Development of loss of function alleles based on CRISPR/CAS9 to study flower and fruit development

Desarrollo de alelos de pérdida de función basados en CRISPR/CAS9 para estudio de desarrollo de flor y fruto

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Abstract

The CRISPR/cas9 system has taken over the methodologies to obtain new alleles as it is based on DNA repair mechanisms activated locally by DNA breakage. The CRISPR/Cas9 complex is driven by a guide RNA conferring specificity of action on a given DNA sequence. The aim of the work is to develop a set of alleles in genes of interest involved in flower and fruit development.

Keywords: genetic engineering; developmental genetics.

Resumen

El sistema CRISPR/Cas9 se ha convertido en la tecnología más útil para obtener alelos nuevos al estar basada en el proceso local de reparación de ADN activado localmente por la rotura del ADN. El complejo CRISPR/Cas9 es guiado por una molécula guía de ARN que le da especificidad de acción sobre una secuencia de ADN concreta. El objetivo del trabajo es desarrollar una colección de alelos en genes de interés relacionados con el desarrollo de la flor y fruto

Palabras clave: ingeniería genética; genética del desarrollo.

1. INTRODUCTION

To date, there are different types of gene editing strategies developed such as, meganucleases, Transcription Activator-Like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFNs) and the CRISPR-Cas9 system. [1, 2]. Unfortunately, TALENs and ZFNs systems are mainly based on protein-DNA interactions which requires cloning of new protein for each target site [3]. In contrast, CRISPR/Cas systems function via an RNA based recognition complex. Thus, by simply altering the guide RNA, Cas9 system can aim towards a new target location. Therefore, this feature makes CRISPR/Cas9 system a crucial element of gene editing strategies.

It has been shown that the CRISPR/Cas9 technology has been successfully used in Petunia and tomato [4-6]. The aim of the PhD proposal is to develop a set of CRISPR/Cas9 based alleles in genes of interest for flower and fruit development. Antirrhinum, petunia, tomato and Arabidopsis plants will be studied.

In this study, we aim to investigate the effects of an interruption with CRISPR/Cas9 precise gene editing technology in the genes naming ETS-related transcription factor (ELF4), Enhancer of benzenoid II (EOBII) and Zeitlupe (ZTL) in order to identify their roles in development and scent

production. Accordingly, both *Anthirrinum majus* (*A. majus*) and *Petunia hybrida* (*P. hybrida*) plants have been selected for the experimental setup.

Therefore, regions related to the mentioned genes have been investigated and the most efficient gRNA for each individual region has been selected in order to design CRISPR/Cas9 based alleles.

2. MATERIALS and METHODS

A. majus hypocotyl transformation and *P. hybrida* leaf transformation protocols have been described below.

2.1 Gene Database development

The flowering and fruit development related genes of *A. majus* and *P. hybrida* species have been selected and obtained from previous studies of our laboratories and online databases.

2.2 Plant Material

The *P. hybrida* and *A. majus,* will be grown in growth chamber conditions and the full development will be held in greenhouse followed by transformation of CRISPR/Cas9 technique. Seeds of *P. hybrida* line Mitchell and *A. majus* will be surface-sterilized. The seeds will be sowed on Murashige and Skoog medium (Duchefa, Haarlem, Netherlands) and will be solidified with 4 g/L of Phytagel (Sigma-Aldrich, Madrid, Spain). The samples will be placed on growth chambers under a photoperiod of 16/8 h of light/dark and 25ºC/18ºC temperature.

2.3 Vector Construction

The vector constructions have been obtained from a private company for both *A. majus* and *P. hybrida* separately according to their individual protocol.

2.4 *A. majus* Hypocotyl Transformation (Agrobacterium)

Transformation will be conducted by using *Agrobacterium tumefaciens* strain EHA105, through PBinPlus agrobacterium vector including the gene of interest (ELF4, EOBII and ZTL) for *A. majus*. Seeds have been put in to the growth chamber (8 h dark 18ºC / 16 h light 25ºC) on MS plates. Seeds will be grown 2 weeks followed by hypocotyl collection and transformation via EHA105. The hypocotyls have been put in new MS contained petri dishes. Plates will be incubated in dark for a week and slowly adapted to the light conditions of growth chamber by removing a sheet layer each day from the top of petri dishes. Hypocotyls have been inserted in to the growth chamber with new MS plates. Plants will be grown until callus formation following verification of gene editing process.

2.5 *P. hybrida* Leaf Transformation (Agrobacterium)

Transformation will be conducted by using *Agrobacterium tumefaciens* strain LBA4404 through PBinPlus agrobacterium vector including the gene of interest (ELF4, EOBII and ZTL) for *P. hybrida*. Seeds have been put in to the growth chamber (8 h dark 18ºC / 16 h light 25ºC) on MS poured containers in vitro. Plants will be grown until a certain point that is identified by early experiments for the leaf collection process. The leaves will be incubated with LBA4404 agrobacterium strain in order to integrate the selected plasmid into the plant genome.

3. RESULTS AND DISCUSSION

The correlation between the genes of interests and the developmental stages, especially circadian clock related pathways, of selected plants are aimed to be observed.

