Front. Biol. China (2006) 2:93–98
DOI 10.1007/s 11515-006-0012-0

RESEARCH ARTICLE

Zhao Xiangqiang, Liang Guohua, Zhou Jingsong
Yan Changjie, Cao Xiaoying, Gu Minghong

Molecular mapping of two semidwarf genes in an *indica* rice variety Aitaiyin3 (*Oryza sativa* L.)

Abstract Genetic analysis established that Aitaiyin3, a dwarf rice variety derived from a semidwarf cultivar Taiyin1, carries two recessive semidwarf genes. By using simple sequence repeat (SSR) markers, we mapped the two semidwarf genes, *sd-1* and *sd-t2* on chromosomes 1 and 4, respectively. *Sd-t2* was thus named because the semidrawf gene *sd-t* has already been identified from Aitaiyin 2 whose origin could be traced back to Taiyin1. The result of the molecular mapping of *sd-1* gene revealed it is linked to four SSR markers found on chromosome 1. These markers are: RM297, RM302, RM212, and OSR3 spaced at 4.7 cM, 0 cM, 0.8cM and 0 cM, respectively. *Sd-t2* was found to be located on chromosome 4 using five SSR markers: two markers, SSR332 and RM1305 located proximal to *sd-t2* are spaced 11.6 cM, 3.8 cM, respectively, while the three distally located primers, RM5633, RM307, and RM401 are separated by distances of 0.4 cM, 0.0 cM, and 0.4 cM, respectively.

Keywords rice, semidwarf gene, SSR markers, molecular mapping

1 Introduction

Rice is one of the most important crops in the world accounting for 50%~80% of the daily diet of approximately half the world's population. Due to rapid increase in population, food shortage has become a worldwide problem. To address this problem, the International Rice Research Institute (IRRI) and other research institutions in Asian countries bred a series of semidwarf varieties by introducing the semidwarf gene, *sd-1* into the tall varieties from the semidwarf varieties such as Dee-geo-gen found in Taiwan Province, China. The semidwarf genes used in rice and wheat breeding are also known as the "Green Revolution Genes". These genes confer semidwarf stature or reduced culm length and have a major effect on harvest index. They also improved lodging resistance and are associated with increased responsiveness to nitrogen fertilizer. These developments contributed substantially to the significant increase in crop yields since 1960s and has averted the chronic food shortage that was the deep concern after the rapid expansion of the world population. The semidwarf genes used for *indica* rice in China were mainly derived from 5 varieties namely: Ai-jue-nan-te, Ai-zi-zhan, Hua-long-shui-tian-gu, Ai-zhong-shui-tian-gu, and Zhong-shan-wu-ming-zhong (Gu and Zhu 1979; Gu 1980; Gu et al., 1988 Xiong et al., 1988; Liang et al., 1995), while those used in southeast Asia were isolated from a Taiwanese variety Dee-geo-woo-gen (Gu 1980). Several researchers reported that these genes are allelic, although they were derived from several varieties (Gu et al., 1988; Xiong et al., 1988; Liang et al., 1995; Kinoshita 1995; Linoshita 1995). The widely utilized semidwarf gene *sd-1* has already been mapped on chromosome 1 (Kinoshita 1995) and that several labs have independently cloned this gene (Monna et al., 2002; Sasaki 2002). Since frequent use of a single semidwarf gene can cause genetic vulnerability to pests and diseases, it is therefore necessary to develop an alternative or new source of semidwarfs to broadening the genetic basis of the semidwarfism.

Our laboratory began screening for new semidwarf genes since 1970s, and we already identified three: *sd-g, sd-t,* and *sd-n* (Liang et al., 1995). Here we report the identification and mapping of a new semidwarf gene *sd-t2* from a dwarf variety Aitaiyin3.

Translated from *Acta Genetica Sinica*, 2005, 32 (2) [译自：遗传学报，2005,32(2)]

Zhao Xiangqiang, Liang Guohua (✉), Zhou Jingsong, Yan Changjie, Cao Xiaoying, Gu Minghong
Key Laboratory of Plant Function Genomics , the Ministry of Education, Agricultural College, Yangzhou Univercity, Yangzhou 225009, China
E-mail:ricegb@yau.edu.cn

2 Materials and methods

2.1 Plant materials and growth condition

A spontaneous *indica* type dwarf variety Aitaiyin 3 and a

tall *indica* type cultivar Nanjing 6 were used in this study. Rice plants were grown in paddies under natural conditions.

