Fine mapping and candidate gene analysis of gl-1, a gene controlling trichome formation in rice

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Abstract: Leaf trichome plays an important role in protecting plants against insect herbivores, loss of water through transpiration and UV irradiation. In our present study, SSSL-W24, a single segment substitution line (SSSL) containing only one chromosome segment of the glabrous japonica cultivar Star bonnet 99 in the genetic background of the pubescent indica cultivar HJX74 showed glabrous leaves and hulls. Genetic analysis of the F2 generation, derived from a cross between SSSL-W24 and HJX74, showed that these traits are controlled by the recessive gene gl-1, which was mapped to the short arm of chromosome 5. Fine mapping and high-resolution linkage analysis using 1585 F3 plants and markers flanking gl-1 were carried out, and the gene was localized to a 35.9 kb region that contains seven annotated genes according to the genome sequence of japonica Nipponbare. Positional cloning of this gene will assist selection of hybrid rice, facilitate the mechanization of agriculture and increase the warehousing capacity of rice.

Key words: Crop genetic breeding; Rice; Trichome; Single segment substitution line; Fine mapping; Glabrous gene;

0 Introduction

Aerial surfaces of most land plants have epidermal hairs called trichome. They are believed to protect plants against insects, microbes, herbivores, and abiotic damages and to assist seed dispersal[1,2]. Furthermore, seed trichomes of Gossypium plants, namely cotton fibers, are the most widely used natural fibers in the textile industry[3].

Glabrous mutants or cultivars are known in various plant species, including *Arabidopsis*[4], rice[5], and maize[6], but few detailed studies on glabrousness were carried out except in *Arabidopsis*.

Trichome patterning in *Arabidopsis* is controlled by several transcription factors. According to their effect on trichome initiation, these transcription factors can be divided into two groups: positive regulators and negative regulators. Positive regulators include the WD-repeat protein *TRANSPARENT TESTA GLABRA1 (TTG1)*[7,8], the R2R3 MYB-type transcription factor *GLABRA1 (GL1)*[9], the basic helix-loop-helix (bHLH) transcription factors *GLABRA3 (GL3)* and *ENHANCER OF GLABRA3 (EGL3)*[10,11], and the homeodomain protein *GLABRA2 (GL2)*[12,13]. The negative regulators include several small single-repeat R3 MYB transcription factors, such as *TRIPTYCHON (TRY)*[14,15], *CAPRICE (CPC)*[16,17], *ENHANCER OF TRY AND CPC 1* and *2 (ETC1 and ETC2)*[18-20], and *TRICHOMELESS1 (TCL1)*[21]. These positive and negative regulators work together to control trichome initiation and patterning in *Arabidopsis*. The R2R3 MYB-type transcription factor *GL1*, a bHLH transcription factor (*GL3* or *EGL3*), and *TTG1* form a complex

Foundations: The National Natural Science Foundation of P.R. China (No. 31200905), The Guangdong Provincial Natural Science Foundation of China (No. 10451064201005483), The Specialized Research Fund for the Doctoral Program of Higher Education (No. 20104404120005), The 40th Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry
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to induce the expression of *GL2*, which in turn induces trichome formation in shoots (reviewed by[22,23]. The small MYB transcription factors *TRY*, *CPC*, *ETC1* and *ETC2*, inhibit the trichome cell type in the shoot, presumably by competing with *GL1* for binding *GL3*, thereby limiting the transcriptional activity of the trichome initiation and patterning activator complex[15,17,24,25].

Glabrous rice is one important ingredient of world seed rice resources, the original variety of which mainly distributes over Yunnan Kweichow Plateau in mainland China, Java island in Indonesia and Africa[26]. It was found to have favorable affinity with *indica* and *japonica* rice, and could overcome the disadvantage of cross-sterility of *indica* and *japonica* rice[26-28]. The American glabrous rice varieties have a high lodging resistance and a higher average efficiency of light energy conversion than Chinese pubescent rice varieties[28]. More interestingly, the allele for glabrous stems and leaves in the *japonica* rice variety, lemont, is associated with a susceptibility to brown planthopper (BPH), indicating that the pubescence in rice might also contribute to the quantitative resistence to BPH[29]. However, in rice, the molecular study of trichome formation is relatively limited. Only a few genes have been preliminarily mapped or studied. The glabrous leaf and hull gene (g*l-1*) was first reported by Yu et al[30], they found the linkage between the RG182 and RG183 in the short arm of chromosome 5 and *gl-1* gene based on the linkage relationship between the rice morphologic markers and RFLP markers, and the genetic distance was 14.3±7.4 cM and 20.9±8.3 cM, respectively. Recently, Wang et al mapped the *gl-1* gene to a 156.84 kb region by using simple sequence repeats (SSR) markers[31]. Qian et al identified a QTL (*qHH*-6) for controlling the lemma hair in chromosome 6, based on the genetic analysis and QTL location for morphological index and its related taxonomic traits of *indica-japonica* DH population[32]. Loss-of-function mutants of *SDG714*, a rice histone H3K9 methyltransferase, generated by RNA interference display a mostly glabrous phenotype as a result of the lack of macro trichomes in glumes, leaves, and culms compared with control plants[33]. *EL2* gene of rice, the homolog of *Arabidopsis SIAMESE (SIM)* gene, was able to rescue the multicellular trichome phenotype of sim mutants of *Arabidopsis* by operating as a cell cycle inhibitor[34].

