Faculty of Agricultural Sciences
Institute of Crop Science
Section of Crop Physiology of Specialty Crops
University of Hohenheim

Prof. Dr. Jens Norbert Wünsche

Physiological and molecular mechanisms of fruitlet abscission in mango

Dissertation submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy
in Agricultural Science

by
Michael Helmut Hagemann
University of Hohenheim

2015

Acknowledgements

I thank Prof. Dr. Wünsche for making this study possible and for his trust in me. His door was always open for discussions and I highly appreciate his support on many different levels. I am also grateful to Prof. Dr. Asch and to Prof. Dr. Weber for reviewing this thesis.

It always was a friendly and nice working environment in our wing of the castle and I thank my colleagues for their valuable suggestions and plenty of technical help. Especially, I thank Dr. Winterhagen and Dr. Hegele for their guidance and long discussions on the topic and beyond. I also appreciate the support from the four B.Sc. students who partly contributed to this thesis, especially M.Sc. Kofler and his committed field work.

I am also grateful for my friends from the Uplands Program. The PhD-time was sometimes challenging but with mutual support we overcame all obstacles in Vietnam and in Germany.

Special thanks to my loving and lovely family – the importance of a secure, inspiring and always exciting childhood cannot be overrated and I am glad that this time is not about to end. I thank my wife Sarah for her support and sacrifices. Thank you for your motivation, encouragement and for your love.

This thesis was accepted as a doctoral dissertation in fulfillment of the requirement for the degree of "Doktor der Agrarwissenschaften" (Dr. sc. agr.) at the Faculty of Agricultural Science at the University of Hohenheim on 19 October 2015.

Date of oral examination: 19 October 2015

Examination committee

Vice-Dean and Head of the Examination Committee:	Prof. Dr. H. Grethe
Supervisor and reviewer:	Prof. Dr. J. N. Wünsche
Co-reviewer:	Prof. Dr. F. Asch
Additional examiner:	Prof. Dr. G. Weber

Table of contents

Acknowledgements ..... **II**

Table of contents ..... **III**

List of figures ..... **VII**

List of tables ..... **XIII**

List of abbreviations ..... **XIV**

1. Introduction **1**

1.1 Mango 1

1.1.1 Origin and botany 1

1.1.2 Production and cultivation practices 2

1.1.3 Specific features of the study area 3

1.2 General description of premature fruit drop in mango 4

1.2.1 Patterns and intensity 4

1.2.2 Possible causes and prevention 6

1.3 Mechanism of fruit abscission 9

1.3.1 Morphological changes 9

1.3.2 Hormonal and molecular control 9

1.4 Ethylene perception 14

1.5 Objectives and hypothesis 15

1.6 Publications 16

2. A new approach for analyzing and interpreting data on fruit drop in mango **17**

2.1 Abstract 17

2.2 Introduction 18

2.3 Material and methods 21

2.3.1 Plant material and experimental sites. 21

2.3.2 Environmental parameters. 21

2.3.3 Experimental design and treatments. 22

2.3.4 Assessment of fruit drop. 23

2.3.5 Statistical analysis. 24

2.4 Results 26

2.4.1 Assessment of fruit drop. 26

2.4.2 Cropping system evaluation. 29

2.4.3 Irrigation. 29

2.4.4 PGR application. 30

2.5 Discussion 31

2.5.1 Fruit drop ... 31
2.5.2 Climatic factors. ... 34
2.5.3 Cropping system ... 34
2.5.4 Irrigation ... 34
2.5.5 PGR. ... 35
2.6 Conclusion ... 36
2.7 Notes ... 36
2.8 Literature cited ... 37
3. Ethephon induced abscission of mango fruitlets − physiological fruit pedicel response ... 41
3.1 Abstract ... 41
3.2 Introduction ... 41
3.3 Material and methods ... 43
3.3.1 Plant material ... 43
3.3.2 Fruit and climate measurements ... 43
3.3.3 Leaf net carbon exchange rate ... 43
3.3.4 RNA extraction and gene expression analysis by quantitative real-time PCR ... 43
3.3.5 Statistical analysis ... 44
3.4 Results ... 45
3.4.1 Environmental conditions during natural fruit drop ... 45
3.4.2 Leaf net carbon exchange rate ... 46
3.4.3 Effectiveness of ethephon as fruit drop inducer ... 46
3.4.4 Ethylene receptors and their response to ethephon ... 47
3.4.5 Fruit detachment force ... 48
3.5 Discussion ... 49
3.6 Conclusion ... 50
3.7 Acknowledgements ... 51
3.8 References ... 51
4. Ethephon induced abscission in mango: physiological fruitlet responses ... 53
4.1 Abstract ... 53
4.2 Introduction ... 53
4.3 Materials and methods ... 56
4.3.1 Plant material and experimental site ... 56
4.3.2 Treatments and experimental design ... 56
4.3.3 Fruitlet drop assessment and sampling ... 56
4.3.4 Gene analysis ... 57
4.3.5 Analysis of soluble carbohydrates ... 59
4.3.6 Polar auxin transport assay ... 60

4.3.7 Statistical analysis 60
4.4 Results 61
4.4.1 Ethephon induced fruitlet abscission 61
4.4.2 Expression of ethylene receptors in the pedicel 62
4.4.3 Expression of ethylene receptors in the fruitlet pericarp 64
4.4.4 Polar auxin transport capacity 65
4.4.5 Analysis of soluble carbohydrates 66
4.5 Discussion 67
4.6 Acknowledgement 72
4.7 Supplementary material 72
4.7.1 Supplementary figures 72
4.7.2 Supplementary tables 77
4.8 References 77

5. Expression and dimerisation of the mango ethylene receptor MiETR1 and different receptor versions of MiERS1 81

5.1 Abstract 81
5.2 Introduction 81
5.3 Material and methods 83
5.3.1 Plant material 83
5.3.2 Sequence isolation and characterisation 84
5.3.3 Gene expression studies by quantitative real-time PCR 84
5.3.4 Transient expression in *Nicotiana benthamiana* 85
5.4 Results and discussion 86
5.4.1 Sequence isolation and characterisation 86
5.4.2 Presence of MiERS1 versions in different mango cultivars 89
5.4.3 Expression analysis by quantitative real-time PCR 89
5.4.4 Mango ethylene receptor localisation in the plant cell 92
5.4.5 Co-localisation of mango ethylene receptors 94
5.4.6 Dimerisation of MiERS1 and the shorter MiERS1 versions 96
5.4.7 Dimerisation analysis by bi-molecular fluorescence complementation (BiFC) assay 98
5.4.8 Summary and conclusion 99
5.5 Acknowledgements 100
5.6 Supplementary material 100
5.6.1 Supplementary tables 101
5.6.2 Supplementary figures 103
5.7 References 107

6. General discussion 111

6.1 The molecular and physiological basis of fruitlet abscission 111

6.2 Linking the theoretical understanding to practical solutions 116
6.3 Does it help to explain fruit drop patterns? 118
6.4 Recommendations for mango production 120
6.4.1 General recommendations 120
6.4.2 Recommendations specific for the study area 121
6.5 Conclusion and outlook 122
7. General references 123
8. Summary 132
9. Zusammenfassung 134
10. Author's declaration 136
11. Curriculum vitae 137

List of figures

Fig. 1.1. Photograph series of the fruit of the cultivar 'Hôi'. (A) ripe fruit, (B) fruit cut open exhibiting the yellow mesocarp and seed, (C) seed and (D) embryo after removal of the endocarp and testa. The several ventrodistally located embryos are characteristic for polyembryonic cultivars (Arndt, 1935)..... 4

Fig. 1.2. Different ways of presenting fruit drop in mango using the example of the cultivar 'Tommy Atkins'. Graph was reproduced and modified based on Nuñez-Elisea and Davenport (1983). Data are presented as (1) the decrease of fruited panicles (straight line, black dot), (2) the decrease of fruit retention related to fruited panicles (dashed line, open dot), (3) the decrease of fruit retention based on total initial panicles (straight line, open dot), (4) and the rate of fruit drop per counting interval (dotted line, star). 5

Fig. 1.3. Scheme of auxin flux in a fruit-bearing panicle of mango. The seed is considered as the main source of auxin. Fruitlet 1: Seed degeneration leads to a decreased auxin efflux. This enhances the sensitivity of the abscission zone (AZ) to ethylene (Roberts et al., 2002) and can possibly also induce the ethylene biosynthesis (Abel et al., 1995), which subsequently induces fruitlet abscission. Fruitlet 2: A fruitlet with a healthy seed is able to maintain a sufficient auxin gradient that decreases the ethylene-sensitivity at the AZ, consequently the fruit persists. Fruitlet 3: The strong auxin efflux from a further developed neighboring fruit leads to the accumulation of auxin in the pedicel. This possibly triggers abscission either by the auxin transport autoinhibition as proposed by Bangerth (1989) or through inducing the ethylene biosynthesis (Abel et al., 1995). Fruitlet 4: The fruitlet has sufficient auxin efflux and persists at the panicle. 10

Fig. 1.4. Model for fruitlet abscission modified after Xie et al. (2011) considering Chen et al. (2005) and Dal Cin et al. (2007, 2009). Ethylene is catalyzed from methionine by the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and the ACC oxidase (ACO). The ACS activity can be modulated by auxin, gibberellins (GA), abscisic acid (ABA), polyamines (PA) or others. In the absence of ethylene the ethylene receptors maintain the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) protein kinase in an active state. The active CTR1 inhibits the ETHYLEN INSENSITIVE-2 (EIN2) signal protein. Ethylene perception leads to a conformational change that inactivate CTR1, which in turn induced the EIN2 transcription factors. EIN2 further induces EIN3 and EIN3-like (EIL), which than induce the activity of transcription factors of the ETHYLENE RESPONSE FACTOR (ERF) family in the nucleus. The ERFs lead to the upregulation of genes of the ethylene signaling, which promotes the ethylene response, while the auxin signaling is downregulated. In the following, the ERFs induce cell-wall degrading enzymes, which finally leads to the detachment of the fruitlet and subsequently to the drop of the fruitlet.... 13

Fig. 2.1. (A) Average fruit retention per panicle (FPP) of 'Hôi' (closed symbols) and 'Tròn' (open symbols) in days after full bloom (DAFB). Each symbol refers to years between 2007 and 2012 and is based on actual fruit counts per panicle. Average fruit retention over that period is also modeled with a sigmoid function (r^2 = 0.85). y_0 is the final fruit retention, whereas y_0+a determines the upper limit of FPP. (B) Slope of the simulated fruit drop curve and the calculated daily rate of fruit drop FD(x). Black arrows indicate x_0, FD_{max}, and FD_{mst}, corresponding with the highest daily fruit losses in absolute terms, the highest FD(x), and the cessation of midseason fruit drop, respectively. 27

Fig. 2.2. Daily rate of fruit drop in six consecutive growing seasons. Gray lines indicate the fruit drop waves by cultivar 'Hôi'. 29

Fig. 3.1. Average maximum and minimum temperature during the flowering and early fruit development period in 2010. Black dots indicate fruit counts of control treatments starting one week after full bloom (7 February 2010). Error bars indicate SEM. 45

Fig. 3.2. Net carbon exchange rate (NCER) and stomatal conductance (SC) at four temperatures during flowering in 2010. 46

Fig. 3.3. Fruit retention during early fruit development in 2010 with application of Ethephon and control treatments at an early (arrow 1) and a late application date (arrow 2). Error bars represent SEM..... 47

Fig. 3.4. Alignment of partial ETR1 and ERS1-like protein sequences with indicated homology relative to the Arabidopsis sequence..... 48

Fig. 3.5. Results of FDF measurements from the first experiment. Average FDF is shown at 2 days before and 1, 3 and 5 days after application of Ethephon and control treatments. 49

Fig. 4.1. The effect of the ethephon treatment 600 ppm (ET600) or 7200 ppm (ET7200) spray applications on average (A) fruitlet retention, (B) fruitlet detachment force of fruitlets detaching at the abscission zone or along the pedicel, (C) percentage of fruitlet detachment at the abscission zone (the remainder to 100% are fruitlets detaching along the pedicel) and (D) seed degeneration in comparison to the control and two days prior to treatment. (A) Horizontal black bar indicates time until 95% of the fruits have abscised in response to ET7200. (B) Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. 62

Fig. 4.2. Expression of the ethylene receptors **(A)** *MiETR1* and **(B)** *MiERS1* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a,b or c). Error bars show standard deviation..... 63

Fig. 4.3. Detection of transcription of the ethylene receptor versions (A) *MiERS1m* and (B) *MiERS1s* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. 64

Fig. 4.4. Expression of the ethylene receptors (A) *MiETR1*and (B) *MiERS1* in the pericarp of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation..... 65

Fig. 4.5. Polar auxin transport (PAT) capacity through the pedicel of pea sized fruitlets. Detection of [^{3}H]-IAA in the receiver block in response to the ethephon treatment with 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation; dpm = disintegrations per minute. [1]sample size (n=3) was too small to perform a statistical test..... 66

Fig. 4.6. Sucrose concentration of pea sized fruitlets after the ethephon treatment 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. 67

Fig. 4.7. Overview of the key fruitlet abscission parameters analyzed in this study. Parameters of ethephon treated fruitlets compared to those of control fruitlets: no significant differences are indicated by a dot, whereas up- or downward pointing arrows indicate significant differences. Different or equal response of the ethephon treatments 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control are indicated by orange and cyan colored symbols, respectively. The parameters are: fruitlet detachment force (FDF), gene expression of the ethylene receptors *MiERS1* and *MiETR1* and their ratios in the pedicel and fruit pericarp, polar auxin transport (PAT), and the concentration of sucrose in the fruit pericarp. 71

Fig. 4.S1. The effect of ethephon treatment 7200 ppm (ET7200) on average (A) fruitlet detachment force of fruitlets detaching at the abscission zone or along the pedicel and (B) percentage of fruitlet detachment at the abscission zone (the remainder to 100% are fruitlets detaching along the pedicel) in comparison to the control and one day prior to treatment. Error bars show standard deviation. Data from 2011. 72

Fig. 4.S2. Expression of the ethylene receptors (A) *MiETR1* and (B) *MiERS1* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 7200 ppm (ET7200) in comparison to the control and one day prior to treatment. Homogeneous subgroups with no

significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2011. 73

Fig. 4.S3. Expression of short versions of the *MiERS1*. (A) *MiERS1m* and (B) *MiERS1s* in the pedicel of pea sized mango fruitlets in response to the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. No statistical test possible due to highly variable sample size. Missing error bar indicates n=1. Data from 2012. 74

Fig. 4.S4. Expression analysis of (A) *MiETR1* and (B) *MiERS1* in the pericarp of pea sized fruitlets in response to the ethephon treatment 7200 ppm (ET7200) in comparison to the control and one day prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2011. 75

Fig. 4.S5. Accumulated [^{3}H]-IAA in the receiver after the ethephon treatment 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation; dpm = disintegrations per minute. Data from 2011. 76

Fig. 4.S6. Concentrations of fructose and glucose of pea sized fruitlets after the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2012. 76

Fig. 5.1. *MiERS1* is expressed in three versions. (A) Structure and domains of the isolated *MiERS1* versions. Domains are boxed in grey, broken line indicate gap in sequence, SNPs and their positions are indicated. Numbers indicate position or sequence length in nucleotides (nt) or amino acids (aa). TM: transmembrane domains, GAF: GAF domain, K: histidine kinase domain, ATP: ATP-binding domain. (B) Alignment of translated cDNA sequences. Numbers indicate sequence length in amino acids. (C) Genomic sequence structure of *MiERS1* versions. Boxes indicate exons and thin lines indicate introns, numbers indicate size of introns in nucleotides. 87

Fig. 5.2. Expression analysis by qPCR of *MiETR1* and the *MiERS1* versions. Means of the level of receptor transcripts in different mango organs and developmental stages relative to a linker sample. *MiETR1* (A) and *MiERS1* (B) are present in 100% of the samples; occurrence of *MiERS1m* (C) and *MiERS1s* (D) in all samples is indicated as percent in relation to tested cDNAs. Expression values of *MiERS1m* and *MiERS1s* refer to samples with detectable expression. Bars indicate standard deviation, except for (C) *MiERS1m*, marble pedicel due to sample size n = 1. 90

Fig. 5.3. Analysis of localisation of MiETR1 and the MiERS1 versions by transient expression in infiltrated *N. benthamiana* leaves. Co-expression of MiETR1 (A) and MiERS1 version YFP-

fusion constructs (D, G, J, yellow) with the ER-marker mCherry-fusion construct (B, E, H, K, red); overlay images of YFP and mCherry channels (C, F, I, L); ER-marker expression as a control without the co-infiltration of receptor constructs (M, N, O). Scale bar 20 µm..... 94

Fig. 5.4. Co-localisation analysis of MiETR1 and the MiERS1 versions by transient expression in infiltrated *N. benthamiana* leaves. Co-expression of MiERS1 versions fused to YFP (A, D, G, yellow) or CFP (B, E, H, blue); Co-expression of MiETR1 fused to YFP (J, M, P, yellow) and MiERS1 versions fused to CFP (K, N, Q, blue); overlay images of YFP and CFP channels (C, F, I, L, O, R). Scale bar 20 µm..... 96

Fig. 5.5. Dimerisation analysis of the MiERS1 versions by transient expression assay in infiltrated *N. benthamiana* leaves. Co-expression of MiERS1 versions and dTM-MiERS1 versions fused to YFP (yellow) and CFP (blue), respectively. Co-expression of the ER-marker fused to mCherry (red). Inset and arrows indicate nuclei displaying specific fluorescence (CFP column, blue); arrowheads indicate diffuse distribution of dTM-MiERS1s (CFP column, blue). dTM: devoid of transmembrane domains, scale bar 20 µm..... 98

Fig. 5.6. Dimerisation analysis of MiETR1 and MiERS1 versions by BiFC assay in infiltrated *N. benthamiana* leaves. Co-expression of MiETR1 and MiERS1 versions fused to splitYFP. dTM: devoid of transmembrane domain, n/cYFP: n- or c-terminal part of splitYFP, scale bar 20 µm. 99

Fig. 5.S1. Scheme of receptor constructs and dimerisation models for transient expression analysis in *N. benthamiana* leaves. (A) YFP fusion-protein to determine cellular localisation, (B) co-localisation (or interaction) of receptors fused to YFP or CFP, (C) dimerisation of YFP and CFP fusion-proteins, (D) BiFC assay with splitYFP-constructs. TM: transmembrane domain (grey) as anchor in the ER membrane, GAF: GAF domain (blue box) for receptor dimerisation, orange: ER membrane, YFP: yellow fluorophore, CFP: blue fluorophore.... 103

Fig. 5.S2. Discrimination of unspecific fluorescence. Autofluorescence signal of necrotic cells detected with the (A) YFP channel, (B) CFP channel, (C) mCherry channel, (D) displayed as overlay image. To guarantee the detection and identification of specific YFP, CFP, and mCherry fluorescence signals, each specimen was checked with the DAPI filter set. With the DAPI filter set specific fluorophore signals are not visible, but autofluorescence of e.g. necrotic cells is bright and clearly visible, which enables the discrimination of specimen with specific fluorophore signals from those with unspecific autofluorescence. Only specimen with specific fluorescence signals were considered for analysis. 104

Fig. 5.S3. Analysis of MiERS1 versions with the TMHMM software tool of translated sequences. Predicted transmembrane domains and orientation within the ER membrane of (A) MiERS1, (B) MiERS1m, (C) MiERS1s. 105

Fig. 5.S4. Presence of *MiERS1s* in genomic DNA of different mango cultivars and comparison to the Hôi *MiERS1s*. (A) Alignment of *MiERS1s* sequence fragments obtained by *MiERS1s*-specific PCR. (B) Schematic display of sequences: boxes indicate areas of significant similarity. Notice the fragment of the additional *MiERS1s* version of 'Mahachanok' revealing gaps in the sequence (indicated by dashes). 107

Fig. 6.1. Hypothetical model for fruitlet abscission in mango based on Botton et al. (2011). During the midseason drop stage fruitlet abscission is mainly a result of inter-organ competition at the tree level for the available resources, mainly assimilates. Newly developing plant organs such as inflorescences, young leaves or shoots are stronger sinks compared to fruitlets. Thus the resources go to the superior sinks which induced nutritional stress in weak sinks. This stress is perceived as sugar starvation at the pericarp level and leads to growth inhibition and activates the ethylene signal cascade. At the seed level both factors, ethylene induced gene regulation and sugar starvation inhibit seed development. Thus the seed is the main source of auxin, the abortion of the seed leads to a lower auxin efflux subsequently to a lower sink signal. At the pedicel level this low auxin efflux increases the sensitivity of the abscission zone (AZ) to ethylene. The abscission process then is activated by ethylene which finally leads to fruitlet drop. 112

Fig. 6.2. Structural difference between the ethylene receptors of mango. All four receptors are anchored in the membrane of the endoplasmic reticulum (ER). The ethylene binding site is located near the N-terminal end (N) within the three conserved transmembrane domains (TM, blue). The GAF-domain (GAF, green) and the HIS-kinase domain (K, purple) are present only in the MiETR1, MiERS1 and the MiERS1m, while the MiERS1m is missing the ATP binding site (ATP, purple). The receiver domain (R; orange) is only present in the MiETR1. The inset shows the possible alternative orientation of the MiERS1 that had a higher probability based on informatical sequence analysis (Chapter 5)..... 115

Fig. 6.3. Photograph of a mango seed or stone weevil (*Sternochetus mangiferae* Fabricius) taken in Tú Nang village (District Yên Châu, Vietnam, 2012). Scale bar = 1 mm. 121

List of tables

Table 2.1. Average daily maximum and minimum ambient temperatures and relative humidity, respectively, from 1 week before bloom to midseason in six consecutive growing seasons. [z] 22

Table 2.2. Overview of experimental characteristics in each of six growing seasons..... 22

Table 2.3. Fruit size dependent time of spray application and concentration of each plant growth regulator. 23

Table 2.4. Literature citations with reference to the termination of post-bloom and mid-season drop. 25

Table 2.5. Fruit per panicle (FPP) at days after full bloom (DAFB) for each growing season and cultivar. 28

Table 2.6. Effect of mango monocropping and mango-maize intercropping systems on number of panicles per tree and number of fruits per panicle in 2012. 29

Table 2.7. Effect of irrigation on fruit retention of mango for the growing seasons 2008 and 2009. 30

Table 2.8. Effect of PGR application for the growing seasons 2008 and 2009. 31

Table 2.9. Correlation coefficients between fruit retention data at the end of post-bloom or mid-season drop with harvest. 33

Table 4.1. Primers specific for mango genes used for quantitative real-time PCR analysis. *β-ACTIN* (*MiACT*), *UBIQUITIN* (*MiUBI*), *α-TUBULIN* (*MiTUB*), *ETHYLENE RESISTANT 1* (*MiETR1*), *ETHYLENE RESPONSE SENSOR 1* (*MiERS1*), and the two *MiERS1* versions *MiERS1m* and *MiERS1s*. (*Mangifera indica* abbreviated as *Mi*). 58

Table 4.S1. Ratio of the ethylene receptors *MiERS1* and *MiETR1* after the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control. Data from 2012. ... 77

Table 5.S1. List of primers for reference gene evaluation and qPCR analysis. 101

Table 5.S2. List of primers for construct cloning. For gateway cloning of fusion protein constructs the attB-recognition site was added as appropriate. 101

Table 5.S3. List of fluorescence fusion protein constructs used for transient expression experiments in *N. benthamiana*. dTM: devoid of transmembrane domain. 102

List of abbreviations[1]

1-MCP	1‑methylcyclopropene
aa	amino acids
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
ACO	ACC oxidases
ACS	ACC synthase
ACT	β-actin
ANOVA	analysis of variance
At	*Arabidopsis thaliana*
ATP	Adenosin triphosphate
AZ	abscission zone
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
BiFC	Bi-molecular fluorescence complementation
cDNA	complementary desoxyribonucleic acid
CFP	cyan fluorescent protein
CO_2	carbon dioxide
CPPU	N-(2-chloro-4-pyridyl)-N-phenylurea
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
DAFB	days after full bloom
DAT	days after treatment
dpm	disintegrations per minute
dTM	devoid of the transmembrane domain
EIL	EIN3-like
EIN2	ETHYLEN INSENSITIVE-2
EIN3	ETHYLEN INSENSITIVE-3
ER	endoplasmic reticulum
ERF	ETHYLENE RESPONSE FACTOR
ERS1	ETHYLENE RESPONSE SENSOR 1
ET	ethephon treatment
ET600	ethephon treatment 600 ppm
ET7200	ethephon treatment 7200 ppm
ETR1	ETHYLENE RESISTANT 1
FAO	Food and Agriculture Organization
FD(x)	daily rate of fruit drop

[1] Standard abbreviation of units following the International Sytem of Units (SI) are not listed.

FDF	fruit/fruitlet detachment force
FD_{max}	maximum value of FD(x)
FD_{mst}	FD(x) midseason termination
FPP	fruit per panicle
FRET	fluorescence resonance energy transfer
GA	gibberellin
GA_3	gibberellic acid 3
GA_{4+7}	formulation congaing gibberellic acid 4 and 7
GAF	cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA
HPLC	high performance liquid chromatography
IAA	indole-3-acetic acid
log_{10}	decadic logarithm
LSD	Fisher's least significant difference
MES	2-(N-morpholino) ethanesulfonic acid
Mi	*Mangifera indica*
NA	not applicable
NAA	1-naphtalene-acetic acid
NCER	net carbon exchange rate
nt	nucleotides
PA	polyamines
PAT	polar auxin transport
PCR	polymerase chain reaction
PGR	plant growth regulator
ppm	part per million
PVPP	polyvinylpolypyrrolidone
qPCR	quantitative real-time PCR
r^2	correlation coefficient
rcf	relative centrifugal force
RNA	ribonucleic acid
ROS	reactive oxygen species
RTE1	REVERSION-TO-ETHYLENE SENSITIVITY1
SI	International System of Units (French: Système International d'Unités)
SNPs	single nucleotide polymorphisms
TM	transmembrane domains
TUB	α‑tubulin
UBI	ubiquitin
YFP	yellow fluorescent protein

1. Introduction

1.1 Mango

1.1.1 Origin and botany

The common mango, *Mangifera indica* L., belongs to the family of Anacardiaceae with most species growing in the tropical and subtropical climate zones. Early Paleocene fossils of members of the genus *Mangifera* have been found in North India, others in Thailand or elsewhere in Southeast Asia, raising the question of its true origin (Mehrotra et al., 1998; Sawangchote et al., 2009; Bompard, 2009). Diversification hot spots of wild mango, however, are to be found in North India and Southeast Asia. The domestication for several millennia in the two regions is likely to be the reason for differences between the "Indian type" mostly monoembryonic and the "Southeast Asian type" consisting of only polyembryonic cultivars (Bompard, 2009). The monoembryonic cultivars evolved in a subtropical monsoonal climate with longer drought periods and higher temperature extremes, while the Southeast Asian polyembryonic cultivars evolved in a tropical or subtropical climate with shorter or no dry seasons (Mukherjee, 1972). Probably as a result of adaptation and breeding, the two mango types have developed fruits of different colors and have different levels of tolerance towards cold temperatures or adverse soil properties (Dinesh et al., 2015; Whiley et al., 1989; Schaffer et al., 2009).

A mango tree produces several flushes of vegetative growth year-round (Davenport, 2009), while flushes of generative growth, i.e. flowering flushes, require the interaction of internal and external factors. Flowering can be induced after an extended period of growth cessation, by periods of drought and/or by low temperatures (3-10°C). Various cultivation practices, for example pruning or nitrogen fertilization, can further be used to improve flowering (Davenport, 2007). Consequently, if no cultivation practices are applied, flowering of mangos is erratic in tropical areas where the prevailing climate is consistent year-round. In the subtropical regions with pronounced dry-seasons and low temperatures mango flowering is abundant and commences typically in the middle of the dry season (Davenport, 2009). The inflorescence of mango is a terminal pyramidal structured panicle, bearing several hundreds of male and perfect (polygamous) flowers (Mukherjee and Litz, 2009). Of these flowers only a few have the ability to develop into fruits, as unsuccessful pollination, embryo abortion, or diseases and pests limit the final number of fruits reaching maturity. Typically only one or no fruit per panicle reach

harvest maturity (Singh et al., 2005). The mango fruit is a drupe that, depending on the cultivar, can reach a length of 30 cm at maturity (Mukherjee and Litz, 2009). The fleshy mesocarp can be consumed unripe, but the flavor and sweetness will only be fully attained in ripe fruits (Brecht and Yahia, 2009). The taste and high nutritional value of mangos might be reasons for their long cultivation history and their great economic importance today (Mukherjee and Litz, 2009).

1.1.2 Production and cultivation practices

With 35 million tons produced in 2010 (FAOSTAT, 2010) mango is the fifth most commonly grown fruit in the tropics and subtropics, ranking only behind banana, watermelon, orange and coconut. The *Food and Agriculture Organization* (FAO) lists 99 mango producing countries, disregarding countries that only produce small quantities, with the nine top producing countries accounting for over four fifths of the global production (Galán Saúco, 2015). The 10-year average (2000 – 2010; FAOSTAT , 2010) shows that India is the main producer of mangos with 12 million tons per year, which accounted for 40% of the global production in 2010, followed by China (13%), Thailand (7%) and Mexico (5%). Interestingly not India, but Mexico is the top mango exporting country, because unlike Mexico India, and also China, produce mainly for their domestic markets (FAOSTAT, 2010; Gunjate, 2009). International trade of mangos is on the increase, especially from the tropical and subtropical regions to temperate regions, i.e. Europe and North America. These markets could be further exploited if the demands regarding fruit quality are met by improving product chains and by the installation of export infrastructures (Galán Saúco, 2015). However, good post-harvest management and high quality source material at the orchard level are also necessary to produce exportable fresh fruit (Galán Saúco, 2004; Gunjate, 2009; Sivakumar et al., 2011).

The yield of mangos fluctuates globally at around 7.5 t ha^{-1}. Yields in industrialized countries are high compared to those in less developed countries. For example yields in the USA and Israel range between 12.5–20.5 t ha^{-1} respectively, while in India they average 6.5 t ha^{-1} (FAOSTAT, 2010). This difference shows that there is a potential for improvement in global mango production, which can be exploited by investing in advanced technologies, especially those involved in post-harvest and orchard management (Galán Saúco, 1997; Galán Saúco, 2004; Gunjate, 2009; Sivakumar et al., 2011).

Mango cultivation requires a well-balanced fertilization strategy (Coelho and Borges, 2004; Galán Saúco, 1997) and pruning to increase light interception (Schaffer and Gaye, 1989) and to reduce humid conditions that promote fungal growth (Arauz, 2000). The combination of fertilization and pruning can also be used to improve synchronization of flowering or to induce off-season production (Davenport, 2007). Another major concern for mango cultivation is that in regions with less than 700 mm of annual precipitation mango production is limited due to insufficient water supply (Galán Saúco, 1997). Especially if precipitation is below 100 mm per month during the dry season, which coincides with the time of early fruit development, plant-stress can be induced. This can leads to yields which are significantly reduced compared to yields of irrigated orchards (Coelho and Borges, 2004; Galán Saúco, 1997).

1.1.3 Specific features of the study area

The prevailing climate in the study region (Yên Châu, Province Sơn La, Vietnam) is characterized by prevailing high temperatures and a long dry season from October to March or April, while the flowering of mango commences already in February (Huong, 2010; Roemer et al., 2011). The yields in the study area have been reported to be lower than the national average of about 8 t ha^{-1} (FAOSTAT, 2010; Huong, 2010). Generally mango production in Vietnam tripled over the last decade (2000 to 2010) to nearly 600 thousand tons (FAOSTAT, 2010). This was achieved mainly in the most productive areas, the lowlands of the Southeast and the Mekong Delta (IFPRI, 2002). In contrast, mango production in the mountainous North of Vietnam is characterized by low-productive home gardening for self-consumption and for being sold in domestic markets (IFPRI, 2002; Trinh et al., 2003). This is likely the result of limited investments and poor management of mango orchards in this area (Huong, 2010; Trinh et al., 2003). However, some local farmers in the study area have started to shift from extensive home gardening to commercially targeted production systems (Huong, 2004; Huong, 2010). Two polyembryonic cultivars are predominantly grown in the study area, the cultivar ‘Hôi’ (Fig.1) and ‘Tròn’ (Yên Châu district, statistic department). The introduction of pruning and pest management practices led to first improvements of mango production in Yên Châu (Huong, 2004). Nevertheless, premature fruit drop has been identified as one of the most severe yield limiting factors for mango production in the study area (Huong, 2010; Roemer et al., 2011).

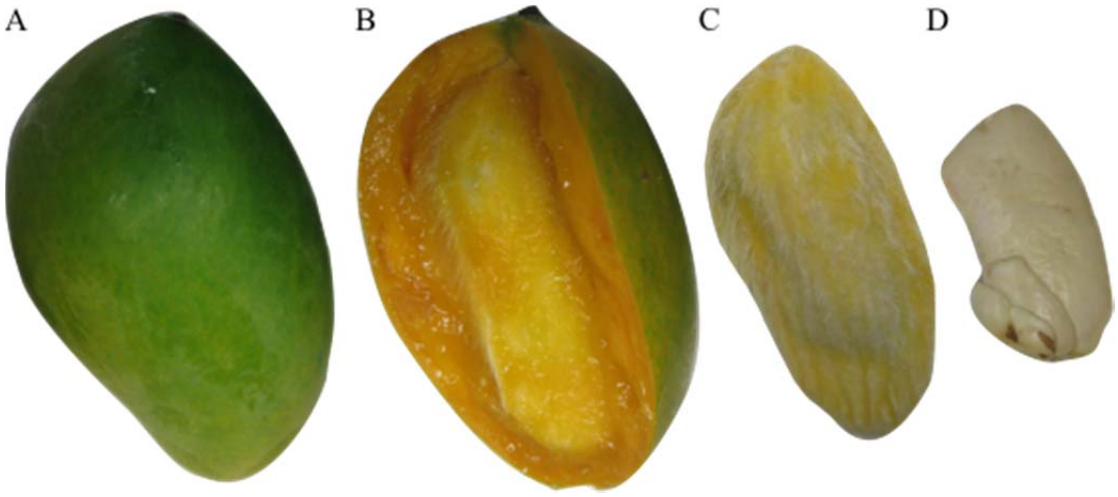


Fig. 1.1. Photograph series of the fruit of the cultivar 'Hôi'. (A) ripe fruit, (B) fruit cut open exhibiting the yellow mesocarp and seed, (C) seed and (D) embryo after removal of the endocarp and testa. The several ventrodistally located embryos are characteristic for polyembryonic cultivars (Arndt, 1935).

1.2 General description of premature fruit drop in mango

Premature fruit drop is a phenomenon that can be observed in several fruit bearing plant species. It has been interpreted as an evolutionary strategy of plants to increase the chances of successful reproduction by matching the crop load to the plant resources (Estornell et al., 2013). In many mango producing regions this self-regulatory mechanism results in limited and thus uneconomic fruit production (Singh et al., 2005).

1.2.1 Patterns and intensity

Due to the impact of premature fruit drop on mango production, several studies have been dedicated to this topic. Among these studies several different approaches have been followed. Four approaches are shown using the example of the mango cultivar 'Tommy Atkins' (Fig. 1.2) based on the study of Nuñez-Elisea and Davenport (1983). Typically, fruit drop is presented as either fruit retention per panicle (Nuñez-Elisea and Davenport, 1983; Roemer et al., 2011) or as decrease of fruit retention per time interval, i.e. rate of fruit drop (Prakash and Ram, 1984; Ram, 1992; Singh and Arora, 1965; Malik et al., 2003). It is important to note that from fruit set to harvest not only the number of fruit per panicle declines but also the number of fruited panicles (fruit bearing panicles) (Fig. 1.2). Consequently, the presentation of fruit drop as "fruit per fruiting panicle" can be misleading if the number of panicles is not provided. Hence,

data should be presented as “fruit per total initial panicles” (Nuñez-Elisea and Davenport, 1983).

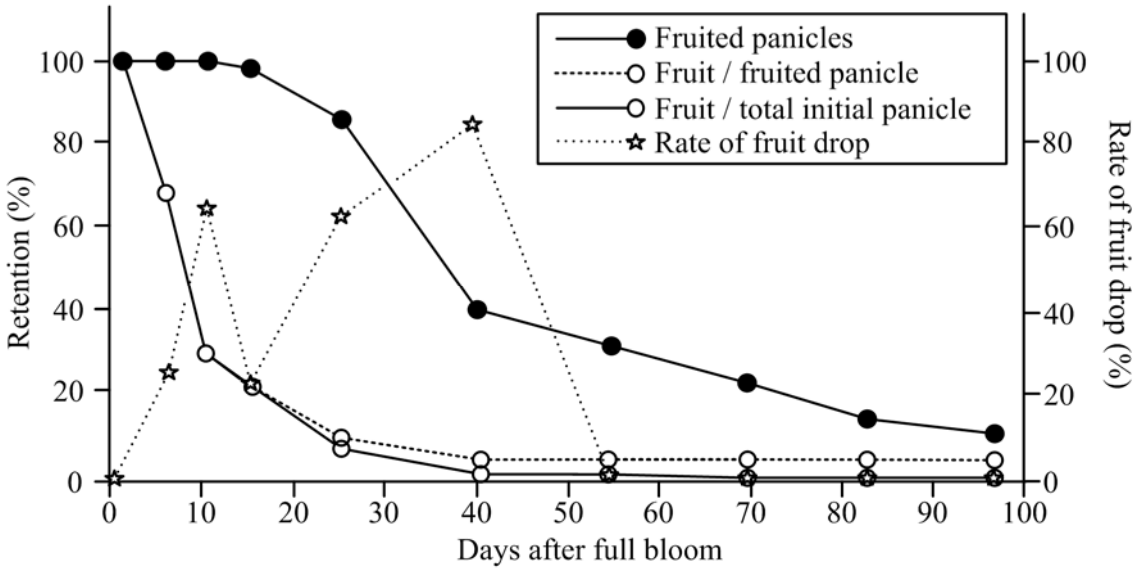


Fig. 1.2. Different ways of presenting fruit drop in mango using the example of the cultivar ‘Tommy Atkins’. Graph was reproduced and modified based on Nuñez-Elisea and Davenport (1983). Data are presented as (1) the decrease of fruited panicles (straight line, black dot), (2) the decrease of fruit retention related to fruited panicles (dashed line, open dot), (3) the decrease of fruit retention based on total initial panicles (straight line, open dot), (4) and the rate of fruit drop per counting interval (dotted line, star).

Presenting rates of fruit drop rather than fruit retention highlights the time periods of high fruit loss. Hence this approach was used to distinguish specific fruit drop stages or waves. Prakash and Ram (1984) for example distinguish between two main waves of fruit drop followed by a phase of negligible fruit loss. Other authors found different patterns (Singh et al., 2005). Consequently a comparison of the fruit drop pattern between studies is difficult and an overall model for fruit drop is missing.

According to a review on fruit drop only about 2% of the initially set fruits reach harvest in mango (Singh et al., 2005). However, depending on the cultivars and cultivation practices applied, higher values of up to 24% are possible (Singh et al., 2005). In two other studies that compared fruit drop in mango cultivars, losses resulted in a broad range of final fruit retention between 0 and 18 % (Guzmán-Estrada, 1996; Ram, 1992). In conclusion there might be potential for increasing the productivity of mango, at least in some cultivar and cultivation practices by reducing fruit drop. In

order to exploit this potential, the causes of excessive premature fruit drop have to be identified and suitable solutions have to be developed.

1.2.2 Possible causes and prevention

In mango, early fruit drop can be caused by factors such as lack of pollination or unsuccessful fertilization of flowers, including reduced pollen viability, poor pollen tube growth, or self-incompatibility (Singh et al., 2005). Embryo disintegration can be an after-effect of problems encountered during fertilization or the result of degradation of embryo sac cells (He et al., 2012). In mangos, such seeds with degenerated embryos have mainly been found in pinhead or pea sized fruitlets (Singh, 1954; Singh, 1961; Singh and Arora, 1965). Interestingly, fruits with degenerated embryos that develop further than the pea stage can reach normal fruit maturity (Singh et al., 2005).

The seeds of mangos can be infested by the larvae of the mango seed or stone weevil (*Sternochetus mangiferae* Fabricius). This insect is typically associated with premature fruit drop, because it deposits its eggs in mango fruitlets to provide the developing larvae with a safe and nutritious food supply (Verghese et al., 2005). However, it is another type of pest, fruit flies of several genera, that accounts for the highest economic losses in mango production. Losses can occur during early fruit development but mostly occur at later stages (Peña et al., 1998). The most economically relevant diseases of mango are anthracnose (*Colletotrichum* spp.), powdery mildew (*Oidium mangiferae* Berthet.) and black spot disease caused either by a fungus (*Alternaria alternate* Fr.) or bacteria of several genera (Arauz, 2000; Gunjate, 2009; Schoeman et al., 1995). These organisms can infest fruitlets and thereby induce fruit drop (Singh et al., 2005). Further pathogens and pests of mango that can induce premature fruit drop in mango are reviewed in dedicated chapters of Litz (2009). Generally, counteractions against pests and pathogens require the application of agrochemicals or, if available, biocontrol agents. Moreover, hygiene and pruning practices throughout orchards are necessary, including the removal of dead plant material and litter from the orchard, to reduce possible sources of re-infection (Arauz, 2000; Peña et al., 1998; Ploetz and Freeman, 2009; Schoeman et al., 1995; Singh et al., 2005).

Carbohydrates required for fruit growth are supplied by leaf photosynthesis (Wardlaw, 1968). Thus the reduction of leaf area, for example by defoliation (McAlister and Krober, 1958; Obeso, 1998; Stephenson, 1980) or shading (Berüter and Droz, 1991; Nzima et al., 1999), reduces resource availability for developing fruits and thereby

inducing fruit drop (Stephenson, 1981). The competition among developing fruitlets and between fruitlets and other sinks leads to the abscission of weaker sinks (Dal Cin et al., 2005; Estornell et al., 2013; Botton et al., 2011). Thus, factors that limit leaf photosynthesis can ultimately induce fruit drop. Mangos reach their highest rates of leaf photosynthesis during the monsoon season with high ambient temperatures and high relative humidity (Elsheery et al., 2007). Low temperatures of 10°C or less can greatly reduce leaf photosynthesis rates, however, these temperatures do only occur in some mango cultivation areas (Allen et al., 2000; Elsheery et al., 2007). Temperature extremes and high humidity can also induce fruit drop by reducing the success of pollination. Unfavorable conditions can decrease the activity of pollinators, mostly flies (Diptera) and beetles (Coleoptera) (Singh, 1954; Singh, 1997). Especially cold temperatures can have physiological effects, as for example a reduction in pollen viability below 10°C (Issarakraisila and Considine, 1994) and abnormal development of flowers below 5°C, which occurs more often in polyembryonic than in monoembryonic varieties (Sukhvibul et al., 1999a; Sukhvibul et al., 1999b). Maintaining orchard temperatures above 10°C by the use of mobile heaters has been successfully applied to improve fruit development (Lakshminarayana and Aguilar, 1975), however, this seems to be a rather uneconomic solution for providing more suitable growing conditions for mangos. A more applicable strategy is to shift the flower and fruit development stage towards the wetter monsoon season by using special pruning and fertilization practices or by the application of plant growth regulators (PGR) as for example gibberellins (Davenport, 2007; Singh, 2009).

Mangos cultivated under subtropical conditions can be affected by extensive dry seasons (Coelho and Borges, 2004; Elsheery et al., 2007). Water is necessary for the transport of nutrients and assimilates through the tree to the developing fruitlets, for maintaining leaf photosynthesis and for evaporative cooling to avoid overheating of plant organs. Subsequently, water limitation can induce fruit drop (Damour et al., 2009; Elsheery et al., 2007) and the effectiveness of irrigation treatments has been proven in several experiments with different mango cultivars under water limited conditions (Singh and Arora, 1965; Bhuyan and Irabagon, 1993; Spreer et al., 2007). The supply of essential nutrients is also important for abundant fruit retention and fruit development (Singh et al., 2005). Therefore, the application of fertilizers to mango trees, especially those providing macronutrients, like nitrogen, phosphorus, and potassium, has been shown to increase yields compared to non-fertilized controls (Syamal and Mishra, 1989). Leaf fertilizers have been shown to be effective in this respect, especially if they

contain boric acid. The micronutrient boron is necessary for pollen and flower development, hence boron deficiencies are likely to promote premature fruitlet abscission (Burondkar et al., 2009; Stino et al., 2011; Singh and Dhillon, 1987). Boron is also important for other plant developmental process as for example hormone signaling of auxin (Blevins and Lukaszewski, 1997), therefore boron might have an indirect effect on the sink strength of young organs.

The application of PGRs is another strategy that has been intensively studied in the context of fruit drop in mangos. The exogenous application of individual polyamines, for example spermine, at fruit set leads to higher fruit retention compared to the control and other polyamines (Malik and Singh, 2006). However, the internal concentration of individual polyamines changes during fruit development, which might explain the variation in response to different polyamine treatments in mangos and other fruit crops (Singh et al., 2005; Xie et al., 2013).

Cytokinins are a group of phytohormones that promote cell division and regulate fruit set and fruit growth (Chen, 1983; Dal Cin et al., 2009; Estornell et al., 2013). Therefore, cytokinin-like PGRs were tested for their potential to regulate crop load, whereas the developmental stage of treated fruits and type of cytokinin applied determine whether the treatment inhibits or induces fruitlet drop (Burondkar et al., 2009; Dennis, 2000; Notodimedjo, 2000). N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) has shown high cytokinin activity (Iwahori et al., 1988) and its application to mango fruitlets at about one month after full bloom leads to significantly increased fruit retention in different mango varieties compared to control treatments (Notodimedjo, 2000).

