Genetic analysis and mapping of gene *fzp*(*t*) controlling spikelet differentiation in rice

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Abstract A mutant of spikelet differentiation in rice called *frizzle panicle* (*fzp*) was discovered in the progeny of a cross between *Oryza sativa* ssp. *indica* cv. V20B and cv. Hua1B. The mutant exhibits normal plant morphology but has apparently fewer tillers. The most striking change in *fzp* is that its spikelet differentiation is completely blocked, with unlimited subsequent rachis branches generated from the positions where spikelets normally develop in wild-type plants. Genetic analysis suggests that *fzp* is controlled by a single recessive gene, which is temporarily named *fzp*(*t*). Based on its mutant phenotype, *fzp*(*t*) represents a key gene controlling spikelet differentiation. Some F_2 mutant plants derived from various genetic background appeared as the "middle type", suggesting that the action of *fzp*(*t*) is influenced by the presence of redundant, modifier or interactive genes. By using simple sequence repeat (SSR) markers and bulked segregant analysis (BSA) method, *fzp*(*t*) gene was mapped in the terminal region of the long arm of chromosome 7, with RM172 and RM248 on one side, 3.2 cM and 6.4 cM from *fzp*(*t*), and RM18 and RM234 on the other side, 23.1 cM and 26.3 cM from *fzp*(*t*), respectively. These results will facilitate the positional cloning and function studies of the gene.

Keywords: rice (*O. sativa* L.), spikelet differentiation, *fzp*, genetics, gene mapping.

Monocots and dicots have diverged for 120 million years. The floral morpha of cereals is unique and much different from that of dicot plants. Nevertheless, it has been found that most genes controlling flower development share a conserved sequence called MADS-box[1]. Therefore, it is likely that monocots and dicots could have similar basic characteristics of flower development, but the mechanisms of genetic regulation for flowering induction and floral differentiation might be different[2,3].

During the past decade, with a great number of flower development mutants discovered in *Arabidopsis* and *Antirrhinum*, two dicot model plants, many genes that play important roles in flower development were identified and characterized. Because mutants play an important role in the studies of functions and interactions of genes controlling flower development, a great deal of

efforts have been made to create and study flower mutants in plants. Rice is a model plant for molecular biology as well as one of the worldwide staple food crops. However, studies on the flower development in cereals, such as rice, have lagged far behind those in dicots. A major reason could be that few flower mutants have been available in cereals.

The transition from vegetative growth to reproductive growth is the most important event of morphogenesis in plants, and the floral bud differentiation is one of the most important stages determining whether the reproductive development can be completed successfully. Up to now, at least five genes for the emergence of floral meristem have been identified in dicots[3]. Mackill et al.[4] reported a mutant called "*frizzle panicle*" (*fzp*) controlled by a recessive gene in rice obtained by treatment of chemical mutagen EMS. The first and second rachis branches of *fzp* develop normally, but no spikelets emerge from the rachis branches. Instead, unlimited subsequent rachis branches develop from the places. Hence, the mutant gene *fzp* must be one of the key genes controlling the spikelet differentiation in rice. Until now, however, no further investigation on the mutant has been reported. We have found a mutant similar to *fzp* from the progeny of a cross between *O. sativa* ssp. *indica* cv. V20B and cv. Hua1B. In this paper, we report results of genetic analysis and gene mapping of *fzp*, which will facilitate gene cloning, gene function analysis and practical application of the mutant.

1 Materials and methods

1.1 Plant materials

The materials used included *fzp* heterozygotes (*indica*), Minghui-77 (*indica*) and Jinghua-8 (*japonica*). The mutant *fzp* was found from the progeny of a cross between V20B and Hua1B. As the mutant cannot produce seeds, the mutant gene *fzp*(*t*) can only be inherited via heterozygous individuals.

1.2 Genetic analysis and mapping populations

Plants with normal phenotype were randomly selected from a plant line segregating at the *fzp* trait and crossed with Minghui77 or Jinghua8 (AA) as well as selfed with 1—2 panicles. F_1 and selfing progeny were planted in the next season. The procedure of constructing genetic populations is shown in fig. 1.

