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Regulator of Weight and Oil of Seed 1 (*RWOS1*) in Soybean: Weighing in on Oil and Yield Trade-offs

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Abstract

Seed weight and oil content are key determinants of soybean value but are difficult to improve simultaneously. Integrative omics identified GmRWOS1, encoding a sodium (Na^+) transporter protein, as a pleiotropic negative regulator. Loss of GmRWOS1 increased seed size, weight, and oil content, highlighting its potential as a target for soybean breeding and gene editing.

Keywords: *GmRWOS1*; Breeding; Glycine max; Functional genomics

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Main text

The seed is both a biological and philosophical unit of life – tiny yet harboring immense potential. In oilseeds, this potential is reflected in key agronomic traits like seed weight and oil content, which together define their agronomic and economic value.

Among oilseed crops, the dominant oil crop, soybeans (*Glycine max*) with high economic relevance contributing > 60% global edible oil production (www.statista.com). Soybean seed weight is correlated with yield and oil content, key agroeconomic traits. Breeding varieties with high grain weight and high oil content has long been a challenge in the seed breeding community [1]. However, seed weight and oil content are typically controlled by complex genetic networks, and these two traits are often negatively correlated. This has made simultaneously improving seed weight and oil content during seed breeding a long-standing challenge.

The regulation of seed size is the result of long-term natural selection, artificial domestication, and improvement, reflecting a delicate balance between survival and utilization. Large seeds facilitate germination and early seedling growth, and are more resilient to harsh environments, while small seeds facilitate widespread dispersal, thus expanding a species' ecological distribution [2]. Manipulating seed size through breeding and biotechnology is not only crucial for increasing yields and global food security,

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but also for optimizing nutrition and industrial utilization. For instance, more than 300 quantitative trait loci (QTLs) related to the regulation of seed weight and oil content have been identified through genome-wide association studies (GWAS) and linkage analysis in soybean (SoyBase, https://soybase.org/). However, the underlying genetic mechanisms controlling seed weight and oil content have only been partially explored, with proteins such as *GmKIX8-1* [3], *Protein, Oil, Weight Regulator 1 (POWER1)* [1].

In the latest study by Yuan et al. [4], multi-omics integration including GWAS, eQTLs (expression QTLs) mapping, and TWAS (Transcriptome-WAS) was used to comprehensively analyze the genetic basis of seed weight and oil content in soybean using 421 diverse soybean accessions of soybean from three continents (Asia, Europe, and North America). As a result, *Regulator of Weight and Oil of Seed 1* (*GmRWOS1*, a sodium pump protein), a causal gene for the GWAS locus was discovered by TWAS.

In brief, a total of 26 loci associated with seed weight and 33 loci associated with oil content by GWAS, and more than 5,276 eQTLs were discovered influencing the expression of over 3,347 genes. Co-expression modules constructed from these loci elucidated how multiple genetic loci synergistically regulates complex phenotypes. Among multiple modules, module IC79 (with 1357 genes) was significantly associated with two traits and controlled by a trans-eQTL hotspot, suggesting the presence of a core regulatory factor. Further analysis revealed that IC79 contains a gene encoding a Na^+ transporter protein, *GmRWOS1*, whose natural haplotype variation was significantly correlated with grain weight and oil content traits, indicating its pleiotropic regulatory functions.

To validate the function of *GmRWOS1* in regulating soybean grain weight and oil content, the authors generated loss-of-function mutant designated Gmrwos1, harboring a frameshift mutation in the third exon of *GmRWOS1*. Comparative phenotypic analysis revealed a significant increase in grain weight, grain size, and oil content in *Gmrwos1* mutant. Concurrently, soybean plants overexpressing *GmRWOS1* under constitutive *35S* promoter were also generated which exhibited opposite phenotypic trends, while plant architecture and grain number per plant remained unchanged (Figure 1). These findings indicate that *GmRWOS1* functions as a negative regulator of seed weight and oil content in soybean.

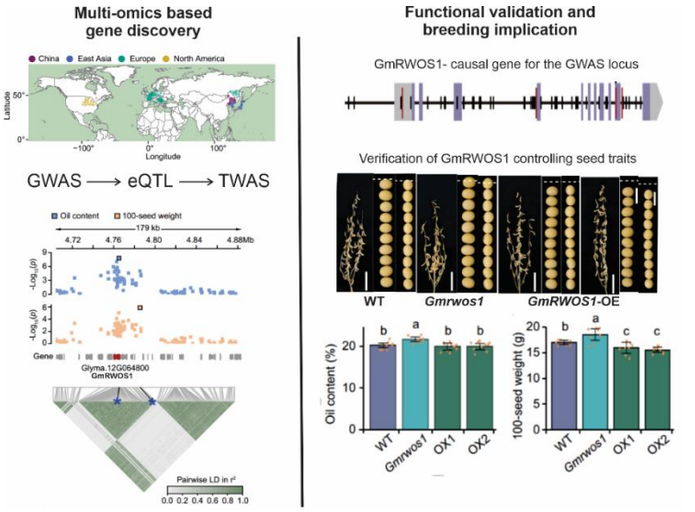


Figure 1. Multi-*omics*-based discovery of *GmRWOS1* in soybean. Altered expression of *GmRWOS1* simultaneously impacts seed weight and oil content as exhibited by CRISPR/Cas9 knockout lines, while overexpression lines exhibit enhanced seed related traits. Thus, this demonstrates a pleiotropic regulatory mechanism for key soybean agronomic traits. Adapted from Yuan et al. [4] Figures 1, 5, 6, and S6.

Yuan et al. [4] combined functional genomics with network analysis to discover a key pleiotropic regulatory factor – the seed weight and oil regulatory gene *GmRWOS1*, that coordinately improves yield and quality. This study not only revealed a key molecular regulator but also provided a strategic framework that can be translated into breeding practices. *GmRWOS1* has become an important candidate target for

molecular breeding and gene editing to improve soybean yield and quality.

Data availability

Not applicable

Acknowledgments

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: GW, MA; data collection: GW; draft manuscript preparation: MA, SR; manuscript revision: MQ, PK. All authors discussed the content, reviewed the results and approved the final version of the manuscript.

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