Transformation of Sabah Traditional Rice for Combating Blast Disease

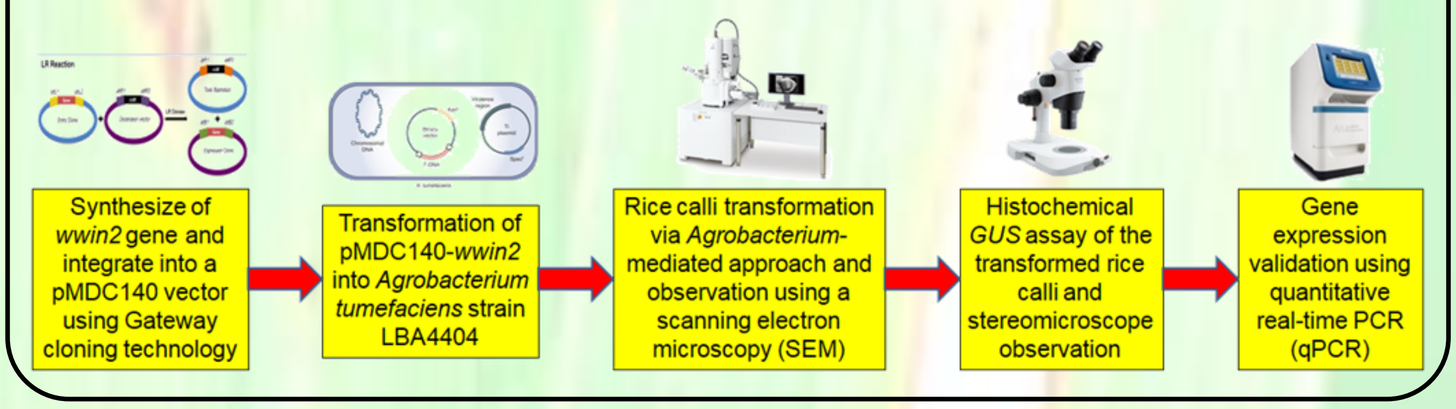
Eric Tzyy Jiann Chong[a], Jovita Jun Wong[a], Zaleha Abdul Aziz[a], Chia Lock Tan[b], Sreeramanan Subramaniam[c], Mariam Abdul Latip[a] and Ping-Chin Lee[a]

[a]Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
[b]Cocoa Biotechnology Research Centre, Malaysian Cocoa Board, Commercial Zone 1, Jalan Norowot, South Kota Kinabalu Industrial Park, 88460 Kota Kinabalu, Sabah, Malaysia
[c]School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden Heights, Penang, Malaysia

Introduction

The worldwide paddy production including the Sabah traditional rice is affected by blast disease which is caused by *Magnaporthe oryzae* fungal infection, resulting in a reduction of 10-30% rice yield annually[1]. Pathogenesis-related class 4 protein such as the wheatwin2 (wwin2) has been reported to significantly defend against a soil-borne fungi infection in tobacco plants[2], but the capability of this protein against *M. oryzae* infections in rice is unclear. Therefore, this study aimed to construct a plasmid containing the *wwin2* gene and transform it into the Sabah traditional rice genome to combat blast disease.

Materials and Methods



Results and Discussion

(A) Integration of the *wwin2* gene into the pMDC140 T-DNA region

The *wwin2* gene was located downstream of the constitutive 2X 35S cauliflower mosaic virus promoter and upstream of the β-glucuronidase (*GUS*) reporter, followed by a nopaline synthase terminator (*nos T*) and the hygromycin resistance (*hptII*) gene (Figure 1). These genes were flanked by left and right borders within the T-DNA region of the pMDC140.

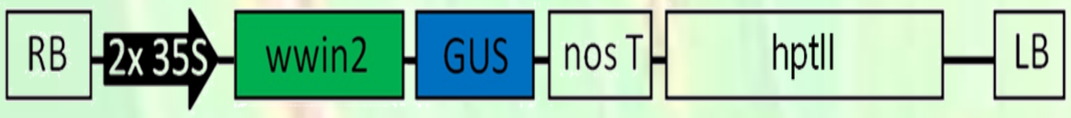


Figure 1. Schematic diagram showing the pMDC140 T-DNA region after the integration of the *wwin2* gene. RB: right border; LB: left border; 2x 35S: 35S promoter region of cauliflower mosaic virus; *wwin2*: wheatwin2 pathogenesis-related 4 gene; GUS: β-glucuronidase reporter gene; nos T: nopaline synthase gene terminator; *hptII*: hygromycin resistance gene.

