## CONTENTS

<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>PageNo.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>List of Tables</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>01</td>
</tr>
<tr>
<td>2.</td>
<td>REVIEW OF LITERATURE</td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>2.1. Conventional propagation</td>
<td>05</td>
</tr>
<tr>
<td></td>
<td>2.1.1. Corm divisions</td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>2.1.2. Removal of leaves and flower spike</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>2.1.3. Corm sizes</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>2.2. In vitro propagation</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>2.2.1. Development of shoot regeneration from different</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.2.2. Callus formation from different explant sources</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.2.3. Regeneration of <em>in vitro</em> cultures</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2.2.4. Rooting of <em>in vitro</em> regenerated cultures</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.2.5. <em>In vitro</em> cormel production</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.2.6. Acclimatization of <em>in vitro</em> regenerants</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2.2.7. Size enhancement studies of cormels</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>2.3. Clonal fidelity</td>
<td>24</td>
</tr>
<tr>
<td>3.</td>
<td>MATERIALS AND METHODS</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3.1. Conventional propagation</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3.1.1. Maximization of corm and cormel production in</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3.1.1.1. Cutting of the corms</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3.1.1.2. Removal of the leaves and flower spike</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3.1.2. Effect of different corm sizes on the production of corms and cormels</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3.1.3. Other conditions for both field experiments</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3.1.3.1. Soil properties of the experimental field</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3.1.3.2. Sowing of corm</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3.1.3.3. Preparation of field and other practices</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>3.1.3.4. Experimental layout</td>
<td>31</td>
</tr>
</tbody>
</table>
3.1.3.5. Data collection and analysis 31

3.2. *In vitro* propagation 32

3.2.1. Plant material 32

3.2.2. Explant sterilization 32

3.2.2.1. Nodal part of inflorescence stem, flower bud and shoot tip of cormel 32

3.2.2.2. Cormels 32

3.2.3. Culture conditions 35

3.2.4. Preparation of culture media 35

3.2.5. *In vitro* production of cormels through direct mode of regeneration 36

3.2.5.1. Shoot regeneration through different explant sources 36

3.2.5.1.1. Preparation of explant 36

3.2.5.1.2. Proliferation of multiple shoots 36

3.2.6. *In vitro* production of cormels through callogenesis 36

3.2.6.1. Initiation of regenerable callus 36

3.2.6.1.1. Preparation of explants 36

3.2.6.1.2. Multiplication of callus 37

3.2.6.1.3. Fresh callus growth 37

3.2.7. Root formation from different explant sources 37

3.2.8. Cormel development from different explant sources 38

3.2.9. Enhancement of cormel size 38

3.2.9.1. Enhancement of cormel size under *in vitro* conditions 38

3.2.9.2. Size enhancement studies of cormels through acclimatization of *in vitro* regenerated cultures 38

3.2.9.2.1. Potting media analysis 39

3.2.10. Layout of the experiment 40

3.3. Clonal fidelity 40

3.3.1. Plant material 40

3.3.2. Genomic DNA extraction 40

3.3.3. Quantification of genomic DNA 41

3.3.4. Polymerase Chain reaction 42

3.3.4.1. Random Amplified Polymorphic DNA 42

3.3.4.1.1. Optimization of PCR conditions 42
3.3.4.1.2. PCR temperature profile 42
3.3.4.2. Inter Simple Sequence Repeat (ISSR) 42
3.3.4.2.1. Optimization of PCR conditions 44
3.3.4.2.2. PCR temperature profile 44
3.3.5. Data analysis 44