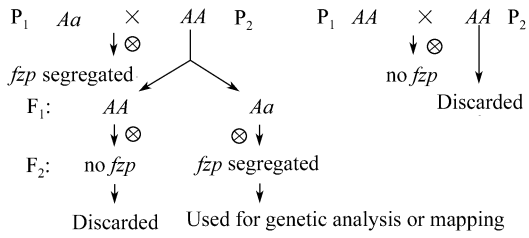

Fig. 1. Construction of F_2 populations for genetic analysis and *fzp*(*t*) mapping.

1.3 Phenotypic observation and genetic analysis

(1) Ratio between the number of wild-type plants and that of *fzp* mutant plants was investigated during the period between heading and milk stage. (2) The morpha of *fzp* mutant panicles was observed.

1.4 DNA pool

The normal DNA pool (Bn) and the mutant DNA pool (Bm) were constructed according to Michelmore et al. (1991)[5].

1.5 SSR analysis

DNA was extracted from fresh leaves using the CTAB method. PCR amplification was conducted following Panaud et al. (1996)[6] with slight modification. A 20 μL PCR system was adopted, containing Mg^{2+} (25 mmol/L) 2.0 μL, PCR buffer (without Mg^{2+}) 2.0 μL, dNTPs (10 mmol/L) 0.3 μL, DNA template (15 ng/μL) 1.4 μL, primer (15 ng/μL) 2.0 μL, TaqE (10 U/μL) 0.15 μL, Sterile H_2O 12.15 μL. The PCR profile was: denaturing at 94℃ for 5 min, followed by 35 cycles of denaturing at 94℃ for 1 min, annealing at 55℃ for 1.5 min (the temperature could be changed for different primers), extension at 72℃ for 2 min, and finally extension for 5 min at 72℃. PCR products were separated on 4% polyacrylamide denaturing gels and bands were revealed by silver-stain following Panaud et al. (1996)[6].

1.6 Gene mapping

The band-type of *fzp* mutant parent was recorded as "1", while that of parent Minghui-77 was recorded as "2" and the heterozygous band-type was recorded as "3". Linkage analysis and mapping of *fzp*(*t*) was performed using the software MAPMAKER/EXP3.0, by referring to the RM marker linkage map published by Temnykh et al.[7].

2 Results

2.1 Phenotype of mutant *fzp*

Before heading, *fzp* mutant and wild-type were not apparently different in plant morpha (including plant size, shape, colour, and plant type, etc.) except for tiller number. Wild-type had 8—12 tillers, while *fzp* mutant had no more than 6 tillers. During panicle differentiation, the first and the second rachis branches of *fzp* appeared to be normal, but from which no spikelets emerged. Instead, they continued to develop into unlimited subsequent small branches, replacing spikelets with masses of rachillas (fig. 2). This suggests that the normally finite floral meristem was converted into infinite panicle meristem. It thus can be deduced that the mutant gene *fzp* starts to be

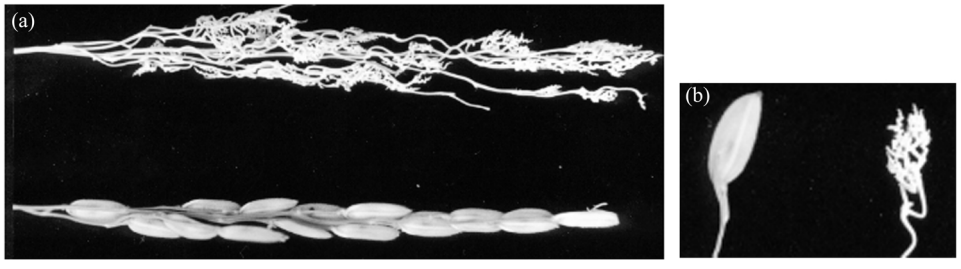

Fig. 2. Comparison of panicle and spikelet between wild-type and *fzp*. (a) A spikelet of *fzp* (top) or wild type (bottom) plant; (b) a floret of *fzp* (right) or wild type (left) plant.

expressed at least as early as during the tillering stage, but its main function is to impede the transition from inflorescence differentiation to spikelet differentiation.

2.2 Genetic analysis of *fzp*

Segregation in four successive progeny generations of a *fzp* heterozygous plant was investigated. It is seen from table 1 that, among the 3121 plants observed, 2348 plants exhibited normal phenotype and 773 exhibited *fzp* phenotype, showing a good fit to the expected ratio of 3∶1 ($X^2_{0.05,\,1} = 3.84$). The ratio did not alter in three cropping seasons. In addition, the phenotype of frizzle panicle was also expressed typically and stably when the mutant was planted in a greenhouse (Fuzhou) through the winter. These results suggest that *fzp* is controlled by a single recessive gene and can be inherited steadily, affected by climate factors (day length, temperature) and cropping conditions. We name the gene *fzp* (*t*).