Gibberellins control diverse aspects of the plant life cycle including fruit development (Yamaguchi, 2008). Gibberellins are therefore used commercially to produce seedless fruits, for example seedless grapes (Varoquaux et al., 2000). Applied to mangos, gibberellins where shown to effectively reduce fruit drop (Benjawan et al., 2006; Chen, 1983; Notodimedjo, 2000; Ram et al., 1983). Another class of phytohormones, auxins, have an important role in maintaining fruits (Estornell et al., 2013; Racskó et al., 2007). For example the application of the synthetic auxin, 1-naphtaleneacetic acid (NAA), at marble fruit stage inhibits fruitlet drop in mangos significantly (Chattha et al., 1999; Naqvi et al., 1990). This is probably caused by the ability of auxins to act as a sink signal thereby allocating carbohydrates to the developing fruitlets instead of to other sinks (Botton et al., 2011; Dhanalakshmi et al., 2003).

Another phytohormone closely associated with fruit drop is ethylene. Ethylene is the signal that triggers the detachment of fruits, consequently the application of substances that inhibit the biosynthesis of ethylene or its perception by mangos has been shown to effectively reduce fruit drop (Malik et al., 2003; Singh and Agrez, 2002). As the above examples show, it is necessary to consider the developmental stage of treated flowers or fruits when using PGRs for the reduction of fruit drop.

1.3 Mechanism of fruit abscission

1.3.1 Morphological changes

The underlying mechanism of premature fruit drop is an energy dependent and highly coordinated process called abscission (Estornell et al., 2013; Sexton and Roberts, 1982). Microscopic studies of the separation layer of abscised leaves, flowers and floral organs of several species by von Mohn (1860) showed that the abscission process requires structural changes at the cellular level. The separation layer in mangos was described as nested within a tissue of specialized cells, the so called abscission zone (AZ) (Barnell, 1939). Later studies showed that the AZ-cells contain dense cytoplasm with a high content of endoplasmic reticulum (ER) leading to a characteristic dark pigmentation (Sexton et al., 1977; Sexton and Roberts, 1982) that also makes the AZ in mango fruit pedicels externally recognizable (Barnell, 1939). When abscission commences, the amount of rough ER increases and vesicles are produced, which presumably contain cell wall degrading enzymes. These vesicles then move outward and fuse with the plasmalemma, thereby releasing their contents (Reid, 1985). Subsequently, the cell walls degrade and swelling of AZ-cells starts. This process allows a plant organ to detach without causing tissue disruptions that could provide entry points for pathogens or pests (Sexton and Roberts, 1982; Roberts et al. 2002). The duration of the abscission process ranges from one hour in flower buds to three days or more in fruit (Sexton and Roberts, 1982). The activation and course of the abscission process is controlled by phytohormones in response to environmental and internal factors such as the availability of resources to the plant (Bangerth, 2000; Lewis et al., 2006; Stephenson, 1981).

1.3.2 Hormonal and molecular control

Plant organ abscission can be under the control of several phytohormones such as auxins, abscisic acid, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonic acid,

polyamines, or other metabolites including reactive oxygen species or carbohydrates (Xie et al., 2013; Estornell et al., 2013). In the current study, however, the focus is on the antagonists auxin and ethylene as well as on carbohydrates due to their importance for the fruitlet abscission in mangos in particular. Ethylene beyond a certain concentration in the AZ triggers abscission of plant organs such as fruits (Brown, 1997; Nuñez-Elisea and Davenport, 1983; Malik et al., 2003; John-Karuppiah and Burns, 2010). Thereby does the AZ-sensitivity to ethylene largely depends on the auxin gradient in the fruit stalk (Fig. 1.3).

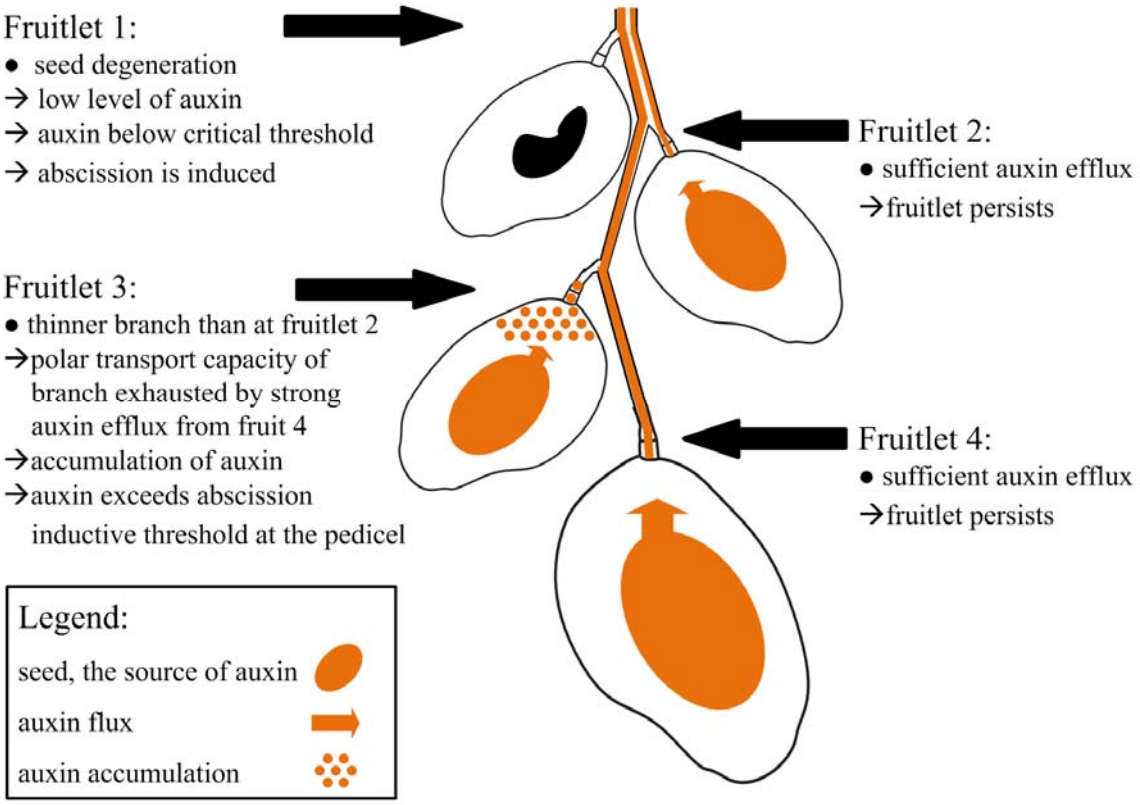


Fig. 1.3. Scheme of auxin flux in a fruit-bearing panicle of mango. The seed is considered as the main source of auxin. Fruitlet 1: Seed degeneration leads to a decreased auxin efflux. This enhances the sensitivity of the abscission zone (AZ) to ethylene (Roberts et al., 2002) and can possibly also induce the ethylene biosynthesis (Abel et al., 1995), which subsequently induces fruitlet abscission. Fruitlet 2: A fruitlet with a healthy seed is able to maintain a sufficient auxin gradient that decreases the ethylene-sensitivity at the AZ, consequently the fruit persists. Fruitlet 3: The strong auxin efflux from a further developed neighboring fruit leads to the accumulation of auxin in the pedicel. This possibly triggers abscission either by the auxin transport autoinhibition as proposed by Bangerth (1989) or through inducing the ethylene biosynthesis (Abel et al., 1995). Fruitlet 4: The fruitlet has sufficient auxin efflux and persists at the panicle.

If the auxin concentration at the AZ is outside of an optimum range, this leads to an increased ethylene sensitivity and synthesis and subsequently to organ abscission (Abel et al., 1995; Estornell et al., 2013; van Doorn and Stead, 1997). The concentration of auxin can be perceived directly by auxin-responsive elements in the promoter of genes that encode for enzymes of the ethylene biosynthesis. For example, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is synthesized by enzymes of the ACC-synthase (ACS) multigene family. In experiments with arabidopsis, the transcripts level of certain ACS isoforms showed to be upregulation within only 25 min in response to extreme concentrations of auxin (Abel et al., 1995). Presuming that the ACS or similar genes of the ethylene evolution have auxin-concentration depending activity also in the pedicel of fruits, a reduced polar auxin transport (PAT) through the pedicel could possibly induce endogenous ethylene evolution directly in the pedicels and in turn trigger abscission in the AZ. Low fruit auxin concentrations as well as low auxin export rates from the distal fruit have both been reported to be concomitant with fruitlet abscission (Else et al., 2004; Prakash and Ram, 1984). High auxin concentration in the pedicel can occur if the fruits compete for carbohydrates (Bangerth, 1989; Bangerth, 2000), which occurs under resource limited conditions (Stephenson, 1981). For example in apples, the dominant carbohydrate-sink within a single inflorescence is usually the biggest fruit. This big fruit can induce the accumulation of auxin in the pedicel of smaller fruits, which results in the autoinhibition of auxin synthesis in the fruitlet followed by the breakdown of auxin export through the AZ with subsequent fruit abscission (Bangerth, 1989; Bangerth, 2000) (Fig. 1.3).

The biosynthesis of ethylene starts with the amino acid methionine, which is catalyzed in two steps into ACC the direct precursor of ethylene (Argueso et al., 2007). ACC in contrast to ethylene can be produced in some distance to its destination. For example, abscission of fruitlets can be induced as a result of water deficiency, which leads to the accumulation of ACC in water-stressed roots. Upon the next rehydration the xylem flow is restored and transports the accumulated ACC to the leaves where is gets oxidized. This last step of the ethylene biosynthesis is catalyzed by ACC oxidases (ACO), which belong to a small multigene family (Barry et al., 1996). These ACOs are tissue specifically expressed and some isoforms increase specifically during fruit ripening (Barry et al., 1996) while others have shown to be upregulated during fruit abscission (John-Karuppiah and Burns, 2010). Once ethylene is synthesized and accumulated in

responsive cells of the AZ it triggers the fruitlet abscission process (Estornell et al., 2013). Ethylene is a general stress hormone, therefore it can be produced also by factors that directly affect a plant organ, such as pathogen attack or wounding and thereby induce abscission (Adie et al., 2007; Estornell et al., 2013). Since ethylene is such an imported factor for fruit abscission it is not surprising that the inhibition of a plant's ethylene receptors can inhibit fruit abscission (Villalobos-Acuña et al., 2010; Yuan and Li, 2008). Examples are silver ions, which substitute copper as receptor cofactor, leading to a non-functional receptor (Beyer, 1976; Binder, 2008) or 1-methylcyclopropene (1-MCP) a substance that competes with ethylene for the ethylene binding site (Rasori et al., 2002).

The binding of ethylene leads to a confirmation change of the receptor that inactivates CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) and allows the signal transduction to proceed (Chen et al., 2005; Hall et al., 2007; Ju and Chang, 2012). In the absence of ethylene, the ethylene response is actively inhibited through the active stage of the protein kinase CTR1 (Binder, 2008). ETHYLEN INSENSITIVE-2 (EIN2) is downstream of CTR1 and transmits the ethylene signal to EIN3 and EIN3-like (EIL) proteins, which are transcription factors und thus located in the nucleus (Chen et al., 2005). The EIL family further activate the transcription of other transcription factors including the ETHYLENE RESPONSE FACTOR (ERF) family, which are direct regulators of ethylene-responsive genes (Chen et al., 2005). The transcript analysis of abscising apple fruitlets has shown that the ethylene response leads to the downregulation transcriptions factors like the *AUXIN/INDOLE-3-ACETIC ACID*, auxin transporters of the *PIN-FORMED* family and further elements of the auxin signalling especially in the seed (Dal Cin et al., 2007; Dal Cin et al., 2009), which is likely to inhibit the seed development. Further genes of the ethylene signalling get upregulated, which represents a positive feed-back loop, were ethylene promotes its own biosynthesis (Stepanova and Alonso, 2009; Estornell et al., 2013). Genes encoding for proteins that facilitate the final fruit detachment are expansins, cellulases and polygalacturonases (Botton et al., 2011; Estornell et al., 2013). Taken together the action of these different ethylene responsive-genes ultimately lead to fruitlet abscission.

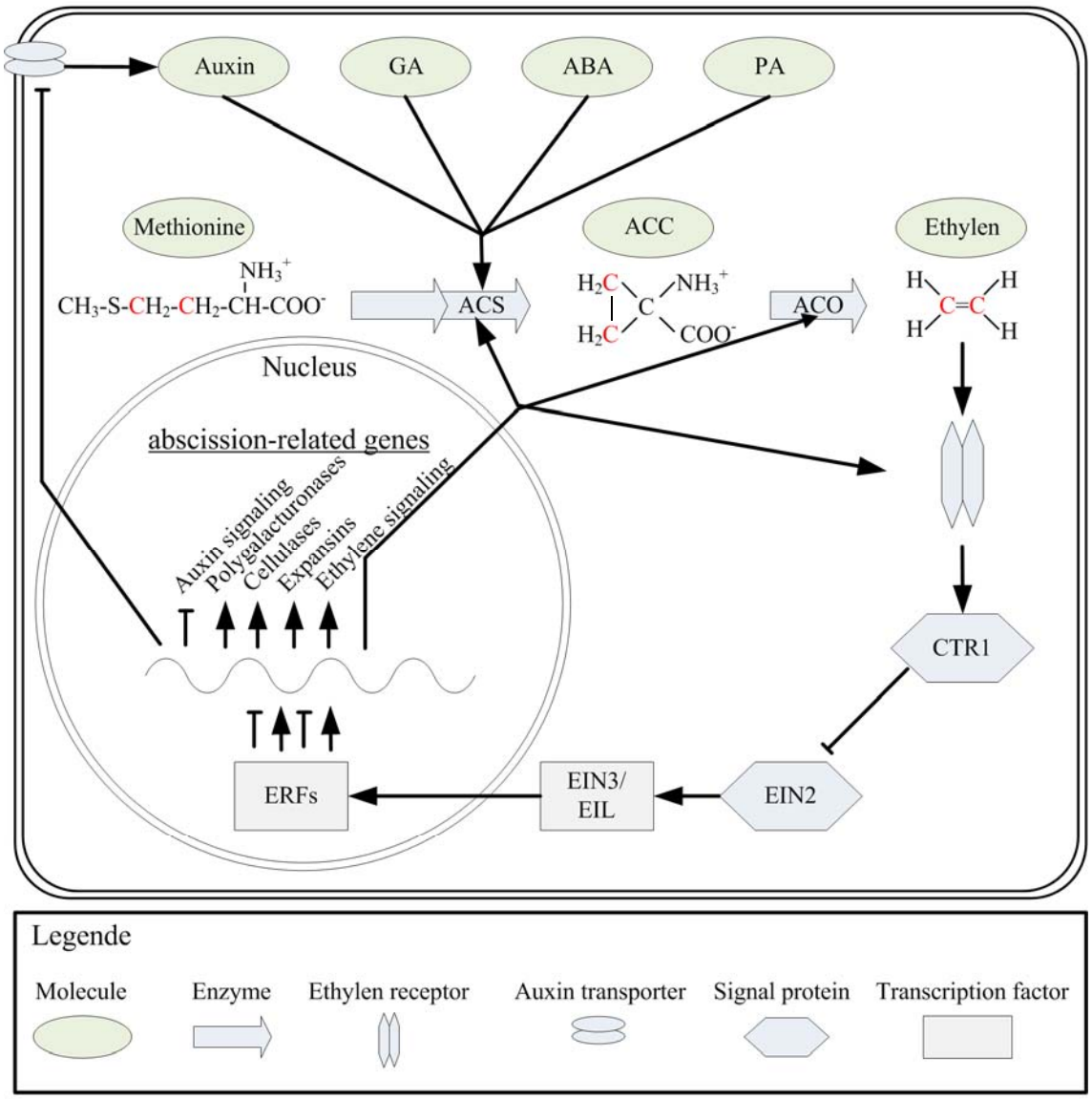


Fig. 1.4. Model for fruitlet abscission modified after Xie et al. (2011) considering Chen et al. (2005) and Dal Cin et al. (2007, 2009). Ethylene is catalyzed from methionine by the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and the ACC oxidase (ACO). The ACS activity can be modulated by auxin, gibberellins (GA), abscisic acid (ABA), polyamines (PA) or others. In the absence of ethylene the ethylene receptors maintain the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) protein kinase in an active state. The active CTR1 inhibits the ETHYLEN INSENSITIVE-2 (EIN2) signal protein. Ethylene perception leads to a conformational change that inactivate CTR1, which in turn induced the EIN2 transcription factors. EIN2 further induces EIN3 and EIN3-like (EIL), which than induce the activity of transcription factors of the ETHYLENE RESPONSE FACTOR (ERF) family in the nucleus. The ERFs lead to the upregulation of genes of the ethylene signaling, which promotes the ethylene response, while the auxin signaling is downregulated. In the following, the ERFs induce cell-wall degrading enzymes, which finally leads to the detachment of the fruitlet and subsequently to the drop of the fruitlet.

1.4 Ethylene perception

Ethylene is involved in various processes in the plant life cycle, including development, gravitropism, stress response, organ abscission and senescence (Adie et al., 2007; Bapat et al., 2010; Estornell et al., 2013; Vandenbussche et al., 2012; Xie et al., 2013). The response to ethylene is mediated by ethylene receptors and various isoforms exist in different species of plants, algae or cyanobacteria (Binder, 2008). These receptors share common features. The receptors assemble to homo- or heterodimers that are stabilized by disulfide bonds (Gao et al., 2008). Further, the receptors are ER-anchored by three N-terminal transmembrane domains encompassing the ethylene binding site (Binder, 2008). The C-terminal site of the receptors is directed towards the cytoplasm (Ju and Chang, 2012) and consists of a GAF-domain, a kinase domain and, in some isoforms, a receiver domain (Binder, 2008). The GAF-domain, named after the proteins of its discoveries (c**G**MP-specific phosphodiesterases, **a**denylyl cyclases and **F**hlA) is necessary for a receptor-receptor interaction (Gao et al., 2008; Ju and Chang, 2012). The receptor-receptor interaction, including dimerisation or receptor clustering, is thought to be a mechanism to modulate the ethylene signalling (Ju and Chang, 2012). The family of ethylene receptors was divided into three subfamilies based on differences in the kinase domains, the presence of an N-terminal signal sequence, and the presence of specific proteins, which have only been described for cyanobacterial receptor isoforms (Binder, 2008). Despite the differences, receptor isoforms are partly functionally redundant and the malfunction of one or more isoform can be compensated by the remaining ones (Binder, 2008; Qu et al., 2007; Shakeel et al., 2012). However, receptor isoforms also have non-overlapping functions and are regulated differently, depending on the plant organ and developmental stage (Binder, 2008; John-Karuppiah and Burns, 2010; Rasori et al., 2002; Sato-Nara et al., 1999; Shakeel et al., 2012; Stepanova and Alonso, 2009), possibly allowing a broad range of modulation of the ethylene response (Stepanova and Alonso, 2009). The downstream elements of the signal transduction are nuclear transcription factors that control the specific gene expression and ultimately lead to a distinct ethylene response (Chen et al., 2005; Etheridge et al., 2005; Ju and Chang, 2012).

1.5 Objectives and hypothesis

The attempt of the current study was to contribute substantially to the current knowledge on fruit abscission, because this plant developmental process is directly linked to crop productivity, especially for mango. Consequently, the overall objective was to understand the underlying mechanism of fruitlet drop in order to provide suitable method for its prevention:

The specific objectives were:

1. to characterize the drop patterns of mango fruit throughout the growing season for defining and studying individual drop stages, which allows the development of stage-specific prevention strategies;

2. to understand the underlying physiological events taking place during the critical fruit drop stage by identifying and studying key fruit parameters;

3. to further the understanding of the molecular triggers of abscission with focus on the ethylene receptors;

4. to provide, based on the results, recommendations for mango producers.

The hypothesis of the study is that fruitlet drop in mango is caused by unfavourable environmental factors in the study region. Thereby is plant stress during mid-season drop the factor that strongly reduces yields of mango. A stress induced limitation of assimilation leads to a carbohydrate deficiency in the fruitlet, the induction of abscission in its pedicel and finally its detachment. This fruitlet drop can be prevented through the reduction of plant stress by manipulating the hormonal control of the abscission process, which likely to increases the productivity of mango.

1.6 Publications

This doctoral thesis consists of four publications. Three of these articles have been published (article I) or submitted (article III, IV) in peer reviewed academic journals. Additionally article II was published in a reviewed conference proceeding. In the following, each publication is presented in one chapter. Within each chapter the reference system, figure style and language (American or British English) was applied according to the authors guidelines of the journal to which the publication was submitted.

Article I

Hagemann, M.H., Roemer, M.G., Kofler, J., Hegele, M., and Wünsche, J.N. 2014. A New Approach for Analyzing and Interpreting Data on Fruit Drop in Mango. *HortSci.* 49**,** 1498-1505. (http://hortsci.ashspublications.org/content/49/12/1498.abstract)

Article II

Hagemann, M.H., Winterhagen, P., Hegele, M., and Wünsche, J.N. 2015. Ethephon induced abscission of mango fruitlets - physiological fruit pedicel response. *Acta Hort.* 1066**,** 109-116. (http://www.actahort.org/books/1066/1066_11.htm)

Article III

Hagemann, M.H., Winterhagen, P., Hegele, M., and Wünsche, J.N. (Submitted to *Frontiers in Plant Science*). Ethephon induced abscission in mango: physiological fruitlet responses.

Article IV

Winterhagen, P., Hagemann, M.H., Wünsche, J.N. (Submitted to the *Journal of Experimental Botany*). Expression and dimerisation of the mango ethylene receptor MiETR1 and different receptor versions of MiERS1

2. A new approach for analyzing and interpreting data on fruit drop in mango[2]

2.1 Abstract

Mango yields are frequently reduced by premature fruit drop, induced by plant stresses during the fruit set period in response to unsuitable climatic or crop management conditions. There are varying strategies for assessing premature fruit drop, which render the comparison and interpretation of published data difficult to draw general conclusions. Therefore, the objective was to provide a mathematical model that is generally valid for describing fruit losses of mango. The model was tested and validated by monitoring the fruit drop for the two local North Vietnamese cultivars, Hôi and Tròn, in different management systems over six consecutive growing seasons: 1) mango–maize intercropping and mango monocropping; 2) irrigation; and 3) plant growth regulator applications with 10 ppm N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU), 40 ppm 1-naphthaleneacetic acid (NAA), and 40 ppm gibberellins (GA_3 and GA_{4+7}). The timely pattern of fruit drop was best described with a sigmoid function ($r^2 = 0.85$) and formed the basis for defining three distinct drop stages. The post-bloom drop, from full bloom to the maximum daily rate of fruit drop [FD(x)], had the highest fruit losses. The following midseason drop stage ends at 1% FD(x), a threshold that is suggested after a comprehensive literature review. Thereafter, during the preharvest drop stage, treatment and cultivar differences appear to remain constant despite continued fruit drop. In contrast to other mango intercropping studies, fruit loss was not greater in the mango–maize intercropping than in the mango monocropping. Irrigation resulted in approximately three times higher fruit retention compared with the non-irrigated control. A single application of NAA at marble fruit stage (BBCH-scale 701) resulted consistently in the highest fruit retention for both cultivars in midseason and at harvest. The model permits the separation between the drop stages, thus allowing the evaluation of 1) natural variation before treatment effects during post-bloom drop; 2) treatment efficacies during midseason drop; and 3) yield forecasting at the beginning of the preharvest stage.

[2]This chapter consist of the correspondent article which was accepted and published by the journal *HortScience*: This article is reproduced with the kind permission of the American Society of Horticultural Science, New Orleans, USA. This article should be cited as: Hagemann, M.H., Roemer, M.G., Kofler, J., Hegele, M., and Wünsche, J.N. 2014. A new approach for analyzing and interpreting data on fruit drop in mango. *HortScience* 49**,** 1498-1505. (http://hortsci.ashspublications.org/content/49/12/1498.short).

Additional index words: fruit retention, intercropping, irrigation, *Mangifera indica*, plant growth regulator

2.2 Introduction

The worldwide production of mango (*Mangifera indica* L.) is frequently reduced by severe losses of fruit numbers throughout the growing season, a phenomenon that is referred to as premature fruit drop (Singh et al., 2005).

Mango produces an abundance of male and polygamous flowers, but only a small proportion of the latter group is successfully pollinated and has the potential for setting fruit (Mukherjee, 1953; Singh et al., 1966). Numerous abiotic and biotic factors reduce pollen viability (Issarakraisila and Considine, 1994), the fertilisation process of the flower and embryo survival (Lakshminarayana and Aguilar, 1975), which are all commonly associated with an extensive fruit drop in early season (Singh et al., 2005). Fruit that retains attracts a greater share of the available tree resources for continued growth and development. Subsequent fruit drop is induced by any factor reducing carbohydrate availability and thus the demand of the growing fruit is not sufficiently matched by its supply (Wünsche and Ferguson, 2005). This carbohydrate imbalance can occur, for example, by air temperatures below 13 °C or exceeding 36 °C due to heavily reduced leaf photosynthesis rates (Issarakraisila and Considine, 1994; Whiley, et al., 1999; Yamada et al., 1996).

For mango, principal phenological growth stages are distinguished (Hernández Delgado et al., 2011) according to the general BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie), however, fruit drop is only described in stage seven with “beginning and end of the physiological fruit drop” when fruits have attained 10% or 30% of final fruit size, respectively. The premature fruit drop stages have been named invariably and there is also no common agreement on the number of drop stages as well as the onset and duration of each. Dahshan and Habib (1985) originally described three distinct stages of premature fruit drop of mango and this classification was also used in the review of Singh et al. (2005). The first stage is referred to as “post-setting drop" and ceases 60 days following “fruit set” (BBCH-scale 619). The second stage is termed “mid-season drop”, characterized by a duration of 15 days with lesser intensity than during the “post-setting drop". The third stage is the “pre-harvest drop” with only moderate losses.

These descriptions of the premature fruit drop, commonly found in the literature, represent some considerable limitations. The onset of each fruit drop stage is based on a "fixed" number of days from "fruit set" as used for example in the review of Singh et al. (2005). Fruit set, however, is variably related to: (1) time after all flowers have dried out at the end of bloom (Malik and Singh, 2003), (2) 14 days after full bloom (DAFB) (Notodimedjo, 2000) or (3) size of fruitlets (Lam et al., 1985). The flowering of mango is a very prolonged and sometimes non-synchronized process, especially in the tropics where erratic flowering is common but also in the subtropics flowering can occur from a few days of an individual flower, 1-2 weeks within a panicle to up to 1 month within a tree canopy (Goguey, 1997; Mukherjee, 1953; Verheij, 1986). Typically, panicles exhibit a hierarchical flowering pattern from distal to proximal position with an overlapping continuum of flowering and fruit development (Mukherjee, 1953; Singh, 1954). The terms "full bloom" and "fruitlet size" are often subjectively assessed by scoring and variably defined, thus do not offer precise occurrences that justify a valid comparison of published fruit set data. The duration of each fruit drop stage is clearly dependent on seasonal, regional and cultivar specific variability and therefore the "fixed time after fruit set" definition might be useful for characterizing the annual drop pattern of a given cultivar in one location, but is not appropriate when comparing multiple data sets. Consequently the main objective of this study is to provide a new approach for interpreting and evaluating fruit drop data, attempting to overcome or at least to alleviate the limitations described above. Consequently, fruit drop of two mango cultivars was monitored in largely different cropping and management systems over six seasons in the Province Sơn La in North Vietnam and data were tested and validated in a mathematical model.

Unfavorable environmental cues, particularly when temperature extremes coincide with severe drought conditions (Elsheery et al., 2007), are thought to be key triggers for the extensive premature fruit drop patterns in this province. Huong (2010) further suggested poor orchard management, in particular insufficient pest management, as an additional cause of fruit drop for the local mango cultivars 'Hôi' and 'Tròn', predominantly cultivated in this region.

Despite extremely low orchard productivity of approximately 1 $t \cdot ha^{-1}$ (Yên Châu, 2008) mango trees are often planted either in monoculture or in intercropping systems with mainly maize in the mountainous Province of Sơn La with steep sloping hill sides. Indeed, tree crops, including mango, provide a more appropriate and sustainable land

use system for steep, deforested slope sites (Roberts-Nkrumah, 2000; Young, 1989), since the cultivation of annual crops in monocropping systems under those topographical conditions will lead to reduced soil fertility and severe soil erosion (Clemens et al., 2010). In particular during the juvenile phase of tree crops, intercropping with annuals is a common practice (Musvoto and Campbell, 1995; Roberts-Nkrumah, 2004). However, it has been reported that some plant species enhance mango fruit drop in intercropping systems (Singh et al., 2005).

Crop management strategies such as irrigation or plant growth regulator (PGR) applications may also offer opportunities for fruit drop prevention. Galán Saúco (1997) suggested that the water requirement of mango is about 100 mm monthly during the fruit development period to ensure good productivity. However, this level is typically lower during early fruit development in the Province of Sơn La (Roemer et al., 2011) with water deficiency particularly prevalent from bloom to mid-season drop due to the lack of precipitation or irrigation sources. Alternatively, applications of PGRs are commonly used for enhancing fruit retention in many perennial fruit crops, including mango. For example, N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) increased fruit retention in different mango cultivars and growing regions (Notodimedjo, 2000; Burondkar et al., 2009). Similar effects were shown when gibberellic acid (GA) was applied either alone or in combination with other PGRs (Benjawan et al., 2006; Chen, 1983; Notodimedjo, 2000; Oosthuyse, 1993, 1995; Ram, 1983; Singh, 2009). Moreover, the synthetic auxin 1-naphthaleneacetic acid (NAA) is another fruit drop reducing PGR with a timely different efficacy; fruit retention is less affected when applied around post-bloom rather than at later fruit developmental stages (Notodimedjo, 2000; Chattha et al., 1999).

In summary, the largely equivocal assessment and description of fruit drop in mango renders the comparison and interpretation of published data, thus making it extremely difficult to draw general conclusions and providing horticultural recommendations. We therefore attempt to offer not only a new approach for assessing fruit drop data, based on a simple mathematical model, but also to newly characterize this process that occurs throughout the growing season from first flower to just prior to harvest. In particular, describing the fruit drop process, we are re-evaluating the appropriateness of existing terminologies in this context and offering more precise benchmarks for onset and duration of each fruit drop stage. For a robust evaluation and validation of our model as

well as the interpretation of the seasonal fruit drop continuum, fruit abscission was monitored over several years in varying mango production systems.

2.3 Material and methods

2.3.1 Plant material and experimental sites.

The experiments were conducted over six consecutive growing seasons from 2007 to 2012 in the Tú Nang commune (lat. 20°37′0″ N, long. 106°4′60″ E) near the township Yên Châu, Province Sơn La, North Vietnam. This is a mountainous region with prevailing monsoon seasonality and 1200 mm of annual precipitation. Mango trees (*Mangifera indica* L.) of the cultivars Hôi and Tròn, ranging between 10 and 15 years of age, were used in several orchards for assessing fruit drop. The tree spacing in all orchards was ≈10 × 10 m. Standard management practices such as, e.g., pruning and plant protection were conducted according to Huong (2004). Soils are classified as either Luvisol or Alisols (FAO, 2006).

2.3.2 Environmental parameters.

Seasonal ambient air temperature and relative humidity were recorded within the center of each of six representative tree canopies in one orchard from 2006 to 2012 using microloggers (HOBO Pro v2; Onset, Bourne, MA). The logger outputs were recorded at 10-min intervals from 1 week before full bloom until midseason, covering the main fruit drop period, and average daily temperatures are shown in Table 2.1. There is little interseasonal variability and the 6-year daily mean was 30 and 17 °C, 89% and 45% for maximum and minimum temperature, and relative humidity, respectively. In the same orchard, soil moisture was measured at weekly intervals from full bloom until midseason in 2008–09 with a profile probe (PR2; Delta-t Devices Ltd., Cambridge, U.K.). The measurements were carried out at 10-cm increments from 10- and 40-cm soil depth 50 cm apart from the trunk of five irrigated and non-irrigated trees, respectively. The profile probe was connected to a handheld data-logging device (Moisture meter HH2; Delta-t Devices Ltd.).

Table 2.1. Average daily maximum and minimum ambient temperatures and relative humidity, respectively, from 1 week before bloom to midseason in six consecutive growing seasons. [z]

	Temperature (°C)		Relative humidity (%)	
Growing season	Maximum	Minimum	Maximum	Minimum
2007	28.1	15.0	88.9	43.2
2008	32.6	21.0	93.0	45.4
2009	31.3	17.3	90.4	38.4
2010	31.3	15.4	89.3	37.2
2011	25.2	16.8	85.9	58.3
2012	29.6	17.4	83.5	44.9

[z] Data were recorded in one orchard in the Tú Nang commune.

2.3.3 Experimental design and treatments.

Seasonal fruit drop was monitored in the same orchard where environmental parameters were monitored. Although the natural fruit drop of 'Hôi' was assessed in all years, fruit drop of both cultivars was also evaluated in response to irrigation and PGR applications in 2008 and 2009 (Table 2.2).

Table 2.2. Overview of experimental characteristics in each of six growing seasons.

Growing season	Full bloom	Cultivar	Treatment(s)	Trees/ cv (n)
2007	15-Feb	Hôi, Tròn	—	10
2008	28-Mar	Hôi, Tròn	Irrigation; PGR	20; 3
2009	06-Feb	Hôi, Tròn	Irrigation; PGR	20; 6
2010	07-Feb	Hôi	—	6
2011	07-Mar	Hôi	—	6
2012	20-Feb	Hôi	Cropping system	>8[z]

[z] In each of three orchards per cropping system.

PGR = plant growth regulator.

The irrigation experiment was conducted using 20 randomly selected trees per cultivar (Table 2.2) Ten trees were irrigated and 10 trees served as non-irrigated controls from ≈6 weeks before flowering until the end of the midseason drop. Trees were irrigated at 3-d intervals for 45 min with a nominal rate of 90 $l \cdot h^{-1}$. The irrigation system included microsprinklers (Gyro Net LR 120; Netafim, Tel Aviv, Israel) placed 30 cm to the tree trunks with water supplied from a rain-fed water tank.

For PGR applications, three trees in 2008 and six trees in 2009 for each cultivar, respectively, were randomly selected. Each of seven PGR treatments was applied to 10 panicles along one branch unit, respectively, on each experimental tree. The treated branches were randomly assigned within the block structure tree. The following PGR formulations were applied either alone or in combination according to manufacturers' specifications: 10 ppm CPPU (Sitofex 10 EC; AlzChem, Trostberg, Germany), 40 ppm GA_3 (ProGibb 40; Valent, Walnut Creek, CA), 40 ppm GA_{4+7} (ProVide 10 SG; Valent), and 40 ppm NAA (Rhodofix; Syngenta, Basel, Switzerland). The treatments were 1) control (water); 2) CPPU; 3) CPPU + GA_3; 4) CPPU + GA_{4+7}; 5) NAA; 6) CPPU + GA_3 + NAA; and 7) CPPU + GA_{4+7} + NAA. All treatments were sprayed at different fruit stages with the surfactant Ethalfix Pro (Syngenta) at a concentration of 5 ppm (Table 2.3). All applications were conducted at predawn to runoff using a pressure-compensated hand sprayer (Gloria; Typ 133, Witten, Germany). Spray drift was prevented by a plastic sheet surrounding the panicle at the time of application.

Table 2.3. Fruit size dependent time of spray application and concentration of each plant growth regulator.

PGR	Concn (ppm)	Fruit stage [z]	Fruit size (mm)	BBCH-scale	Time of application (DAFB)	
					2008	2009
CPPU	10	Pinhead	6	619	Apr 09 (12)	Feb 13 (7)
GA	40	Pea	11	n.a.	Apr 11 (14)	Feb 25 (19)
NAA	40	Marble	20	701	Apr 17 (20)	Mar 04 (26)

[z] Fruit stages as described by Malik and Singh (2003).

DAFB = days after full bloom; CPPU = N-(2-chloro-4-pyridyl)-N′-phenylurea; GA = gibberellin; NAA = 1-naphthaleneacetic acid; NA = not applicable.

In addition, 'Hôi' monocropping and 'Hôi'–maize intercropping were compared at three orchard sites, respectively, in 2012. Each site was used for at least 12 years in the current cropping system. At least eight trees were selected in each orchard, giving a total of 54 trees (Table 2.2).

2.3.4 Assessment of fruit drop.

For counts of fruit retention, 10 (12 in 2012) healthy-appearing panicles were tagged at random for each tree or treatment shortly after full bloom. Counts started not later than

3 weeks after full bloom (greater than 90% of all panicles with less than 20% unbroken buds; approximately BBCH-scale 615) at weekly intervals. Fruit retention is expressed as the average fruit number per initially tagged panicles [fruit per panicle (FPP)]. In all years, fruit counts discontinued between 60 and 80 DAFB, ≈1 month before harvest, except in 2008 and 2011 when fruit retention was recorded until commercial harvest. According to the outlined inconsistent fruit drop definition, resulting from the use of "post-setting" as the denominator, fruit retention data are based on days after full bloom but not related to the BBCH-scale for reasons stated in the introduction.

2.3.5 Statistical analysis.

Seasonal fruit drop was evaluated by analyzing counts of fruit retention per panicle at each assessment date. The timely patterns of average fruit retention were described with best fit regression functions using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA). The mean data of each counting date were used for the regression analysis. The parameterized linear, polynomial, exponential decay, sigmoid, and logistic functions were compared for the best fit based on the adjusted correlation coefficient (r^2). Assumptions for the regression analysis, normality and variance homogeneity, were checked. The seasonal curves of FPP were best described with a four-parametric sigmoid function:

$$\mathrm{FPP(x)} = y_0 + \frac{a}{1+e^{-\left(\frac{x-x_0}{b}\right)}} \qquad [1]$$

where x is the dependent variable DAFB. The parameter y_0 is diverging to positive infinity, marking the final fruit retention with assuming no changes until harvest. The sum y_0+a determines the upper limit of FPP(x) with divergence to negative infinity, matching the initial value of FPP. The parameter b is the steepness of the slope of the sigmoid function, whereas x_0 is its point of inflection, corresponding with the highest daily fruit losses in absolute terms. The constant e represents the Euler's number. The FD(x) was calculated according to Zucconi et al. (1978):

$$\mathrm{FD(x)} = 100\text{-}\left(\frac{\mathrm{FPP}_{(x+1)}}{\mathrm{FPP}_{(x)}} * 100\right) \qquad [2]$$

FD(x) was plotted and the maximum value (FD_{max}) was determined from the graph. Moreover, a threshold was determined from data in the literature (Table 2.4), at which the midseason fruit drop is described to be ended [FD(x) midseason termination

(FD_{mst})]. The appropriateness of x_0, FD_{max}, and FD_{mst} for comparing sets of fruit drop data from the literature was tested.

Table 2.4. Literature citations with reference to the termination of post-bloom and mid-season drop.

Publication	Days after full bloom	
	Post-bloom	Midseason
Thimmappaiah and Suman, 1987	29[z]	59[z]
Asif et al., 2002	35[z]	—
Bhuyan and Irabagon, 1993	21	—
Guzman-Estrada, 1996	—	39–64[zy]
Lam et al., 1985	—	56[z]
Núñez-Elisea and Davenport, 1983	—	54[z]
Prakash and Ram, 1984	14	35
Singh and Arora, 1965	—	42
Singh et al., 2005	74[z]	89[z]
Average	35	56

[z] Authors refer to "days after fruit set"; thus, to allow a comparison among various studies, 14 days were added to convert the time unit to DAFB according to the time duration cited by Notodimedjo (2000).

[y] Range due to several cultivars being evaluated in that study.

The effects of cropping system, irrigation, and PGR applications on fruit retention were evaluated shortly after full bloom (BBCH-scale 615) and at the end of post-bloom (approximately BBCH-scale 701) and midseason drop (approximately BBCH-scale 703), respectively, by pairwise comparison of the means at a probability level of $P \leq 0.05$ (SAS 9.3; SAS Institute Inc., Cary, NC). However, after the first counting dates (greater than four), FPP did not follow normal distribution as a result of an increasingly high proportion of panicles bearing no fruit. Consequently, the positively skewed data, following a Poisson distribution, were evaluated with a generalized linear mixed model procedure (SAS Proc Glimmix) using a logarithmic link function and correction for the overdispersion of the errors (Bolker et al., 2009). Treatment means were estimated using the *lsmeans* statement in Proc Glimmix, which compensates for the unbalance in the data set (more observations in some treatments than in others). The model assumptions were checked by examining the residual plots.

2.4 Results

2.4.1 Assessment of fruit drop.

The best approximation of the mean data of the two cultivars in all six seasons and for both cultivars could be achieved by a sigmoid function with an r^2 of 0.85 (Fig. 2.1A). For a single season and cultivar, the data were best modeled with a sigmoid curve ($r^2 \geq$ 0.93; Table 2.5). Time units other than DAFB have also been tested for increasing the accuracy of the fruit drop simulation overall growing seasons. The concepts evaluated were degree-days with base air temperatures 10, 13, and 15 °C (Issarakraisila and Considine, 1994; Whiley et al., 1988) and growth hours, the sum of all hours between 10, 13, or 15 °C and 36 °C. However, the resulting r^2 values were lower compared with those derived from using DAFB (data not shown). The slope of the simulated fruit drop curve shows a minimum at 23 DAFB where the sigmoid curve has its inflection point (Fig. 2.1B). FD(x), calculated from Eq. [2], showed a maximum at 31 DAFB (FD_{max}) and dropped below a threshold of 1.2% at 56 DAFB (FD_{mst}). The initial FPP (y_0+a) varied considerably, depending on cultivar and season, and was 5-fold greater in 2009 than in 2011 for 'Hôi', but this cultivar resulted not consistently in a higher initial FPP compared with 'Tròn' (Table 2.5). It is worth mentioning that there is no correlation between initial FPP and final fruit retention, because, e.g., a high y_0+a can lead to relatively high or low y_0 and vice versa. The duration between full bloom and the average occurrence of x_0 is 3 weeks in which ≈50% of the set fruit have dropped (Table 2.5). Subsequently, the drop intensity continuously to increase until FD_{max} is attained when on average only one-third of the fruit is still retained. The time period between full bloom and FD_{max} refers to the post-bloom drop stage that ends averagely at 31 DAFB. The following stage, the midseason drop, is characterized by a continuously decreasing fruit drop intensity until FD_{mst} is reached at 56 DAFB (Table 2.5). There are slightly more fruit retained per panicle in 'Hôi' than in 'Tròn' but FPP is on average less than one. Thereafter, the average number of fruit per panicle is only moderately changing throughout the preharvest drop stage. For example, fruit retention of 'Hôi' decreased from 0.4 to 0.3 FPP and from 1.2 to 1.1 FPP in 2008 and 2011, respectively, from the end of midseason drop until harvest. The average FD(x) over six consecutive seasons follows a single wave with the main fruit losses occurring between 20 and 40 DAFB (Fig. 2.1B). In contrast, evaluating individual seasons indicates that fruit drop can occur in more than one wave (Fig. 2.2), except in 2011 and 2012 with only one

wave. In most seasons, the last fruit drop wave has a smaller amplitude than the previous one.

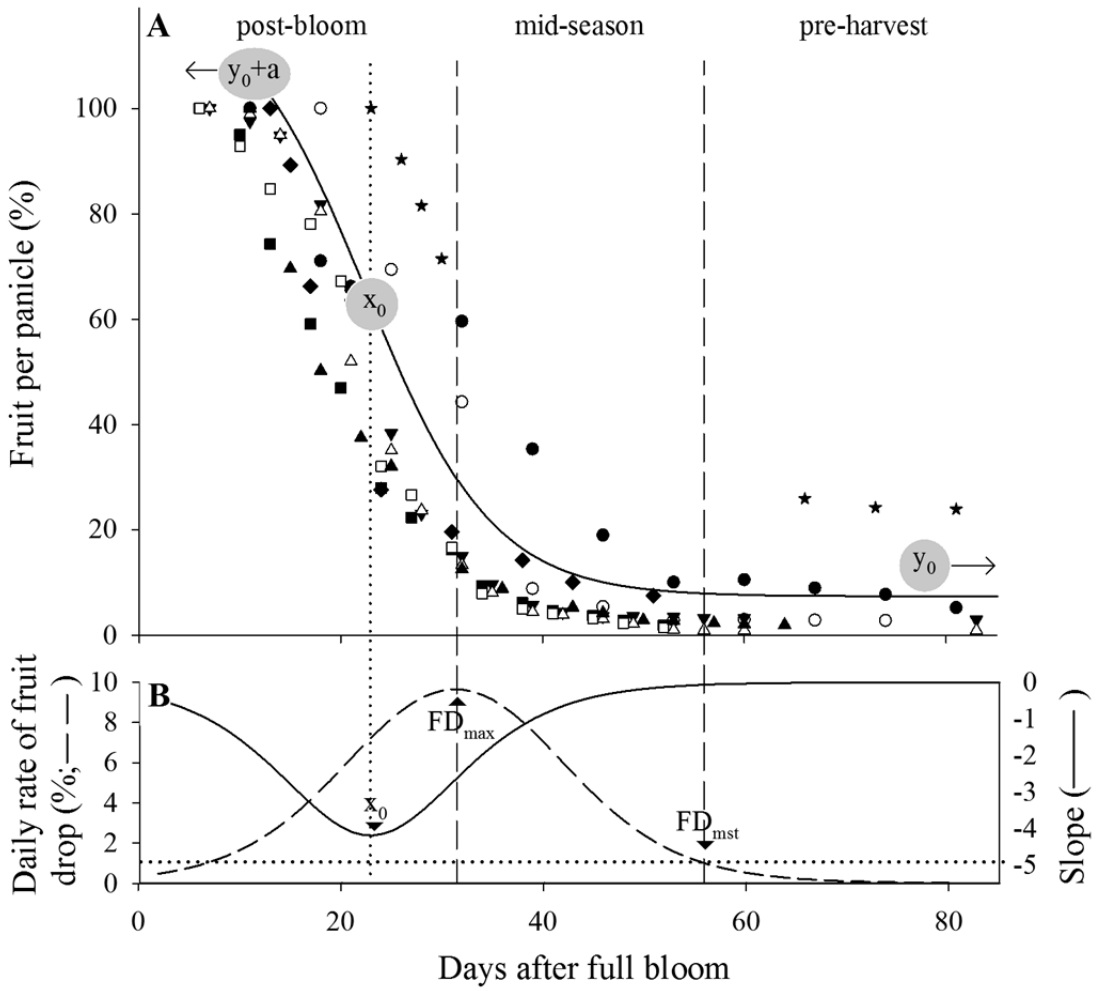


Fig. 2.1. (A) Average fruit retention per panicle (FPP) of 'Hôi' (closed symbols) and 'Tròn' (open symbols) in days after full bloom (DAFB). Each symbol refers to years between 2007 and 2012 and is based on actual fruit counts per panicle. Average fruit retention over that period is also modeled with a sigmoid function (r^2 = 0.85). y_0 is the final fruit retention, whereas y_0+a determines the upper limit of FPP. (B) Slope of the simulated fruit drop curve and the calculated daily rate of fruit drop FD(x). Black arrows indicate x_0, FD_{max}, and FD_{mst}, corresponding with the highest daily fruit losses in absolute terms, the highest FD(x), and the cessation of midseason fruit drop, respectively.