In our study, we constructed a rice library of 1,529 single segment substitution lines (SSSLs), by using HJX74, an elite *indica* variety from South China, as recipient and 26 accessions including 14 *indica* and 12 *japonica* collected worldwide as donors[35,36]. One of our SSSL lines, SSSL-W24, derived from crosses by using HJX74 as the recurrent and Star bonnet 99 as the donor showed glabrous leaves and hulls just like the donor parent Star bonnet 99 did. Preliminary mapping of these traits revealed that the glabrousness in SSSL-W24 was controlled by a single recessive gene in the short arm of chromosome 5, in consistent with *gl-1* locus reported previously[30,31]. Then, we carried out fine mapping and localized *gl-1* to a 35.9 kb target region that contains seven annotated genes according to the genome sequence of *japonica* Nipponbare. The potential importance and utilization of *gl-1* gene in rice breeding is also discussed.

1 Materials and methods

1.1 Plant materials

SSSL-W24 is one of a set of 1,529 single segment substitution lines (SSSLs), which was developed from backcross progenies (BC_3F_3) derived from a cross between an elite *indica* variety from South China, HJX74, as the recurrent parent and an American *japonica* glabrous rice, Star bonnet 99, as the donor parent. The SSSL-W24 plants containing only one chromosome segment from Star bonnet 99 showed glabrous leaves and hulls when compared with the recurrent parent HJX74. The Star bonnet 99 segment was located at approximately 12.7 cM intervals in chromosome 5, delimited by the RM122 and PSM202 markers.

A small F_2 population consisting of 250 plants was obtained by selfing the F_1 plants generated from SSSL-W24 'female' and HJX74 'male' parents in the early season of 2006. Heterozygous plants were selected to produce a larger F_3 population for fine mapping of *gl-1*. In total, 1,585 F_3 plants were grown in the early season of 2007. Each plant was genotyped using molecular markers and phenotyped by visual examination at the maturation stage. The F_4 recombinant lines derived from the F_3 population were used for progeny testing.

All materials were planted and maintained in the experimental farm of South China Agricultural University, Guangzhou, China. The seeds were planted in a seed bed and transplanted to the field. The planting density was 16.67 cm between plants in a row, and the rows were 20.00 cm apart. Field management, including irrigation, fertilizer application and pest control, followed essentially the normal agricultural practice.

1.2 DNA extraction and PCR amplification

Microquantities of DNA were extracted from fresh leaves of each individual using a previously reported method[37]. DNA from the parental plants were extracted using the CTAB method[38]. The PCR profile used for amplification was basically according to the protocol described previously[39], and InDel marker amplification was performed as described[40]. PCR products were resolved on 6% non-denaturing polyacrylamide gel and subjected to the silver staining procedure as described[41].

1.3 Molecular marker developmenmt

SSR markers were developed by using the sequence of the delimited region from the International Rice Genome Sequencing Project (IRGSP) database (http://rgp.dna.affrc.go.jp/IRGSP/index.html). Suitable SSR sequences were identified using the online SSR identification tool SSRIT (http://www.gramene.org/microsat/). Primers for the amplification of target SSRs were designed using software Primer Premier 5.0 (Premier Biosoft International, http://www.premierbiosoft.com). Additionally, Insertion-Deletion (InDel) markers were developed by selecting suitable InDels (insertion or delection of 5-20 bp) in the delimited region from the genome-wide DNA polymorphism database of rice[42]; http://shengh-uan.shnu.edu.cn/ricemarker). Sequences of ~300 bp around the InDels were then

chosen and PCR primers were designed which amplified products ranged from 90 to 250 bp for analysis by polyacrylamide gel electrophoresis. Twelve newly developed Indel or SSR markers were listed in Tab. 1.

