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DNL1, encodes cellulose synthase-like D4, is a major QTL for plant height and leaf width in rice (*Oryza sativa* L.)

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ABSTRACT

To better understand the genetic of rice agronomic traits, we selected two different rice germplasms in phenotypes, Xian80 and Suyunuo, to construct genetic population for QTL analysis. A total of 25 QTLs for six traits were found in a 175 F_2 population. Major QTLs, *qPH12*, *qLW12.2*, *qLL12* and *qGW12.1*, explaining 50.00%, 57.08%, 15.41% and 22.51% phenotypic variation for plant height, leaf width, leaf length and grain width, respectively, were located on the same interval of chromosome 12 flanking SSR markers RM519 and RM1103. In consideration of the great effects on plant height and leaf width, the locus was named *DNL1* (*Dwarf and Narrowed Leaf 1*). Using a segregating population derived from F_2 heterozygous individuals, a total of 1363 dwarfism and narrowed-leaf individuals was selected for screening recombinants. By high-resolution linkage analysis in 141 recombination events, *DNL1* was narrowed to a 62.39 kb region of InDel markers ID12M28 and HF43. The results of ORF analysis in target region and nucleotide sequence alignment indicated that *DNL1* encodes cellulose synthase-like D4 protein, and a single nucleotide substitution (C2488T) in *dnl1* result in decrease in plant height and leaf width. Bioinformatical analysis demonstrated that a conserved role for *OsCSLD4* in the regulation of plant growth and development. Expression analysis for *OsCSLDs* showed *OsCSLD4* highly expressed in roots, while other *CSLD* members had comparatively lower expression levels. However, no clear evidence about *CSLD4*/*DNL1* expression was associated with its function.

1. Introduction

Rice plant architecture is crucial for grain yield, and is determined by many factors, including plant height and leaf morphology. Plant height and leaf width are considered as complex traits which are major related to lodging resistance, nutrient utilization and photosynthetic efficiency [1,2]. Although extensive investigation for the two traits has been enforced, and considerable quantitative trait loci (QTLs) have been identified (http://archive.gramene.org/db/qtl/), few have been isolated until now.

Using dwarf mutants and/or narrowed-leaf mutants in rice have shown that these genes associated with dwarfism and/or narrowed-leaf are mostly related to the biosynthesis and regulation of the phytohormone. For example, the rice *Dwaf 1* gene, which encodes the GTP-binding protein, might be involved in gibberellin signal transduction [3], *dwarf 61* is the rice homolog of the Arabidopsis *BRI1* gene, which encodes a putative BR receptor kinase [4], *dwarf 53* acts as a repressor of the strigolactone signaling pathway [5,6]. Rice *narrow leaf 1* was related to polar auxin transport activity [7]. In addition, *narrow leaf 7* encodes a flavin-containing monooxygenase, belonging to YUCCA family, is involved in auxin biosynthesis [8]. Rice *narrow leaf 2* and *narrow leaf 3* loci encode WUSCHEL-related homeobox 3A (OsWOX3A), which was conjectured involved in organ development by regulated auxin synthesis and/or transports genes [9].

Among plant kingdom, rigid plant cell wall represent key determinants of overall plant form, plant growth and development, and the responses to biotic and abiotic stresses [10]. Cellulose, hemicelluloses, and pectins are the major components of plant cell wall [11]. In higher plants, cellulose, which consists of β-1,4-glucan chains, is believed to be synthesized at the plasma membrane by rosette complexes, comprising six subunits, presumably one rosette complexes containing 36 individual cellulose synthase (CESA) proteins [12]. CESA, belonging to glycosyltransferase family 2 (GT 2) enzyme, has eight transmembranes (TMs), 2 TMs toward the N terminus and 6 TMs close to the C terminus, a conserved 'D, D, D, QXXRW' motif and a glycosyltransferase (GT) domain between the two TMs clusters [13]. Cellulose synthase-like (CSL) are hypothesized to involved in the synthesis of the back-bones of hemicelluloses [14]. CSL proteins sharing significant sequence

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similarity with CESA proteins and classified into nine subfamilies (CSLA–H and CSLJ) [15]. Several CSL families function had been described. CslA family members encode mannan synthases [16], CSLC proteins relate to the synthesis of xyloglucan [17], while CSLF and CSLH involved in mixed-linkage glucan synthases [18,19]. Of the CSL subfamily, CSLD shown closely related to the CESA subfamily, which was assumed CSLDs may also function as cellulose synthases [20]. KOJAK/AtCSLD3 was the first identified members of CSLDs in Arabidopsis and was believed to plays a role in root hair growth in Arabidopsis [21,22]. AtCSLD2 is also required for normal root hair growth, but appears to be required at a later stage of hair development than AtCSLD3 [23]. OsCSLD1 may be the functional ortholog of KOJAK/AtCSLD3 as its root-specific expression pattern and csld1 mutation develop abnormal root hairs in rice [24]. ZmCSLD1 was essential for plant cell division in maize, especially during early phases of cross-wall formation. zmcsld1 mutant performed narrow-organ and warty phenotypes due to altered cell division reduces cell sizes and cell number [25]. Using mutant alleles of the Arabidopsis *CSLD1* and *CSLD4* genes revealed that CSLD1 and CSLD4 play important roles in pollen tube growth, possibly by participating in pollen tube cellulose synthesis [26].