Table 2.5. Fruit per panicle (FPP) at days after full bloom (DAFB) for each growing season and cultivar.

Growing season and cultivar		r^2 [z]	y_0+a [y]	x_0 [x]		FD_{max} [w]		FD_{mst} [v]	
			FPP	DAFB	FPP	DAFB	FPP	DAFB	FPP
2007	Hôi	0.98	10.9	23	5.6	49	1.7	95	0.3
	Tròn	0.95	11.0	27	5.4	43	1.0	76	0.1
2008	Hôi	0.99	8.8	23	4.9	29	1.8	45	0.4
	Tròn	1.00	10.4	22	5.2	31	1.3	52	0.2
2009	Hôi	1.00	28.5	16	14.9	30	3.5	59	0.5
	Tròn	0.99	18.7	22	9.5	30	2.9	51	0.5
2010	Hôi	0.99	9.1	19	5.0	22	3.1	35	1.1
2011	Hôi	1.00	5.7	31	3.3	35	2.4	50	1.2
2012	Hôi	0.93	18.9	21	10.2	29	3.5	49	0.8
Average			13.6	23	7.1	33	2.4	56	0.6

[z] correlation coefficient of regression analysis (FPP * DAFB) using a sigmoid function

[y] initial value of FPP at zero DAFB

[x] highest daily fruit loss in absolute terms

[w] maximum value of FD(x)

[v] termination of midseason fruit drop

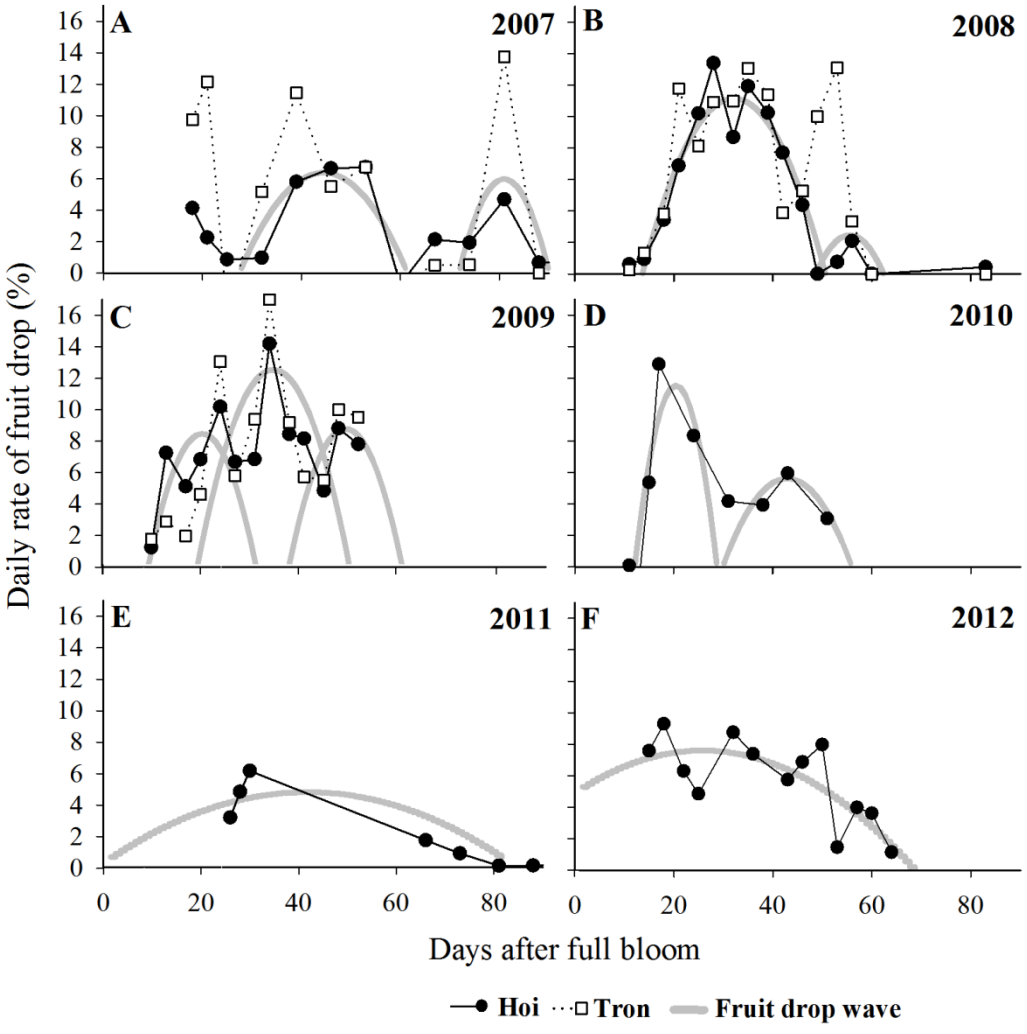


Fig. 2.2. Daily rate of fruit drop in six consecutive growing seasons. Gray lines indicate the fruit drop waves by cultivar 'Hôi'.

2.4.2 Cropping system evaluation.

The number of panicles per tree and the average number of fruit per panicle at each of the three fruit drop stages were not significantly different between the mango–maize intercropping system and the mango monocropping system (Table 2.6).

Table 2.6. Effect of mango monocropping and mango-maize intercropping systems on number of panicles per tree and number of fruits per panicle in 2012.

Treatment	Bloom (8 DAFB)	Post-bloom (32 DAFB)	Midseason (50 DAFB)
1. No. of panicles per tree			
Monocropping	494a [z]	149a	—
Intercropping	592a	162a	—
2. No. of fruits per panicle			
Monocropping	8.5a	2.1a	0.7a
Intercropping	9.1a	2.3a	0.5a

[z] Means followed by the same letter (a – d) in each column do not differ significantly ($P \leq 0.05$).

DAFB = days after full bloom.

2.4.3 Irrigation.

Fruit per panicle at the end of midseason drop was significantly increased in both cultivars by irrigation when compared with the control (Table 2.7). Interestingly, this effect was not seen in 2009 at the earlier stages, except for 'Hôi' at post-bloom. Fruit retention was considerably higher in 2009 compared with 2008. At harvest in 2008, irrigated trees retained 3-fold more fruit than non-irrigated trees in both cultivars. Despite these significant cropload differences, fruit length and fruit weight were similar in both treatments, resulting in on average 8- and 10-cm long and 180- and 230-g heavy fruit for 'Tròn' and 'Hôi', respectively. In general, soil moisture was 20% to 30% higher for irrigated trees compared with untreated controls. Moreover, soil moisture was higher in 2008 than in 2009 as a result of 196 mm precipitation in 2008 compared with only 1 mm in 2009.

Table 2.7. Effect of irrigation on fruit retention of mango for the growing seasons 2008 and 2009.

Treatment	Fruit per panicle						
	Bloom		Post-bloom		Midseason		Harvest
	2008 (7 DAFB)	2009 (6 DAFB)	2008 (32 DAFB)	2009 (31 DAFB)	2008 (49 DAFB)	2009 (52 DAFB)	2008
1. Hôi							
Control	9.1 c[z]	23.7 a	0.9 c	3.8 b	0.3 b	0.4 b	0.3 c
Irrigation	10.5 b	22.8 a	3.2 a	4.6 a	1.7 a	2.8 a	0.8 a
2. Tròn							
Control	9.8 bc	19.2 b	0.8 c	3.1 b	0.1 c	0.3 b	0.1 d
Irrigation	11.4 a	18.2 b	2.5 b	5.0 a	1.3 a	2.4 a	0.7 b

[z] Means followed by the same letter (a – d) in each column do not differ significantly ($P \leq 0.05$).

DAFB = days after full bloom.

2.4.4 PGR application.

Fruit retention at the end of bloom, before PGR treatment, was significantly greater and more variable in 2009 than in 2008 (Table 2.8), indicating naturally, presumably environmentally induced differences. Nevertheless, all PGR applications resulted in greater numbers of fruit per panicle after the midseason drop when compared with the control treatment, regardless of cultivar and year (Table 2.8). This effect was already evident for the cultivar 'Tròn' at the end of the post-bloom drop. Overall, the PGRs increased midseason fruit retention 2-fold in 2008, whereas it was 5- to 10-fold in 2009 compared with the controls. It is noteworthy that NAA and CPPU reduced most effectively the fruit drop, but the combination of both or either of these PGRs with GA did not lead to higher fruit retention. Similar to the irrigation experiment, the cultivar 'Hôi' tended to have higher fruit retention than 'Tròn', particularly in 2009.

Table 2.8. Effect of PGR application for the growing seasons 2008 and 2009.

Treatment	Fruit per panicle						
	Bloom		Post-bloom		Midseason		Harvest
	2008 (4 DAFB)	2009 (6 DAFB)	2008 (32 DAFB)	2009 (31 DAFB)	2008 (49 DAFB)	2009 (52 DAFB)	2008
1. Hôi							
Control	12.3 a[z]	16.0 ab	4.1 a	3.7 c	1.4 d	0.4 d	0.2 c
CPPU	12.6 a	15.1 bc	4.1 a	5.9 a	3.1 b	2.6 a	0.6 b
CPPU+GA_3	12.4 a	15.2 bc	4.7 a	5.3 b	2.6 c	2.5 a	0.3 c
CPPU+GA_{4+7}	11.8 a	17.2 a	3.6 a	3.7 c	2.4 c	0.9 c	0.1 c
NAA	11.5 a	14.0 c	4.0 a	3.0 c	3.6 a	2.3 a	0.8 a
NAA+CPPU +GA_3	12.6 a	14.6 bc	3.3 a	5.9 a	2.6 c	2.4 a	0.6 b
NAA+CPPU +GA_{4+7}	13.0 a	16.5 ab	4.0 a	5.1 b	2.4 c	1.3 b	0.3 c
2. Tròn							
Control	12.3 a	20.1 a	2.6 c	1.6 d	1.6 d	0.1 d	0.3 bc
CPPU	12.2 a	21.1 a	4.1 a	4.8 a	3.5 ab	1.6 a	0.3 c
CPPU+GA_3	12.1 a	15.4 ac	3.8 a	4.5 a	2.6 c	0.9 b	0.3 c
CPPU+GA_{4+7}	12.3 a	17.8 ab	3.3 b	2.5 c	2.7 c	0.5 c	0.4 bc
NAA	11.5 a	19.2 a	4.0 a	3.7 b	3.7 a	1.5 a	0.7 a
NAA+CPPU +GA_3	12.2 a	14.9 bc	3.7 ab	4.0 b	3.0 bc	1.4 a	0.6 ab
NAA+CPPU +GA_{4+7}	12.3 a	15.1 c	3.8 ab	2.7 c	3.0 bc	0.3 cd	0.5 bc

[z] Means followed by the same letter (a – d) in each column and for each variety do not differ significantly ($P \leq 0.05$, n = 30 in 2008; n = 60 in 2009).

DAFB = days after full bloom; CPPU = N-(2-chloro-4-pyridyl)-N′-phenylurea; GA = gibberellin; NAA = 1-naphthaleneacetic acid.

2.5 Discussion

2.5.1 Fruit drop.

The seasonal decrease of fruit retention in mango was described mathematically with a sigmoid function (Fig. 2.1) for two cultivars over several growing seasons. The sigmoidal shape is typical for many biological growth and developmental processes, including mango fruit growth (Ram, 1983). Other mathematical functions, like for example the exponential decay, did not describe adequately the data scattering of fruitlet retention around bloom (Fig. 2.1A) as a result of the number of set fruit approximately equaling the number of abscised fruit until all panicle have fully expanded (Mukherjee,

1953; Singh, 1954). Thereafter, fruit retention decreases with the steepest slope (x_0) at ≈3 weeks after full bloom (Fig. 2.1B), representing the highest daily fruit loss in absolute terms. Approximately 1 to 2 weeks later, the FD_{max} is highest, marking the end of the post-bloom drop stage (Table 2.5) and corresponding well with average cited cessation dates of this drop stage (Table 2.4). By the end of the post-bloom drop, more than two-thirds of all fruit per panicle have dropped, presumably as a result of observed embryo degeneration and parthenocarpic fruit as one causal factor and indeed Singh and Arora (1965) found degenerated ovules (shrivelled seeds) in half of the abscised fruit. However, during midseason drop, they reported hardly any cases of embryo degeneration despite the occurrence of high fruit losses, which amounted to ≈20% to 30% in the current study (Table 2.5). A physiological explanation of the midseason drop might be the shift from cell division during post-bloom drop to cell enlargement in midseason, which is accompanied by increased carbohydrate requirement of the fruit panicles. Defoliation experiments in citrus (Mehouachi et al., 1995; Ruiz et al., 2001) have indicated that the carbohydrate demand during early fruit growth is met by fruit photosynthesis and reserves in the fruiting wood, whereas midseason fruit growth depends increasingly on carbohydrates partitioned from leaves and therefore assimilate import through the fruit peduncle. Moreover, sucrose as the key translocation sugar in many plant species is predominantly transported to tissues with high auxin concentrations as shown, e.g., in ^{14}C labeling experiments (Dhanalakshmi et al., 2003). In mango, the accumulation of fruit auxins starts with the onset of the midseason drop and peaks ≈42 DAFB (Prakash and Ram, 1984), thus presumably acting as a strong sink signal for carbohydrate import into the fruit. Chattha et al. (1999) and Notodimedjo (2000) provide further support of this sink strength notion, showing that fruit losses are more effectively prevented in midseason than in the early season with exogenously applied synthetic auxins. This might be the result of augmenting the flow of endogenous auxin across the performed separation tissue layer of the pedicel beyond a critical fruit drop-inducing concentration (Sexton and Roberts, 1982). During the following preharvest drop stage (Fig. 2.1), the number of fruit per panicle changes little, likely because the carbohydrate demand by the fruit is matched by its supply and fewer environmental stresses occurring at that time of the growing season (Roemer et al., 2011).

In contrast to fruit retention data (Fig. 2.1A), FD(x) results in one to three waves that occur between post-bloom and midseason drop (Figs. 2.1B and 2). This was described earlier for mango as well as for other fruit trees, including litchi and orange (Guzman-

Estrada, 1996; Prakash and Ram, 1984; Yuan and Huang, 1988; Zucconi et al., 1978). Some polyembryonic mango cultivars, like for example 'Hôi' and 'Tròn', are more cold-sensitive than monoembryonic cultivars (Elsheery et al., 2007; Sukhvibul et al., 2000). Consequently, cold-adapted mango cultivars are better suited for cultivation in the subtropical climate of northern Vietnam, where ambient temperatures below 15 °C with "zero growth" in mango (Whiley et al., 1988) occur frequently. However, there was no correlation between FD(x) and high/low ambient temperatures and/or low relative humidity as was proposed as a cause for premature fruit drop in tree crops (Roemer et al., 2011; Yuan and Huang, 1988; Zucconi et al., 1978).

It has been reported that the fruit set at the end of the post-bloom drop is already pre-determining the final yield at harvest (Thimmappaiah and Suman, 1987) and evaluating the data of Notodimedjo (2000) allows the same conclusion. This notion, however, is not in agreement with the current findings and other results in the literature (Guzman-Estrada, 1996; Stino et al., 2011) (Table 2.9). In contrast, fruit retention at the end of the midseason fruit drop stage was in good agreement with that at harvest in 2008 (Table 2.9) and this is confirmed by other studies (Bhuyan and Irabagon, 1993; Guzman-Estrada, 1996; Notodimedjo, 2000). Subsequently, the midseason fruit retention data are useful for yield estimates and evaluating the effect of fruit drop prevention treatments on final yield.

Table 2.9. Correlation coefficients between fruit retention data at the end of post-bloom or mid-season drop with harvest.

		Treat-		Post-bloom		Midseason	
Publication	Cultivar	ment	Sum	DAFB	r^2	DAFB	r^2
Thimmappaiah and Suman, 1987	13	1	13	29[z]	**0.43**[y]	59	0.26
Notodimedjo 2000	1	7	7	35	**0.69**	56	**0.59**
Stino et al. 2011	6	5	30	29	0.15		
Current study (PGR)	2	7	14	32	0.00	49	**0.42**
Current study (irrigation)	2	2	4	32	**0.95**	49	**0.98**
Guzman-Estrada, 1996	5	1	5	39	0.03	64	**0.33**
Bhuyan and Irabagon, 1993	1	12	12	21	0.16	56	**0.83**

[z] Authors refer to "days after fruit set"; thus, to allow a comparison among various studies, 14 days were added to convert the time unit to DAFB according to the time duration cited by Notodimedjo (2000).

y Bold coefficients indicate a moderate or strong correlation.

DAFB = days after full bloom; PGR = plant growth regulator.

2.5.2 Climatic factors.

Early fruit development was characterized by daily fluctuations of cold nights and hot daytime temperatures (Table 2.1) as well as strong, hot, and dry winds as described in detail by Roemer et al. (2011). According to earlier findings, wind velocity is unlikely to provide sufficient force necessary for the detachment of healthy fruit and therefore may only force unhealthy fruit with loosely adhering junctions to drop (Singh et al., 2005; Singh and Arora, 1965). However, dry conditions seem to be detrimental to orchard productivity of mango because irrigation increased fruit retention significantly. The average yield of ≈1 $t \cdot ha^{-1}$ is, however, still much lower compared with the 8 $t \cdot ha^{-1}$ achieved on average internationally (FAOSTAT, 2012) as well as in the key Vietnamese mango production areas of the Mekong Delta and the southeast (IFPRI, 2002).

2.5.3 Cropping system.

The review of Singh et al. (2005) describes that intercropping can induce increased fruit drop in mango. This is not in agreement with the current study, in which a mango–maize intercropping system had no detrimental effects on the number of panicles per tree and number of fruit per panicle when compared with monocropping systems (Table 2.6). This may be explained by farmers paying more attention to tree management practices in intercropping systems, because of more frequent field visits and thus observation time (Roberts-Nkrumah, 2000).

2.5.4 Irrigation.

The positive effect of irrigation on fruit retention (Table 2.7) is in good agreement with other irrigation studies of mango (Larson et al., 1989; Spreer et al., 2009). In the current study, irrigation started before bloom because earlier studies have shown greater flower abundance and fruit set on irrigated than on non-irrigated trees (González et al., 2004). Care was taken that water was not applied too early in the dry season because it was reported that a greater amount of vegetative buds rather than flower buds are produced (Coelho and Borges, 2004). Indeed, the number of fruit per panicle around bloom in 2008 was only approximately half of that in 2009, presumably the result of more precipitation and 3 weeks earlier irrigation commencement in 2008 than in 2009. Alternatively, the yearly fruit retention differences might also be explained by the

formation of more vegetative flushes in 1 year and the irregular bearing habit of mango (Davenport, 2006; Huong, 2010). The differences between irrigated and non-irrigated trees were much higher after midseason drop than post-bloom drop in agreement with the concept that drought causes reduced rates of leaf net photosynthesis and thus carbon supply limitations to the developing fruit particularly in midseason (Damour et al., 2009). In general, irrigation is an effective method for reducing fruit drop. However, the construction of an irrigation system proves to be difficult in the mountainous regions of Sơn La because most orchards are far away from water sources and in most cases there is little infrastructure that would allow long-distance or uphill transport of water (personal communication, participants of the Tú Nang Mango Grower Workshop 2012).

2.5.5 PGR.

The applications of PGRs was also a successful strategy for increasing fruit retention (Table 2.8) and resulted in similar or even higher fruit retention values than the irrigation treatment. The spray application of CPPU between 7 and 12 DAFB was earlier than the use of all other PGRs and the enhancing effect on fruit retention was already noticeable during the post-bloom drop. Because Burondkar et al. (2009) showed a positive effect of CPPU on leaf chlorophyll content in mango, it can be deduced that leaf net photosynthesis and subsequently the amount of carbohydrates available in support of fruit growth were also increased and this in turn might have prevented fruit drop. CPPU, like their natural analogs, is known for promoting cell division and is therefore used for increasing fruit growth, e.g., in kiwi production (Iwahori et al., 1988; Mok and Mok, 2001). Indeed, the harvest in 2008 showed a 20% increased fruit size for CPPU-treated mango when compared with the control fruits (data not shown). In addition, cytokinins promote vascular tissue differentiation, therefore increasing the transport capacity of resources into the fruit, which might have strengthened CPPU-treated fruits and thus reduced fruit drop. At the end of the midseason drop, all PGR treatments had significantly higher fruit retention compared with the control. Nevertheless, CPPU and NAA resulted consistently in the highest midseason fruit retention for both cultivars, but the NAA treatment had the greatest number of fruit per panicle at harvest compared with all other PGR treatments (Table 2.8). Based on these findings, both substances can be recommended to growers as an effective measure for fruit drop prevention.

2.6 Conclusion

The presented mathematical model for the seasonal fruit drop pattern of mango allowed the partitioning into three distinct stages: the post-bloom, the midseason, and the preharvest drop, respectively. Each stage was characterized by specific parameters that explained the variability in fruit drop, induced by cultivar, season, and treatment, and permits the evaluation of the 1) natural variation before the treatment effect during post-bloom drop; 2) treatment efficacy during midseason drop; and 3) yield forecasting at the beginning of the preharvest stage. Moreover, the model proposes a systematic and standardized approach for distinguishing between the drop stages, thus making the comparison between published data more precise and consistent. The results of the experiments lead to useful and applicable general recommendations for mango growers to alleviate fruit drop, particularly under the prevailing growing conditions in northern Vietnam: 1) mango–maize intercropping systems are not disadvantageous over mango monocropping; and 2) pre-bloom irrigation when a required infrastructure is available or, alternatively, spray applications of NAA.

2.7 Notes

Financial support provided by the Deutsche Forschungsgemeinschaft (SFB 564) and the Fiat Panis Foundation is greatly appreciated. We thank Dr. Juan Carlos Laso Bayas for statistical consulting and Dr. Pham T. Huong for her professional advice as well as for facilitating good relationships with local mango grower in the study area. We are grateful to Dr. Patrick Winterhagen for valuable scientific discussion and reading the manuscript.

From a thesis submitted by Michael H. Hagemann as partial fulfillment of the requirements for the PhD degree.

Received for publication July 14, 2014.

Accepted for publication October 12, 2014.

2.8 Literature cited

Asif, M., M. Usman, B. Fatima, M.I. laskani, and M.M. Khan. 2002. Fruit set and drop behaviour of three commercial cultivars of mango. Pak. J. Agri. Sci. 39:129-131.

Benjawan, C., P. Chutichudet, and T. Chanaboon. 2006. Effect of Gibberellin (GA_3) on fruit yield and quality of Kaew mango (*Mangifera indica* L.) cv. Srisaket 007 in Northeast Thailand. Pakistan J. Biol. Sci. 9:1542-1546.

Bhuyan, M.A.J. and J.A. Irabagon. 1993. Effect of fertilizer, potassium nitrate and irrigation on fruit drop of 'Carabao' mango. South Indian Hort. 41:315-321.

Bolker, B.M., M.E. Brooks, C.J. Clark, S.W. Geange, J.R. Poulsen, M.H.H. Stevens, and J.-S.S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127-135.

Burondkar, M.M., B.B. Jadhav, and M.B. Chetti. 2009. Post-flowering morpho-physiological behavior of Alphonso mango as influenced by plant growth regulators, polyamine and nutrients under rainfed conditions. Acta Hort. 820:425-432.

Chattha, G.A., M.A. Anjum, and A. Hussain. 1999. Effect of various growth regulators on reducing fruit drop on mango (*Mangifera indica* L.). Int. J. Agri. Biol. 1:288-289.

Chen, W.-S. 1983. Cytokinins of the developing mango fruit: isolation, identification, and changes in levels during maturation. Plant physiol. 71:356-361.

Clemens, G., S. Fiedler, N.D. Cong, N. Van Dung, U. Schuler, and K. Stahr. 2010. Soil fertility affected by land use history, relief position, and parent material under a tropical climate in NW-Vietnam. Catena. 81:87-96.

Coelho, E.F. and A.L. Borges. 2004. Irrigation and fertirrigation of mango. Acta Hort. 645:119-128.

Dahshan, D.I. and S. Habib. 1985. Seasonal changes in endogenous auxin like substances in relation to fruit drop in mango. Proc. Egypt. Bot. Soc. 4:769-780.

Damour, G., M. Vandame, and L. Urban. 2009. Long-term drought results in a reversible decline in photosynthetic capacity in mango leaves, not just a decrease in stomatal conductance. Tree Physiol. 29:675-684.

Davenport, T.L. 2006. Pruning strategies to maximize tropical mango production from the time of planting to restoration of old orchards. HortScience. 41:544-548.

Dhanalakshmi, R., T.G. Prasad and M. Udayakumar. 2003. Is auxin a diffusible signal mediating abscission of recessive sinks? Plant Sci. 164:689-696.

Elsheery, N.I., B. Wilske, J.-L. Zhang, and K.-F. Cao. 2007. Seasonal variations in gas exchange and chlorophyll fluorescence in the leaves of five mango cultivars in southern Yunnan, China. J. Hort. Sci. Biotechnol. 82:855-862.

FAO, 2006. World reference base for soil resources: A framework for international classification, correlation and communication. World soil resources report No. 103. Food Agric. Organ.

FAOSTAT, 2012. Statistical database of the Food Agric. Organ. 20 Jul 2013. http://faostat.fao.org.

Galán Saúco, V. 1997. Horticultural practices of Mango. Acta Hort. 455:391-400.

Goguey, T. 1997.Architectural approach of the mechanisms of canopy growth and flowering of mango trees. Acta Hort. 455:124-131.

González, A., P. Lu, and W. Müller. 2004. Effect of pre-flowering irrigation on leaf photosynthesis, whole-tree water use and fruit yield of mango trees receiving two flowering treatments. Sci. Hort. 102:189-211.

Guzman-Estrada, C. 1996. Fruit drop and yield of five mango cultivars in Southern Sinaloa. Acta Hort. 455:459-464.

Huong, P.T. 2004. Impacts of thinning and pre-harvest bagging on growth, yield and fruit's appearance of mango grown in Sap Vat commune, Yên Châu district, Sơn La province. J. Sci. Dev. 5:234-328.

Huong, P.T. 2010. Some measures for rehabilitation of neglected mango orchards in Yên Châu, Sơn La. J. Sci. Dev. 8:69-75.

Hernández Delgado, P. M., M. Aranguren, C. Reig, D. Fernández Galván, C. Mesejo, A. Martínez Fuentes, V. Galán Saúco and M. Agusti. 2011. Phenological growth stages of mango (*Mangifera indica* L.) according to the BBCH scale. Sci. Hort. 130:536-540.

IFPRI, 2002. Fruits and vegetables in Vietnam: Adding value from farmer to consumer. Int. Food Policy Res. Inst. Washington D.C. USA.

Issarakraisila, M. and J.A. Considine. 1994. Effects of temperature on pollen viability in mango cv. 'Kensington'. Ann. Bot. 73:231-240.

Iwahori, S., S. Tominaga, and T. Yamasaki. 1988. Stimulation of fruit growth of kiwifruit, *Actinidia chinensis* Planch., by N-(2-chloro-4-pyridyl)-N'-phenylurea, a diphenylurea-derivative cytokinin. Sci. Hort. 35:109-115.

Lakshminarayana, S. and P.H. Aguilar. 1975. Effect of orchard heating in reducing parthenocarpic fruit in 'Haden' mango. Proc. Fl. St. Hort. Soc. 88:502-505.

Lam, P.F., K.H. Ng, D. Omar, and Y. Talib. 1985. Fruit-drop and growth, respiration and chemical changes in 'Golek' mango. Malaysian Agr. Res. Dev. Inst. bul. Penyelidikan. 13:8-14.

Larson, K.D., B. Schaffer, and F.S. Davies. 1989. Effect of irrigation on leaf water potential, growth and yield of mango trees. Proc. Fl. St. Hort. Soc. 102:226-228.

Malik, A.U. and Z. Singh. 2003. Abscission of mango fruitlets as influenced by biosynthesis of polyamines. J. Hortic. Sci. Biotechnol. 78:721-727.

Mehouachi, J., D. Serna, S. Zaragoza, M. Agusti, M. Talon, and E. Primo-Millo. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. Plant Sci. 107:189-197.

Mok, D.W.S.and M.C. Mok. 2001. Cytokinin metabolism and action. Annu. Rev. Plant Physiol. Mol. Biol. 52:89-118.

Mukherjee, S. 1953. The mango - Its botany, cultivation, uses and future improvement, especially as observed in India. Econ. Bot. 7:130-162.

Musvoto, C. and B. Campbell. 1995. Mango trees as components of agroforestry systems in Mangwende, Zimbabwe. Agroforest. Syst. 32:247-260.

Núñez-Elisea, R. and T.L. Davenport. 1983. Abscission and ethylene production in mango (*Mangifera indica* L.) fruit cv. Tommy Atkins. Proc. Fla. Hort. Soc. 96:185-188.

Notodimedjo, S. 2000. Effect of GA_3, NAA and CPPU on fruit retention, yield and quality of mango (cv. Arumanis) in East Java. Acta Hort. 509:587-600.

Oosthuyse, S.A. 1993. Effect of spray application of KNO_3, urea and growth regulators on the yield of 'Tommy Atkins' mango. South African Mango Grow. Assn Yrbk. 13:58-62.

Oosthuyse, S.A. 1995. Effect of post-bloom aqueous spray application of GA_3, NAA, and CPPU on fruit retention, fruit size and yield in Tommy Atkins and Heidi mango. South African Mango Grow. Assn. Yrbk. 15:31-33.

Prakash, S. and S. Ram. 1984. Naturally occurring auxins and inhibitor and their role in fruit growth and drop of mango 'Dashehari'. Sci. Hort. 22:241-248.

Ram, S. 1983. Hormonal control of fruit growth and fruit drop in mango cv. 'Dashehari'. Acta Hort. 134:169-178.

Roberts-Nkrumah, L.B. 2000. The establishment and growth of young mango trees in on-farm hillside trials in Trinidad, W.I. Acta Hort. 509:705-712.

Roberts-Nkrumah, L.B. 2004. Evaluation of young fruit tree performance in hillside trials in Trinidad and Tobago. Acta Hort. 638:459-464.

Roemer, M.G., M. Hegele, J.N. Wünsche, and P.T. Huong. 2011. Possible physiological mechanisms of premature fruit drop in mango (*Mangifera indica* L.) in Northern Vietnam. Acta Hort. 903: 999-1006.

Ruiz, R., A. García-Luis, C. Monerri, and J.L. Guardiola. 2001. Carbohydrate availability in relation to fruitlet abscission in citrus. Ann. Bot. 87:805-812.

Sexton, R., Roberts, J.A., 1982. Cell biology of abscission. Ann. Rev. Plant Physiol. 33, 133-162.

Spreer, W., S. Ongprasert, M. Hegele, J.N. Wünsche, and J. Müller. 2009. Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes. Agr. Water. Manage. 96:574-584.

Singh, R.N. 1954. Studies on the floral biology and subsequent developments of fruits in the mango (*Mangifera indica* L.) varieties of Dashehari and Langra. Indian J. Hort. 11:69-88.

Singh, R. N., P. K. Majumdar, and D. K. Sharma. 1966. Sex-expression in mango (*Mangifera indica* L.) with reference to prevailing temperature. Proc. Amer. Soc. Hort. Sci. 89:228-229.

Singh, R.N. and K.S. Arora. 1965. Some factors affecting fruit drop in mango (*Mangifera indica* L.). Indian J. Hort. 35:196-205.

Singh, Z., A. Malik, and T.L. Davenport. 2005. Fruit drop in mango. Hort. Rev. 31:111-153.

Singh, Z. 2009. Gibberellin type and time of application influence fruit set and retention in mango. Acta Hort. 820:407-412.

Stino, R.G., S.A.M.A. El-Wahab, and S.A. Habashy and R.A. Kelani. 2011. Productivity and fruit quality of three mango cultivars in relation to foliar sprays of calcium, zinc, boron or potassium. J. Hort. Sci. Ornam. Plant. 3:91-98.

Sukhvibul, N., A.W. Whiley, M.K. Smith, and S.E. Hetherington. 2000. Susceptibility of mango (*Mangifera indica* L.) to cold-induced photoinhibition and recovery at different temperatures. Aust. J. Agric. Res. 51:503-513.

Thimmappaiah and C.L. Suman. 1987. Sex ratio in relation to fruit-set and fruit yield in mango. Punjab Hort. J. 27:8-11.

Verheij, E.W.M. 1986. Towards a classification of tropical fruit trees. Acta Hort. 175:137-150.

Whiley, A.W., J.B. Saranah, T.S. Rasmussen, E.C.Winston, and B.N. Wolstenholme. 1988. Effect of temperature on growth of ten cultivars of mango with relevance to production in Australia. Proc. 4th Austral. Conf. Tree and Nut Crops: 176-185.

Whiley, W.A, Searle, C, Schaffer, B, Wolstenholme, and N. B. 1999. Cool orchard temperatures or growing trees in containers can inhibit leaf gas exchange of avocado and mango. J. Amer. Soc. Hort. Sci. 124:46-51.

Wünsche, J.N. and Ferguson I.B. 2005. Crop Load Interactions in Apple. Hort. Rev. 31:231-290.

Yamada, F., H. Fukamachi, and T. Hidaka. 1996. Photosynthesis in longan and mango as influenced by high temperatures under high irradiance. J. Jpn. Soc. Hort. Sci. 64:749-756.

Yên Châu, 2008. Statistical Data of Yên Châu District. Statistical Department, unpublished.

Young, A. 1989. Agroforestry for soil conservation. CAB International. Wallingford, UK.

Yuan, R., and H. Huang. 1988. Litchi fruit abscission: its patterns, effect of shading and relation to endogenous abscisic acid. Sci. Hort. 36:281-292.

Zucconi, F., S.P. Monselise, and R. Goren. 1978. Growth abscission relationships in developing orange fruit. Sci. Hort. 9:137-146.

3. Ethephon induced abscission of mango fruitlets − physiological fruit pedicel response[3]

3.1 Abstract

Fruitlet abscission is a severe problem in mango (*Mangifera indica*) production; especially in the mountainous area of North Vietnam where hot and dry winds during long periods of drought cause severe premature fruit loss. Low rates of leaf net photosynthesis of averagely 8 µmol CO_2/m^2*s are typical for drought stressed mango trees. Presumably due to the declined carbon assimilation, fewer carbohydrates are available for fruit growth and development. Such stress can induce the fruit abscission process that involves an ethylene dependent signal cascade, including the examined ethylene receptors *MiETR-1* and *MiERS-1*, sequence homologues to Arabidopsis *ETR1* and *ERS1*, respectively. In the current study, ethephon spray applications on single clusters were used to induce artificially the abscission process in mango, leading to a rapid reduction of the fruit detachment force (FDF) and subsequent high rate of fruit drop. Both ethylene receptors showed a different expression pattern in the pedicel abscission zone in response to the ethephon treatment, whereas transcript levels in the untreated control remained unchanged. A high proportion (70%) of the variation in FDF of visually healthy appearing fruit in both treatments was explained by fruit length and pedicel diameter. The results clearly indicate, that the up-regulation of the examined ethylene receptors leads to a reduction of FDF and subsequently measured fruit drop.

3.2 Introduction

The global increase in mango production of 30% from 1999 to 2009 was overall achieved by increasing the production area but not by higher yields (FAO, 2010). While in some regions yields are much higher than the global average of 8 t/ha (FAO, 2010), low yields with less than 4 t/ha occur e.g. in commercial orchards in Australia (González et al., 2004). Low yields are also typical for mango production in the region of Son La province in North Vietnam. One of the main local cultivars is 'Hoi', which is

[3]This chapter consist of the correspondent article which was accepted and published by the journal *Acta Horticulturae*. The post-print version of the article is reproduced with the kind permission of the International Society of Horticultural Science, Leuven, Belgium. This article should be cited as: Hagemann, M.H., Winterhagen, P., Hegele, M., and Wünsche, J.N. 2015. Ethephon induced abscission of mango fruitlets - physiological fruit pedicel response. *Acta Horticulturae* 1066**,** 109-116. (http://www.actahort.org/books/1066/1066_11.htm)

heavily affected by low yields but has great economic potential due to its regional popularity (Huong, 2010). In general, causes of low yields are diseases, pests or severe climatic conditions often leading to fruit drop. Fruit drop during early developmental stages is a common phenomenon for mango and often referred to as *initial*, *post-setting* or *post-bloom drop* (Anila and Radha, 2003; Singh et al., 2005). Huong (2010) suggested poor mango orchard management and in particular insufficient pest management as one cause for the post-bloom drop of 'Hoi' mango. In addition, climatic factors like extremely high temperatures and dry winds occurring during the period of early fruit development are thought to be additional stressors causing the abscission of premature fruitlets (Roemer et al., 2011). A reduced carbohydrate supply to fruitlets has been proposed as a crucial factor for early drop in general (Brown, 1997). Such carbohydrate shortage might be caused by a decline in leaf photosynthesis as has been reported for mango under drought conditions (Elsheery et al., 2007). For citrus, as example, successive defoliation and thus reduced photosynthesis clearly reduced carbohydrate availability to fruitlets (Gomez-Cadenas et al., 2000). The general model for abscission of generative organs as reviewed by van Doorn and Stead (1997) assumes, that the sensitivity to ethylene is suppressed by an auxin controlled mechanism, which depends on the intensity of auxin efflux from a healthy organ, such as fruitlets, leaves or flowers. A reduced auxin flow leads to an increased sensitivity for ethylene in a layer of predetermined cells within the fruit stalk called abscission zone. The abscission process is activated beyond a certain ethylene concentration (Brown, 1997). The activation starts by binding of ethylene to ethylene responding receptors which leads to the inactivation of the ethylene response inhibition (Binder, 2008). Concerning the abscission process, the ethylene response includes transcription factors which induce cell re-differentiation as well as production and secretion of cell wall degrading enzymes that lead to fruit loosening and thus fruit drop (Binder, 2008; Roberts et al., 2002). The up-regulation of transcript levels of specific ethylene responding receptors is an early signal for ethylene response activation and has been reported also for apple and peach (Dal Cin et al., 2008; Rasori et al., 2002).

In the present study net leaf photosynthesis was measured as net carbon exchange rate (NCER) to determine whether mango leaf CO_2 assimilation is reduced to an extent that is typical for mango under severe climatic conditions. Moreover, abscission related ethylene receptors and their timely pattern of expression during abscission were examined. Finally, fruit parameters were evaluated which may help to distinguish healthy appearing and about-to-abscise fruit.

3.3 Material and methods

3.3.1 Plant material

The study was conducted on two commercial orchards near the township of Yen Chau in the Tu Nang commune (Son La Province, Vietnam). Twelve-year old mango trees, cv. 'Hoi', were used in the experiment. All trees received the same fertilizer and plant protection management. Three trees were used for the first experiment conducted on one orchard with application at the 2 February 2010. For the second experiment conducted on the second orchard at 3 March 2010 the number of trees was extended to six due to a reduced crop load as result of fruit drop. Spray solutions of either Ethephon (Flordimex 420, 17.14 ml/l, 7200 ppm) or water control were applied to 30 panicle clusters per treatment, whereas 10 and 5 clusters were sprayed per tree in the first and second experiment, respectively. Six fruits each from six different panicles were picked two days before treatment and one, three and five days after.

3.3.2 Fruit and climate measurements

Fruit detachment force was recorded with a force gauge PCE-FM50 (PCE, Germany). The diameter, length and weight of each sample fruit as well as pedicel diameter were measured. Relative humidity and temperature was recorded using 12 HOBO v2 data loggers (Onset, USA), installed at upper and lower part in northern and southern directions on four representative trees on one of the experimental orchards.

3.3.3 Leaf net carbon exchange rate

Saturated NCER ($\geq$800 $\mu mol/m^2*s$) of healthy and fully expanded leaves was measured during early fruitlet development between 25 January and 11 February in 2010 at ambient air temperatures of about 15, 20, 25 and 30°C. Measurements were conducted using a Parkinson leaf chamber connected to a CIRAS-1 unit (PP-Systems, USA). CO_2 concentration was set to 380 ppm and water vapor pressure was adjusted to ambient conditions. At each measure date four leaves on each of four trees were recorded.

3.3.4 RNA extraction and gene expression analysis by quantitative real-time PCR

A 4 mm fragment of the pedicel including the abscission zone were cut using a double razor blade and samples were snap frozen in liquid nitrogen. Frozen fruit stalks were

ground under liquid nitrogen to fine powder of which 100 mg subsamples were taken for extractions. RNA was extracted with MasterPure Plant RNA Purification Kit (Epicentre, USA) following the manufacturer´s recommendations with the modification of adding polyvinylpolypyrrolidone (PVPP) to the plant lysis solution in order to reduce influence of phenolic compounds. The successful elimination of genomic DNA was tested by using the extracts as template in a standard PCR with actin primers. RNA concentration was measured with the Nanodrop 1000 photospectrometer (Thermo Fischer, USA) and RNA quality was checked by standard gel electrophoresis. RNA samples were stored at -80°C until further analysis. The cDNA synthesis from mRNA was conducted with TaqMan Reverse Transcription Kit (Applied biosystems, USA) following the manufacturer´s protocol. For cDNA synthesis 500 ng of RNA was used in each reaction. Quality of cDNA was tested by quantitative real-time PCR (qPCR) with actin specific primers. cDNA was stored at -20°C until further analysis.

Primers for the MiETR1 were designed based on a mango ETR1 sequence (Genbank ID: AF227742.1). Conserved regions of ERS-like sequences from woody plants and Arabidopsis were identified by alignments to design degenerated primers. Nested PCRs were performed to verify sequence specificity before cloning. The PCR products were ligated into the pGEM-T vector (Promega, VIC, Australia) following the manufacturer´s recommendations. Colony PCR with gene specific primers was performed to verify positive clones for subsequent plasmid extraction (QIAPrep Miniprep, Qiagen, Germany) and sequencing (GATC, Germany).

Real-time PCR was performed with a Rotor-Gene 6000 cycler (Corbett, Australia) using the following conditions: initial denaturation for 3 min at 95°C, followed by 40 cycles of 20 s at 95°C (denaturation), 20 s at 58°C (annealing) and 20 s at 72°C (extension), followed by a melt curve from 60°C to 99°C in 0,5°C steps. The fragment size of the PCR product was checked by standard gel electrophoresis. Primer efficiency was calculated and verified according to Peirson et al. (2003). Relative expression (Delta Delta C_T) of the target genes MiETR1 and MiERS1 were analyzed with the Rotor-Gene 6000 real-time analyzer software v1.7 (Corbett, Australia).

3.3.5 Statistical analysis

Statistical analysis was conducted with SPSS 17,0 (IBM, USA). All measured and analyzed parameters were included for a cluster analysis for the identification of health-characteristics. The cluster of data sets corresponding to healthy-appearing fruits was

then used for multiple linear regression analysis with FDF as depending variable. The resulting equation was used for FDF prediction.

3.4 Results

3.4.1 Environmental conditions during natural fruit drop

The examined time period ranged from full bloom (>90% of the panicles had >80% of the buds are flowering) to the end of post-bloom drop. Three periods of high day temperatures and peak temperatures above 40°C were interrupted by two cold snaps with temperatures below 13°C (Fig. 3.1). One week after full bloom the fruit set was averagely eight fruits per panicle. Fruit number declined rapidly after the first cold snap to one fruit at the beginning of April, representing the end of the period of severe premature fruit losses.

