Institute of Genetics and Developmental Biology
Chinese Academy of Sciences

UPL2/LARGE2

Increased Seed Number in grain crops

Increasing yield components by mutations or edits to reduce UPL2 function

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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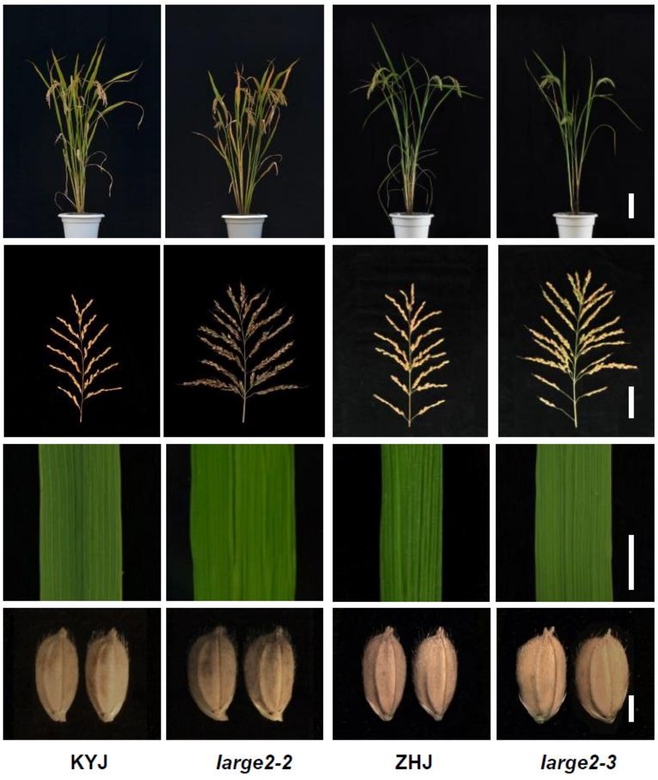
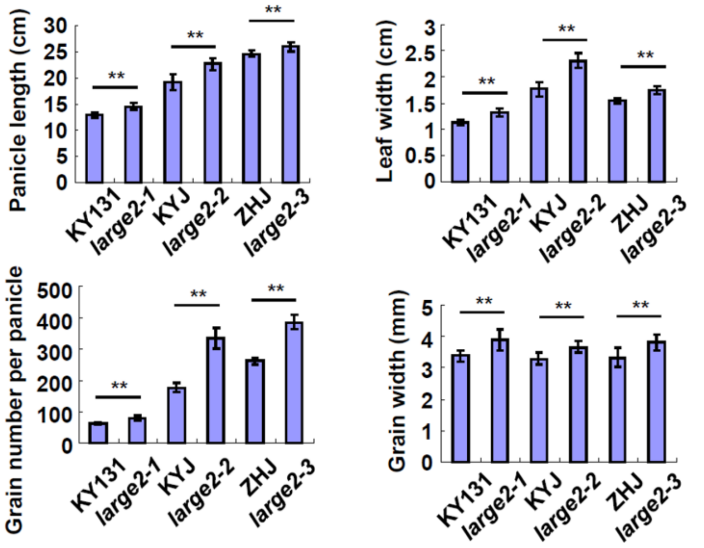
LARGE2 regulates inflorescence size and hence is a major determinant of grain number. *Large2* mutants of rice have larger panicles with increased grain number, wider grains and leaves, and thicker stems. Dr Yunhai Li and his team at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, have discovered that LARGE2 encodes a HECT-domain E3 ubiquitin ligase UPL2.

The IGDB team isolated novel panicle size mutants by mutagenesis using, different mutagens and different *japonica* rice background genotypes, namely Kongyu131 (KY131, sodium azide), Kuanyejing (KYJ, EMS and cobalt 60) and Zhonghuajing (ZHJ, cobalt 60). Nine of the large-panicle mutants were found to be in the same gene and were named as *large2-1* to *large2-9*. The number of primary and secondary panicle branches were increased, resulting in markedly increased grain numbers per panicle. Grains were wider and leaf width was also increased. Plant height was slightly reduced but stems were thicker than wild type control.



