



NPT1 / OTUB1

Novel High Yielding Rice Ideotype

10% yield increase, tested over 3 years

**Genome editing target for increased yield
in a wide range of crops**

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Patent Literature

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Researchers at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing have cloned and characterized a QTL in rice that is alone responsible for a “new plant type” (NPT) rice crop ideotype with distinctive architecture, having *larger panicles, fewer sterile tillers, stronger culms and higher yield*. This gene encodes a deubiquitinating enzyme, OTUB1, never before associated with a phenotype in plants but which is *highly conserved across plants and other species*.

Reducing the expression or activity of OTUB1 (though not its complete loss) increases grain yield via effects on a number of important yield component traits. This opens the way for increased crop yields using a variety of strategies, including genome editing, and in a range of crop species.

Xiangdong Fu and colleagues identified the single “NPT” QTL in a study of rice recombinant inbred lines showing that the QTL was responsible for enhanced grain number, reduced tiller number and thicker culms. Positional cloning was carried out and identified a previously uncharacterized rice gene, predicted to encode a deubiquitinating enzyme with homology to mammalian OTUB1, a protein associated with regulation of p53 stability and DNA damage repair. The IGDB team were able to describe a number of sequence variations distinguishing the *npt1* allele from the conventional allele – some being SNPs in the coding region, one intronic Indel and one a SNP in the promoter.

Expression analysis using RT-PCR and histochemical techniques showed that peak abundance of OsOTUB1 occurs in the shoot meristem and young panicles and that the gene is strongly expressed in vascular tissue, the root cap and quiescent centre cells. Comparison of OsOTUB1 downregulation achieved by CRISPR/Cas editing of OsOTUB1 with transgenic over expression indicated that reduction of OsOTUB1 levels and/or activity is associated with the high yielding NPT ideotype architecture.

Haplotype analysis revealed that the high yielding *npt1* allele has not been exploited by breeders of elite *indica* and temperate *japonica* rice varieties. The IGDB team examined the effect of combining *npt1* with the high yielding *dep1-1* allele which has been heavily used by rice breeders in China. In a comparison of NILs, ***npt1/dep1-1*** lines flowered at the same time as ***NPT1/dep1-1*** lines, but had fewer tillers, larger shoot apical meristems, more vascular bundles, thicker culms, thicker parenchyma/sclerenchyma cell walls and over three successive field trial seasons the ***npt1/dep1-1*** lines yielded 10.4% more than ***NPT1/dep1-1*** combinations.

In a separate project at IGDB, Yunhai Li and colleagues identified the same gene as being responsible for a “wide and thick” grain phenotype identified in a mutant screen for grain size variants.

Xiangdong Fu’s team have gone on to confirm that OsOTUB1 has deubiquitinase activity, being able to cleave Lys48- and Lys63-linked tetra ubiquitin. They also showed that **OTUB1 orthologues from rice, maize, barley and even mouse are functionally interchangeable**. In humans, OTUB1 interacts with UBC13, an E2 enzyme for Lys63-linked ubiquitination, to inhibit double strand break-induced chromatin ubiquitination, and the IGDB group has shown that the same interactions occur between OsOTUB1 and OsUBC13. Overexpression of OsUBC13 causes a similar phenotype to plants carrying *npt1/reduced OTUB1* alleles, whereas RNAi downregulation of OsUBC13 causes the negative phenotypes seen by overexpressing OsOTUB1.

