A WUSCHEL-like homeobox gene, OsWOX3B responses to NUDA/GL-1 locus in rice

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Abstract

Background: Most of the rice varieties are pubescent. However, the presence of trichomes is an undesirable characteristic in rice production because trichomes can cause atmospheric pollution. The use of glabrous rice varieties represents a solution to this problem. Yunnan Nuda Rice, a glabrous cultivar that constitutes approximately 20% of rice germplasms in Yunnan can provide important recourse for breeding of glabrous rice varieties.

Results: The “Nuda” phenotype in Yunnan Nuda Rice was found to be controlled by a single recessive allelic gene within the well-characterized GL-1 locus. A high-resolution genetic and physical map was constructed using 1,192 Nuda individuals from the F2 population that was delivered from the cross between the Yunnan Nuda variety HMK and the pubescent TN1 variety. The NUDA/GL-1 gene was mapped to a 28.5 kb region containing six annotated genes based on the Nipponbare genomic sequence. By comparing the sequences and expression patterns of different pubescent and glabrous varieties, LOC_Os05g02730, a WUSCHEL-like homeobox gene (OsWOX3B) was identified as the candidate gene. This hypothesis was confirmed by RNA interference (RNAi) and transgenic complementation. Trichome deficiency in RNAi lines was associated with increased efficiency of grain packaging but did not affect the main agronomic traits.

Conclusion: NUDA/GL-1 locus encodes OsWOX3B gene.

Keywords: Yunnan nuda rice, Nuda/glabrous, Map-based cloning, WUSCHEL-like homeobox gene OsWOX3B

Background

Trichomes are ubiquitous in land plants (Southwood 1986; Werker 2000). The density, morphology, and chemical composition of leaf hairs vary widely and these factors contribute to their diverse physiological, physical and chemical functions (Southwood 1986; Werker 2000). However, the presence of trichomes is an undesirable characteristic in rice production due to the generation of dust during harvesting and grain manipulating processes. Trichomes are important causes of atmospheric pollution and these pollutants can cause acute and chronic irritation of the eyes, skin, and respiratory tract, which subsequently result in intolerable itching and allergy, and even longstanding diseases such as Rice Miller’s Syndrome (Lim et al. 1984). Therefore, the use of glabrous rice varieties, which are trichome-deficient on the leaves and glumes, represents a solution to this problem.

Natural and artificial glabrous mutants have been identified in model plants such as Arabidopsis (Karkkainen and Agren 2002) and cereal crop species including rice (Foster and Rutger 1978), maize (Moose et al. 2004), wheat (Leisle 1974) barley (Sato and Takeda 1992) oats (Sarkarung and Collins 1977) pearl millet (Kumar and Andrews 1993) and sorghum (Gibson and Maiti 1983). In particular, trichome formation in Arabidopsis has been used as a model system to study various developmental and cellular mechanisms in plants (Hulskamp 2004; Marks 1997). With mutagenesis screens, dozens of genes involved in trichome initiation, spacing, and shape have been identified in Arabidopsis (Marks 1997; Hulskamp and Schnittger 1998 Serna and Martin 2006 Szymanski et al. 2000). These genes encode several classes
of transcriptional factors, including MYB (GLI) (Larkin et al. 1993; Oppenheimer et al. 1991), WD-40 (TTG1) (Walker et al. 1999), bHLH (GL3/EGL3) (Payne et al. 2000) and HD-ZIP (GL2) (Johnson et al. 2002; Szymanski et al. 2000). Recent studies of Arabidopsis have shown a complex that consists of GL1-GL3/EGL3- TTG1 promote GL2 expression to regulate hair cell differentiation (Payne et al. 2000; Lloyd et al. 2000). Several other MYB proteins, including CYC, TRY (Schellmann et al. 2002) and ETC1 (Kirik et al. 2004), compete with GL1 by interacting with bHLH proteins to repress trichome initiation on the leaf (Schellmann et al. 2002). However, no glabrous genes have been cloned in cereal crop species to date.

Glabrous rice varieties have been identified in different locations worldwide including America, Yunnan in China and regions of south-eastern and southern Asia. Two loci (GL-1 and GL-2) for glabrous leaf and hull characteristics have been identified by classic genetic analysis. Two complementary genes (Hla and Hlb) have been identified for long pubescence on leaves, and one gene (Hg) has been identified for long pubescence on floral hulls (Nagao et al. 1960). Among these genes, only the GL-1 gene from American glabrous rice has been mapped to chromosome 5 with the use of restriction fragment length polymorphism (RFLP) markers (Yu et al. 1995). This locus was further narrowed to a region of approximately 157 kb (Wang et al. 2009) and 54 kb (Li et al. 2010) by two independent research groups.

