

## Table of Contents

	<b>Page</b>
<b>Acknowledgements</b>	<b>i</b>
<b>Abstract</b>	<b>ii</b>
<b>Abstrak</b>	<b>iii</b>
<b>Table of Contents</b>	<b>iv</b>
<b>List of Tables</b>	<b>x</b>
<b>List of Figures</b>	<b>xi</b>
<b>Abbreviations</b>	<b>xiv</b>
<b>Chapter 1 Introduction</b>	
<b>1.1</b> <i>Cryptocoryne willisii</i> species and plant transformation.....	<b>1</b>
<b>1.2</b> Objectives of the study.....	<b>2</b>
<b>1.3</b> Strategies.....	<b>2</b>
<b>Chapter 2 Literature reviews</b>	
<b>2.1</b> The aquatic plants.....	<b>3</b>
<b>2.2</b> The <i>Cryptocoryne</i> species.....	<b>5</b>
<b>2.2.1</b> Cultivation and tissue culture of	
<i>Cryptocoryne</i> species.....	<b>8</b>

	<b>Page</b>
<b>2.3</b> Gene transfer to plants.....	<b>10</b>
<b>2.3.1</b> Methods of gene transfer to plants	
<b>2.3.1.1</b> Direct gene transfer.....	<b>11</b>
<b>2.3.1.2</b> Indirect gene transfer – <i>Agrobacterium</i> mediated transformation.....	<b>12</b>
<b>2.3.1.3</b> Ti plasmid of <i>A. tumefaciens</i> and the T – DNA.....	<b>13</b>
<b>2.3.1.4</b> Factors influencing the success of Agrobacterium-mediated transformation.....	<b>14</b>
<b>2.3.1.5</b> The T – DNA transferring machinery and mechanism.....	<b>16</b>
<b>2.3.1.6</b> Vectors for <i>Agrobacterium</i> – mediated transformation.....	<b>20</b>
<b>2.3.1.5.2</b> The pCAMBIA vectors.....	<b>22</b>
<b>2.3.2</b> Genetic transformation in aquatic plants.....	<b>24</b>
<b>2.4</b> The reporter systems.....	<b>25</b>
<b>2.4.1</b> The $\beta$ – glucuronidase reporter gene.....	<b>27</b>
<b>2.4.2</b> The green fluorescent protein.....	<b>27</b>
<b>2.5</b> Molecular assessment of transformant	
<b>2.5.1</b> Polymerase Chain Reaction (PCR).....	<b>32</b>
<b>2.5.2</b> Southern Blotting.....	<b>32</b>

	<b>Page</b>
<b>Chapter 3 Materials and Methods</b>	
<b>3.1 Plant tissue culture</b>	
<b>3.1.1 Maintenance and subculturing of tobacco</b> <i>(Nicotiana benthamiana)</i> explants.....	34
<b>3.1.2 Determination of the best media for shoot</b> induction of <i>C. willisii</i> .....	35
<b>3.1.3` Determination of hygromycin concentration for</b> selecting of transformed <i>C. willisii</i> .....	35
<b>3.2 Constructs and vectors</b>	
<b>3.2.1 Growth of <i>Agrobacterium tumefaciens</i></b> harbouring binary system LBA4404 and pCAMBIA1304.....	36
<b>3.2.2 Preparation of <i>Agrobacterium</i> glycerol stock.....</b>	37
<b>3.2.3 Minimal inhibitory concentration (MIC) of</b> <i>Agrobacterium</i> .....	37
<b>3.2.4 Preparation of plasmid DNA from</b> <i>Agrobacterium</i> .....	38
<b>3.3 <i>Agrobacterium</i>-mediated plant transformation</b>	
<b>3.3.1 Post – Cocultivation.....</b>	40
<b>3.4 Verification of putative transformants</b>	
<b>3.4.1 Fluorescent microscopic visualization of green</b> fluorescent proteins (GFP).....	42

	<b>Page</b>
<b>3.4.2</b> GUS assessments	
<b>3.4.2.1</b> Histochemical staining.....	<b>42</b>
<b>3.4.2.2</b> GUS fluorometric assay.....	<b>43</b>
<b>3.5</b> Confirmation of transformation /Molecular assessment	
<b>3.5.1</b> Plant DNA (Deoxyribonucleic acid)	
extraction and quantification.....	<b>45</b>
<b>3.5.2</b> DNA Quantification.....	<b>46</b>
<b>3.5.3</b> Polymerase chain reaction (PCR)	
<b>3.5.3.1</b> Primer design for <i>mgfp5</i> gene.....	<b>47</b>
<b>3.5.3.2</b> PCR condition and system.....	<b>47</b>
<b>3.5.3.3</b> Optimization of duplex amplification of <i>mgfp5</i> genes.....	<b>49</b>
<b>3.5.4</b> Agarose gel electrophoresis.....	<b>49</b>
<b>3.5.5</b> Southern Blotting.....	<b>50</b>

## **Chapter 4 Results and Discussion**

<b>4.1</b> The effects of 6-BA on <i>Cryptocoryne willisii</i> shoots induction.....	<b>53</b>
<b>4.2</b> <i>Agrobacterium tumefaciens</i> strain LBA4404 harbouring the binary vector pCAMBIA1304 .....	<b>59</b>

	<b>Page</b>
<b>4.2.1</b> Growth curve.....	<b>59</b>
<b>4.2.2</b> <i>Agrobacterium</i> cell density count.....	<b>61</b>
<b>4.2.3</b> Minimal inhibitory concentration (MIC) of <i>Agrobacterium</i> .....	<b>63</b>
<b>4.2.4</b> Screening of <i>A. tumefaciens</i> strain LBA4404	
<b>4.2.4.1</b> harbouring the binary vector	
pCAMBIA1304 Preparation of plasmid	
DNA from bacteria culture.....	<b>66</b>
<b>4.3</b> <i>Agrobacterium</i> -mediated plant transformation	
<b>4.3.1</b> Transformation of <i>Nicotiana benthamiana</i> .....	<b>68</b>
<b>4.3.2</b> Transformation of <i>C. willisii</i> .....	<b>69</b>
<b>4.3.2.1</b> Effects of infection and co-cultivation period.....	<b>71</b>
<b>4.3.3</b> Histochemical assessment of tobacco and <i>C. willisii</i> .....	<b>78</b>
<b>4.3.4</b> GFP visualisation in putative transformants	
<b>4.3.4.1</b> Tobacco.....	<b>83</b>
<b>4.3.4.2</b> GFP visualisation in transformed <i>C. willisii</i> explants.....	<b>84</b>

	<b>Page</b>
<b>4.4</b> Molecular assessments	
<b>4.4.1</b> Primer design and optimization of PCR condition.....	<b>86</b>
<b>4.4.2</b> Screening of <i>Agrobacterium</i> and confirmation of plasmid DNA by PCR.....	<b>88</b>
<b>4.4.3</b> PCR assessment on putative transformed plants	
<b>4.4.3.1</b> Tobacco.....	<b>89</b>
<b>4.4.3.2</b> PCR confirmation of <i>C. willisii</i> transformation.....	<b>91</b>
<b>4.4.4</b> Restriction digest of genomic DNA and Southern blotting .....	<b>93</b>
 <b>Chapter 5 Conclusion</b>	
<b>5.0</b> Conclusion.....	<b>97</b>
 <b>References</b>	
 <b>Appendixes</b>	