Identification and Genetic Characterization of a Novel Tillering Dwarf Semi-Sterile Mutant \textit{tdr1} in Rice

Bingrui Sun\textsuperscript{1,2*}, Tingyou Huang\textsuperscript{3*}, Chongyun Fu\textsuperscript{1,2#}

\textsuperscript{1}Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China  
\textsuperscript{2}Guangdong Provincial Key Laboratory of New Technology in Rice Breeding, Guangzhou, China  
\textsuperscript{3}Mianyang Academy of Agricultural Sciences, Mianyang, China  

Email: fcyyd2901@126.com

Abstract

Tillering and plant height are important components of plant architecture and grain production in rice. We identified a novel high tillering, dwarf and semi-sterile mutant, as named \textit{tdr1} in a rice maintainer line E20 derived from the cross between 
IR68888B and Luxiang 90. The investigation of tiller dynamic in the \textit{tdr1} line displayed 3 different phases: rapid increasing of tillers in the vegetative growth stage, producing no new tillers in the transition stage from the vegetative growth to reproductive growth, and regeneration of new tillers after heading. The assay of hormones showed the significant reduction of brassinolide level and no change of the levels of gibberellic acid, cytokinin and strigolactone in the \textit{tdr1} line. Genetic analysis indicated the phenotype of high tillering, dwarfism and semi-sterility is controlled by a recessive gene in several different segregation populations. The \textit{TDR1} gene was mapped in the 105 kb interval between RM3288 and RM6590 on chromosome 4. Cloning of \textit{TDR1} gene would provide a new opportunity to uncover the molecular mechanism of the development of plant height and tiller in rice.

Keywords

Rice, High Tillering, Dwarf, Semi-Sterile, Mapping

1. Introduction

Plant height and tillering are two important traits of plant architecture affecting grain yields in rice. Dwarfism improves plant lodging resistance to increase grain

*The co-first authors contributed equally to this work.
yield and harvest index [1]. Rice tillering determines the number of panicles per plant. Excessive tillering generally causes a high rate of unproductive tiller, small panicle size and poor setting rate [2]. It was proved that there was a negative correlation between tiller number and plant height [3].

Tillers originated from axillary buds in the axil of leaves and axillary buds are usually dormant after their formation. The outgrowth of axillary buds is regulated by the interaction of environmental and endogenous factors [4]. In rice, it is reported that the growth and development of tillers were associated with plant hormones such as auxin, cytokinin, gibberellins (GA), brassinosteroid and strigolactone [5]-[11]. A series of high-tillering dwarf mutants have been identified and characterized in rice in detail and some underlying genes have been cloned [12]-[17].

In this study, we identified a novel mutant plant tdr1 with high-tillering, dwarf and semi-sterile phenotypes from a rice maintainer line E20 derived from the cross between IR68888B and Luxiang 90. We investigated its phenotype and the response to plant hormones and performed mapping analysis.

2. Materials and Method

2.1. Plant Materials

In the spring of 2006, we found a mutant with multi-tillers, dwarfism and semi-sterility (named as tdr) in the F4 progeny line E20 from the cross between IR68888B (female parent) and Luxiang 90 (male parent). We also created several F1 populations derived from the crosses between the tdr1 line (female parent) and other indica lines E20, 931, IR68888B and Luxiang 90 (male parents).

2.2. Phenotypic Characterization and Assays of Phytohormone Level

In order to characterize the mutant phenotype, the mutant tdr, its wild type, and 9311 were grown in paddy field in Mianyang, Sichuan. A total of 30 plants from each of the above three lines were used to investigate several traits including tiller number, plant height, inter-node length, kilo-grain weight and pollen fertility.

Because the phenotypes of multi-tiller, dwarfism and semi-sterility were generally associated with plant hormones, exogenous gibberellins (1 × 10^{-4} mol/L GA3) and sterile water as control were sprayed for the tdr line, its wild type and 9311 at elongation stage in the greenhouse. The length of panicle, 1st, 2nd, 3rd, 4th and 5th upper internodes were measured for 10 plants each line at mature stage. Simultaneously, phytohormone levels were scored for young seedling using the ELISA kits (AndyGene) of four plant hormones including gibberellic acid (GA), cytokinin (CTK), brassinolide (BR) and strigolactone (SL) according to the corresponding protocols (http://www.andygene.com/index.php).

