Table of contents

Introduction **18**

1. Plant-made antibodies. *19*

2. Plant-made antibodies in the context of oral passive immunization. *19*

3. Edible plant organs as a platform for production of antibodies and other mucosal therapeutics. *21*

4. Secretory IgA as a target molecule for oral passive immunotherapy. *22*

5. Practical considerations for antibody production in plants. *24*

5.1 Subcellular Localization *24*

5.2 Glycosylation *25*

5.3 Antibody Degradation *26*

6. Passive immunization against rotavirus in edible fruits as a proof of concept. *27*

7. References *30*

Objectives **35**

Chapter 1 ..... **37**

Neutralizing antibodies against rotavirus produced in transgenically labelled purple tomatoes. **37**

1. Introduction *38*

2. Results *40*

2.1 Design and selection of human IgA genes for expression in tomato fruits *40*

2.2 Transgenic fruits accumulate high levels of mAb *41*

2.3 Anti-VP8 activity is maintained in late ripening fruits in the form of Fab' fragments* *43*

2.4 Minimally processed tomato-based products show strong anti-VP8 activity* *45*

2.5 Minimally processed fruit samples show strong rotavirus neutralization activity *47*

2.6 Rosea1 and Delila transgenes can be used to confer identity preservation to IgA-expressing tomatoes *50*

3. Discussion *52*

4. Experimental procedures *57*

4.1 DNA constructs and vectors *57*

4.2 Tomato transformation, plant material and sample preparation *58*

4.3 VP8 rotavirus surface protein production* *58*

4.4 ELISAs for the detection of VP8 binding activity and recombinant immunoglobulin A determination 59*

4.5 SDS-PAGE and Western blot analysis 60

4.6 Protein SSL7 affinity purification 60

4.7 Neutralization assays 60

5. Acknowledgements 61

6. References 62

7. Supplementary material 65

Chapter 2 68

Evaluation of unintended effects in the composition of tomatoes expressing a human immunoglobulin A against Rotavirus. 68

1. Introduction 69

2. Results 72

2.1 Evaluation of IgA content in the fruit. 72

2.2 Identification of differentially expressed proteins in IgA-tomatoes by 2D-DIGE and LC-MSMS. 74

2.3 Metabolic profiling for the evaluation of unintended effects by UPLC-MS. 77

3. Discussion 85

4. Experimental Procedures 88

4.1 Plant material 88

4.2 Protein extraction, SDS-PAGE, Western blot and ELISA tests 88

4.3 2D DIGE analysis 89

4.4 Protein identification 89

4.5 UPLC-QTOF 90

4.6 Statistical data analysis 91

5. Acknowledgements 92

6. References 93

7. Supplementary material 96

Chapter 3 99

Combinatorial analysis of secretory Immunoglobulin A (sIgA) expression in plants 99

1. Introduction 100

2. Results 102

2.1 GoldenBraid-assisted multigene assembly of 16 versions of secretory IgA 102

2.2 Transient expression in Nicotiana benthamiana of 16 versions of sIgA against Rotavirus 104

2.3 Detailed characterization and purification of the HCα1-LCλ-JC-SCkdel combination ... *107*
3. Discussion ... *110*
4. Experimental Procedures ... *113*
4.1 Cloning and assembly of modular parts ... *113*
4.2 Strains and growth conditions ... *114*
4.3 Plant transient transformation ... *114*
4.4 Plant material and sample preparation ... *114*
4.5 VP8 Rotavirus surface protein production* ... *114*
4.6 ELISAs for the quantification and detection of VP8 binding activity of IgA and sIgA* *115*
4.7 SDS-PAGE and Western blot analysis ... *115*
4.8 SSL7 affinity purification ... *116*
5. Acknowledgments ... *116*
6. References ... *117*
7. Supplementary material ... *120*
General Discussion ... **121**
1. IgA expression levels ... *122*
2. Formulation and stability in the mucosa ... *124*
3. Safety considerations: Identity preservation and transgenic unintended effects. ... *125*
4. Production of sIgA in fruits. ... *126*
5. Final remarks ... *127*
6. References ... *129*
Conclusions ... **132**