Genetic analysis and gene mapping of a rice few-tillering mutant in early backcross populations (*Oryza sativa* L.)

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Abstract A rice mutant, *G069*, characteristic of few tiller numbers, was found in anther culture progeny from the F_1 hybrid between an *indica-japonica* cross, *Gui630×02428*. The mutant has another two major features: delayed tillering development and yellowing apex and margin on the mature leaves. As a donor parent, *G069* was further backcrossed with the recurrent parent, *02428*, for two turns to develop a BC_2F_2 population. Genetic analysis in the BC_2F_2 population showed that the traits of few-tillering and yellowing apex and margin on the mature leaves were controlled by one recessive gene. A pool of equally mixed genomic DNA, from few-tillering individual plants in BC_2F_2, was constructed to screen polymorphism with simple sequence repeat (SSR) markers in comparison with the *02428* genome. One SSR marker and three restriction fragment length polymorphism (RFLP) markers were found possibly linked with the recessive gene. By using these markers, the gene of few-tillering was mapped on chromosome 2 between RFLP marker C424 and S13984 with a genetic distance of 2.4 cM and 0.6 cM, respectively. The gene is designated *ft1*.

Keywords: ***Oryza sativa*** **L., few-tillering, gene mapping.**

Tillering is a major characteristic of rice. Tiller number is usually thought to be controlled by quantitative trait loci (QTLs). Some researches showed that tiller number in cereal was decided by dominant or epistatic gene, but more studies revealed that it is mainly controlled by additive effect of the genes. Xu et al.[1] studied rice tillering at different developmental stages and found that high tillering ability was inherited as a partial dominant character and regulated by two or more partially dominant genes. It appeared to them that an identical polygenic system was responsible for the genetic control of tillering at different growth stages as well as the final productive tiller number though, with the growth of a rice plant, relative contributions of effective nonadditive genes action and environmental factors to the variation decreased while those of additive gene action increased. Wu[2] also found five time-related loci on chromosome 1, 3 and 5, respectively. Wu[3] mapped two QTLs controlling rice tillering on chromosome 4 and 12 using RFLP markers. It seems that few-tillering is not controlled by one single gene.

The number of productive tillers per rice plant is an important development trait that is involved in the formation of grain yield in rice. Recently developed super-rice has a standard mould: few tillers and high frequency of productive tiller and heavy weight of a single panicle[4]. Here we report a new rice mutant, *G069*, with the maximum tiller number less than 4, which was found in the doubled haploid progeny from anther culturing the F_1 hybrid of an *indica-japonica* cross, *Gui630* × *02428*. It is noted that the mutant has two other features: delayed tillering and yellowing apex and margin on mature leaves. The leaf apex and margin become yellow when one subsequent new leaf extends fully, and the process of yellowing continues until the topmost leaf turns yellow but it never extends to a whole mature leaf. In this study, the characters are proved to be controlled by one recessive gene and the relevant gene is mapped on chromosome 2 in a backcross population of early generation with molecular marker.

1 Materials and methods

1.1 Identification of few-tillering mutant

In order to find whether few-tiller trait of the rice mutant depends on cultivating conditions, the rice plants of *G069* were tested in the fields at two different planting densities, 26 cm×16 cm and 53 cm×16 cm, or with two different amounts of fertilizer (nitrogen), 255 kg and 510 kg per hectare as top dressing. The *02428* plants were grown as control. Investigations of tiller development and other phenotypes were made at 7-day intervals since 10 days after transplantation.

1.2 Construction of a few-tillering segregation population

The donor parent G069 crossed with the recurrent parent *02428*. The few-tillering rice plant in F_2 population was selected and backcrossed with *02428*, and then the BC_2F_2 population was developed from BC_2F_1 for mapping the relevant gene of few-tillering.

1.3 Construction of few-tillering gene pool and molecular marker analysis

Fresh leaves from 40 few-tillering plants in the BC_2F_2 population were collected and mixed in equal amount for extraction of total DNA. The extracted DNA was used as few-tillering gene pool to compare with genomes of *02428* for screening polymorphism using SSR markers. The primers of SSR markers were synthesized according to the primer sequences reported by Chen et al.[5] SSR analysis was conducted as described by Panaud et al.[6] The reaction product was segregated in 3% agarose gel and stained by ethidium bromide. Extraction of rice DNA and RFLP analysis were carried out according to McCouth et al.[7] Eight kinds of restriction enzyme: *Eco*R I, *Bam*H I, *Hin*d III, *Sca* I, *Eco*R V, *Bgl* II, *Dra* I and *Xba* I were used for DNA digestion. The RFLP probes were provided by Rice Genome Project of Japan[8].

1.4 Linkage analysis

Linkage analysis of polymorphic SSR and RFLP markers and few-tillering locus was performed in the BC_2F_2 population. The segregation data was processed and the location of the

few-tillering locus was determined using the software MAPMAKER 3.0[9].