Yunnan is a center of rice landrace diversity. It is reported that about 20% of the Yunnan landraces are glabrous rice varieties, known as Yunnan Nuda Rice (Qin et al. 1997). In this study, the glabrous phenotypes of Yunnan upland Nuda rice landrace HMK were characterized. By genetic analysis, the glabrous phenotype of HMK was shown to be conferred by a pair of recessive genes, and this locus was allelic with the reported GL-1 locus. Furthermore, the Nlida/GL1 gene was cloned using a map-based approach and identified as a WUSCHEL-like gene (LOC_Os05g02730) belonging to the homeodomain (HD) subfamily.

**Results**

**Characterization of Yunnan nuda rice HMK variety**

The presence of trichomes is ubiquitous in rice varieties. Leaf and grain phenotypes of the pubescent TN1 variety and the glabrous Yunnan Nuda Rice HMK variety were examined using stereomicroscopy and scanning electron microscopy (SEM).

Trichomes were observed on the upper (adaxial) surface and along the veins of the leaf blade and on glumes of pubescent rice varieties, such as TN1 (Figures 1a, 1b). Three types of trichomes were observed under SEM of the leaf: macro, micro and glandular hairs (Figure 1e). Macro hairs were located on silica cells over a thin vascular bundle, whereas micro and glandular hairs were located along the stomata cells or adjacent to motor cells (Figure 1f). Very few macro hairs were observed on the lower (abaxial) surface of the leaf (Figure 1g). On the glumes, hairs were distributed on the valleys between two swelled longitudinal veins (Figure 1h).

Both the leaf and hull are glabrous and smooth in Yunnan Nuda Rice variety HMK (Figures 1c and 1d). Small bulges were present in place of macro hairs on the leaves (Figure 1i), although glandular hairs and occasional micro hairs were still observed (Figure 1j). There were no macro hairs on the lower (abaxial) surface.
Genetic analysis and fine mapping of the NUDA locus

The genetic basis of the Nuda phenotype was analyzed. All F1 hybrids resulting from the cross between TN1 and a Yunnan Nuda Rice variety, HMK, exhibited a trichomous leaf phenotype. One-hundred and fifty-eight F2 individuals were screened and 120 trichomous leaf individuals and 38 glabrous individuals were identified. A three to one ($\chi^2 = 0.076$, $\chi^2_{0.05} = 3.84$) segregation ratio was determined. These results indicated that the Nuda phenotype was controlled by a single pair of recessive genes.

American rice varieties commonly exhibit a glabrous phenotype similar to that of Yunnan Nuda Rice. The GL-1 gene conferring this phenotype has been mapped to the start of chromosome 5 (Chr. 5). To determine whether NUDA is allelic to GL-1, the HMK variety was crossed with the glabrous American variety, Lemont. All F1 plants exhibited a glabrous phenotype (Figure 2 a-c), which indicated that the NUDA locus is allelic to GL-1.

Simple sequence repeat (SSR) markers located at the start of Chr. 5 were used for the primary mapping of NUDA. Linkage analysis of 158 F2 individuals showed linkage of the RM17713 and RM13 markers with the NUDA locus at distances of 7.7 and 7.2 cm, respectively (Figure 2d). The NUDA locus was mapped between markers GL2 and GL8 (Figure 2d). A high-resolution genetic and physical map for the fine mapping of NUDA was constructed by the analysis of 11 newly developed markers, including 10 insertion/deletion (InDel) markers and one single nucleotide polymorphism (SNP) marker in 1,192 F2 Nuda individuals derived from the TN1 and HMK cross (Table 1). The NUDA locus was mapped to a 28.5 kb interval between markers GL7 and GLSNP (Figure 2e). Six annotated genes were identified in this defined interval based in P1 artificial chromosome (PAC) clone AP001111 from the Nipponbare variety. These were LOC_Os05g02710 (retrotransposon protein), LOC_Os05g02720 (hypothetical protein), LOC_Os05g02730 (WUSCHEL-related homeobox 3B), LOC_Os05g02740 (expressed protein), LOC_Os05g02750 (membrane protein) and LOC_Os05g02754 (expressed protein) (Figure 2f).

NUDA/GL-1 candidate genes

Genomic sequences of the candidate region defined by the markers GL7 and GLSNP were retrieved from the sequence of Nipponbare PAC clone P0699E04 (AP001111) and 9311 contig Ctg015203 (AAAA02015203). A large deletion including LOC_Os05g02710 and LOC_Os05g02720 in 9311 was confirmed by PCR and sequence analyses, indicating that these genes do not encode NUDA/GL-1 in the pubescent Nipponbare and 9311 varieties (Figure 2g).
The remaining four candidate genes were identified as follows: LOC_05g02750 encodes a bacterial-type ATP binding cassette (ABC) transporter protein involved in efflux transport activity specific for UDP-glucose and aluminum tolerance in rice (Ma et al. 2009); LOC_05g02740 and LOC_05g02754 encode expressed proteins with unknown functions; and LOC_05g02730, a WUSCHEL-like homeobox gene (OsWOX3B).