Here, we cloned and characterized a new QTL, *DNL1*, a novel allele of *cellulose synthase-like D4*, regulates plant height and leaf width. Our findings provide further investigation of OsCSLD4 through plant morphogenesis.

2. Materials and methods

2.1. Plants materials and field experiments

Indica rice germplasm Xian80 presents dwarfism, narrowed and shorted leaves, accompanied by the characteristics of small grain, and extremely early heading. Xian80 was crossed with a Japonica variety Suyunuo (SYN) to generate F_1 and 175 F_2 plants for QTL mapping. Subsequently, each heterozygous F_2 plants were chosen to generate a segregating population by marker-based selection. A total of 1363 dwarfism and (or) narrowed-leaf individuals (recessive) were selected out of the segregating population for fine mapping. Nine rice accessions including the parents (Table 4) were used for target gene sequencing and alignment. Rice materials were cultivated and examined under natural field conditions during growing season in Nanjing, China. Plant height, leaf length and width were observed at 2 weeks after heading. The distance from the ground to the top of panicle was measured as plant height (cm). The average of 3 flag leaf widths/lengths at their widest point each plant was calculated as leaf width/length (0.1 cm). Grain length/width was measured by electronic vernier caliper (0.01 mm). Heading date was recorded the days from seeding to heading.

2.2. Molecular marker development

Simple sequence repeat (SSR) markers were designed as described on the Gramene database (http://www.gramene.org/). Insert and deletion (InDel) markers for fine-mapping were newly developed by comparison of DNA sequences between japonica cultivar Nipponbare and indica cultivar 93-11 at target region (Table 1).

2.3. Linkage and QTL analysis

A total of 140 polymorphic SSR markers even distribution on rice genome was used to identified the genotypes of 175 F_2 plants at their DNA templates by PCR. The PCR procedure was as following: 95 °C for 5 min, followed by 32 cycles of 95 °C for 30 s, annealing temperature for 30 s, 72 °C for 40 s, and a final elongation step at 72 °C for 10 min. The PCR products were analyzed on 6–10% agarose gels. Software IciMapping3.3 (www.isbreeding.net/) was used for genetic map construction and QTL analysis. LOD > 2.5 presents the threshold of QTL along with the composite interval mapping method.

2.4. Fine mapping

Considering that the dwarf and narrow leaf performed recessive, 1363 dwarf and narrow-leaf plants were selected out of segregating population for fine mapping *DNL1*. Recombinants were screened by SSR markers RM519 and RM6947 (flanking *DNL1*). To further narrowed down the *DNL1*, InDel markers newly designed within the target region was screened in recombinants. The plant height and leaf width of selected recombinants were further confirmed by progenies test. The candidate genes from Xian80, SYN, and other rice accessions genomic DNA were amplified, sequenced and compared.

2.5. Sequence collection, alignment and phylogenetic tree construction of CSLD subfamily

CSLD subfamily sequence of rice (*Oryza sativa* L.) and Arabidopsis (*Arabidopsis thaliana*) were downloaded from Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/) and JCVI (http://www.jcvi.org/). BLASTP searches against the National Center for Biotechnology Information (NCBI,

Table 1
Primers in this study.

Markers	Sense primer (5′–3′)	Anti-sense primer (5′–3′)	Product size (bp)	Intention
RM519	AATTTCCGCGAAATCAGCATCC	TCATCTGGACAGTCGAGGTACGC	125	Fining mapping
RM6947	ATTAAACGTCCACTGCTGGC	GCTAGGTTAGTGGTGCAGGG	155	
RM28466	CCGACGAAGAAGACGAGGAGTAGCC	AGGCCGGAGAGCAATCATGTCG	99	
HF17	AGTATAGCCAAGTTCATCGC	TTCGAATTTGGTTAGACACA	135	
HF28	AACCTATAAAGCCGAGCTG	ATTAATTCCTCCATTCCCC	130	
HF29	TGCAGCTCTAATTGTCACTG	CCGGCTACAGTGTTGTAGAT	153	
HF36	CTATGACGGATGTATGCCA	ATGCTAACAACATGCGAGTA	140	
HF43	AGGTGAGAGATGAACTCCG	GCAAGCACATGAACAAAGTA	130	
HF44	ATTTTAACTTGAACGGAGCA	TTTGTTATCCCAGAGACCTG	156	
Id12M5	TGAGGTAAAAGGGACGAATA	CAGACACCACATGCATAAAT	144	
ID12M28	AAATCCAATTTCTTGTGGTG	GCATTCACCTGTGTATTCCT	177	
dnl1-2	TCCATTCACATGAGAGATCCT	TGATACACAAACAATCGCTTA	4300	Gene cloning
qCSLD1	AGATGAAGGTTCTCCAGCGCATC	TTGAGCGTCTGCACGATGAAC	133	Gene expression
qCSLD2	TGTCCTTAGCCCATACAGGCTTC	TGCAAGGAATAGACCAAGAACAGC	64	
qCSLD3	TTCTCCGGCTTCTTCATCGTCCAG	GGTCATGGTGAGGAGGTAGCAAAG	66	
qCSLD4	ACAGGTTGTTGATTGCGATTCGG	ATGAGAAGGCGAACCACACCTC	132	
qCSLD5	TCAAGTGGACGTCGCTCTTCATC	ACGAGCGCGATGATGTTGATCC	64	

http://www.ncbi.nlm.nih.gov/) were performed using OsCSLD4 sequences as queries to collect CSLD subfamily in other plant species. Multiple sequence alignment of CSLD proteins was carried out using the ClustalX 2.1 program (http://www.clustal.org/download/current/) with default parameters and then displayed on ESPript3.x-ENDscript2 (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). MEGA6 program (http://www.megasoftware.net/) was used to constructed neighbor-joining tree with 1000 replications bootstrap method using the pairwise deletion option. Protein domains were identified using SMART (http://smart.embl-heidelberg.de/) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/).

