Contents

Summary i

Acknowledgement iv

Abbreviations v

Index of Figures vii

Index of Tables ix

1 Introduction 1

2 Materials and Methods 7

2.1 Media, reagents and chemicals 7

2.1.1 Media for fungi 7

2.1.2 Reagents and buffers for DNA extraction 8

2.1.3 Reagents and buffers for PCR 9

2.1.4 Media and reagents for cloning and sequencing 12

2.1.4.1 Media for propagation of *Escherichia coli* 12

2.1.4.2 Reagents for cloning and sequencing 13

2.1.5 Solution and buffers for agarose gel electrophoresis 14

2.1.6 Other chemicals and kits 15

2.1.7 Fungal isolates 16

2.2 Detection of *Ganoderma* in oil palm based on PCR 19

2.2.1 Preparation of oil palm root and fungal samples 19

2.2.2 DNA extraction methods 20

2.2.3 Determination of DNA concentration by optical density measures 21

2.2.4 Primer design and evaluation 22

2.2.4.1 Primer design and PCR conditions 22

2.2.4.2 Determination of detection limit of oil palm *Ganoderma* DNA and specificity of primers 23

2.3 Development of molecular diagnosis for identification and differentiation of oil palm *Ganoderma* against other *Ganoderma* species derived from laccase, ITS region, IGS1 region and Mn-SOD genes 24

2.3.1 DNA extraction 24

2.3.2 Primer design and PCR conditions 25

2.3.2.1 Laccase gene 25

2.3.2.2 ITS, IGS1 regions, Mn-SOD gene and restriction fragment length polymorphisms (RFLP) analyse 26

2.3.3 Cloning of PCR products 27

2.3.3.1 Cloning of laccase genes into plasmid pTZ19R 27

2.3.3.2 Cloning of ITS. IGS1 and Mn-SOD gene using the TOPO cloning kit 29

2.3.4 Sequencing of plasmid inserted with PCR product 30

2.3.5 Nucleotide sequence data analysis 31

3 Results **35**
3.1 Detection of oil palm *Ganoderma* 35
3.1.1 Primer designs from ITS region and determination of detection limits of the generated primers 35
3.1.2 Cross reaction test of saprophytic fungi by using three primer sets 37
3.1.3 Detection of *Ganoderma* DNA from naturally infected oil palm roots 40
3.2 Identification and differentiation of oil palm *Ganoderma* based on laccase genes 42
3.2.1 Primer design and PCR amplification of *Ganoderma* DNA using the primer pairs designed from the laccase genes 42
3.2.2 Characterization of the nucleotide sequences of PCR products of oil palm *Ganoderma* isolate BS amplified using primer pairs Lac 2a-Lac 2b, Lac 2a-Lac 2r and Lac 2f-Lac 2b 49
3.2.3 Evaluation of primer specificity of Lac 2a-Lac 2b in the PCR amplification of oil palm *Ganoderma* DNA 59
3.2.3.1 PCR amplifications using primer pair Lac 2a-Lac 2b with different concentrations of *Taq* polymerase and $MgCl_2$ 59
3.2.3.2 PCR Amplification of the DNA fragment of *Ganoderma* Lac 1700 (designation pU1700) using the primer pair Lac 2a-Lac 2b at different temperatures, concentrations of *Taq* polymerase and $MgCl_2$. 60
3.2.4 Primer specificity for oil palm *Ganoderma* designed from specific nucleotide sequence corresponding to conserved amino acid sequence of ILHCHI and introns of *Ganoderma* Lac 1700 fragment 61
3.3 Identification and differentiation of oil palm *Ganoderma* based on ITS, Mn-SOD and IGS1 genes 66
3.3.1 ITS region and ITS-RFLP analyses 66
3.3.1.1 ITS region sequences 66
3.3.1.2 Restriction fragment length polymorphism (RFLP) analysis for characterizing the PCR product of the ITS region of oil palm *Ganoderma* 66
3.3.2 Species-specific primer design for oil palm *Ganoderma* and PCR amplification 71
3.3.3 Specificity tests of the primer sets IT1-IT2 and IT1-IT3 for *Ganoderma* 76
3.3.4 Phylogenetic analysis of oil palm *Ganoderma* based on ITS1 and ITS2 regions 79
3.4 Identification of oil palm *Ganoderma* based on the Mn-SOD gene sequence 82
3.4.1 Primer design and PCR amplification 82
3.4.2 Phylogenetic analysis of oil palm *Ganoderma* 85
3.5 Identification and differentiation of oil palm *Ganoderma* based on the IGS1 region 89
3.5.1 IGS1 sequence and IGS1-RFLP analysis 89

3.5.1.1 Determination of the IGS1 region of 13 *Ganoderma* isolates 89
3.5.1.2 Nucleotide sequences of the IGS1 region from 13 *Ganoderma* isolates 89
3.5.2 IGS1-RFLP analyses for oil palm *Ganoderma* 96
3.5.3 Species-specific primer design for oil palm *Ganoderma* isolates 98
3.5.4 Phylogeny analysis of the IGS1 region 100

4 Discussion **103**
4.1 Detection of oilpalm *Ganoderma* 103
4.2 Identification and differentiation of oil palm *Ganoderma* 105

5 Perspectives **116**

6 Zusammenfassung **117**

7 References **120**

8 Appendixes **134**

Curriculum vitae

Declaration (in German)