The sequences and expression patterns of these four genes were investigated to identify the NUDA/GL-1 candidate gene. The sequences of these genes were identical to those of Nipponbare. Therefore, the expression patterns of these genes were further investigated in leaves and young panicles from HMK and TN1 varieties. A similar expression pattern of LOC_05g02740 and LOC_05g02750/LOC_05g02754 was observed in both varieties. LOC_05g02730 expression was detected in the panicles of TN1 but not HMK, although expression of this gene was not detected at vegetative stage in either variety (Figure 3). These results implicated LOC_05g02730 (OsWOX3B) as the NUDA/GL-1 candidate gene.

RNAi and genetic complementation of the NUDA/GL-1 gene
RNAi and genetic complementation analyses were performed to confirm that OsWOX3B corresponded to NUDA/GL-1. The transfer of an RNAi construct designed to repress OsWOX3B expression into Nipponbare resulted in transgenic lines that exhibited a glabrous phenotype in leaves (Figure 4a-d) and hulls (Figure 4e-h) from T0 generation. Furthermore, a 4.2 kb fragment including the promoter and coding region of OsWOX3B was cloned into pCAMBIA1301 and transferred into HMK. Pubescent transgenic rice plants with trichomes on the leaves were obtained, although the shapes of the trichomes were different and the alignments of the cells were disordered, which probably were due to the ectopic expression of OsWOX3B in transgenic plants (Figure 4i-k). These results indicated that the OsWOX3B corresponded to the NUDA/GL-1 gene and was responsible for the glabrous phenotype in HMK Nuda rice.

NUDA/GL-1/OsWOX3B encodes a member of WOX3 subfamily
Both the 5’ and 3’ ends of the NUDA/GL-1/OsWOX3B cDNA were obtained by the rapid amplification of cDNA ends (RACE) from the mRNA extracted from the panicles of the pubescent variety. The full length of NUDA/GL-1/OsWOX3B cDNA is 1019 bp, including an open reading frame with 780 bp, a 5’-untranslated region (UTR) with 132 bp and a 3’-UTR with 107 bp (Figure 5a). By comparing the sequences of the full length cDNA and the relevant genomic region, two introns were identified (Figure 5b). The putative protein contains a homeodomain (HD), which is a DNA-binding domain and a WUSCHEL domain, which indicated it...
belonged to the *WUSCHEL*-like homeobox (WOX) gene family (Figure 5a).

Fourteen *OsWOX* genes and fifteen *AtWOX* genes were identified based on previous studies (Dai et al. 2007; Haecker et al. 2004 Nardmann et al. 2007 Vandenbussche et al. 2009 Zhang et al. 2010) and BLAST results in this study. BLAST searches of the NCBI Non-redundant Protein Sequences Database (http://blast.ncbi.nlm.nih.gov/) were performed using the homeodomain sequence of *AtWUS* (*At2g17950*) (Haecker et al. 2004) as a query. Phylogenetic analysis of these genes and four maize WOX genes, which belong to the same subgroup of *OsWOX3*, was performed based on the sequences of the homeodomain regions. *OsWOX3B* was shown to be a homolog of *AtWOX3/PRS* (Figure 5c). In contrast to *Arabidopsis*, three rice genes and four maize genes were identified in the WOX3 subfamily. The phylogenetic tree map indicated that genes of WOX3 subfamily were divided into several subgroups. *LOC_Os11g01130* (also known as *OsNS2* or *OsWOX3*) (Zhang et al. 2010; Dai et al. 2007), *LOC_Os12g01120* (also known as *OsNS* or *OsNS1*) (Nardmann et al. 2007; Zhang et al. 2010), *ZmNS1* and *ZmNS2* were tightly related and close to *AtWOX3/PRS*. *NUDA/GL-1/OsWOX3B* was in a separate subgroup (Figure 5c).

The expression patterns of *NUDA/GL-1/OsWOX3B* in the root, shoot apical meristem (SAM), leaf sheath, leaf blade and panicle from the TN1 and HMK varieties were detected by RT-PCR. The expression of this gene could be detected in the panicle but not in the root, SAM, sheath or blade in pubescent rice TN1. In Nuda Rice HMK, no expression was detected in each tested tissue (Figure 6).