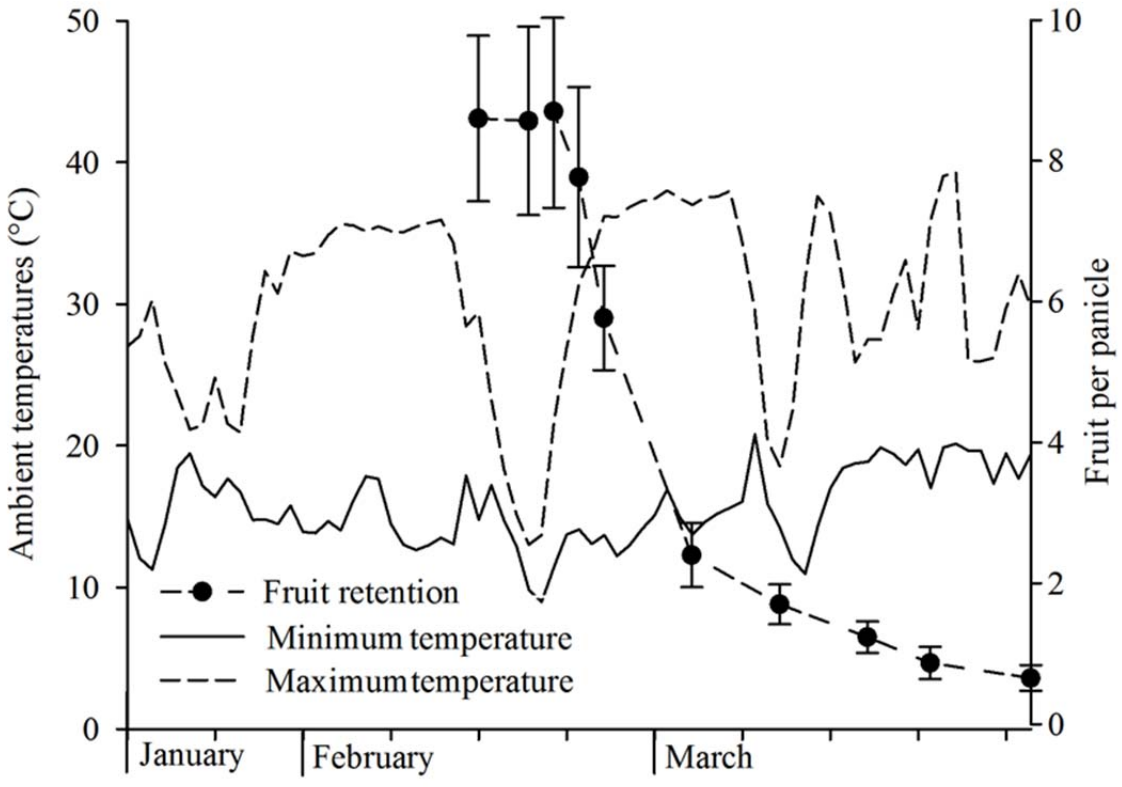


Fig. 3.1. Average maximum and minimum temperature during the flowering and early fruit development period in 2010. Black dots indicate fruit counts of control treatments starting one week after full bloom (7 February 2010). Error bars indicate SEM.

3.4.2 Leaf net carbon exchange rate

The average NCER during flowering was 8 µmol CO_2/m^2*s (Fig. 3.2). Leaf CO_2 assimilation and stomatal conductance was not affected by leaf temperature (Fig. 3.2). However, highest stomatal conductance was found at the lowest leaf temperature.

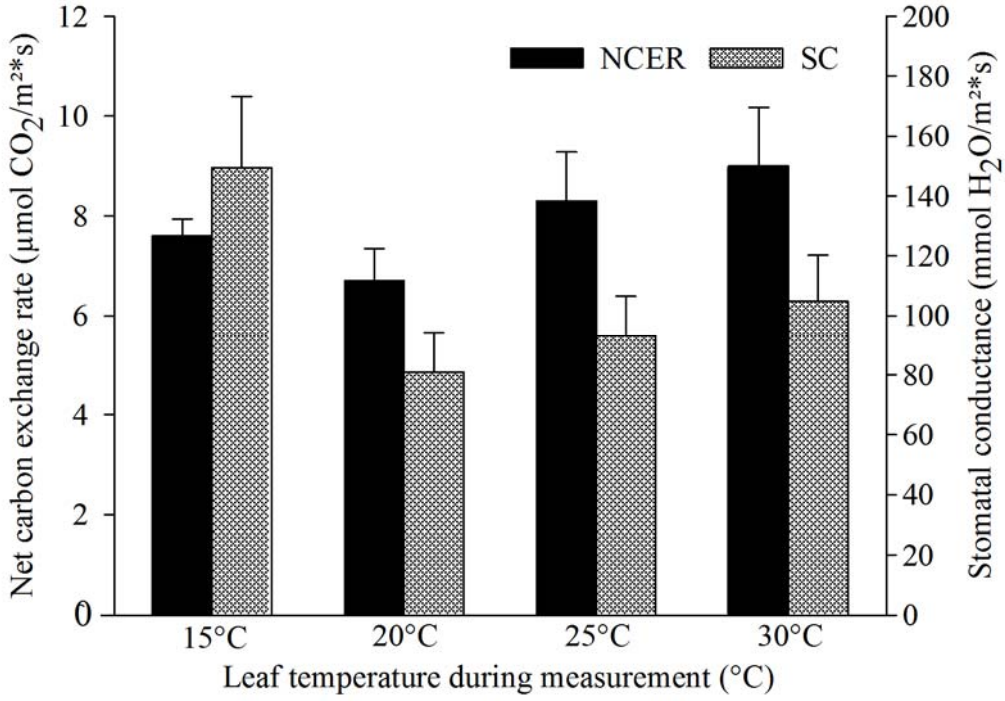


Fig. 3.2. Net carbon exchange rate (NCER) and stomatal conductance (SC) at four temperatures during flowering in 2010.

3.4.3 Effectiveness of ethephon as fruit drop inducer

The application of Ethephon to fruitlets of 5 mm in diameter resulted in a rapid decline of fruit retention, resulting in less than 1 fruit per panicle after 1 week and total fruit loss after two weeks (Fig. 3.3). Ethephon application to 11 mm large fruitlets resulted in an even faster drop and depleting within one week. Control panicles treated with water yielded in one fruit per panicle at early and late application time.

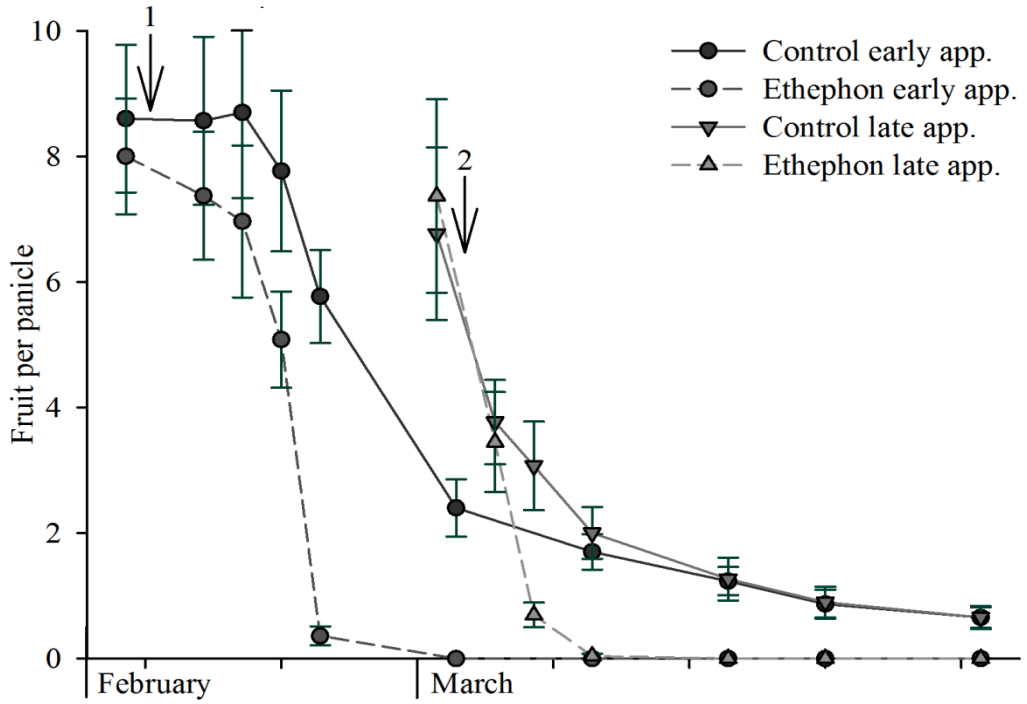


Fig. 3.3. Fruit retention during early fruit development in 2010 with application of Ethephon and control treatments at an early (arrow 1) and a late application date (arrow 2). Error bars represent SEM.

3.4.4 Ethylene receptors and their response to ethephon

The isolated partial sequences for MiETR1 and MiERS1 of mango cv. 'Hoi' were analysed by BLAST search and partial protein sequence alignment with the Arabidopsis ethylene receptors resulted in an amino acid homology of 64% and 70% for the MiETR1 and MiERS1, respectively (Fig. 3.4). The alignment with other woody plants showed high amino acid homologies among all tested sequences. The Ethephon treatment did not lead to a *MiETR1* up-regulation at any date after treatment. In contrast the *MiERS1* showed a several fold transcript up-regulation one day after the Ethephon treatment. This high transcript accumulation of the *MiERS1* was reduced again to the initial level 3 and 5 days after treatment (data not shown).

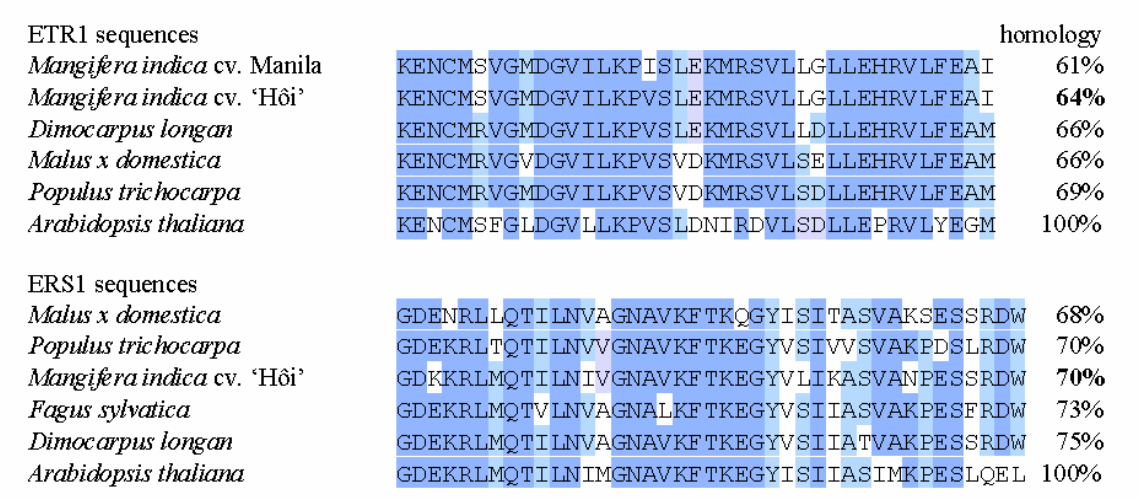


Fig. 3.4. Alignment of partial ETR1 and ERS1-like protein sequences with indicated homology relative to the Arabidopsis sequence.

3.4.5 Fruit detachment force

The FDF was not significant declined one day after the Ethephon treatment. However, FDF was nearly entirely lost at day 3 and day 5 after application (Fig. 3.5). Statistical cluster analysis of all data sets and by including all parameters from individual fruits helped to classify a data set of healthy appearing fruits. From linear regressions made with all parameters of the healthy appearing fruit cluster the combination of pedicel diameter and fruit length could describe best the variation in FDF (70%). The equation of the linear regression is

FDF = (Length * 27,21 + Pedicel diameter * 299,233 - 170,27).

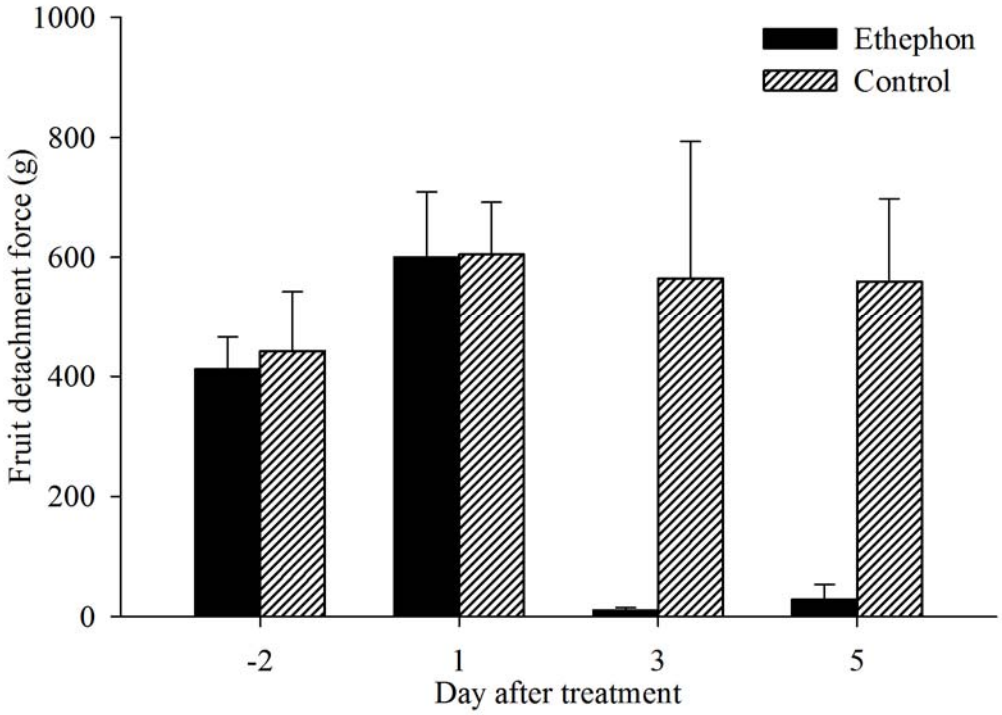


Fig. 3.5. Results of FDF measurements from the first experiment. Average FDF is shown at 2 days before and 1, 3 and 5 days after application of Ethephon and control treatments.

3.5 Discussion

The main features of the climate during early fruit development in Son La Province is drought along with alternating ambient temperature extremes as recorded in 2011 (Fig. 3.1) and found with previous climate records (Roemer et al., 2011). These climatic conditions are assumed to be the initial cause for the heavy fruitlet drop after flower abscission in mid-February to only one fruit at the end of March in 2011. Drop pattern from eight to one fruits per panicle has previously been reported for several mango cultivars and usually continues with a less intense drop at the late fruit development until harvest (Roemer et al., 2011; Singh and Arora, 1965; Singh et al., 2005). Whereas Roemer et al. (2011) and Huong (2010) point out the combination of drought and hot weather as main abiotic stressors, the occurring of cold snaps should also be taken into account as a critical factor. Mango as a chilling-sensitive crop has been shown to respond to cold conditions with a medium to long term decline in leaf photosynthesis (Whiley et al., 1999; Allen et al., 2000) especially when combined with extended drought periods (Elsheery et al., 2007). Leaf NCER measurement of 'Hoi' mango during dry season corresponds to results of several mango varieties that showed a

reduction from 18 µmol CO_2/m^2*s during rainy season compared to about 9 µmol CO_2/m^2*s during dry season (Elsheery et al., 2007). Therefore, a reduced photosynthesis rate for 'Hoi' mango under abiotic stress suggests reduced carbohydrate availability for fruitlets and thus inducing fruit abscission.

Ethephon was used to induce fruit drop at early fruit developmental stages, allowing the analysis of the abscission mechanism within the range of closely attached to about-to-abscise mango fruit pedicels. The ethylene receptor MiETR1 plays a role in wounding and ripening (Martínez et al., 2001), but did not show any response at the transcript level to the Ethephon treatment. However, MiERS1 revealed a strong up-regulation in response to the Ethephon treatment suggesting a prevailing role in the fruit abscission mechanism. Ish-Shalom et al. (2011) showed similar results for the mango cv. 'Kent' by using a less than a tenth of the Ethephon concentration used in their study. Because monoembryonic varieties as 'Kent' (Crane and Campbell, 1994) are less related to polyembryonic cultivars as 'Hoi', results suggest that these early steps in abscission are conserved throughout the species *Mangifera indica*.

The Ethephon application was defined as starting point for inducing abscission, therefore, the sequence of events related to ethylene receptor regulation and FDF were measured. An almost complete loss of FDF was found 3 days after treatment (Fig. 3.5), however, FDF was not yet reduced 1 day after treatment when MiERS1 was already up-regulated. This indicates that MiERS1 is regulated before the abscission zone is activated, suggesting that this gene is an early abscission factor in the abscission process and up-stream of cell separation. The FDF prediction might be used in future applications as a fruit parameter to define fruits as either about-to-abscise or closely attached "healthy" fruits.

3.6 Conclusion

In mango cv. 'Hoi' the post-bloom fruit drop is reliably inducible by the plant growth regulator Ethephon, leading to the up-regulation of the ethylene receptor MiERS1. It is proposed that this receptor plays a role up-stream of the cell separation process within the activated abscission zone, leading to a reduced FDF and subsequent fruit drop. FDF approximation can be used as further criteria for describing the sequence of events during post-bloom mango fruit drop.

3.7 Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft (DFG) through the Sonderforschungsbereich 564 (The Upland Program) is greatly appreciated.

3.8 References

Anila, R. and Radha, T. 2003. Studies on fruit drop in mango varieties. J. Trop. Agr., 41:30-32.

Allen, D.J., Ratner, K., Giller, Y.E., Gussakovsky, E.E., Shahak, Y. and Ort, D.R. 2000. An overnight chill induces a delayed inhibition of photosynthesis at midday in mango (*Mangifera Indica* L.). J. Exp. Bot., 51:1893-1902.

Binder, B.M. 2008. The ethylene receptors: Complex perception for a simple gas. Plant Science, 175:8-17.

Brown, K.M. 1997. Ethylene and abscission. Physiol. Plant., 100:567-576.

Crane, J.H. and Campbell, C.W. 1994. The mango. Fact sheet, Horticultural Sciences Department, Florida cooperative extension service, Institute of Food and Agricultural Sciences, University of Florida, HS-2:1-6.

Elsheery, N.I., Wilske, B., Zhang, J.-L. and Cao, K.F. 2007. Seasonal variations in gas exchange and chlorophyll fluorescence in the leaves of five mango cultivars in southern Yunnan, China. J. Hortic. Sci. Biotech., 82:855-862.

Ish-Shalom, M., Dahan, Y., Maayan, I. and Irihimovitch, V. 2011. Cloning and molecular characterization of an ethylene receptor gene, MiERS1, expressed during mango fruitlet abscission and fruit ripening. Plant Physiol. Biochem., 49:931-936

Dal Cin, V., Danesin, M., Botton, A., Boschetti, A., Dorigoni, A. and Ramina, A. 2008. Ethylene and preharvest drop: the effect of AVG and NAA on fruit abscission in apple (*Malus domestica* L. Borkh). Plant Growth Regul., 56:317-325.

FAO, 2010. Food Agriculture Organization of the United Nations. FAOSTAT database collection. http://faostat.fao.org

Gómez-Cadenas, A., Mehouachi, J., Tadeo, F.R., Primo-Millo, E. and Talon, M. 2000. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. Planta, 210:636-643.

González, A., Lu, P. and Müller, W. 2004. Effect of pre-flowering irrigation on leaf photosynthesis, whole-tree water use and fruit yield of mango trees receiving two flowering treatments. Sci. Hort., 102:189-211.

Huong, P.T. 2010. Some measures for rehabilitation of neglected mango orchards in Yen Chau, Son La. J. Sci. Dev., 8:69-75.

Martínez, P.G., Gómez, R.L. and Gómez-Lim, M.A. 2001. Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. J. Plant Physiol., 158:101-108.

Peirson, S.N., Butler, J.N. and Foster, R.G. 2003. Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Res., 31:e73.

Rasori, A., Ruperti, B., Bonghi, C., Tonutti, P. and Ramina, A. 2002. Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. J. Exp. Bot., 53:2333-2339.

Roberts, J.A., Elliott, K.A. and Gonzalez-Carranza, Z.H. 2002. Abscission, dehiscence, and other cell separation process. Annu. Rev. Plant Bio., 53:131-158.

Roemer M.G., Hegele, M., Huong, P.T. and Wünsche, J.N. 2011. Possible Physiological Mechanisms of Premature Fruit Drop in Mango (*Mangifera indica* L.) in Northern Vietnam. Acta Hort., 903:999-1006.

van Doorn, W.G. and Stead, A.D. 1997. Abscission of flowers and floral parts. J. Exp. Bot, 48:821-837.

Singh, R. and Arora, K.S. 1965. Some factors affecting fruit drop in mango (*Mangifera indica* L.). Indian J. Agric. Sci., 35:196-205.

Singh, Z., Malik, A.U. and Davenport, T.L. 2005. Fruit drop in mango. Hort. Rev., 31:111-153.

Whiley, A, W., Searle, C, Schaffer, B. and Wolstenholme, B.N. 1999. Cool orchard temperatures or growing trees on containers can inhibit leaf gas exchange of avocado and mango. J. Am. Soc. Hortic. Sci., 124:46-51.

4. Ethephon induced abscission in mango: physiological fruitlet responses[4]

4.1 Abstract

Fruitlet abscission of mango is a severe production problem worldwide. A detailed physiological and molecular characterization of fruitlet abscission in mango is required to describe the onset and time-dependent course of this process. To identify the underlying key mechanisms of abscission, ethephon was applied at two concentrations (600 ppm, 7200 ppm) during the mid-season drop stage of mango. Both ethephon treatments (ET) reduced significantly the capacity of polar auxin transport (PAT) in the pedicel at 1 day after treatment (DAT) and thereafter compared to untreated pedicels. The transcript levels of the ethylene receptor genes *MiETR1* and *MiERS1* were significantly upregulated already at 1 DAT in the ET7200 while only at 2 DAT in the ET600 when compared to the control fruitlets. Specifically, a significant increase of *MiETR1* in the pericarp at 2 DAT and of *MiERS1* in the pedicel at 2 and 3 DAT was induced by ET600. In contrast, both genes were significantly upregulated by ET7200 immediately at 1 DAT and thereafter, except *MiETR1* in the pedicel. Moreover, two novel short versions of the *MiERS1* were identified and were detected more often in the pedicel of treated than untreated fruitlets at all sampling times. Sucrose concentration in the fruitlet pericarp was not affected by both ethephon treatments at 1 DAT while it was significantly lower at 2 DAT than in control fruitlets. However, at 3 DAT, the sucrose concentration remained low in the ET7200 but was similar to the control fruitlets in the ET600. In conclusion, it is postulated that the ethephon-induced abscission process commences with a reduction of the PAT capacity in the pedicel, followed by an upregulation of ethylene receptors and finally a decrease of the sucrose concentration in the fruitlets.

4.2 Introduction

Fruit drop is a yield-limiting factor for the production of several specialty crops, for example sweet cherry (Blanusa et al., 2005), litchi (Kuang et al., 2012), or mango (Singh et al., 2005). Plant organ shedding or abscission is a highly coordinated process

[4]This chapter consist of an unpublished manuscript that has been submitted to the journal Frontiers in Plant Science.

governed by the interplay of several plant metabolites, in particular phytohormones, carbohydrates and polyamines (Sexton and Roberts, 1982; Malik and Singh, 2003; Xie et al., 2013). Understanding the regulation of genes encoding proteins for synthesis, perception, and transport of these metabolites and subsequently induced physiological changes at cellular level associated with the abscission process is of paramount importance for increasing the productivity of horticultural crops. This fundamental knowledge can be specifically utilized for devising practical solutions, ranging from marker-assisted genotype selection to crop management strategies using for example effective and growth stage dependent applications of plant growth regulators and irrigation water (Estornell et al., 2013).

Of particular concern in many mango production systems worldwide is the extensive fruitlet drop. This major production constraint has been extensively studied at the orchard level (Singh et al., 2005) and was also a key research objective by Hagemann et al. (2014) who investigated the potential use of plant growth regulators, irrigation techniques and cropping systems for improving fruit retention in mango. Both, biotic and abiotic factors have been frequently suggested as the key triggers for inducing fruitlet drop in mango (Singh et al., 2005). Biotic factors are mainly the lack of pollination or fertilization of flowers and pest or disease pressure that subsequently lead to seed degeneration (Singh and Arora, 1965; Estornell et al., 2013). Abiotic factors associated with fruitlet drop are extensive drought periods, extreme ambient air temperatures or dry and strong winds (Burondkar et al., 2000; Singh et al., 2005; Hagemann et al., 2014; Hagemann et al., 2015). These factors likely reduce the auxin efflux from as well as the carbohydrate influx to the fruitlet, thus the demand of the growing fruitlets could not be sufficiently matched by its supply (Wünsche and Ferguson, 2005; Estornell et al., 2013). This was shown for example in litchi, where branch girdling and defoliation, clearly limiting the carbohydrate supply to the fruitlets, resulted in a decrease of fruitlet auxin concentration which in turn led to abscission (Kuang et al., 2012). This result supports the theory for mango that a reduced basipetal transport of seed-derived auxin through the pedicel (Chacko et al., 1970; Prakash and Ram, 1984; Roemer et al., 2011) and the subsequently increased sensitivity for ethylene in the pedicel abscission zone (AZ) induces fruitlet abscission (Estornell et al., 2013).

Ethylene is perceived by binding to two sub-families of specific ethylene receptors which control the downstream signal cascade (see reviews of Binder, 2008 and Stepanova and Alonso, 2009). Five ethylene receptors have been identified in the model

plant arabidopsis (Binder, 2008) and homologous genes were subsequently described for several crop plants, e.g. six receptors in tomato (Alexander and Grierson, 2002), nine in apple (Ireland et al., 2012), and at present two in mango (Martínez et al., 2001; Ish-Shalom, et al., 2011). Based on assessing the triple-response to varying degrees of ethylene perception of arabidopsis mutants, it was found that a malfunction of one or more receptors can mostly be compensated by the other receptors, however, double mutants of the receptors *ETHYLENE RESISTANT 1* (*AtETR1*) and *ETHYLENE RESPONSE SENSOR 1* (*AtERS1*) exhibits the most severe deficiencies (Binder, 2008). These experiments on receptor functionality led to the development of a hierarchical model resulting in *AtETR1* and *AtERS1* being the predominant receptors. The plant ethylene response is regulated by receptor specific elements, as for example the *REVERSION-TO-ETHYLENE SENSITIVITY 1* (*AtRTE1*) that exclusively modulates the function of the *AtETR1* (Shakeel et al., 2013), or by receptor-receptor interaction through building homo- and heterodimers or clusters of higher complexity (Gao et al., 2008). Given the numerous regulatory mechanisms of the ethylene response, it is surprising that increased ethylene production in horticultural fruit trees, induced by wounding, girdling or ethephon application, results in similar patterns of receptor transcription. It is remarkable that fruitlet and mature fruit abscission seems always associated with a strong upregulation of *ERS1* but not of *ETR1* in pedicels of mango (Ish-Shalom et al., 2011), orange (John-Karuppiah and Burns, 2010), peach (Rasori et al., 2002), and apple (Dal Cin et al., 2008).

Ethephon is an ethylene releasing chemical and commonly used to induce thinning of fruitlets or to facilitate the fruit harvesting process (Dennis, 2000; Ish-Shalom et al., 2011; John-Karuppiah and Burns, 2010). In the presence of ethylene, the cells within the fruit pedicel AZ produce cell wall degrading enzymes, thereby inducing the disintegration of the separation layer in the AZ and ultimately leading to the drop of the fruit (Leslie et al., 2007). Ethephon has previously been used to study the regulation of the mango ethylene receptors *MiERS1* and *MiETR1* during the fruitlet abscission process in laboratory-based experiments (Ish-Shalom et al., 2011). Consequently, the aim of the present study was to investigate the physiological and molecular mechanisms of ethephon-induced fruitlet abscission in mango under field conditions. In particular, emphasis was given to analyzing carbohydrate concentration, polar auxin transport (PAT) capacity and the transcription of ethylene receptors of individual fruitlets and pedicels before and after ethephon spray applications. Moreover, new ethylene receptor versions were identified and their expression patterns interpreted.

4.3 Materials and methods

4.3.1 Plant material and experimental site

Experiments were conducted over two consecutive fruit growth cycles in 2011 and 2012 in the Tú Nang commune (20°37'0N, 106°4'60E) near the township Yên Châu, Province Sơn La, North Vietnam. The mango (*Mangifera indica* L.) trees of the local cultivar 'Hôi' were between 10 and 15 years of age. For orchard management details see Hagemann et al. (2014).

4.3.2 Treatments and experimental design

To investigate the physiological and molecular mechanism of fruitlet (pea size) abscission in mango, fruitlet drop was induced by ethephon spray applications during the critical mid-season drop stage. Consequently, there was a greater probability that all fruitlets investigated at each sampling time were at a similar abscission stage. In 2011 and 2012, twelve trees were randomly selected for each of the following treatments: water control and two ethephon (Flordimex 420, Spiess Urania, Germany) concentrations, the ethephon treatment 7200 ppm (ET7200) and the ethephon treatment 600 ppm (ET600). The latter treatment was added in 2012 to compare the results to those of Ish-Shalom et al. (2011). All treatments were sprayed to run-off with 5 ppm surfactant (Ethalfix ® Pro, Syngenta, Switzerland) using a low-pressure handhold sprayer (Gloria, Typ 133, Witten, Germany). For each experimental tree, healthy appearing panicles were randomly tagged at one week after full bloom (≥ 90% of all panicles are at least to 80% flowering). For each treatment, six trees with 10 panicles each were used for assessing fruit drop, whereas six trees with 40 panicles each were used for taking fruit samples.

4.3.3 Fruitlet drop assessment and sampling

Fruit retention was recorded every two days for the first four counting dates and weekly thereafter and expressed as the average fruit number of all initially tagged panicles. Sampling for gene expression and carbohydrate analysis commenced about two days (2±1) prior to treatment and continued 1, 2 (only in 2012) and 3 days after treatment (DAT). At each sampling day, 12 fruitlets (averagely 2 fruitlets from 1 panicle per tree) were collected for each treatment at noontime. First, the fruitlet detachment force (FDF), using a gauge (PCE-FM50, Germany) and the location of the detachment at the

AZ or along the pedicel were determined. In addition, diameter, length and weight of each sampled fruitlet and the pedicel diameter at mid-position were measured. Fruitlets were then cut in half and the seed was either scored healthy or degenerated when symptoms of degradation, discoloration or shrivel were noticed. From each fruitlet, the following parts were sampled for analysis: (1) a 4 mm long pedicel fragment, including the AZ, for gene expression analysis (2) a fruitlet wedge for the gene expression analysis and (3) the remaining fruitlet pericarp for the carbohydrate analysis. All samples were immediately snap frozen in liquid nitrogen and stored until further processing at -80 °C for gene expression analysis and at -30 °C for carbohydrate analysis.

Sampling for polar auxin transport (PAT) assay commenced about two days (2±1) prior to treatment and continued only at 2 DAT in 2011, whereas at 1 and 3 DAT in 2012. At each sampling day, 6 panicles (1 panicle per tree) were collected per treatment at noontime. The cut end of each panicle was placed in a falcon tube filled with water and transported in sealed styrofoam boxes to the laboratory within 2 h of sampling. Two fruitlets per panicle served for taking records of diameter, length and weight as well as pedicel diameter at mid-position. The AZ was sampled by cutting 4 mm to either side of the AZ with two parallel mounted razor blades and processed as described in section 2.6.

4.3.4 Gene analysis

4.3.4.1 RNA extraction and cDNA synthesis

Frozen fruitlet pedicels and pericarp were ground in liquid nitrogen to fine powder. Total ribonucleic acid (RNA) was extracted from 100 mg subsamples with the MasterPure Plant RNA Purification Kit (Epicentre, USA), following the manufacturer´s recommendations. In addition, to reduce the phenolic compounds from the fruitlet pericarp, polyvinylpolypyrrolidone was added in the first step of the extraction process. Genomic desoxyribonucleic acid (DNA) was eliminated with DNase1 and this was subsequently tested by polymerase chain reaction (PCR). RNA samples were stored at -80 °C until complementary DNA (cDNA) synthesis using the TaqMan Reverse Transcription Kit (Applied biosystems, USA), following the protocol of the manufacturer. For cDNA synthesis 500 ng of total RNA was used for each reaction. cDNA quality was tested by quantitative real-time PCR (qPCR), using a Rotor-Gene 6000 cycler (Corbett, Australia), with the following conditions: initial denaturation at

3 min and at 95 °C; 40 cycles of denaturation (20 s, 95 °C), annealing (20 s, 58°C) and extension (20 s, 72 °C); followed by a melt curve from 60 °C to 99 °C in 0,5 K steps.

4.3.4.2 Gene identification

Specific primers for *MiETR1* were designed (Genbank ID: AF227742.1; Table 4.1). Conserved regions of *ERS*-like sequences from woody plants and arabidopsis were identified by alignments to design degenerate primers. Nested PCRs were performed to verify sequence specificity before cloning. The PCR products were ligated into the pGEM-T vector (Promega, VIC, Australia) following the manufacturer´s recommendations. After blue-white selection, a colony PCR with gene specific primers (Table 4.1) was performed to verify positive clones for subsequent plasmid extraction (QIAPrep Miniprep, Qiagen, Germany) and sequencing (GATC, Germany). Using degenerate primers to identify the homologue to the arabidopsis *AtERS1*, three different versions of mango *ERS1* were confirmed by sequencing: a version with the full length sequence (*MiERS1*) that is comparable to the *AtERS1*, a medium sized *MiERS1m* with a length of 1203 nucleotides, and a short *MiERS1s* with a length of 561 nucleotides. The sequences were confirmed to be *MiERS1*-like by BLAST search using the NCBI online tool (http://blast.ncbi.nlm.nih.gov) and following the recommendations of Samach (2012).

Table 4.1. Primers specific for mango genes used for quantitative real-time PCR analysis. *β-ACTIN* (*MiACT*), *UBIQUITIN* (*MiUBI*), *α-TUBULIN* (*MiTUB*), *ETHYLENE RESISTANT 1* (*MiETR1*), *ETHYLENE RESPONSE SENSOR 1* (*MiERS1*), and the two *MiERS1* versions *MiERS1m* and *MiERS1s*. (*Mangifera indica* abbreviated as *Mi*).

Gene	Forward primer (5´to 3´)	Reverse primer (5´to 3´)	Amplicon size (bp)
MiACT	CCCTGAAGAGCACCCA	AGTTGTACGACCACTGGC	156
MiUBI	AAGATCCAGGACAAGGAGG	GGACCAGGTGGAGCG	125
MiTUB	ATCAACTACCAGCCACC	CCTTCCTCCATACCCTCAC	184
MiETR1	CCAAGGAGAATTGCATGAG	GGCAGCTTGCTCCTC	141
MiERS1	TGGCGACAAGAAACGACTG	GCCAGTCTCTTGAAGACTC	116
MiERS1m	GCGCTGTAATGAACCATGA	TCTTTGGTATCGTGTTGTC	151
MiERS1s	TCTAGTGTCATGTCTAACTGC	GTGCTACCTTTGTCAAGC	115

4.3.4.3 Gene expression studies

The transcription levels of *MiETR1* and the three versions of *MiERS1* were analyzed by qPCR. The efficiency of each primer pair was determined with DART tool (Peirson et al., 2003). Primer specificity was confirmed by melt curve analyses for each individual run and by sequencing of the resulting amplicons. Relative expression of the target genes was analyzed with the efficiency corrected ΔΔCt-method using the DART tool (Peirson et al., 2003). A pool-sample, composed of 1 µl cDNA, was used in each run as a reference for the relative gene expression and as a standard for the different runs. Three potential reference genes, *β-ACTIN* (*MiACT*), *α-TUBULIN* (*MiTUB*), and *UBIQUITIN* (*MiUBI*), were evaluated for their expression stability in the pericarp and pedicel from control and ET7200. The reference gene analysis was performed with the BestKeeper tool (Pfaffl et al., 2004). *MiACT* was selected as reference gene because it revealed the highest expression stability, indicated by the lowest standard deviation of the absolute regulation coefficient: 1.55 for *MiUBI*, 1.53 for *MiTUB*, 1.51 for *MiACT*.

4.3.5 Analysis of soluble carbohydrates

The concentration of fruit soluble carbohydrates was analyzed for all fruitlets that were used for gene expression studies in 2012. Individual fruitlets were freeze-dried and ground to a homogenous powder under liquid nitrogen with an impact ball mill (CryoMill, Retsch, Germany). A subsample of 50 mg was taken and re-suspended in 950 µl bi-distilled water, diluted 1:4 and vortexed thoroughly for 1 min. The debris was removed by centrifugation (5 min, 18.000 rcf, 20 °C) and 750 µl were collected from the supernatant. Because of the high content of organic acids in the sample, which are disturbance variables in the analytical process, acids were removed from the sample fraction with a strong anion exchange column (Strata-X-A 33u, Phenomenex, CA, USA). Therefore, the columns were pre-conditioned with 8 ml of 0.1 M sodium hydroxide followed by 2 ml of water. The sample was then transferred to the column, eluted with 3 ml water and concentrated to a dry pellet with a rotary evaporator set-up (RC1022, RVT4104, VLP120; Thermo Fisher Scientific Inc., MA, USA). The pellet was re-suspended in 600 µl of water, filtered through a nylon filter with a pore size of 0.45 µm (Wicom, Germany) and injected into the high performance liquid chromatography (HPLC) sampler (Bischoff, Germany). The HPLC setup consisted of a guard column, Hamilton PRP-X400, and a main column, Hamilton HC-75 Ca2+ (Hamilton, NV, USA), connected to a refractometric detector (Model 8120; Bischoff,

Germany). The carbohydrate separation was done isocratically with bi-distilled water as mobile phase facilitated by two HPLC-pumps (HPLC-Compact-Pump2250, Bischoff, Germany). The analysis conditions were 80 °C at a flow rate of 1.2 ml min^{-1}. The amounts of glucose, fructose, and sucrose were quantified using respective standards (Sigma-Aldrich, MO, USA).

4.3.6 Polar auxin transport assay

To assess the basipetal (polar) auxin transport, the basal end of the fruitlet pedicel was placed onto 96 well microplates (Greiner bio-one, Germany), however, only 24 wells per plate were used due to reasons of practicality. Each well contained 300 µl solidified buffer with 0.05 M 2-(N-morpholino) ethanesulfonic acid (MES), adjusted to pH 5.2, and 1.2% Agar-Agar. A donor block with a volume of 50 µl, shaped as concave disc and consisting of MES buffered 1.5% Agar-Agar, was immediately placed onto the apical side of the pedicel. The acropetal auxin transport was also determined by using 12 additional pedicels in reverse orientation. A droplet of 10 µl [^{3}H]-IAA (indole-3-acetic acid with a specific activity of 962 GBq $mmol^{-1}$; Amersham plc, UK) was applied into the cavity of the donor block. Each plate was placed in a dark box with 100% relative humidity and incubated for 8 h at 25 °C. After the incubation, the donor block, the pedicel and the agar of the receiving well (receiver block) were placed into different plastic scintillator vials and stored at -20 °C until extraction. For extraction 2 ml of scintillation liquid (Quickzint 212, Zinsser Analytic, Germany) was added to each vial and the samples were incubated at room temperature for 10 days on a rotary shaker at 200 rpm. Thereafter, the [^{3}H]-IAA activity was measured in a liquid scintillation counter (Tri-Carb 3110 TR, PerkinElmer, USA) for 5 min.

4.3.7 Statistical analysis

The effects of the ethephon treatments on the expression level of ethylene receptors and the concentration of soluble fruit carbohydrates were evaluated by pairwise comparison of the means at a probability level of $p \leq 0.05$ and the Fisher's least significant difference (LSD) (SAS 9.3; SAS Institute Inc., Cary, NC, USA). Model assumptions (normality and variance homogeneity) for the analysis of variance (ANOVA) were checked by examining the residual plots. For analysis of the ethylene receptor expressions, a transformation with the common logarithm was used to stabilize the

variance at high expression levels (Rocke and Durbin, 2001), however, the untransformed means are presented in the figures. The results of the PAT experiment and of the FDF measurements did not meet the assumption of variance homogeneity, thus an ANOVA based on ranks (Dunn's post test) was used to identify differences between treatment groups. In all models various covariates were tested for significant influences on treatment effects.

4.4 Results

4.4.1 Ethephon induced fruitlet abscission

Both ethephon concentrations induced an immediate and a much stronger fruitlet abscission than the control treatment (Fig. 4.1A). However, 95% of all fruitlets abscised within 8 days after ET7200 application, whereas it required 6 additional days for ET600 treated fruitlets to reach this level. It is important to note that while ET7200 defruited completely all panicles within one month, the ET600 resulted in 2% fruitlets per panicle (Fig. 4.1A). The FDF was significantly reduced by approximately 85% in the ET7200 at 1 DAT and in the ET600 at 2 DAT, respectively, when compared to the control (Fig. 4.1B, Supplementary Fig. 4.S1). The FDF in the ET7200 remained extremely low at 2 DAT and was zero at 3 DAT, whereas in the ET600 at 3 DAT it was similar to that of controls (Fig. 4.1B). While all ET7200 treated fruitlets detached at the AZ, a close to 100% abscission at the AZ occurred only at 2 DAT for ET600 fruitlets (Fig. 4.1C). This corresponds in all cases with extremely low FDF values (Fig. 4.1B). However, the ET600 application detached only about 50% fruitlet at the AZ at 1 and 3 DAT, which corresponds with relatively high FDF values due to higher detachment forces needed to pull-off the remaining 50% fruitlet somewhere along the pedicel. In contrast, approximately 30% of the controls detached at the AZ, thereby about 70% broke at different locations of the pedicel (Fig. 4.1C). These results are in good agreement with the findings in the previous year (2011), specifically, an ET7200 induced continuous decrease of FDF to zero concomitantly with an increase in fruitlet detachment at the AZ to 100% at 3 DAT (Supplementary Fig. 4.S1). Overall, about one third of all fruitlets evaluated showed visible symptoms of seed degeneration; however, this did not seem to be related to the ethephon treatments (Fig. 4.1D).

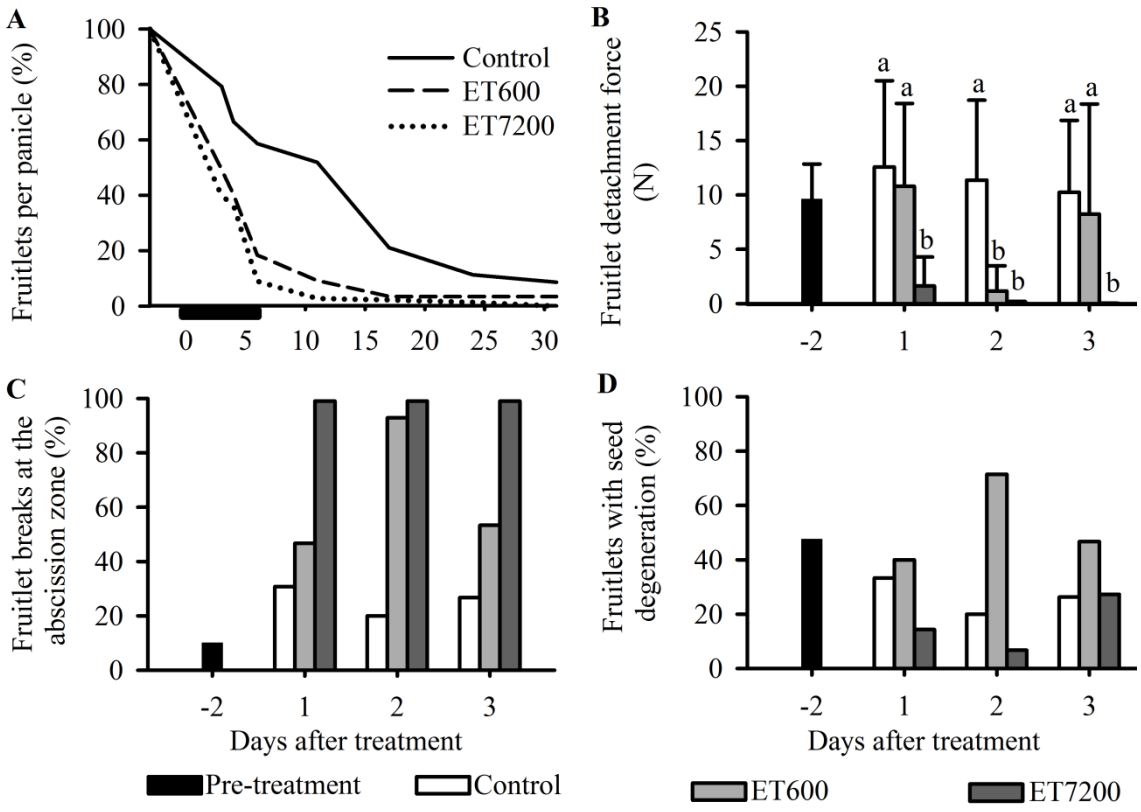


Fig. 4.1. The effect of the ethephon treatment 600 ppm (ET600) or 7200 ppm (ET7200) spray applications on average (A) fruitlet retention, (B) fruitlet detachment force of fruitlets detaching at the abscission zone or along the pedicel, (C) percentage of fruitlet detachment at the abscission zone (the remainder to 100% are fruitlets detaching along the pedicel) and (D) seed degeneration in comparison to the control and two days prior to treatment. (A) Horizontal black bar indicates time until 95% of the fruits have abscised in response to ET7200. (B) Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation.