2.6. RNA extraction and expression analysis

Total RNA was extracted from various plant tissues in Xian80 and SYN and was converted into first strand cDNA. Quantitative real-time (qRT-PCR) was carried out to amplify the transcripts of *OsCSLDs* in rice with 39 PCR cycles. 18s rRNA was also amplified as the control. The gene-specific primers were listed on Table 1.

3. Results

3.1. DNL1 is a major QTL for plant height and leaf width

The parental cultivars, XIian80 and Suyunuo (SYN), displayed significant differences in all the traits examined (Fig. 1A–F, Table 2). Xian80 has the characteristics of dwarfism, narrow and short leaf, small grain, and extremely early heading. In contrast, SYN shows normal morphologic characters with large grains. Compared to SYN, the length of each internode in Xian80 is almost uniformly shortened, resulting in an elongation pattern of internodes similar to SYN (Fig. 1G). Both plant height and leaf width of F_1 appeared dominant effects, and frequency distributions in F_2 population were displayed continuous and bimodal as cut-off scores of 105 cm and 1.4 cm for plant height and leaf width, respectively (Fig. 1H). Also, high correlations between the two traits was observed in F_2 population ($R^2 = 0.6^{**}$, $P \leqslant 0.01$). Accordingly it can be assumed that these two traits were controled by a common genetic factor.

To dissect the genetic factors of plant height, leaf width and length, grain length and width, and heading date, QTL analysis was carried out in F_2 population, and twenty five QTLs for these traits were identified, which suggested that these traits were controlled by QTL locus (Table 3). Among these, four QTLs, *qPH12*, *qLW12.2*, *qLL12*, and *qGW12.1* were located on the same genome region of chromosome 12 between SSR markers RM519 and RM1103. The four QTLs explained 50.00%, 57.08%, 15.41%, and 22.51% phenotypic variation for plant height, leaf width and length, and grain width, respectively. Moreover, SYN alleles had dominant and positive effects on these traits. These results indicated that there might exist a major gene on chromosome 12 both influencing the four traits. Considering that *qPH12* and *qLW12.2* had significant genetic effects on plant height and leaf width, and had a high correlation between the two traits, the locus was named as *Dwarf and Narrowed leaf 1*, *DNL1*.

3.2. Fine mapping of DNL1

DNL1 was first located to a 22.98 cM genetic distance on chromosome (Fig. 2A). To further narrow down *DNL1* locus, the heterozygous individuals of F_2 population were selected out, based on the genotype of SSR markers RM519 and RM1103, to develop a segregating population for fine mapping. From this population, a total of 1363 dwarfism and/or narrowed-leaf individuals was selected and screened for recombinants by SSR markers RM519 and RM6947 (a marker outboard RM1103), and 145 genetic recombination events was found. High-resolution linkage analysis in the 141 recombination events was performed, using InDel markers within interval of RM519 and RM6947, and *DNL1* was narrowed to a 62.39 kb region of InDel markers ID12M28 and HF43 (Fig. 2B). Crucial recombination events occured within (and nearby) the target region were further verified by the phenotypes of their progenies. According to Rice Genome Annotation Project, 9 Opening Reading Frames (*ORFs*) exist there. Of which, ORF5 has two exons, and encodes a member of CSLD (Cellulose synthase-like D protein) subfamily, named as *OsCSLD4*, which play important roles in cell-wall formation and plant growth. (Fig. 2C). On the basis, *CSLD4* was determined as the candidate gene of *DNL1*.

3.3. One nucleotide substitution C (2488) T resulted in dwarf and narrowed-leaf phenotype

To verify that *OsCSLD4* is responsible for *DNL1* phenotype, nucleotide sequence alignment of *OsCSLD4* was performed between Xian80 and SYN. It uncovered the presence of 6 sites of 7 nucleotide differences at *OsCSLD4* exons. The nucleotide transitions from TC (SYN) to AA (Xian80) (TC637, 638AA) in the 1st-exon, from C (SYN) to T (Xian80) (C2488T) and A (SYN) to G (Xian80) (A3845G) in the 2nd exon, caused amino acid residue changes from serine to asparagine (S213N), proline to serine (P746S), and asparagine to serine (N1198S), respectively. Three other nucleotide substitutions did not result in amino acid variations (Fig. 2D and E).

In order to find the functional site, nucleotide sequence analysis of *CSLD4* was further carried out in 9 rice varieties (including the parents) that well covered genetic diversity. 12 sites of single nucleotide polymorphism (SNP1 to SNP12) and one insert and deletion polymorphism (InDel1) were identified (Table 4). Five SNP sites, SNP1, SNP2, SNP5, SNP8, and SNP12, lead to amino acid change in *OsCSLD4*. The site of C to T change at nucleotide 2844 (C2488T) (SNP8) only occurred in Xian80, and four other SNP sites in various varieties are not associated with the phenotype. Despite the 12-consecutive-nucleotide deletion result in a 3-amino-acid deletion of OsCSLD4 in 93-11 and Huagengxian74, it does not cause the dwarfism and narrow-leaf phenotype. Consequently, it is crucial for the DNL1 phenotype in Xian80 that the SNP8 (C2488T) caused an amino acid residue changes from proline to serine (P746S).