**Trichome deficiency increases efficiency of grain packaging**

The agronomic traits of Nipponbare and three *OsWOX3B/NUDA/GL-1* RNAi transgenic lines were investigated. No significant differences were observed in terms of plant height, tiller number, specklet number per panicle, seed setting percentage and grain weight in RNAi lines compared with Nipponbare (Additional file 1: Table S1). These results indicated that *OsWOX3B/NUDA/GL-1* RNAi had no effect on these agronomic traits. Similarly, no significant differences were observed in grain size and shape, including grain length, grain width, grain thickness and ratio of length and width in RNAi lines compared with Nipponbare. These findings indicated that *OsWOX3B/NUDA/GL-1* RNAi did not affect the size and shape of grains (Figure 7a, Additional file 2: Figure S1). However, the efficiency of grain packaging was increased in the three RNAi transgenic lines as measured by the number and weight of dried grains in a 100 ml volume (Nipponbare, 2,119.2 ± 23.0 grains weighing 49.6 ± 0.5 g; three *OsWOX3B/NUDA/GL-1* RNAi transgenic lines: 2,432.7 ± 18.4 grains weighing 57.8 ± 0.5 g; 2,406.5 ± 9.1 grains weighing 57.6 ± 0.2 g and 2,431.9 ± 9.2 grains weighing 57.9 ± 0.2 g) (Figure 7b).

**Discussion**

The glabrous trait is beneficial for rice production due to the consequent reduction in dust pollution during grain...
harvesting, drying and processing. Furthermore, it has been observed that the bulk density of the glabrous rice varieties was higher (Rutger and Mackill 2001). Theoretically, the lack of trichomes can make the glabrous kernels packed closer because the spaces occupied by the trichomes are released. It is clear that the size and shape of the grains also affect the grain packaging efficiency. In this study, the grain size and shape of OsWOX3B RNAi lines were similar with those of wild type plants. These strictly isogenic materials provided convincing evidence that lack of glum hair truly increased the efficiency of grain packaging. Glabrous rice varieties have been found in many locations worldwide and glabrous germplasm has been used to develop glabrous varieties. Studies suggest the existence of at least two loci that confer the glabrous phenotype in leaves and hulls although they have not been cloned. In this study, the NUDA/GL-1/OsWOX3B gene was cloned by using a map-based approach and the locus was shown to encode a WUSCHEL-like homeobox gene (OsWOX3B) by RNAi and complementation transgenic studies.

Trichome is an excellent model system to study cell fate determination, cell cycle regulation, cell polarity and cell expansion in the model plant species, Arabidopsis. Recently, a number of genes that control the initiation and morphogenesis of trichomes have been identified.

Figure 5 NUDA/GL-1/OsWOX3B encodes a member of WOX3 subfamily. a Sequences of cDNA and amino acid of NUDA/GL-1/OsWOX3B, the homeodomain is underlined, and the WUSCHEL domain is framed. b The splicing pattern of NUDA/GL-1/OsWOX3B, the open bar indicates the introns, the diagonal bar indicates the ORF, the gray bar indicates the UTRs. c Phylogenetic analysis of WOX genes. Neighbor-joining trees of WOX proteins from rice (Os), Arabidopsis (At) and maize (Zm). For the neighbor-joining tree, 1,000 boot-strap samples were generated to assess support for the inferred relationships.

Figure 6 RT-PCR of OsWOX3B/NUDA/GL-1 in the root, SAM, leaf sheath, leaf blade and panicle from TN1 and HMK. R, root; SM, SAM; S, leaf sheath; L, leaf blade.
using this model (Serna and Martin 2006; Szymanski et al. 2000), many of which encode several classes of transcriptional factors including MYB, WD-40, bHLH and HD-ZIP. However, experimental evidence has suggested that this model is limited to plants that belong to the Rosid division of the Eudicots (Serna and Martin 2006). The PALE ALEURONE COLOUR (PAC) gene in maize that encodes a WD repeat protein can complement the glabrous phenotype of *ttg1* mutant in Arabidopsis. Interestingly, the *pac* mutant has no trichome phenotype (Carey et al. 2004). In this study, a WUSCHEL-like homeobox (WOX) gene was identified that controlled trichome development on the leaf and hull. These results suggested a separate pathway for trichome development in monocot plants and provided new evidence for the convergent evolution of trichome development pathways.

The WOX genes, which are specifically expressed in plants, form a large family that is a subgroup of the homodomain (HD)-containing transcription factors. WOX members characterized to date are involved in regulating diverse aspects of development. LOC_Os11g01130 (*Wox3/OsNS2*) is involved in the direct repression of YAB3, which is required for rice leaf development (Dai et al. 2007). OsWOX11 is an auxin- and cytokinin-responsive gene and is required to activate shoot-borne crown root development (Zhao et al. 2009). Ectopic expression of QHB affects normal shoot and leaf development (Kamiya et al. 2003). In this study, a member of the *WOX3* subfamily was demonstrated to be involved in trichome development.

