

# Contents

1. Introduction	1
1.1. Distribution of tea (worldwide and in India)	3
1.2. Agro-Climatic conditions required for tea cultivation (Tea board of India)	3
1.2.1. Air Temperature	4
1.2.2. Soil temperature	4
1.2.3. Rainfall	5
1.2.4. Humidity	5
1.2.5. Solar radiation	6
1.2.6. Day length	6
1.2.7. Altitude	6
1.2.8. Hail	6
1.3. Methods of propagation	6
1.4. Processing and marketing of Tea	7
1.5. Diseases of tea	8
1.6. Uses of Tea ( <i>Camellia sinensis</i> )	8
1.7. Tea Genetic Resources, conservation and its yield worldwide	9
1.7.1. Tea Genetic resources and conservation	9
1.7.2. Tea Yield	9
1.8. Problem areas in Tea	10
2. Review of literature	13
2.1 History of Tea	14
2.1.1. History of tea world wide	14
2.1.2. History of Tea in India	16
2.2. Taxonomy of tea	16
2.3. Genome diversity	17
2.4. Economic importance and health benefits	18

2.5. Conventional propagation and breeding	18
2.6. DNA fingerprinting study of tea	21
2.6.1. Morphological markers	21
2.6.2. Cytological markers	21
2.6.3. Biochemical markers	22
2.6.4. Isozymes markers	23
2.7. Molecular marker technology	25
2.7.1. Restriction Fragment Length Polymorphism (RFLP)	25
2.7.2. Random Amplified Polymorphic DNA (RAPD)	25
2.7.2.1. Germplasm characterization	26
2.7.2.2. Detection of genetic fidelity among <i>in-vitro</i> raised plants by RAPD analysis	27
2.7.2.3. Cultivar identification	28
2.7.3. DNA Amplification fingerprinting (DAF)	28
2.7.4. Amplification Fragment Length Polymorphism (AFLP)	29
2.7.5. Microsatellites	30
2.7.6. Organelle DNA analysis	31
2.8. Various Molecular marker techniques applied to <i>Camellia</i> -Milestones	32
2.9. <i>In vitro</i> culture studies in tea	34
2.10. Histological study	38
2.11. Genetic transformation study of tea	39
2.11.1. <i>Agrobacterium</i> mediated genetic transformation	40
2.11.2. <i>Agrobacterium rhizogenes</i>	43
2.11.3. Biolistic	43
2.12. Gene constructs and vectors	44
2.12.1. Herbicide tolerance genes	44
2.12.2. Insect tolerance genes	44
2.12.3. Seed storage protein genes	44
2.12.4. Coat protein genes for virus protection	45
2.12.5. Light regulated gene	45
2.13. Phenotypic characteristics of transformed plant	45
3. Material and Methods	47

3.1. Plant Material	48
3.2. DNA Fingerprinting Study	48
3.2.1. Tea DNA extraction	48
3.2.2. Purification of Tea DNA	50
3.2.3. Quantification of Tea DNA	51
3.2.3.1. Spectrophotometric measurement	51
3.2.3.2. Gel Analysis	51
3.2.4. RAPD (Random Amplified Polymorphic DNA) of Tea ( <i>Camellia sinensis</i> )	51
3.2.4.1. RAPD-PCR Amplification	51
3.2.4.2. RAPD Data Analysis	52
3.2.5. PCR-RFLP (Restriction Fragment Length Polymorphism) analysis of Tea ( <i>Camellia sinensis</i> )	53
3.2.5.1. Primer used for trnL-trnF (“Taberlet”) region (Taberlet et al., 1991) of the tea genome	53
3.2.5.2. PCR-RFLP (Restriction Fragment Length Polymorphism) Amplification	53
3.2.5.3. PCR-RFLP product Restriction digestion	54
3.2.5.4. PCR-RFLP Data analysis	55
3.2.6. Sequencing of PCR-RFLP amplification products	55
3.2.6.1. Purification of 10 PCR products for sequencing	56
3.2.6.2. Sequence analysis and GenBank submission	56
3.2.7. Amplification of <i>rbcL</i> -ORF106, chloroplast DNA (all of <i>rbcL</i> plus spacer between it and ORF106 exon)	57
3.2.7.1. PCR Amplification	57
3.2.7.2. PCR product Restriction digestion and analysis	57
3.2.8. Microsatellite Markers study	58
3.2.8.1. Primers Used	58
3.2.8.2. PCR- Amplification	58
3.3. <i>In vitro</i> culture studies	59
3.3.1. Establishment of callus cultures	59