Tab. 1 Markers developed for *gl-1* mapping

Marker	Type	Sequence	Product size (bp)
PSM578	SSR	F: GGACTGGCTCTGCCTCGTTA	155
		R: GCAGGAGTGAGGGAGTAAGAA	
PSM581	SSR	F: TGCGTGTATGAGTTTGCTG	204
		R: CTAGTGATGGAAAAGGAGAAG	
PSM760	InDel	F: CAACTGTGCACCCAGGGA	97
		R: CAACACGACCAGCAAGGC	
PSM761	InDel	F: CAATGTTGAAGGTGGTGTA	161
		R: ACTGCTGACGTTTTGAGAC	
PSM762	InDel	F: TTTGCCCCTCCACCCACA	183
		R: GTCATAGGCGGCGCTATCA	
PSM764	InDel	F: ACGGGCTTGCGATTTATCTG	138
		R: GTCAACTAGAACATGGTCCAGC	
PSM781	InDel	F: TGATTGAACACGCCGTGC	164
		R: TGTTTGGAGGCCATCACG	
PSM782	InDel	F: TTGCGACCTGATTGTTCTTC	275
		R: CCTCACCATTATGACCTTTACC	
PSM784	InDel	F: CAGCTCCGCCTCCTTCCTC	233
		R: TCGGCACTCCACCTGATGTAA	
PSM794	SSR	F: TCCCCAGATCCCGCGTAACC	176
		R: GGCAAAAGGGCAAAGAAAGG	
PSM796	SSR	F: TTCTGCCTGCCTCCATCCTC	100
		R: GCCATCGCATTGCATTTCAC	
PSM798	SSR	F: GCGGCACGGCAACACTCT	204
		R:CCGATGCGGGCGACTTCT	

1.4 Linkage map and gene mapping

All the available markers were used for the construction of a linkage map and for map-based cloning of *gl-1*. Linkage analysis based on the 250-plant F2 population was conducted using Mapmaker/Exp3.0[43] to determine the order of the markers and the genetic distance between every two adjacent markers in the target region. In each recombinant family, the genotypes for all markers in the target region were determined and homozygous recombinants were identified. The recombinant plants were further confirmed by progeny testing.

2 Results

2.1 Detection and preliminary mapping of *gl-1*

SSSL-W24 exhibited glabrous leaves and hulls (Fig. 1), and it carried only one chromosomal segment of about 12.7 cM between the markers RM122 and PSM202 derived from the genome of

Star bonnet 99 on the short arm of chromosome 5 in the genetic background of HJX74. Hence, we speculated that this target Star bonnet 99 chromosomal segment co-segregated with the glabrous leaf and hull phenotype. The small F_2 population, consisting of 250 individual plants derived from the cross between SSSL-W24 and HJX74, were used to confirm our speculation. The 250 plants were genotyped using SSR markers on the substituted segment and phenotyped by visual examination at the maturation stage. The F_1 plants derived from the cross between SSSL-W24 and HJX74 had the same phenotype as HJX74; 62 of 250 F_2 plants showed the SSSL-W24 phenotype (3:1 ratio; χ^2= 0.02, P > 0.95) (Tab. 2), indicating that the glabrous plant was controlled by a single recessive gene, the locus of which was in consistent with that of *gl-1* reported previously[30,31]. Therefore, these data confirmed that the chromosomal segment containing the recessive gene *gl-1* from Star bonnet 99 was responsible for the glabrous leaf and hull phenotype.

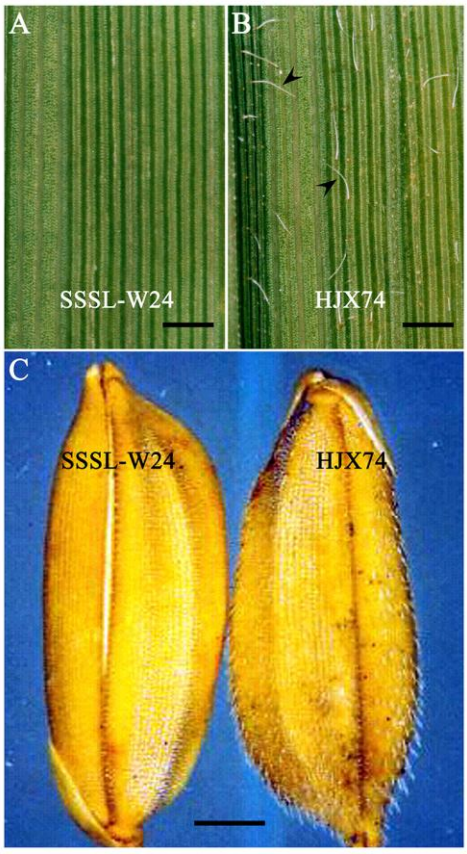

Fig. 1 Phenotypic characterization of glabrous traits in SSSL-W24 and HJX74. Trichome formation is absent in leaves and hulls for SSSL-74 (A and C left), but is normal for HJX74 (B and C right). Arrowheads indicate the pubescence present in leaf surface of HJX74. Scale bar equals to 200 μm for A and B, and 1mm for C.

Tab. 2 Phenotype segregation of the F_2 mapping population derived from SSSL-W24 and HJX74

Phenotype	No. of Plants	χ^2 value (3:1)
Glabrousness	62	0.02
Pubescence	188	
Total	250	

To preliminarily map the *gl-1* position, 6 RM markers were selected, and 6 SSR as well as 5

InDel markers were designed in the *gl-1* region, but only 2 SSR and 3 InDel revealed polymorphisms between the two parents. Primer sequences, marker types, map positions, and amplified lengths of these newly developed polymorphic markers are listed in Tab. 1. By using these 5 polymorphic markers, the *gl-1* gene was validated and mapped primarily to a 1.2 cM region between the markers PSM581 and PSM760, with genetic distance of 0.6 cM and 0.6 cM, respectively (Fig. 2A).