(B) Observation on *Agrobacterium*-transformed rice callus surface using SEM

A clear fibril material formation produced by *Agrobacterium* on the surface of rice callus was observed under SEM (Figure 2a). A formation of the mucilaginous layer resulting from an unsystematic proliferation in the growing callus that created a hole and exposed the inner cell to the outer environment was also clearly seen on the surface of the callus (Figure 2b), which allowing the entrance of *Agrobacterium* into the inner part of the callus for T-DNA delivery.

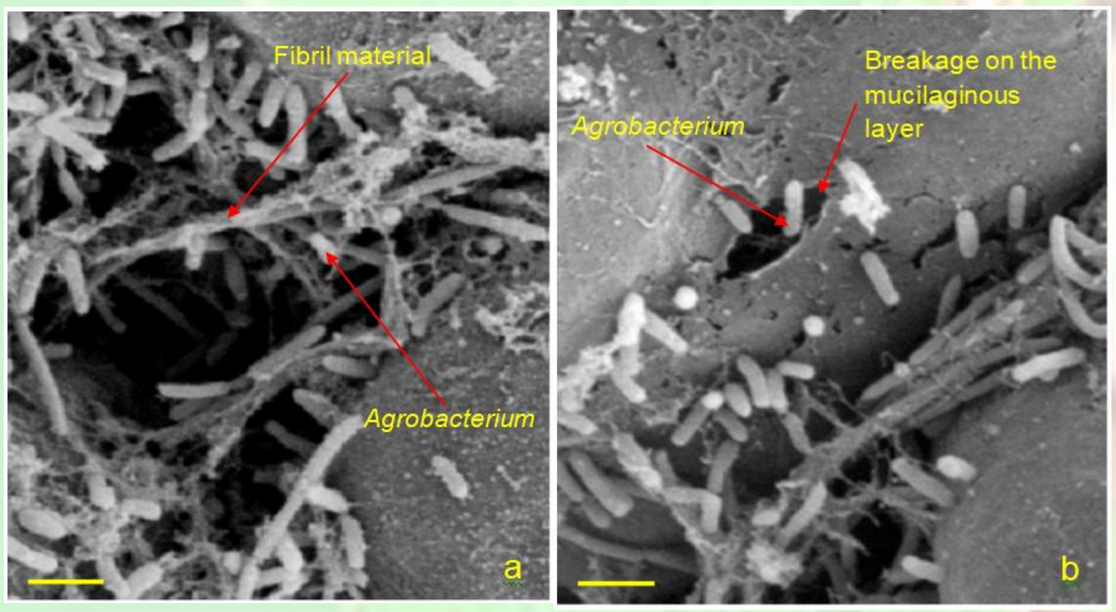


Figure 2. Attachment of *Agrobacterium* on the rice callus surface under SEM. (a) *Agrobacterium* attached to the cell membranous layer, anchoring the rice callus with fibril material (magnification = 8000x, bar = 2.0 μm). (b) *Agrobacterium* entered into the holes formed on the mucilaginous layer at the outer surface of the callus, facilitating the T-DNA transfer process (magnification = 8000x, bar = 2 μm).

(C) Histochemical *GUS* assay and stereomicroscope observation

The transient *GUS* expression in the transformed calli after immersed in GUS buffer for up to 48 hr at 37°C is shown in Figure 3. This study obtained a high frequency of calli with positive *GUS* stain (93.3% ± 0.01), with approximately 36.7% of the calli were with deep blue staining intensity (scoring = XXX). No blue spot was observed in the control callus. Previous studies reported less than 65% of the transgenic rice calli were positive for *GUS* stain when using different vector backbones[3,4], indicating that the newly constructed pMDC140-*wwin2* vector in this study provides a high transformation success rate and at the same time able to express *GUS* consistently in the transformed calli.

