Triticeae Genomics and Genetics, 2025, Vol.16, No.2, 72-78
http://cropscipublisher.com/index.php/tgg

Case Study Open Access

CRISPR/Cas9-Mediated Editing of *TaGW2* to Enhance Grain Size in Wheat

Xingzhu Feng
Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China
Corresponding email: xingzhu.feng@hibio.org

Triticeae Genomics and Genetics, 2025, Vol.16, No.2 doi: 10.5376/tgg.2025.16.0008
Received: 12 Feb., 2025
Accepted: 22 Mar., 2025
Published: 06 Apr., 2025

Preferred citation for this article:
Feng X.Z., 2025, CRISPR/Cas9-mediated editing of *TaGW2* to enhance grain size in wheat, Triticeae Genomics and Genetics, 16(2): 72-78 (doi: 10.5376/tgg.2025.16.0008)

Abstract Wheat (*Triticum aestivum*) is a major food crop in the world, and its grain weight is one of the important traits that determine its yield. The *TaGW2* gene is widely considered to be a key negative regulator of wheat grain size. With the development of CRISPR/Cas9 gene editing technology, targeted modification of the *TaGW2* gene has become an important molecular breeding strategy for improving wheat grain weight. In this study, the structural characteristics and expression patterns of the *TaGW2* gene were systematically analyzed, an efficient CRISPR/Cas9 editing system was designed, mutant materials were constructed, and their grain phenotypes were deeply evaluated. The study showed that the *TaGW2* knockout mutant showed significant improvements in grain length, grain width, and 1000-grain weight, and had no adverse effects on plant height and growth period. This study collected and summarized actual editing cases from multiple authoritative institutions such as the Chinese Academy of Agricultural Sciences, CSIRO in Australia, and Nagoya University in Japan, verifying the wide applicability and breeding potential of *TaGW2* editing in different genetic backgrounds. In this study, CRISPR/Cas9 technology was used to precisely edit the wheat *TaGW2* gene in order to enhance the length, width, and 1000-grain weight of the grain, thereby improving the yield potential of wheat.

Keywords Wheat; *TaGW2* gene; CRISPR/Cas9 editing; Grain weight improvement; Molecular breeding

1 Introduction

The yield of wheat is mainly related to grain weight, which is generally expressed as "thousand-grain weight" (TGW). The larger the grain weight, the higher the yield, so increasing grain weight has always been a key goal in breeding (Wang et al., 2018).

TaGW2 is a gene that controls grain weight. It produces a protein called "RING finger E3 ubiquitin ligase". This gene is a "negative regulator", which means that it inhibits grain enlargement. If this gene is knocked out or expressed less, the grain will become larger and the yield will increase. However, if this gene is expressed too much, although it can enhance drought resistance, it may reduce yield under ideal planting conditions (Wang et al., 2018; Li et al., 2023). This gene also plays a role in crop response to adverse environments. It not only affects yield, but is also related to stress resistance. Now, CRISPR/Cas9 technology can be used to "edit" this gene very accurately. Researchers have successfully created plants with single, double, and triple mutations, all of which can make the grains larger, and this change can be inherited (Zhao et al., 2024). Recently, research has also improved CRISPR editing methods, such as the ability to modify multiple genes at a time and more efficient gene introduction methods. These new advances make the application of this technology in wheat breeding faster and more accurate.

This study reviews the structure and expression of *TaGW2*, summarizes the latest progress in using CRISPR/Cas9-mediated *TaGW2* gene editing technology to increase wheat grain size, and explores the challenges, opportunities, and future development directions of this technology in wheat breeding. This study emphasizes the potential of CRISPR/Cas9 technology as a transformative tool for sustainable genetic improvement of crops, hoping to better utilize the targeted editing technology of the *TaGW2* gene to increase wheat yield.