2.2 Development of rice mapping population

The dwarf variety Aitaiyin was crossed to Nanjing 6 in 1999. The F1s were pollinated in Hainan in the spring of 2000. The parents, F_1s, and offsprings were planted in Yangzhou in summer of 2000.

2.3 Identification of the semdwarf lines with new semidwarf gene

Sixty semidwarf plants in the F_2 of Nanjing6 crossed with Aitaiyin3 were selected at random. Selected plants were then backcrossed with another semidwarf variety Nanjing11 in the spring of 2001 in Hainan to identify progenies with the newly introduced semidwarf gene. In 2001, F_1s and progenies of the selected lines were planted and their heights were recorded. If the F_1s were significantly taller than the semidwarf variety, then the related semidwarf lines might carry a new semidwarf gene, otherwise, the semidwarf lines would probably just carry the *sd-1* gene.

2.4 DNA Preparation and SSR analysis

Total genomic DNA samples from three parents were extracted from leaves using the method of McCouch, et al(1988). Rice genomic DNA of each individual of the mapping population was prepared from leaves using a modified 2×CTAB method mentioned by Murray, and Thompson (1980) Genomic DNA was dissolved in double distilled water and used as a template for PCR analysis.

All PCR reactions were performed in and MJ PTC-100 thermocycler (Waltham, Mass.). The basic SSR procedure is as follows: 1 μM of primers, 200 μM of each dNTP, 50 ng of DNA template, 2 mM $MgCl_2$, 2.5 μL 10 × buffer (supplied by Sangon Inc.), 1 U of Taq polymerase, final volume was adjusted to 25μL using double distilled water. The amplification reactions were performed using the following profile: 94℃ for 5 min, 32 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 1.5 min, with a final extension step of 72℃ for 10 min. Amplicons were separated on 3.0% agarose gels and visualized under UV light after ethidium bormide staining. The gels were then photographed or scanned with a Bio-Rad DOC-1000 scanner (Hercules, Calif.). If no polymorphisms were detected on the agarose gels, the amplicons were separated on a 6% PAGE sequencing gel run at 1200 V for 2~3 h following a pre-electrophoresis for 30 min. The bands were visualized using silver-staining method.

2.5 Molecular marker development and linkage analysis

SSRs were identified by using the online version of Simple Sequence Repeat Identification Tool (SSRIT) available at http://www.gramene.org/db/searches/ssrtool. Primer pairs flanking the SSRs were designed using Primer Premier 5.0. Primers were 18~22 nucleotides long, devoid of secondary structure or consecutive tracts of a single nucleotide, with a GC content around 50% (Tm approximately 55℃), and preferably should have G or C at the 3' end. Primers were synthesized by Shenghai Shengon Inc. and were used to identify the polymorphisms between the parents. Primer pair that provided a correct linkage relationship to *sd-t2* was used as a new marker for the further mapping.

Linkage relationship and map distances (in cM) were estimated using the program MAPMAKER/ 3.0b(Temnykh 2001).

3 Results

3.1 The inheritance of the dwarfism of Aitaiyin 3

The median height of Aitaiyin 3 was 59.5 cm±2.0 cm (10 plants), 162.6 cm±1.9 cm for Nanjing 6, and 172.7cm ±2.0cm for that of F_1. In the F_2 generation, 1034 were tall, 638 were senidwarfs, and 132 were dwarfs. This fitted well in the segregation ratio of 9:6:1(χ^2=5.84,v=2) indicating that there were two dwarf genes present in Aitaiyin 3.