A. majus plants have been transformed with Cas9 vector in order to produce Cas protein required for CRISPR/Cas9 system to function. *P. hybrida* plants also have been planted for further leaf collection followed by leaf transformation with *Agrobacterium tumefaciens*.

4. CONCLUSIONS

The simplicity and sustainability of CRISPR/Cas9 system makes it a valuable genome editing tool amongst other genome editing methods. The gene editing techniques having the ability to accelerate plant breeding by enabling the precise gene modifications. CRISPR/Cas9 technology is specifically important amongst all other gene editing technologies due to its ability to modify multiple traits simultaneously [7]. Moreover, the technique does not require any protein engineering processes.

In this Phd thesis, we aim to obtain CRISPR/Cas9 based alleles targeting the ELF4, EOBII and ZTL on both *A. majus* and *P. hybrida* plant genomes in order to investigate their response in the manners of developmental genetics.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1] Li T., Huang S., Jiang W.Z., Wright D., Spalding M.H., Weeks D.P., Yang B. 2011. TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. Nucleic Acids Res 39: 359–372.

[2] Mahfouz M.M., Li L., Shamimuzzaman Md., Wibowo A., Fang X., Zhu J.-K. 2011. De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. Proc Natl Acad Sci U S A 108: 2623–2628.

[3] Feng Z., Zhang B., Ding W., Liu X., Yang D.-L. Wei P., Cao F., Zhu S., Zhang F., Mao Y. Zhu, J.-K., 2013. Efficient genome editing in plants using a CRISPR/Cas system. Cell Res 23: 1229–1232.

[4] Manchado-Rojo M., Weiss J., Egea-Cortines M., 2014. Validation of Aintegumenta as a gene to modify floral size in ornamental plants. Plant Biotechnology Journal 12: 1053–1065.

[5] Zhang B., Yang X., Yang C., Li M., Guo Y. 2016. Exploiting the CRISPR/Cas9 System for Targeted Genome Mutagenesis in Petunia. Sci Rep 6.

[6] Jacobs T.B., Zhang N., Patel D., Martin G.B. 2017. Generation of a Collection of Mutant Tomato Lines Using Pooled CRISPR Libraries. Plant Physiology 174: 2023–2037.

[7] Bortesi L., Fischer R. 2015. The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnology Advances 33: 41–52.

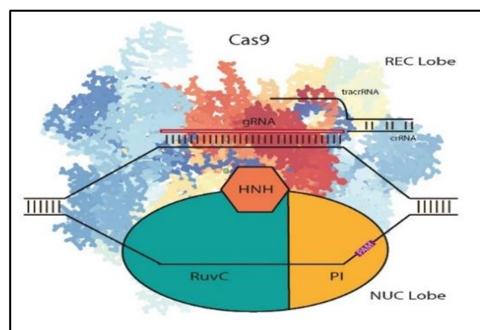

Figure 1. The representative image of RNA guided DNA cleavage by CRISPR/Cas9 system.

ELF4 Petunia axiliaris

Name	Score	Sequence	Strand	Position	GC
>Paxil_ELF4 Peaxi162Scf00825g00024.1	0.3467	GTTGTTGCTCTCATCTTCAATGG	-	406	45 %
>PhELF4silenced Peaxi162Scf00825g00233.1	0.4402	AGATCACGACTTCAGTATGGAGG	+	265	45 %
>Paxil_ELF4_ Peaxi162Scf00825g00022.1	0.7515	TGTCGTGTGATCTTGACTGATGG	-	149	50 %

ELF4 A. majus

Name	Score	Sequence	Strand	Position	GC
>ELF4_Amajus_347b_scaffold	0.6121	GGAGTAAAGCGACACCACTTTGG	-	264	50 %

EOBII Petunia axiliaris

Name	Score	Sequence	Strand	Position	GC
Peaxi162Scf00080g00064	0.5414	TTATGGCTCGGACGTACTTGAGG	+	803	50 %

EOBII A. majus gRNA sequences

Name	Score	Sequence	Strand	Position	GC
>Chr3 Chr3:34135221..34136156 (+ strand) class=gene length=936	0.4245	GGAAGAGTTGCAGATTACGGTGG	+	374	50 %
>Chr5 Chr5:56923523..56924575 (+ strand) class=gene length=1053	0.3571	AGTTTGGTGAGTAGTGACGGTGG	+	863	50 %

ZTL Petunia axiliaris

Name	Score	Sequence	Strand	Position	GC
>PaxilZTL lcl|Peaxi162Scf01124g00126.1	0.6138	GGATTGAGATACCAGTGACGTGG	+	1353	50 %

ZTL A. majus

Name	Score	Sequence	Strand	Position	GC
S20-2_N413_trimmed_contig_7988 ADO1_ARATHRecName	0.5889	CTTCTGTTGGTTCTGTTTGTCGG	-	1764	50 %
>S20-2_N413_trimmed_contig_7067 ADO1_ARATHRecName	0.7652	CGTCGTATCGTTATCCGTGATGG	-	2286	50 %

Figure 2. List, sequence and GC contents of designed gRNA constructs related to the ELF4, ZTL, EOBII genes according to their species.