In addition to *OsNS2*, several other *WOX3* genes have been investigated. *AtWOX3/PRS* is expressed in the periphery of the shoot meristem and recruits founder cells from all meristem layers to form lateral domains of both vegetative and floral organs. Ectopic expression of *PRS* forms multicellular bulges on the stem and sepal (Matsumoto and Okada 2001). The maize duplicate genes *narrow sheath 1* (*NS1*) and *narrow sheath 2* (*NS2*) function in a lateral domain of shoot apical meristems (Nardmann et al. 2004). Mutant leaves miss a large domain, including the leaf margin and mutant internodes are shortened on the marginal side of the stem (Nardmann et al. 2007). *NS* and *PRS* show conserved function during the recruitment of organ founder cells from the lateral domain of plant meristems (Nardmann et al. 2004). However, none of these genes are involved in trichome development. The presence of orthologs in monocots, such as *OsWOX3B* in rice, which root outside *NS/PRS* branching, indicated the occurrence of duplication after the separation of monocot and dicot species (Nardmann et al. 2007). The expression patterns of duplicated no-*NS/PRS* branching paralogs were different from those of *NS/PRS* branching paralogs, which indicates subfunctionalization (Nardmann et al. 2007). The new function identified in *WOX3* members outside *NS/PRS* branching in this study also supports the occurrence of subfunctionalization in this gene family.

In this study, the expression of *OsWOX3B* was only detected in the panicle of TN1. It is reported that *PRS* expresses only at a specific developmental stage (Matsumoto and Okada 2001). The negative detection of *OsWOX3B* in leaf in this study could also be due to the spatiotemporal expression of this gene. Interestingly, *OsWOX3B* expression was detected in the rice panicle of TN1 but not HMK rice. The transgenic *OsWOX3B* RNAi lines displayed a glabrous phenotype. These results suggested that *OsWOX3B* expression was suppressed in Yunnan Nuda Rice. However, no
differences were identified in the coding or promoter sequences of this gene in comparisons of the HMK and pubescent Nipponbare varieties. It can be speculated that the glabrous phenotype of Yunnan Nuda rice results from epigenetic variations, such as DNA methylation.

Glabrous germplasms have been used to develop dust pollution free rice varieties. In this study, no differences were identified in the OsWOX3B gene sequences of pubescent rice and glabrous Yunnan Nuda rice, which would provide an advantageous functional molecular marker for breeding purposes. However, a dominantly inherited glabrous phenotype is obtained by RNAi suppression of OsWOX3B, thus indicating a novel future strategy for the development of glabrous rice, particularly glabrous hybrid rice.

Conclusion

Yunnan Nuda Rice exhibited a typical glabrous phenotype. The Nuda phenotype was shown to be controlled by a pair of recessive genes allelic with the well-characterized GL-1 locus. The NUDA/GL-1 locus was fine mapped to a 28.5 kb region. A WUSCHEL-like homeobox gene (OsWOX3B) was confirmed as the candidate gene by RNAi and complementation analyses.

Methods

Plant materials

In this study, Yunnan Nuda Rice variety HMK was used to identify the NUDA gene. The American glabrous rice variety with gl-1 gene Lemont was used for allelic tests. The pubescent indica variety TN1 was crossed with HMK to construct the mapping population. The pubescent variety Nipponbare was used as the receptor of OsWOX3B RNAi. All plants were grown in paddy fields in Shanghai, Hangzhou China during the summer and Hainan, China during the winter.

Scanning electron microscope analysis

Leaves were dissected, fixed overnight at 4°C in FAA (formalminglacial acetic:70% ethanol = 1:1:18) and dehydrated in a graded ethanol series (50, 70, 80, 90, 95 and 100%). Samples were critical-point-dried, mounted, sputter-coated with platinum and observed and photographed using a scanning electron microscope (JSM-6360LV; JEOL Ltd, Tokyo).

DNA extraction and molecular marker analysis

Rice genomic DNA was extracted from fresh leaves according to the CTAB method (Murray and Thompson 1980). SSR markers information was obtained from Gramene (www.gramene.org). To construct a high-density linkage map for fine mapping in the target region, new insertion/deletion (InDel) markers and single nucleotide polymorphism (SNP) markers were developed according to the sequence differences between indica var. 93–11 and japonica var. Nipponbare (http://www.ncbi.nlm.nih.gov). Primers flanking the InDel and SNP polymorphisms were designed using the Primer Premier 5.0 program and tested on the parent varieties.

Linkage map and gene mapping

Two newly developed markers and two polymorphic SSR markers (RM17713 and RM13) were used to construct a linkage map of NUDA/GL-1 using Mapmaker/Exp 3.0 (Lander et al 1987) on the basis of the 158-plant F2 population. The marker order and the genetic distance between every two adjacent markers were determined in the target region on chromosome 5. These informative molecular markers and newly developed markers were used for genotyping each plant of the F2 population to identify recombinants in the target region. The linkage relationship between markers and the NUDA/GL-1 locus was analyzed. By assaying the recombinant events, the NUDA/GL-1 locus was narrowed down to a 28.5 kb region on the AP001 111 PAC clone.