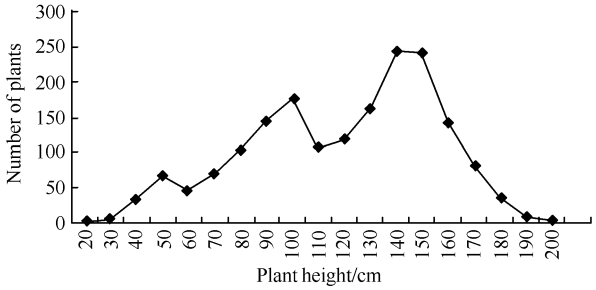


Fig.1 The plant height segregation of the F_2 generation of Aitaiyin3×Nanjing6

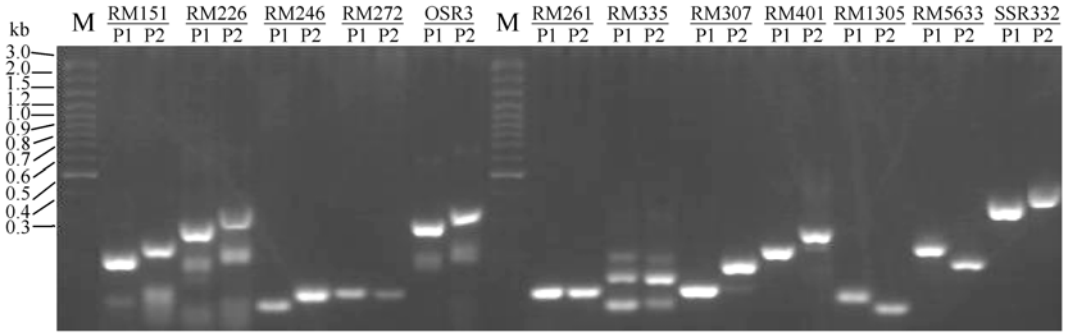
3.2 Identification of the semidwarf lines with new semidwarf gene

Aitaiyin 3 is a natural mutant derived from Taiyin1, a variety from Thailand. Most *indica* rice varieties used in the southeast Asian region carry a semidwarf gene *sd-1* derived from a Taiwan ese variety Dee-geo-woo-gen. There were two semidwarves (dwarf genes) in Aitaiyin 3. To test whether *sd-1* is also present in Aitaiyin 3, the semidwarf lines derived from the F_2 population of Nanjing6 crossed with Aitaiyin 3 were backcrossed to Naning11 which is known to contain the semidwarf gene *sd-1*. About half of F_1s were semidwarfs and carry the semidwarf gene *sd-1*, while the other half of the line were tall. These results indicate the presence of a new semidwarf gene in Aitaiyin 3 which was named *sd-t2* to differentitiate from another semidwarf gene derived from the related Aitaiyin 1 cultivar.

The new lines containing the newly identified semidwarf gene were also studied using linkage analysis (data not shown).

3.3 Selection of polymorphic markers between Nanjing6 and Aitaiyin 3

In order to map the semidwarf genes in Aitaiyin3, 350 SSR markers distributed on 12 chromosomes in rice were used for polymorphic analysis and linkage analysis. Of the 350 SSR markers, 71 were found to be polymorphic between the two parental strains. Only polymorphic markers were used to analyze a small population containing 14 tall and 14 dwarf plants (Fig.2). Twelve markers located at chromosome 1 and 4 markers on chromosome 4 were found tobe cosegregated with the dwarf genes. This suggests that the two dwarf genes are indeed located at chromosomes 1 and 4.

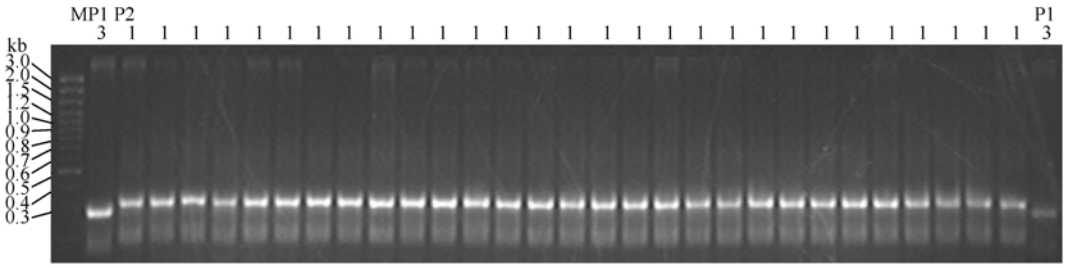


Note: P_1 is Nanjing6; P_2 is Aitaiyin3

Fig.2 The amplified result of some SSR markers between the parents

3.4 The molecular mapping of the semidwarf gene *sd1*

Four polymorphic markers located at the long arm of chromosome 1 were used to map *sd-1* in 122 dwarf plants from the F_2 derived from crosses between Aitaiyin 3 and Nanjing 6. It appeared that the SSR marker OSR 3 was tightly linked to the one of dwarf genes *sd-1*. Linkage analysis indicated that *sd-1* was linked to RM297, RM302, RM212, and OSR3 SSR markers whose genetic distance with each other are 0.5cM, 0.0cM, 2.0cM, 2.0cM, respectively. Fine mapping of the *sd-1* gene is not yet performed since the gene still has been cloned.

