TAC1

(green dot) is a loose plant architecture rice variety with *TAC1* genotype. The red dot is a heterozygous state. The gray point is negative CK.

Plant architecture identification of rice varieties

The plant architecture was observed and investigated at the heading stage. A compact plant architecture denotes that each tiller is compact, and this type of architecture is found in rice materials LiangfengB, TaifengB, and Gui316 (Fig. 2). A loose plant architecture has a certain angle for each tiller, and this characteristic is observed in Shen08S, GR076, and RP07-1 (Fig. 3). The results of sequence verification by Sanger-sequencing confirmed the SNP variation are correctly corresponding to phenotypes in our material (Fig. 4). LiangfengB, TaifengB, and Gui316, which is compact plant architecture, was the *tac1* allele (G) type; and Shen08S, GR076, and RP07-1, which is loose plant architecture, was the *TAC1* allele (A) type. The plant architectures of 93 rice materials were investigated (Table 2). Results showed that 23 rice materials had a compact plant architecture, whereas 70 rice materials had a loose plant architecture.

Polymorphism of PM-TAC1 among rice material

The PM-TAC1 fluorescent molecular markers developed herein were used to detect the 93 rice varieties. The amplification results of each rice material were classified according to fluorescent signals (Table S2, Fig. 1). The genotypes of each rice material were classified based on scatter diagram (Table 2). The scatter diagram intuitively classified the corresponding genotype of each material. The green dot represents the sample of HEX fluorescence signal, a loose *TAC1* genotype; the blue dot denotes the sample of FAM fluorescence signal, a compact *tac1* genotype. A comparison of the statistical data revealed that the phenotypic differentiation of the marker PM-TAC1 was consistent with the field phenotypes statistics (Table 2), indicating that the fluorescent molecular marker PM-TAC1 quickly and accurately detected the SNP variations.

Utilization of PM-TAC1 in rice breeding

Backcross breeding was conducted using L204 (compact plant architecture) and R998 (loose plant architecture) to breed the compact plant architecture material (Fig. 5A). PM-TAC1 was used to detect 378 randomly selected individual plants in the BC₂F₃

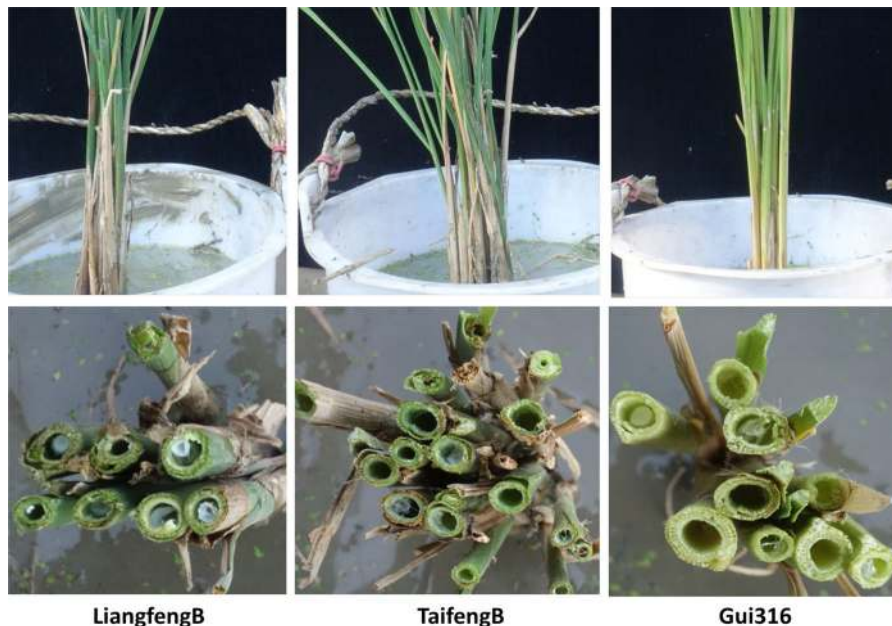
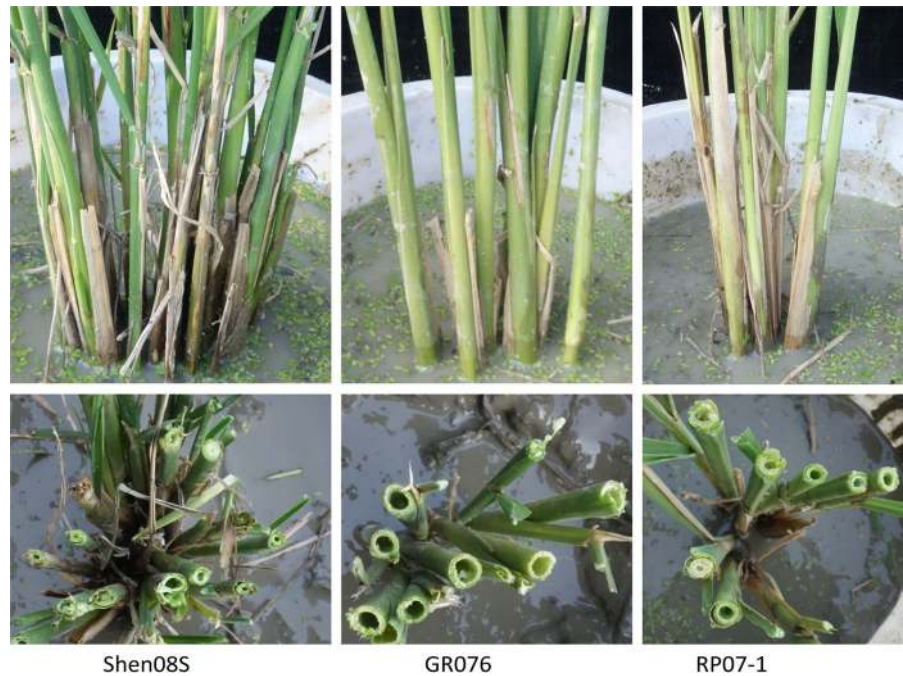