RNA isolation, RT-PCR analysis and RACE

Total RNA was extracted from various rice tissues using EASYspin (Yuanpinghao, Tianjin). The extracted RNA was treated with RNase-free DNaseI (Frementas) to eliminate genomic DNA contamination according to the protocols recommended by the manufacturer.

First strand cDNA was synthesized from 2.5 μg total RNA using the RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, K1622). RT-PCR was performed with pairs of locus specific primers as follows:

LOC_Os05g02730, forward: 5'-CTTACACACCACGCTACTAACC-3', reverse, 5'-CTAGACGACGTATGCTGCTCT-3';
LOC_Os05g02740, forward: 5'-TGGGTITTCCTGCAAAAACACT-3', reverse 5'-CTACCGCCAGCCTTTGTGA-3';
LOC_Os05g02750/ LOC_Os05g02754, forward: 5'-TGTTTCATACCCACGATCTGC-3', reverse 5'-TGAAAGAAGCTAGGGAGGA-3';

Rice OsActin1 control gene, forward: 5'-TGCTATGTACGTCGCCATCCAG-3', reverse: 5'-AATGAGTAAACCACGCTCCGCT-3').

To obtain the full length-cDNA of LOC_Os05g02730, 5' and 3' RACE were undertaken by using 5'RACE System for Rapid Amplification of cDNA Ends Version 2.0 (Invitrogen, Catalog no.18374-058) and 3'–Full RACE Core Set Ver.2.0 (TaKaRa, Code: D314). The GSP1 primer for 5' RACE is: 5'-CTAGACGACGTCA
TGCTGCTCT-3’. The GSP1 primer for 3’ RACE is: 5’-CTTACACCACCAGCTACTACTACC-3’.

In silico analysis of NUDA/GL-1/OsWOX3B protein
Conserved domain in NUDA/GL-1/OsWOX3B protein was searched in NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Blast search parameters were set as the default.

To search the WOX genes in Arabidopsis and rice, BLAST was performed using the Blastp program to search the NCBI Non-redundant Protein Sequences Database (http://blast.ncbi.nlm.nih.gov/) using the homeodomain sequence of AtWUS as a BLAST query. The collected amino acid sequences were aligned using the CLUSTALW program (http://www.ddbj.nig.ac.jp/search/clustalw-j.html) with standard parameters. A phylogenetic tree was generated with the neighbor-joining method and 1,000 bootstrap samples were generated to assess support for the inferred relationships.

Vector construction and transformation
LOC_Os05g02730 was amplified with its 2 kb native promoter from Nipponbare genome DNA using primers 02730-clone-f (5’-GGGTGTAACCTGCTGCGGACT-3’) and 02730-clone-r (5’-CATTTCCATCCATAGCGTTGA-3’). The 4.2 kb PCR product was cloned into the pMD18T (TaKaRa) vector and sequenced. The verified clone was digested with HindIII and EcoRI and the DNA fragment was cloned into the gateway RNAi vector pANDA35HK (Miki et al. 2005) (http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cloning/Gateway-Cloning/GatewayC-Misc/Protocols.html#bp). The complementary and RNAi vectors were introduced into Agrobacterium tumefaciens into HMK and Nipponbare, respectively, by the Gateway-Cloning/GatewayC-Misc/Protocols.html#bp). To construct the LOC_Os05g02730 RNAi vector, the third exon was amplified using primer 02730-RNAI-f (5’-GGGGACCACTTTGTACAAGAAAGCTGGGTCC-3’) and 02730-RNAI-r (5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTC-3’). The 4.2 kb PCR product was cloned into the pMD18T (TaKaRa) vector and sequenced. The verified clone was digested with HindIII and EcoRI and the DNA fragment was cloned into the gateway RNAi vector pANDA35HK (Miki et al. 2005) (http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cloning/Gateway-Cloning/GatewayC-Misc/Protocols.html#bp). The complementary and RNAi vectors were introduced into Agrobacterium tumefaciens-mediated transformation under selection with hygromycin at 50 mg/l (Toki 1997). The RNAi lines were verified with primers Guslinker-f (5’-CATGAAAGATGCCGACCTAGC-3’) and Guslinker-r (5’-ATCCACGCGGTTACTCCG-3’).