4. RESULTS AND DISCUSSION 46

4.1. Conventional propagation 47
  4.1.1. Maximization of corm and cormel production 47
    4.1.1.1. Corm and cormel weight 47
    4.1.1.2. Size and number of corms and cormels 52
      4.1.1.2.1. Grading of the obtained corms and cormels 54
    4.1.1.3. Discussion 57
    4.1.1.4. Conclusion 58
  4.1.2. Effect of various corm sizes on corm and cormel production 59
    4.1.2.1. Vegetative and floral parameters 59
    4.1.2.2. Corm and cormel weight 61
    4.1.2.3. Size and number of corms and cormels 64
      4.1.2.3.1. Grading of the obtained corms and cormels 66
    4.1.2.4. Discussion 70
    4.1.2.5. Conclusion 72

4.2. In vitro propagation 73
  4.2.1. In vitro production of cormels through direct regeneration 73
    4.2.1.1. Shoot regeneration by using different explant sources 73
      4.2.1.1.1. Selection of better stage/size in each explant 73
      4.2.1.1.2. Shoot regeneration through nodal cultures 73
      4.2.1.1.3. Shoot regeneration through whole cormels of different size 74
      4.2.1.1.4. Shoot regeneration through cormel sprouts 80
      4.2.1.1.5. Shoot regeneration from cormel sections 86
    4.2.1.2. Proliferation of multiple shoots from different explant sources 86
      4.2.1.2.1. Choice of plant material and proliferation media 86
      4.2.1.2.2. Multiple shoots from different explant sources 89
      4.2.1.3. Root formation in shoots derived from different explant sources 89
4.2.1.3.1. Choice of plant material and root induction media
4.2.1.3.2. Root induction in shoots derived from different explant sources
4.2.1.3.3. Number of roots regenerated from shoots of different explant sources
4.2.1.4. *In vitro* production of cormels in plantlets derived from different explant sources
4.2.1.4.1. Cormel induction
4.2.1.4.2. Number of cormels

4.2.2. *In vitro* production of cormels through callogenesis
4.2.2.1. Callus induction from different explant sources
4.2.2.1.1. Callus induction from shoot tip of cormel
4.2.2.1.2. Callus induction from cormel slices
4.2.2.2. Multiplication of callus induced from different Explant sources
4.2.2.2.1. Multiplication of callus from shoot tip of cormel
4.2.2.2.2. Multiplication of callus from cormel slices
4.2.2.3. Fresh callus growth from different explant sources
4.2.2.3.1. Fresh callus growth form shoot tip of cormel
4.2.2.3.2. Fresh callus growth from cormel slices
4.2.2.4. Shoot regeneration from calli of different explant sources
4.2.2.4.1. Shoot regeneration from shoot tip of cormel and cormel slices
4.2.2.4.2. Number of shoots regenerated from calli of shoot tip of cormel and cormel slices
4.2.2.5. Root regeneration in shoots developed from different explant sources
4.2.2.5.1. Root regeneration in shoots derived from shoot tip of cormel
4.2.2.5.2. Root regeneration in shoots from cormel slices
4.2.2.6. Production of cormels
4.2.2.6.1. Cormel induction from shoot tip of cormel
4.2.2.6.2. Cormel induction from cormel slices
4.2.3. Grading of in vitro produced cormels 123

4.2.4. Size enhancement studies of cormels by using in vitro regenerated plantlets 130

4.2.4.1. Size enhancement studies of cormels by using cormel induction medida 130

4.2.4.2. Cormel size enhancement studies and survival percentage of in vitro regenerants by using various potting media 130

4.2.4.2.1. Cormel diameter of acclimatized plantlets 136

4.2.5. Discussion 139

4.2.6. Conclusion 150

4.3. Economical analysis 153

4.3.1. Conventional methods of propagation 153

4.3.2. In vitro propagation 153

4.4. Determining clonal fidelity 157

4.4.1. Random Amplified Polymorphic DNA 157

4.4.2. Molecular characterization with RAPD markers 160

4.4.3. Molecular characterization with ISSR markers 160

4.4.4. Discussion 168

4.4.5. Conclusion 169

5. SUMMARY 170

LITERATURE CITED 175

APPENDICES 191