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# Chromosome mapping, molecular cloning and expression analysis of a novel gene response for leaf width in rice

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ABSTRACT

Genetic analysis revealed that narrow leaf, small panicle, thin and slender stems as well as low fertility rate of an *Indica* rice variety were recessive traits and controlled by a single gene. Applying map-based cloning strategy, a novel narrow leaf gene, which was named *nal11* was delimited to an interval of 58.3 kb between the InDel markers N10 and InD5016. There are 9 genes in the mapping interval, and only a heat shock DNAJ protein encode gene (*Os07g09450*) has a specific G to T SNP, which was occurred at the last base of the second exon of *Os07g09450* in ZYX. 5′ and 3′ RACE result shown that there were two transcripts in *NAL11*, and the SNP in *nal11* leads to a variable shear of mRNA. In addition, this type of mRNA alternative splicing together with a stop codon closely followed the SNP which caused termination of translation destroyed the DNAJ domain of *nal11*'s product. These results suggested that the heat shock *DNAJ* gene was most likely to be the candidate gene of *nal11*. The results of RT-PCR and real-time PCR further verified that the SNP in the ZYX-*nal11* gene affects mRNA splicing pattern. Phenotype of ZYX may be caused by a statistically significant reduction in the total number of small veins in leaf, size and number of small vascular bundles and cells in stems, similar to several previous reported mutations. The basic molecular information we provide here will be useful for further investigations of the physiological function of the heat shock *DNAJ* gene, which will be helpful in better understanding the role of the DNAJ family in regulation of plant type traits such as leaf width of rice.



## 1. Introduction

Rice is a major cereal food source for more than half of the world's population [17]. After two times of the green revolution, rice yield has been greatly improved. However, after entering into the twenty-first century, the world population continues to expand, soil erosion and degeneration caused decrease of arable land, sustained tension of agricultural water resources in many areas, global warming and the future demand for bio-energy has made the food crisis become more and more emergent. In order to improve rice yield, plant type breeding combined with heterosis has become a consensus strategy to break through the bottleneck of high yield breeding. Excellent plant morphology has high utilization rate of light energy, which is the basis of high yield. As an important food crop, rice leaves are not only the main vegetative organs, but also play an important role in plant type and yield [18]. The shape and size of rice leaves are closely associated with photosynthetic efficiency. Therefore, through the improvement of rice leaf morphology to cultivate new varieties with ideal plant type has become a hot research topic [3,7,9].

Narrow leaf usually accompanied by upright, these two traits are beneficial to all layers of the blades to fully absorb light energy. Therefore, narrow leaf is one the most important traits on leaf morphology traits research. To date, several narrow leaf genes have been identified. The *NAL1* gene encodes a putative trypsin-like serine and cysteine protease, which affects polar auxin transport as well as the vascular patterns. In accordance with reduced leaf blade width, leaves of *nal1* contain a decreased number of longitudinal veins [11]. *NAL2* encodes an identical OsWOX3A transcriptional activator. *NAL3* is a paralogous gene of *NAL2*. Both of them involved in organ development, including lateral-axis outgrowth and vascular patterning of leaves, lemma and palea morphogenesis

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in spikelets, and development of tillers and lateral roots. Narrow leaves of *nal2*/*nal3* resulted mainly from reduced lateral-axis outgrowth with fewer longitudinal veins and much more, larger bulliform cells. *NAL2/3* alters the expression levels of several leaf-development associated genes. Moreover, several auxin transport related genes were also significantly changed [27]. *NAL7/COW1*, encodes a flavin-containing monooxygenase which involved in auxin biosynthesis, and it was a homologue to *YUCCA*. The number and size of bulliform cells was reduced in *nal7* [13]. The *NAL9* gene encodes a protein homologous to the *Arabidopsis* ClpP6 subunit that targeted to the chloroplast. The number of small vascular bundles was reduced in the *nal9* blades [31]. *Narrow leaf and dwarf1* (*nd1*) was located on chromosome 12 and encodes the cellulose synthase-like protein D4 (OsCSLD4). Reduction of xylan content and cellulose caused structural defects in primary walls indicate that OsCSLD4 plays important roles in cell-wall formation and plant growth. Later study found that *narrow and rolled leaf1* (*nrl1*), *slender leaf1* (*sle1*), *dwarf and narrowed leaf1* (*dnl1*), *OsCD1* and *OsCSLD4* are