2 Structure and Expression Characteristics of *TaGW2*

2.1 Gene structure and conserved sequence analysis

TaGW2 is a gene related to grain size. It encodes a protein called "E3 ubiquitin ligase". This protein has a special "ring finger" structure, which is critical to its function. In hexaploid wheat, this gene has three versions, namely *TaGW2*-6A, 6B and 6D. Its coding sequence is 1 275 bases, which can produce a protein containing 424 amino acids. Studies have found that the coding part of this gene is not much different in different wheat varieties, that is, it is very conservative. But its promoter region is more different, especially in the 6A and 6B versions. These differences affect grain size and weight and are the reason for the formation of different haplotypes (Su et al., 2010; Qin et al., 2014). The C-terminus of the protein, that is, the LXLX region near the end (amino acids 376 to 424), is particularly important for identifying the target protein. This segment helps *TaGW2* bind to other proteins involved in grain development, thereby exerting its effects (Lü et al., 2022).

2.2 Expression patterns in different tissues and developmental stages

TaGW2 is always expressed in wheat. All three versions (6A, 6B, and 6D) are expressed, but with different intensities. Generally speaking, 6A is expressed the most, 6B is in the middle, and 6D is the least, especially at the critical stage of grain development (Qin et al., 2014). This gene is not only expressed in grains, but can also be seen in leaves, stems and other parts, indicating that it plays a wide range of roles in the growth and development of wheat (Figure 1) (Su et al., 2010; Zhang et al., 2022).

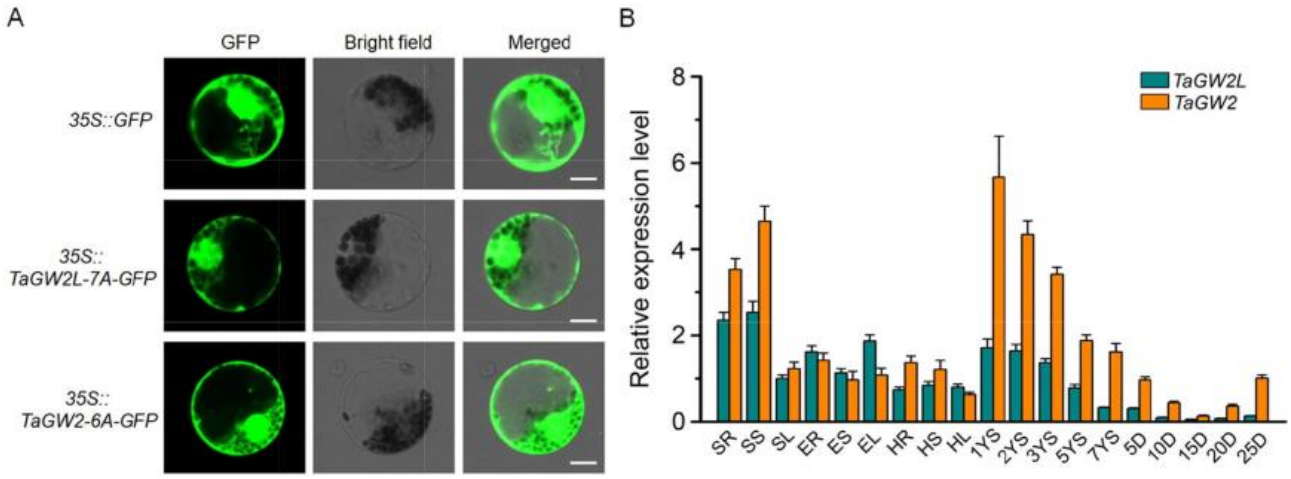

Figure 1 Subcellular localization and spatiotemporal expression of *TaGW2*L and *TaGW2* in wheat (Adopted from Zhang et al., 2022)
Image caption: (A) Subcellular localization of *TaGW2*L and *TaGW2* in wheat protoplasts. Scale bars, 10 μm. (B) Expression pattern analysis of *TaGW2*L and *TaGW2* in wheat. SR, seedling roots; SS, seedling stems; SL, seedling leaves; ER, roots at elongation stage; ES, stems at elongation stage; EL, leaves at elongation stage; HR, roots at heading stage; HS, stems at heading stage; HL, leaves at heading stage; 1YS-7YS, 1 cm-7 cm young spikes; 5D-25D, grains at 5-25 days after pollination. Values are presented as mean ± SD (Adopted from Zhang et al., 2022)

2.3 Spatiotemporal expression during key grain developmental stages

During grain development, the expression of *TaGW2* is particularly active during the rapid cell division period and the late filling period. This suggests that it may play a regulatory role in endosperm cell growth and nutrient accumulation (Bednarek et al., 2012; Qin et al., 2014). Studies have found that the expression of this gene is related to the width of the grain. The lower the expression, the larger the grain and the higher the thousand-grain weight (TGW) (Su et al., 2010). The variation in its promoter region will further affect the expression level of this gene at different stages, thereby affecting the final grain size.