Fig. 2 Rice varieties with a compact plant architecture

Fig. 3 Rice varieties with a loose plant architecture



population (Table S3, Fig. 5B). In the scatter diagram, the corresponding genotype of each material is accurately classified to the loose *TAC1* genotype (the green dot), the compact *tac1* genotype (the blue dot) and the *TAC1/tac1* heterozygous genotype (the red dot). The genotyping is accurately to select the target plant architecture (Fig. 5C, Fig. 5D). The results demonstrated that PM-TAC1 can be effectively used in plant architecture molecular breeding of rice.

Discussion

PM-TAC1 is suitable for plant architecture breeding of rice

The realization of ideal plant architecture is an ideal state of design breeding (Zhang 2007). A large tiller angle increases leaf shade and reduces photosynthesis efficiency. By contrast, a small tiller angle makes the plant structure more effective. A reasonable tillering angle is a key factor for cultivating the ideal plant architecture to achieve high-yield breeding. High-yield rice populations require a loose and moderate plant architecture. An appropriate tiller angle is also conducive to enhancing rice photosynthesis, improving resistance to lodging and sheath blight disease

(Sun et al. 2019), and improving plant yield and rice harvest index. Moreover, tiller angle directly determines the planting density of rice and has a considerable influence on field population yield.

The tiller angle gene, which controls the extremely dispersed or erect types of rice, has little practical breeding value. By contrast, QTLs with certain effects have great value in plant architecture improvement. The major QTL *TAC1* can increase the appropriate tillering angle of rice lines, a characteristic that is conducive to reducing the harm of sheath blight and has no adverse effects on other agronomic traits (Yu et al. 2007; Jiang et al. 2012). The results of the present study demonstrated that *TAC1* can lead to a reasonable tillering angle and can be used for plant architecture improvement of indica and japonica rice varieties. SNP variations from A to G at the 3' junction of intron 4 of *TAC1* changed the plant architecture of rice from loose to compact (Yu et al. 2007). SNP variations in this locus can be used as a functional molecular marker in rice breeding. Based on these SNP variations, we successfully developed the PARMS marker PM-TAC1 of *TAC1*. This marker has achieved accurate genotyping in 93 rice materials (Fig. 1). The genotypes were consistent with the phenotypic results, and the marker was effectively applied to the plant architecture breeding of indica hybrid rice. The loose

Shen08S	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
RP07-1	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
R998	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
GR076	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
L204	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
LiangfengB	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
TaifengB	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC
Gui316	AAAATTTCTGGCCGACAATACGTCTTGATCAGGAACTGACTAAAGAAATTCCTTTCACC

