



## Identification of a Gravitropism-Deficient Mutant in Rice

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**Abstract:** A gravitropism-deficient mutant *M96* was isolated from a mutant bank, generated by ethyl methane sulfonate (EMS) mutagenesis of indica rice accession ZJ100. The mutant was characterized as prostrate growth at the beginning of germination, and the prostrate growth phenotype ran through the whole life duration. Tiller angle and tiller number of *M96* increased significantly in comparison with the wild type. Tissue section observation analysis indicated that asymmetric stem growth around the second node occurred in *M96*. Genetic analysis and gene mapping showed that *M96* was controlled by a single recessive nuclear gene, tentatively termed as *gravitropism-deficient M96* (*gdM96*), which was mapped to a region of 506 kb flanked by markers RM5960 and InDel8 on the long arm of chromosome 11. Sequencing analysis of the open reading frames in this region revealed a nucleotide substitution from G to T in the third exon of *LOC\_Os11g29840*. Additionally, real-time fluorescence quantitative PCR analysis showed that the expression level of *LOC\_Os11g29840* in the stems was much higher than in the roots and leaves in *M96*. Furthermore, the expression level was more than four times in *M96* stem than in the wild type stem. Our results suggested that the mutant gene was likely a new allele to the reported gene *LAZY1*. Isolation of this new allele would facilitate the further characterization of *LAZY1*.

**Key words:** plant architecture; gravitropism; *LAZY1*; gene mapping; mutant

Plant architecture is one of the significant factors associated with rice yield. Erect plant architecture is considered to be the ideal plant type and continuously selected by farmers and breeders (Kovach et al, 2007). Previous studies have shown that gravity is a significant external factor during plant growth and development, and contributes to morphogenesis and physiological function of the plant. Plant response to gravity is called gravitropism or geotropism, and can be divided into four sequential steps: gravity perception, signal formation in the gravity perceptive cell, intracellular and intercellular signal transduction, and asymmetric cell elongation between the upper and lower sides of the responding organs (Fukaki et al, 1996).

The normal gravitropism (shoot negative gravitropism and root positive gravitropism) is necessary for plant

morphological development and biological function (Dong, 2014). Most plant species show the root positive gravitropism for the uptake of water and minerals while exhibit the shoot negative gravitropism in favor of photosynthesis and fertilization preferably (Song et al, 2006). Mutants with defective gravitropism from *Arabidopsis* vary in their response to gravity and can be divided into five different classes: class I, mutants that show an abnormal gravitropic response in inflorescence stems only; class II, mutants that show defective gravitropism in both inflorescence stems and hypocotyls, but normal gravitropism in roots; class III, mutants that show defective gravitropism in the roots, hypocotyls and inflorescence stems; class IV, mutants that have defective gravitropism in hypocotyls and roots but not in inflorescence stems; class V, mutants that exhibit

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only a defective gravitropic phenotype in roots (Tasaka et al, 1999). Gravitropism has been also observed in rice, and a few number of gravitropism-related genes have been identified and characterized such as *LAZY1* (*LAI*) (Li et al, 2007), *PROSTRATE GROWTH1* (*PROG1*) (Jin et al, 2008), *LOOSE PLANT ARCHITECTURE1* (*LPA1*) (Liu et al, 2016) and *spk(t)* (Miyata et al, 2005). Among these genes, *LAZY1* controls rice shoot gravitropism through regulating polar auxin transportation (Li et al, 2007) and *LAZY1*-dependent and -independent signaling pathways have been identified in coleoptiles (Yoshihara and Iino, 2007). *PROG1* encodes a single Cys<sub>2</sub>-His<sub>2</sub> zinc-finger protein (Tan et al, 2008), and predominantly expresses in the axillary meristems (Jin et al, 2008). It is believed that *PROG1* lost its function during the evolutionary process in the ancestor of modern cultivated rice. *LPA1*, encoding a plant-specific INDETERMINATE DOMAIN protein, influences plant architecture by affecting the gravitropism of leaf sheath pulvinus and lamina joint (Wu et al, 2013). In fact, *LPA1* determines lamina joint bending by suppressing auxin signaling that interacts with C-22-hydroxylated and 6-deoxo brassinosteroids in rice (Liu et al, 2016). *spk(t)* is thought to be a gene responsible for the stub spreading phenotype in Kasalath but yet to be isolated (Miyata et al, 2005).

In the present study, we identified a prostrate growth mutant *M96* from an ethane methyl sulfonate (EMS)-induced rice accession ZJ100 mutant bank. Here, we present the results on characterization of the mutant phenotype, genetic analysis, gene mapping and candidate gene prediction. Our results showed that the prostrate growth phenotype of *M96* is controlled by a single recessive gene which is likely a new allele to *LAZY1*. Isolation of this new allele, tentatively termed as *gravitropism-deficient M96* (*gdM96*), would facilitate the further characterization of *LAZY1*.

## MATERIALS AND METHODS

### Rice materials

The *M96* mutant was obtained from an EMS-induced

indica rice accession ZJ100 mutant bank. This mutant has been selfed for more than 10 generations, and the target trait has been stably expressed in both the greenhouse and field conditions in Fuyang, Zhejiang Province and Lingshui, Hainan Province, China.

### Methods

#### Gravity response analysis

For gravity response experiment, the seeds of mutant *M96* and the wild type ZJ100 were dehusked and surface sterilized with 75% ethanol for 2 min and 30% bleach for 15 min, and then washed five times with autoclaved distilled water. The sterilized seeds were then planted in plates containing 1/2 MS medium (pH 5.8) and 0.5% Plant Preservative Mixture (PPM<sup>TM</sup>, Beijing QiWei YiCheng Tech Co., Ltd., Beijing, China) for 5 d under continuous light or continuous dark at 28 °C in a growth chamber (Panasonic, MLR-352H-PC, Osaka, Japan), respectively. Consequently, the seedlings were placed horizontally in the same conditions for 24 h.

#### Exogenous hormone treatment

For hormone treatment, the sterilized seeds were planted in 1/2 MS medium (pH 5.8) and 0.05% Plant Preservative Mixture (PPM<sup>TM</sup>, Beijing QiWei YiCheng Tech Co., Ltd., Beijing, China) supplemented with different concentrations of exogenous hormones (Table 1) and grew in a growth chamber (Panasonic, MLR-352H-PC, Osaka, Japan) for 5 d at 28 °C with 14 h light and 10 h dark each day. Plant hormones 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid 3 (GA<sub>3</sub>) were purchased from Sigma-Aldrich Co., ST. Louis, USA.

#### Tissue microstructure

Optical microscopic observation of stems was performed with plants at the heading stage grown in the paddy fields. The second internodes above the ground were cut longitudinally and fixed in 2.5% glutaraldehyde overnight. The optical microscopic observation was carried out as described by Zhang et al (2007).

#### Genetic analysis and gene mapping

*M96* mutant was used as the female parent and

**Table 1. Concentrations of exogenous hormones.**

Hormone type	Treatment					
	1	2	3	4	5	6
2,4-dichlorophenoxyacetic acid	0.1	0.1	0.5	1.0	2.0	4.0
Gibberellic acid 3	0.5	1.0	2.0	4.0	10.0	20.0

mg/L

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