Project Abstract

Project Title:	Develop a New Transgene Free Editing System for Gene Function Validation and Breeding	
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CRISPR/Cas genome editing can be a powerful tool to generate resistant mutants by knocking out susceptible genes (S-genes), which can be used to improve wheat resistance to Fusarium head blight (FHB). However, a major challenge for genome editing is to eliminate off-target edits and transgenes in the edited plants to avoid GMOs regulatory concerns. Recently, transient expression of Cas-gRNA ribonucleoprotein complex (RNP) has proven to be effective to solve these issues. Nanoparticle (NP) has proven to be one of the most promising materials for gene delivery in numerous biotechnological applications. However, the inheritable edited mutation using NPs has not been reported in wheat. Our overall goals are to develop a genome editing method that produces transgene-free mutant plants without genotype preference and to study candidate genes' functions on FHB resistance and create new sources of resistance to FHB and other diseases using the edit system.

The major objectives in this proposal are to 1) develop an NP-mediated RNP gene delivery system for genome editing, 2) use the new editing system to validate the function of *Rht-B1* on FHB resistance, and 3) investigate FHB-resistance mechanisms of *Rht-B1*. The outcomes will be 1) a new transgene-free genome editing method available for breeding and gene function studies, 2) confirmation of *Rht-B1* effects on FHB resistance to provide a guideline for effective use of *Rht-B1* in breeding, and 3) understanding of interaction between DELLA and GID1 on FHB resistance. In this proposal, 1) we will evaluate and identify the best sized NPs for gene delivery, and use the meristematic cells of imbibed wheat seeds as the target tissue for gene delivery and *TaHRC* as the target gene to desin gRNA; 2) the effect of *Rht-B1* on FHB resistance will be validated by knocking out each and both of DELLA and GRAS domains of *Rht-B1* and phenotype the mutants for FHB resistance to determine their functions using the new editing system; 3) The protein interaction between the DELLA motif and GID1 will be studied using BiFC and colocalization assays *in planta* and co-transformation assay in yeast. The new transgene-free genome editing protocol will be publicly available for breeding and gene function studies through publications; the mutants generated from this study will be available on request for studying gene functions of *Rht-B1* on other traits, and information on the interaction between DELLA and GID1 on FHB resistance will be publicly available through publications.