2.2 Fine mapping of *gl-1*

For further refining the position of *gl-1*, a larger F_3 segregating population containing 1,585 plants derived from the F_2 heterozygous plants in which the region around the *gl-1* locus was heterozygous was used to identify recombinant events between the *gl-1* locus and tightly linked markers.

Based on the available genomic sequence information of the reference Nipponbare and 93-11, the genetic interval between PSM581 and PSM760 covers ~ 186 kb, including three overlapping BAC clones OJ1654_B10, P0496H07 and P0699E04. A combination of SSRIT (http://www.gramene.org/microsat/) searching and genome sequence alignment focused on this region generated a further 5 SSR and 7 InDel markers, of which 3 SSR and 4 InDel markers were informative between HJX74 and Star bonnet 99 (Tab. 1). Thus, together with the markers PSM581 and PSM760, 9 markers were available for further fine mapping of *gl-1* gene. The subsequent recombinant screening work identified 22 recombinants in the target region from the 1,585-plant F_3 population and the *gl-1* locus was more precisely positioned in the interval between PSM796 and PSM781 (Fig. 2B) in detecting one recombinant event that occurred between marker PSM796 and *gl-1* (Fig. 2C), and other two recombinants occurred between marker PSM781 and *gl*-1 (Fig. 2C). The genotype and phenotype of each recombinant were confirmed by progeny analysis. Since PSM796 and PSM781 were produced from sequence information of the BAC clones P0496H07 and P0699E04, respectively (Fig. 2B), the sequences of the two contiguous contigs were analyzed with the Sequencer Program (Gene Code Corporation, Mich., USA) to further confirm the overlapping sequences and the physical distance between the *gl-1* gene and these closely linked markers. Consequently, the genomic region containing the *gl-1* locus was narrowed down to an 35.9 kb fragment defined by PSM796 at 940 kb and PSM781 at 975.9 kb on chromosome 5 (http://www.gramene.org) (Fig. 2B).

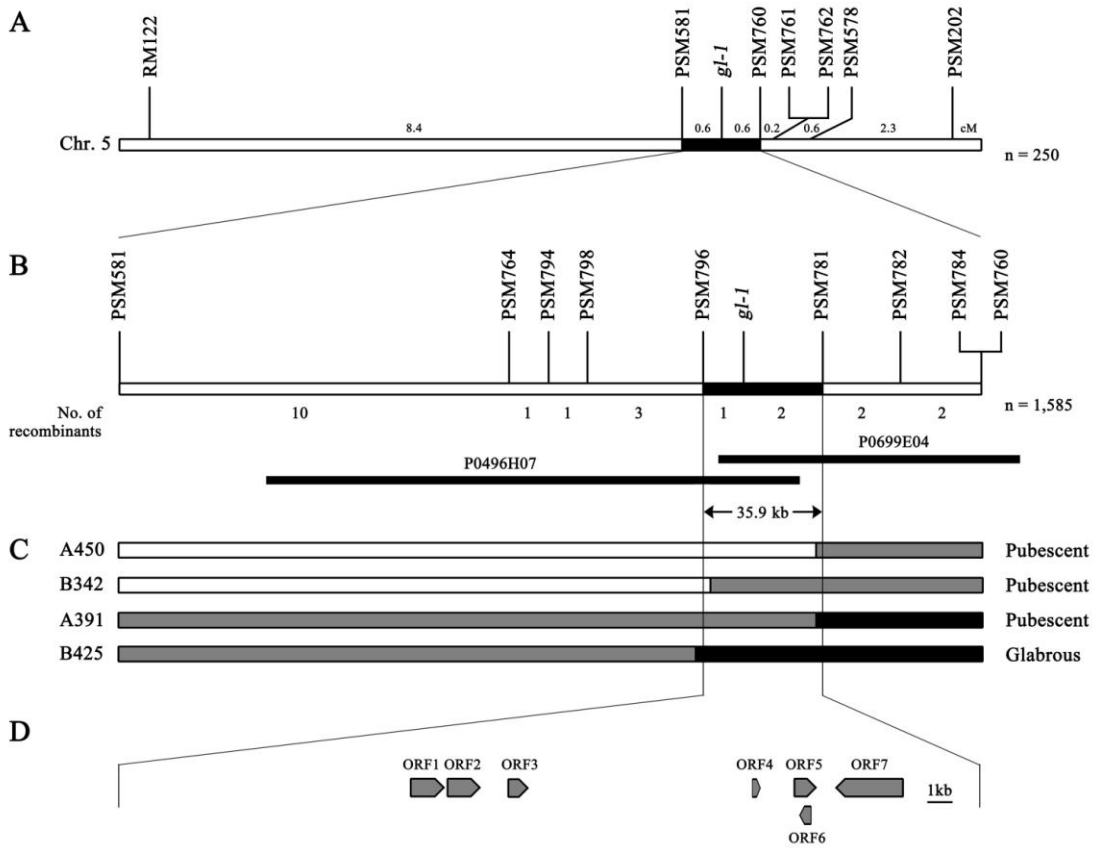

Fig. 2 Genetic and physical maps of the *gl-1* gene and candidate gene analysis.