Table 2
Data collection of main agronomic traits of Xian80, SYN, F_1 and F_2 progeny.

Traits	Xian80	SYN	F_1	F_2	
				Variation	Average
Grain length (mm)	7.82 ± 0.39	10.09 ± 0.39	8.39 ± 0.201	7.53–10.25	8.58 ± 0.273
Grain width (mm)	2.51 ± 0.14	3.61 ± 0.14	3.42 ± 0.079	2.63–3.87	3.38 ± 0.047
Plant height (cm)	50.4 ± 4.04	142.8 ± 1.30	156.3 ± 3.407	38.9–153.9	111.3 ± 22.262
Leaf length (cm)	19.7 ± 0.83	37.8 ± 2.20	47.51 ± 5.387	19.2–41.3	28.71 ± 6.041
Leaf width (cm)	0.80 ± 0.11	1.80 ± 0.07	1.96 ± 0.149	0.7–2.0	1.5 ± 0.298
Heading date (d)	55	89	92	48–98	72 ± 13

Table 3
QTL detected in F_2 population.

Traits	QTLs	Chr.	Position	Left Marker	Right Marker	LOD	PVE(%)	Add	Dom
Leaf width (LW)	*qLW1*	1	207.00	RM1068	RM3362	3.53	3.78	0.0735	0.0126
	qLW9.1	9	6.00	RM23788	RM7212	6.28	6.14	−0.0964	0.0036
	qLW9.2	9	45.00	RM5657	RM566	5.88	5.33	0.0849	0.0211
	qLW12.1	12	100.00	RM28166	RM519	5.76	7.45	−0.0429	0.1182
	qLW12.2	12	123.00	RM519	RM1103	37.22	57.08	−0.2710	0.1837
Leaf length (LL)	*qLL2*	2	115.00	RM6122	RM6295	3.05	5.56	2.1996	−0.3891
	qLL4	4	205.00	RM1155	RM3367	4.07	10.54	−2.9698	1.2911
	qLL8	8	0.00	RM337	ID8-4	2.81	5.09	1.8411	0.3942
	qLL12	12	125.00	RM519	RM1103	6.70	15.41	−3.4305	0.8352
Plant height (PH)	*qPH2.1*	2	105.00	RM13439	RM13682	10.41	14.65	−11.8217	−2.3428
	qPH2.2	2	115.00	RM6122	RM6295	4.48	5.73	7.2408	−1.8404
	qPH8	8	0.00	RM337	ID8-4	2.89	3.62	−0.0620	7.6742
	qPH12	12	123.00	RM519	RM1103	24.83	50.00	−19.4473	12.4641
Grain width (GW)	*qGW2*	2	205.00	RM13353	RM13439	3.32	14.59	−0.1025	−0.0901
	qGW3	3	49.00	RM282	RM15144	3.00	13.33	−0.0974	−0.1053
	qGW12.1	12	134.00	RM519	RM1103	3.32	22.51	−0.1361	0.1827
	qGW12.2	12	154.00	RM3226	RM1227	3.59	17.94	−0.1292	0.1065
Grain length (GL)	*qGL3*	3	75.00	RM15144	ID3-2	7.20	26.61	−0.3964	−0.2526
	qGL7	7	139.00	RM3826	RM5426	4.15	19.61	0.3514	−0.0030
Heading date(HD)	*qHD1*	1	185.00	ID1-3	RM5389	3.62	5.71	2.5229	−1.0176
	qHD5	5	11.00	RM5796	RM7444	7.39	14.35	−3.7732	1.7111
	qHD6	6	3.00	RM8109	RM588	7.05	11.16	3.3293	−1.9248
	qHD7	7	128.00	RM5405	RM3826	3.76	5.46	−2.8360	−0.0751
	qHD10	10	59.00	RM8201	RM1108	5.11	11.87	−3.4664	−1.5188
	qHD12	12	62.00	RM1337	RM28029	3.59	5.48	−2.4736	0.6303

Table 4
Nature variation of *OsCSLD4* in 9 rice varieties/lines.

Accessions	Subsp	InDel1	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	Leaf width	Plant height
		307-318	328	637,638	684	915	1757	1878	1911	2488	3062	3426	3522	3845	(cm)	(cm)
Nipponbare	japonica	GCGGCGGCGGAG	C	TC	C	T	G	A	C	C	C	G	T	A	1.4	108
Suyunuo	japonica	GCGGCGGCGGAG	C	TC	C	T	G	A	C	C	C	G	T	A	1.8	142.8
Xian80	indica	GCGGCGGCGGAG	C	AA	C	C	G	A	T	T	C	G	C	G	0.8	50.4
93-11	indica	deletion	G	AA	T	C	G	G	C	C	C	T	C	A	2.2	116
IR97	indica	GCGGCGGCGGAG	C	AA	C	C	G	A	T	C	C	G	T	G	2.5	101.4
Huagengxian74	indica	deletion	C	AA	C	C	A	G	C	C	T	T	T	A	1.8	94.8
88-1516	indica	GCGGCGGCGGAG	C	TC	C	T	G	A	C	C	C	G	T	A	1.4	100.2
Rizyoto	indica	GCGGCGGCGGAG	C	TC	C	T	G	A	C	C	C	G	T	A	1.5	115.2
Slo-16	indica	GCGGCGGCGGAG	C	TC	C	T	G	A	C	C	C	G	T	A	1.6	127.4
Amio acid change		AEAA(101-104) deletion	V110L	S213N	-	-	R502H	-	-	P746S	-	-	-	N1198S		

The numbers stand for the positions of these polymorphic sites in *OsCSLD4* genomic sequence. "–" indicated the SNP did not result in amino acid change. The red boxes mean SNPs or deletion and yellow box is a SNP only detected in *dnl1* of Xian 80.