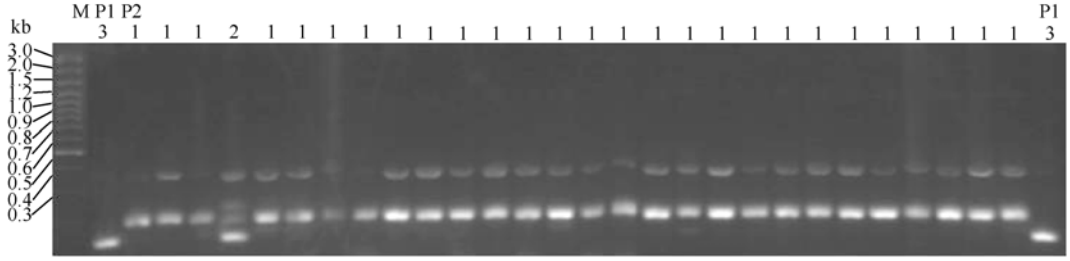


Note: P1 is Nanjing6; P2 is Aitaiyin3; M is 100 bp DNA ladder plus; Numbers are the genotype symbols for the individuals

Fig.3 The parental polymorphism of *SSR* marker OSR3 and its segregation in some F_2 dwarf individuals

3.5 The molecular mapping of the semidwarf gene *sdt-2*

Two SSR markers, RM307 and RM401 were used to map *sd-t2* in 122 dwarf plants in F_2 population. The linkage analysis of RM307 and *sd-t2* is shown in Fig.4. The semidwarf gene *sd-t2* was mapped proximal of RM307 and RM401 SSR markers with both 0.4 cM apart. The semidwarf gene *sd-t2* was originally thought to be located at the top of the short arm of chromosome 4 since the old map says that RM401 is located at the same region. In our previous experiments we based designing SSR markers on the old map, however, we did not see any new markers that were tightly linked to *sd-t2*.In the new map of rice genome, the SSR marker RM401 was relocated to the centromere region of chromosome 4. Since no semidwarf or dwarf genes have been previously reported in that region, we therefore conclude that *sd-t2* is a new member of the semidwarf gene family.



Note: P1 is Nanjing6; P2 is Aitaiyin3; M is 100 bp DNA ladder plus; Numbers are the genotype symbols for the individuals

Fig.4 The parental polymorphism of *SSR* maker RM307 and its segregation in some F_2 dwarf individuals

3.6 Fine mapping of the semidwarf gene *sd-t2*

Fine mapping of gene of interest is the key step in a map-based cloning approach. To finely map *sd-t2* locus, 47 new SSR markers were developed in the region based on the available rice genome sequence. The PCR products could be obtained with 22 of the 47 newly developed SSR markers (Table 1). SSR332 was determined to be polymorphic between the two parents and was found to be linked to *sd-t2* with the genetic distance of 15.4 cM. Moreover, it is located between RM307 and RM401 markers. The newly developed SSR markers by McCouch, *et al* were also used to map *sd-t2*. Two markers, RM5633 and RM1305 were found to be polymorphic. All the polymorphic markers linked to *sd-t2* were used to finely map the *sd-t2*. The map is shown in Fig.5. More markers are currently in development in our laboratory to further refine the mapping of *sd-t2*.

