THESIS

TRANSFORMATION OF MAIZE ANTHOCYANIN REGULATORY GENES, *B-PERU* AND *C1*, INTO ORCHID

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A Thesis Submitted in Partial Fulfilment of
the Requirments for the Degree of
Doctor of Philosophy (Genetics)
Graduate School, Kasetsart University
2006

ISBN
974-16-2357-7

Chidchanok Suwanaketchanatit 2006: Transformation of maize anthocyanin regulatory genes, *B-peru* and *C1*, into orchid. Doctor of Philosophy (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Assistant Professor Pattana Srifah Huehne, Ph.D. 103 pages.
ISBN: 974-16-2357-7

The effecting factors for particle gun bombardment were studied in *Dendrobium* Jaquelyn Thomas. Three different plasmids, pActin1-D, pAHC27, and P2K7, containing different promoters, Act-1, Ubi-1, and CaMV35S, respectively, fused to *β-glucuronidase* (*gus*) gene and Nos terminator were introduced into the orchid sepal. The Gus activity under the control of Act-1 and CaMV35S promoters showed high level of expression, while Ubi-1 promoter was poor. Transgenic orchids were established by co-bombardment of pMNK1005 and pBpSA13 or pC1SA12. The pMNK1005 contained *hygromycin phosphotransferase* (*hgh*) gene fused to *green fluorescent protein* (*gfp*) genes driven by a Ubi-1 promoter. The plasmids pBpSA13 and pC1SA12 consist of *B-peru* and *C1* genes of maize transcription factors, respectively, under controlled by Act-1 promoter. The transgenic orchid was sequential screened on medium supplemented with 5mg/l, 25mg/l, and 30mg/l hygromycin. The high transformation efficiency of 19.87% and no-chimera cells were obtained. The integration of the transgene in chromosome of transgenic plantlets was confirmed by PCR and Southern blot hybridization. The results of co-transformation with two groups of transcription factor gene families showed the accumulation of red-purple pigments in the epidermal layer of the leaf sheaths of independent transgenic lines compared with the green tissues of the un-transformed plantlet. Northern blot and RT-PCR analysis were utilized in determine expression of the genes involved in the anthocyanin biosynthesis.

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ACKNOWLEDGMENTS

I would like sincerely like to acknowledge Thailand Research Fund (TRF) and National Center for Genetic Engineering and Biotechnology (BIOTEC) for supporting this research.

I am profound to my thesis advisor Assistant Professor Pattana Srifah Huehne, Associate Professor Surin Peyachoknagul, Assistant Professor Chitrapan Piluek, and Dr. Tawansak Pawsang for their direction, suggestion, and encouragement. I am indebted to Dr. Saluk parnsiri of Kasetsart University re Research and Development Institute (KURDI) who have supplied the particle gun and Dr. Jaruwan Chartisathian of Biotechnology Research and Development office, Development of Agriculture who have supplied the fluorescence microscope. I am also grateful to all friends in my Department of Genetics and my close friends who always cheer me up.

Finally, my appreciation devotes to my father, my mother, and my relatives, who stand beside me for giving heartful supports.

Chichanok Suwanaketchanatit

May 2006