A. Linkage map of chromosome 5 constructed using 250 F_2 incividuals. The *gl-1* gene was mapped to the region between markers PSM581 and PSM760. Numbers show genetic distance between adjacent markers.

B. Fine mapping of the *gl-1* gene. The *gl-1* gene was restricted to the region between markers PSM796 and PSM781 using a total of 1585 plants from segregated populations. The number of recombinants between the markers and *gl-1* is indicated under the linkage map. Physical distance between the markers PSM796 and PSM781 is also indicated in the picture. The black horizontal lines represent BAC clones of Nipponbare with the accession numbers as indicated.

C. The genotypes and phenotypes of the four recombinants (A450, B342, A391 and B425) between PSM581 and PSM760. The white and black regions indicate the segments from HJX74 and Star bonnet 99, respectively, while the gray regions indicate heterozygote.

D. Candidated region of the *gl-1* locus and the annotated gene in japonica Nipponbare from RGAP (http://rice.plantbiology.msu.edu/).

2.3 Putative genes in the 35.9 kb DNA fragment

According to available sequence annotation databases (http://rice.plantbiology.msu.edu/; http://rgp.dna.affrc.go.jp/), there are seven annotated genes (LOC_Os05g02710, LOC_Os05g02720, LOC_Os05g02730, LOC_Os05g02740, LOC_Os05g02750, LOC_Os05g02754, and LOC_Os05g02760) in the 35.9 kb target region (Fig. 2D and Tab. 3). Of these genes, five are with unknown function, and the remaining ones consist of LOC_Os05g02730, an expressed homeobox domain containing protein, and LOC_Os05g02750, a putative ABC transporter without an ATPase domain, whose homolog *ALS3* in *Arabidopsis* is required for aluminum (Al) tolerance.

Tab. 3 Candidate genes for *gl-1*

ORF	**Candidate gene**	**Description**
ORF 1	LOC_Os05g02710	Putative unclassified retrotransposon protein
ORF 2	LOC_Os05g02720	Hypothetical protein
ORF 3	LOC_Os05g02730	Homeobox domain containing protein, expressed
ORF 4	LOC_Os05g02740	Unknown protein, expressed
ORF5	LOC_Os05g02750	Putative ABC transporter, expressed
ORF 6	LOC_Os05g02754	Unknown protein, expressed
ORF 7	LOC_Os05g02760	Unknown protein, expressed

3 Discussion

The most significant finding in our study was the delimitation of the *gl-1* gene in Star bonnet 99, which had effects on trichome formation in rice, to a DNA fragment of approximately 35.9 kb in length. Identification of quantitative trait loci (QTL) has become a popular topic in genetics in recent years, and QTL mapping has become a very important tool for finding the genes that regulate complex quantitative traits. F_2 populations, recombinant inbred lines (RILs) and doubled haploid lines (DHs) have been used widely for primary mapping. Advanced populations such as near isogenic lines (NILs), chromosomal segment substitution lines (CSSLs), and single segment substitution lines (SSSLs) can be used for fine mapping QTLs to a locus as a Mendelian factor by blocking genetic background noise[37,44]. We developed a library of single segment substitution lines (SSSLs) from crosses between HJX74 and 26 rice accessions collected worldwide to clarify the genetic basis of complex traits of agronomic importance[35,36,45]. This library has been used for mapping and cloning of *GIF1*, a gene required for carbon partitioning during early grain-filling of rice[46]. Therefore, this library is suitable for detecting the genes or QTLs for complex traits. Actually, a total of more than 2,000 genes or QTLs were found by whole genome surveying.

Based on the available sequence annotation database (http://rice.plantbiology.msu.edu/; http://www.rgp.dna.affrc.go.jp/), the candidate gene for *gl-1* was identified. There are seven predicted genes in the target region according to the genome sequence of Nipponbare (*O. sativa* ssp. *japonica*). Among them, the most likely candidate was LOC_Os05g02730, an expressed homeobox domain containing protein, reminding us the *GL2* gene in *Arabidopsis*, which was the only homeodomain protein taking charge in the *Arabidopsis* trichome development. *GL2*, the mutation of which caused a glabrous phenotype, plays a key role in trichome formation in mediating the input of different trichome patterning genes into trichome differentiation machinery[47]. In addition to the homeodomain, *GL2* contains a START (steroidogenic acute regulatory protein-related lipid transfer) domain that has a putative steroid-binding activity, but this START domain is missing in the expressed LOC_Os05g02730 protein. Actually, the role of the START domain in *GL2*'s function in trichome formation is unclear. As shown in *Arabidopsis gl2-1* mutant, the *GL2* truncated protein lacking the START domain still retain a partial activity responsible for the formation of rudimentary trichomes[48].