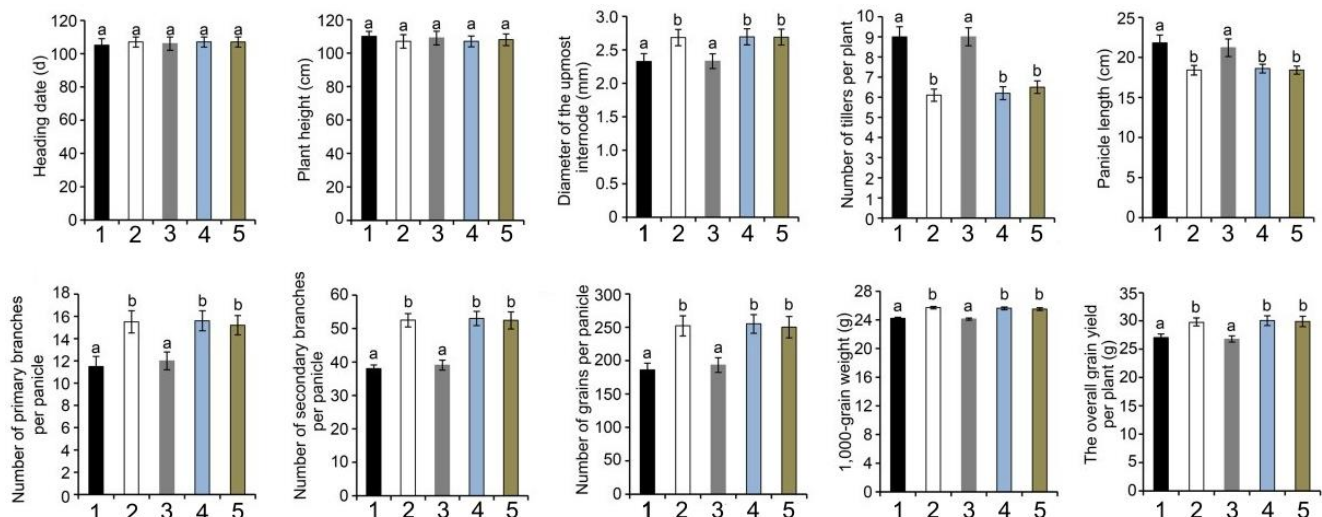
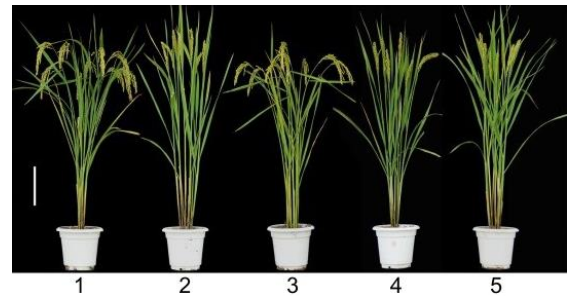
Y2H studies with OsOTUB1 identified a wide range of candidate interactors but including OsSPL14, which is a SQUAMOSA promoter-binding protein-like (SBP-domain) transcription factor known to affect tiller number, culm thickness and grain number. *In planta* studies confirmed that OsSPL14 and OsOTUB1 interact *in vivo*, and deletion studies confirmed that the conserved SBP domain is necessary and sufficient for such interaction to occur. The inventors show that OsOTUB1 and OsSPL14 act antagonistically to control plant architecture through regulation of common target genes. Further, the IGDB group showed that OsOTUB1 interacts with the full set of rice SBP-domain transcription factors.

The IGDB teams describe the highly conserved nature of OTUB among crop species and beyond, and provide analysis of conserved structural and functional domains. They describe specific genome editing strategies for crops such as **maize, wheat, soybean and brassica oilseeds**.

Fig 1. The effect of functional OsOTUB1 on plant architecture and grain yield in paddy field trials

1) Zhonghua11, japonica rice WT control; 2) ZH11-*npt1*: ZH11 NIL with *npt1* allele; 3) and 4) Transgenic ZH11-*npt1* expressing different spliced forms of OTUB1, respectively, from native OsOTUB1 promoter; 5) Genome-edited rice line with reduced function OTUB1.

Data shown as mean \pm s.e.m. ($n=288$). All phenotypic data were measured from the paddy-grown plants under normal cultivation conditions. The presence of the same lowercase letter denotes a non-significant difference between means ($P < 0.05$).



In summary:

- * Genome editing (and transgenic) approaches to increase yield in various crop species are provided
- * Enhance plant ideotype and increase grain yield by modifying OTUB1
- * Additional alternative strategies by overexpressing UBC13 or SPL14
- * A range of novel genome edited *npt1/OTUB1* mutants for direct use in rice breeding, also rice NIL's containing *npt1* crossed into *dep1* backgrounds
- * Methods for screening germplasm

The OTUB1 technology is patented by PBL on behalf of IGDB. **For more information or licensing interest, please contact PBL.**

References:

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