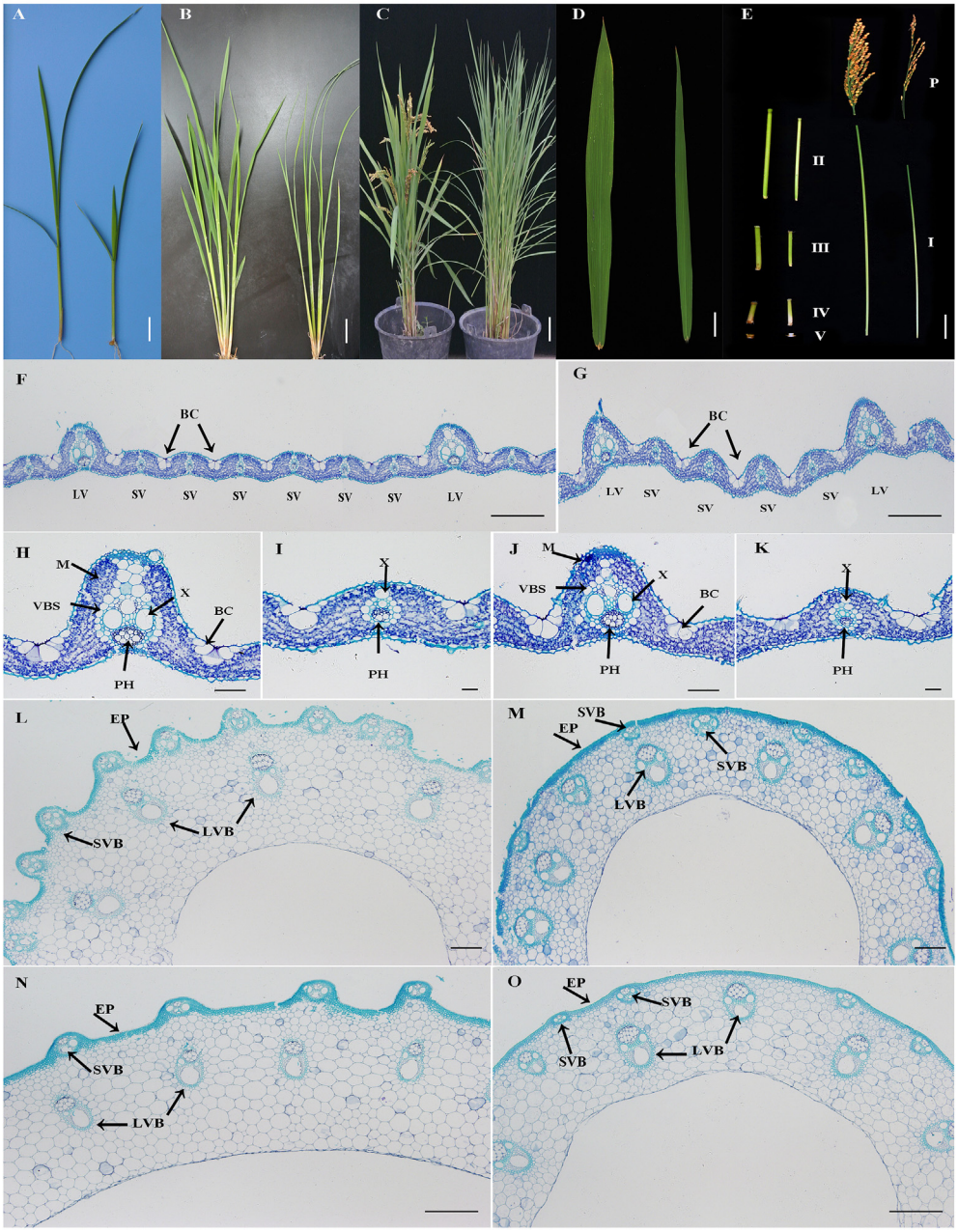


**Fig. 1.** Gross morphology of 02428 and ZYX plants and paraffin section observation of 02428 and ZYX leaves and stems at mature stage.
A-C, Gross morphology of 02428 (left) and ZYX (right) plants at trefoil stage, tillering stage and maturation stage; D, Flag leaf of 02428 (left) and ZYX (right); E, Panicle and internodes of 02428 (left) and ZYX (right), P indicate Panicle, I to V indicate the corresponding internodes from top to bottom; F, Transverse section of 02428 mature stage leaf, LV indicate large vein, SV indicate small vein, BC indicate bulliform cells; G, Transverse section of ZYX mature stage leaf; H, Enlarged LV of 02428, M indicate mesophyll cell, VBS indicate vascular bundle sheath, X indicate xylem, PH indicate phloem; I, Enlarged SV of 02428, X indicate xylem, PH indicate phloem; J, Enlarged LV of ZYX, M indicate mesophyll cell, VBS indicate vascular bundle sheath, X indicate xylem, PH indicate phloem; K, Enlarged SV of ZYX, X indicate xylem, PH indicate phloem; L, Transverse section of 02428 neck-panicle internode, EP indicate epidermis, LVB indicate large vascular bundles, SVB indicate small vascular bundles; M, Transverse section of ZYX neck-panicle internode; N, Transverse section of 02428 internode II, EP indicate epidermis, LVB indicate large vascular bundles, SVB indicate small vascular bundles; O, Transverse section of ZYX internode II; Bars: A, 1 cm; B, 5 cm; C, 10 cm; D, 5 cm; E, 5 cm; F, 100 μm; G, 100 μm; H, 50 μm; I, 20 μm; J, 50 μm; K, 20 μm; L, 50 μm; M, 50 μm; N, 100 μm; O, 100 μm.