We have shown here that rice trichome formation is influenced by *gl-1* and we speculate that LOC_Os05g02730 is a strong candidate for the underlying *gl-1* locus. Among the other six candidates, LOC_Os05g02750 encodes a putative ABC transporter without an ATPase domain. The homolog of LOC_Os05g02750 in *Arabidopsis*, *ALS3*, is required for aluminum (Al) tolerance and may function to redistribute accumulated Al away from sensitive tissues in order to protect the growing root from the toxic effects of Al[49]. The other five candidates are with unknown function. LOC_Os05g02710 encodes a putative unclassified retrotransposon protein, containing a transposase DDE domain. LOC_Os05g02720, LOC_Os05g02740, LOC_Os05g02754 and LOC_Os05g02760 encode hypothetical or expressed proteins without any conserved domain. Of course, the gene underlying the *gl-1* phenotype might have other candidates in light of the information available. Our analysis was conducted based on the genome sequence of the cultivated japonica rice Nipponbare, while *gl-1* was derived from Star bonnet 99. We can not rule out the possibility of gene loss in Nipponbare compared with Star bonnet 99 in the *gl-1* region. Therefore, we are conducting ongoing studies to completely sequence the region of the Star bonnet 99 and HJX74 genomes corresponding to the 35.9 kb target region in Nipponbare in order to determine the candidate. At the same time, complement testing is being performed using these seven candidate genes, and studies of genetic basis and function of *gl-1* are underway.

The *gl-1* gene can be utilized to improve rice breeding in many aspects. The visible traits have many potential advantages in rice genetic improvement because breeders still utilize morphological traits as selection markers in conventional rice breeding processes[50]. With the development of hybrid rice in China, its seed purity has become more and more important. Male sterile or restorer lines with visible morphological traits are very useful in identifying seed purity and have shown great potential in hybrid rice breeding programs. As a physical marker, the glabrousness trait can be introgressed into sterile or restorer lines with the objective of identify hybrids.

In addition, glabrous rice with a smooth leaf and hull is considerd to have health benefits, since the lack of trichomes on leaves and glumes results in less dust and less skin irritation and itching among workers during harvesting, threshing, drying and milling of rice[51]. Furthermore, Glabrous rice is propitious for mechanized manipulation of modern agriculture[28], and the capacity of storage of which is 10%～15% more than varieties which have lemma hair[27].

4 Conclusion

In this paper, we delimited the *gl-1* gene in Star bonnet 99 to a DNA fragment of approximately 35.9 kb in length, including seven putative ORFs. The genetic and morphological information obtained in this study would be very useful for final successful cloning of *gl-1* gene, and for elucidating the mechanisms of trichome formation in rice and understanding its differences in the genetical controls in rice and *Arabidopsis*. In addition, our continuing work on genetic basis and function of *gl-1* will also certainly be used to assist selection of hybrid rice, facilitate the mechanization of agriculture and increase the warehousing capacity of rice through approaches such as marker-assisted selection (MAS), metabolic engineering and transgenic modification.

Acknowledgements

This study was finacially supported by grants from the National Natural Science Foundation of P.R. China (31200905), the Guangdong Provincial Natural Science Foundation of China (10451064201005483), the Specialized Research Fund for the Doctoral Program of Higher Education (20104404120005), and the 40th Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