Table 1 Inheritance mode of *fzp* in three cropping seasons

Cropping season	Population	Normal plants	*fzp*	X^2 (3:1)
Early (1998)	345	268	77	1.32
Middle (1999)	258	185	73	1.49
Late (1998)	437	339	98	1.54
Early (2000)	1224	922	302	0.07
Early (2001)	857	634	223	0.48
Total	3121	2348	773	0.09

In the F_2 population derived from the cross between *fzp* heterozygotes (*FZPfzp*) and Jinghua8 or Minghui77, the ratio of normal plants to *fzp* was also 3∶1 (see table 2), verifying again that *fzp* was controlled by a single recessive gene. In the F_2 progeny of *FZPfzp*/Minghui77, the mutant phenotype was typical, but in the F_2 progeny of *Fzpfzp*/Jinghua8, the mutant phenotype in some of the mutant plants was markedly attenuated into a “middle type”. The “middle-type” plants could produce frizzle panicles, but they also generated a few normal spikelets, which could develop into seeds. Since *FZPfzp* and Minghui77 are *indica* rice while Jinghua8 is *japonica* rice, the results imply that the extent of genetic background difference between parents could affect the exhibition of *fzp* phenotype.

Table 2 Effects of genetic background on the inheritance of *fzp*

Hybrid combination	Year	Population	Wild-type plants	*fzp* plants	X^2 (3∶1)	“middle-type” plants	“middle-type” plants/*fzp*
FZPfzp/Minghui77	1999	248	190	58	0.34	0	0
(*indica* / *indica*)	2001	451	341	110	0.09	0	0
FZPfzp/Jinghua8	1999	105	80	25	0.08	4	0.16
(*indica* / *japonica*)	2001	375	286	89	0.32	17	0.19

2.3 SSR marker analysis

180 pairs of SSR primers evenly distributed in the rice genome were selected from a total of 314 published ones for analyzing SSR polymorphisms between the *fzp* mutant and Minghui77, and 76 (42.2%) pairs revealed polymorphism. The 76 pairs of SSR primers were then used to de-

tect polymorphisms between the normal DNA pool and the mutant DNA pool. An SSR marker on chromosome 7, RM172, was found polymorphic between the two DNA pools, suggesting that RM172 might be linked with *fzp(t)*. To verify the result, 49 mutants selected from the F_2 population were assayed with the RM172 primers. 46 mutants exhibited the PCR band-type of *fzp*, while the rest 3 showed the band-type of heterozygote (fig. 3). The result confirms the linkage of RM172 to *fzp*(*t*). Three other markers, RM248, RM234 and RM18, were found to be linked with *fzp* (*t*) in further analysis on the SSR markers nearby RM172 on chromosome 7.

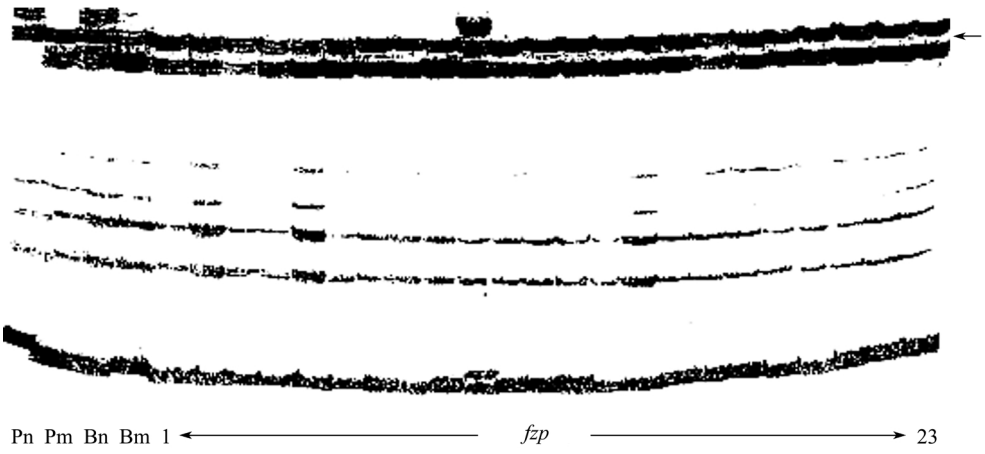

Fig. 3. PCR products (amplified by primer RM172) of the parent *fzp* (Pm) and Minghui-77 (Pn), the normal (Bn) and mutant (Bm) DNA pools and some mutants (1-23) from the F_2 population. Arrow indicates a recombinant progeny.