3.4. Structure analysis of DNL1

Database search indicated that DNL1 is Cellulose synthase-like protein D4 protein, belonging to glycosyltransferase 2 family (GT2). OsCSLD4 contains a Zf-Ring 4 domain, a Cellulose_synt, two transmembrane domains (TM1 and TM2) toward N terminus, and six transmembrane domain (TM3 to TM8) at C terminus (Fig. 3A). The Cellulose_synt, which was designated as 'cellulose synthase catalytic subunits', has a GT domain and a 'D, DCD, D, QXXRW' motif between TM2 and TM3. The 'DCD' residues were considered as the active site of GT domain. Many *OsCSLD4* mutants have been characterized previously, which were quite different with *dnl1* (P746S), happened on the GT domain (Fig. 3A). We did a multiple sequence alignment of GT2 domain in *dnl1* against with CSLD subfamily members in rice, Arabidopsis, Physcomitrella patens and other plant species. The results shown that the GT domain was highly conserved in all selected CSLD subfamily members (Fig. 3B), demonstrated that the proline to serine substitution

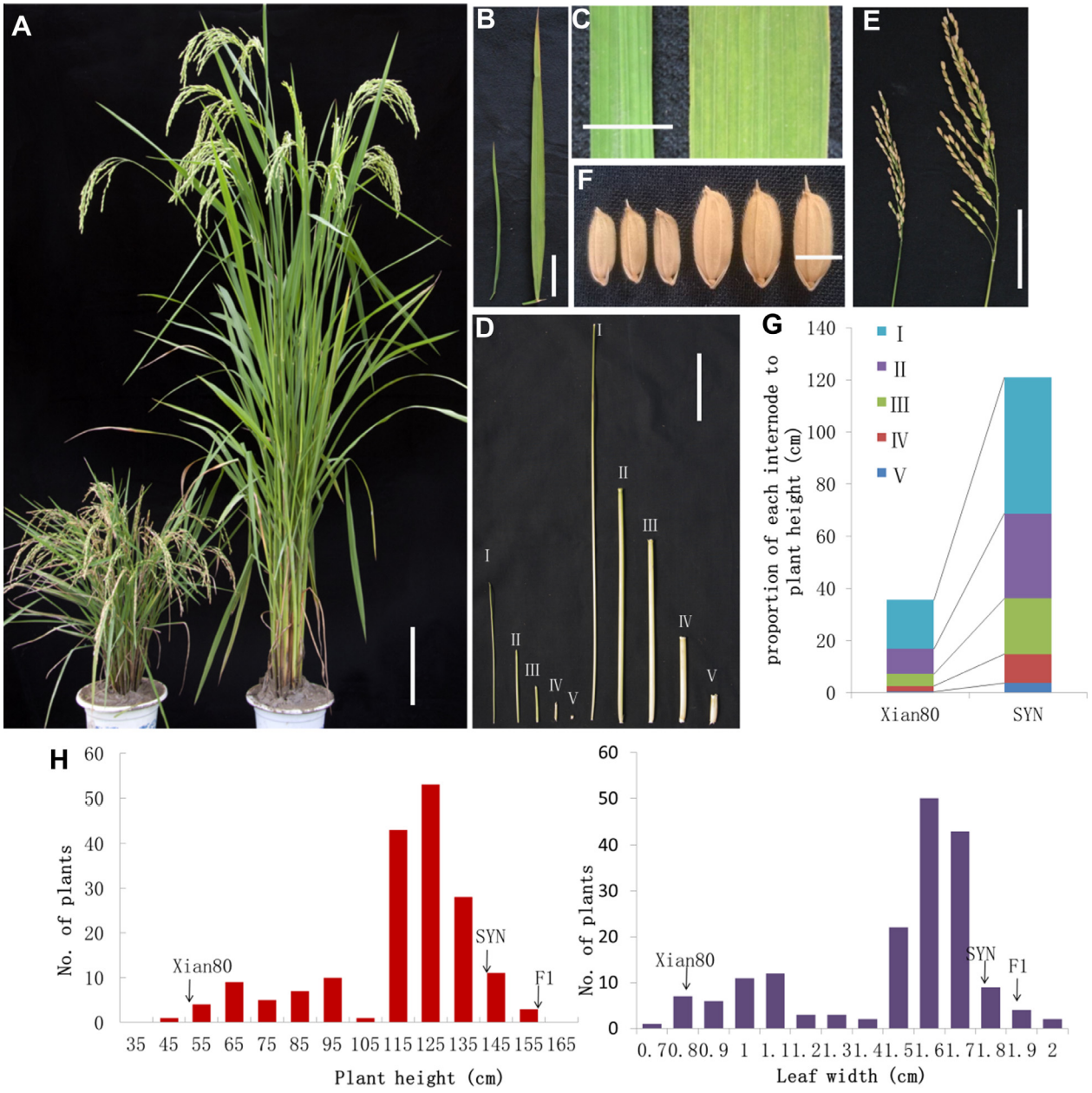

Fig. 1. Phenotype of Xian80 (left) and Suyunuo (SYN) (right), and frequency distribution of leaf width and plant height in F_2 population. (A) The phenotype of Xian80 (left) and SYN (right) at maturing stage, bar = 10 cm; (B) flag leaves of Xian80 (left) and SYN (right) at heading stage, bar = 10 cm; (C) variation of flag leaf width between Xian80 (left) and SYN (right) at heading stage, bar = 1 cm. (D) Features of internodes (I, II, III, IV and V) among Xian80 (left) and SYN (right), bar = 10 cm; (E) main panicles of Xian80 (left) and SYN (right), bar = 10 cm; (F) grains of Xian80 (left) and SYN (right), bar = 5 mm. (G) Comparison of each internodes to plant height; (H) frequency distribution of leaf width and plant height in F_2 population.

(P746S) was a rare mutation and the GT domain of OsCSLD4 is essential for rice plant growth and development.

Phylogenetic tree was generated based on multialignment among these CSLD proteins with neighbor-joining (NJ) methods by MEGA6 software. The phylogenetic tree was separated into five major clades due to their similarities in rice, however the CSLDs of *P. patens* and CSLD6 of *A. thaliana* form their own clades. Except clade I and V, each of the three clades, II to IV, contains at least one CSLD of each plant, demonstrated that ancient gene duplication events and the four genetic positions are shared across these plants. The OsCSLD4 was placed in clade I with CSLD5_ARATH, A.tacschii_CSLD4 and MmCSLD5, suggesting that they may have conserved function cross plant kingdom.

3.5. Expression levels of OsCSLD subfamily altered in dnl1

To investigate the tissue-specific expression patterns of *OsCSLD* genes in rice, total RNA from roots, leaves, stems, nodes, and young panicles were isolated and amplified via qRT-PCR. In SYN, the five *OsCSLD* genes were expressed in all organs and exhibited different expression patterns (Fig. 4A). *OsCSLD4* displayed highest expression levels in roots, while *OsCSLD1*, *OsCSLD2*, *OsCSLD3*, and *OsCSLD5* expressed at lower levels. *OsCSLD3* and *OsCSLD5* exhibited relatively higher expression in panicles. In roots, compared to SYN, however, the expression levels of *OsCSLD4* and *OsCSLD5* were down regulated in Xian80. In contrast, *OsCSLD1*, *OsCSLD2*, and *OsCSLD3* were up regulated (Fig. 4B). In conclusion, the SNP8 mutation in Xian80