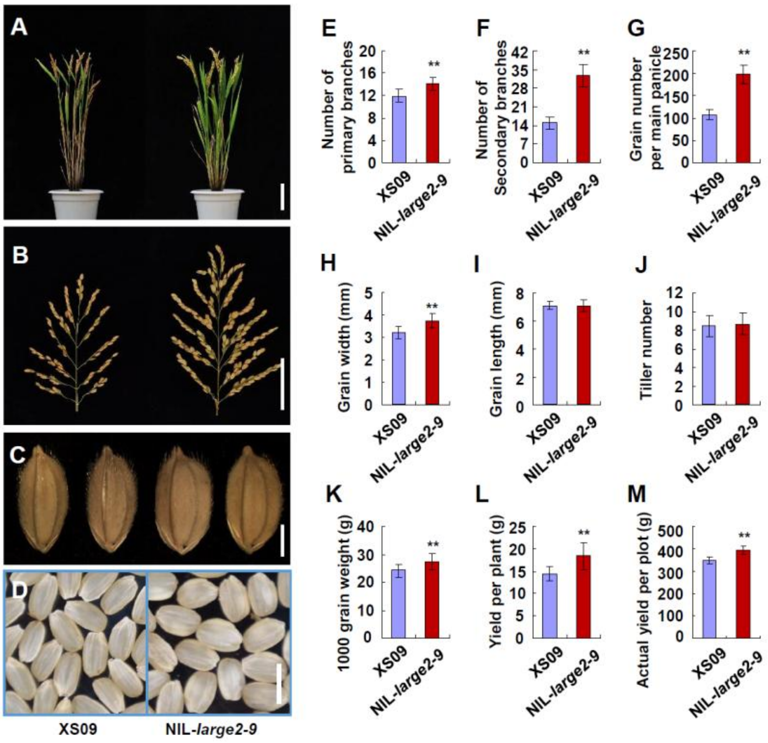


The LARGE2 gene was cloned by whole-genome resequencing of pooled F2 segregants in two different crosses involving *large2* mutants x WT, locating multiple mutants in the same gene – **UPL2**. RNAi transgenic plants were made, confirming the downregulation/silencing of the candidate gene confers the same phenotype, of increased panicle size and grain number, as the *large2* mutants.

LARGE2 encodes the 405kD E3 ubiquitin ligase UPL2. Eight of the *large2* mutants result in loss or truncation of the HECT domain, which lies in the C-terminal region of UPL2, indicating that the mutants are loss-of-function alleles. This was confirmed by conducting functional ubiquitination studies. Homologs of LARGE2/UPL2 are found in a **wide range of organisms and appear to be evolutionarily highly conserved**.

The IGDB team have gone on to show that LARGE2 is **highly expressed in young panicles and grains**, and acts by repressing meristematic activity. LARGE2 regulates the size of shoot apical meristems and inflorescence meristems, with large2 mutants having increased meristematic activity and increased expression of meristem activity marker genes (which are conserved between species as diverse as Arabidopsis and rice)

LARGE2 physically associates *in vivo* with APO1, a positive regulator of panicle size and grain number. LARGE2 modulates the stability of APO1, and genetic analysis indicates that LARGE1 functions with both APO1 and APO2 in a common pathway. APO1 and APO2 regulate panicle size and hence grain number by influencing the precocious conversion of inflorescence meristems to spikelet meristems in rice. The F-box protein APO1 shares similarity with Arabidopsis UFO and the transcription factor APO2 is the orthologue of Arabidopsis LFY. Loss-of-function mutants of *apo1* and *apo2* have small panicles with reduced grain number, while gain-of-function APO alleles, such as STRONG CULM2 (SCM2) have large panicles and thick culms.