Agronomic traits and evaluation of efficiency of grain packaging
The agronomic traits of the Nipponbare and the homzygous T2 generation of three independent OsWOX3/NUDA/GL-1 RNAi transgenic lines were individually surveyed during the summer of 2011 in Songjiang, Shanghai. Factors that included plant height, tillering number, grains per panicle, seed setting rate and 1,000-grain weight (g) were measured. The packaging efficiencies of the Nipponbare and the three RNAi transgenic lines were evaluated by measuring the number and weight of dried grains (about 10% of seed moisture contents) in 100 ml volume. Grain was harvested from four plants of each line. Mean values were compared and analyzed using a t-test.

Additional files

**Additional file 1: Table S1.** Evaluation of agronomic traits of Nipponbare and Nuda RNAi transgenic lines. The agronomic traits of the Nipponbare and three OsWOX3/NUDA/GL1 RNAi transgenic lines were evaluated by measurements of the plant height, tillering number, grains per panicle, seed setting rate, and 1,000-grain weight.

**Additional file 2: Figure S1.** The size and shape of the grains of Nipponbare and Nuda RNAi transgenic lines. a Grain length of Nipponbare and Nuda RNAi transgenic lines. b Grain thickness of Nipponbare and Nuda RNAi transgenic lines. c Grain width of Nipponbare and Nuda RNAi transgenic lines. d The grain length/width ratio of Nipponbare and Nuda RNAi transgenic lines.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HZ and KP performed the characterization of Yunnan nuda rice, mapping, cloning and analyzing the NUDML-G2-2/OsWOX3B gene. YW and YP performed the rice transformation. FH and LW performed the investigation of the agronomic traits. BH and QQ conceived the proposal, corrected the drafted manuscript. ST generated financial support for the study and corrected the final manuscript. All authors read and approved the final manuscript.