2 Results and analysis

2.1 Morphological characters of *G069*

Field investigation of rice plants of *G069* through generations and in different cultivating conditions showed that the few-tillering plants were always accompanied by yellowing leaf apex and margin. Compared with normal rice plants, the tillering speed of few-tillering plants was slower and the maximum tiller number was smaller. Tiller development of few-tillering plants was delayed for approximately 6 days in comparison with the normal plants (table 1). The tillering speed of *G069* was 0.16—0.20 tiller per day (*TPD*) while the speed of the normal plant was 0.55—1.27 *TPD*, which may partly account for few tillers of *G069*. The average tiller number per plant in the field with a high level of fertilizer was 3.83 tillers while in the field with a general level of fertilizer the number was 3.3 tillers. The tiller number per plant in the condition with the general fertilizer level and at the normal plant density was 2.42 tillers, while the number was 2.82 at the high fertilizer level or at the small plant density. Therefore, the effects of fertilizer level and planting density on the few-tillering plants of BC_2F_1 generation was rather small, indicating that the few-tillering trait is mainly controlled by heredity and less influenced by cultivating conditions.

Table 1 Tiller development of generations

Generation		Parent *02428*	Parent Gui630	Donor parent *G069*	BC_2F_1	BC_2F_2	BC_2F_2
		normal	normal	mutant	normal	normal	mutant
Period of investigation	May 27	2.28[a)]	4.26	0.63	2.56	1.09	0
	June 3	7.15	12.85	1.88	2.67	4.96	1.12
	June 10	11.05	19.77	2.93	11.56	7.84	2.06
	June 17	11.82	22.03	3.3	11.67	10.04	2.11

May 27：10 days after transplantation of the seedling. a) tiller number.

2.2 Genetic analysis of few-tillering

Genetic analysis of few-tillering was conducted in early backcross populations derived from donor parent *G069*×recurrent parent *02428*. The tiller number of the F_1 plants was as normal as that of *02428*, and the other phenotypes of BC_1F_1 and BC_2F_1 generations were normal, too. BC_2F_2 population was obtained through selfing of BC_2F_1 plants. The tillering trait in BC_2F_2 population (584 plants) was segregated with a double-peak distribution (fig. 1), and the two peak values were near the tiller numbers of *02428* and *G069*. The few-tillering group (0—3 tillers) and the high-tillering group (4—26 tillers) were classified according to the minimum value between the two peaks in the distribution profile. It was noted that all few-tiller plants (137 plants) in BC_2F_2 had yellowing apex and margin on the mature leaves while the high-tillering plants (447) had normal leaves. The proportion of the normal and mutant plant was equal to the segregation ratio of 3：1 (X_c^2=0.6598< $X_{0.05,1}^2$=3.84), indicating that the few-tillering trait and the yellowing phe-

nomenon were controlled by a recessive gene.

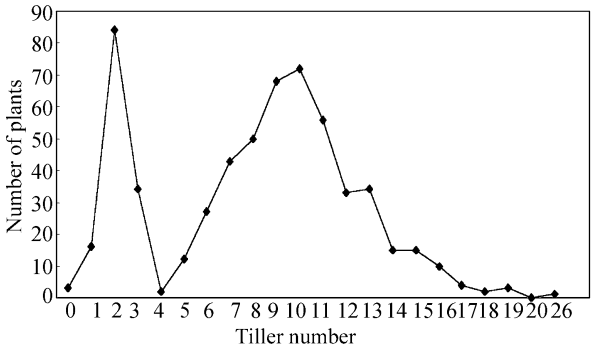

Fig. 1. Frequency of tiller distribution in BC_2F_2.

2.3 Polymorphism screening of the few-tillering gene pool and *02428*

Equal amounts of genomic DNAs from 40 few-tillering individual plants in the BC_2F_2 population were mixed together to construct a few-tillering gene pool (*FTGP*). A total of 146 SSR markers were used to screen polymorphisms between *FTGP* and *02428*. Only the marker *RM263* on chromosome 2 could reveal the polymorphism. Then 10 RFLP markers around this SSR were used to further screen polymorphisms between *FTGP* and *02428*, and found that three of them (*C424*, *S13984*, *R480A*) were polymorphic (fig. 2). So these molecular markers are possibly linked to the gene for few-tillering.