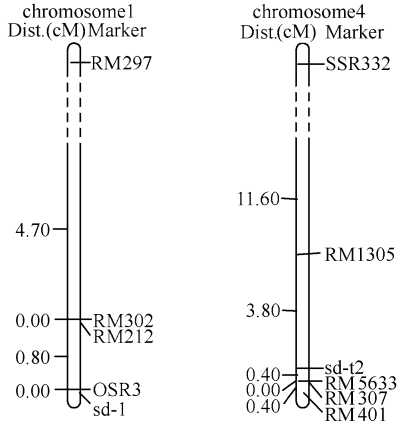


Fig.5 The molecular mapping result of *sd1* and *sd-t2*

Table 1 Some *SSR* markers developed on the chromosome 4 of rice in this study

Makers	Forward sequences(5′→3′)	Reverse sequences(5′→3′)	Gene Bank acc. no.	Amplified region/bp
SSR1	CAACTCTTTGACGCCCAC	AATACCGAAATCACACCAAGT	AL606593	4262~4581
SSR3	CGTTTTTTTTCATTTCCCCT	TGGCACATCATCATCATCAC		71688~71925
SSR8	CTGCTCGGGAGTTACTGACC	CAACGAAACAGGGTACAAACAC		45955~46254
SSR9	CGGCAGTCGGTTTGGCAT	GTCTATTTTGCCTCCTCCGTG	AL606642	105084~105424
SSR10	GCTGAGGAGCCCCCACTAT	GTGCTCTCGTGTGCCAAACT		109978~110450
SSR11	GTCCACCTTTCGGGTCTGAG	GTGCGGGTTGGGTAATGG		38305~38628
SSR12	GGCATCAAATCTCCGTTCG	CGGCGTCAAACATTTAGGG	AL606689	119607~119864
SSR13	GTTCGTAACCCCCCTGTAGT	GTCGGTCCTTCCTCTTCTG		122603~123113
SSR15	TAGTAGCAACCAACGGGACG	GGAAGAGAGGGAAGAGTGGG		13942~14154
SSR16	GAAATGAAAGAGGAAGAAGAGAG	CACTAGCGGTCCTTAGACTTG	AL662935	29749~30039
SSR17	GGAGCAGCCAGCCACTAAAG	ACGAGCACCTCCGAAGACTG		116715~117245
SSR18	CTACCTCTTGTCACCGCTGC	GAAAGAAAGAAAGAAAGGAGTTGC		119640~119852
SSR307	GTTAGGGAAGGGAAGGCGACCAT	TTCCTCACCCACTAAGCCACATCG	AL731602	38160~38550
SSR310	CCTAGCACTCGCTGGAACACC	AGCCTTACCCTTTGGCGTTG	AL731603	165124~165697
SSR311	GCTTGATTCACATGCTCCTTGCTC	GGACCCTCTTGTCATCCGCTCT	AL731635	5745~6164
SSR312	TACAGATCCGTATTTGAGTTGG	TTCGGTCTGAATCATCATAGGC		45386~46315
SSR313	TTCGGGATGGATTTATCAGGAC	CCTATGTGAGTGGGAACTAAAGC		66011~66592
SSR314	GAATTAAACCACCTCAGGAATGAC	TCAGAGCGAGCAGTTAGTTTCC	AL606450	101805~102563
SSR315	TGGTGGTGGCGGTGGACATA	CCAGAGGCGGAGAAGGAAGAA		18384~18833
SSR318	GAGGAGGGTGATACCGAAGA	TCTCGGGTGAAATGCTACAA	AL606616	70485~71052
SSR319	TCGAAACATGAATGATTTAGCT	CTATGGTCAAAACTCAGAAGGC		84649~85146
SSR332	TTCTTCAAGGCAGCGATTTC	CCATGCTGCTTTGTCTATTT	AL662985	60717~61112

4 Discussion

Currently more than 60 dwarf genes have been identified in rice (mostly in *japonica* variety), but some of which are the same genes or allelic and most of these genes do not have a direct practical value in rice breeding (Kinoshita 1995). About 15 semidwarf genes have been identified in both *indica* and *japonica* rice varieties, including *sd-1* which is still currently the dominant semidwarf gene used in *indica* rice breeding. Since semidwarf genes are infrequent in the identified rice stocks, we focused our efforts in finding new semidwarf genes. In the recent years, four semidwarf genes non-allelic to *sd-1* have been identified namely: *sd-g, sd-t ,sd-t2, and sd-n.* (Liang et al., 1994; 1995; Jiang et al., 2002).

More dwarf genes having been isolated and characterized in mqny plants, all of which affect the synthesis of gibberellins, brassinosteroids, auxins, and polyamines. They also alter the composition and rate of synthesis of the cell wall and also the glycosyltransferase levels in plants (Hedden and Phillips 2000; Schaller 2003; Xu et al., 1995; Peng et al., 1999; Ikeda et al., 2001; Hedden et al., 2002; Schomburg et al., 2003; Ashikari et al., 1999; Itoh et al., 2001). The green revolution genes, *sd-1* in rice and *Rht* in wheat are known to reduce height and are capable of boosting the yields significantly. The molecular basis for semidwarfism seems to be different. In *sd-1,* semidwarism is a result of GA 20-oxidase gene mutation which causes a deficiency in the amount of bioactive GA in elongating stems. Whereas *Rht* genes are negative regulators of GA signaling that in the mutant are no longer modulated by GA. Unlike *Rht* in semidwarf wheat, *sd-1* in semidwarf rice still retained its ability to respond to applications of bioactive gibberellins. This suggests the presence of still uncharacterized semidwarf genes that could be used for rice breeding (Monna et al., 2002; Sasoki et al., 2002; McCouch et al., 1988; Muvray and Thompson 1980; Temnykh et al., 2001; Lancler et al., 1987).