3 CRISPR/Cas9 System Design and Editing Strategy

3.1 Target site selection and gRNA construction

To edit *TaGW2*, the first step is to find a suitable target. Generally, a more conservative region of this gene is selected, which makes it easier to interrupt its function. gRNA (guide RNA) is specially designed to

simultaneously recognize three versions of *TaGW2* in wheat (i.e. 6A, 6B, and 6D). We will use some computational tools to help with the design, which can reduce accidental damage to other genes (Wang et al., 2018). In order to knock out multiple versions of genes at the same time, multiple gRNAs can be strung together and connected with tRNA. In this way, a transcript can carry multiple gRNAs, which will be accurately cut in the cell, improving the overall editing efficiency (Xie et al., 2015).

3.2 Construction of editing vectors and system validation

Usually, we will put the CRISPR/Cas9 system into a binary vector. This vector contains the Cas9 protein, which is driven by a strong promoter (such as the Ubi promoter in maize), and a gRNA module controlled by the wheat U6 promoter (Zhang et al., 2019). We use Agrobacterium to transfer this system into wheat cells, which is more stable than the gene gun and can reduce the number of transformations and gene silencing (Zhang et al., 2019). In transgenic wheat, we will check whether Cas9 and gRNA are expressed normally and confirm whether the target site is successfully edited.

3.3 Mutant identification and mutation type analysis

We use PCR to amplify the target region of *TaGW2* and then use sequencing to confirm whether mutations have occurred. In wheat, the most common mutation is "deletion", which sometimes exceeds 10 base pairs. Homozygous mutants or biallelic mutants can sometimes be obtained in the first generation (T0), and sometimes need to wait until the next few generations to appear (Zhang et al., 2019). The CRISPR/Cas9 system can continue to work in the next generation, sometimes generating new mutations in the offspring, which can bring more genetic changes (Wang et al., 2018). In order to ensure that this system does not cut randomly, we also perform off-target checks. Many studies have found that as long as the gRNA is well designed, off-target problems will hardly occur or cannot be detected at all.

4 Phenotypic Changes and Grain Weight in *TaGW2* Mutants

4.1 Analysis of grain length, width, and thousand-kernel weight

Studies have found that *TaGW2* gene mutations can increase grain size and thousand-grain weight (TGW). Compared with the wild type, single-gene mutations can increase grain length by about 2%, width by 2%-3%, and TGW by 5%-7% (Simmonds et al., 2016; Wang et al., 2018; Bi et al., 2024). If two genes mutate together, the effect is more obvious, and TGW can be increased by 10%-12%. When all three genes mutate, the increase in TGW is the largest, reaching 16%-21% (Zhang et al., 2018). This increase is quite stable in different wheat varieties and environments. Moreover, the more mutations there are, the more obvious the increase in grain weight is. This shows that these genes have an additive effect. In particular, the two alleles *TaGW2*-6B and *TaGW2*-6A have a greater effect on grain width and weight (Yang et al., 2012).

4.2 Effects of gene editing on plant architecture and growth period

Although we mainly want to improve grain traits by editing *TaGW2*, it sometimes affects other agronomic traits. Studies have found that some *TaGW2* mutations are related to early heading and early maturity of wheat, and may also change plant height and ear structure (Jaiswal et al., 2015). However, most edited varieties have no major changes in the number of spikelets and grains. In other words, these mutations mainly make the grains themselves larger and do not affect the appearance of the whole plant much (Simmonds et al., 2016). Sometimes there is a small "trade-off": the grain weight increases, but the number of grains per ear may decrease slightly. But overall, the yield can still be maintained, or even higher (Zhai et al., 2017; Vicentin et al., 2024).

