Journal of Integrative Agriculture 2018, 17(5): 1057–1065

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Phenotypic analysis of a *dwarf and deformed flower3* (*ddf3*) mutant in rice (*Oryza sativa* L.) and characterization of candidate genes

WANG Yu-peng, TANG shuang-qin, WU Zhi-feng, SHI Qing-hua, WU Zi-ming

Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education/Collaborative Innovation Center for the Modernization Production of Double Cropping Rice/College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, P.R.China

Abstracts

Dwarf mutants are the crucial resources for molecular biology research and rice breeding. Here, a rice mutant, *dwarf and deformed flower3 (ddf3)*, was identified in tissue culture of *Oryza sativa cv*. Dongjin. Compared with wild type, the *ddf3* mutant exhibited severe dwarfism, a greater number of tillers and significantly decreased fertility. In addition, leaf length, panicle length, and grain length, were significantly shorter. All internodes of *ddf3* were shorter than those of wild type, and histological analysis revealed that internode cell elongation was significantly inhibited in *ddf3*. In the *ddf3* mutant, pollen activity was significantly decreased, and the development of most stigmas was abnormal. Genetic analysis indicated that the *ddf3* mutant phenotypes are controlled by a single or tightly linked nuclear genes. Using an F_2 mapping population generated from a cross between *ddf3* and Yangdao 6 (9311), the *DDF3* gene was mapped to a 45.21-kb region between insertion-deletion (InDel) markers M15 and M16 on the long arm of chromosome 7. Sequencing revealed a 13.98-kb-deletion in this region in the *ddf3* mutant genome that resulted in the complete or partial deletion of *ZF* (DHHC type zinc finger protein), *EP* (expressed protein), and *FH2* (actin-binding FH2 domain-containing protein) genes. Quantitative RT-PCR analyses revealed that in wild type, the transcript levels of *FH2* were almost the same in all organs, while *ZF* was mainly expressed in the panicle, and no expression of *EP* was detected in any organ. Based on these results, *ZF* and *FH2* could be potential *DDF3* candidate genes involved in the regulation of rice morphology and flower organ development. Our work has laid the foundation for future functional analysis of these candidate genes and has provided a profitable gene resource for rice breeding for increased fertility in the future.

Keywords: rice, *dwarf and deformed flower 3*, mapping, DHHC-type, actin-binding FH2 domain

1. Introduction

Rice is an important food crop that feeds over half of the world's population, and it is also one of the most important monocot model systems because of characteristics such as a small sequenced genome and a mature *Agrobacterium*-mediated genetic transformation system. Rice production is correlated with multiple factors, such as tiller number, grain number per panicle, grain size, and plant height. Plant height,

Received 24 May, 2017 Accepted 8 August, 2017
Correspondence WU Zi-ming, Tel: +86-791-83828113, Fax: +86-791-83813877, E-mail: wuzmjxau@163.com

doi: 10.1016/S2095-3119(17)61770-2

which is established during the vegetative growth periods, plays a crucial role in plant architecture, photosynthetic efficiency, nutrient absorption, and lodging resistance (Krag and Nielsen 1989; Wang *et al*. 2016). Generally, taller plants are advantageous for maintaining a higher photosynthetic efficiency, but have reduced lodging tolerance; dwarf plants have increased lodging tolerance, but severe dwarfism affects the development of other organs, which is not conducive to the improvement of rice yield (Ding *et al*. 2015; Zhao *et al*. 2015; Zhang *et al*. 2016). The appropriate plant height, therefore, is a critical factor in improving rice yield.