Dwarf and semidwarf genes families have been identified in many plants. Mutation of one gene in the family always leads only to a modest reduction in bioactive GAs, which causes a relatively benign height reduction in semidwarf plants since other genes in the family can partially rescue the mutation. Known examples are in *sd-1* and *ga5* mutants in rice and *Arabidopsis,* respectively. It would be very interesting to find new genes in the family and to determine the molecular basis on how their expression affect plant physiology that result to a semidwarf productivity. The *sd-t2* gene we report here may be the key to help elucidate the signal transduction events that affect GA biosynthesis and increase in our understanding of events might lead to improvement of plant phenotypes thourgh genetics. The fine mapping and cloning of the gene reported here are in progress.

Acknowledgements This project was supported by the National Natural Science Foundation of China (No. 30270705 and 3990086) and the National High Technology Program (No. 2003CB114303).

References

Ashikari M., Wu J., Yano M., Sasaki T., Yoshimura A., Rice gibberellin-insensitive dwarf mutant gene Dwarf 1 encodes the subunit of GTP-binding protein. Proc. Natl. Acad. Sci. U.S.A., 1999, 96(18): 10284–10289

Gu M.-H. and Zhu L.-H., Genetic analysis of the allelism of several dwarf genes in Indica rice (*Oryza sativa* L.), Hereditas, 1979, 1(6):10–13[顾铭洪，朱立宏，几个矮秆籼稻矮秆基因等位关系的初步分析，遗传，1979，1(6)：10–13]

Gu M.-H., Pan X.-B., Li X.，Dong G.-C., Isolation and identification of a new semidwarf gene inIndica rice (*Oryza sativa* L.). Scientica Agricultura Sinica, 1988, 21(1): 33–40[顾铭洪，潘学彪，李欣，董桂春，一种籼稻矮源的分离和遗传鉴定，中国农业科学，1988，21(1)：33–40]

Gu M.-H., The dwarf resources and their utilization in rice breeding, J. of Jiangsu Agriculture College, 1980, 1(1): 40–44[顾铭洪，矮源及其在水稻育种上的应用，江苏农学院学报，1980,1(1)：40–44].

Hedden P. and Phillips A.-L., Gibberellin metabolism: new insights revealed by the genes, Trends Plant Sci., 2000, 5(12): 523–530

Hedden P., Phillips A.-L., Rojas M.-C., Carrera E., Tudzynski B., Gibberellin biosynthesis in plants and fungi: a case of convergent Evolution? Plant Growth Regul, 2002, 20(4): 319–331

Ikeda A., Ueguchi-Tanaka M., Sonoda Y., Kitano H., Koshioka M., Futsuhara Y., Matsuoka M., Yamaguchi J., *Slender* rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the Height-regulating gene *GAI/RGA/RHT/D8*. Plant Cell, 2001, 13(5): 999–1010

Itoh H., Ueguchi-Tanaka M., Sentoku N., Kitano H., Matsuoka M., Kobayashi M., Cloning and functional analysis of two gibberellin 3 beta-hydroxylase genes that are differently expressed during the growth of rice, Proc. Natl. Acad. Sci. U.S.A., 2001, 98(15): 8909–8914

Jiang G.-H., Liang G.-H., Zhai W.-X., Gu M.-H., Lu R.-L., Xu J.-C., Zhu L.H., Genetic mapping of a new semidwarf gene, sd-t(t), in indica rice and estimating of the physical distance of the mapping region. Sci. China (series C), 2002, 32(3): 193–199[江广怀，梁国华，翟文学，顾铭洪，鲁润龙，朱立宏，籼稻中矮秆基因 *sd-t* 的遗传定位及定位区间物理距离的估计，中国科学(C 辑), 2002,32(3)：193–199]

Kinoshita T., Report of committee on gene symbolization, nomenclature and linkage groups, Rice Genetics Newsl., 1995, 12: 9–97.