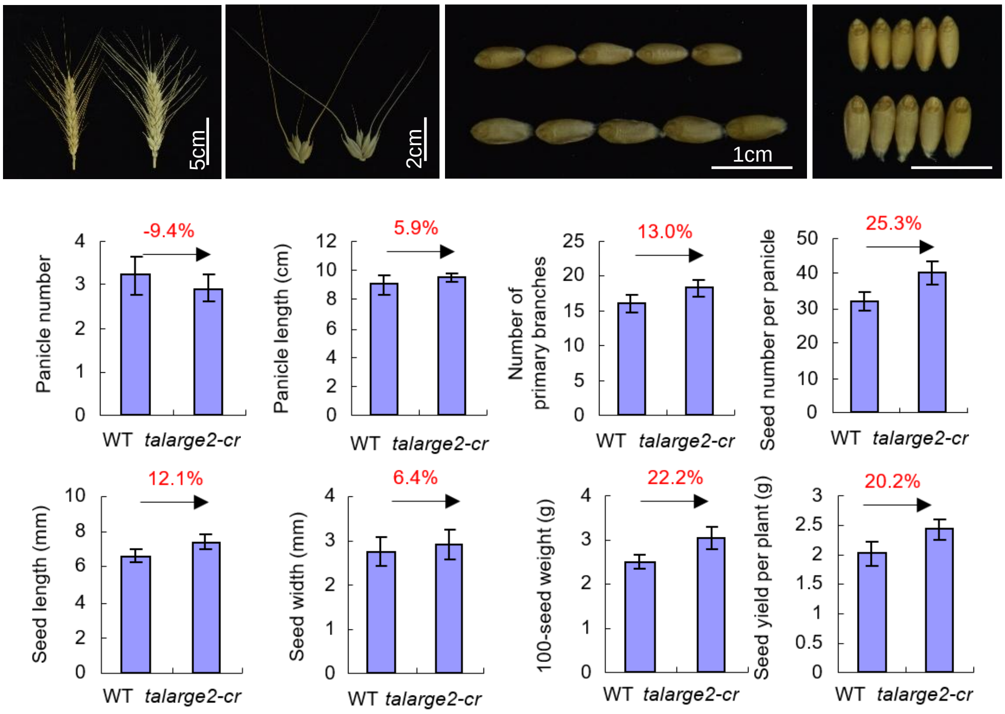
(Left) Introgression of the large2-9 mutation into the *japonica* variety Xiushui09 (XS09) **increases grain yield in field-grown plants.**

The finding that LARGE 2 encodes a functional HECT-domain E3 ligase, UPL2, which acts functionally on APO1/2, reveals a **novel mechanism to manipulate inflorescence size and grain number/yield in grain crops** by mutagenesis and/or gene editing.

The LARGE2/UPL2 technology is patented by PBL on behalf of IGDB.

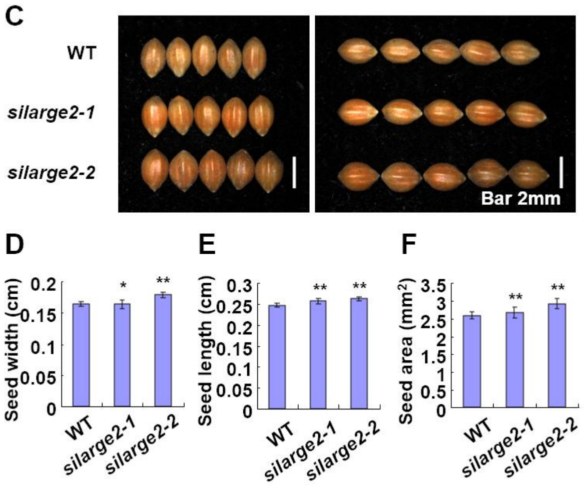
UPDATE APRIL 2023

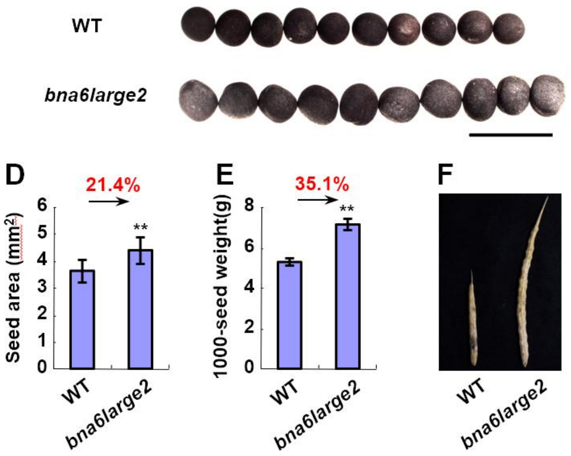
The inventors have extended the practical demonstration of the UPL2/LARGE2 technology into **wheat, millet and oil-seed rape**. Gene-edited mutants of the **wheat** orthologue TaLARGE2 have larger spikes with more, heavier seeds resulting in increased yield of seed per plant. The edits successfully targeted all three TaLARGE2 loci (a, b and d):



(Above) Mutation of the wheat homologous gene *TaLARGE2* by CRISPR-Cas9 system results in larger panicles and seeds, as well as increased seed yield.

Similarly, truncation of the LARGE2 orthologues in **millet** (below, left) and **oil-seed rape** (below, right) results in a large-seed phenotype:





For more information or licensing interest, please contact PBL.

References:

Luojiang Huang *et al* (2021). The LARGE2-APO1/APO2 regulatory module controls panicle size and grain number in rice. *The Plant Cell*; 33(4): 1212-1228. https://doi.org/10.1093/plcell/koab041