3.3.2. Regeneration of plants from callus tissues	60
3.3.3. Formation of Somatic embryos and whole plantlet from cotyledon explants on Murashige and Skooge medium containing various hormones	60
3.4. Histological observations of the cultured cotyledons	61
3.5. Genetic transformation study	61
3.5.1. <i>Agrobacterium</i> mediated transformation of Tea	61
3.5.1.1. Bacterial Strains and vector used	61
3.5.1.2. Optimization of parameters required for genetic transformation of Somatic embryos from cotyledonary explants used in the present study	62
3.5.1.3. Transformation protocol	63
3.5.2. Particle Bombardment- Mediated Transformation of tea	64
3.5.2.1. Bacterial Strain and vector	64
3.5.2.2 Plasmid Isolation from the strain pZP200KC using SIGMA miniprep isolation kit according to the instructions manual	65
3.5.2.3. Transformation Protocol (Christou 1996)	66
3.5.2.4. Ready to shoot	67
3.6. Assay of putative transformants achieved by both types of gene transfer methods	68
3.6.1. Assay of <i>GUS</i> activity	68
3.6.2. Isolation of transformed plant DNA	68
3.6.3. Polymerase chain reaction characterization	69
4. Results and Discussion	71
4.1. DNA fingerprinting study	72
4.1.1. Tea DNA extraction, purification and quantification	72
4.1.1.1. Tea DNA extraction	72
4.1.1.2. Tea DNA purification	72
4.1.1.3. Tea DNA quantification	72
4.1.2. RAPD analysis	72
4.1.2.1. RAPD PCR product agarose gel analysis	72
4.1.2.3. RAPD PCR product data analysis using NTSYSpc software	77
4.1.3. PCR-RFLP analysis	79
4.1.3.1. RFLP PCR product agarose gel analysis	79

4.1.3.2. PCR-RFLP product restriction digestion agarose gel analysis	79
4.1.3.3. PCR-RFLP product data analysis using POPGENE freeware software	80
4.1.3.4. PCR-RFLP product data analysis using NTSYSYpc software	82
4.1.3.5. Sequence analysis using Clustal W (Thompson <i>et al.</i> , 1994) and GenBank submission	82
4.1.4. <i>rbcL</i> -ORF106, chloroplast DNA (all of <i>rbcL</i> plus spacer between it and ORF106 exon) analysis	83
4.1.4.1. Chloroplast DNA PCR product agarose gel analysis	83
4.1.5. Microsatellite markers study analysis	86
4.1.6. Comparative account of DNA fingerprinting study	87
4.2. <i>In vitro</i> culture studies	92
4.2.1. Establishment of callus cultures	92
4.2.2. Regeneration of plant from callus cultures	92
4.2.3. Formation of Somatic embryos and whole plantlet from cotyledon culture	92
4.2.3.1. Somatic embryo formation	93
4.2.3.2. Plantlet regeneration from embryos formed	94
4.2.4. Transfer of plants to Soil	94
4.3. Histological observations of the cultured cotyledons	94
4.4. Genetic transformation study	95
4.4.1. <i>Agrobacterium</i> mediated transformation of Tea	95
4.4.1.1. <i>GUS</i> activity of putative transformed somatic embryos from cotyledons and embryo-derived plants	97
4.4.1.2. Molecular characterization of the transformed plants	97
4.4.1.3. Establishment of transformed plants	98
4.4.1.4. Transfer of transformed plants to Soil	98
4.4.2. Particle Bombardment- Mediated Transformation of tea	98
4.4.2.1. <i>GUS</i> activity of putative transformed embryogenic callus and embryo-derived plants	98
4.4.2.2. Molecular characterization of the transformed plants	99
4.4.2.3. Establishment of transformed plants	99

CONTENTS

4.4.2.4. Transfer of transformed plants to Soil	99
4.5. <i>Agrobacterium</i> mediated transformation of tea vs. Particle mediated bombardment of tea	100
5. Conclusion	101
6. References	103
7. Appendix	i

---