4.3 Correlation analysis between phenotype and genotype

The number of copies of *TaGW2* is negatively correlated with grain size and TGW. In other words, the more copies are mutated, the larger and heavier the grains are (Wang et al., 2018; Zhang et al., 2018). Genetic variation in the promoter region can also affect expression levels, which may be one of the reasons for the phenotypic differences between different varieties (Qin et al., 2014; Jaiswal et al., 2015). Now, we can use SNPs or haplotypes related to *TaGW2* for molecular marker-assisted selection, so that we can more quickly select lines with larger grain weight and higher yield (Yang et al., 2012). Different combinations of homologous genes and the special reactions between varieties show that the relationship between genotype and phenotype is very critical. Understanding these relationships will help to better optimize breeding strategies (Bi et al., 2024).

5 Comparison of Editing Effects in Different Genetic Backgrounds

5.1 Research findings from the Crop Science Institute, Chinese Academy of Agricultural Sciences

Researchers from the Chinese Academy of Agricultural Sciences found that after editing *TaGW2* homologous genes in different wheat varieties, the grains became larger and the thousand-grain weight (TGW) was significantly improved. However, the increase was different for different varieties. For example, when single mutations were made in the A, B and D genomes, the mutation effect of the B and D genomes was the best. The two varieties Paragon and Bobwhite performed best in this regard. The study also found that if two or even three homologous genes were mutated, the improvement of TGW would be more obvious, reaching 16% to 21%. This difference may be related to the gene expression level of the variety itself. This shows that genetic background plays a big role in the results of gene editing (Simmonds et al., 2016; Wang et al., 2018; Zhang et al., 2018).

5.2 Multi-gene editing experiments conducted by CSIRO, Australia

CSIRO, Australia, and its partners conducted multi-gene editing experiments. They used CRISPR/Cas9 to simultaneously edit *TaGW2* and other agronomic trait-related genes in hexaploid wheat. The experimental results showed that after knocking out all three homologous *TaGW2* genes, the grains became larger, TGW was also greatly improved, and these traits could be stably inherited (Figure 2). More importantly, this editing system can continue to work for several generations. They also hybridized these edited lines with other wheat varieties to further transfer these excellent traits. This shows that this method can be used in different genetic backgrounds and is a very efficient breeding method (Wang et al., 2018).

