

## 1.0 INTRODUCTION

### 1.1 *Cryptocoryne willisii* species and plant transformation

The cultivation of ornamental aquatic plants has emerged as a profitable and viable commercial venture. They are important to increase the aesthetic value of the aquarium as well as to improve oxygenation and maintain the healthy condition for the fishes in the aquarium tank. The *Cryptocoryne* species received great welcome from the aquarium hobbyist and culturist. *Cryptocoryne willisii* is among the valuable species easily cultivated and required only light management. However, *C. willisii* seldom produce flowers (Rataj and Horeman, 1977). Not only this limits the aesthetic value of this plant but it also hampers the production of new varieties. This can be achieved by plant transformation method whereby the flowering transition process can be induced to trigger the florigen, CONSTANS (CO) to signal for floral induction (Ayre and Turgeon, 2004). Hence, a reliable transformation system is desirable for *C. willisii*.

Reporter genes are chaperones that facilitate transgenes expression assessments and easy scored indicators of plant transformation. Commonly used reporters genes include genes encoding for  $\beta$ -glucuronidase (GUS), green fluorescent protein (GFP), chloramphenicol acetyl transferase (CAT),

luciferase (LUC) and protein involved in regulation of anthocyanin biosynthesis. In this study, GUS and GFP reporter genes were exploited for optimisation of transformation parameters and establishment of efficient *Agrobacterium*-mediated transformation system for *C. willisii*. There was no report of transformation on this plant species yet neither with direct nor indirect transformation methods.

## 1.2 Objectives of the study

- 1) To establish a reliable and optimum transformation system for *Cryptocoryne willisii* using *Agrobacterium tumefaciens* strain LBA4404 harbouring the binary vector pCAMBIA1304.
- 2) To identify the expression level of reporter genes in *Cryptocoryne willisii*.

## 1.3 Strategies

- 1) Use of tobacco plants as positive control for the vectors.
- 2) Optimization of *Agrobacterium*-mediated transformation parameters for introducing reporter genes into *C. willisii*.