References

[1] Larkin JC, Brown ML, Schiefelbein J. How do cells know what they want to be when they grow up? Lessons from epidermal patterning in Arabidopsis[J]. Annu Rev Plant Biol, 2003, 54: 403-430.
[2] Werker E. Trichome diversity and development[J]. Adv Bot Res, 2000, 31: 1-35.
[3] Wilkins TA, Rajasekaran, K., and Anderson, D.M. Cotton biotechnology[J]. Crit Rev Plant Sci, 2000, 53: 511-550.
[4] Koornneef M, Dellaert LW, van der Veen JH. EMS- and radiation-induced mutation frequencies at individual loci in Arabidopsis thaliana (L.) Heynh[J]. Mutat Res, 1982, 93(1): 109-123.
[5] Foster K W RJN. Independent segregation of semi-drwafing genes and a gene for pubescence in rice[J]. Heredity, 1978, 69: 137-138.
[6] Moose SP, Lauter N, Carlson SR. The maize macrohairless1 locus specifically promotes leaf blade macrohair initiation and responds to factors regulating leaf identity[J]. Genetics, 2004, 166(3): 1451-1461.
[7] Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW. The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root[J]. Dev Biol, 1994, 166(2): 740-754.
[8] Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, Marks MD, Gray JC. The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein[J]. Plant Cell, 1999, 11(7): 1337-1350.
[9] Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD. A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules[J]. Cell, 1991, 67(3): 483-493.
[10] Payne CT, Zhang F, Lloyd AM. GL3 encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1[J]. Genetics, 2000, 156(3): 1349-1362.
[11] Zhang F, Gonzalez A, Zhao M, Payne CT, Lloyd A. A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis[J]. Development, 2003, 130(20): 4859-4869.
[12] Rerie WG, Feldmann KA, Marks MD. The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in Arabidopsis[J]. Genes Dev, 1994, 8(12): 1388-1399.
[13] Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW. The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana[J]. Development, 1996, 122(4): 1253-1260.
[14] Schnittger A, Folkers U, Schwab B, Jurgens G, Hulskamp M. Generation of a spacing pattern: the role of triptychon in trichome patterning in Arabidopsis[J]. Plant Cell, 1999, 11(6): 1105-1116.
[15] Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, Beermann A, Thumfahrt J, Jurgens G, Hulskamp M. TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis[J]. Embo J, 2002, 21(19): 5036-5046.
[16] Wada T, Kurata T, Tominaga R, Koshino-Kimura Y, Tachibana T, Goto K, Marks MD, Shimura Y, Okada K. Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation[J]. Development, 2002, 129(23): 5409-5419.
[17] Wada T, Tachibana T, Shimura Y, Okada K. Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC[J]. Science, 1997, 277(5329): 1113-1116.
[18] Esch JJ, Chen MA, Hillestad M, Marks MD. Comparison of TRY and the closely related At1g01380 gene in controlling Arabidopsis trichome patterning[J]. Plant J, 2004, 40(6): 860-869.
[19] Kirik V, Simon M, Huelskamp M, Schiefelbein J. The ENHANCER OF TRY AND CPC1 gene acts redundantly with TRIPTYCHON and CAPRICE in trichome and root hair cell patterning in Arabidopsis[J]. Dev Biol, 2004, 268(2): 506-513.
[20] Kirik V, Simon M, Wester K, Schiefelbein J, Hulskamp M. ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichome development of Arabidopsis[J]. Plant Mol Biol, 2004, 55(3): 389-398.
[21] Wang S, Kwak SH, Zeng Q, Ellis BE, Chen XY, Schiefelbein J, Chen JG. TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in Arabidopsis[J]. Development, 2007, 134(21): 3873-3882.
[22] Schiefelbein J. Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot[J]. Curr Opin Plant Biol, 2003, 6(1): 74-78.
[23] Pesch M, Hulskamp M. Creating a two-dimensional pattern de novo during Arabidopsis trichome and root hair initiation[J]. Curr Opin Genet Dev, 2004, 14(4): 422-427.
[24] Hulskamp M, Misra S, Jurgens G. Genetic dissection of trichome cell development in Arabidopsis[J]. Cell,