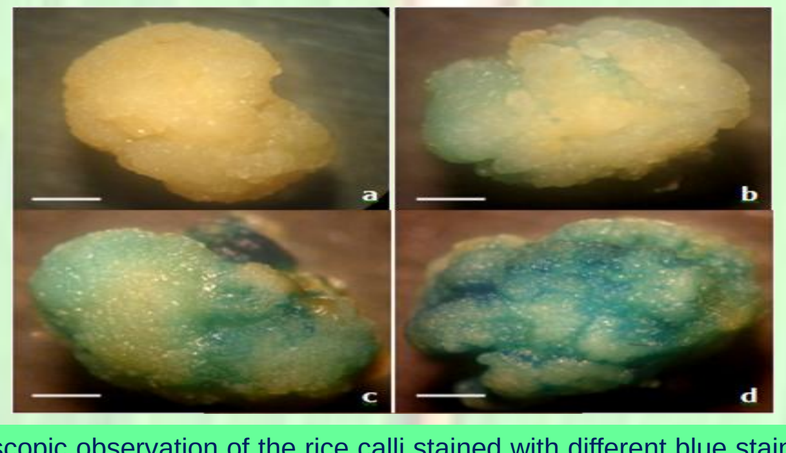


Figure 3. Microscopic observation of the rice calli stained with different blue stain intensities after GUS buffer immersion (magnification = 20x, bar = 0.15 cm). (a) Non-transformed callus (Scoring: -). (b) Callus with light blue stain (scoring: X). (c) Callus with medium blue stain (scoring: XX). (d) Callus with deep blue stain (scoring: XXX).

(D) The expression of *wwin2* and *hptII* genes in the transformed calli

The expressions of *wwin2* (mean C_T value = 29.79 ± 0.36 in transformed callus 1 and 24.28 ± 0.16 in transformed callus 2) and *hptII* (mean C_T value = 27.64 ± 0.26 in transformed callus 1 and 22.70 ± 0.93 in transformed callus 2) genes were detected in transformed calli, and C_T values for both genes were underdetermined in non-transformed callus (Table 1). The expressions of the sucrose phosphate synthase (*sps*) gene as an endogenous reference were detected in both transformed and non-transformed calli.

Table 1. The C_T values of the *wwin2*, *hptII*, and *sps* genes in gene expression analysis

Gene	Mean C_T value ± S.D.*		
	Transformed callus 1	Transformed callus 2	Non-transformed callus (control)
wwin2	29.79 ± 0.36	24.28 ± 0.16	Underdetermined
hptII	27.64 ± 0.26	22.70 ± 0.93	Underdetermined
sps	30.18 ± 1.60	22.20 ± 0.95	20.73 ± 0.75

*Each value represents the mean ± standard deviation (S.D.) in 3 replicates.

Conclusions

In conclusion, this study constructed a vector containing the *wwin2* gene with a high transformation rate and capable of consistently expressing GUS and *wwin2* in the transformed Sabah traditional rice calli. Subsequent analyses are needed to verify the defense mechanism of the wwin2 protein towards rice blast disease.

Acknowledgements

We would like to thank the local farmers for providing the seeds for this study. This study was supported by the Ministry of Higher Education, Malaysia under the Exploratory Research Grant Scheme (ERGS0031-STG-1/2013).

References

1. Nalley L, Tsiboe F, Durand-Morat A, et al. 2016. *PLoS One* **11**(12): e0167295.
2. Fiocchetti F, D'Amore R, De Palma M, et al. 2008. *Plant Cell Tiss. Organ Cult.* **92**: 73-84.
3. Chakraborty M, Reddy PS, Narasu ML, et al. 2016. *Physiol. Mol. Biol. Plant* **22**: 51-60.
4. Wakasa Y, Ozawa K, Takaiwa F. 2012. *Plant Cell Rep.* **31**: 2075-2084.

UMS UNIVERSITI MALAYSIA SABAH
LEMBAGA KOKO MALAYSIA • MALAYSIAN COCOA BOARD •
USM UNIVERSITI SAINS MALAYSIA

Further Information

Please contact Prof. Dr. Lee Ping Chin (E-mail: leepc@ums.edu.my; Tel: +60-88-320000 ext. 100101) for further details. Please scan the QR code to visit our website for current research activities.