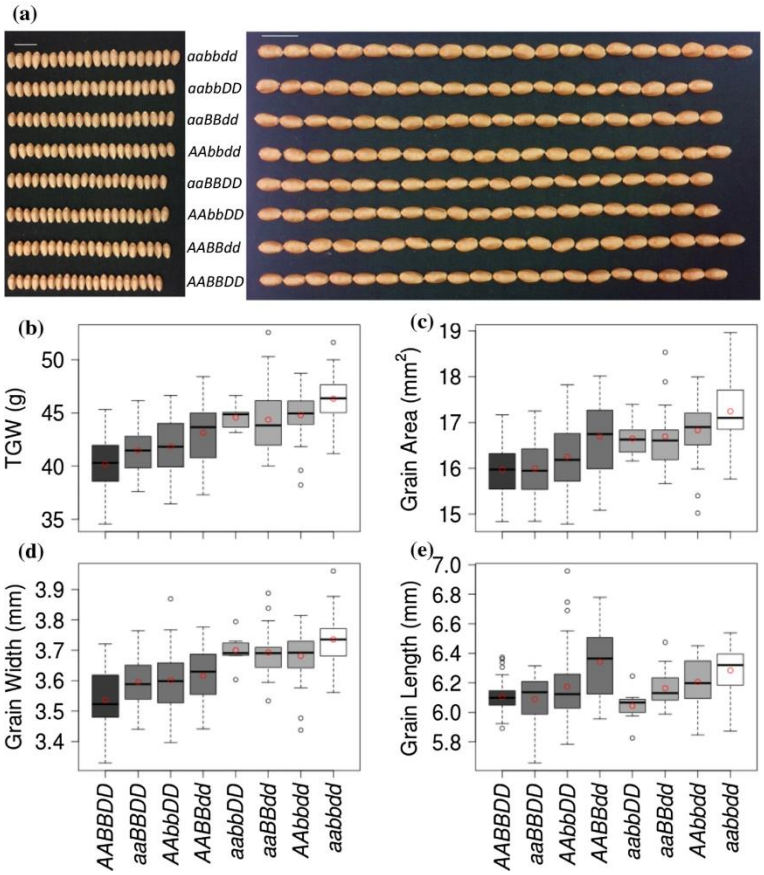

Figure 2 The effects of single-, double-, and triple- KO mutations in the *TaGW2* gene homoeologues on the grain morphometric and TGW traits in Bobwhite. The image of twenty seeds from wild-type, single-, double- and triple- mutant plants (scale bar 1 cm). b-e Box and whisker plots show the distribution of TGW (b), grain area (c), grain width (d), and grain length (e) for wild-type and mutant wheat lines. The datasets from Bobwhite and the T0 progeny plants carrying wild-type *TaGW2* alleles were combined because they did not show statistical differences. The mean value for each genotype is shown as a red circle. The genotypes of the *TaGW2* homoeologues are shown in all panels with lower and uppercase letters corresponding to the mutant and wild-type alleles, respectively, for the A, B, and D genome homoeologues (color figure online) (Adopted from Wang et al., 2018)

5.3 Field evaluation of mutants by Nagoya University, Japan

The team at Nagoya University in Japan conducted field trials. They introduced *TaGW2* mutations into tetraploid and hexaploid wheat, such as splice acceptor site mutations in *TaGW2*-A1. These mutations significantly increased wheat grain weight, grain width and grain length under various environments. Moreover, they did not affect the number of spikelets or grains. This shows that editing *TaGW2* can not only increase yield, but also has no negative effects (Simmonds et al., 2016).

Editing *TaGW2* does have a significant effect. However, the performance will vary between different varieties and different homologous genes. From global research, editing multiple genes together will have a better effect (Simmonds et al., 2016; Wang et al., 2018; Zhang et al., 2018).

6 Integration of CRISPR/Cas9 with Breeding Pipelines for Grain Weight Improvement

6.1 Combination with genomic selection (GS) for selection accuracy

CRISPR/Cas9 can accurately edit key genes such as *TaGW2*. But if it is used with genomic selection (GS), the breeding effect will be better. GS uses genome-wide markers to predict which plants have potential. In this way, breeders can select plants that not only have the target edited gene but also have a good genetic background. This combination can find materials with larger grains and higher yields more quickly. This method has been proven to be effective in other crops (Awan et al., 2022; Ahmar et al., 2023).

6.2 Integration with high-throughput phenotyping (HTP) platforms

High-throughput phenotyping (HTP) can quickly measure the grain size, weight and other agronomic traits of many wheat plants using machines and sensors. If HTP is combined with CRISPR/Cas9 editing, the best performing mutants can be quickly screened. This will allow for faster evaluation of the effects of these edits in different environments and genetic backgrounds. This approach will make the breeding process simpler and more efficient, and ensure that only the most promising lines advance to the next stage of promotion (Awan et al., 2022; Ahmar et al., 2023).

6.3 Introgression of edited traits into commercial cultivars

Once we have found CRISPR-edited lines with heavy grains and good traits, we can use conventional hybridization methods to introduce these good genes into existing commercial varieties. In this process, marker-assisted selection can also improve efficiency. Rapid breeding and accelerated generation advancement technology can also make this process faster. This approach has been successful in other cereals. This shows that this strategy is also promising for wheat, and can help us breed high-quality non-GMO wheat varieties suitable for large-scale planting (Liang et al., 2017; Awan et al., 2022).