Shen08S	TTTTGCAGGATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
RP07-1	TTTTGCAGGATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
R998	TTTTGCAGGATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
GR076	TTTTGCAGGATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
L204	TTTTGC GG GATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
LiangfengB	TTTTGC GG GATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
TaifengB	TTTTGC GG GATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAGAAACATC
Gui316	TTTTGC GG GATTTGTTGGATCCATCCTCACGCTTCGGATTCCTTGCTCAAGAAAACATC
***** *****	
Shen08S	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGCTGTTGTT
RP07-1	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGCTGTTGTT
R998	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGCTGTTGTT
GR076	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGCTGTTGTT
L204	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGGTGCTGTTGTT
LiangfengB	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGGTGCTGTTGTT
TaifengB	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGGTGCTGTTGTT
Gui316	CATGCATATCGTCGACAGCGTTCGTTTCAGTCTCTTCCTTTTGGTTGTTGGGGCTGTTGTT
***** *****	

Fig. 4 Sequence verification of the SNP variations. The SNP variation in the box is exploited by the PM-TAC1 markers

plant architecture of the rice variety L204 was improved and an L204 material with compact plant architecture was obtained via molecular marker assisted breeding (Fig. 5).

PM-TAC1 based on PARMs is a highly efficient marker for MAS breeding

The development of functional molecular markers of target trait genes is an important aspect of directional

improvement breeding(Xu and Crouch 2008). As the most potential marker of the third-generation molecular markers, SNP markers have developed rapidly and been widely used in genetic analysis. Gel electrophoresis-based molecular markers such as single-strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DDGE), cleaved amplified polymorphic sequence (CAPS), and allele-specific PCR (AS-PCR) is limited because electrophoresis is laborious, time-consuming, requires

Table 2 PM-TAC1 marker genotypes and plant architecture phenotypes of 93 rice varieties

No	Variety	Genotype	Phenotype	No	Variety	Genotype	Phenotype
P1	MeiB	<i>TAC1</i>	Loose	P48	Lixiang2	<i>TAC1</i>	Loose
P2	82B	<i>TAC1</i>	Loose	P49	R308	<i>TAC1</i>	Loose
P3	112B	<i>TAC1</i>	Loose	P50	R318	<i>TAC1</i>	Loose