1994, 76(3): 555-566.
[25] Esch JJ, Chen M, Sanders M, Hillestad M, Ndkium S, Idelkope B, Neizer J, Marks MD. A contradictory GLABRA3 allele helps define gene interactions controlling trichome development in Arabidopsis[J]. Development, 2003, 130(24):5885-5894.
[26] Yan ZB. Analysis for compatibility in some smooth rice varieties of USA[J]. Seed, 1998, 4: 8-10. (In chinese).
[27] Guo LB, Luo LJ, Zhong DB, Yu XQ, Mei HW, Wang YP, Ying CS. Evaluation, improvement and utilization on some selected american rice cultivars[J]. Journal of Agricultural Science, 1999, 5): 201-206. (In chinese).
[28] Hu PS, Tang SQ, Luo J, Huang FS. Utilization of american glabrous rice and breeding of super-high-yield varieties[J]. Acta Agronomica Sinica, 1999, 25(1): 32-38. (In chinese).
[29] Xu XF, Mei HW, Luo LJ, Cheng XN, Li ZK. RFLP-facilitated investigation of the quantitative resistance of rice to brown planthopper (Nilaparvata lugens)[J]. Theor Appl Genet, 2002, 104(2-3): 248-253.
[30] Yu ZH, McCouch SR, Tanksley SD, Kinoshita T, Sato S. Association of morphological and RFLP markers in rice (Oryza sativa L.)[J]. Genome, 1995, 38(3):566-574.
[31] Wang D, Sun SX, Gao FY, Lu XJ, Li ZH, Ren GJ. Mapping a rice glabrous gene using simple sequence repeat markers[J]. Rice Sci, 2009, 16(2): 93-98.
[32] Qian Q, He P, Zheng X, Chen Y, Zhu L. Genetic analysis of morphological index and its related taxonomic traits for classification of indica/japonica rice[J]. Sci China C Life Sci, 2000, 43(2): 113-119.
[33] Ding Y, Wang X, Su L, Zhai J, Cao S, Zhang D, Liu C, Bi Y, Qian Q, Cheng Z, Chu C et al. SDG714, a histone H3K9 methyltransferase, is involved in Tos17 DNA methylation and transposition in rice[J]. Plant Cell, 2007, 19(1): 9-22.
[34] Peres A, Churchman ML, Hariharan S, Himanen K, Verkest A, Vandepoele K, Magyar Z, Hatzfeld Y, Van Der Schueren E, Beemster GT, Frankard V et al. Novel plant-specific cyclin-dependent kinase inhibitors induced by biotic and abiotic stresses[J]. J Biol Chem, 2007, 282(35): 25588-25596.
[35] Talukar A, Zhang GQ. Construction and characterization of 3-S Lines, an alternative population for mapping studies in rice (Oryza sativa L.)[J]. Euphytica, 2007, 156: 237-246.
[36] Xi ZY, He FH, Zeng RZ, Zhang ZM, Ding XH, Li WT, Zhang GQ. Development of a wide population of chromosome single-segment substitution lines in the genetic background of an elite cultivar of rice (Oryza sativa L.)[J]. Genome, 2006, 49(5): 476-484.
[37] Shan JX, Zhu MZ, Shi M, Gao JP, Lin HX. Fine mapping and candidate gene analysis of spd6, responsible for small panicle and dwarfness in wild rice (Oryza rufipogon Griff.)[J]. Theor Appl Genet, 2009, 119(5):827-836.
[38] Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA[J]. Nucleic Acids Res, 1980, 8(19): 4321-4325.
[39] Panaud O, Chen X, McCouch SR. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.)[J]. Mol Gen Genet, 1996, 252(5): 597-607.
[40] Li WT, Zeng RZ, Zhang ZM, Ding XH, Zhang GQ. Fine mapping of locus S-b for F1 pollen sterility in rice (Oryza sativa L.)[J]. Chinese Sci Bull, 2006, 51: 675-680.
[41] Li WT, Zeng RZ, Zhang ZM, Zhang GQ. Mapping of S-b locus for F1 pollen sterility in cultivated rice using PCR based markers[J]. Acta Bot Sin, 2002, 44: 463-467.
[42] Shen YJ, Jiang H, Jin JP, Zhang ZB, Xi B, He YY, Wang G, Wang C, Qian L, Li X, Yu QB et al. Development of genome-wide DNA polymorphism database for map-based cloning of rice genes. Plant Physiol, 2004, 135(3): 1198-1205.
[43] Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations[J]. Genomics, 1987, 1(2): 174-181.
[44] Xing YZ, Tang WJ, Xue WY, Xu CG, Zhang Q. Fine mapping of a major quantitative trait loci, qSSP7, controlling the number of spikelets per panicle as a single Mendelian factor in rice[J]. Theor Appl Genet, 2008, 116(6): 789-796.
[45] Liu G, Zeng R, Zhu H, Zhang Z, Ding X, Zhao F, Li W, Zhang G. Dynamic expression of nine QTLs for tiller number detected with single segment substitution lines in rice[J]. Theor Appl Genet, 2009, 118(3): 443-453.
[46] Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, He W, Lu B, Lin H, Ma H et al. Control of rice grain-filling and yield by a gene with a potential signature of domestication[J]. Nat Genet, 2008, 40(11): 1370-1374.
[47] Ishida T, Kurata T, Okada K, Wada T. A genetic regulatory network in the development of trichomes and root hairs[J]. Annu Rev Plant Biol, 2008, 59: 365-386.
[48] Di Cristina M, Sessa G, Dolan L, Linstead P, Baima S, Ruberti I, Morelli G. The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development[J]. Plant J, 1996, 10(3): 393-402.
[49] Larsen PB, Geisler MJ, Jones CA, Williams KM, Cancel JD. ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in Arabidopsis[J]. Plant J, 2005, 41(3): 353-363.
[50] Wang WY, Ding HF, Li GX, Jiang MS, Li RF, Liu X, Zhang Y, Yao FY. Delimitation of the PSH1(t) gene for rice purple leaf sheath to a 23.5 kb DNA fragment[J]. Genome, 2009, 52(3): 268-274.
[51] Lee SY, Cheong JI, Kim TS. Production of doubled haploids through anther culture of M1 rice plants derived from mutagenized fertilized egg cells[J]. Plant Cell Rep, 2003, 22(3): 218-223.

水稻表皮毛形成基因 gl-1 的精细定位和候选基因分析

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摘要：叶表皮毛对于保护植物免受昆虫攻击和防止水分散失具有非常重要的作用。本研究从 Star bonnet 99 为供体，HJX74 为受体的单片段代换系中鉴定得到一个控制水稻表皮毛的隐性基因 gl-1。通过利用该单片段代换系和 HJX74 构建的 F2 群体对 gl-1 进行精细定位，将其定位于第 5 染色体上 35.9 kb 的物理距离内，包含 7 个注释基因。对该基因的克隆将有助于杂交水稻的筛选除杂，水稻的机械化操作水平的提高和水稻的仓储效率的提升。

关键词：作物遗传育种；水稻；表皮毛；单片段代换系；精细定位；光身基因；
中图分类号：S5 农作物