Lander E.-S., Green P., Abrahamson J., Barlow A., Daly M. J., Lincoln S.-E., Newburg L., MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, 1987, 1(2): 174–181

Liang C.-Z., Gu M.-H., Pan X.-B., Liang G.-H., Zhu L. H., RFLP tagging of a new semidwarf gene in rice, Theor. Appl. Genet., 1994, 88: 898–900.

Liang G.-H., Pan X.-B., Gu M.-H., Ji C.-Q., Isolation and identification of a semidwarf gene from an indica rice variety Aitaiyin 2. Chinese J. Rice Science, 1995, 9(5): 189–192[梁国华，潘学彪，顾铭洪，稔朝球，矮泰引一种半矮秆基因的分离和鉴定，中国水稻科学，1995，9(5)：189–192]

McCouch S.-R., Kotcket G., Yu Z.-H., Molecular mapping of rice chromosome, Theor. Appl. Genet., 1988, 76: 815–829

McCouch S.-R., Teytelman L., Xu Y., Lobos K.-B., Clare K., Walton M., Fu B., Maghirang R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R., DeClerck G., Schneider D., Cartinhour S., Ware D., Stein L., Development and mapping of 2240 new SSR markers for rice, DNA Res., 2002, 9(6): 199–207

Monna L., Kitazawa N., Yoshino R., Suzuki J., Masuda H., Maehara Y., Tanji M., Sato M., Nasu S., Minobe Y., Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis, DNA Res., 2002, 9: 11–17

Mori M., Nomura T., Ooka H., Ishizaka M., Yokota T., Sugimoto K., Okabe K., Kajiwara H., Satoh K., Yamamoto K., Hirochika H., Kikuchi S., Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis, Plant Physiol., 2002, 130(3): 1152–1161

Murray M.-G. and Thompson W.-F., Rapid isolation of high molecular weight plant DNA, Nucl. Acid Res., 1980, 8: 4321–4325

Peng J., Richards D.-E., Hartley N.-M., Murphy G.-P., Devos K.-M., Flintham J.-E., Beales J., Fish L.-J., Worland A.-J., Pelica F., Sudhakar D., Christou P., Snape J.-W., Gale M.-D., Harberd N.-P., "Green revolution genes" encode mutant gibberellin response modulators, Nature, 1999, 400(6741): 256–261

Sasaki A., Ashikari M., Ueguchi-Tanaka M., Itoh H., Nishimura A., Swapn D., Ishiyama K., Saito T., Kobayashi M., Khush G.-S., Kitano H., Matsuoka M., A mutant gibberellin-synthesis gene in rice, Nature**,** 2002, 416: 701–702

Sato Y., Sentoku N., Miura Y., Hirochika H., Kitano H., Matsuoka M., Loss-of-function mutations in the rice homeobox gene *OSH15* affect the architecture of internodes resulting in dwarf plants. EMBO J., 1999, 18(4): 992–1002.

Schaller H., The role of sterols in plant growth and development, Prog. Lipid Res., 2003, 42(3): 163–175

Schomburg F.-M., Bizzell C.-M., Lee D.-J., Zeevaart J.-A., Amasino R.-M., Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants, Plant Cell, 2003, 15(1): 151–163

Temnykh S., DeClerck G., Lukashova A., Lipovich L., Cartinhour S., McCouch S.-R., Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential, Genome Res., 2001, 11: 1441–1452

Xiong Z.-M. Min S.-K., Cheng S.-H., Genetic analysis and utilization of the dwarf resources in China, Rice Rev. Abstr.,1988, 7(4): 1–5[熊振民，闵绍楷，程式华，我国水稻矮源的研究与利用，水稻文摘，1988，7(4)：1–5]

Xu Y.-L., Li L., Wu K., Peeters A.-J., Gage D.-A., Zeevaart J.-A., The *GA5* locus of Arabidopsis thaliana encodes a multifunctional gibberellin 20-oxidase: molecular cloning and functional expression, Proc. Natl. Acad. Sci. U.S.A.,. 1995, 92(14): 6640–6644

Yamamuro C., Ihara Y., Wu X., Noguchi T., Fujioka S., Takatsuto S., Ashikari M., Kitano H., Matsuoka M., Loss of function of a rice *brassinosteroid insensitive1* homolog prevents internodes elongation and bending of the lamina joint. Plant Cell, 2000, 12(9): 1591–1605