P4	186B	<i>TAC1</i>	Loose	P51	R319	<i>TAC1</i>	Loose
P5	9311B	<i>TAC1</i>	Loose	P52	R402	<i>TAC1</i>	Loose
P6	II-32B	<i>tac1</i>	Compact	P53	R463	<i>TAC1</i>	Loose
P7	II-33B(an)	<i>tac1</i>	Compact	P54	R527	<i>TAC1</i>	Loose
P8	BoB	<i>tac1</i>	Compact	P55	IRBB5	<i>TAC1</i>	Loose
P9	BoIIB	<i>tac1</i>	Compact	P56	R5867	<i>TAC1</i>	Loose
P10	FuyiB	<i>tac1</i>	Compact	P57	R7571	<i>TAC1</i>	Loose
P11	Gang46B	<i>tac1</i>	Compact	P58	R774	<i>TAC1</i>	Loose
P12	GufengB	<i>tac1</i>	Compact	P59	R789	<i>TAC1</i>	Loose
P13	Guang8B	<i>TAC1</i>	Loose	P60	R795	<i>TAC1</i>	Loose
P14	HenfengB	<i>TAC1</i>	Loose	P61	R9516	<i>TAC1</i>	Loose
P15	Hua2048B	<i>tac1</i>	Compact	P62	R087	<i>TAC1</i>	Loose
P16	Jin23B	<i>tac1</i>	Compact	P63	R087	<i>TAC1</i>	Loose
P17	LiangfengB	<i>tac1</i>	Compact	P64	RP07-1	<i>TAC1</i>	Loose
P18	ShanB	<i>tac1</i>	Compact	P65	Bing4114	<i>TAC1/tac1</i>	Loose
P19	Shen95B	<i>TAC1</i>	Loose	P66	Ce253	<i>TAC1</i>	Loose
P20	TaifengB	<i>tac1</i>	Compact	P67	Duoxi1	<i>TAC1</i>	Loose
P21	TeB	<i>tac1</i>	Compact	P68	R998	<i>TAC1</i>	Loose
P22	TianfengB	<i>tac1</i>	Compact	P69	Gui3158	<i>TAC1</i>	Loose
P23	WufengB	<i>tac1</i>	Compact	P70	Gui316	<i>tac1</i>	Compact
P24	BoIIB	<i>tac1</i>	Compact	P71	Gui501	<i>TAC1</i>	Loose
P25	YexiangB	<i>TAC1</i>	Loose	P72	Gui826	<i>TAC1</i>	Loose
P26	YixiangB	<i>tac1</i>	Compact	P73	Gui965	<i>TAC1</i>	Loose
P27	Yu19B	<i>TAC1</i>	Loose	P74	Gui99	<i>TAC1</i>	Loose
P28	Yue4B	<i>TAC1</i>	Loose	P75	GuiR100	<i>TAC1</i>	Loose
P29	ZhenfengB	<i>tac1</i>	Compact	P76	Huazhan	<i>TAC1</i>	Loose
P30	ZhongjiuB	<i>tac1</i>	Compact	P77	Minghui63	<i>TAC1</i>	Loose
P31	ZhongzheB	<i>TAC1</i>	Loose	P78	Yahui2155	<i>TAC1</i>	Loose
P32	LongS	<i>TAC1</i>	Loose	P79	Guike1	<i>TAC1</i>	Loose
P33	JingS	<i>TAC1</i>	Loose	P80	Guiyu9	<i>TAC1</i>	Loose
P34	Y58S	<i>TAC1</i>	Loose	P81	Wushansimiao	<i>TAC1</i>	Loose
P35	Shen08S	<i>TAC1</i>	Loose	P82	2428	<i>TAC1</i>	Loose
P36	He620S	<i>TAC1</i>	Loose	P83	Nante	<i>TAC1</i>	Loose
P37	1892S	<i>TAC1</i>	Loose	P84	Zhenguiyai	<i>TAC1</i>	Loose
P38	9311	<i>TAC1</i>	Loose	P85	Gumei4	<i>TAC1</i>	Loose
P39	838R(L)	<i>TAC1</i>	Loose	P86	BP60	<i>TAC1</i>	Loose
P40	Fuhui838	<i>TAC1</i>	Loose	P87	Jinweiai	<i>tac1</i>	Compact
P41	GR076	<i>TAC1</i>	Loose	P88	Kongyu131	<i>tac1</i>	Compact
P42	GS2	<i>TAC1</i>	Loose	P89	Dayu23	<i>TAC1</i>	Loose
P43	IRBB7	<i>TAC1</i>	Loose	P90	L204	<i>tac1</i>	Compact
P44	R128	<i>TAC1</i>	Loose	P91	LCL	<i>TAC1</i>	Loose
P45	R138	<i>TAC1</i>	Loose	P92	Hongnanzao	<i>TAC1</i>	Loose