7 Concluding Remarks

Site-directed editing of the *TaGW2* gene homolog in wheat using CRISPR/Cas9 technology has been shown to significantly affect grain size and thousand-grain weight (TGW). The study found that the more sites that were mutated, the more obvious the improvement in TGW. TGW increased in single, double, and triple mutations, with the triple mutant increasing TGW by 16% to 21%. However, this phenotypic effect also varies depending on the wheat variety and the location of the edit in the genome. This suggests that the expression level of *TaGW2* may be different in different varieties. Overall, these results prove that *TaGW2* is a gene that suppresses grain size, and "knocking it out" can help increase yield.

CRISPR/Cas9 technology can quickly and stably change key yield genes like *TaGW2*. It can also reduce the possibility of off-target effects, allowing the modified traits to continue in future generations. This technology can also edit multiple genes at once, which is very helpful for improving complex traits. These advances also show that gene editing technology can be gradually applied to conventional wheat breeding, helping us breed varieties with strong adaptability and high yield.

In order to maximize the advantages of CRISPR/Cas9, future breeding work will also need to combine it with genomic selection, high-throughput phenotyping, and efficient gene introduction methods. This combined strategy

will accelerate the discovery and utilization of superior alleles, thereby rapidly increasing wheat yields. It is also important to continue studying the different functions of *TaGW2* and its interactions with other yield genes. This will further optimize breeding methods and help to breed new high-yield and stress-resistant wheat varieties. CRISPR/Cas9 targeted editing of *TaGW2* is a precise, powerful, and scalable method. It not only increases grain weight and yield, but is also suitable for integration into modern breeding systems, and has great potential.

Acknowledgments

I appreciate Dr Xu from the Hainan Institution of Biotechnology for her assistance in references collection and discussion for this work completion.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ahmar S., Hensel G., and Gruszka D., 2023, CRISPR/Cas9-mediated genome editing techniques and new breeding strategies in cereals-current status, improvements, and perspectives, Biotechnology Advances, 69: 108248.
https://doi.org/10.1016/j.biotechadv.2023.108248

Awan M., Pervaiz K., Rasheed A., Amin I., Saeed N., Dhugga K., and Mansoor S., 2022, Genome edited wheat- current advances for the second green revolution, Biotechnology Advances, 60: 108006.
https://doi.org/10.1016/j.biotechadv.2022.108006

Bednarek J., Boulaflous A., Girousse C., Ravel C., Tassy C., Barret P., Bouzidi M., and Mouzeyar S., 2012, Down-regulation of the *TaGW2* gene by RNA interference results in decreased grain size and weight in wheat, Journal of Experimental Botany, 63(16): 5945-5955.
https://doi.org/10.1093/jxb/ers249

Bi C., Wei C., Li J., Wen S., Zhao H., Yu J., Shi X., Zhang Y., Liu Q., Zhang Y., Li B., and You M., 2024, A novel variation of *TaGW2*-6B increases grain weight without penalty in grain protein content in wheat (*Triticum aestivum* L.), Molecular Breeding, 44: 1-20.
https://doi.org/10.1007/s11032-024-01455-y

Jaiswal V., Gahlaut V., Mathur S., Agarwal P., Khandelwal M., Khurana J., Tyagi A., Balyan H., and Gupta P., 2015, Identification of novel SNP in promoter sequence of *TaGW2*-6A associated with grain weight and other agronomic traits in wheat (*Triticum aestivum* L.), PLoS ONE, 10(6): e0129400.
https://doi.org/10.1371/journal.pone.0129400

Li S., Zhang Y., Liu Y., Zhang P., Wang X., Chen B., Ding L., Nie Y., Li F., Ma, Z., Kang Z., and Mao H., 2023, The E3 ligase TaGW2 mediates transcription factor TaARR12 degradation to promote drought resistance in wheat, The Plant Cell, 36(3): 605-625.
https://doi.org/10.1093/plcell/koad307

Liang Z., Chen K., Li T., Zhang Y., Wang Y., Zhao Q., Liu J., Zhang H., Liu C., Ran Y., and Gao C., 2017, Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nature Communications, 8: 14261.
https://doi.org/10.1038/ncomms14261