2.3. Extraction of DNA and Mapping of TDR Gene

DNA was prepared using the modified hexadecyltrimethylammonium (CTAB)
method. Ten normal plants and 10 dwarf plants from the F2 population derived from tdr and 9311 were used to create the dominant and recessive bulks, respectively. Fine mapping of TDR gene was conducted with SSR and InDel markers. InDel markers were designed according to the genomic sequences of indica line 9311 and japonica line Nipponbare.

3. Results

3.1. Phenotypic Characterization of tdr1 Line

The mutant tdr showed dwarfism, high tillering ability, small grain and low setting rate (Figure 1). Ten plants from each lines tdr, its wild type and 9311 were characterized. Compared with the wild type, the maximum number of tillers in tdr1 line is up to 81.4 and its plant height is not enough half (45.5 cm) of the plant height of its wild type (109.86 cm). Its kilo-grain weight is 15.36 gram, the setting rate is only 32.47% and lots of unstained pollens were observed.

To fully understand tillering dynamics of tdr1 line, we continuously investigated the number of tillers of 10 plants for tdr1 line, its wild type and 9311 every 5 days after transplantation. We found that the tdr1 line has rapidly increased the tiller number since June 13th (Figure 2) and the number of tillers reached a plateau for the tdr1 line on July 8th. For the wild type and 9311, the number of tillers have become immobile since June 28th, but new tillers occurred again on about August 7th (the heading day of tdr1 line) for the tdr1 line, which indicates that the transition from vegetative to reproductive stages could inhibit the growth of axillary buds and the end of the transition phase could relieve the dormancy of axillary buds in the tdr1 line again.

3.2. Effect Evaluation of Exogenous Gibberellin and Phytohormones Determination

In previous reports, GA plays an important role for plant height and tillering. In order to understand the effect of GA for the tdr1 line, we treated the tdr1 line, its wild type and 9311 with exogenous GA3 (1 × 10^{-4} mol/L). We found that exogenous GA can increase the plant height for the above three lines, but showed different effect for the three lines (Figure 3). For the tdr1 line, the 2nd and 3rd upper internodes were significantly elongated, but its plant height was not fully recovered. For its wild type, the length of the 1st, 2nd and 3rd upper internodes were remarkably increased. For 9311, the panicle, 1st, 2nd, 3rd and 4th internodes were significantly elongated. These above results indicate that the phenotype of the tdr1 line is not caused by the signal transduction pathway of GA.

To fully understand the change of phytohormones in the tdr1 line, we measured the content of phytohormones such as gibberellins (GA), cytokinin (CTK), brassinolide (BR) and strigolactone (SL) in the tdr line and its wild type. Compared with in the wild type (GA 113.02 pmol/L, CTK 41.99 pmol/L, SL 37.08 pmol/L), the levels of GA (109.34 pmol/L), CTK (43.05 pmol/L) and SL (35.18 pmol/L) did not significantly change in the tdr1 line, but the level of BR (165.22 pmol/L)
Figure 1. The variant phenotype of the tdr line including plant height, the size of anthers and seeds and the fertility of pollen.

Figure 2. The dynamics of tiller number of the tdr line, its wild type and 9311.

pmol/L) remarkably decreased in the tdr line (Figure 4), implying the biosynthesis of brassinolide might play roles for the phenotype of tdr line.

3.3. Genetic Segregation of tdr Phenotype

To determine the inheritance pattern of the phenotype of high-tillering, dwarfism and semi-sterility in the mutant tdr line, we used the tdr line and four normal semi-dwarf lines with different genetic background such as its wild type E20, 9311, IR68888B and Luxiang 90 to construct several F2 populations and 1
Figure 3. Effects of exogenous GA on the length of different internodes and panicles in tdr1 line, its wild type and 9311.

Figure 4. The assays of four phytohormones including GAs, CTKs, BRs and SLs in the leaves of the tdr1 line and its wild type.

backcross population (BC$_1$F$_1$). We scored plant height of the parent lines, their F$_1$, F$_2$ and BC$_1$F$_1$ progenies and investigated the segregation ratios of plant height in F$_2$ and BC$_1$F$_1$ populations (Table 1). All the F$_1$ plants from the four crosses showed the wild-type phenotype, and all of these F$_2$ progenies have a segregation ratio of 3:1 between wild-type and mutant plants ($\chi^2 < 3.84$), indicating this phenotype is controlled by a recessive gene.

