LIÈGE université
Gembloux
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 HU Bowen,

Titulaire d'un diplôme de *master of agriculture*,

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

DOCTEUR EN SCIENCES AGRONOMIQUES ET INGENIERIE BIOLOGIQUE,

le 17 décembre 2018, à 10h30 précises (personne ne sera admis après cette heure),

en l'auditorium PhV (Physiologie Végétale, bât. 48),

Avenue Maréchal Juin, 13, à 5030 GEMBLOUX.

Cette dissertation originale a pour titre :

« Molecular breeding and gene function verifying for cucumber fruit traits ».

Le jury est composé comme suit :

Présidente : Prof. M. VANDENBOL, Professeur ordinaire,
Membres : Prof. H. VANDERSCHUREN (Promoteur), Prof. H. SANWEN (Copromoteur - CAAS, Chine), Prof. S. MASSART, Prof. L. LASSOIS, Prof. Y. LI (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

Summary

Cucumber is a widely cultivated vegetable with high economic benefit. The assembled draft genome sequence and variation map generated by deep resequencing provided big data to advantage cucumber serving as the model plant for sex determination, long-distance trafficking and plant viruses disease research. However, the underlying molecular basis of some important horticultural traits, such as peel color, is still unknown. Besides, Cucurbitaceous species are well known to be one of the most refractory plants for transformation, which makes many meaningful traits hardly to be verified reliably in cucumber by reverse genetics, or makes identified gene cannot be applied well in molecular breeding.

In this dissertation, the classical research methods of forward genetics and reward genetics was adopted. Initially, we discovered a cucumber mutant showing distinct light green exocarp of immature fruit from our EMS mutant library. By combining bulked-segregant analysis (BSA) and transient RNA interference, we first identified *Csa7G051430* responsible for light green skin. Additionally, We successfully obtained transgenic cucumber using an inbred line rather than an F1 hybrid as previously used. We further optimized the CRISPR/Cas9 system by using stronger CsU6 promoter and a GFP tag to facilitate selection both the transformants and transgene-free mutants among the progeny. Thirdly, With these optimized procedures, we first verified the gene function of *CsWIP1* which plays an important role in sex determination in cucumber and generated transgene-free gynoecious cucumber plants from a commercially valuable inbred line.

The main contents and results are as follows:

(1) An ACCUMULATION AND REPLICATION OF CHLOROPLASTS 5 gene mutation

A mutant showing light green exocarp was discovered from ethylmethane sulfonate (EMS) mutagenized cucumber line 406 with dark green exocarp. Genetic analysis showed the mutant phenotype is conferred by a single recessive gene, here designated as lgp (light green peel). By re-sequencing of bulked segregants, we identified the candidate gene Csa7G051430 encoding ACCUMULATION AND REPLICATION OF CHLOROPLASTS 5 (ARC5) that plays a vital role in chloroplast division in Arabidopsis. A single nucleotide polymorphism (SNP) causing amino acid alteration in the conserved GTPase domain of Csa7G051430 showed co-segregation with the altered phenotype. Furthermore, a transient RNA interference of this gene resulted in reduced number and enlarged size of chloroplasts, which were also observed in the lgp mutant. These evidences support that the non-synonymous SNP in Csa7G051430 is the causative mutation for the light green peel. This study provides a new allele for cucumber breeding for light green fruits and additional resource for the study of chloroplast development.

(2) Improving transformation protocol and optimizing CRISPR/Cas9 system in cucumber

To find a way to improve the genetic transformation efficiency, GFP (green fluorescent protein) was used as a reporter during *Agrobacterium* infection and in situ hybridization of *CsSTM* was carried out. The result suggested the regenerated adventitious shoot was originate from cells in deeper layers where *Agrobacterium* can barely arrived by immerse infection. To enhance infection, a simple syringe was used for vacuum infiltration. Additionally, hemin was used to promote the rooting of transgenic shoots, which solved the problem that addition of auxin promoted chlorosis of the transgenic shoots. The transformation protocol established in this study was used to perform CRISPR/Cas9-mediated knocking out of three genes and the transformation efficiency approached 1.00‰. Aside from cucumber, the transgenic and genome-editing approach has been validated in melon, so it would be possible that this approach can be widely used in Cucurbitaceae species.

(3) Engineering non-transgenic gynoecious cucumber using CRISPR/Cas9 system

We verified the function of an important sex determination gene *CsWIP1* and obtained transgene-free gynoecious cucumber by utilizing the improved efficient transformation protocol and optimized CRISPR/Cas9 system. *Cswip1* T0 mutants displayed gynoecious phenotype, with the upper nodes bearing only female flowers, which indicated *CsWIP1* acts as an inhibitor of carpel development in cucumber. Three types of deletions were found in T0 plant line 4 which displayed a high mutation rate 64.3% without off target. Homozygous and transgene-free *Cswip1* T2 mutants were obtained from self-pollinating of heterozygous T1 mutants generated by crossing-pollinating between T0 and wild-type. Compared with monoecious wild-type plants, the *Cswip1* mutants had seven times female flowers more than wild-type. Development of gynoecious cucumber by transgenic approach provide a stable and fast strategy to convert monoecious lines to gynoecious lines.