Lü Q., Li L., Meng Y., Sun H., Chen L., Wang B., and Li X., 2022, Wheat E3 ubiquitin ligase *TaGW2*-6A degrades TaAGPS to affect seed size, Plant Science, 320: 111274.
https://doi.org/10.1016/j.plantsci.2022.111274

Qin L., Hao C., Hou J., Wang Y., Li T., Wang L., Ma Z., and Zhang X., 2014, Homologous haplotypes, expression, genetic effects and geographic distribution of the wheat yield gene *TaGW2*, BMC Plant Biology, 14: 107.
https://doi.org/10.1186/1471-2229-14-107

Simmonds J., Scott P., Brinton J., Mestre T., Bush M., Del Blanco A., Dubcovsky J., and Uauy C., 2016, A splice acceptor site mutation in *TaGW2*-A1 increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains, Theoretical and Applied Genetics, 129: 1099-1112.
https://doi.org/10.1007/s00122-016-2686-2

Su Z., Hao C., Wang L., Dong Y., and Zhang X., 2010, Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.), Theoretical and Applied Genetics, 122: 211-223.
https://doi.org/10.1007/s00122-010-1437-z

Vicentin L., Canales J., and Calderini D., 2024, The trade-off between grain weight and grain number in wheat is explained by the overlapping of the key phases determining these major yield components, Frontiers in Plant Science, 15: 1380429.
https://doi.org/10.3389/fpls.2024.1380429

Wang W., Simmonds J., Pan Q., Davidson D., He F., Battal A., Akhunova A., Trick H., Uauy C., and Akhunov E., 2018, Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of *TaGW2* homoeologues to grain size and weight in wheat, Theoretical and Applied Genetics, 131: 2463-2475.
https://doi.org/10.1007/s00122-018-3166-7

Xie K., Minkenberg B., and Yang Y., 2015, Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system, Proceedings of the National Academy of Sciences, 112: 3570-3575.
https://doi.org/10.1073/pnas.1420294112

Yang Z., Bai Z., Li X., Wang P., Wu Q., Yang L., Li L., and Li X., 2012, SNP identification and allelic-specific PCR markers development for *TaGW2*, a gene linked to wheat kernel weight, Theoretical and Applied Genetics, 125: 1057-1068.
https://doi.org/10.1007/s00122-012-1895-6

Zhai H., Feng Z., Du X., Song Y., Liu X., Qi Z., Song L., Li J., Li L., Peng H., Hu Z., Yao Y., Xin M., Xiao S., Sun Q., and Ni Z., 2017, A novel allele of *TaGW2*-A1 is located in a finely mapped QTL that increases grain weight but decreases grain number in wheat (*Triticum aestivum* L.), Theoretical and Applied Genetics, 131: 539-553.
https://doi.org/10.1007/s00122-017-3017-y

Zhang D., Zhang X., Xu W., Hu T., Ma, J., Zhang Y., Hou J., Hao C., Zhang X., and Li T., 2022, *TaGW2*L, a GW2-like RING finger E3 ligase, positively regulates heading date in common wheat (*Triticum aestivum* L.), The Crop Journal, 10(4): 972-979.
https://doi.org/10.1016/j.cj.2021.12.002

Zhang Y., Li D., Zhang D., Zhao X., Cao X., Dong L., Liu J., Chen K., Zhang H., Gao C., and Wang D., 2018, Analysis of the functions of *TaGW2* homoeologs in wheat grain weight and protein content traits, The Plant Journal, 94: 857-866.
https://doi.org/10.1111/tpj.13903

Zhang Z., Hua L., Gupta A., Tricoli D., Edwards K., Yang B., and Li W., 2019, Development of an Agrobacterium-delivered CRISPR/Cas9 system for wheat genome editing, Plant Biotechnology Journal, 17: 1623-1635.
https://doi.org/10.1111/pbi.13088

Zhao S., Han X., Zhu Y., Han Y., Liu H., Chen Z., Li H., Wang D., Tian C., Yuan Y., Guo Y., Si X., Wang D., and Ji X., 2024, CRISPR/CasΦ2-mediated gene editing in wheat and rye, Journal of Integrative Plant Biology, 66(4): 638-641.
https://doi.org/10.1111/jipb.13624