Table 2 continued

No	Variety	Genotype	Phenotype	No	Variety	Genotype	Phenotype
P46	R1439	<i>TAC1</i>	Loose	P93	Honggui6	<i>TAC1</i>	Loose
P47	R273	<i>TAC1</i>	Loose				

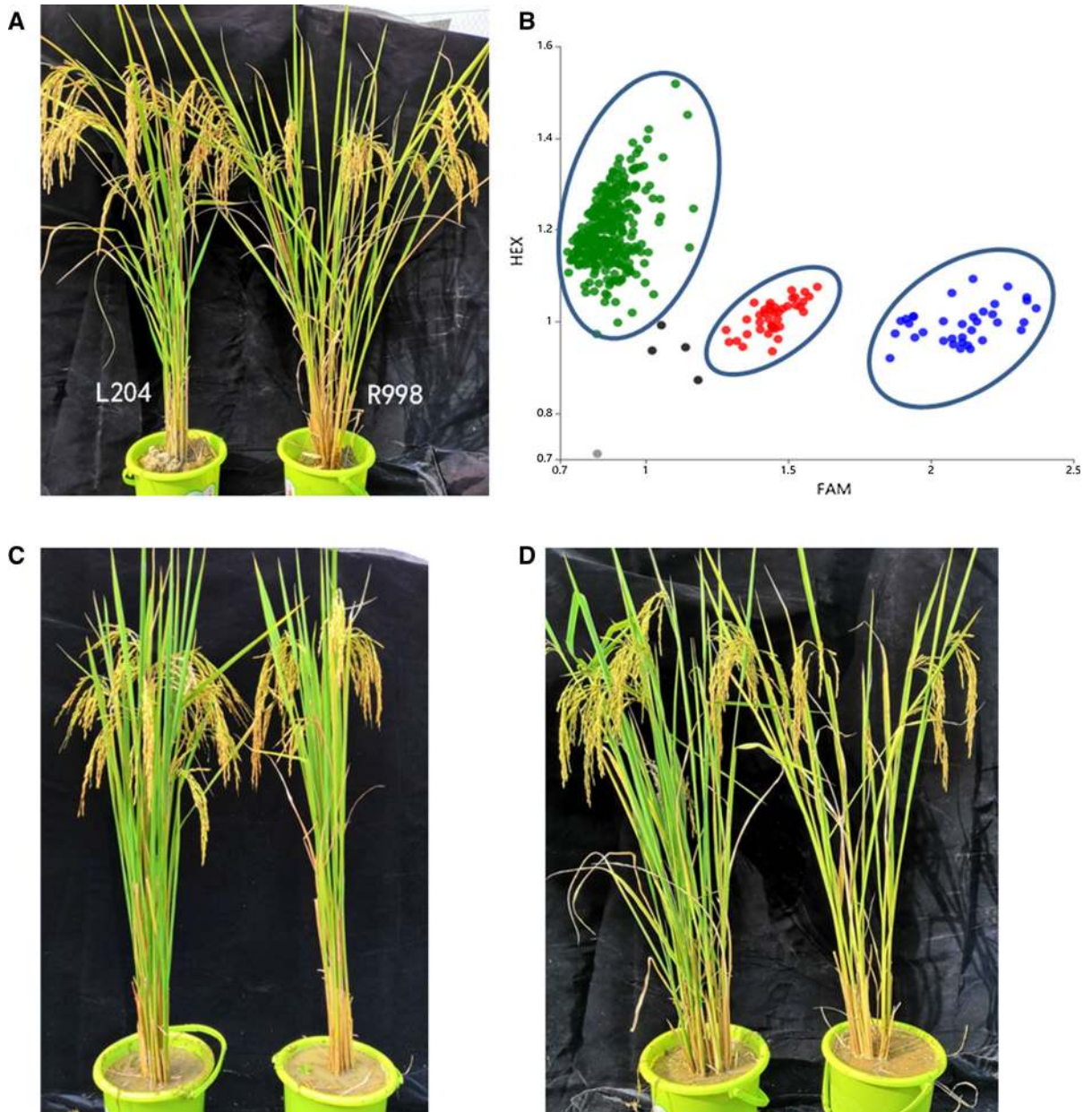


Fig. 5 PM-*TAC1* genotypes and plant architecture phenotypes of the BC_2F_3 breeding population. **A.** plant architecture of the two parents L204 and R998. **B.** PM-*TAC1* genotyping of 378 randomly selected individual plants in the BC_2F_3 population. **C.**

compact plant architecture of *tac1* genotype in the BC_2F_3 population. **D.** loose plant architecture of *TAC1* genotype in the BC_2F_3 population.

toxic reagents, and complicated. The gel-free SNP marker system such as direct sequencing, DNA chips, denaturing high-performance liquid chromatography (DHPLC), mass spectrometry detection technology, and high resolution melting (HRM) is high throughput and automated detection methods, which is too expensive to afford for most breeders (Thomson 2014; Batley 2015). Compared with these methods, PARMS based on fluorescence detection has the advantages of simple operation, short operation period, and low cost. Moreover, this technique can detect SNPs and short indel loci (Zhang et al. 2019a, b; Lu et al. 2020). In PARMS, two different universal primers are added to the end of the primers. With the participation of universal fluorescent primers in the amplification system, different genotypes are amplified to obtain different fluorescent groups. The detection samples are amplified by PCR only once without electrophoresis detection, and the amplification data are directly obtained from the original plate by a fluorescent scanner. After software analysis, the amplification data and the *TAC1* genotypic data are obtained. Our results demonstrated that PM-TAC1 by PARMS is a highly efficient marker for MAS breeding (Fig. 5).

Conclusion

In this study, the *TAC1* fluorescence marker PM-TAC1 was developed via the PARMS technology. This fluorescence marker can be detected at any stage of rice growth. It is simple, fast, inexpensive, and can obtain accurate results. Moreover, this marker can realize closed tube operation. This functional marker of the rice tiller angle gene *TAC1* was successfully developed and effectively used in breeding new plant architecture breeding of rice.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiments comply with the current laws of the country in which they were performed.

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