4.4.2 Expression of ethylene receptors in the pedicel

Both ethephon treatments led to a specific receptor transcription pattern in the pedicel, with little response of *MiETR1* and a strong upregulation of *MiERS1* (Fig. 4.2, Supplementary Fig. 4.S2). *MiETR1* was not significantly regulated by ET7200, except at 1 DAT in 2012 (Fig. 4.2A, Supplementary Fig. 4.S2). In contrast, the expression of *MiERS1* shows a strong response to both ethephon concentrations. ET7200 led to a six and three times higher expression level at 1 DAT in 2011 and 2012, respectively, compared to the control (Fig. 4.2B, Supplementary Fig. 4.S2B). The ET7200 induced *MiERS1* upregulation remained higher than the control at the following sampling days, although this was not significant at 3 DAT in 2011 (Fig. 4.2B, Supplementary

Fig. 4.S2B). The ET600 led to an increasingly stronger *MiERS1* transcription, with a significant *MiERS1* upregulation at 2 and 3 DAT but not at 1 DAT unlike in the case of the ET7200 (Fig. 4.2B).

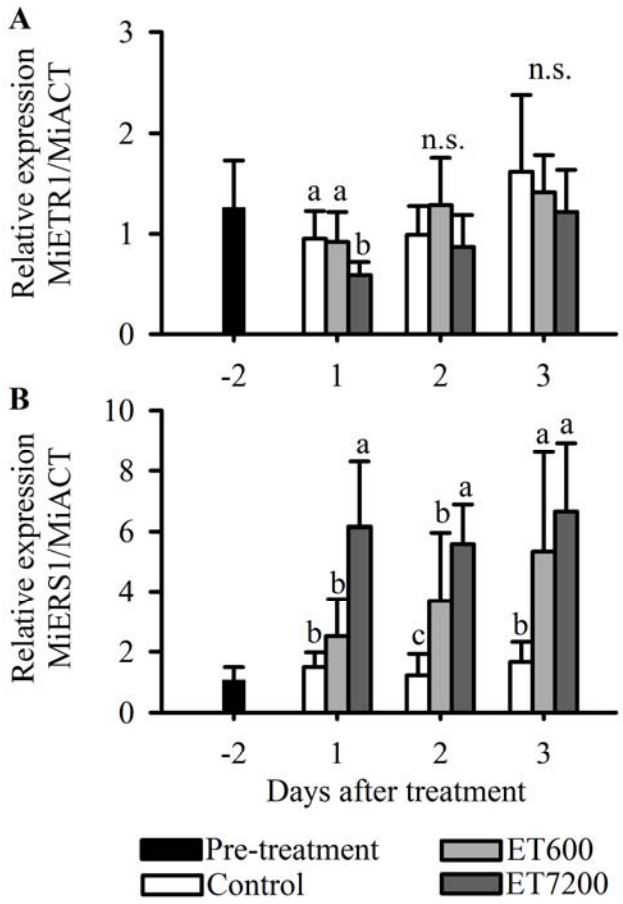


Fig. 4.2. Expression of the ethylene receptors **(A)** *MiETR1* and **(B)** *MiERS1* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a,b or c). Error bars show standard deviation.

Three homologues of the arabidopsis ethylene receptor *AtERS1* have been identified. According to a BLAST analysis all three *MiERS1* versions, are highly similar (identity values of 98 to 99%) to the two full length *MiERS1* GenBank accessions (JN851132.1, JF323582.1). These two accessions derived from the cultivar 'Kent', thus the 1 to 2% sequence differences are likely a result of a few nucleotide polymorphisms between the cultivars 'Hôi' and 'Kent'. The 'Hôi' *MiERS1* full length has a coding sequence of 1890 nucleotides while the other versions, *MiERS1m* and *MiERS1s* are shorter with 1203

nucleotides and 561 nucleotides, respectively. In contrast to the *MiERS1*, which was detected in all samples (100%), transcripts of *MiERS1m* and *MiERS1s* could only be detected in a much reduced number of samples, although *MiERS1m* was more frequently detected than *MiERS1s* (Fig. 4.3). Nevertheless, transcripts of both shorter receptor versions were detected more often in pedicels of treated fruitlets than in controls (Fig. 4.3). The regulation of *MiERS1m* and *MiERS1s* in the pedicel appears to be erratic, therefore a statistical analysis was not possible (Supplementary Fig. 4.S3).

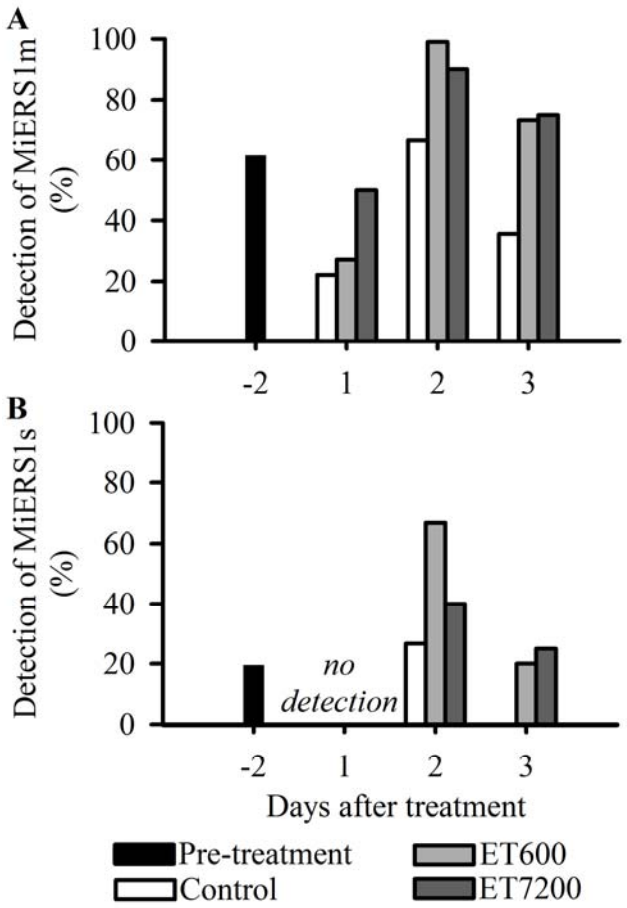


Fig. 4.3. Detection of transcription of the ethylene receptor versions (A) *MiERS1m* and (B) *MiERS1s* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment.

4.4.3 Expression of ethylene receptors in the fruitlet pericarp

The two receptors *MiETR1* and *MiERS1* were expressed in the fruitlet pericarp with a similar timely pattern in both experimental years (Fig. 4, Supplementary Fig. 4.S4). Both receptors were significantly upregulated at all DAT following the ET7200 application compared to the control. In contrast, the ET600 led to a significant

upregulation of the *MiETR1* only at 2 DAT (Fig. 4A, Supplementary Fig. S4A), while the transcription level of *MiERS1* was similar to that of the controls at all sampling dates (Fig. 4B, Supplementary Fig. 4.S4B). Both short versions of *MiERS1* were rarely detected in the fruitlet pericarp (data not shown) and consequently analysis of these receptors versions was not further pursued.

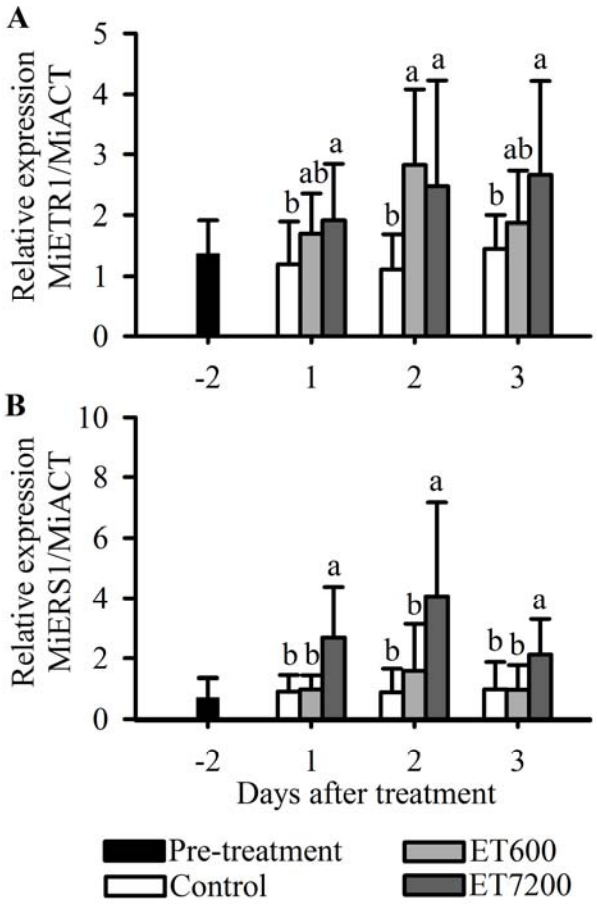


Fig. 4.4. Expression of the ethylene receptors (A) *MiETR1*and (B) *MiERS1* in the pericarp of pea sized mango fruitlets in response to the ethephon treatment 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation.

4.4.4 Polar auxin transport capacity

The acropetal transport capacity of 40±20 dpm was always significantly lower than the PAT capacity of pedicels from control fruitlets. Both ethephon concentrations effectively decreased the PAT capacity of the pedicel at each sampling time (Fig. 5, Supplementary Fig. 4.S5); however, ET7200 reduced the PAT capacity to a greater extent than the ET600.

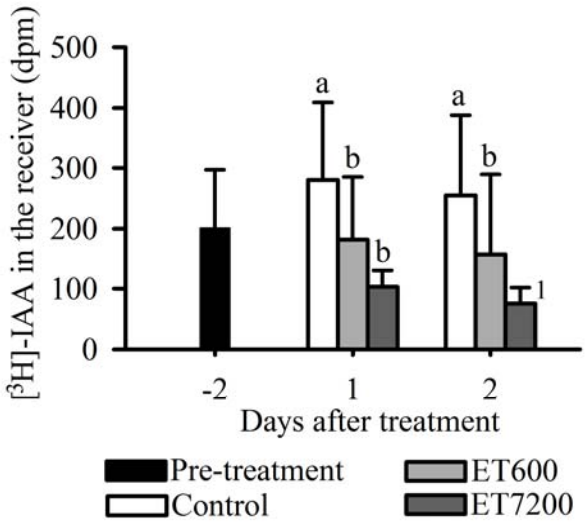


Fig. 4.5. Polar auxin transport (PAT) capacity through the pedicel of pea sized fruitlets. Detection of [^{3}H]-IAA in the receiver block in response to the ethephon treatment with 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation; dpm = disintegrations per minute. [1]sample size (n=3) was too small to perform a statistical test.

4.4.5 Analysis of soluble carbohydrates

Among all the analyzed carbohydrates, a clear response to both ethephon treatments was only found for sucrose, indicated by significantly lower concentrations in treated fruitlets than those in controls at 2 DAT (Fig. 4.6, Supplementary Fig. 4.S6). While the sucrose concentration in ET7200 treated fruitlets remained low at 3 DAT, it was not different between ET600 treated fruitlets and controls. Ethephon did not affect the concentration of fructose in the fruitlets (Supplementary Fig. 4.S6A). Fruitlet concentration of glucose was significantly increased only at 3 DAT by ET600 compared to the control, whereas was not affected by ET7200 (Supplementary Fig. 4.S6B).

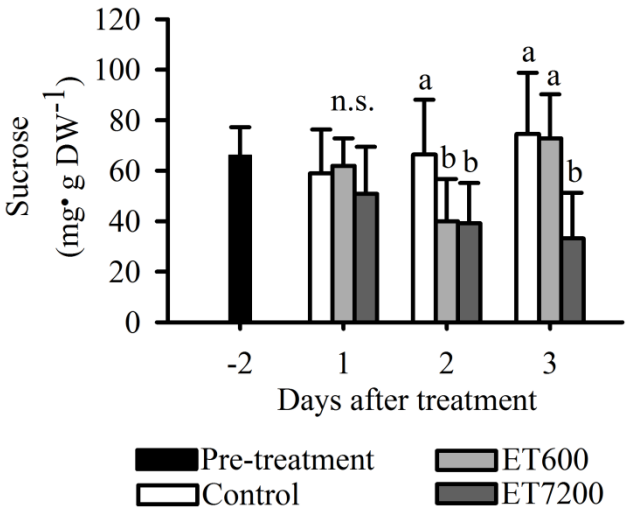


Fig. 4.6. Sucrose concentration of pea sized fruitlets after the ethephon treatment 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation.

4.5 Discussion

The current study supports earlier findings (Malik et al., 2003) that ethephon induces fruitlet abscission in mango is a concentration dependent response: the ET7200 led to a complete loss of fruitlets while approximately 3% of fruitlets were retained in the ET600 at 1 month after spray application (Fig. 4.1A). This clearly indicates that the fruitlet abscission response to ET600 is less pronounced and hence proportionally fewer about-to-abscise fruitlets with a greater FDF value were sampled at 3 DAT when compared to the ET7200.

Irrespective of the treatment applied, low FDF values were symptomatic for fruitlets breaking at the abscission zone (Hagemann et al., 2015; Hagemann et al., in press) and are indicative of an advanced abscission process. Nutritional stress during embryogenesis, leading to seed degeneration, was previously found as another symptomatic cause of fruitlet abscission (Singh, 1961; Botton et al., 2011). However, despite 30% of the fruitlets containing degenerated seeds, it appeared to be related neither with the point of detachment (data not shown) nor with the ethephon treatments (Fig. 4.1D). Nevertheless, the ET7200 must have induced specific morphological changes at the cellular level within 24 h that led to low FDF values and fruitlets detaching at the AZ, the weakest point along the pedicel (Fig. 4.1B, C). In contrast, this response was only seen 48 h after the ET600. Indeed, microscopy studies of Barnell as

early as in 1939 showed that cellular changes within the AZ of mango pedicels, e.g. meristematic activity and swelling of cell walls, allow a fruitlet to separate with a clean break. Moreover, the action of cell wall degrading enzymes and an increase of turgor pressure are necessary for fruitlet detachment and prior to that, specific genes must have been differentially expressed to induce the AZ (Roberts et al., 2002).

Ethylene receptors were examined as the target genes since the ethylene signaling pathway has been linked to the induction of the AZ and fruitlet abscission (Xie et al., 2013). Of the two ethylene receptors so far described for mango, the *MiETR1* has been reported to be upregulated in the pericarp but not in the pedicel of fruitlets induced for abscission (Martínez et al., 2001; Ish-Shalom et al., 2011). In contrast, *MiERS1* has been reported to be upregulated in the pedicel but not in the pericarp of abscission-induced fruitlets (Ish-Shalom et al., 2011). The current results confirm the findings of Ish-Shalom et al. (2011) that ethephon does not upregulate *MiETR1* but *MiERS1* in the pedicel by using the more sensitive qPCR method instead of the Northern blot (Dean et al., 2002). The about five-times higher concentration than the one used by Ish-Shalom et al. (2011), 1400 ppm *vs.* 7200 ppm, led to at least 48 h longer upregulation of the *MiERS1* (Fig. 4.2, Supplementary Fig. 4.S2). In general, the *ERS1* responds with an upregulation in the fruitlet pedicels and leaf petioles of different tree crops, including mango, within 24 h of an abscission inducing treatment (Rasori et al., 2002; John-Karuppiah and Burns, 2010; Ish-Shalom et al., 2011). These results corroborate the hypothesis that the role of the *ERS1* in organ abscission is highly conserved in plants. The newly identified short *MiERS1* versions *MiERS1m* and the *MiERS1s* may also be associated with fruitlet abscission because their probability of detection and their expression level were higher in pedicels of ethephon treated and thus abscising fruitlets than in untreated controls (Fig. 4.3, Supplementary Fig. 4.S3).

The *MiETR1* upregulation in the pericarp of ethephon treated fruitlets was more pronounced following the ET7200 compared to the ET600 (Fig. 4.4A). The ET600 induced significant upregulation of the *MiETR1* but not of the *MiERS1* in the pericarp, corresponds to the findings of Ish-Shalom et al. (2011). It is important to note that Ish-Shalom et al. (2011) applied a higher concentration of ethephon at a lower temperature (1400 ppm at 20 °C) compared to the current study (600 ppm at 29 °C), hence both studies are comparable due to the temperature-depending effect of ethephon (Yuan and Burns, 2004). In contrast, the ET7200 led to a significant upregulation of both ethylene receptors in the pericarp of fruitlets from 1 DAT onwards (Fig. 4.4).

Thus, the expression pattern of both receptors clearly indicates an ethephon (ethylene) concentration dependent response. It is likely that ET7200 induced a greater endogenous autocatalytic ethylene synthesis, which largely contributes to a longer lasting and significantly greater ethylene receptor response. It may also be that the ethylene sensitivity threshold of the AZ is in part maintained through the ET7200 application despite a 50% ethephon degradation within 1 DAT (Domir and Foy, 1978). In the natural abscission process, it is suggested that fruitlet-derived ethylene is synthesized in the pericarp and diffused to the AZ (Núñez-Elisea and Davenport, 1986; Malik et al., 2003) where it induces the upregulation of ethylene receptors (Stepanova and Alonso, 2009) prior to the induction of the abscission process. These findings lead to the hypothesis that during natural abscission, ethylene receptors are first upregulated in the fruitlet and then in the pedicel (Hagemann et al., in press). Chemical induction of the abscission by ethephon would result in a simultaneous upregulation of ethylene receptors in fruitlets and pedicels (Fig. 4.2, 4.4).

Another key element of the abscission process is the auxin signaling (Xie et al., 2013), which was expressed as PAT capacity in the present study. Untreated mango fruitlets transported only 5% of the radioactively labelled auxin through an 8 mm long pedicel within 8 h, while it was 38% through 4 mm sweet cherry pedicles within 3 h (Else et al., 2004) and 5-13% through 15 mm lupine hypocotyls within 8 h (Sánchez-Bravo et al., 1992). In mango the vascular system is in close association with resin canals and exudates rich in carbohydrates and phenolic compounds cause a rapid sealing of the cut surface (Lima Filho, 2004; Joel, 1981), thus likely reducing the PAT capacity. However, a sealing of the cut surface was prevented by immediately placing a physiologically-buffered agar block on the cut surfaces. Both ethephon treatments reduced the PAT capacity of mango fruitlet pedicels within 24 h (Fig. 4.5) which supports earlier findings that the transcript of an auxin efflux carrier responsible for the basipetal auxin transport (Friml, 2003) was reduced within 24 h of ethylene treatment (Dal Cin et al., 2009). Experiments with Arabidopsis seedlings showed that ethylene biosynthesis pathway enzymes respond to varying auxin concentrations (Abel et al., 1995), suggesting that a reduced PAT through the pedicel can also induce endogenous ethylene evolution in pedicels and in turn trigger abscission in the AZ.

Carbohydrate deficiency is another plausible cause of fruitlet abscission (Xie et al., 2013), however, few data of carbohydrate concentrations in mango fruitlets during the main fruitlet drop stage at pea to marble size are available. Defoliation experiments with

citrus have clearly shown that low sucrose concentration in fruitlets cause fruitlet abscission (Mehouachi et al., 1995) and in agreement with this finding, also low concentration of sucrose in mango fruitlet seem to be related to the abscission inducing treatment (Fig. 4.6, Supplementary Fig. 4.S6). Sucrose concentration in the pulp of mature mango fruit ranged from 46 to 114 mg g^{-1} dry weight, depending on cultivar, ripening stage and method used for analysis (Thanaraj et al., 2009). Moreover, it was shown earlier for mango that sucrose is the main translocation carbohydrate in support of fruit growth (Chauhan and Pandey, 1984). It is suggested that the ethephon-induced reduction of sucrose concentration in fruitlets at 2 DAT is triggered by reduced auxin signaling that subsequently reduces the sink strength for carbohydrate import into the fruitlet commencing at 1 DAT (Fig. 4.6).

In conclusion, the data suggest that the ethephon-induced fruitlet abscission follows a different sequence of events compared to the natural abscission process. In the latter case, resource deficiency, e.g. carbohydrate supply limitations for fruitlet growth, or seed degeneration with auxin signaling disruption are primary physiological causes (Hagemann et al., in press; Xie et al,. 2013). In contrast, the ethephon-induced fruitlet abscission process responds initially with a reduction of the PAT capacity in the pedicel, followed by an upregulation of ethylene receptors and then a decline in sucrose concentration; physiological markers that were not linked to seed degeneration. Ethephon spray applications at the high concentration caused a faster abscission of mango fruitlets at the AZ than the low ethephon concentration. This might be due to a more rapid saturation of ethylene receptor binding sites in the pedicel by the high ethephon concentration, which presumably also causes a greater autocatalytic ethylene production in the pericarp and the pedicel.

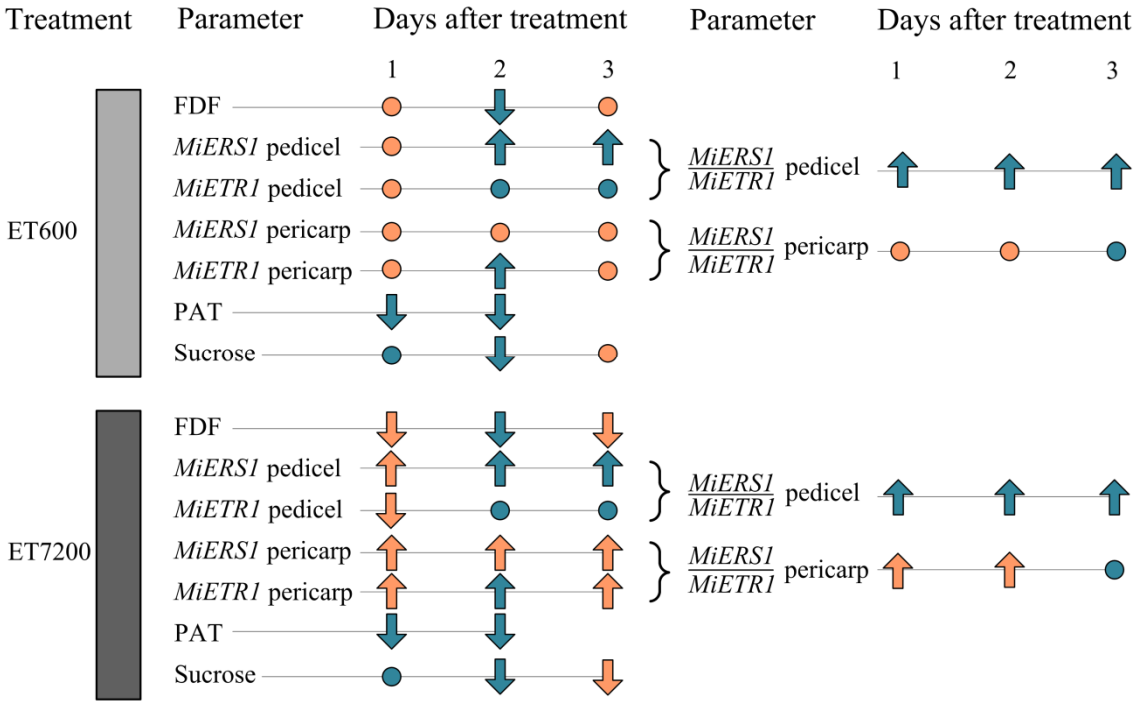


Fig. 4.7. Overview of the key fruitlet abscission parameters analyzed in this study. Parameters of ethephon treated fruitlets compared to those of control fruitlets: no significant differences are indicated by a dot, whereas up- or downward pointing arrows indicate significant differences. Different or equal response of the ethephon treatments 600 ppm (ET600) and 7200 ppm (ET7200) in comparison to the control are indicated by orange and cyan colored symbols, respectively. The parameters are: fruitlet detachment force (FDF), gene expression of the ethylene receptors *MiERS1* and *MiETR1* and their ratios in the pedicel and fruit pericarp, polar auxin transport (PAT), and the concentration of sucrose in the fruit pericarp.

An alternative explanation is provided by Dal Cin et al. (2005) who first suggested that a greater ERS1/ETR1 ratio in both the pedicel AZ and the fruit cortex (pericarp) is a decisive trigger for fruitlet abscission during the midseason drop stage in apple. This notion was also suggested for mango (Ish-Shalom et al., 2011); however, specific evidence is provided in the present study with higher *MiERS1/MiETR1* ratios in the pericarp and the pedicel of ET7200-treated fruitlets than those of control fruitlets (Fig. 4.7; Supplementary Table 4.S1). In contrast, ET600 induced an increased *MiERS1/MiETR1* ratio in the pedicel but not in the pericarp, suggesting that the receptor regulation in the pericarp is not the primary determining factor in both ethephon-inducing fruitlet abscission treatments (Fig. 4.7; Supplementary Table 4.S1). However, the one-day earlier reduction of FDF in the ET7200 than the ET600 might be associated with the higher *MiERS1/MiETR1* ratios in both pedicel and pericarp. Following the *MiERS1/MiETR1* ratio concept, the ethephon-induced fruitlet abscission process

commences with a reduction of the PAT capacity and an upregulation of ethylene receptors.

4.6 Acknowledgement

The research was financially supported by the Deutsche Forschungsgemeinschaft (DFG) through the Sonderforschungsbereich 564. The authors thank Dr. Daniel Neuwald for his support by the carbohydrate analysis. They also thank Dr. Juan Carlos Laso Bayas for the statistical advice.

4.7 Supplementary material

4.7.1 Supplementary figures

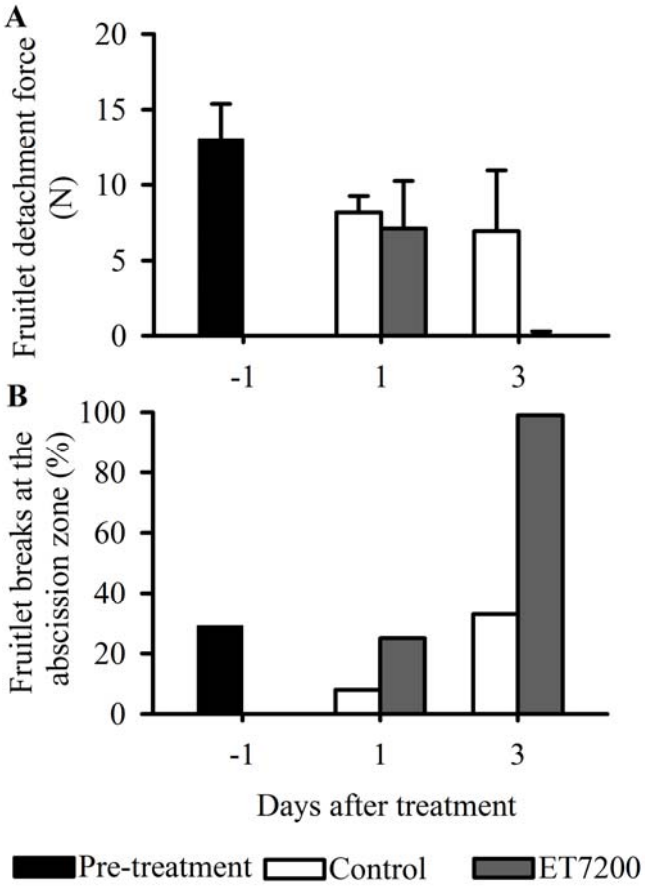


Fig. 4.S1. The effect of ethephon treatment 7200 ppm (ET7200) on average (A) fruitlet detachment force of fruitlets detaching at the abscission zone or along the pedicel and (B) percentage of fruitlet detachment at the abscission zone (the remainder to 100% are fruitlets detaching along the pedicel) in comparison to the control and one day prior to treatment. Error bars show standard deviation. Data from 2011.

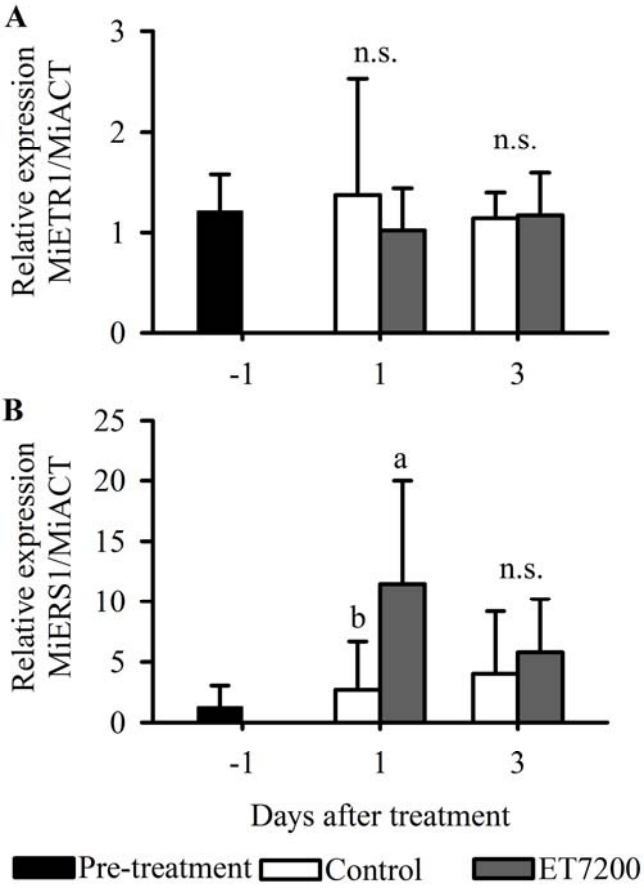


Fig. 4.S2. Expression of the ethylene receptors (A) *MiETR1* and (B) *MiERS1* in the pedicel of pea sized mango fruitlets in response to the ethephon treatment 7200 ppm (ET7200) in comparison to the control and one day prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2011.

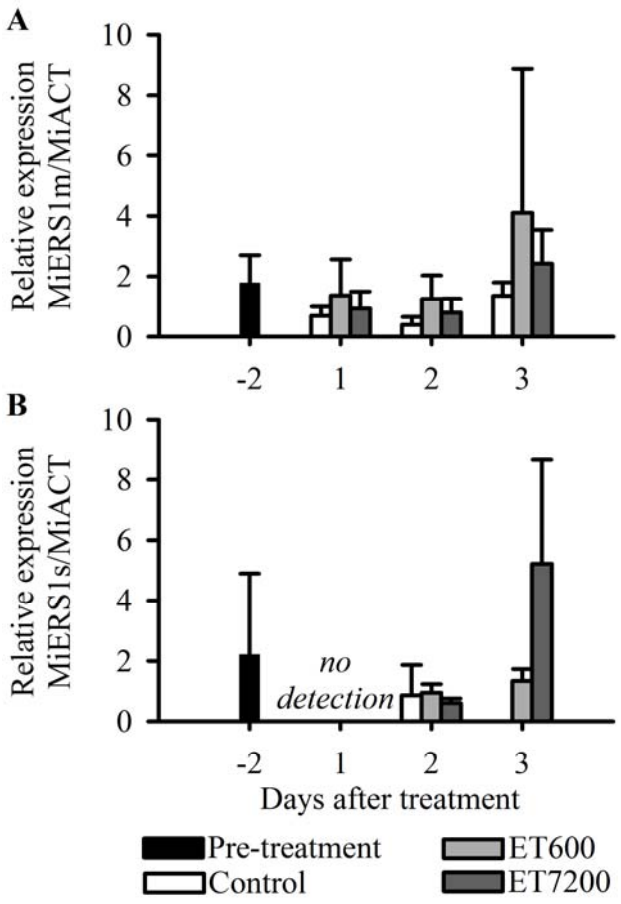


Fig. 4.S3. Expression of short versions of the *MiERS1*. (A) *MiERS1m* and (B) *MiERS1s* in the pedicel of pea sized mango fruitlets in response to the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. No statistical test possible due to highly variable sample size. Missing error bar indicates n=1. Data from 2012.

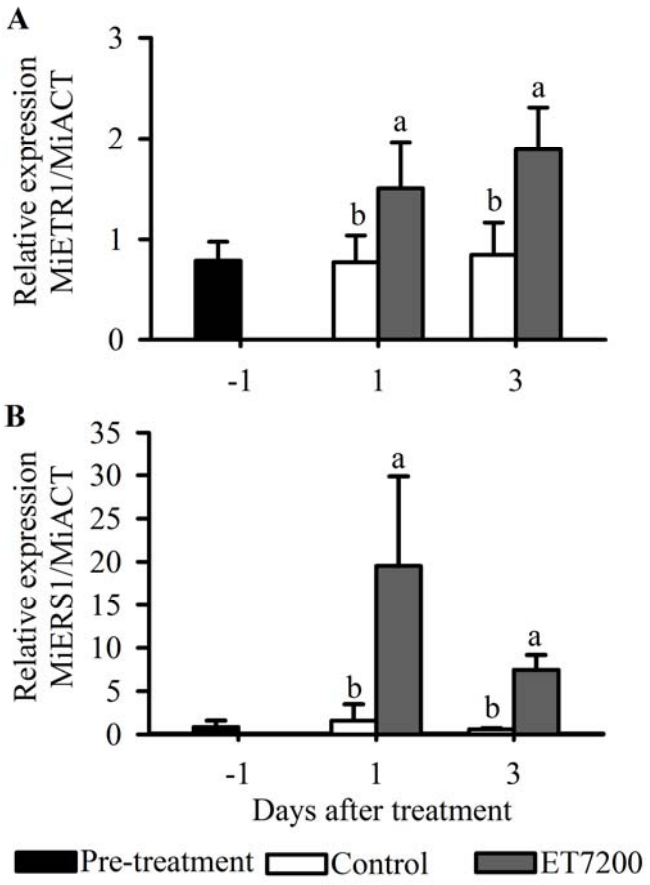


Fig. 4.S4. Expression analysis of (A) *MiETR1* and (B) *MiERS1* in the pericarp of pea sized fruitlets in response to the ethephon treatment 7200 ppm (ET7200) in comparison to the control and one day prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2011.

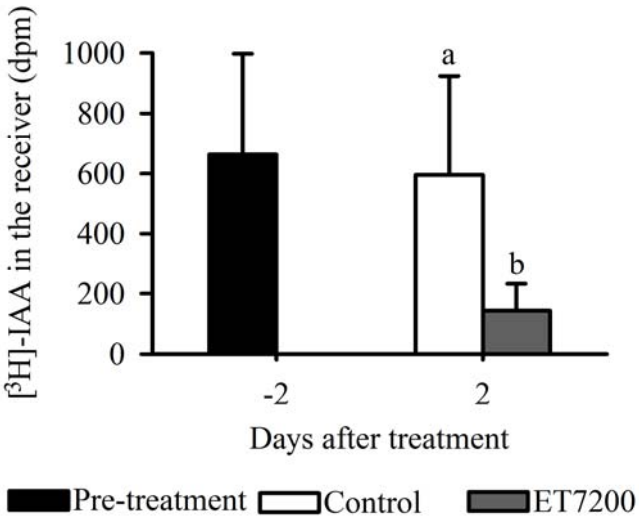


Fig. 4.S5. Accumulated [^{3}H]-IAA in the receiver after the ethephon treatment 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation; dpm = disintegrations per minute. Data from 2011.

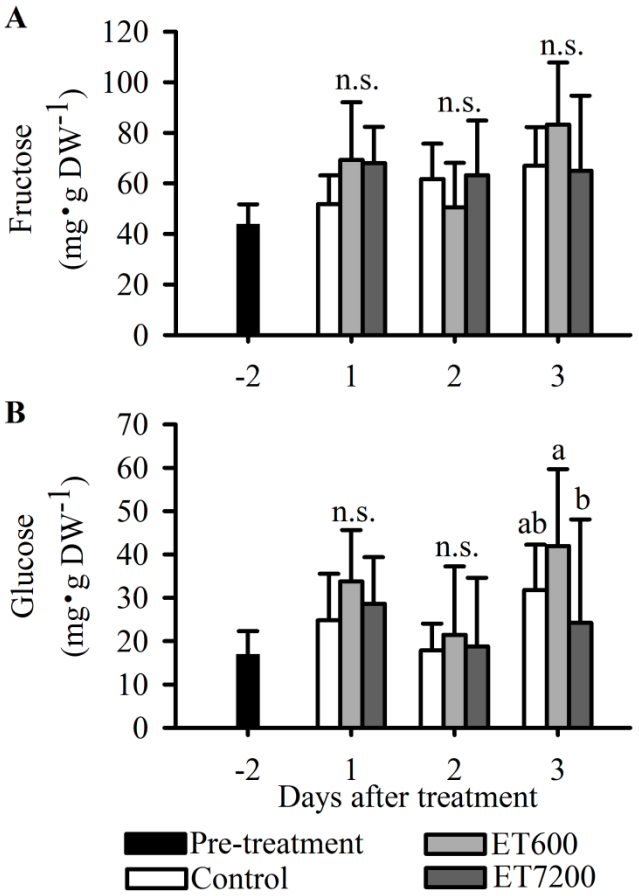


Fig. 4.S6. Concentrations of fructose and glucose of pea sized fruitlets after the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control and two days prior to treatment. Homogeneous subgroups with no significant difference ($p \leq 0.05$) are indicated by same letters (a or b). Error bars show standard deviation. Data from 2012.

4.7.2 Supplementary tables

Table 4.S1. Ratio of the ethylene receptors *MiERS1* and *MiETR1* after the ethephon treatments 600 ppm (ET600) or 7200 ppm (ET7200) in comparison to the control. Data from 2012.

Parameter	Treatment	Days after treatment		
		1	2	3
MiERS1/MiETR1 pedicel	Control	1.6	1.2	1.0
	ET600	2.7	2.9	3.8
	ET7200	10.4	6.4	5.5
MiERS1/MiETR1 pericarp	Control	0.8	0.8	0.7
	ET600	0.6	0.6	0.5
	ET7200	1.4	1.6	0.8

4.8 References

Abel, S., Nguyen, M.D., Chow, W., and Theologis, A. (1995). ASC4, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. J. Biol. Chem. 270, 19093–19099. doi: 10.1074/jbc.270.32.19093

Alexander, L., and Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *J. Exp. Bot.* 53**,** 2039–2055. doi: 10.1093/jxb/erf072

Barnell, E. (1939). Studies in tropical fruits: V. Some anatomical aspects of fruit-fall in two tropical arboreal plants. *Annu. Bot.* 3**,** 77–89.

Binder, B.M. (2008). The ethylene receptors: complex perception for a simple gas. *Plant Sci.* 175**,** 8–17. doi: 10.1016/j.plantsci.2007.12.001

Blanusa, T., Else, M.A., Atkinson, C.J., and Davies, W.J. (2005). The regulation of sweet cherry fruit abscission by polar auxin transport. *Plant Growth. Regul.* 45**,** 189–198. doi: 10.1007/s10725-005-3568-9

Botton, A., Eccher, G., Forcato, C., Ferrarini, A., Begheldo, M., Zermiani, M., Moscatello, S., Battistelli, A., Velasco, R., and Ruperti, A. (2011). Signaling pathways mediating the induction of apple fruitlet abscission. *Plant physiol.* 155, 185–208. doi: 10.1104/pp.110.165779

Burondkar, M.M., Rajput, J.C., Waghmare, G.M., Jamadagni, B.M., and Chavan, S.A. (2000). Recurrent flowering: a new physiological disorder in Alphonso mango. *Acta Hort.* 509**,** 669–674.

Chacko, E.K., Kachru, R.B., and Singh, R.N. (1970). Changes in levels of acidic and neutral growth promoters during fruit development in Dashehari mango (*Mangifera indica* L.). *J. Hort. Sci.* 45**,** 341–349.

Chauhan, P.S., and Pandey, R.M. (1984). Relative $^{14}CO_2$ fixation by leaves and fruits, and translocation of ^{14}C-sucrose in mango. *Sci. Hort.* 22**,** 121–128. doi: 10.1016/0304-4238(84)90091-8

Dal Cin, V., Danesin, M., Boschetti, A., Dorigoni, A., and Ramina, A. (2005). Ethylene biosynthesis and perception in apple fruitlet abscission (*Malus domestica* L. Borck). *J. Exp. Bot.* 56**,** 2995-3005. doi: 10.1093/jxb/eri296

Dal Cin, V., Danesin, M., Botton, A., Boschetti, A., Dorigoni, A., and Ramina, A. (2008). Ethylene and preharvest drop: the effect of AVG and NAA on fruit abscission in apple (*Malus domestica* L. Borkh). *Plant Growth Regul.* 56**,** 317–325. doi: 10.1007/s10725-008-9312-5

Dal Cin, V., Velasco, R., and Ramina, A. (2009). Dominance induction of fruitlet shedding in *Malus* x *domestica* (L. Borkh): molecular changes associated with polar auxin transport. *BMC Plant Biol.* 9**,** 139. doi: 10.1186/1471-2229-9-139

Dean, J.D., Goodwin, P.H., and Hsiang, T. (2002). Comparison of relative RT-PCR and northern blot analyses to measure expression of β-1,3-glucanase in *Nicotiana benthamiana* infected with *Colltotrichum destructivum*. *Plant Mol. Biol. Rep.* 20**,** 347–356. doi: 10.1007/BF02772122

Dennis, F.G., Jr. (2000). The history of fruit thinning. *Plant Growth Regul.* 31**,** 1–16. doi: 10.1023/A:1006330009160

Domir, S.C., and Foy, C.L. (1978). A study of ethylene and CO_2 evolution from ethephon in tobacco. *Pest. Biochem. Physiol* .9**,** 1–8. doi: 10.1016/0048-3575(78)90058-5

Else, M.A., Stankiewicz-Davies, A.P., Crisp, C.M., and Atkinson, C.J. (2004). The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention. *J. Exp. Bot.* 55**,** 2099–2109. doi: 10.1093/jxb/erh208

Estornell, L.H., Agustí, J., Merelo, P., Talón, M., and Tadeo, F.R. (2013). Elucidating mechanisms underlying organ abscission. *Plant Sci.* 199–200**,** 48-60. doi: 10.1016/j.plantsci.2012.10.008

Friml, J. (2003). Auxin transport – shaping the plant. *Curr. Opin. Plant Biol.* 6**,** 7–12. doi: 10.1016/S1369526602000031

Gao, Z., Wen, C.-K., Binder, B.M., Chen, Y.-F., Chang, J., Chiang, Y.-H., Kerris, R.J., Chang, C., and Schaller, G.E. (2008). Heteromeric interactions among ethylene receptors mediate signaling in arabidopsis. *J. Biol. Chem.* 283**,** 23801–23810. doi: 10.1074/jbc.M800641200

Hagemann, M.H., Roemer, M.G., Kofler, J., Hegele, M., and Wünsche, J.N. (2014). A new approach for analyzing and interpreting data on fruit drop in mango. *HortSci.* 49**,** 1498–1505.

Hagemann, M.H., Winterhagen, P., Hegele, M., and Wünsche, J.N. (2015). Ethephon induced abscission of mango fruitlets – physiological fruit pedicel response. *Acta Hort.* 1066**,** 109–116.

Hagemann, M.H., Winterhagen, P., Roemer, M.G., Hegele, M., and Wünsche, J.N. (in press). Proposed physiological mechanism of mango fruitlet abscission. *Acta Hort*.

Ireland, H.S., Guillen, F., Bowen, J., Tacken, E.J., Putterill, J., Schaffer, R.J., and Johnston, J.W. (2012). Mining the apple genome reveals a family of nine ethylene receptor genes. *Postharvest Biol. Tec.* 72**,** 42–46. doi: 10.1016/j.postharvbio.2012.05.003

Ish-Shalom, M., Dahan, Y., Maayan, I., and Irihimovitch, V. (2011). Cloning and molecular characterization of an ethylene receptor gene, *MiERS1*, expressed during mango fruitlet abscission and fruit ripening. *Plant Physiol. Biochem.* 49**,** 931–936. doi: 10.1016/j.plaphy.2011.05.010

Joel, D.M. (1981). The duct systems of the base and stalk of the mango fruit. *Bot.Gaz.* 142**,** 329–333.

John-Karuppiah, K.-J., and Burns, J.K. (2010). Expression of ethylene biosynthesis and signaling genes during differential abscission responses of sweet orange leaves and mature fruit. *J. Am. Soc. Hort. Sci.* 135**,** 456–464.

Kuang, J.-F., Wu, J.-Y., Zhong, H.-Y., Li, C.-Q., Chen, J.-Y., Lu, W.-J., and Li, J.-G. (2012). Carbohydrate stress affecting fruitlet abscission and expression of genes related to auxin signal transduction pathway in litchi. *Int. J. Mol. Sci.* 13**,** 16084–16103. doi: 10.3390/ijms131216084

Leslie, M.E., Lewis, M.W., and Liljegren, S.J. (2007). "Organ Abscission," in *Annual Plant Reviews Volume 25: Plant Cell Separation and Adhesion,* eds. J.A. Roberts & Z.H. Gonzalez-Carranza. (Oxford: Blackwell Publishing Ltd), 106–136. doi: 10.1002/9780470988824.ch6

Lima Filho, J.M.P. (2004). Methodology for water potential measurement on mango using the pressure chamber. *Acta Hort.* 645**,** 459–461.

Malik, A.U., and Singh, Z. (2003). Abscission of mango fruitlets as influenced by biosynthesis of polyamines. *J. Hort. Sci. Biotech.* 78**,** 721–727.

Malik, A.U., Agrez, V., and Singh, Z. (2003). Fruitlet abscission of mango in relation to ethylene. *J. Hort. Sci. Biotech.* 78**,** 458–462.

