**TABLE OF CONTENTS**

**INTRODUCTION** 1

1. Production of recombinant proteins in plants 2

2. Plant-made antibodies 4

3. Expression systems for plant-made-antibodies 5

3.1. Stable gene transformation 6

3.2. Transient gene expression 7

4. Considerations for antibody productions in plants 12

4.1. Glycosilation 12

4.2. Subcellular location 14

4.3. Plantibody degradation 15

4.4. Downstream processing 15

5. New strategies for recombinant antibody production based on different antibody formats 17

6. New strategies based on antibody cocktails 18

7. Pluribody Technology 22

8. References 25

Objectives 36

**CHAPTER 1 Production of Infliximab in plant: Comparative analysis of different antibody formats** 38

1. Introduction 39

2. Results 41

2.1. Construction of several Infliximab formats by GoldenBraid 41

2.2. Anti-human TNF-α antibodies production in N. benthamiana leaves and in tomato fruit 42

2.3. Functional comparison of the different anti- TNF-α formats 46

2.4. Comparison of the TNF-α neutralization activity of the different anti-TNF-α antibody formats 48

3. Discussion 49

4. Materials and methods 53

4.1. Cloning and assembly of DNA parts 53

4.2. Strains and growth conditions 53

4.3. Nicotiana benthamiana transient expression 53

4.4. Tomato stable transformation 54

4.5. Sample preparation 54

4.6. ELISA for the detection of human TNF-α binding activity and recombinant immunoglobulin determination 55

4.7. SDS-PAGE and Western blot analysis 55

4.8. Affinity chromatography purification 56

4.9. Human U937 cell viability 56

5. References 56

6. Supplementary material 61

6.1. Supplementary Table 1. Primer sequences for DNA constructs 61

**CHAPTER 2: Plants as expression system for manufacturing monoclonal antibody cocktail** 63

1. Introduction 64

2. Results 67

2.1. Construction of the three individual antibodies comprising zmapp with GoldenBraid 67

2.2. Transient expression in Nicotiana benthamiana of three individual antibodies and a three-antibodies cocktail against EVOB 69

3. Discussion 72

4. MATERIALS AND METHODS 75

4.1. Cloning and assembly of pieces 75

4.2. Strains and growth conditions 76

4.3. Nicotiana benthamiana transient expression 76

4.4. Sample preparation 76

4.5. Elisa for the detection of anti-glycoprotein of EBOV activity of individual and mAb mixture 77

4.6. SDS-PAGE and Western blot analysis 77

5. References 78

6. Supplementary material 82

6.1. Supplementary Table 1. Primer sequences for DNA constructs 82

**CHAPTER 3 Functional evaluation of plant-derived-mAb cocktail against a snake venom** 83

1. Introduction 84

2. Results and discussion 87

2.1. Production and purification of polyclonal plant-derived recombinant antibody in N. benthamiana leaves 87

2.2. Immunoreactivity of antivenoms 88

2.3. Toxic and enzymatic activities and their neutralization by antivenom 90

3. Discussion 94

4. Materials and methods 97

4.1. Infiltration of N. benthamiana leaves 97

4.2. Extraction and purification mAb mixture from plant leaf tissue 97

4.3. SDS-PAGE 98

4.4. ELISA 98

4.5. Determination of the immunoreactivity profile 99

4.6. Neutralization of hemorrhagic activity 100

4.7. Neutralization of intraperitoneal lethality activity 100

4.8. Neutralization of intravenous lethality activity **¡Error! Marcador no definido.**

4.9. Neutralization of phospholipase A2 (PLA2) activity 100

4.10. Neutralization of coagulant activity 101

4.11. Neutralization of protease activity 101

5. References 101

**General Discussion** 107

1. Recombinant plant-derived-mAb expression levels 108

2. Manufacturing plant-derived-mAb cocktail. 111

3. Final remarks 115

4. Refences 116

**Conclusions** 120