During the last 20 years, considerable progress has been made in elucidating the regulatory mechanisms controlling plant height in rice. Previous studies have shown that plant hormones such as gibberellin (GAs) (Daviere and Achard 2013), brassinolide (BRs) (Ye *et al*. 2011), and strigolactone (SLs) (Screpanti *et al*. 2016), and genes associated with cell wall synthesis, cellular differentiation, and cell elongation (Yang *et al*. 2011), are the main factors controlling plant height. Mutations in these genes inhibit the development of stem internodes, leading to a dwarf phenotype. So far, the genes known to be related to plant dwarfism are mainly involved in GA, BR, and SLs biosynthesis and signal transduction. Additionally, some genes that are not associated with hormone metabolic pathways, such as *OsKinesin-13A* (Deng *et al*. 2014), *OSH15* (Sato *et al*. 1999), *OsGLP1* (Banerjee and Maiti 2010), and *DTH8* (Wei *et al*. 2010), have been found to influence plant height.

Although determined during the vegetative growth stage, plant height has a significant effect on reproductive traits, such as heading date, flower development, and rice quality (Song *et al*. 2008; Duan *et al*. 2012a, b). Many dwarf mutants are associated with the abnormal flower development. For example, a mutation in *DDF1*, which encodes an F-box protein, leads to much shorter plant height compared with wild type and significant structural abnormalities in florets (Duan *et al*. 2012b). The *ddf2* mutant has a severe dwarf phenotype and defects in spikelet and floral organs (Zhang *et al*. 2015). *DEFORMED FLORAL ORGAN1* (*DFO1*) was identified as a rice epigenetic repressor. The *dfo1* mutant has a small stature and defects in palea identity (Zheng *et al*. 2015). Although a few genes have been reported to control both plant height and floral organ development, flower-deformation/dwarf genes remain largely undiscovered.

To gain further insight into the relationship between plant height and flower development and the molecular basis for these phenotypes, we identified and characterized the *dwarf and deformed flower 3* (*ddf3*) mutant, which in addition to a severe dwarf phenotype, exhibits pollen abortion and pistils with multiple stigmas. The mutant gene was mapped between insertion-deletion (InDel) markers M15 and M16 on the long arm of chromosome 7, which corresponds to an approximate physical distance of 45.21 kb. Sequencing revealed a 13.98-kb deletion in the *ddf3* mutant genome, which caused the deletion of three genes. Our work has laid the foundation for further gene cloning and functional analysis of *DDF3*.

2. Materials and methods

2.1. Plant materials and growth conditions

A dwarf mutant, which was named *dwarf and deformed flower3* (*ddf3*), was identified in the progeny of an *Oryza sativa* cultivar Dongjin plant derived from tissue culture transformation. We confirmed that the mutant phenotypes were stably inherited after five years of cultivation in natural condition. To map the mutant locus, we generated an F_2 mapping population derived from a cross between the *ddf3* mutant and the cultivar Yangdao 6 (9311). For phenotypic characterization, microscopic observation, analysis of floral organ development, mapping, and qRT-PCR assays, *ddf3*, wild-type (WT) Dongjin and the F_2 mapping population were cultivated in an experimental field at Nanchang (28°41´N, 115°55´E) during the normal growing season.

2.2. Phenotypic characterization

At the heading stage, plant height, leaf length, leaf width, and internode length were measured in the mutant and WT. Yield-related traits, including panicle length, no. of grains per panicle, seed-setting rate, grain size, and 1 000-grain weight, were measured at harvest. All trait measurements are presented as the average from 10 plants.

2.3. Histological analysis of internodes

At the heading stage, the middle sections of the second internodes were collected from the *ddf3* mutant and WT and then fixed in FAA (formalin:acetic acid:50% ethanol, 2:1:17 (v/v)) overnight. After a series of dehydration and infiltration steps, the tissues were embedded in paraffin (Paraplast Plus; Sigma-Aldrich). The embedded tissues were cut into 8 μm sections with a microtome (Leica RM2265, Germany). Then the paraffin was removed from the sections with xylene. This was followed by a dehydration through an ethanol gradient, and toluidine blue staining. The stained sections were observed and photographed with a Nikon 50i microscope (Nikon, Japan).

2.4. Analysis of pollen activity and stigma development

The I_2-KI staining method was used to detect pollen activity as described previously (Jiang *et al*. 2007). Briefly, the