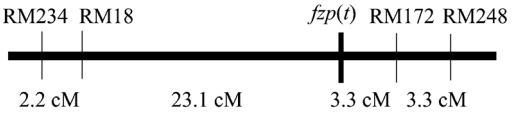

Fig. 4. Linkage among *fzp* (*t*) and SSR markers on chromosome 7.

Based on the above data, a regional linkage map of *fzp* (*t*) was constructed using the software MAPMAKER/EXP3.0 (fig. 4). It is shown that RM172 and RM248 are 3.2 cM and 6.4 cM apart from *fzp* (*t*), respectively, on one side, while RM18 and RM234 are 23.1 cM and 26.3 cM apart from *fzp*(*t*), respectively, on the other side.

3 Discussion

3.1 The effects of genetic background on the spikelet differentiation in *fzp*

It was observed that spikelets in the parental *fzp* mutants were always completely degenerated, and the mutant trait was never partly or completely reversed to the wild-type no matter what the cropping season was, suggesting that environments could not change or attenuate the expression of the *fzp* phenotype under the parental genetic background. The same result was also observed in all the *fzp* plants in the F_2 population derived from the cross between *fzp* heterozygotes (*indica*) and Minghui-77 (*indica*). However, in the F_2 population derived from the cross of *fzp*

heterozygotes with Jinghua-8 (*japonica*), the *fzp* phenotype in some mutant plants was obviously attenuated into "middle type". The "middle-type" plants had the same panicle phenotype as *fzp* except that they could generate a few normal spikelets and, therefore, produce a few seeds. It can thus be deduced that the emergence of "middle-type" plants might be determined by the extent of difference between parental genetic backgrounds. There could be three causes: (1) effects of redundant genes; (2) effects of modifying genes; and (3) effects of gene interactions.

The effects of redundant genes are common in plant reproductive development. The complementary functions of redundant genes may vary in different genetic backgrounds. When the expression of redundant genes is weak, their effect could not reach the threshold value for floral meristem, thus, the "middle-type" phenotype cannot emerge. When the expression of redundant genes reaches the threshold value, a few spikelets will be able to develop in the *fzp* mutant, making it appear as a "middle type" [8]. Hence, the extent of difference of genetic background between parents determines whether there will be "middle-type" plants in their segregating progeny. Studies have shown that many key genes involved in the reproductive development in *Arabidopsis* and *Antirrhinum* are redundant in function[9—13].

3.2 Pleiotropy of genes in plant development

The present study has shown that the spikelet differentiation in *fzp* is completely blocked and the tiller number in the mutant is also significantly reduced. This suggests that the expression of *fzp*(*t*) might begin before the tillering stage. Therefore, *fzp*(*t*) has dual functions affecting both the vegetative development and the reproductive development, but the degrees of its effects on the two stages are different.

Many studies have indicated that although the major functions of most key genes controlling the reproductive development in plant are to determine the identities of meristems and floral organs, those genes may also be involved in the control of vegetative growth[14], ovule development[15], seed coat development[16], root development[17], embryogenesis[18], symbiotic induction and stress resistance[19]. Some studies have suggested that some key genes controlling the reproductive development in plant could function in different developmental stages, different positions and different organs. The *SaMADSD* gene isolated by Bonhomme et al.[20] from *Sinapis alba* affects both inflorescence development and floral organogenesis. The *PFG* gene isolated by Richard et al.[21] from *Penniua* can be expressed in almost all tissues and developmental stages (except for root, pistil and seedling). More than twenty *AGL* (*AG-like*) sequences have been identified and characterized in *Arabidopsis*. These sequences are expressed in different developmental stages, but their functions are apparently different. Purugganan et al.[22] suggest from an evolutionary viewpoint that some *MADS-box* genes in plant may have pleiotropy, playing different roles from different laterals and at different levels.

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