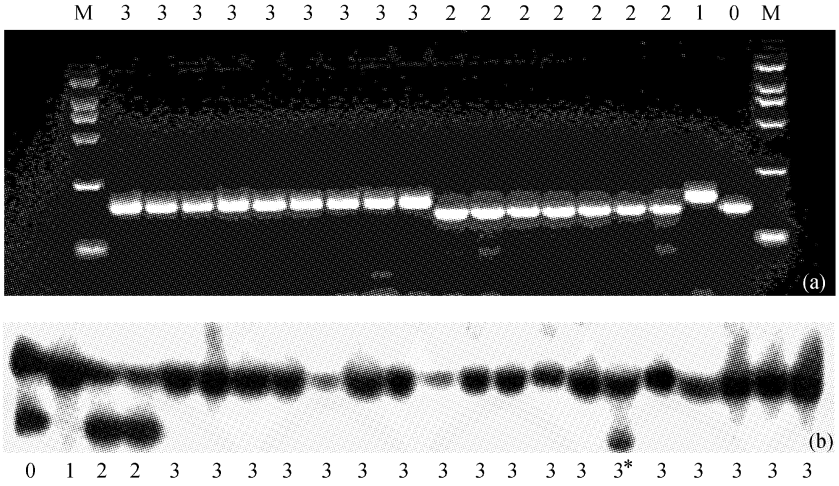

Fig. 2. Linkage analysis of few-tillering trait and molecular marker. (a) Polymorphism of the SSR marker, RM263, between *02428* and the few-tillering gene pool (*FTGP*) and its segregation among F_2 population. (b) Polymorphism of the probe C424/*Eco*R V between *02428* and the few-tillering gene pool (*FTGP*) and its segregation among F_2 population. M, Marker; 0, recurrent parent *02428*; 1, the few-tillering gene pool (*FTGP*); 2, the higher tiller plant of F2; 3, the few tiller plant of F_2.

2.4 Genetic mapping of the gene for few-tillering

The four positive markers, one SSR and three RFLPs, discovered in screening *FTGP* and *02428*, were used to analyze 137 individual plants in the BC_2F_2 population. All of them showed co-segregation with the few-tillering trait with a few recombinants. Only one and five recombinants were found between the few-tillering locus and two markers, S13984 and C424, respectively. A partial linkage map around the few-tillering locus on chromosome 2 was constructed according to the segregation data (fig. 3). The recessive gene of few-tillering was mapped between the marker C424 and S13984 with a genetic distance of 2.4 cM and 0.6 cM, respectively.

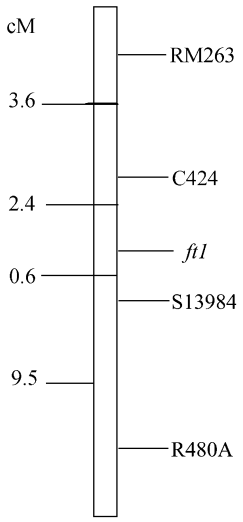

Fig. 3. The linkage map of few-tillering gene (*ft1*) on Chr. 2.

3 Discussion

Backcross can decrease the proportion of a donor parent genome in its progenies and the genomes of the backcross progenies will gradually approach the genome of recurrent parent. In theory, 75% of the few-tillering plant's genome would come from *02428* after *G069* backcrossed with *02428* for two turns. Nevertheless, the mutant *G069* in itself was from the anther culture progeny of *Gui630*×*02428* and it contained 55.6% of *02428* genome in terms of molecular marker constitution (Li. et al., personal communication, 1994). So the genome proportion of 02428 in the few-tillering plants of BC_2F_2 reached 90% on average. Therefore, in *FTGP* all marker sites except few-tillering locus and its two linked regions had a proportion of 90% coming from *02428* genome if the pool was large enough. Because of the competition of genomic templates between allelic sites of *FTGP* in a polymerase chain reaction (PCR), the marker sites from *02428* genome and the few-tillering locus from *gui630* had the superiority in template numbers to be amplified to much greater extent a counterpart site, from which the amounts of amplified products might be too small to be detected. So *FTGP* could be considered as a near isogenic line of *02428* when the polymorphisms between *FTGP* and the recurrent parent *02428* was screened by using SSR marker, and the resultant polymorphic SSR polymorphism could be linked to the target gene. In the present study, the constructed *FTGP* was made up of 40 individual plants in the BC_2F_2 population and a positive SSR marker RM263 on chromosome 2 was found by screening polymorphisms between this pool and *02428*, with a total number of 146 SSRs. Linkage analysis in the BC_2F_2 population proved that the marker RM263 was linked with the few-tillering locus. Further RFLP analysis using the markers near RM263 successfully located the gene for few-tillering on chromosome 2 between RFLP marker C424 and S13984. Therefore, the strategy of construction of a genomic mixture pool from individual plants with one recessive gene in an early backcross population is a very efficient way to screen out the molecular markers, both in PCR based markers and RFLP markers, possibly linked to the target gene. This can be applied to similar studies in other plants.

By now five genes of reduced culm number (RCN) have been registered in rice, of which the genes of *RCN-1* and *RCN-2* were all located on chromosome 6, and *RCN*-5 was on chromosome 4[10]. But there has been no report on any mutants like G069. In addition to reduced culm number, the mutant G069 has two other major features, delayed tillering development and yellowing apex and margin on the mature leaves, cosegregating with the few-tiller trait in backcross populations. Therefore, the few-tiller trait and the yellowing of leaf apex should be the two effects of the same

single mutant gene. Since no RCN gene has been mapped on rice chromosome 2, this few-tillering gene must be a new one, which is designated *ft1*.

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