Martínez, P.G., Gómez, R.L., and Gómez-Lim, M.A. (2001). Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. *J. Plant Physiol.* 158**,** 101–108. doi: 10.1078/01761610122258602

Mehouachi, J., Serna, D., Zaragoza, S., Agusti, M., Talon, M., and Primo-Millo, E. (1995). Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107**,** 189-197. doi: 10.1016/0168-9452(95)04111-7

Núñez-Elisea, R., and Davenport, T.L. (1986). Abscission of mango fruitlets as influenced by enhanced ethylene biosynthesis. *Plant Physiol.* 82**,** 991–994. doi: 10.1104/pp.82.4.991

Peirson, S.N., Butler, J.N., and Foster, R.G. (2003). Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. *Nucleic Acids Res.* 31**,** e73. doi: 10.1093/nar/gng073

Pfaffl, M.W., Tichopad, A., Prgomet, C., and Neuvians, T.P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper – Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* 26**,** 509–515. doi: 10.1023/B:BILE.0000019559.84305.47

Prakash, S., and Ram, S. (1984). Naturally occurring auxins and inhibitor and their role in fruit growth and drop of mango Dashehari. *Sci. Hort.* 22**,** 241–248. doi: 10.1016/0304-4238(84)90057-8

Rasori, A., Ruperti, B., Bonghi, C., Tonutti, P., and Ramina, A. (2002). Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. *J. Exp. Bot.* 53**,** 2333–2339. doi: 10.1093/jxb/erf097

Roberts, J.A., Elliott, K.A., and Gonzalez-Carranza, Z.H. (2002). Abscission, dehiscence, and other cell separation processes. *Annu. Rev. Plant Biol.* 53**,** 131–158. doi: 10.1146/annurev.arplant.53.092701.180236

Rocke, D.M., and Durbin, B. (2001). A model for measurement error for gene expression arrays. *J. Comput. Biol.* 8**,** 557–569. doi: 10.1089/106652701753307485

Roemer, M.G., Hegele, M., Wünsche, J.N., and Huong, P.T. (2011). Possible physiological mechanisms of premature fruit drop in mango (*Mangifera indica* L.) in Northern Vietnam. *Acta Hort.* 903**,** 999–1006.

Samach, A. (2012). Congratulations, you have been carefully chosen to represent an important developmental regulator! *Ann. Bot.* 111**,** 329-333. doi: 10.1093/aob/mcs161

Sánchez-Bravo, J., Ortuño, A.M., Botía, J.M., Acosta, M., and Sabater, F. (1992). The decrease in auxin polar transport down the lupin hypocotyl could produce the indole-3-acetic acid distribution responsible for the elongation growth pattern. *Plant Physiol.* 100**,** 108–114. doi: 10.1104/pp.100.1.108

Sexton, R., and Roberts, J.A. (1982). Cell biology of abscission. *Annu. Rev. Plant Physiol.* 33**,** 133–162. doi: 10.1146/annurev.pp.33.060182.001025

Shakeel, S.N., Wang, X., Binder, B.M., and Schaller, G.E. (2013). Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family. *AoB Plants* 5. doi: 10.1093/aobpla/plt010

Singh, R.N., and Arora, K.S. (1965). Some factors affecting fruit drop in mango (*Mangifera indica* L.). *Indian J. Agric. Sci.* 35**,** 196–205.

Singh, Z., Malik, A., and Davenport, T.L. (2005). "Fruit drop in mango," in *Horticultural Reviews,* ed. J. Janick. (Oxford, UK: John Wiley & Sons, Inc.), 111–153. doi: 10.1002/9780470650882.ch3

Singh, U.R. (1961). Studies in the fruit drop of mango. IV Embryo development, its degeneration and studies on fruit-pedicel and abscission zone. *Hort. Adv.* 5**,** 218–227.

Stepanova, A.N., and Alonso, J.M. (2009). Ethylene signaling and response: where different regulatory modules meet. *Curr. Opin. Plant Biol.* 12**,** 548–555. doi: 10.1016/j.pbi.2009.07.009

Thanaraj, T., Terry, L.A., and Bessant, C. (2009). Chemometric profiling of pre-climacteric Sri Lankan mango fruit (*Mangifera indica* L.). *Food Chem.* 112**,** 786–794. doi: 10.1016/j.foodchem.2008.06.040

Wünsche, J.N., and Ferguson, I.B. (2005). "Crop load interactions in apple," in *Horticultural Reviews,* ed. J. Janick. (Oxford: John Wiley & Sons, Inc.), 231–290. doi: 10.1002/9780470650882.ch5

Yuan, R., and Burns, J.K. (2004). Temperature factor affecting the abscission response of mature fruit and leaves to CMN-pyrazole and ethephon in 'Hamlin' oranges. *J. Am. Soc. Hort. Sci.* 129**,** 287-293.

Xie, R.J., Deng, L., Jing, L., He, S.L., Ma, Y.T., Yi, S.L., Zheng, Y.Q., and Zheng, L. (2013). Recent advances in molecular events of fruit abscission. Biol. Plantarum 57, 201–209. doi: 10.1007/s10535-012-0282-0

5. Expression and dimerisation of the mango ethylene receptor MiETR1 and different receptor versions of MiERS1[5]

5.1 Abstract

Different versions of the mango ethylene receptor MiERS1 were identified and the analysis indicates that, in addition to MiERS1, two short versions of this receptor (MiERS1m, MiERS1s), representing truncated proteins with central deletions of functional domains, are present in mango. The expression pattern was investigated and it was found, that the two short receptor versions reveal a different expression pattern compared to *MiERS1*, and that they are highly variably transcribed. With transient expression assays using fluorescent fusion proteins, the localisation in leaf cells of the tobacco model and the dimerisation behaviour of the receptors were determined. MiERS1, MiETR1, and the short MiERS1 receptor versions are anchored in the endoplasmic reticulum (ER) membrane and co-localise with each other and with an ER-marker. Furthermore, ectopic expression of the shorter MiERS1 versions induces a re-organisation of the ER resulting in accumulation of ER bodies. Dimerisation assays suggest that both short MiERS1 receptor versions can bind to proteins located in the ER. Bi-molecular fluorescence complementation (BiFC) assays indicate, that MiERS1m can dimerise with itself and with MiERS1, but not with MiETR1. However, dimerisation of MiERS1s with the known mango ethylene receptors could not be detected, although it was located in the ER membrane system.

Key words: ethylene receptor, dimerisation, receptor diversification, short ERS1 versions, endoplasmic reticulum, ER bodies.

5.2 Introduction

Ethylene signalling is involved in various plant physiological processes including biotic and abiotic stress response, fruit ripening, organ abscission and senescence, seed germination and plant development. Endogenous ethylene synthesis depends on 1-aminocyclopropane-1-carboxylate (ACC) as a substrate produced by the ACC synthase, and in a following step on the autocatalytic activity of ACC oxidase as previously reviewed (Chae and Kieber, 2005; Yang and Hoffmann, 1984). Ethylene receptors are

[5]This chapter consist of an unpublished manuscript that has been submitted to the *Journal of Experimental Botany*.

anchored to the endoplasmic reticulum (ER) membrane and perceive ethylene, resulting in their inactivation due to binding the ligand, which triggers the signal cascade via the release of CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) (Binder, 2008; Bleecker *et al.*, 1998; Chen *et al.*, 2005; Etheridge *et al.*, 2005; Ma *et al.*, 2006; Mayerhofer *et al.,* 2012; Stepanova and Alonso, 2009). In this conserved pathway ethylene receptors directly contribute to signalling by binding and controlling CTR1, a cytosolic Ser/Thr kinase, which acts as a negative regulator (Binder, 2008; Clark *et al.,* 1998; Etheridge *et al.*, 2005; Mayerhofer *et al.*, 2012; Stepanova and Alonso, 2009). Downstream in the ethylene signalling pathway transcription factors are involved and control the specific gene expression, that finally leads to a distinct ethylene response (Chen *et al.*, 2005; Etheridge *et al.*, 2005).

Ethylene receptors play a key role in the ethylene signalling pathway in plants and are divided into two subfamilies: arabidopsis contains, besides the members ERS1 and ETR1 of subfamily I, the receptors ERS2, ETR2, and EIN4 of the subfamily II (Bleecker *et al.*, 1998; Hua *et al.*, 1998; Sakai *et al.*, 1998). The different receptors generally show some, however incomplete, redundancy in their function, but the ethylene receptors of the subfamily I appear to be essential and their action cannot be replaced by the activity of the subfamily II members, which was demonstrated by experiments with arabidopsis loss-of-function mutants (Qu *et al.*, 2007). Recent research on the arabidopsis model provides detailed information on ethylene signalling and receptor function, intermolecular interaction and fine tuning of signalling (Hall *et al.*, 2012; Liu and Wen, 2012; Mayerhofer *et al.*, 2012; Stepanova and Alonso, 2009). By conducting a series of complementary experiments, the role of ethylene receptors was characterised in the yeast model in more detail and it was demonstrated, that the receptors interact and are capable of modulating each other's signal output (Gao *et al*., 2008). It was found that ethylene receptors of both receptor families interact not only as homo- or heterodimers, but they can act also as a higher order protein complex, which is thought to be crucial for defining ethylene sensitivity and precise adjustments of the ethylene response (Chen *et al.*, 2010; Gao *et al.*, 2008; Grefen *et al.*, 2008).

The investigation of ethylene receptors from different plant species supports the overall high conservation of this important signalling system. However, additional ethylene receptor isoforms have been described from other plants, increasing the receptor diversity and putative regulatory possibilities in ethylene signalling (Binder, 2008; Ireland *et al.*, 2012; John-Karuppiah and Burns, 2010). The number and versions of the

identified receptors varies and several crop plants host more receptors than the model plant arabidopsis, revealing variability due to isoforms or by alternative splicing (Bassett *et al.*, 2002; Bustamante-Porras *et al.*, 2007; Ireland *et al.*, 2012). Because of the increased variability of ethylene receptors and the consequently added possibilities of receptor interaction, it could be presumed that regulation of the ethylene signalling and response pathways might be even more complex in perennial plant species like tomato, coffee, apple, or peach (Bassett *et al.*, 2002; Bustamante-Porras *et al.*, 2007; Ireland *et al.*, 2012; Kevany *et al.*, 2007). Despite the increasing economic importance of mango (Galán Saúco, 2015) and the prevailing role of ethylene signalling for its production only limited information is available on ethylene perception (Hagemann *et al.*, 2015; Ish-Shalom *et al.*, 2011; Martínez *et al.*, 2001).

5.3 Material and methods

5.3.1 Plant material

Mango trees of the cultivar 'Hôi' (n = 6) grown in a commercial orchard located in Yên Châu district, Vietnam were used as a source for plant material (Hagemann *et al.*, 2014). Samples ($n \geq 3$) of various tissues were collected for expression analysis based on the phenological growth stages according to the BBCH scale (Hernández Delgado *et al.*, 2011): (1) premature leaves and (2) their peduncles (BBCH = 115) as well as (3) old leaves and (4) their peduncles (BBCH = 119); (5) premature flowers (BBCH = 517) as well as mature flowers and floral parts (BBCH = 615) including (6) male flowers and (7) their stamen (separately collected), (8) perfect (hermaphrodite) flowers; fruitlets of (9) pinhead (~6 mm, BBCH = 619), (10) pea (~11 mm, BBCH = not applicable), or (11) marble size (~20 mm, BBCH = 710); and the corresponding (12) pedicels of pea sized and (13) marble sized fruitlets. All plant samples were immediately snap frozen in liquid nitrogen and stored until further extraction and analysis. For a comparative investigation of genomic sequences, leaves of the mango cultivar 'Tròn' were collected from the same orchard in Yên Châu and fruit exocarp samples of other mango cultivars were obtained from fruit purchased at fresh markets: 'Namdocmai', 'Mahachanok' (local fresh market in Thailand) 'Tommy Atkins', 'Keitt', 'Palmer' (import from Brazil for German fresh market). *Nicotiana benthamiana* plants were grown in a climate chamber under a light/dark period of 16/8 h at 24 °C for five weeks and used for agrobacteria infiltration and transient gene expression experiments.

5.3.2 Sequence isolation and characterisation

Total RNA was extracted after grinding samples under liquid nitrogen using the Masterpure Plant RNA kit (Epicentre, WI, USA) as recommended by the manufacturer. Sequences of cDNA were obtained by using First Choice RLM-RACE (Life Technologies, CA, USA) following manufacturer´s guidelines. To obtain genomic DNA the MasterPure Plantleaf DNA kit (Epicentre, WI, USA) was used for extraction as recommended. Sequences were amplified by PCR using Phusion HiFi Polymerase (Finnzymes, Finland) and cloned for sequencing and further analysis. Obtained ethylene receptor sequences were confirmed by online BLAST search at NCBI and ExPASy. Subsequent bioinformatical analysis was performed using the analysis tools InterPro (Hunter *et al.*, 2012) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) for protein domain prediction, and NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/) for splicing site prediction in genomic sequences, respectively (Brunak *et al.*, 1991; Hebsgaard *et al.*, 1996).

5.3.3 Gene expression studies by quantitative real-time PCR

For synthesis of cDNA the Taqman RT kit (Applied Biosystems, MA, USA) and 0.5 µg RNA per reaction were used after manufacturer´s recommendation. For gene expression analysis quantitative real-time PCR (qPCR) was performed using the RotorGene SYBR Green PCR Kit (Qiagen, Germany) and the RotorGene 6000 cycler (Corbett, Australia). After evaluation, mango actin (*MiACT*) was chosen as reference gene for the qPCR analysis of the genes of interest (Supplementary material and methods 5.S1). Specific primers (Supplementary Table 5.S1) were designed for the mango target genes and amplification products were sequenced to confirm specificity. All runs were performed at 95 °C/3 min initial denaturation, followed by 40 cycles at 95 °C/20 s denaturation, 58 °C/20 s annealing, 72 °C/20 s elongation, and a final melting curve with 0.5 K steps from 60 °C to 99 °C. Gene expression was analysed with the efficiency corrected ΔΔCt method using the DART tool as recommended (Peirson *et al.*, 2003). A pool-sample was composed from 1 µl of cDNA of each tissue sample and used in the different runs as an intra- and interrun standard. For statistical analysis the gene expression was evaluated by analysing untransformed and $\log_{10}$-transformed data of the relative expression of the target genes. The correlation coefficient and the R^2 were calculated using SigmaPlot 11.0 (Systat Software Inc., CA, USA).

5.3.4 Transient expression in *Nicotiana benthamiana*

Using appropriate primer combinations (Supplementary Table 5.S2) ethylene receptor sequences were cloned via the pDONR221 vector and the gateway cloning system (Life technologies, CA, USA) into the binary vectors pH7CWG2 and pK7YWG2 (Karimi *et al.*, 2002), and into the splitYFP binary vector system pB4-GWnYFP/cYFP (Hino *et al.*, 2011; Tanaka *et al.*, 2012) (Supplementary Table 5.S3, Supplementary Fig. 5.S1). Agrobacteria were transformed with the final constructs for subsequent infiltration of *N. benthamiana* leaves. For infiltration assays fusion protein constructs with yellow and cyan (blue) fluorescence proteins (YFP, CFP), respectively, were used to perform (co-)localisation and dimerisation analysis of MiETR1 and the different MiERS1 versions *in planta*. Following constructs (Supplementary Table S3, Supplementary Fig. S1) were used: (i) ethylene receptor sequences fused to YFP or CFP for analysis of the localisation and the co-localisation in the cell; (ii) constructs with receptors **d**evoid of the **t**rans**m**embrane domain (dTM) fused to CFP for testing, if those truncated receptors interact and co-localise with membrane-anchored complete receptors fused to YFP; (iii) splitYFP-constructs for bimolecular fluorescence complementation (BiFC) studies to analyse specific dimerisation of the receptors. Corresponding splitYFP-constructs containing the dTM-ethylene receptors were designed to avoid unspecific splitYFP complementation simply due to close neighbouring in the ER membrane. Expression of a positive control reporter construct with fluorescent protein under control of the 35S promoter resulted in a very bright signal with a different distribution in the cell compared to the receptor fusion proteins (data not shown).

For transient expression experiments with (split)fluorescence protein fusion-constructs, agrobacteria containing a construct with the silencing inhibitor pGJ p19 and agrobacteria containing an ER-marker fused to mCherry (CD3-959) were co-infiltrated (Nelson *et al.*, 2007; Voinnet *et al.*, 2003; Winterhagen *et al.*, 2009). The co-infiltration experiments were performed in all appropriate combinations and repeated at least three times with similar results. Fluorescence signal was analysed two days after infiltration by confocal laser scanning microscopy (Zeiss LSM 700, Zeiss, Germany). Sequential scanning and an adjusted channel setup with appropriate light paths verified specificity of fluorescence signals and minimized the signal of possible fluorescence resonance energy transfer (FRET) or background in YFP- and CFP-fusion protein co-infiltration assays. The DAPI filter set (Zeiss, Germany) was used to control each specimen to discriminate tissue with unspecific autofluorescence based on senescing cells or locally

developing necrosis (Supplementary Fig. 5.S2). Images were acquired and processed with the Zeiss ZEN2010 software package (Zeiss, Germany) and Adobe Photoshop (Adobe Systems, CA, UAS), respectively.

5.4 Results and discussion

5.4.1 Sequence isolation and characterisation

To obtain cDNA of *MiERS1* and *MiETR1* from the mango cultivar 'Hôi', primers were designed based on the mango sequences Genbank ID: JF323582.1 and AF227742.1, respectively (Ish-Shalom *et al.*, 2011; Martínez *et al.*, 2001). Besides the expected PCR products of full-length *MiERS1* and *MiETR1*, additional amplicons of shorter *MiERS1*-like versions were found. The PCR products were cloned and sequencing resulted in the identification of the expected full-length sequences of *MiETR1* (Genbank ID KP057214) with 2220 nucleotides (nt), and *MiERS1* (Genbank ID KJ735092) with 1890 nt length, respectively. In addition, two further *MiERS1* versions, a medium sized with 1203 nt (*MiERS1m*, Genbank ID KJ735093) and a small one with 561 nt (*MiERS1s*, Genbank ID KJ735094) length, respectively, were identified revealing deletions of central parts compared to *MiERS1* (Fig. 5.1A). In comparison to the arabidopsis *ERS1* sequence and using the NetGene2 tool (Brunak *et al.*, 1991; Hebsgaard *et al.*, 1996) for splicing site prediction, it was found that the coding sequences of the shorter *MiERS*-like versions *MiERS1m* and *MiERS1s* are possibly not products of alternative splicing, as the sequences were not assembled at the predicted splicing sites, but within exons. *MiERS1m* has a deletion from nt 1178 within the second exon, missing the exons three and four and finishes with a short 3´ terminal part of the last 25 nts from the fifth exon. *MiERS1s* consists of a first part from nt 1 to 384 of the first exon, then exons two to four are missing and the sequence finishes with the nt 177 3´-terminal end of the fifth exon. Silent mutations were identified in the full-length *MiERS1* based on single nucleotide polymorphisms (SNPs) at positions A/C138 and A/G225 (Fig. 5.1A), suggesting that at least two *MiERS1* sequences are expected to be present in the mango genome, which is corroborated by previous findings (Ish-Shalom *et al.*, 2011). For both shorter *MiERS1* versions only the SNP combination C138/G225 was found, which might be an indication that the genomic origin of these versions is the corresponding full-length *MiERS1* gene.

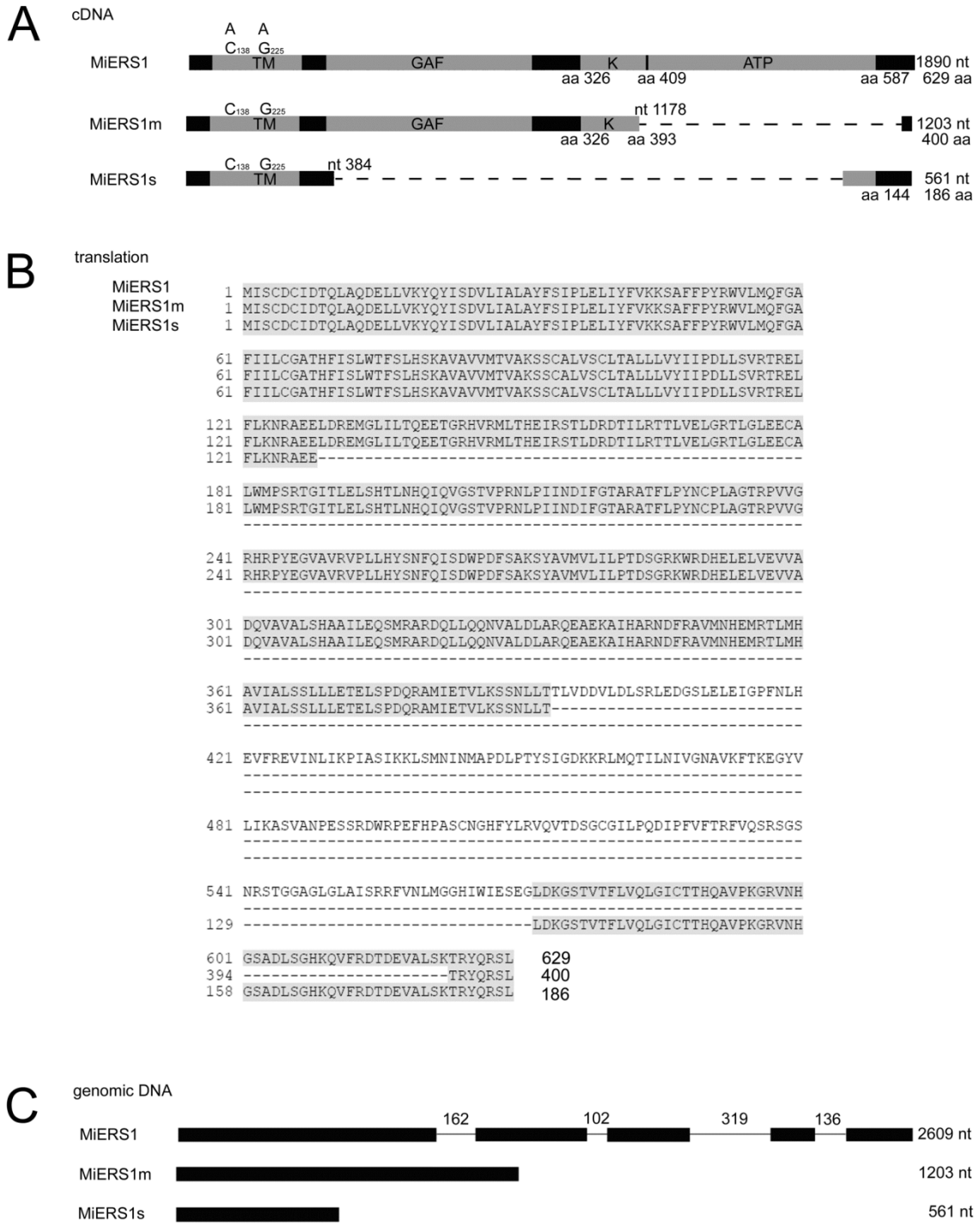


Fig. 5.1. *MiERS1* is expressed in three versions. (A) Structure and domains of the isolated *MiERS1* versions. Domains are boxed in grey, broken line indicate gap in sequence, SNPs and their positions are indicated. Numbers indicate position or sequence length in nucleotides (nt) or amino acids (aa). TM: transmembrane domains, GAF: GAF domain, K: histidine kinase domain, ATP: ATP-binding domain. (B) Alignment of translated cDNA sequences. Numbers indicate sequence length in amino acids. (C) Genomic sequence structure of *MiERS1* versions. Boxes indicate exons and thin lines indicate introns, numbers indicate size of introns in nucleotides.

In silico translation of the shorter versions revealed that the sequences MiERS1m and MiERS1s are in frame resulting in putative proteins, which are devoid of certain functional domains in comparison to MiERS1 (Fig. 5.1A, B). MiERS1m is missing the ATP-binding domain that acts as phosphor acceptor and is described to be necessary for CTR1 interaction (Bleecker *et al.*, 1998; Clark *et al.*, 1998). The receptor contains the GAF domain (amino acids 158 to 307) and the main part of the histidine kinase (amino acids 326 to 409) as identified by InterPro analysis (Hunter *et al.*, 2012). These sequence features might indicate that the degenerated version MiERS1m possibly depends on dimerisation with full-length ethylene receptors to be functional and may influence the signal output of receptor complexes. All those above mentioned domains are missing in MiERS1s, and the InterPro analysis did not recognise the remnants of the C-terminal part of the truncated ATP-binding domain, which - considering the known ethylene signalling pathway - causes doubts on the functionality of MiERS1s. However, the N-terminal end, including the sensor domain for ethylene binding, is conserved in all three MiERS1 versions and reveals the characteristic transmembrane domains (TM) as predicted by analysis with the TMHMM tool (Supplementary Fig. 5.S3). Based on the TMHMM analysis and previously described ethylene receptors from other plants (Bleecker *et al.*, 1998; Grefen *et al.*, 2008; Ma *et al.*, 2006; Zhong *et al.*, 2008), MiERS1 and MiERS1m are thought to be anchored in the ER membrane and strictly oriented with the N-terminus facing the lumen, while the C-terminus is protruding into the cytosol. However, for MiERS1s it is predicted by the TMHMM tool with a slightly higher probability that the orientation of this receptor protein in the ER membrane is reversed (Supplementary Fig. 5.S3C). Especially considering the missing GAF domain for receptor dimerisation, the absence of the histidine kinase and ATP-binding domains, and the possibly reversed orientation on the ER membrane, a function of MiERS1s in the CTR1-dependent ethylene signal pathway is questionable, but it may be involved in alternative ethylene signalling pathways, such as discussed by Zhang *et al.* (2014).

As indicated above, the transcripts of the shorter MiERS1 versions possibly are not results from alternative or aberrant splicing events and the sequences were expected to be present in the 'Hôi' genome. PCR and sequencing analysis confirmed the presence of all three *MiERS1* versions in the genomic DNA (Fig. 5.1C). Sequencing of the full-length *MiERS1* (Genbank ID KJ735095) revealed that four introns are located at the predicted and conserved sites. However, the genomic *MiERS1m* and *MiERS1s* sequences do not contain any introns and they were confirmed in the genomic DNA as expressed.

5.4.2 Presence of MiERS1 versions in different mango cultivars

Besides the obligatory full version of *MiERS1*, it was also tested if the shorter *MiERS1* versions are present in the genome of other mango cultivars, or if these versions are an unique and specific feature of 'Hôi'. The *MiERS1m* version was detected by PCR only in 'Hôi', which might indicate that *MiERS1m* is not present in the other cultivars or that the primers (designed for the 'Hôi' *MiERS1m* sequence) are not specific for *MiERS1m* of the other tested cultivars. However, versions of *MiERS1s* could be identified by PCR with the specific primers in the genomic DNA of other mango cultivars (Supplementary Fig. 5.S4): 'Tròn', 'Namdocmai', 'Keitt', and 'Mahachanok'. These *MiERS1s* versions were confirmed by sequencing and are highly similar (>97%) to the sequence of 'Hôi'. In the cultivars 'Tommy Atkins' and 'Palmer' both short *MiERS1* versions could not be confirmed, despite using nested PCR and different primer combinations. Short *MiERS1* versions are not known for other plants and their existence is not limited to the local mango varieties of Northern Vietnam, therefore, it is postulated that the presence of the short ethylene receptors is a specific feature for mango or certain mango cultivars.

5.4.3 Expression analysis by quantitative real-time PCR

The qPCR analysis of the ethylene receptors in various mango tissues and plant organs at different developmental stages shows that the expression level of *MiETR1* (Fig. 5.2A) is relatively homogenous throughout the set of samples, while *MiERS1* (Fig. 5.2B) and the two smaller versions *MiERS1m* (Fig. 5.2C) and *MiERS1s* (Fig. 5.2D) are heterogeneously expressed ranging over two orders of magnitude. Furthermore, *MiETR1* and *MiERS1* transcripts were present in each tested sample (Fig. 5.2A, B), but transcripts of both shorter *MiERS1* versions were not always detectable and overall they displayed a highly erratic transcription pattern in most tissues: *MiERS1m* was found in approximately 78% and MiERS1s in approximately 54% of all tested cDNAs, respectively (Fig. 5.2C, D). However, there was a significant positive correlation detected between the *MiERS1m* and the *MiERS1s* expression with a R^2 of 40% or 57% for the untransformed or $\log_{10}$-transformed data, respectively.

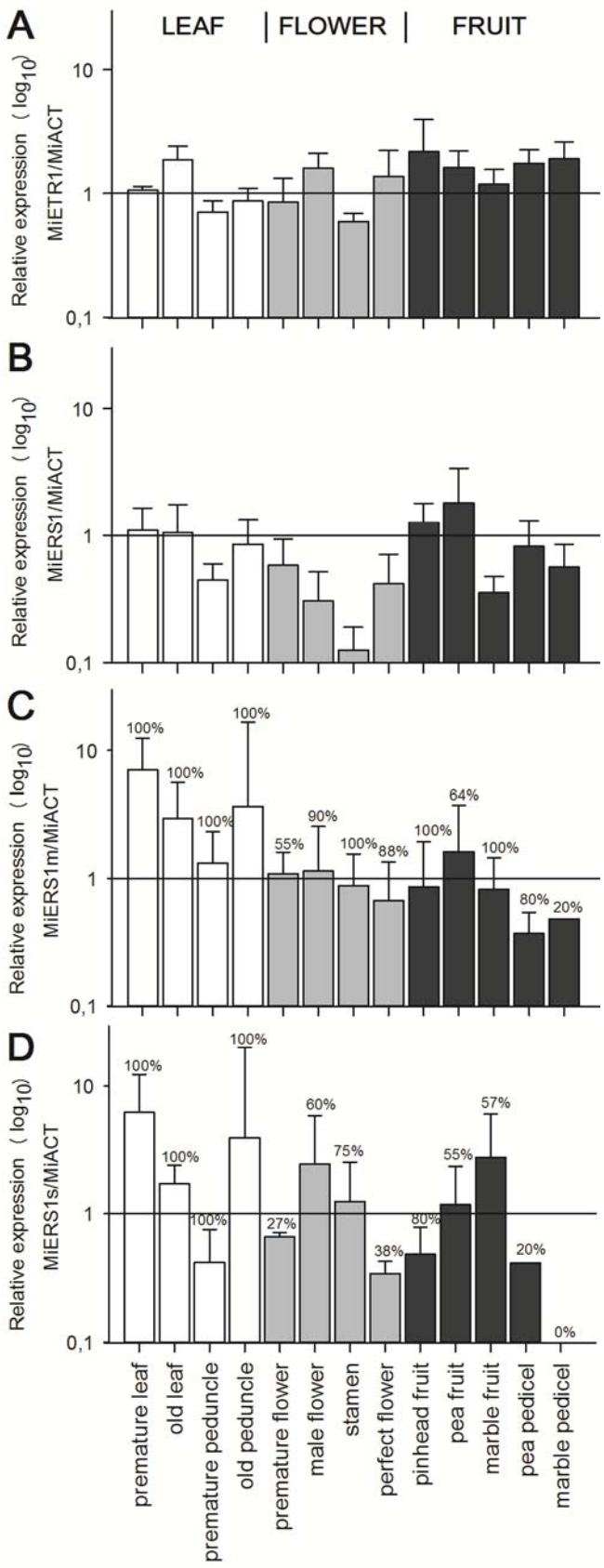


Fig. 5.2. Expression analysis by qPCR of *MiETR1* and the *MiERS1* versions. Means of the level of receptor transcripts in different mango organs and developmental stages relative to a linker sample. *MiETR1* (A) and *MiERS1* (B) are present in 100% of the samples; occurrence of *MiERS1m* (C) and *MiERS1s* (D) in all samples is indicated as percent in relation to tested

cDNAs. Expression values of *MiERS1m* and *MiERS1s* refer to samples with detectable expression. Bars indicate standard deviation, except for (C) *MiERS1m*, marble pedicel due to sample size n = 1.

MiETR1 (Fig. 5.2A) and *MiERS1* (Fig. 5.2B) are similarly expressed in leaves and leaf peduncle abscission zones. The transcription level of *MiERS1m* (Fig. 5.2C) and *MiERS1s* (Fig. 5.2D) was on average highest in premature leaves compared to all other tested tissues. Furthermore, transcription levels of the two shorter receptor versions were higher on average in the peduncles of old leaves compared to those of premature leaves. However, the expression level was highly variable and future studies of leaf abscission might clarify, if the short receptor versions are involved in the leaf abscission process.

The highest variability of the relative expression level of *MiETR1* and *MiERS1* was found in the floral tissues. It appears, that especially *MiERS1* (Fig. 5.2B) has a tendency to be expressed at an overall lower level in flowers in comparison to fruits and leaves. ERS1-mediated ethylene signalling has a prevailing role in flower development (Yamasaki *et al.*, 2003). It is suggested by Yamasaki *et al.* (2003) that MiERS1 might promote female pistil primordia development and inhibits stamen development in hermaphrodite cucumber flowers. Indeed, in mango the lowest expression level of *MiERS1* was found in stamen compared to all tested tissues (Fig. 5.2B). However, the expression levels of *MiERS1m* and *MiERS1s* in stamen was high in contrast to *MiERS1* (Fig. 5.2C, D), suggesting that these short receptor versions promote the development of stamen.

MiETR1 is stably expressed throughout the examined fruit stages (Fig. 5.2A), which is in agreement with results for *ETR1* expression in the fruit of other plant species, like peach and persimmon, showing that *ETR1* transcription remains at a certain level, while the *ERS1*-homologues are regulated during fruit development (Pang *et al.*, 2007; Rasori *et al.*, 2002). In mango, *MiERS1* might also be involved in early fruit development, as the expression level increases from the (perfect) flower stage to the pea fruit stage, however, *MiERS1* transcription level is decreased in the marble fruit stage (Fig. 5.2B). This result is corroborated by data from muskmelon, where an increase of *ERS1* expression during early fruit development has previously been reported (Sato-Nara *et al.*, 1999). Similarly, an increased level of *ERS1* transcription in persimmon fruitlets at an early fruit developmental stage (that corresponds to mango pea size stage) was

described, which was followed by a decreased transcript level in later fruit stages (Pang *et al.*, 2007). In agreement with the work of Pang *et al.* (2007) and Sato-Nara *et al.* (1999) it is suggested for mango fruitlets, that the accumulation of MiERS1 could reduce the ethylene sensitivity, as a high amount of ethylene receptors can protect cells from undergoing the ethylene response, which is also in agreement with the model based on experiments with arabidopsis and described by Liu and Wen (2012). High levels of endogenous ethylene occur during premature mango fruitlet abscission (Malik *et al.*, 2003), and high levels of ethylene receptors may protect those fruitlets against the ethylene effects which are not designated for abscission.

Considering *MiERS1m* and *MiERS1s* detection in different tissues, their variable transcription levels and erratic expression in most tissues suggest, that these short receptor versions are regulated very specifically, organ and stage dependent, and may have different functions compared to MiERS1.

5.4.4 Mango ethylene receptor localisation in the plant cell

After infiltration of *N. benthamiana* leaves specific fluorescence signal for all receptor-YFP fusion protein constructs (Fig. 5.3A, D, G, J) was found in a similar pattern like for the ER-marker (Nelson *et al.*, 2007) (Fig. 5.3B, E, H, K; mCherry display). This indicates that MiETR1 and all MiERS1 versions are anchored in the ER membrane system. Because of the known localisation of ethylene receptors described from other plants (Grefen *et al.*, 2008; Ma *et al.*, 2006; Zhong *et al.*, 2008) and the presence of the conserved transmembrane domains, a co-localisation with the co-infiltrated ER-marker was expected. The specific fluorescence signal for MiETR1-YFP mainly matches the pattern of the ER-marker (Fig. 5.3C), however, the pattern of MiERS1 versions indicates that these receptors are not evenly distributed within the ER membrane system. This is visualised by overlay images, where the differences of the fluorescence signals are obvious (Fig. 5.3F, I, L). Frequently, an intensive YFP specific signal was detected in ER-related bodies or clusters, which appeared in several cells (Fig. 5.3D, G, J). These subcellular structures were mostly also labeled with the ER-marker (Fig. 5.3K, L; insets) and especially found in leaves co-infiltrated with the MiERS1-YFP versions, but were rarely found in cells co-expressing MiETR1-YFP, and never in controls infiltrated only with the ER-marker and the silencing inhibitor p19 (Fig. 5.3N, O).

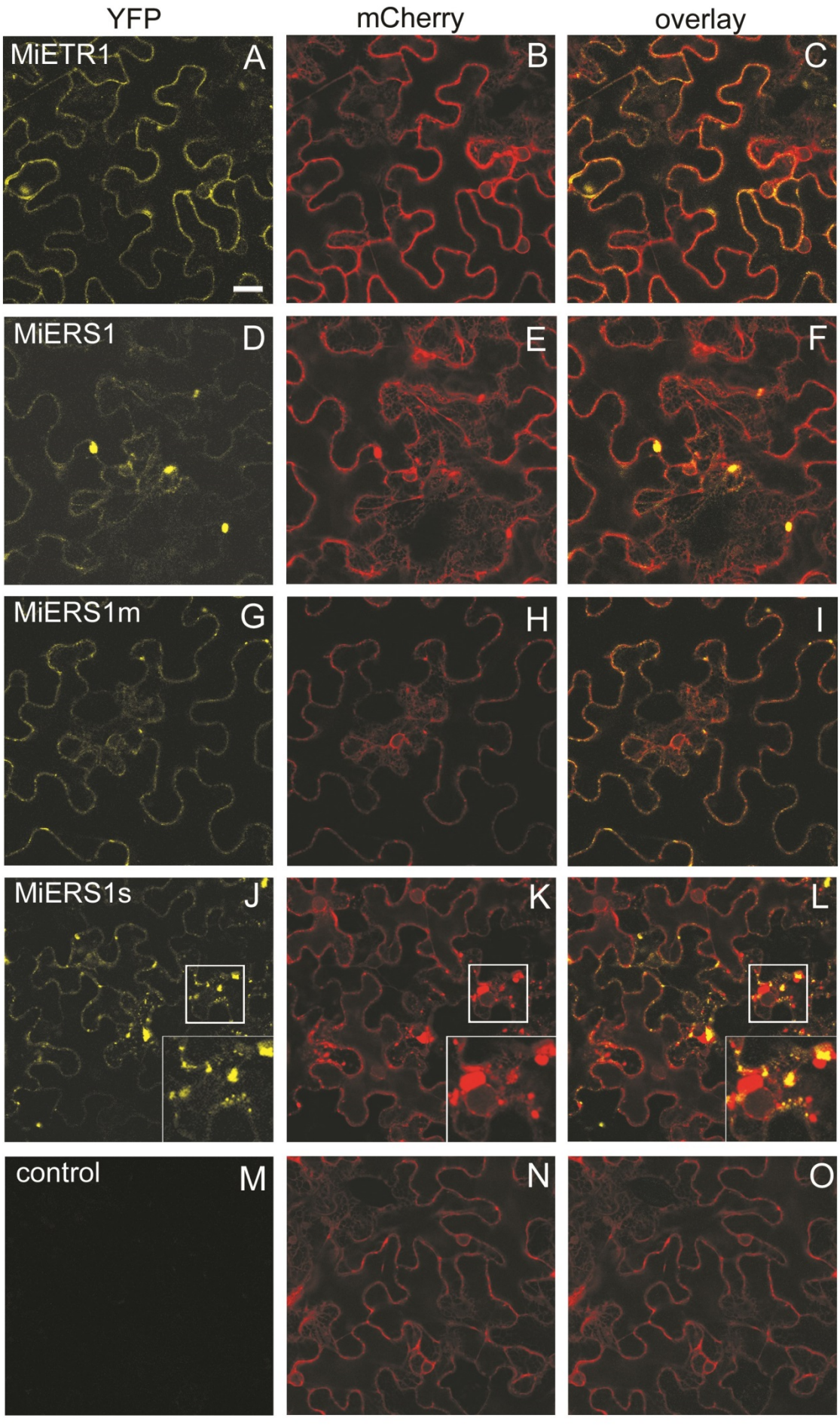

YFP
mCherry
overlay
MiETR1
A
B
C
MiERS1
D
E
F
MiERS1m
G
H
I
MiERS1s
J
K
L
control
M
N
O

Fig. 5.3. Analysis of localisation of MiETR1 and the MiERS1 versions by transient expression in infiltrated *N. benthamiana* leaves. Co-expression of MiETR1 (A) and MiERS1 version YFP-fusion constructs (D, G, J, yellow) with the ER-marker mCherry-fusion construct (B, E, H, K, red); overlay images of YFP and mCherry channels (C, F, I, L); ER-marker expression as a control without the co-infiltration of receptor constructs (M, N, O). Scale bar 20 µm.

Consequently, the appearance of the observed ER bodies seems to be linked to the overexpression of the mango ethylene receptors, and their accumulation was especially increased in cells expressing the MiERS1 versions. Interestingly, these ER clusters, of which many but not all displayed bright YFP-specific fluorescence, were often localised closely to the nucleus and they were especially prominent and more abundant in cells (co-)expressing MiERS1s constructs (Fig. 5.3J, K, L; insets). The ER clusters accumulated around the nucleus (increasingly from one to three days after infiltration, data not shown) and the formation of these clusters might indicate a stress response or senescence, as ethylene is known to play a role in stress signalling and cell death (Lim *et al.*, 2007; Satoh *et al.*, 2008). Stress induced ER bodies and senescence derived ER structures clustering around the nucleus two to three days after treatment were described previously (Farage-Barhom *et al.*, 2011; Matsushima *et al.*, 2002). In consideration of this, it is suggested that the endogenous ethylene signal pathway of the (co-)infiltrated cells probably is disturbed by mango ethylene receptor expression, which could enforce a stress response and cause re-organisation of the ER.

5.4.5 Co-localisation of mango ethylene receptors

To test if the different receptors occupy the same ER compartments the receptor-YFP (or CFP) fusion constructs were co-infiltrated. The results indicate that MiETR1 and all MiERS1 versions generally co-localise (Fig. 5.4). In most cases the co-expressed fusion proteins are present in the same ER structures and are also accumulating in the ER bodies.