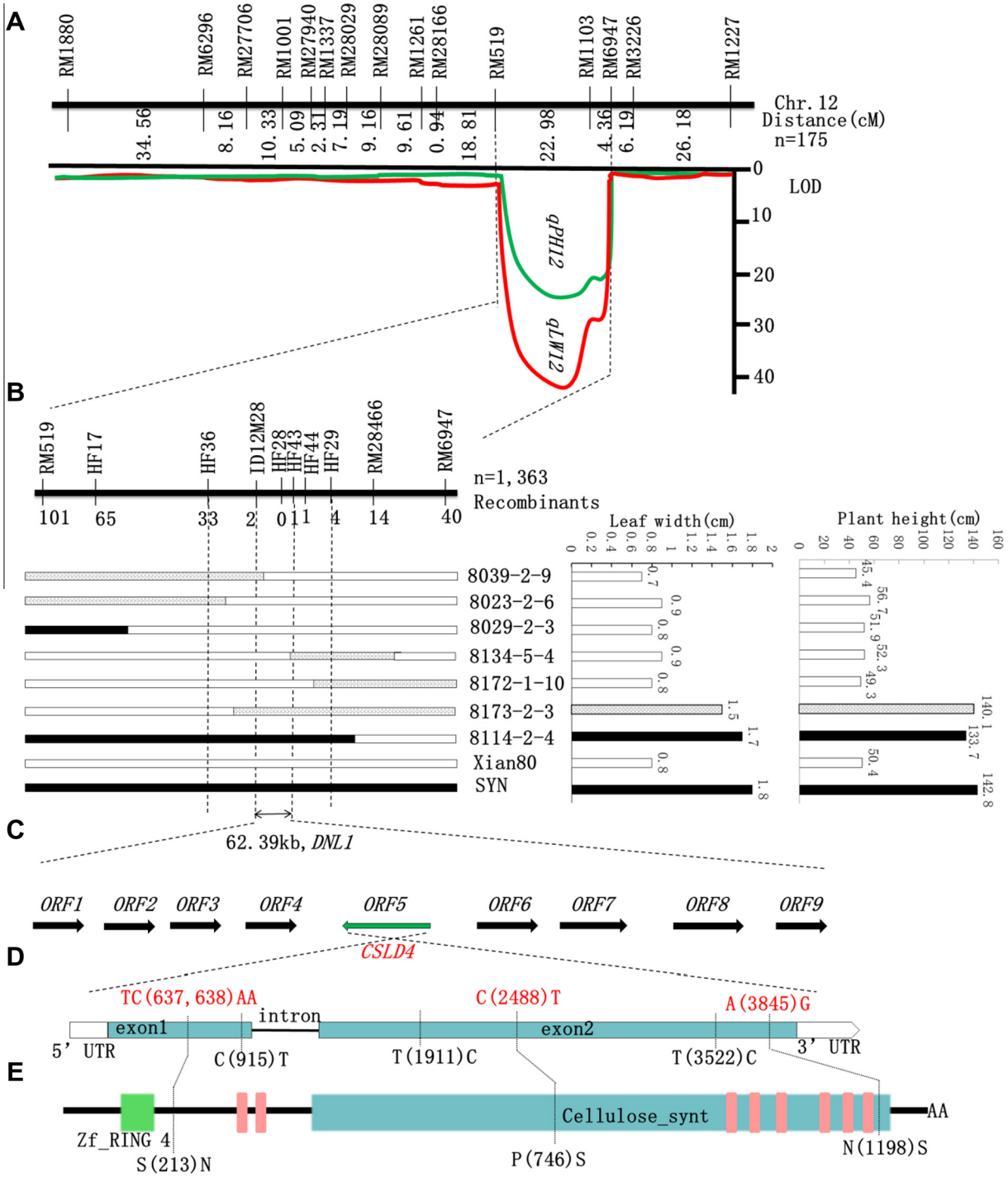

Fig. 2. Map-based cloning of *DNL1* and mutation site analysis of *OsCSLD4*. (A) Location of *DNL1* on rice chromosome 12. The numbers below the black bar indicate genetic distances (cM) between markers. Red and green peak indicates LOD score of QTL for leaf width and plant height, respectively. (B) High-resolution linkage analysis of *DNL1* locus. The numbers of recombinants between the gene and markers were showed below the linkage map. Black solid and hollow bars represent homozygous chromosomal segments for SYN and Xian80, and grille for heterozygote, respectively. Serial numbers indicated the key recombinants. (C) Predicted open reading frames (ORFs) in *DNL1* locus on Rice Genome Annotation Project (RGAP). RGAP indicated 9 ORFs in this region, ORF5 is *OsCSLD4*. The arrows indicated the ORF1 to ORF9 (Loc_Os12g36850, Loc_Os12g36860, Loc_Os12g36870, Loc_Os12g36880, Loc_Os12g36890, Loc_Os12g36900, Loc_Os12g36910, Loc_Os12g36920, Loc_Os12g36930). (D) Structure of *OsCSLD4* and natural variations between Xian80 and SYN. (E) Structure of OsCSLD4 Protein. The red boxes represent transmembrane domains, dotted lines indicate the mutant positions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

disturbed the expression levels of *OsCSLDs*, but the cooperative relationships of *OsCSLD4* with other *OsCSLDs* in rice were unclearly.

4. Disscussion

Rice is not only a model plant of monocots but also one of the most important crops in the world. During the past decades, numerous dwarf mutants, termed as '*d*' mutants in rice have been described and investigated because of their genetic importance of plant growth and development. Saving reduce plant height, the dwarf mutants also displayed mutational leaves. For instance, *GID2* encodes a F-box protein and acts as a regulator of Gibberellin signaling. Similar to *d1* mutant, the leaf blades of *gid2* mutants are wider and darker green than the wild-type plants [27,28].

