LIÈGE université
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Agro-Bio Tech

Le Corps professoral de

Gembloux Agro-Bio Tech - Université de Liège vous prie

de lui faire l'honneur d'assister à la défense publique de la dissertation originale que

Monsieur SUN Yongwei,

Titulaire d'nn *master of crop genetics and breeding,*

présentera en vue de l'obtention du grade et du diplôme de

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 26 juin 2017, à 13 heures précises (personne ne sera admis après cette heure),

en l'auditorium Zl (Zoologie, bâtiment 9),

Passage des Déportés, 2 à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Development and applications of CRISPR-Cas9 and RNAi for rice and wheat agronomic traits improvement »

Le jury est composé comme suit :

Présidente : Prof. M.-L. FAUCONNIER, Présidente du Département AGROBIOCHEM,
Membres : Prof. F. FRANCIS (Promoteur), Prof. L. XIA (Copromoteur – CAAS, Chine), Prof. M. ONGENA, Prof. H. VANDERSCHUREN, Prof. F. VERHEGGEN, Prof. J. CHEN (CAAS, Chine).

UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH – SERVICE INSCRIPTIONS ET APPARITORAT
Passage des Déportés, 2 - 5030 Gembloux - Belgique
Téléphone +32(0)81 62 23 45 - Fax +32(0)81 62 21 68 - www.gembloux.ulg.ac.be

Abstract

Genome editing technologies enable precise modifications of DNA sequences *in vivo* and offer a great promise for harnessing plant genes in crop improvement. The precise manipulation of plant genomes relies on the induction of DNA double-strand breaks (DSBs) by sequence-specific nucleases (SSNs) to initiate DNA repair reactions that are based on either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ can result in frame-shift mutations that often create genetic knockouts. These knockout lines are useful for functional and reverse genetic studies but also have applications in agriculture. HDR has a variety of applications as it can be used for gene replacement, gene stacking, and for creating various fusion proteins. An overview of development and applications of precise GT in plants using SSNs systems is presented in Chapter I.

Cereals high in amylose content (AC) and resistant starch (RS) offer potential health benefits. Starch branching enzyme (SBE) plays a major role in determining the fine structure and physical properties of starch. Here, we use CRISPR/Cas9 technology to generate targeted mutagenesis in *SBEI* and *SBEIIb* in rice. The frequencies of obtained homozygous or bi-allelic mutant lines with indels in *SBEI* and *SBEIIb* in T_0 generation were from 26.7 to 40%. *SbeII* mutants showed higher proportion of long chains presented in debranched amylopectin, significantly increased AC and RS content to as higher as 25.0% and 9.8%, respectively. The details of results are presented in Chapter IV.

Complete knockouts and loss-of-function mutations are very valuable in defining gene functions, their applications in crop improvement are somewhat limited because many agriculturally important traits are conferred by point mutations or a change of gene expression levels. Development of a technology that enables gene replacement rather than gene inactivation will greatly facilitate plant breading. In this study, we report an efficient method to introduce multiple discrete point mutations in the rice *ALS* gene using CRISPR/Cas9-mediated homologous recombination. We not only generated homozygous herbicide resistance rice plants in one generation, but also demonstrated that the presented strategy is feasible and effective in precise gene replacement by using CRISPR/Cas9 system to facilitate crop genetic improvement. The details of results are presented in Chapter V.

Aphids are major agricultural pests which cause significant yield losses of wheat each year in China. Present attempts to improve the aphid resistance of wheat through conventional breeding in China are being confronted by slow progress due to lack of aphid resistant wheat germplasm, the complexity of plant-aphid interactions and the rapid development of resistant pest biotypes. An overview of the engineering of plants for aphid resistance is presented in chapter II. We identify a gene (*23028*) related to aphid ingestion and digestion from the aphid, *S. avenae*. Aphids fed on plant material expressing double-stranded RNA (dsRNA) specific to *23028* and *C002* show a decline in growth, reproduction and survival rates. The details of results presented in the chapter VI.