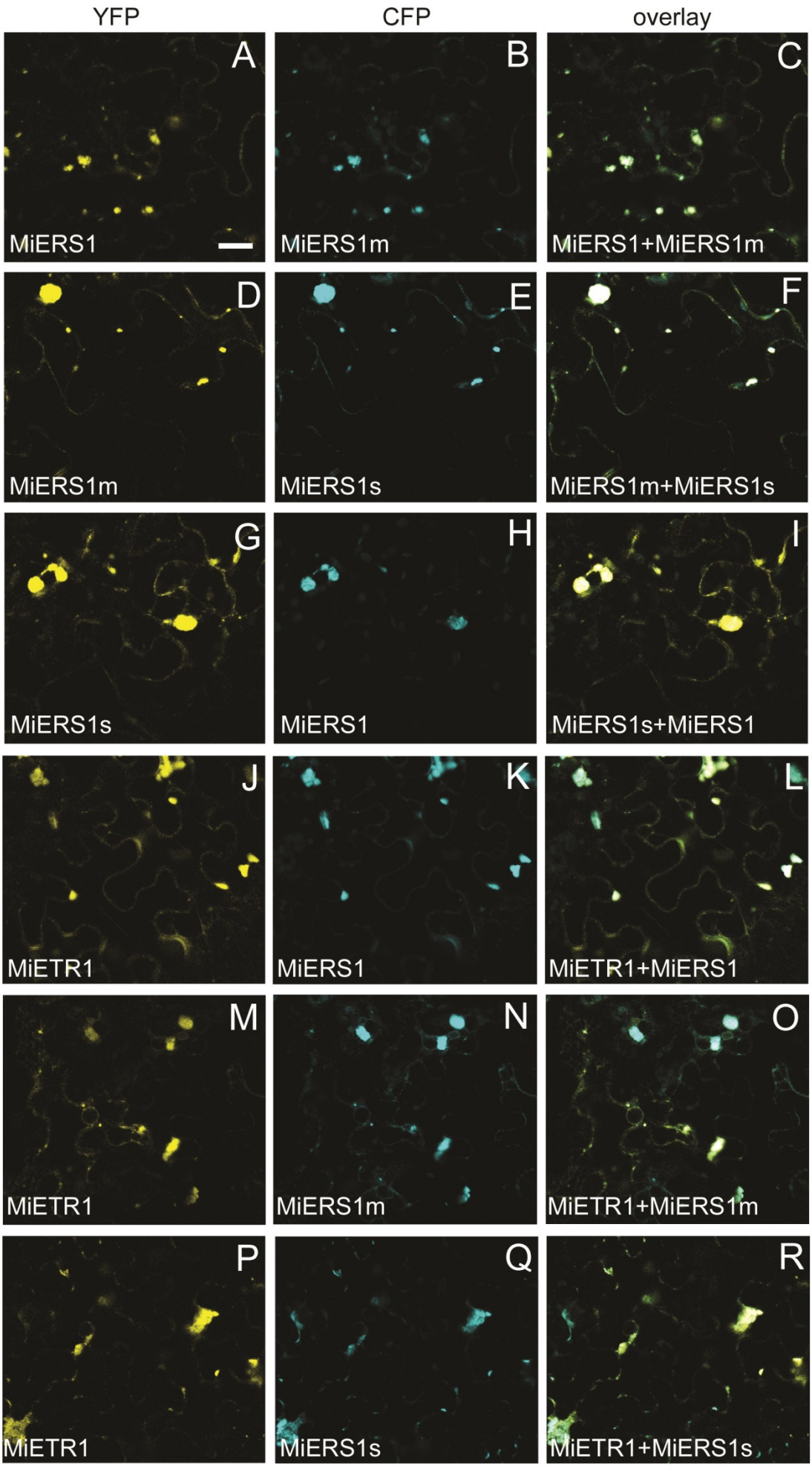
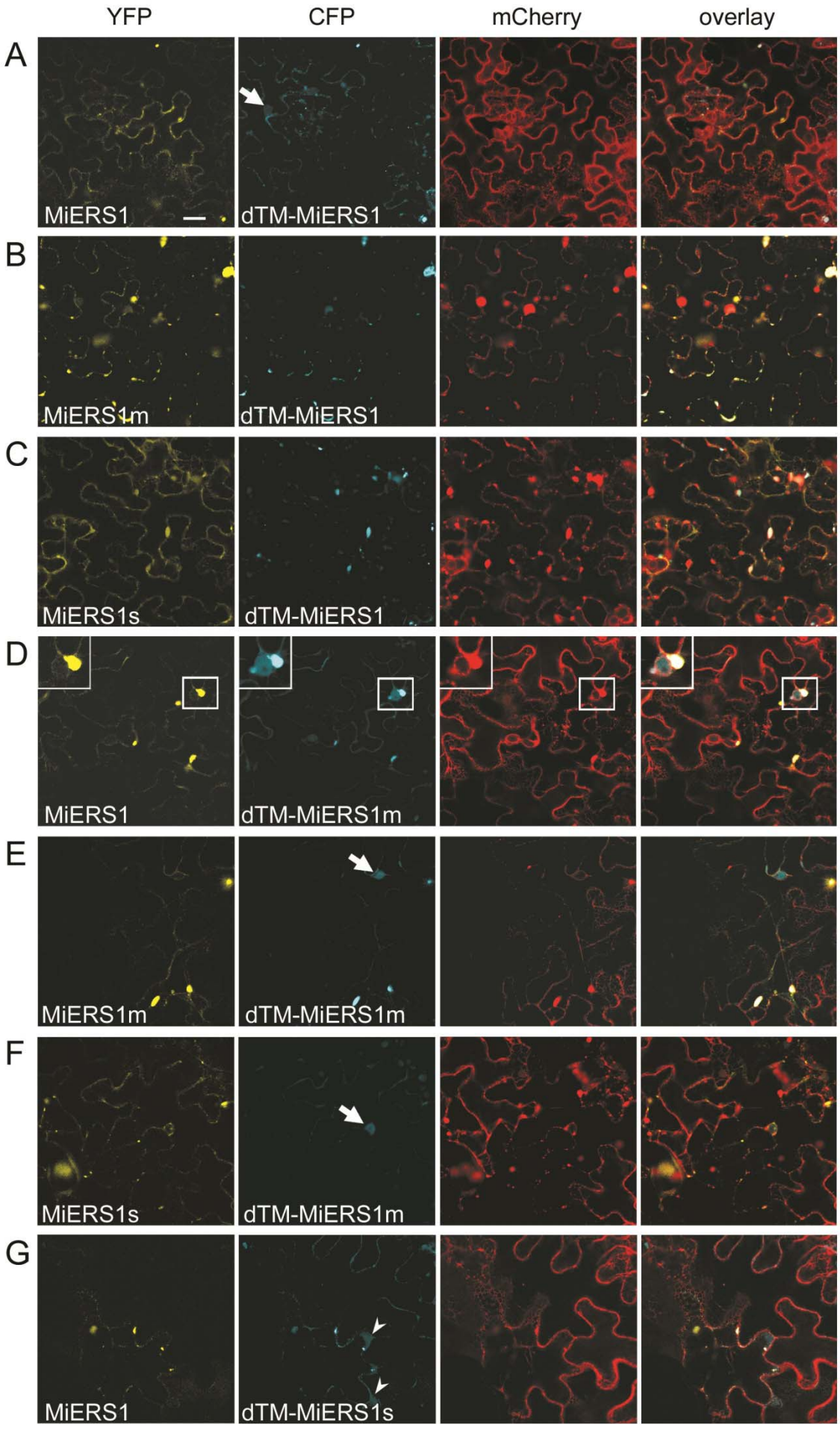

YFP
CFP
overlay
A
B
C
MiERS1
MiERS1m
MiERS1+MiERS1m
D
E
F
MiERS1m
MiERS1s
MiERS1m+MiERS1s
G
H
I
MiERS1s
MiERS1
MiERS1s+MiERS1
J
K
L
MiETR1
MiERS1
MiETR1+MiERS1
M
N
O
MiETR1
MiERS1m
MiETR1+MiERS1m
P
Q
R
MiETR1
MiERS1s
MiETR1+MiERS1s

Fig. 5.4. Co-localisation analysis of MiETR1 and the MiERS1 versions by transient expression in infiltrated *N. benthamiana* leaves. Co-expression of MiERS1 versions fused to YFP (A, D, G, yellow) or CFP (B, E, H, blue); Co-expression of MiETR1 fused to YFP (J, M, P, yellow) and MiERS1 versions fused to CFP (K, N, Q, blue); overlay images of YFP and CFP channels (C, F, I, L, O, R). Scale bar 20 μm.

5.4.6 Dimerisation of MiERS1 and the shorter MiERS1 versions

To analyse dimerisation behaviour of MiERS1 versions dTM-receptor-CFP fusion proteins, which should not be able to anchor themselves in the ER membrane system, were co-expressed with the receptor-YFP fusion proteins (Fig. 5.5). The dTM-receptors were mainly found in the ER structures and usually co-localised with the co-infiltrated ER-marker and with the YFP fusion constructs of the complete receptors. This cellular distribution suggests that dTM-receptors interact with dimerisation partners bound in the ER-membrane system, and those binding partners could be the co-infiltrated receptors (fused to YFP), or possibly might also be endogenous membrane bound proteins of *N. benthamiana*. However, this result is especially surprising for MiERS1s, as this receptor version does not contain any known domain for protein interaction and the localisation of dTM-MiERS1s in the ER membrane was not expected. Frequently, for the dTM-receptor fusion proteins - and mostly found for dTM MiERS1m and dTM MiERS1s - a less intense but CFP-specific signal was detected in nuclei (Fig. 5.5, CFP column in A, D-I, indicated by inset or arrows). Further, diffuse fluorescence signals of dTM-MiERS1s were also determined in cell compartments not labelled with the ER-marker (Fig. 5.5, CFP column in G, indicated by arrowheads). This non-ER localisation was never found for complete receptor fusion proteins, which indicates that - due to the lack of the membrane-anchor and possibly not finding a binding partner for dimerisation - the dTM-receptors are not consequently targeted to the ER and display partly an unspecific distribution.


YFP
CFP
mCherry
overlay
A
MiERS1
dTM-MiERS1
B
MiERS1m
dTM-MiERS1
C
MiERS1s
dTM-MiERS1
D
MiERS1
dTM-MiERS1m
E
MiERS1m
dTM-MiERS1m
F
MiERS1s
dTM-MiERS1m
G
MiERS1
dTM-MiERS1s

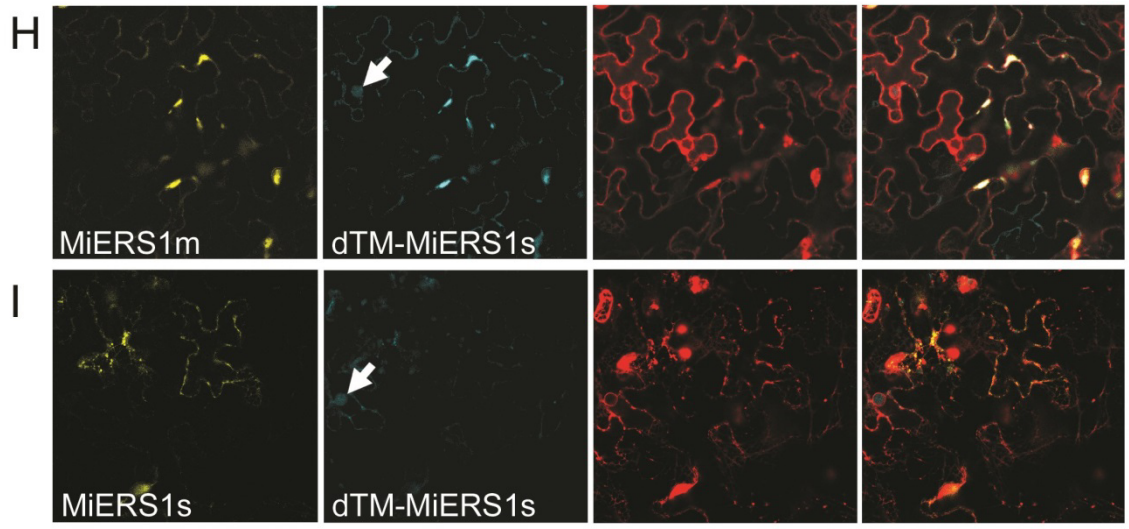


Fig. 5.5. Dimerisation analysis of the MiERS1 versions by transient expression assay in infiltrated *N. benthamiana* leaves. Co-expression of MiERS1 versions and dTM-MiERS1 versions fused to YFP (yellow) and CFP (blue), respectively. Co-expression of the ER-marker fused to mCherry (red). Inset and arrows indicate nuclei displaying specific fluorescence (CFP column, blue); arrowheads indicate diffuse distribution of dTM-MiERS1s (CFP column, blue). dTM: devoid of transmembrane domains, scale bar 20 µm.

5.4.7 Dimerisation analysis by bi-molecular fluorescence complementation (BiFC) assay

To determine all possible combinations for homo-/heterodimerisation of all MiERS1 versions, BiFC experiments (Fig. 5.6) with splitYFP-constructs were conducted to have a control over false positive fluorescence signal due to a simple and close co-localisation or unspecific protein interaction in ER structures. The BiFC analysis revealed that MiERS1 and MiERS1m can interact as homo- and heterodimers (Fig. 5.6A-C), which supports the hypothesis that these MiERS1 versions possibly modulate receptor transmitted signals. A BiFC assay considering the dimerisation of MiERS1 versions with MiETR1 was also performed and resulted in fluorescence signals only for the receptor combination with the full-length MiERS1 and MiETR1 (Fig. 5.6D). The BiFC analysis concerning MiERS1s did not result in a specific fluorescence signal in any combination, indicating that this version might not be able to dimerise with itself or with the other receptors (data not shown). The result suggests that the detected co-localisation or dimerisation of MiERS1s in the previous assays is not based on specific binding to co-expressed receptor fusion proteins, but may be due to interaction with other proteins located in the ER.

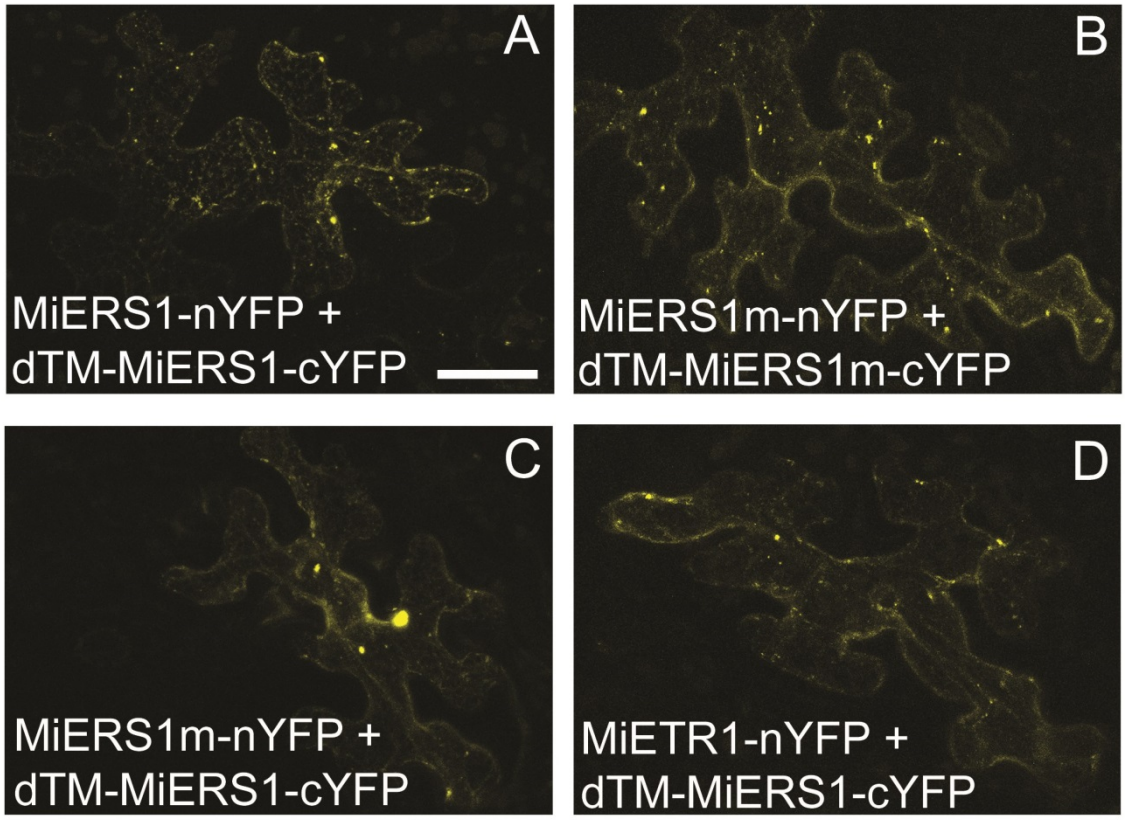


Fig. 5.6. Dimerisation analysis of MiETR1 and MiERS1 versions by BiFC assay in infiltrated *N. benthamiana* leaves. Co-expression of MiETR1 and MiERS1 versions fused to splitYFP. dTM: devoid of transmembrane domain, n/cYFP: n- or c-terminal part of splitYFP, scale bar 20 µm.

5.4.8 Summary and conclusion

In addition to the known mango ethylene receptors MiERS1 and MiETR1, short versions of MiERS1 were identified, which reveal deletions of crucial domains for protein function. Concerning the sequence characteristics and dimerisation analysis, MiERS1m could play a role in the ethylene signalling pathway and might be able to modify signal output in receptor complexes. MiERS1m itself is missing the domain necessary for ATP-binding and for interaction with CTR1, therefore, MiERS1m-mediated ethylene signalling might depend on dimerisation with ethylene receptors containing all functional domains. The transcription level of MiERS1m is differently regulated in various tissues compared to the full-length ethylene receptor and transcripts are not present in all samples. This could be an indication for MiERS1m playing a specific role in dependence on the developmental or physiological stage of an individual plant organ. MiERS1s, like the other ethylene receptors, is confirmed to be located in the ER membrane system, however, the absence of critical domains, the predicted

orientation in the ER membrane, and the results of the BiFC analysis suggest that MiERS1s may not be involved in the CTR1-dependent ethylene signal transduction, but possibly in alternative pathways. This notion, the transcription pattern and the presence of MiERS1s in the genome of several mango cultivars indicates a specific function of this receptor version.

5.5 Acknowledgements

The research was funded by the DFG via the SFB564. The authors thank Shoji Mano and Daniel Van Damme for generously providing the BiFC binary vectors and the ER-marker construct, respectively. For arabidopsis seeds and support with agrobacteria and plant transformations the authors are grateful to Moritz Nowack.

5.6 Supplementary material

Material and methods 5.S1. Reference gene evaluation.

To our knowledge no comparative analysis of reference genes for mango has been published, therefore, fragments of the candidate reference genes β-actin, α-tubulin and ubiquitin were isolated from mango and tested. A pool-sample as a reference sample (n = 30 sample cDNAs) and individual samples from each tissue ($n \geq 2$, N = 30) were randomly chosen for the reference gene evaluation. Based on the lowest value of the primer efficiency corrected fold change regulation, which was calculated through a multiple comparison test with the tool BestKeeper (Pfaffl et al., 2004), the candidate reference genes were evaluated. The resulting standard deviations of the absolute regulation coefficient were 1.9 for ubiquitin, 1.8 for α-tubulin and 1.6 for β-actin. Conclusively, β-actin was identified as the most stably expressed gene to be preferred as reference for the qPCR analysis. Primers used for reference gene evaluation and gene specific qPCR analysis are listed in supplementary Table S1.

Supplementary references

Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper - Excel-based tool using pair-wise correlations. *Biotechnology Letters* 26, 509-515.

5.6.1 Supplementary tables

Table 5.S1. List of primers for reference gene evaluation and qPCR analysis.

Gene	primer sequence (F)	primer sequence (R)	amplicon size [nt]
MiTUB	5′-ATC AAC TAC CAG CCA CC-3′	5′-CCT TCC TCC ATA CCC TCA C-3′	184
MiUBI	5′-AAG ATC CAG GAC AAG GAG G-3′	5′-GGA CCA GGT GGA GCG-3′	125
MiACT	5′-CCC TGA AGA GCA CCC A-3′	5′-AGT TGT ACG ACC ACT GGC-3′	156
MiERS1	5′-TGG CGA CAA GAA ACG ACT G-3′	5′-GCC AGT CTC TTG AAG ACT C-3′	116
MiERS1m	5′-GCG CTG TAA TGA ACC ATG A-3′	5′-TCT TTG GTA TCG TGT TGT C-3′	151
MiERS1s	5′-TCT AGT GTC ATG TCT AAC TGC-3′	5′-GTG CTA CCT TTG TCA AGC-3′	115
MiETR1	5′-CCA AGG AGA ATT GCA TGA G-3′	5′-GGC AGC TTG CTC CTC-3′	142

Table 5.S2. List of primers for construct cloning. For gateway cloning of fusion protein constructs the attB-recognition site was added as appropriate.

primer name	primer sequence
MiERS1_F	5′-ATGATATCTTGTGATTGCATTGATAC-3′
dTM-MiERS1_F	5′-CTGAAGAACAGGGCTGAAGAG-3′
MiERS1_R	5′-GTCAAGGCTTCTTTGGTATC-3′
MiERS1m_R	5′-TTGGTATCGTGTTGTCAAG-3′
MiERS1s_R	5′-GTGCTACCTTTGTCAAGC-3′
MiETR1_F	5′-ATGGAGTCTTGCAACTGCAT-3′
MiETR1_R	5′-GGTTATGGCCTCGAATAGA-3′

Table 5.S3. List of fluorescence fusion protein constructs used for transient expression experiments in *N. benthamiana*. dTM: devoid of transmembrane domain.

Expressed cassette under control of 35S promoter	Binary vector
MiERS1-CFP	pH7CWG2
MiERS1m-CFP	pH7CWG2
MiERS1s-CFP	pH7CWG2
MiERS1-YFP	pK7YWG2
MiERS1m-YFP	pK7YWG2
MiERS1s-YFP	pK7YWG2
dTM-MiERS1-CFP	pH7CWG2
dTM-MiERS1m-CFP	pH7CWG2
dTM-MiERS1s-CFP	pH7CWG2
MiERS1-nYFP	pB4-GWnYFP
MiERS1-cYFP	pB4-GWcYFP
MiERS1m-nYFP	pB4-GWnYFP
MiERS1m-cYFP	pB4-GWcYFP
MiERS1s-nYFP	pB4-GWnYFP
MiERS1s-cYFP	pB4-GWcYFP
dTM-MiERS1-nYFP	pB4-GWnYFP
dTM-MiERS1-cYFP	pB4-GWcYFP
dTM-MiERS1m-nYFP	pB4-GWnYFP
dTM-MiERS1m-cYFP	pB4-GWcYFP
dTM-MiERS1s-nYFP	pB4-GWnYFP
dTM-MiERS1s-cYFP	pB4-GWcYFP
MiETR1-CFP	pH7CWG2
MiETR1-YFP	pK7YWG2
MiETR1-nYFP	pB4-GWnYFP
MiETR1-cYFP	pB4-GWcYFP

5.6.2 Supplementary figures

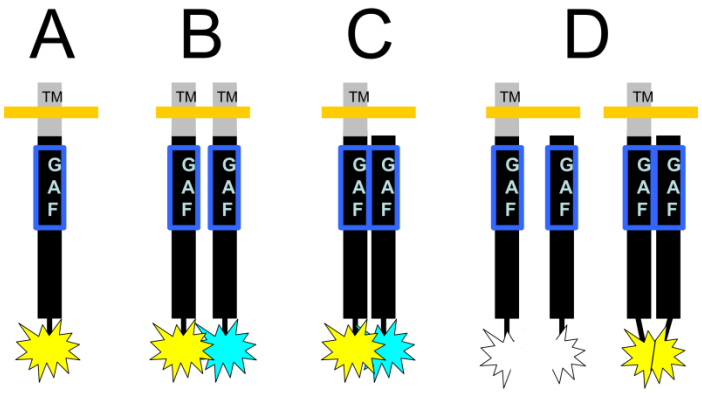


Fig. 5.S1. Scheme of receptor constructs and dimerisation models for transient expression analysis in *N. benthamiana* leaves. (A) YFP fusion-protein to determine cellular localisation, (B) co-localisation (or interaction) of receptors fused to YFP or CFP, (C) dimerisation of YFP and CFP fusion-proteins, (D) BiFC assay with splitYFP-constructs. TM: transmembrane domain (grey) as anchor in the ER membrane, GAF: GAF domain (blue box) for receptor dimerisation, orange: ER membrane, YFP: yellow fluorophore, CFP: blue fluorophore.

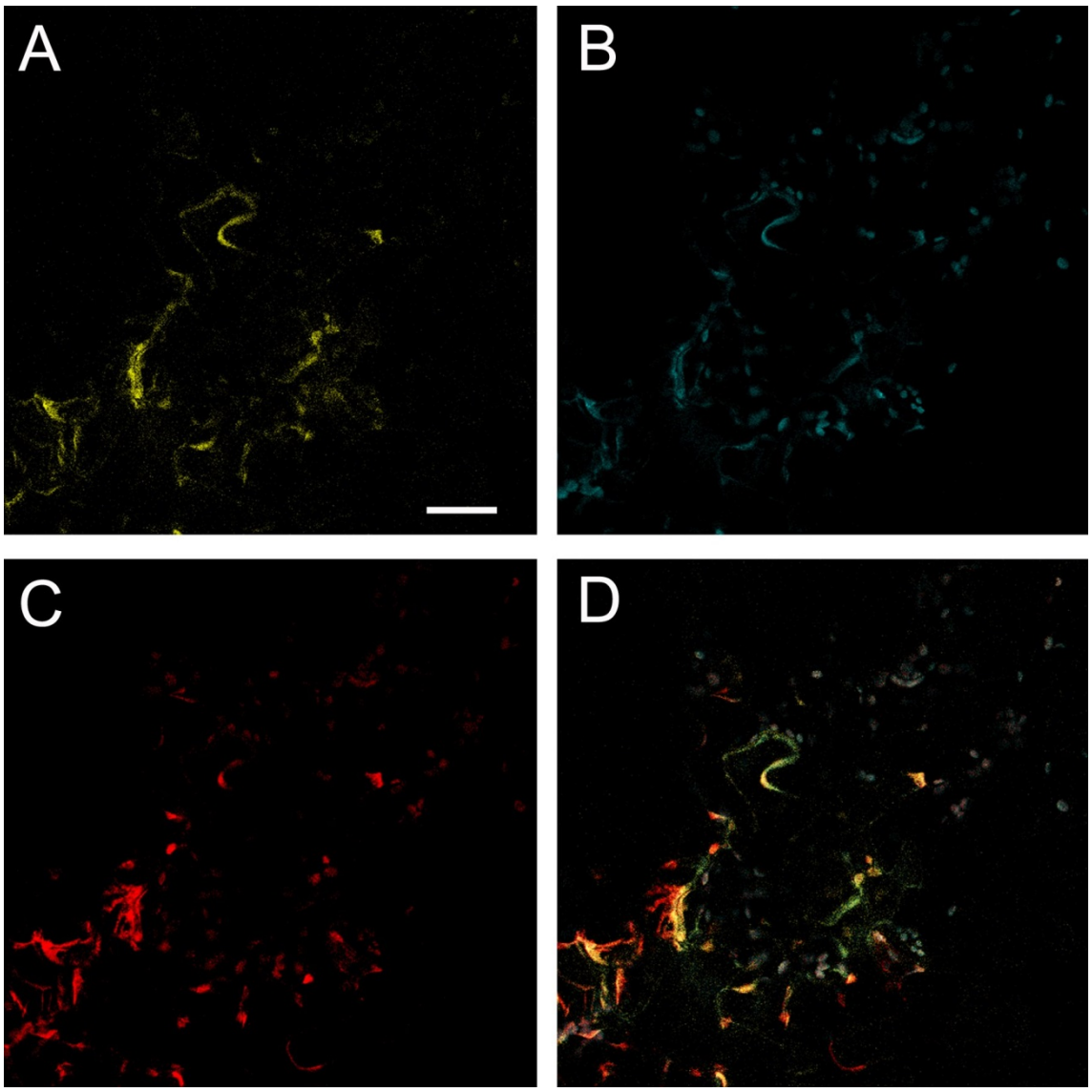


Fig. 5.S2. Discrimination of unspecific fluorescence. Autofluorescence signal of necrotic cells detected with the (A) YFP channel, (B) CFP channel, (C) mCherry channel, (D) displayed as overlay image. To guarantee the detection and identification of specific YFP, CFP, and mCherry fluorescence signals, each specimen was checked with the DAPI filter set. With the DAPI filter set specific fluorophore signals are not visible, but autofluorescence of e.g. necrotic cells is bright and clearly visible, which enables the discrimination of specimen with specific fluorophore signals from those with unspecific autofluorescence. Only specimen with specific fluorescence signals were considered for analysis.

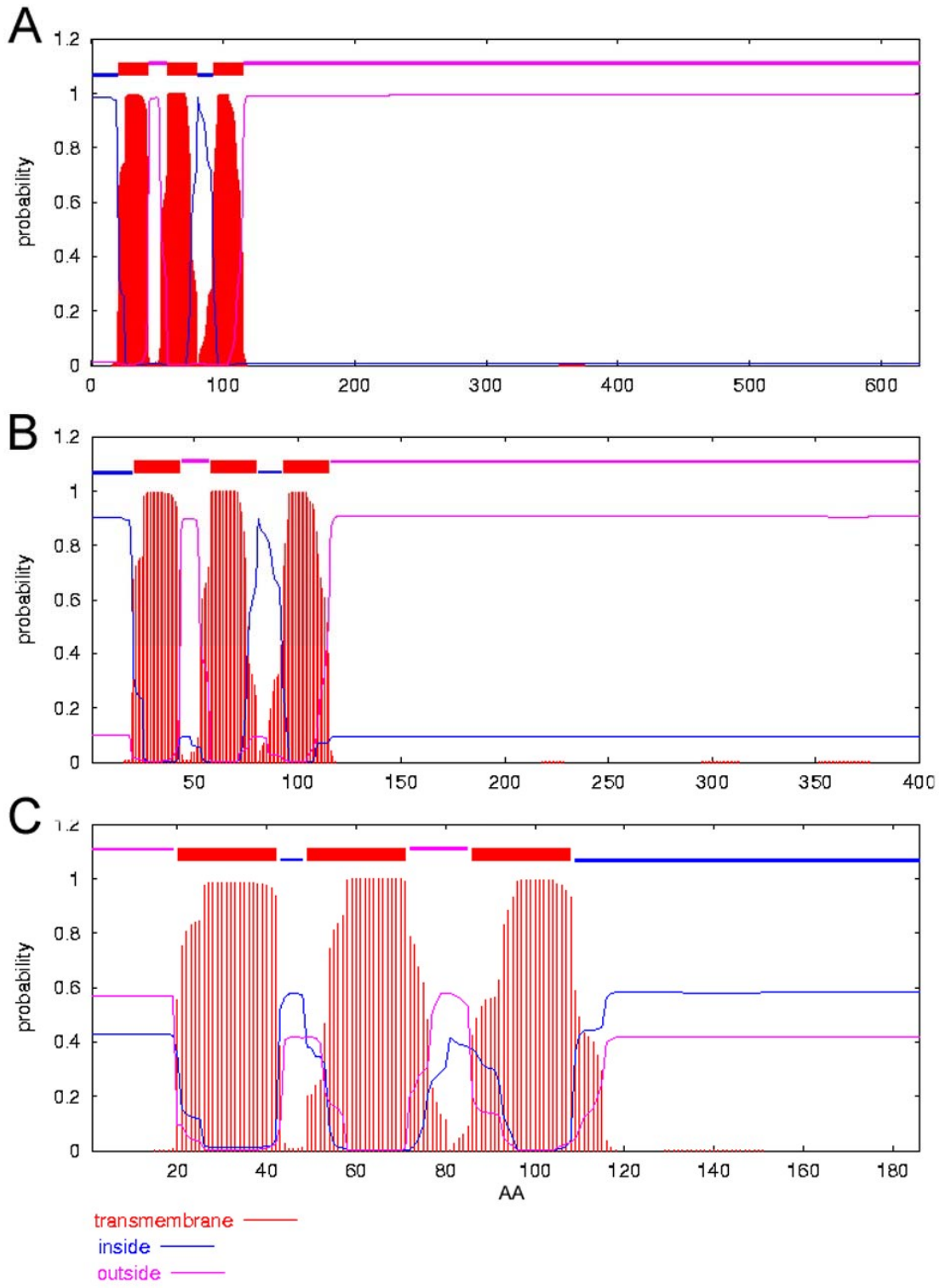
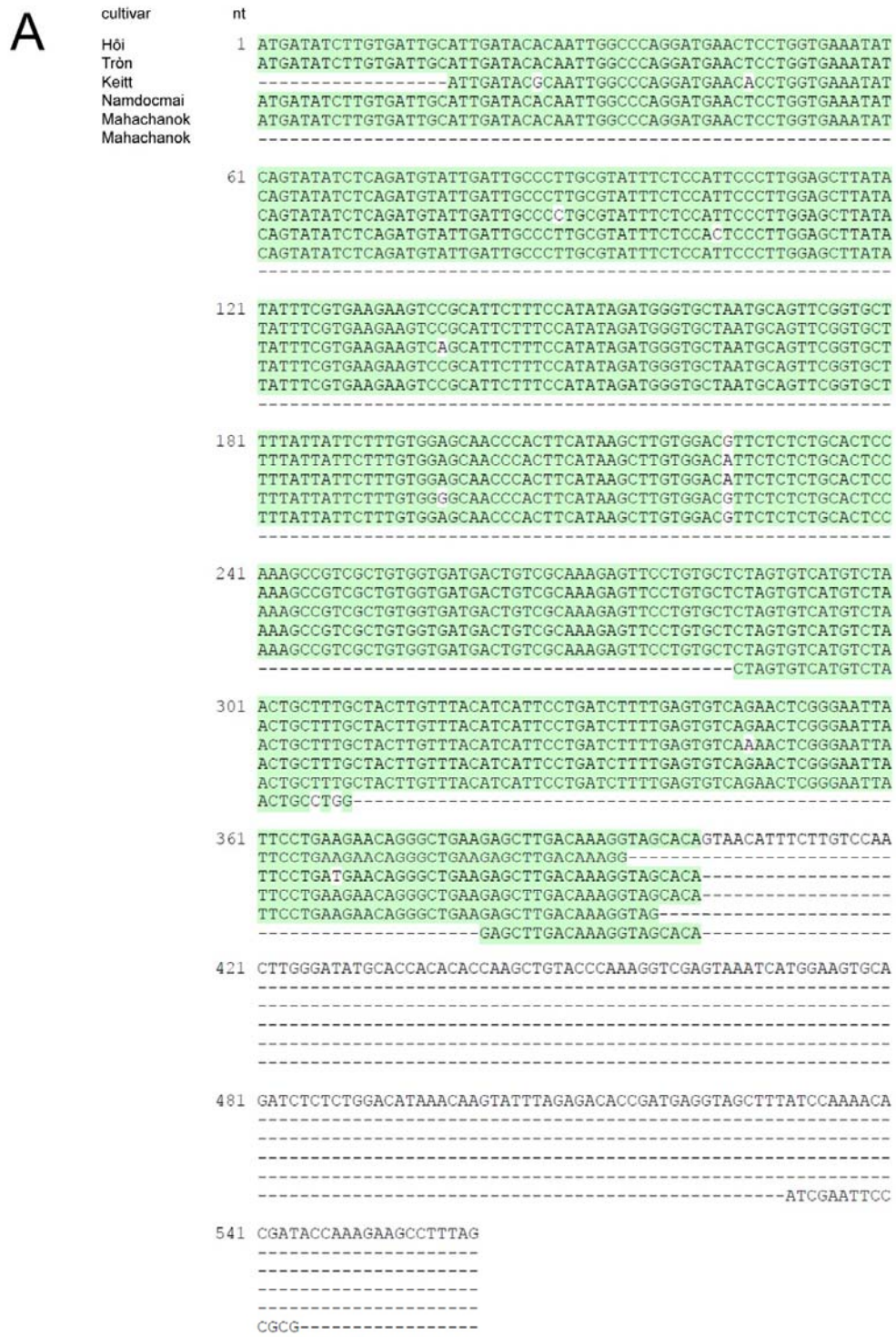


Fig. 5.S3. Analysis of MiERS1 versions with the TMHMM software tool of translated sequences. Predicted transmembrane domains and orientation within the ER membrane of (A) MiERS1, (B) MiERS1m, (C) MiERS1s.


A
cultivar
nt
Hôi
Tròn
Keitt
Namdocmai
Mahachanok
Mahachanok
1 ATGATATCTTGTGATTGCATTGATACACAATTGGCCCAGGATGAACTCCTGGTGAAATAT
ATGATATCTTGTGATTGCATTGATACACAATTGGCCCAGGATGAACTCCTGGTGAAATAT
------------------ATTGATACGCAATTGGCCCAGGATGAACACCTGGTGAAATAT
ATGATATCTTGTGATTGCATTGATACACAATTGGCCCAGGATGAACTCCTGGTGAAATAT
ATGATATCTTGTGATTGCATTGATACACAATTGGCCCAGGATGAACTCCTGGTGAAATAT
61 CAGTATATCTCAGATGTATTGATTGCCCTTGCGTATTTCTCCATTCCCTTGGAGCTTATA
CAGTATATCTCAGATGTATTGATTGCCCTTGCGTATTTCTCCATTCCCTTGGAGCTTATA
CAGTATATCTCAGATGTATTGATTGCCCCTGCGTATTTCTCCATTCCCTTGGAGCTTATA
CAGTATATCTCAGATGTATTGATTGCCCTTGCGTATTTCTCCACTCCCTTGGAGCTTATA
CAGTATATCTCAGATGTATTGATTGCCCTTGCGTATTTCTCCATTCCCTTGGAGCTTATA
121 TATTTCGTGAAGAAGTCCGCATTCTTTCCATATAGATGGGTGCTAATGCAGTTCGGTGCT
TATTTCGTGAAGAAGTCCGCATTCTTTCCATATAGATGGGTGCTAATGCAGTTCGGTGCT
TATTTCGTGAAGAAGTCAGCATTCTTTCCATATAGATGGGTGCTAATGCAGTTCGGTGCT
TATTTCGTGAAGAAGTCCGCATTCTTTCCATATAGATGGGTGCTAATGCAGTTCGGTGCT
TATTTCGTGAAGAAGTCCGCATTCTTTCCATATAGATGGGTGCTAATGCAGTTCGGTGCT
181 TTTATTATTCTTTGTGGAGCAACCCACTTCATAAGCTTGTGGACGTTCTCTCTGCACTCC
TTTATTATTCTTTGTGGAGCAACCCACTTCATAAGCTTGTGGACATTCTCTCTGCACTCC
TTTATTATTCTTTGTGGAGCAACCCACTTCATAAGCTTGTGGACATTCTCTCTGCACTCC
TTTATTATTCTTTGTGGGGCAACCCACTTCATAAGCTTGTGGACGTTCTCTCTGCACTCC
TTTATTATTCTTTGTGGAGCAACCCACTTCATAAGCTTGTGGACGTTCTCTCTGCACTCC
241 AAAGCCGTCGCTGTGGTGATGACTGTCGCAAAGAGTTCCTGTGCTCTAGTGTCATGTCTA
AAAGCCGTCGCTGTGGTGATGACTGTCGCAAAGAGTTCCTGTGCTCTAGTGTCATGTCTA
AAAGCCGTCGCTGTGGTGATGACTGTCGCAAAGAGTTCCTGTGCTCTAGTGTCATGTCTA
AAAGCCGTCGCTGTGGTGATGACTGTCGCAAAGAGTTCCTGTGCTCTAGTGTCATGTCTA
AAAGCCGTCGCTGTGGTGATGACTGTCGCAAAGAGTTCCTGTGCTCTAGTGTCATGTCTA
--CTAGTGTCATGTCTA
301 ACTGCTTTGCTACTTGTTTACATCATTCCTGATCTTTTGAGTGTCAGAACTCGGGAATTA
ACTGCTTTGCTACTTGTTTACATCATTCCTGATCTTTTGAGTGTCAGAACTCGGGAATTA
ACTGCTTTGCTACTTGTTTACATCATTCCTGATCTTTTGAGTGTCAAAACTCGGGAATTA
ACTGCTTTGCTACTTGTTTACATCATTCCTGATCTTTTGAGTGTCAGAACTCGGGAATTA
ACTGCTTTGCTACTTGTTTACATCATTCCTGATCTTTTGAGTGTCAGAACTCGGGAATTA
ACTGCCTGG---
361 TTCCTGAAGAACAGGGCTGAAGAGCTTGACAAAGGTAGCACAGTAACATTTCTTGTCCAA
TTCCTGAAGAACAGGGCTGAAGAGCTTGACAAAGG-------------------------
TTCCTGATGAACAGGGCTGAAGAGCTTGACAAAGGTAGCACA------------------
TTCCTGAAGAACAGGGCTGAAGAGCTTGACAAAGGTAGCACA------------------
TTCCTGAAGAACAGGGCTGAAGAGCTTGACAAAGGTAG----------------------
----------------------GAGCTTGACAAAGGTAGCACA------------------
421 CTTGGGATATGCACCACACACCAAGCTGTACCCAAAGGTCGAGTAAATCATGGAAGTGCA

----
481 GATCTCTCTGGACATAAACAAGTATTTAGAGACACCGATGAGGTAGCTTTATCCAAAACA

--ATCGAATTCC
541 CGATACCAAAGAAGCCTTTAG

CGCG----------------
B
cultivar
Hôi
Tròn
Keitt
Namdocmai
Mahachanok
Mahachanok
561 nt

Fig. 5.S4. Presence of *MiERS1s* in genomic DNA of different mango cultivars and comparison to the Hôi *MiERS1s*. (A) Alignment of *MiERS1s* sequence fragments obtained by *MiERS1s*-specific PCR. (B) Schematic display of sequences: boxes indicate areas of significant similarity. Notice the fragment of the additional *MiERS1s* version of 'Mahachanok' revealing gaps in the sequence (indicated by dashes).

5.7 References

Bassett CL, Artlip TS, Callahan AM. 2002. Characterization of the peach homologue of the ethylene receptor, PpETR1, reveals some unusual features regarding transcript processing. Planta 215, 679–688.

Binder BM. 2008. The ethylene receptors: Complex perception for a simple gas. *Plant Science* 175, 8–17.

Bleecker AB, Esch JJ, Hall AE, Rodriguez FI, Binder BM. 1998. The ethylene-receptor family from Arabidopsis: structure and function. *Philosophical Transactions of the Royal Society B: Biological Sciences* 353, 1405–1412.

Brunak S, Engelbrecht J, Knudsen S. 1991. Prediction of Human mRNA Donor and Acceptor Sites from the DNA Sequence. *Journal of Molecular Biology* 220, 49–65.

Bustamante-Porras J, Campa C, Poncet V, Noirot M, Leroy T, Hamon S, de Kochko A. 2007. Molecular characterization of an ethylene receptor gene (CcETR1) in coffee trees, its relationship with fruit development and caffeine content. *Molecular Genetics and Genomics* 277, 701–712.

Chae HS, Kieber JJ. 2005. *Eto Brute?* Role of ACS turnover in regulating ethylene biosynthesis. *Trends in Plant Science* 10, 291–296.

Chen YF, Etheridge N, Schaller GE. 2005. Ethylene signal transduction. *Annals of Botany* 95, 901–915.

Chen YF, Gao Z, Kerris RJ, 3rd, Wang W, Binder BM, Schaller GE. 2010. Ethylene receptors function as components of high-molecular-mass protein complexes in Arabidopsis. PLoS One 5, article e8640. doi: 10.1371/journal.pone.0008640

Clark KL, Larsen PB, Wang X, Chang C. 1998. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. *Proceedings of the National Academy of Sciences* 95, 5401–5406.

Etheridge N, Chen YF, Schaller GE. 2005. Dissecting the ethylene pathway of Arabidopsis. *Briefings in Functional Genomics and Proteomics* 3, 372–381.

Farage-Barhom S, Burd S, Sonego L, Mett A, Belausov E, Gidoni D, Lers A. 2011. Localization of the Arabidopsis senescence- and cell death-associated BFN1 nuclease: from the ER to fragmented nuclei. *Molecular Plant* 4, 1062–1073.

Galán Saúco V. 2015. Current situation and future prospects of worldwide mango production and market. *Acta Horticulturae* 1066, 69–84.

Gao Z, Wen CK, Binder BM, Chen YF, Chang J, Chiang YH, Kerris RJ, 3rd, Chang C, Schaller GE. 2008. Heteromeric interactions among ethylene receptors mediate signaling in Arabidopsis. *Journal of Biological Chemistry* 283, 23801–23810.

Grefen C, Stadele K, Ruzicka K, Obrdlik P, Harter K, Horak J. 2008. Subcellular localization and in vivo interactions of the *Arabidopsis thaliana* ethylene receptor family members. *Molecular Plant* 1, 308–320.

Hagemann MH, Roemer M, Kofler J, Hegele M, Wünsche JN. 2014. A new approach for analyzing and interpreting data on fruit drop in mango. *HortScience* 49, 1498–1505.

Hagemann MH, Winterhagen P, Hegele M, Wünsche JN. 2015. Ethephon induced abscission of mango fruitlets – Physiological fruit pedicel response. *Acta Horticulturae* 1066, 109–116.

Hall BP, Shakeel SN, Amir M, Haq NU, Qu X, Schaller GE. 2012. Histidine kinase activity of the ethylene receptor ETR1 facilitates the ethylene response in Arabidopsis. *Plant Physiology* 159, 682–695.

Hebsgaard SM, Korning PG, Tolstrup N, Engelbrecht J, Rouze P, Brunak S. 1996. Splice site prediction in *Arabidopsis thaliana* DNA by combining local and global sequence information. *Nucleic Acids Research* 24, 3439–3452.

Hernández Delgado PM, Aranguren M, Reig C, Fernández Galván D, Mesejo C, Martínez Fuentes A, Galán Saúco V, Agustí M. 2011. Phenological growth stages of mango (*Mangifera indica* L.) according to the BBCH scale. *Scientia Horticulturae* 130, 536–540.

Hino T, Tanaka Y, Kawamukai M, Nishimura K, Mano S, Nakagawa T. 2011. Two Sec13p homologs, AtSec13A and AtSec13B, redundantly contribute to the formation of COPII transport vesicles in *Arabidopsis thaliana*. *Bioscience, Biotechnology and Biochemistry* 75, 1848–1852.

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM. 1998. EIN4 and ERS2 are members of the putative ethylene receptor gene family in Arabidopsis. *Plant Cell* 10, 1321–1332.

Hunter S, Jones P, Mitchell A, *et al.* 2012. InterPro in 2011: new developments in the family and domain prediction database. *Nucleic Acids Research* 40, D306–312.

Ireland HS, Guillen F, Bowen J, Tacken EJ, Putterill J, Schaffer RJ, Johnston JW. 2012. Mining the apple genome reveals a family of nine ethylene receptor genes. *Postharvest Biology and Technology* 72, 42–46.

Ish-Shalom M, Dahan Y, Maayan I, Irihimovitch V. 2011. Cloning and molecular characterization of an ethylene receptor gene, MiERS1, expressed during mango fruitlet abscission and fruit ripening. *Plant Physiology and Biochemistry* 49, 931–936.

John-Karuppiah KJ, Burns KJ. 2010. Expression of ethylene biosynthesis and signaling genes during differential abscission responses of sweet orange leaves and mature fruit. *Journal of the American Society for Horticultural Science* 135, 456–464.

Karimi M, Inze D, Depicker A. 2002. GATEWAY vectors for agrobacterium-mediated plant transformation. *Trends in Plant Science* 7, 193–195.

Kevany BM, Tieman DM, Taylor MG, Cin VD, Klee HJ. 2007. Ethylene receptor degradation controls the timing of ripening in tomato fruit. *Plant Journal* 51, 458–467.

Lim PO, Kim HJ, Nam HG. 2007. Leaf senescence. *Annual Review of Plant Biology* 58, 115–136.

Liu Q, Wen CK. 2012. Arabidopsis ETR1 and ERS1 differentially repress the ethylene response in combination with other ethylene receptor genes. *Plant Physiology* 158, 1193–1207.

Ma B, Cui ML, Sun HJ, Takada K, Mori H, Kamada H, Ezura H. 2006. Subcellular localization and membrane topology of the melon ethylene receptor CmERS1. *Plant Physiology* 141, 587–597.

Malik AU, Agrez V, Singh Z. 2003. Fruitlet abscission of mango in relation to ethylene. *Journal of Horticultural Science and Biotechnology* 78, 458–462.

Martínez PG, Gomez RL, Gomez-Lim MA. 2001. Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. *Journal of Plant Physiology* 158, 101–108.

Matsushima R, Hayashi Y, Kondo M, Shimada T, Nishimura M, Hara-Nishimura I. 2002. An endoplasmic reticulum-derived structure that is induced under stress conditions in Arabidopsis. *Plant Physiology* 130, 1807–1814.