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References
Carey CC, Strahle JT, Selinger DA, Chandler VL (2004) Mutations in the pale aleurone color1 regulatory gene of the Zea mays anthocyanin pathway have distinct phenotypes relative to the functionally similar TRANSPARENT TESTA GLABRA1 gene in Arabidopsis thaliana. Plant Cell 16:450–464
Dai M, Hu Y, Zhao Y, Liu H, Zhou D (2007) A WUSCHEL-LIKE HOMEBOX gene Represses a YABBY gene expression required for rice leaf development. Plant Physiol 144:380–390
Foster KW, Rutger JN (1978) Independent segregation of semi-dwarfing genes and a gene for pubescence in rice. J Hered 69:137–138
Gibson PT, Malay RM (1983) Trichomes in segregating generations of Sorghum matings. I. Inheritance of presence and density. Crop Sci 23:73–75
Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of WOX genes mark cell fate decisions during...
early embryonic patterning in Arabidopsis thaliana. Development 131:657–668
Hulskamp M, Schnittger A (1998) Spatial regulation of trichrome formation in Arabidopsis thaliana. Semin Cell Dev Biol 9:213–220
Hulskamp M (2004) Plant trichomes: a model for cell differentiation. Nat Rev Mol Cell Biol 5:471–480
Johnson CS, Kolevski B, Smyth DR (2002) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell 14:1359–1375
Kamiya N, Nagasaki H, Morikami A, Sato Y, Matsuoka M (2003) Isolation and characterization of a rice WUSCHEL-type homeobox gene that is specifically expressed in the central cells of a quiescent center in the root apical meristem. Plant J 35:420–441
Karkkainen K, Agren J (2002) Genetic basis of trichome production in Arabidopsis lyrata. Hereditas 136:219–226
Kirk V, Simon M, Wester K, Schieffelin J, Hulskamp M (2004) ENHANCER of TRY and CPC 2 (ETC2) reveals redundancy in the region-specific control of trichrome development of Arabidopsis. Plant Mol Biol 55:389–398
Kumar KA, Andrews DJ (1993) Genetics of quantitative traits in pear-millet - a Review. Crop Sci 33:1–20
Lander ES, Green P, Abrahamsson J, Barlow A, Daly MJ, Lincon SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkages of experimental and natural populations. Genomics 1:174–181
Larkin JC, Oppenheimer DG, Pollock S, Marks MD (1993) Arabidopsis GLABRUS1 gene requires downstream sequences for function. Plant Cell 5:1739–1748
Leisle D (1974) Genetics of leaf pubescence in wheat. Crop Sci 14:173–174
Li W, Wu J, Weng S, Zhang D, Zhang Y, Shi C (2010) Characterization and fine mapping of the glabrous leaf and hull mutants (gl1) in rice (Oryza sativa L.). Plant Cell Rep 29:617–627
Lim HH, Domnala Z, Jorginder S, Lee SH, Lim CS, Abu Bakar CM (1984) Rice millers’ syndrome: a preliminary report. Br J Ind Med 41:445–449
Lloyd AM, Payne CT, Zhang F (2000) GL3 encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1. Genetics 156:1349–1362
Ma JF, Huang CF, Yamaji N, Mitani N, Yano M, Nagamura Y (2009) A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell 21:655–667
Marks MD (1997) Molecular genetic analysis of trichrome development in Arabidopsis. Annu Rev Plant Physiol Plant Mol Biol 48:137–163
Matsumoto N, Okada K (2001) A homeobox gene, PRESSED FLOWER, regulates lateral axis-dependent development of Arabidopsis flowers. Genes Dev 15:3355–3364
Miki D, Itoh R, Shimamoto K (2005) RNA silencing of single and multiple members in a gene family of rice. Plant Physiol 138:1903–1913
Moose SP, Lauter N, Carlson SR (2004) The maize macrohair1 locus specifically promotes leaf blade macrohair initiation and responds to factors regulating leaf identity. Genetics 166:1451–1461
Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325
Nagao S, Takahashi M, Kioshita T (1960) Genetical studies on rice plant, XXV: Inheritance of three morphological characters, pubescence of leaves and floral glumes, and deformation of empty glumes. Journal of the Faculty of Agriculture, Hokkaido University 5:299–314
Nardmann J, J, J, Wier W, Scalon MJ (2004) The maize duplicate genes narrow sheath1 and narrow sheath2 encode a conserved homeobox gene function in a lateral domain of shoot apical meristems. Development 131:2827–2839
Nardmann J, Zimmermann R, Durantini D, Kranz E, Wier W (2007) WD40 gene phylogeny in Poaceae: a comparative approach addressing leaf and embryo development. Mol Biol Evol 24:2474–2484
Oppenheimer DG, Herman PL, Sivakumaran S, Esch J, Marks MD (1991) A myb gene required for leaf trichome differentiation in Arabidopsis is expressed in stipules. Cell 67:483–493
Payne CT, Zhang F, Lloyd AM (2000) GL3 encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1. Genetics 156:1349–1362
Qin F, Zhang D, Lin X, Xie Y (1997) Classification of Yunnan Nuda compatible varieties. J Huasheng Agricultural University 16:320–324
Rutger JN, Mackill DJ (2001) Application of Mendelian genetics in rice breeding. In: Rice Genetics IV, Proceedings of the Fourth International Rice Genetics Symposium, 22–27 October 2000, Los Baños, Philippines. Science Publishers, Inc and Los Baños (Philippines): International Rice Research Institute, Enfield, NH (USA), p 29
Sarkarung S, Collins F (1977) Inheritance of leaf pubescence in oats. Agron Abstr 77:7
Sato K, Takeda K (1992) Genetic analysis of large trichomes on the barley leaf. J. Genet 86:3-30
Scalon MJ, Schneebberger RG, Freeling M (1996) The maize mutant narrow sheath fails to establish leaf margin identity in a meristematic domain. Development 122:1683–1691
Schellmann S, Schnittger A, Kirk V, Wada T, Okada K, Beermann A, Thumfahrt J, Jurgens G, Hulskamp M (2002) TRPYTHION and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J 21:5296–5306
Serna L, Martin C (2006) Trichomes: different regulatory networks lead to convergent structures. Trends Plant Sci 11:274–280
Southwood R (1986) Plant surfaces and insects - an overview. In: Juniper B, Southwood R (eds) Insects and the plant surface. Edward Arnold (Publishers) Ltd, London, Sydney & Baltimore, pp 1–22
Szymanski DB, Lloyd AM, Marks MD (2000) Progress in the molecular genetic analysis of trichome initiation and morphogenesis in Arabidopsis. Trends Plant Sci 5:214–219
Toki S (1997) Rapid and efficient Agrobacterium-mediated transformation in rice. Plant Mol Biol Rep 15:16–21
Vandenbussche M, Horstman A, Zethof J, Koes R, Rijkersma AS, Gerats T (2009) Differential transcription of WOX transcription factors for lateral development and organ fusion in Petunia and Arabidopsis. Plant Cell 21:2269–2283
Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch J, Marks MD, Graya JC (1999) The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. Plant Cell 11:1337–1350
Wang D, Sun S, Fa G, Lu X, Li Z, Ren G (2009) Mapping a rice glabrous gene using simple sequence repeat markers. Rice Sci 16:93–98
Weker E (2000) Trichome diversity and development. Adv Bot Res 31:1–35
Yu ZH, Mccouch SR, Kinosita T, Sato S, Tankesly SD (1995) Association of morphological and RFLP markers in rice (Oryza-Sativa L). Genome 38:565–574
Zhang X, Zong J, Liu J, Yin J, Zhang D (2010) Genome-wide analysis of WOX gene family in rice, sorghum, maize, Arabidopsis and poplar. J Integr Plant Biol 52:1016–1026
Zhao Y, Hu Y, Dai M, Huang L, Zhou DX (2009) The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. Plant Cell 21:736–748

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