

LIST OF FIGURES

	Page
Figure 2.1: Botanical drawing of <i>C. willisii</i>	6
Figure 2.1: General Ti – plasmid map.....	15
Figure 2.3: <i>Agrobacterium</i> - mediated gene transferring mechanisms. With every steps described in the text in boxes.....	19
Figure 2.4: The pCAMBIA1304 vector.....	23
Figure 2.5: Transition of meristematic cells from vegetative phase to reproductive phase.....	35
Figure 3.1: An illustration of set-up for Southern transfer.....	52
Figure 4.1: Effects of different concentration of 6-BA on multiple shoots formation frequencies of <i>C. willisii</i> . Error bars corresponding to standard deviation where n = 5. Different letters indicate values that are significantly different at 95 % confidence level using Duncan multiple comparison test.....	58
Figure 4.2: Rooted plantlet of <i>C. willisii</i>	59
Figure 4.3: Growth curve of <i>A. tumefaciens</i> strain LBA4404 harbouring the binary vector pCAMBIA1304.....	61
Figure 4.4: Viable cell count of <i>A. tumefaciens</i> strain LBA4404 harbouring pCAMBIA1304. Cell density was counted based on CFU per ml which represents the bacteria colony derived form a single ancestor of <i>A. tumefaciens</i> in one ml broth culture.....	63

Page

- Figure 4.5:** Agarose gel electrophoresis visualisation of plasmid DNA extracted from *A. tumefaciens* strain LBA4404 harbouring the binary vector pCAMBIA1304. The expected size of pCAMBIA1304 is 12361bp.....68
- Figure 4.6:** Fluorometric assay of GUS enzyme activity for transformed *C. willisii* with different infection times (2, 4, 6, 8, 10 min) and cocultivated with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304 for 1 day. Different letter indicated that the mean values are significantly contrasted at 95 % confidence level. One way ANOVA analysis of the data obtained was summarised in Appendix 13. Error bars represent the standard deviations where n = 3.....75
- Figure 4.7:** Fluorometric assay of GUS enzyme activity for transformed *C. willisii* with different infection times (2, 4, 6, 8, 10 min) and co-cultivated with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304 for 2 days. Different letter indicated that the mean values are significantly contrasted at 95 % confidence level. One way ANOVA analysis of the data obtained was summarised in Appendix 14..... 76

Page

- Figure 4.8:** Fluorometric assay of GUS enzyme activity for transformed *C. willisii* with different infection times (2, 4, 6, 8, 10 min) and cocultivated with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304 for 3 days. One way ANOVA analysis showed that the results obtained for this experiment was not significant at 95% confidence level (Appendix 15)..... 77
- Figure 4.9:** Fluorometric assay of GUS enzyme activity for transformed *C. willisii* with different infection times (2, 4, 6, 8, 10 min) and cocultivated with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304 for 4 days. Different letter indicated that the mean values are significantly contrasted. One way ANOVA analysis of the data obtained was summarised in Appendix 16..... 78
- Figure 4.10:** Pictures showing GUS histochemical staining in tobacco explants transformed with *A. tumefaciens* strain LBA4404 harbouring the binary vector pCAMBIA1304..... 81
- Figure 4.11:** Histochemical staining of transformed *C. willisii*. Blue-stains were indicated by arrows..... 83
- Figure 4.12:** GFP visualisation on transformed tobacco explants. Left: Explants viewed in normal light. Right: Explants viewed in UV light (blue-light).....84

Page

- Figure 4.13:** GFP visualisation on transformed *C. willisii* explants.
Left: Explants viewed in normal light. Right: Explants viewed in UV light (blue-light).....86
- Figure 4.14:** Picture showing the negative control of non-transformed *C. willisii* leaf explant viewed under UV (blue) light. Leaf explant appeared red as a result of auto-fluorescence from chlorophyll..... 87
- Figure 4.15:** Gradient PCR of *mgfp5* reporter gene of plasmid DNA with different annealing temperature..... 89
- Figure 4.16:** Screening of *A. tumefaciens* LBA4404 harbouring pCAMBIA1304 prior to plant transformation experiments...91
- Figure 4.17:** PCR confirmation of the presence *mgfp5* gene in transformed tobacco callus explants..... 93
- Figure 4.18:** PCR confirmation of *C. willisii* transformation..... 94
- Figure 4.19:** Digestion and Southern Blotting of transformed *C. willisii* genomic DNA. (a). Digested genomic DNA (Sample S1, S2, S3, S4, S5 and S6) separated on 1% (w/v) agarose gel. Figure showed membrane after Southern Hybridization being carried out with *mgfp5* gene specific probes.....96