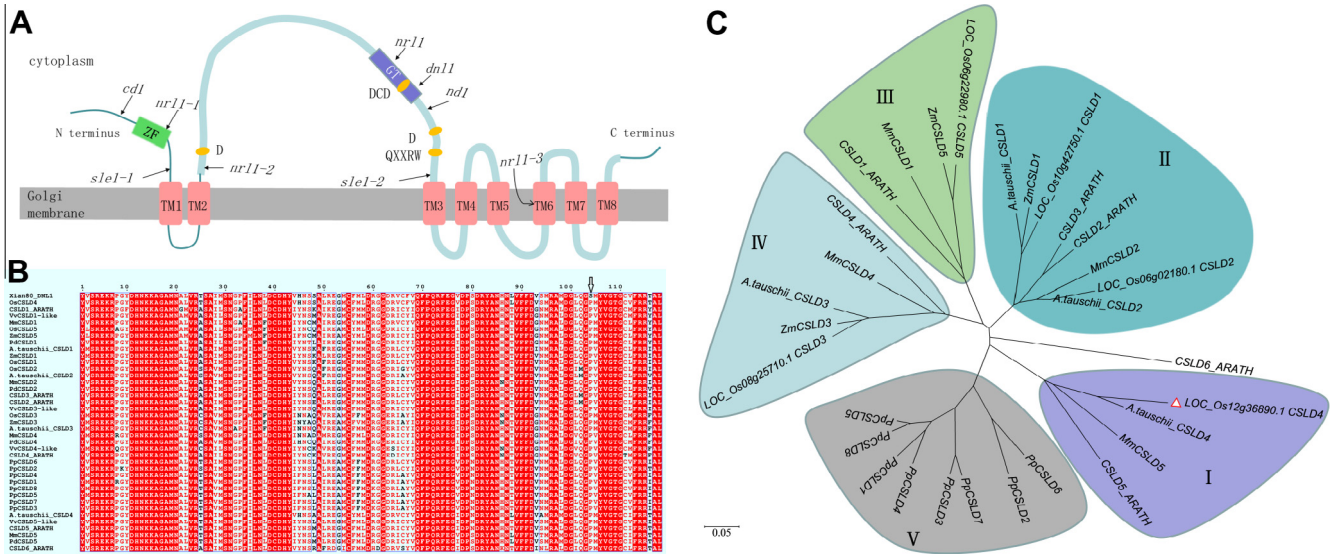

Fig. 3. Structural and evolutional analysis of cellulose synthase-like D proteins. (A) Domain structure of OsCSLD4 protein. Italics indicate OsCSLD4 mutant alleles in previously studies. ZF, zinc-finger Ring domain; D, aspartic acid; C, glycine; Q, glutamine; R, arginine; W, tryptophan; X, any amino acid; GT, glycosyl transferase domain; TM, transmembrane domain. (B) Sequence multialignment of GT domain on CSLD proteins among rice and other plant species. The arrow showed the proline (746) residue. Black star pointed the mutation amino acid proline 746 residue. Abbreviation are rice (Os), *Arabidopsis thaliana* (ARATH), *Aegilops tauschii* (*A. tauschii*), *Zea mays* (Zm), *Musa acuminata* (Mm), *Physcomitrella patens* (Pp), *Phoenix dactylifera* (Pd), *Vitis vinifera* (Vv). (C), Unrooted neighbor-joining tree constructed from the multiple sequence alignment.

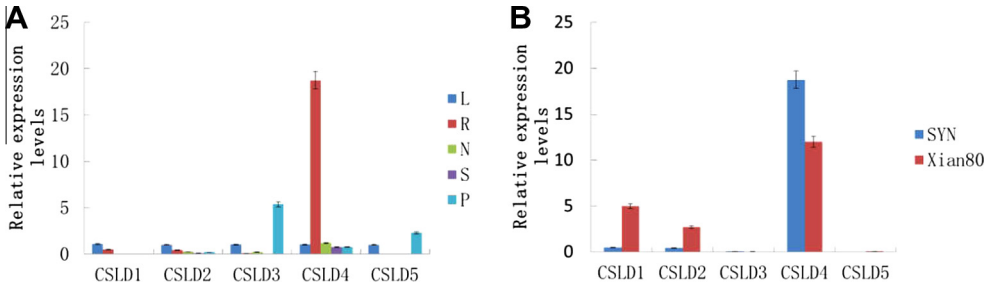

Fig. 4. Expression patterns of *OsCSLD* subfamily in SYN and Xian80. (A) The levels of *OsCSLD* mRNA in different organs of SYN. The abbreviation stand for leaves, roots, nodes, stems, and panicles for SYN (left), respectively. (B) qRT-PCR analysis of *OsCSLD* mRNA in SYN and Xian80.

d61 displayed erect leaves [29], *TDD1* is a tryptophan- (Trp-) and IAA-deficient mutant which exhibited dwarf and narrowed leaf [30]. In this study, we reported a rice landrace Xian80 shown dwarf phenotype combined with narrowed and shorted leaves, small grains, and early heading. Genetics and QTL analysis revealed that height and leaf width were controlled by a major locus *DNL1*. Map-based cloning revealed that *DNL1* encodes OsCSLD4, claimed with CSLD subfamily. Nucleotide variance analysis of nine rice accessions and multi-alignment of *OsCSLD4* with its homologs indicated that one single nucleotide substitution, C to T (C2488T) (SNP8), is the mutational site in *dnl1*. It is notable that several studies had reported some characteristic of *OsCSLD4* (Fig. 3A). *nd1* mutant site located at nucleotide 2894 leaded to alanine to valine substation at the 965th amino acid residue [31], *nrl1-3* (G3360A) resulted in a premature stop codon [32], while *nrl1* [33], *sle1-2* [34] and *cd1* [35] had a 58 bp deletion, 44 bp deletion, and 8 bp insertion brought about frameshift, respectively. Therefore, we demonstrated that the *dnl1* is a novel allele of *OsCSLD4*, and plays an essential role in rice growth and development.

There are five CSLD members in rice, namely OsCSLD1–OsCSLD5, and six in *A. thaliana*, AtCSLD1–AtCSLD6. AtCSLD2 and AtCSLD3 were considered as two closely related CSLD homologs in *A. thaliana* have redundant functions during both root hair and female gametophyte development [36]. It had proved that both AtCSLD1 and AtCSLD4 play important roles in pollen tube growth, and functionally redundant [26]. In our study, OsCSLD1, OsCSLD2, AtCSLD2 and AtCSLD3 were classified into clade II, while OsCSLD5 and AtCSLD1, OsCSLD3 and AtCSLD4 were suited at clade III and IV, respectively. However, OsCSLD4 and AtCSLD5 were placed in the same clade. These results indicated that these CSLD proteins in each clade might have conserved specific developmental roles. Loss of *AtCSLD5* expression results in reduced plant height, root hair elongation, xylan and homogalacturonan synthase activity. *AtCSLD5* highest expressed at the phase of growth in which stem elongation occurs [37]. *dnl1* has the similarity phenotype with *Atcsld5*. *OsCSLD4* was highest expressed in roots suggested OsCSLD4 might also function in roots. Some evidences proved that the cooperative activities of AtCSLD2, AtCSLD3 and AtCSLD5 were required for normal Arabidopsis development [38]. Other *OsCSLD* genes expressed at lower levels in SYN. However, the expression patterns of *OsCSLD* in Xian80 were altered. Our results will provide further insights into the function of the CSLD subfamily in rice.

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