3.4. The Mapping of TDR1 Gene

Genetic analysis showed that the phenotype of high-tillering, dwarfism and semi-sterility was controlled by a recessive gene. We selected the F$_1$ population
from the cross between 9311 and the tdr1 line to map the TDR gene. Thirty nine SSR markers showed the polymorphism between 9311 and tdr1 line in about 600 used SSR markers evenly distributed on 12 chromosomes in rice. We used these polymorphic SSR markers to perform the linkage analysis for 392 F₂ recessive plants and finally the underlying gene TDR1 was located in the 2.1 cM interval between RM252 and RM303 on Chr.4 (Figure 5, Table 2).

For fine mapping of the TDR1 gene, we further screened the polymorphic markers between RM252 and RM303 based on the Nipponbare reference genome and obtained 7 polymorphic markers in this interval. The 1620 recessive plants in the F₂ population were used to fine map the TDR1 gene. Finally, the TDR1 gene was mapped in the 105.4 kb interval between RM3288 and RM6590. Based on the Nipponbare reference genome (https://rapdb.dna.affrc.go.jp/), there are 20 annotated genes including D17/HTD1 (Os04g0550600) and OsSPL7 (Os04g0551500) (Table 3).

4. Discussion

Recently lots of high-tillering dwarf rice mutants were reported and some underlying genes were mapped and cloned. Phytohormones play important roles for plant growth and development. In this study, the tdr1 mutant displayed the capability of high tillering, dwarfism, small seeds and the reduction of fertility. The tdr1 line was treated with exogenous GA3 and its height was not fully recovered, which indicates the tdr1 mutant is independent of the GA pathway. The
The linkage map of TDR1 gene.

Table 2. Lists of the primers used for mapping the underlying gene TDR1.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM252</td>
<td>TTCGCTGACGTGATAGGTTG</td>
<td>ATGACTTGATCCCGAGAACG</td>
</tr>
<tr>
<td>RM303</td>
<td>GCATGGCCAAATATTAAAGG</td>
<td>GGTTGGAAATAGAAGTTCCGGT</td>
</tr>
<tr>
<td>RM1018</td>
<td>ATCTTGCCACCTGCACCAC</td>
<td>TGTGACTGCTTTTCTGTCCGC</td>
</tr>
<tr>
<td>ID12</td>
<td>TGCCAAATAGATCGCTGA</td>
<td>ACCAACGACAGATTAGTG</td>
</tr>
<tr>
<td>RM6589</td>
<td>AAGTTCACAACACGTCGTGC</td>
<td>CGACGCTGTTGACAGCC</td>
</tr>
<tr>
<td>RM3288</td>
<td>CTCGTACCGTCAAAAGACCC</td>
<td>AATCTGGAGGGCAGTCGCA</td>
</tr>
<tr>
<td>RM6590</td>
<td>TTGCAGTGCTGAGAGGAGG</td>
<td>CACATGTCATCTCACACCC</td>
</tr>
<tr>
<td>RM3820</td>
<td>CTCGTACAGTCAGGACACAG</td>
<td>GTGGCTTTCTAATGGTGGG</td>
</tr>
<tr>
<td>RM470</td>
<td>TCCTCATCGGCTTCTCTTTTC</td>
<td>AGAACCCTTTCTACGTACAG</td>
</tr>
</tbody>
</table>

assay of phytohormone level also showed that this mutant phenotype was not related with GA, CTK and SL and could be caused by the reduction of BR. It is proved that BR plays crucial roles in the development of lateral organs including lateral organogenesis, plant height, seed size and fertility [18] [19] [20]. On the aspect of tiller dynamics, the tdr1 line rapidly increases of tillers in the vegetative growth phase, stops producing new tillers in the transition phase from the vegetative growth to reproductive growth, and regenerated new tillers after heading. The htd1 mutant has been reported to still keep the high tillering capacity [21].

