Functional analysis of the wheat ortholog of OsGW2, an E3 ligase potentially involved in grain development

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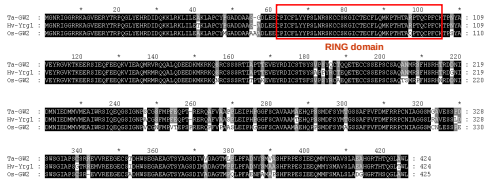
The Ubiquitin-dependent proteolytic pathway (UPS) is a widely spread regulatory mechanism in plants and is described in numerous developmental processes including seed development. In this pathway, three enzymes are involved : E1, E2 and E3. The latter, E3 ubiquitin ligase, target specifically proteins for degradation by 26S proteasome. Recently a QTL controlling rice grain width and weight[1], GW2, was identified. GW2 encodes a previously unknown RING-E3 ligase that acts as a novel negative regulator of cell division during rice grain development. In this study, we identified the GW2 ortholog in wheat and showed that it is a functional E3 ubiquitin ligase.

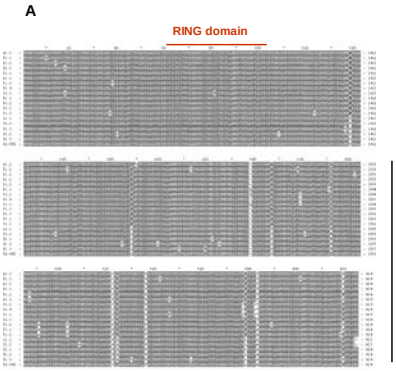
Using the OsGW2 primers, we identified the GW2 ortholog in wheat, *Triticum aestivum* cv.Récital. TaGW2 shares 94% and 87% amino acid sequence identity with homologous sequences Yrg1 in barley and OsGW2 in rice, respectively. The previously unknown RING-C5HC2 domain is conserved.

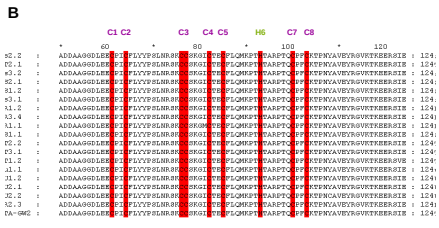


1. TaGW2 is carried by homeologous genomes A, B and D in hexaploid wheat.

T. aestivum cv Récital is a hexaploid wheat (AABBDD) derived from crosses between the wheat diploid genotypes *T.urartu* (AA), *Aegilops speltoides* (BB) and *Aegilops tauschii* (DD). We identified TaGW2 in these three genotypes indicating that each homeologous genome of *T.aestivum* carries at least one TaGW2 copy.

One TaGW2 expressed copy from *T.aestivum* cv Récital was selected and used for biochemical studies.

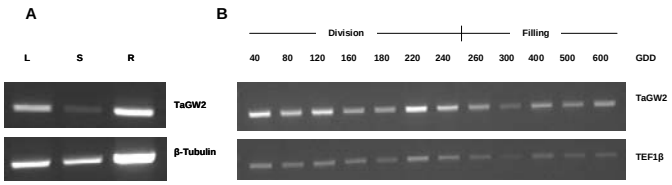




A-The amino acid alignement of these TaGW2 sequences showed a perfect conservation of the RING-C5HC2 domain in N-Ter between the different genotypes but some variations in the putative target protein recognition domain in C-Ter.

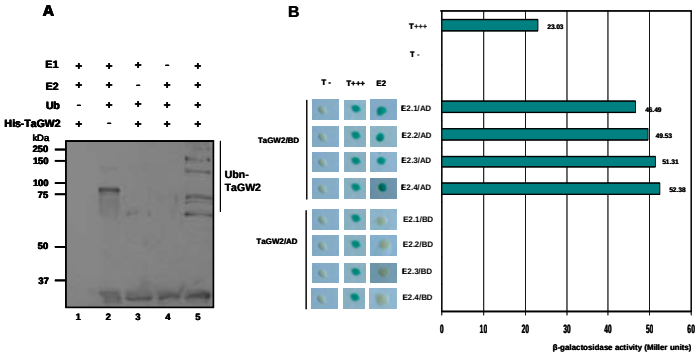
B- A close view of the RING-C5HC2 domain.

2. TaGW2 transcript presents a constitutive expression in wheat.



TaGW2 transcript accumulation was studied by RT-PCR in wheat leaves (L), stems (S) and roots (R) (**A**), and grains over different developmental stages (**B**). This result showed that TaGW2 mRNA is expressed constitutively in various wheat organs.

3. TaGW2 is a functional E3 ubiquitin ligase *in vitro.*



Several studies have reported that RING-type proteins could carry out self-ubiquitination[2] *in vitro*, i.e form polyubiquitin chains on itself in the absence of any target protein designed for degradation. To investigate whether TaGW2 is a functional E3 ligase, we tested an affinity-purified fusion protein HIS-TaGW2 for this self-ubiquitination property (A) and for potential interaction with wheat E2 (B).

A : *In vitro* E3 ubiquitin ligase assay of the TaGW2 protein. In the presence of E1, E2 (UbcH5b) and ubiquitin (Ub), HIS-TaGW2 is able to ubiquitinate itself (lane 5) whereas in the absence of any E1, E2 or E3 enzymes, no obvious protein ubiquitination is detected.

B : Yeast two-hybrid assay of TaGW2 and four wheat E2. X-gal screening (left panel) and CPRG test for β-galactosidase activity (right panel) showed a strong protein-protein interaction between TaGW2 and each wheat E2.

Taken together, these results demonstrate that TaGW2 is a functional E3 ubiquitin ligase *in vitro*.

CONCLUSION : In this study, we have cloned GW2 ortholog in wheat, *T. aestivum* cv. Récital and our data show that TaGW2 is a functional E3 ubiquitin ligase *in vitro*. The TaGW2 transcript seems to display a constitutive expression in various wheat organs. Moreover, TaGW2 is carried by each homeologous genome. The target protein of TaGW2 remains to be found and we will investigate it by global approach as pull-down assay or yeast two-hybrid screening. In order to discern TaGW2 function in wheat grain development, RNAi and overexpression experiments were initiated.

1- *Song XJ, Huang W, Shi M, Zhu MZ, Lin HX* (2007) A QTL for rice width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature genetics 39:623-630

2- *Stone SL, Hauksdóttir H, Troy A, Herschleb J, Kraft E, Callis J* (2005) Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis. Plant physiology 137:13-30