In our paper, the underlying gene TDR1 was located in the about 105 kb region of chromosome 4. In this candidate region, 20 genes are annotated and includes D17/HTD1 and OsSPL7 causing the phenotype of high tillering and dwarfism. D17/HTD1 was proved to be associated with strigolactone biosynthesis [22] [23]. However, our hormone assays also indicates that there is no significant change for strigolactone in the tdr1 line and its wild type. OsSPL7 was reported as a target of miR156f and binds directly the OsGH3.8 promoter to regulate tiller and plant height, and the miR156f/OsSPL7 pathway was involved in the regulation of plant architecture mediated by auxin [24]. Currently, it is
Table 3. The candidate genes in the mapping interval of TDR1 gene.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Position</th>
<th>Annotation information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Os04g0550200</td>
<td>chr04:27539878..27540865</td>
<td>Pathogenesis-related transcriptional factor and ERF domain containing protein. OsERF34</td>
</tr>
<tr>
<td>Os04g0550300</td>
<td>chr04:27544416..27545300</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>Os04g0550400</td>
<td>chr04:27551423..27560074</td>
<td>E3 ligases of H2Bub1, Transcriptional regulation of anther development. OsHUB1</td>
</tr>
<tr>
<td>Os04g0550500</td>
<td>chr04:27563230..27566440</td>
<td>Similar to N-acetyl glutamate kinase 2</td>
</tr>
<tr>
<td>Os04g0550600</td>
<td>chr04:27567824..27570449</td>
<td>Negative regulation of the outgrowth of axillary buds, Strigolactones biosynthesis. D17/HTD1</td>
</tr>
<tr>
<td>Os04g0550700</td>
<td>chr04:27570555..27572699</td>
<td>Uncharacterised conserved protein UCP012943 domain containing protein</td>
</tr>
<tr>
<td>Os04g0550800</td>
<td>chr04:27575661..27576994</td>
<td>Major intrinsic protein family protein</td>
</tr>
<tr>
<td>Os04g0550833</td>
<td>chr04:27576034..27576647</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>Os04g0550866</td>
<td>chr04:27595181..27595879</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>Os04g0550900</td>
<td>chr04:27595492..27596105</td>
<td>Aquaporin TIP2-3</td>
</tr>
<tr>
<td>Os04g0551200</td>
<td>chr04:27605166..27608318</td>
<td>Similar to Cytoplasmic malate dehydrogenase</td>
</tr>
<tr>
<td>Os04g0551300</td>
<td>chr04:27608423..27612487</td>
<td>Similar to Growth regulator like protein</td>
</tr>
<tr>
<td>Os04g0551400</td>
<td>chr04:27609034..27611514</td>
<td>Non-protein coding transcript</td>
</tr>
<tr>
<td>Os04g0551500</td>
<td>chr04:27614776..27618001</td>
<td>Squamosa promoter-binding-like protein 7. OsSPL7</td>
</tr>
<tr>
<td>Os04g0551550</td>
<td>chr04:27614946..27617739</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>Os04g0551600</td>
<td>chr04:27627478..27628262</td>
<td>Zinc finger, FYVE/PHD-type domain containing protein</td>
</tr>
<tr>
<td>Os04g0551700</td>
<td>chr04:27633216..27637876</td>
<td>PAP fibrillin family protein</td>
</tr>
<tr>
<td>Os04g0551800</td>
<td>chr04:27638063..27643857</td>
<td>Similar to T-complex protein 1, alpha subunit (TCP-1-alpha) (CCT-alpha)</td>
</tr>
<tr>
<td>Os04g0552000</td>
<td>chr04:27645701..27647185</td>
<td>Barwin-related endogulcanase domain containing protein</td>
</tr>
<tr>
<td>Os04g0552066</td>
<td>chr04:27646851..27647183</td>
<td>Hypothetical protein</td>
</tr>
</tbody>
</table>

found that there is the interaction between auxin and brassinosteroid regulating plant growth and development. Auxin could promote the expression of *DWARF4*, a crucial hydroxylase for BR biosynthesis to control endogenous BR level and also inhibit the binding of BZR1 to the promoter of *DWARF4* [25] [26]. In our study, the level of BRs is significantly reduced in the *tdr1* line, compared with its wild type. Because the level of auxin was not tested, we can not know whether the auxin-BR interaction was destroyed to cause the phenotype of high tillering, dwarfism and semi-sterility in the *tdr1* line.

**Acknowledgements**

This work was supported in part by grants from Sichuan Applied Basic Research Project (2015JY0061), Natural Science Foundation of Guangdong Province, China (2017A030310094) and Science and Technology Program of Guangzhou, China (201707010218).

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.
References