Mayerhofer H, Panneerselvam S, Mueller-Dieckmann J. 2012. Protein kinase domain of CTR1 from *Arabidopsis thaliana* promotes ethylene receptor cross talk. *Journal of Molecular Biology* 415, 768–779.

Nelson BK, Cai X, Nebenfuhr A. 2007. A multicolored set of *in vivo* organelle markers for co-localization studies in Arabidopsis and other plants. *Plant Journal* 51, 1126–1136.

Pang JH, Ma B, Sun H-J, Ortiz GI, Imanishi S, Sugaya S, Gemma H, Ezura H. 2007. Identification and characterization of ethylene receptor homologs expressed during fruit development and ripening in persimmon (*Diospyros kaki* Thumb.). *Postharvest Biology and Technology* 44, 195–203.

Peirson SN, Butler JN, Foster RG. 2003. Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. *Nucleic Acids Research* 31, article e73. doi: 10.1093/nar/gng073

Qu X, Hall BP, Gao Z, Schaller GE. 2007. A strong constitutive ethylene-response phenotype conferred on Arabidopsis plants containing null mutations in the ethylene receptors ETR1 and ERS1. *BMC Plant Biology* 7, article 15. doi:10.1186/1471-2229-7-3

Rasori A, Ruperti B, Bonghi C, Tonutti P, Ramina A. 2002. Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. *Journal of Experimental Botany* 53, 2333–2339.

Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM. 1998. ETR2 is an ETR1-like gene involved in ethylene signaling in Arabidopsis. *Proceedings of the National Academy of Sciences* 95: 5812–5817.

Sato-Nara K, Yuhashi KI, Higashi K, Hosoya K, Kubota M, Ezura H. 1999. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiology* 120, 321–330.

Satoh S, Watanabe M, Chisaka K, Narumi T. 2008. Suppressed leaf senescence in chrysanthemum transformed with a mutated ethylene receptor gene mDG-ERS1(etr1-4). *Journal of Plant Biology* 51, 424–427.

Stepanova AN, Alonso JM. 2009. Ethylene signaling and response: where different regulatory modules meet. *Current Opinion in Plant Biology* 12, 548–555.

Tanaka Y, Kimura T, Hikino K, Goto S, Nishimura M, Mano S, Nakagawa T. 2012. Gateway vectors for plant genetic engineering: overview of plant vectors, application for bimolecular fluorescence complementation (BiFC) and multigene construction. In: Barrera-Saldana HA (ed) Genetic Engineering - Basics, New Applications and Responsibilities. pp 35–58.

Voinnet O, Rivas S, Mestre P, Baulcombe D. 2003. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant Journal* 33, 949–956.

Winterhagen P, Dubois C, Sinn M, Wetzel T, Reustle GM. 2009. Gene silencing and virus resistance based on defective interfering constructs in transgenic *Nicotiana benthamiana* is not linked to accumulation of siRNA. *Plant Physiology and Biochemistry* 47, 739–742.

Yamasaki S, Fujii n, Takahashi H. 2003. Characterization of ethylene effects on sex determination in cucumber plants. *Sexual Plant Reproduction* 16, 103–111.

Yang SF, Hoffmann NE. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* 35, 155–189.

Zhang J, Yu J, Wen CK. 2014. An alternate route of ethylene receptor signaling. *Frontiers in Plant Science* 5, article 648. doi: 10.3389/fpls.2014.00648

Zhong S, Lin Z, Grierson D. 2008. Tomato ethylene receptor-CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum. *Journal of Experimental Botany* 59, 965–972.

6. General discussion

6.1 The molecular and physiological basis of fruitlet abscission

Plants are sessile organisms in a constantly changing environment, therefore they have evolved strategies enabling them to adapt to unfavorable conditions. One of these strategies is abscission, the coordinated detachment of plant organs which, for example, enables a plant to eliminate tissues infected with pathogens or pests or to adapt to seasonal conditions such as drought stress (Xie et al., 2013). Fruit abscission in particular has three main functions, to disperse the plants' progeny (Giovannoni, 2004), to detach fruits with developmental disorders, and to overcome nutritional plant stress (Racskó et al., 2007). In chapter 2 it has been proposed that abscission is induced as the result of a combination of temperature extremes and drought which lead to nutritional stress within the tree. This is referred to as "physiological fruitlet drop" and, based on transcriptomic studies in apple, a model was developed with four steps, starting with the tree, then the fruit pericarp, the seed and finally the pedicel (Botton et al., 2011) (Fig. 6.1).

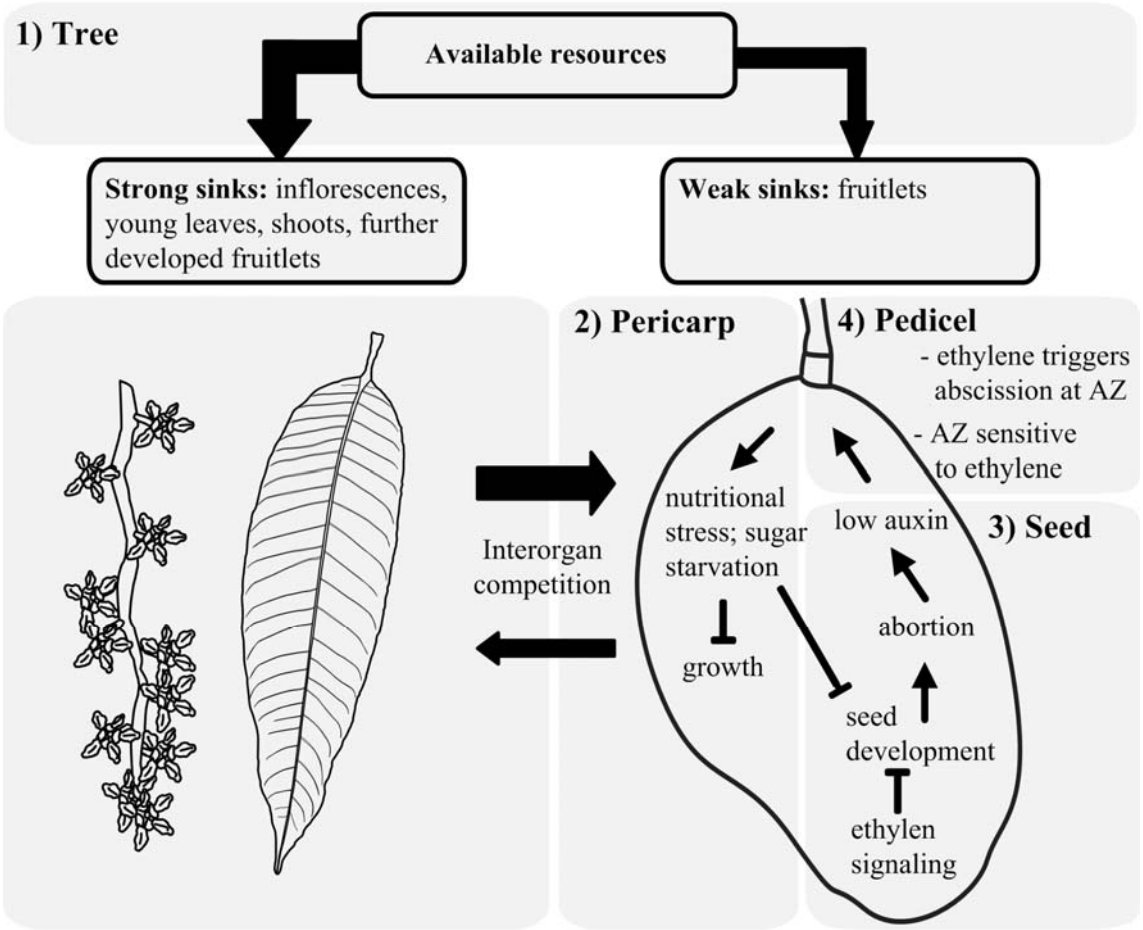


Fig. 6.1. Hypothetical model for fruitlet abscission in mango based on Botton et al. (2011). During the midseason drop stage fruitlet abscission is mainly a result of inter-organ competition at the tree level for the available resources, mainly assimilates. Newly developing plant organs such as inflorescences, young leaves or shoots are stronger sinks compared to fruitlets. Thus the resources go to the superior sinks which induced nutritional stress in weak sinks. This stress is perceived as sugar starvation at the pericarp level and leads to growth inhibition and activates the ethylene signal cascade. At the seed level both factors, ethylene induced gene regulation and sugar starvation inhibit seed development. Thus the seed is the main source of auxin, the abortion of the seed leads to a lower auxin efflux subsequently to a lower sink signal. At the pedicel level this low auxin efflux increases the sensitivity of the abscission zone (AZ) to ethylene. The abscission process then is activated by ethylene which finally leads to fruitlet drop.

Following this notion at the first level, a tree under nutritional stress is unable to support all fruitlets, which induces competition for assimilates (Bangerth, 2000; Botton et al., 2011). In the subtropics mango trees can be under nutritional stress as result of low leaf net carbon exchange rates (NCER) during the dry season (Fig. 3.2; Elsheery et al., 2007). In agreement, the irrigation treatment leads to higher fruit retentions compared to non-irrigated trees, suggesting that the productivity of non-irrigated trees is limited by water availability (Table 2.7). The physiological drop model for apple further suggests that, at the fruit level, nutritional stress first occurs in the pericarp, due to an upregulation of abscisic acid (ABA) and ethylene-related genes as well as by a downregulation of gibberellin (GA) related genes (Botton et al., 2011). The results of the current study for mango support this model, especially regarding the role of ethylene-related genes. In naturally abscising mango fruitlets, compared to non-abscising fruitlets, ethylene receptors were found to be upregulated in the pericarp but not in the pedicel AZ (Hagemann et al., in press). When comparing parameters between abscising and non-abscising fruitlets, it is unlikely that all abscising mango fruitlets investigated will be in a similar abscission stage. It is therefore more likely that statistical differences will be detected between parameters that start to change early in the course of abscission than between those that start to change later. Therefore the upregulation of ethylene receptors in the pericarp found by Hagemann et al. (in press), represents an early parameter of fruit abscission, although, as shown in chapters 3 and 4, the simultaneous induction of abscission by the treatment with ethephon results in the upregulation of ethylene receptors in both, the pedicel AZ and the pericarp (Fig. 4.2 and

4.4). This suggests that the upregulation of ethylene receptors is a key element of mango fruitlet abscission in both tissues and, further, that in accordance with the model of Botton et al. (2011), the abscission signal is perceived first in the pericarp and then in the AZ. In apple fruitlets, the abscission signal, nutritional stress in the pericarp, leads to the accumulation of sucrose in abscising fruitlets (Botton et al., 2011). The authors suggest that this sucrose accumulation is a reaction to sugar starvation and can be interpret as a signal inducing stress response and senescence. In contrast, abscising mango fruitlets show lower sucrose concentration compared to non-abscising fruitlets during the midseason drop stage irrespective of whether abscission was induced by ethephon or occurred naturally (Chapter 2 and 3; Hagemann et al., *in press*). The differences in fruitlet sucrose concentration could either depend on different signaling of the species, or be related to the fruit stage (Mehouachi et al., 1995); so the apple fruitlets would possibly have been shifted further from the cell division to the cell enlargement and carbohydrate storage building phase compared to the mango fruitlets at the time of midseason drop (Chapter 2). Despite these possible differences, the seed is thought to be the source of the potential sink signal auxin in fruits (Sexton and Roberts, 1982; Taylor and Whitelaw, 2001; Singh et al., 2005; Botton et al., 2011). Based on the physiological drop model, if nutritional stress in the fruit pericarp exceeds a threshold then the induction of abscission moves to the next level, the seed. Here, an upregulation of genes involved in reactive oxygen species (ROS) signaling commences and the level of hydrogen peroxide increases, which in turn leads to a decrease in auxin export (Botton et al., 2011). Seed degeneration during midseason drop, however, could not be associated with fruitlet abscission in mango (Chapter 4; Hagemann et al., *in press*), while ethephon induced abscission shows the involvement of auxin in mango fruitlet abscission as the polar auxin export (PAT) capacity decreases rapidly within 24h after treatment (Chapter 4). Based on the data in this study, it is not clear if the PAT capacity and thus the auxin signal breakdown precedes the ethylene receptor upregulation in the pedicel AZ as proposed in the model of Botton et al. (2011), because both parameters indicate the induction of the abscission process at 1 DAT. In general, the fruitlet during midseason drop shows several similarities to the proposed mechanism developed for the physiological drop of apple, thus further research should cover fruitlet abscission during all fruit developmental stages, including the study of the ROS, ABA and GA signaling.

In addition to the physiological fruitlet drop, which is induced by resource limitations at the tree level (Estornell et al., 2013; Botton et al., 2011), a fruitlet can induce its own abscission if the seed-derived auxin efflux is disrupted (Estornell et al., 2013; Xie et al.,

2013). In mango, this can occur for example through insects which directly feed on the seed as for example the larvae of the mango seed weevil do (*Sternochetus mangiferae* Fabricius) (Verghese et al., 2004). Abscission can also be induced by ethylene caused by insects feeding on the fruit (Bleecker and Kende, 2000). Wounding of mango fruitlets in particular has been shown to induce ethylene receptor transcription in the mesocarp and, thereby, is likely to induce fruit ethylene production (Martínez et al, 2001). Ethylene can also originate directly from a pathogen, *Fusarium mangiferae* Britz, Wingfield and Marasas, the causal agent of mango malformation (Ansari et al., 2013). All these factors are likely to increase fruit ethylene production and the receptor transcription, which was found to precede fruitlet abscission (Malik et al., 2003; Hagemann et al., *in press*). The ethylene from abscising fruitlets diffuses to the pedicel AZ (Nuñez-Elisea and Davenport, 1986; Malik et al., 2003) and additionally induces the degradation of the seed, which in turn leads to a reduced auxin efflux, resulting in higher ethylene sensitivity in the AZ (Botton et al., 2011; Estornell et al., 2013; Xie et al., 2013).

Irrespective from where the abscission-inducing signal originates, proximal (at the tree level) or distal (at the fruit level), the final step of the abscission process is at the AZ level, where the ethylene receptors perceive ethylene (Estornell et al., 2013). Following the ethylene signal transduction model the binding of ethylene to ethylene receptors triggers a pathway that directly leads to the expression of abscission executing proteins (Torre et al., 2006; Taylor, 2001). The results of the current study verify and extend earlier findings in mango (Ish-Shalom et al., 2011) that for example the *MiETR1* is upregulated only in the fruitlet while the *MiERS1* is upregulated mainly in the pedicel (Fig. 4.2 and 4.4). Furthermore, two additional *MiERS1* isoforms could be identified in the current study and their transcription patterns were analyzed in different tissues. The results suggest that these receptor versions probably play a role in flower and fruit development as well as in fruitlet abscission (Chapter 4 and 5). The existence of these new receptor versions and possible receptor-receptor interaction presented in chapter 5, suggest that the fruitlet abscission regulation at ethylene perception level is more complex than previously known. This notion is in agreement with the concept based on experiments with arabidopsis, that receptor interaction can modulate the ethylene signal output (Gao, 2008; Grefen, 2008; Chen, 2010). The presented data show that the receptors of mango can also form homodimers (MiERS1 and MiERS1m) or heterodimers (MiETR1-MiERS1; MiERS1-MiERS1m) (Fig. 5.4 and 5.6), which possibly enables the formation of higher order protein clusters. Indeed, Chen et al.

(2010) suggest that generally not individual receptors but receptors organized in a protein complex are the functional units for ethylene perception and signal transduction. The authors showed that AtETR1 builds small cluster units of constant size while the AtERS1 builds cluster units that can increase in size following ethylene perception (Chen et al., 2010). Three out of four ethylene receptors identified in the current study contain the GAF domain (Fig. 6.2) that is required for receptor interaction (Gao et al., 2008), however, the MiERS1s could be associated with a receptor cluster possibly through binding proteins that were predicted to exist in arabidopsis AtERS1 clusters (Chen et al., 2010). Further it has been shown in experiments with arabidopsis mutants that receptors with deletions in the GAF-domain still could contribute to the ethylene signal output possibly though a CTR-1 independent signal pathway (Zhang et al., 2014).

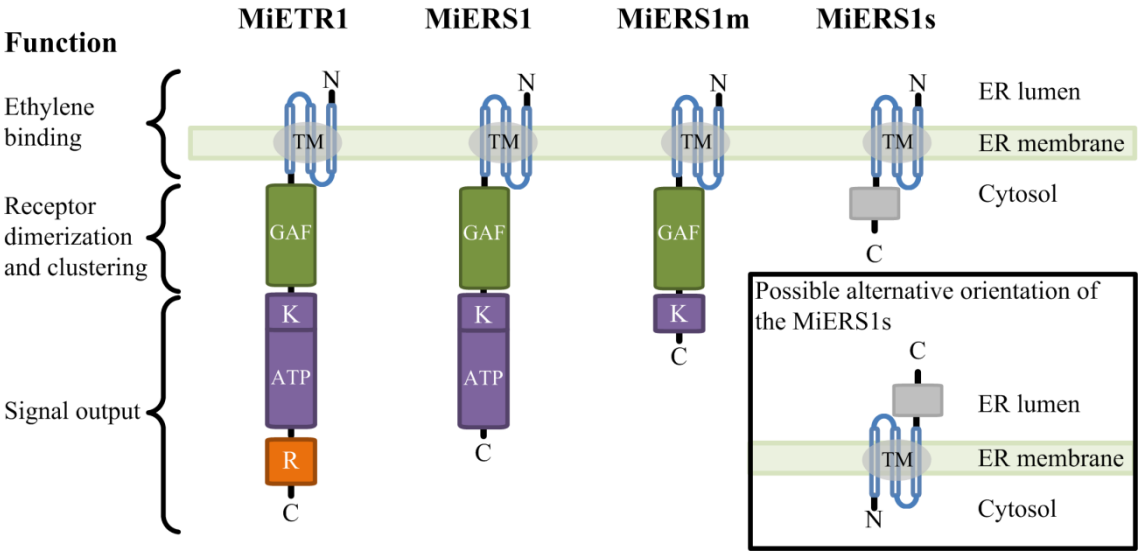


Fig. 6.2. Structural difference between the ethylene receptors of mango. All four receptors are anchored in the membrane of the endoplasmic reticulum (ER). The ethylene binding site is located near the N-terminal end (N) within the three conserved transmembrane domains (TM, blue). The GAF-domain (GAF, green) and the HIS-kinase domain (K, purple) are present only in the MiETR1, MiERS1 and the MiERS1m, while the MiERS1m is missing the ATP binding site (ATP, purple). The receiver domain (R; orange) is only present in the MiETR1. The inset shows the possible alternative orientation of the MiERS1 that had a higher probability based on informatical sequence analysis (Chapter 5).

From the finding that the intensity of ERS1 gene regulation, in terms of transcript amount, is on an overall higher level compared to the ETR1 regulation, shown for mango and peach (Chapter 4; Rasori et al., 2002), it can also be concluded that the abundance of ERS1 receptor protein is accordingly higher and forms the mentioned receptor clusters. Gao et al. (2008) suggest that ERS1 clusters can laterally transmit the

signal of detection of an ethylene molecule to associated receptors and thereby amplify the signal and subsequently induce the ethylene response even at low ethylene concentrations. As an example, in fruit abscission, modifications of the ethylene signal transduction could be the basis of the different responses of leaf and fruit to the induction of abscission by the same concentration of ethephon, which has, for example, been shown for citrus (John-Karuppiah and Burns, 2010).

In addition to the before mentioned abscission process, an ethylene independent organ abscission might exist. Evidence for this hypothesis is that some cultivars of apple are not susceptible to external ethylene or that ethylene perception mutants of tomato show a delayed abscission (Sun et al., 2009; Xie et al., 2013). However, for the fruitlet abscission of most mango and other fruit cultivars, fruitlet abscission seems to depend on ethylene and a deep understanding of the process is important for optimizing fruit production systems (Singh et al., 2005; Bapat et al., 2010; Xie et al., 2013).

6.2 Linking the theoretical understanding to practical solutions

Abscission-related traits are of great interest for fruit breeders, a salient example being that of the JOINTLESS gene in tomato. Tomato plants with mutated versions of this gene fail to develop pedicel abscission zones, a trait that leads to less post-harvest spoilage. While jointless fruits remain at the plant and can be manually detached at the pedicel-fruit junction, jointed fruitlets often detach at the pedicel-stem AZ prior to picking. The remaining fruit pedicel of jointed fruits can lead to puncturing of other fruits for example in the harvest container and thereby reduce the fruit quality and promote pathogens infections (Zahara and Scheuerman, 1988; Mao et al., 2000). The transformation of crops with mutated ethylene receptor genes can also be used to extend shelf life (Satoh et al., 2008) or the RNA-antisense technique can reduce fruit abscission as shown with LeETR1-antisense transformed tomato (Bapat et al., 2010). Abscission-related traits also exist in fruit trees, for example in apple, and might be based on a defective ethylene perception (Sun et al., 2009). In crops with extensive fruit abscission such as mango, these traits are in the focus of breeding (Krishna and Singh, 2007). Based on the presented data, promising target genes for the development of cultivars with low abscission rates are possibly the MiERS1 versions (Chapter 4 and 5), because these receptors were shown to respond particularly well in tissues associated with fruit detachment, i.e. the pedicel AZ (Chapter 3 and 4).

The following evidence that a resource deficiency is a prevailing factor limiting mango production, at least in the current study, has been presented:

1. Fruitlet abscission is lower in irrigated than in non-irrigated trees (Chapter 2), an effect likely to be due to the reduction of drought stress and consequent increased carbohydrate supply (Damour et al., 2009).
2. Low photosynthesis rates have been measured (Chapter 3) that are typical for drought-stressed mango (Elsheery et al., 2007).
3. It has been shown that levels of sucrose, the main supply carbohydrate in mango, are reduced during fruitlet abscission (Chapter 4; Hagemann et al., in press).

The conclusion that can be drawn from these findings is that the reduction of drought stress is critical for enhancing mango production especially during the dry season in sub-tropical regions such as the study area. Production can be increased either qualitatively or quantitatively (Schulze et al., 2013), however, in the current study (Chapter 2), differences in fruit quantity, but not in quality, were detected. Further, it was suggested that irrigation can increase the flower and fruitlet abundance in mango (Spreer et al., 2009) and, indeed, the number of fruits was higher in panicles of irrigated than in non-irrigated trees after the post-bloom and midseason drop stage (Fig. 2.7). Thus, it is important to note that the pattern and the commencement of irrigation seem to have profound consequences for the efficacy of the treatments. Roemer et al. (2011) started irrigation at full bloom (2007) while in the later study on the same orchard and with similar conditions irrigation was commenced at 56 (2008) and 37 (2009) days prior to full bloom (Chapter 2). The results show that the pre-bloom irrigation led to significantly higher fruit retention compared to trees that were irrigated later after blooming had started (Roemer et al., 2011) or trees that were not irrigated at all (González et al., 2004; Chapter 2) However, a period of drought and low temperatures initiate the development of generative (flower) buds (Coelho and Borges, 2004; Davenport, 2009). Therefore early irrigation could be a factor that determines an previously uncommitted bud to develop into a vegetative (leaf) bud instead of into a generative bud, especially if irrigation is applied during the bud initiation phase (Lu and Chacko, 2000; Coelho and Borges, 2004; Crane et al., 2009; Davenport. 2009).

As well as the timing of irrigation, the timing of plant growth regulator (PGR) applications also has consequences for flower and fruitlet development of mango. A PGR that is highly efficient in reducing fruitlet abscission is the synthetic auxin

1-naphthaleneacetic acid (NAA) when applied at 3-4 weeks (Naqvi et al., 1990; Chapter 2) or 6 weeks (Chattha et al., 1999) after full bloom. The exact time of application seems to be crucial. Notodimedjo (2000) applied similar concentrations of NAA to mango trees compared to the current study at two weeks after full bloom, but no positive effects on fruitlet retention were shown. The ability of NAA to increase fruit retention if applied when fruitlets are pea to marble sized is probably due to an increase in the sink strength of developing fruitlets which attracts carbohydrates (Botton et al., 2011; Dhanalakshmi et al., 2003). This increase of carbohydrates in the fruitlet could in turn promote the synthesis of seed-derived auxin and could thereby prevent the induction of abscission in the pedicel AZ (Sexton and Roberts, 1982; Taylor and Whitelaw, 2001; Singh et al, 2005; Botton et al, 2011). It is important to note that in apple production NAA application at flowering is used as a thinning treatment (Dennis, 2000; Stover et al., 2001). Stover et al. (2001) suggest that the fruitlet-thinning effect of low rates of NAA applied at flowering is based on an NAA-mediated initiation of recurrent flower buds by modulating the GA-distribution and/or by reduction of leaf photosynthesis rates. This can lead to inter-organ competition for tree resources, where developing buds typically exhibit a higher sink strength (Botton et al., 2011). In mango recurring flowers buds have also been identified as cause of fruitlet abscission that affects only pea sized but no later fruit stages (Burondkar et al., 2000). No studies on the use of NAA as fruit-thinning treatment was found in mango, hence it is unclear whether NAA causes similar thinning effects as reported for apple (Dennis, 2000; Stover et al., 2001). However, at early fruit developmental stages, NAA seem to lack the ability to prevent fruitlet abscission (Notodimedja, 2000).

6.3 Does it help to explain fruit drop patterns?

Compared to the abundance of flowers developed, many fruit species produce only a small portion of mature fruits, as a result of fruitlet abscission (Stephenson, 1981). Typically, fruitlet abscission does not occur at a constant rate, but rather in drop waves or stages that were distinguished previously for mango (Table 2.4), litchi (Yuan and Huang, 1988), orange (Zucconi et al., 1978) or apple (Abruzzese et al., 1995; Dal Cin et al., 2005). In the current study, three fruit drop stages have been described in detail for mango, the post-bloom, the midseason and the preharvest drop stage (Chapter 2). A drop stage analogous to the post-bloom drop has also been reported for apple (Abruzzese et al., 1995). This period is characterized by fruitlets abscising as a result of

a late response to an unsuccessful pollination or fertilization (Singh et al., 2005) and by developmental disorders that lead to embryo degeneration (Luckwill, 1953A; Luckwill, 1953B; Singh and Arora, 1965). A resource deficiency was suggested to play only a minor role at the post-bloom drop stage (Chapter 2), thus, fruitlet demand for carbohydrates is met by fruitlet photosynthesis and reserves from the fruiting wood (Mehouachi et al., 1995; Ruiz et al., 2001). In the midseason drop stage, the demand of fruitlets for carbohydrates increases, hence, fruitlets start to build up stores of starch (Mehouachi et al., 1995; Ruiz et al., 2001). The midseason drop stage of mango (Chapter 2) corresponds to the so called "physiological" drop that was described for apple (Abruzzese et al., 1995; Dal Cin et al., 2005; Botton et al., 2011). This stage is different from the senescence-driven abscission of mature ripe fruits (Bangerth, 2000) and fruit losses during the midseason drop stage that are based on fruitlet thinning practices for adjusting the crop load to the available resources (Botton et al., 2011; Estornell et al., 2013). In the pre-harvest drop stage, fruit losses are marginal in mango (Chapter 2), however, PGR-treatments during this stage can be used to improve fruit quality and storability, which is important for mango growers who want to access international markets (Sivakumar, et. al., 2011; Galán Saúco, 2015). Research on apple is currently exploiting the potential of sprayable 1-MCP applied at the pre-harvest stage and results show that it can extend fruit storability and post-storage quality (Elfving et al., 2007). Therefore, this treatment has a potential to also improve these parameters in mango.

In summary, fruit quality and quantity can be improved by treating fruitlets during different developmental stages by adjustment of the crop load through PGR application, irrigation, or other horticultural practices (Dennis, 2000; Davenport, 2007). The success of the crop load regulatory treatment depends on numerous factors, among them weather extremes, alternate bearing, or pest infestation. Therefore, mathematical models have been developed that take these factors into account and implement them in decision support services for commercial fruit growers that helps them to choose the optimal management practice. The well-established MaluSim model, for example, calculates a carbon balance based on leaf area, crop load, radiation and climatic data, which is used to decide if and to what extent fruitlet thinning should be applied in apple (Lakso and Robinson, 2015). For the development of a similar model for mango it would also be important to consider the fruit drop stage, because fruitlet abscission, especially at the post-bloom drop stage, is not or only to a limited extent dependent on the tree´s carbon supply (Chapter 2 and 3). Also the effect of PGR treatments can be

different, depending on the time of application and the fruit developmental stage as discussed in Chapter 6.2.

6.4 Recommendations for mango production

A thorough review on the best practice for mango production is given in Litz et al. (2009). Here the main recommendations that can be drawn from the current study are presented.

6.4.1 General recommendations

Intercropping of mango together with maize does not increase fruitlet abscission, at least not under the conditions of one experimental season (Chapter 2). Furthermore, intercropping of mango with different vegetables has been shown to increase the profitability and/or soil fertility of mango orchards in different agroclimates (Musvoto and Campbell, 1995; Singh et al., 2015) and therefore can be generally recommended. Especially in the juvenile phase, that is, the first 4 years when trees do not produce fruits, the intercrop provides a background source of income (Roberts-Nkrumah, 2004; Singh et al., 2015).

Irrigation, especially irrigation applied with micro-sprinklers at 3 to 6 weeks prior to full bloom can relieve plant stress during an extensive drought period and thereby increase fruit production (Chapter 2, 6.2). Irrigation with micro-sprinklers has also been shown to lead to higher profits compared to the more labor-intensive manual hose irrigation that is typical for Southeast Asian mango orchards (Schulze et al., 2013). Under conditions where water is limited, more sophisticated techniques such as deficit irrigation and partial root zone drying can further improve productivity, hence, these methods are more water-efficient compared to full irrigation with micro-sprinklers (Spreer et al., 2009).

PGR application, in particular the application of 40 ppm NAA to marble-sized (~20 mm) fruitlets can reduce abscission and lead to higher yields (Chapter 2). Therefore it is important to note that a single application of NAA is more effective than NAA used in combinatory treatments (Chapter 2). In a previous study the combination of CPPU (10 ppm) and GA_3 (40 ppm) also led to consistently higher fruit retention compared to the control (Oostuyse, 1993), however, this effect was not observed in the current study. This shows that the effect of PGRs has to be tested under specific

agroclimatic conditions and for the specific cultivar. Consequently recommendations cannot easily be generalized.

6.4.2 Recommendations specific for the study area

The mountainous study area (Yên Châu, Province Sơn La, Vietnam) is characterized by the shift from traditional agriculture with home gardening for self-consumption to a marked-oriented system with cash crop cultivation (Clemens et al., 2010). This shift also affects mango production and recently farmers have started to establish commercially-targeted production systems in the region (Huong, 2004; Huong, 2010). The introduction of a general orchard management including pruning and pest management has already led to improvements in mango production in Yên Châu (Huong, 2004). The main pathogens and pests of the study region are: anthracnose (*Colletrotrichum gloeosporioides* [Penz] Sacc), powdery mildew (*Oidium mangiferae* Berthet), fungal black blight (*Capnodium mangiferum* Cooke), leaf hoppers (*Penthimia* spp.), and *Bactrocera dorsalis* Hendel (Huong, 2004; Mango Grower Workshop in Tú Nang, District Yên Châu, Province Sơn La, Vietnam, 2012). Additionally, the occurrence of the mango seed weevil (*Sternochetus mangiferae* Fabricius) has been reported in the study region (Fig. 6.3). This pest is explicitly responsible for fruit abscission (Verghese et al., 2004).

Fig. 6.3. Photograph of a mango seed or stone weevil (*Sternochetus mangiferae* Fabricius) taken in Tú Nang village (District Yên Châu, Vietnam, 2012). Scale bar = 1 mm.

Consequently, a general improvement of orchard management is necessary to increase mango production in the area. This includes better hygiene and pruning practices for reducing possible sources of re-infection as well as a system of pest management that focuses on the prevailing pests (Arauz, 2000; Peña et al., 1998; Ploetz and Freeman, 2009; Schoemann, 1995; Singh et al., 2005).

The intercropping of mango and maize is recommended for the study region for increasing the profitability of mango (Singh et al., 2015) and further to counteract the soil-degrading effect of maize monoculture with the soil-preserving properties of tree crops (Roberts-Nkrumah, 2000; Roberts-Nkrumah, 2004; Young, 1989).

The use of irrigation with micro-sprinklers has been shown to effectively reduce fruitlet abscission compared to the untreated trees. Therefore, irrigation can be recommended. However, due to infrastructure limitation this is not currently applicable and therefore the application of NAA (40 ppm) at late post-bloom drop stage is a well-tested alternative for the reduction of fruitlet abscission in the study area (Chapter 2).

The polyembryonic cultivars currently grown in Yên Châu district certainly have the advantage of bearing true-to-type so that each tree produces fruit of predictable properties and similar quality. On the other hand, polyembryonic cultivars are more sensitive to the dry and cold climatic conditions (Elsheery et al., 2007; Sukhvibul et al., 2000), which can occur in the study region (Chapter 2 and 3). Therefore alternative monoembryonic cultivars should be considered for future plantings.

6.5 Conclusion and outlook

Fruitlet abscission is a major yield-limiting factor in the study region. It is also a serious production constraint for mango production world-wide. Therefore, the topic is in the mainstream of research and the current study has provided not only additional practical solutions for reducing fruitlet abscission in mango, but a new approach for improved and more standardized interpretation of data.

The study on fruitlet abscission of mango could verify earlier findings on the involvement of ethylene receptors in the process, and also two novel *ERS1* versions could be identified, raising the question of their role in the abscission process. These short *MiERS1* versions are certainly new aspects of ethylene signaling and might contribute to the modification of the ethylene response. Thus further research in basic or applied science is required in this area.

The use of different concentrations of an abscission-inductive treatment led to a probable sequence of events starting with ethylene perception in the pedicel, followed by reduced auxin signaling, then a reduced concentration of fruit carbohydrates and fruitlet drop. This knowledge is important for further understanding the natural process of fruitlet abscission in mango in order to prevent fruitlet abscission inducing factors, and thereby improving crop production.

7. General references

Abel, S., Nguyen, M.D., Chow, W., and Theologis, A. (1995). ASC4, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana*. *J. Biol. Chem.* 270**,** 19093-19099. doi: 10.1074/jbc.270.32.19093

Abruzzese, A., Mignani, I., and Cocucci, S.M. (1995). Nutritional status in apples and June drop. *J. Am. Soc. Hort. Sci.* 120**,** 71-74.

Adie, B., Chico, J.M., Rubio-Somoza, I., and Solano, R. (2007). Modulation of plant defenses by ethylene. *J. Plant Growth Regul.* 26**,** 160-177. doi: 10.1007/s00344-007-0012-6

Allen, D.J., Ratner, K., Giller, Y.E., Gussakovsky, E.E., Shahak, Y., and Ort, D.R. (2000). An overnight chill induces a delayed inhibition of photosynthesis at midday in mango (*Mangifera indica* L.). *J. Exp. Bot.* 51**,** 1893-1902. doi: 10.1093/jexbot/51.352.1893

Ansari, M.W., Shukla, A., Pant, R.C., and Tuteja, N. (2013). First evidence of ethylene production by *Fusarium mangiferae* associated with mango malformation. *Plant Sigal. Behav.* 8**,** e22673. doi: 10.4161/psb.22673

Arauz, L.F. (2000). Mango anthracnose: economic impact and current options for integrated management. *Plant Dis.* 84**,** 600-611. doi: 10.1094/PDIS.2000.84.6.600

Argueso, C., Hansen, M., and Kieber, J. (2007). Regulation of ethylene biosynthesis. *J. Plant Growth Regul.* 26**,** 92-105. doi: 10.1007/s00344-007-0013-5

Arndt, C.H. (1935). Notes on polyembryony and multiple shoots from the seed in *Mangifera indica*. *Am. J. Bot.* 22**,** 26-30.

Bangerth, F. (1989). Dominance among fruits/sinks and the search for a correlative signal. *Physiol. Plantarum* 76**,** 608-614. doi: 10.1111/j.1399-3054.1989.tb05487.x

Bangerth, F. (2000). Abscission and thinning of young fruit and their regulation by plant hormones and bioregulators. *Plant Growth Regul.* 31**,** 43-59. doi: 10.1023/A:1006398513703

Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R., and Nath, P. (2010). Ripening of fleshy fruit: Molecular insight and the role of ethylene. *Biotech. Adv.* 28**,** 94-107. doi: 10.1016/j.biotechadv.2009.10.002

Barnell, E. (1939). Studies in tropical fruits: V. Some anatomical aspects of fruit-fall in two tropical arboreal plants. *Ann. Bot.* 3**,** 77-89.

Barry, C.S., Blume, B., Bouzayen, M., Cooper, W., Hamilton, A.J., and Grierson, D. (1996). Differential expression of the 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. *Plant J.* 9**,** 525-535. doi: 10.1046/j.1365-313X.1996.09040525.x

Benjawan, C., Chutichudet, P., and Chanaboon, T. (2006). Effect of gibberellin (GA_3) on fruit yield and quality of Kaew mango (*Mangifera indica* L.) cv. Srisaket 007 in Northeast Thailand. *Pakistan J. Biol. Sci.* 9**,** 1542-1546. doi: 10.3923/pjbs.2006.1542.1546

Berüter, J., and Droz, P. (1991). Studies on locating the signal for fruit abscission in the apple tree. *Sci. Hort.* 46**,** 201-214. doi: 10.1016/0304-4238(91)90043-X

Beyer, E.M., Jr. (1976). A potent inhibitor of ethylene action in plants. *Plant Physiol.* 58**,** 268-271. doi: 10.1104/pp.58.3.268

Bhuyan, M.A.J., and Irabagon, J.A. (1993). Effect of fertilizer, potassium nitrate and irrigation on fruit drop of 'Carabao' mango. *South Indian Hort.* 41**,** 315-321.

Binder, B.M. (2008). The ethylene receptors: Complex perception for a simple gas. *Plant Sci.* 175**,** 8-17. doi: 10.1016/j.plantsci.2007.12.001

Bleecker, A.B., and Kende, H. (2000). Ethylene: A gaseous signal molecule in plants. *Annu. Rev. Cell Dev. Biol.* 16**,** 1-18. doi: doi:10.1146/annurev.cellbio.16.1.1

Blevins, D.G., and Lukaszewski, K.M. (1998). Boron in plant structure and function. *Ann. rev. plant physiol. plant mol. biol.* 49**,** 481-500. doi: 10.1146/annurev.arplant.49.1.481

Bompard, J.M. (2009). "Taxonomy and systematics," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 19-41.

Botton, A., Eccher, G., Forcato, C., Ferrarini, A., Begheldo, M., Zermiani, M., Moscatello, S., Battistelli, A., Velasco, R., Ruperti, B., and Ramina, A. (2011). Signaling pathways mediating the induction of apple fruitlet abscission. *Plant Physiol.* 155**,** 185-208. doi: 10.1104/pp.110.165779

Brecht, J.K., and Yahia, E.M. (2009). "Postharvest physiology," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 484-528.

Brown, K.M. (1997). Ethylene and abscission. *Physiol. Plant.* 100**,** 567-576. doi: 10.1111/j.1399-3054.1997.tb03062.x

Burondkar, M.M., Rajput, J.C., Waghmare, G.M., Jamadagni, B.M., and Chavan, S.A. (2000). Recurrent flowering: A new physiological disorder in Alphonso mango. *Acta Hort.* 509**,** 669-674.

Chattha, G.A., Anjum, M.A., and Hussain, A. (1999). Effect of various growth regulators on reducing fruit drop on mango (*Mangifera indica* L.). *Int. J. Agri. Biol.* 1**,** 288-289.

Chen, W.-S. (1983). Cytokinins of the developing mango fruit: isolation, identification, and changes in levels during maturation. *Plant physiol.* 71**,** 356-361. doi: 10.1104/pp.71.2.356

Chen, Y.-F., Etheridge, N., and Schaller, G.E. (2005). Ethylene signal transduction. *Ann. Bot.* 95**,** 901-915. doi: 10.1093/aob/mci100

Chen, Y.-F., Gao, Z., Kerris, R.J., Iii, Wang, W., Binder, B.M., and Schaller, G.E. (2010). Ethylene receptors function as components of high-molecular-mass protein complexes in arabidopsis. *PLoS ONE* 5**,** e8640. doi: 10.1371/journal.pone.0008640

Clemens, G., Fiedler, S., Cong, N.D., Van Dung, N., Schuler, U., and Stahr, K. (2010). Soil fertility affected by land use history, relief position, and parent material under a tropical climate in NW-Vietnam. *CATENA* 81**,** 87-96. doi: 10.1016/j.catena.2010.01.006

Coelho, E.F., and Borges, A.L. (2004). Irrigation and fertirrigation of mango. *Acta Hort.* 645**,** 119-128.

Crane, J.H., Salazar-García, S., Lin, T.-S., De Queiroz Pinto, A.C., and Shü, Z.-H. (2009). "Crop production: Management," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 432-483.

Dal Cin, V., Boschetti, A., Dorigoni, A., Velasco, R., and Ramina, A. (2007). "Ethylene involvement in auxin transport during apple fruitlet abscission (*Malus domestica* L. Borck)," in *Advances in Plant Ethylene Research,* ed. A.R.E. Al. (Dordrecht: Springer Netherlands), 89-93.

Dal Cin, V., Danesin, M., Boschetti, A., Dorigoni, A., and Ramina, A. (2005). Ethylene biosynthesis and perception in apple fruitlet abscission (*Malus domestica* L. Borck). *J. Exp. Bot.* 56**,** 2995-3005. doi: 10.1093/jxb/eri296

Dal Cin, V., Velasco, R., and Ramina, A. (2009). Dominance induction of fruitlet shedding in *Malus* x *domestica* (L. Borkh): molecular changes associated with polar auxin transport. *BMC Plant Biol.* 9**,** 139. doi: 10.1186/1471-2229-9-139

Damour, G., Vandame, M., and Urban, L. (2009). Long-term drought results in a reversible decline in photosynthetic capacity in mango leaves, not just a decrease in stomatal conductance. *Tree Physiol.* 29**,** 675-684. doi: 10.1093/treephys/tpp011

Davenport, T.L. (2007). Reproductive physiology of mango. *Brazilian J. Plant Physiol.* 19**,** 363-376. doi: 10.1590/S1677-04202007000400007

Davenport, T.L. (2009). "Reproductive physiology," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 97-169.

Dennis, F.G., Jr. (2000). The history of fruit thinning. *Plant Growth Regul.* 31**,** 1–16. doi: 10.1023/A:1006330009160

Dhanalakshmi, R., Prasad, T.G., and Udayakumar, M. (2003). Is auxin a diffusible signal mediating abscission of recessive sinks? *Plant Sci.* 164**,** 689-696. doi: 10.1016/S0168-9452(03)00008-6

Dinesh, M.R., Vasugi, C., and Ravishankar, K.V. (2015). Morphological, molecular characterization and breeding for biotic and abiotic stress in mango (*Mangifera indica* L.). *Acta Hort.* 1066, 37-46.

Elfving, D.C., Drake, S.R., Reed, A.N., and Visser, D.B. (2007). Preharvest applications of a sprayable 1-methylcyclopropene in the orchard for management of apple harvest and postharvest condition. *HortSci.* 42**,** 8.

Else, M.A., Stankiewicz-Davies, A.P., Crisp, C.M., and Atkinson, C.J. (2004). The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention. *J. Exp. Bot.* 55**,** 2099–2109. doi: 10.1093/jxb/erh208

Elsheery, N.I., Wilske, B., Zhang, J.-L., and Cao, K.-F. (2007). Seasonal variations in gas exchange and chlorophyll fluorescence in the leaves of five mango cultivars in southern Yunnan, China. *J. Hortic. Sci. Biotech.* 82**,** 855-862.

Estornell, L.H., Agustí, J., Merelo, P., Talón, M., and Tadeo, F.R. (2013). Elucidating mechanisms underlying organ abscission. *Plant Sci.* 199–200**,** 48-60. doi: 10.1016/j.plantsci.2012.10.008

Etheridge, N., Chen, Y.-F., and Schaller, G.E. (2005). Dissecting the ethylene pathway of arabidopsis. *Brief. Funct. Genomic. Proteomics* 3**,** 372-381. doi: 10.1093/bfgp/3.4.372

FAOSTAT, 2010. Statistical database of the Food Agric. Organ. Accessed on 10 Dec 2012. http://faostat.fao.org

Galán Saúco, V. (1997). Horticultural practices of mango. *Acta Hort.* 455**,** 391-400.

Galán Saúco, V. (2004). Mango production and world market: current situation and future prospects. *Acta Hort.* 645**,** 107-116.

Galán Saúco, V. (2015). Current situation and future prospects of worldwide mango production and market. *Acta Hort.* 1066**,** 69-84.

Gao, Z., Wen, C.-K., Binder, B.M., Chen, Y.-F., Chang, J., Chiang, Y.-H., Kerris, R.J., Chang, C., and Schaller, G.E. (2008). Heteromeric interactions among ethylene receptors mediate signaling in arabidopsis. *J. Biol. Chem.* 283**,** 23801–23810. doi: 10.1074/jbc.M800641200

Giovannoni, J.J. (2004). Genetic regulation of fruit development and ripening. *Plant Cell* 16**,** S179-S180. doi: dx.doi.org/10.1105/tpc.019158

González, A., Lu, P., and Müller, W. (2004). Effect of pre-flowering irrigation on leaf photosynthesis, whole-tree water use and fruit yield of mango trees receiving two flowering treatments. *Sci. Hort.* 102**,** 189-211. doi: 10.1016/j.scienta.2003.12.011

Grefen, C., Städele, K., Růžička, K., Obrdlik, P., Harter, K., and Horáka, J. (2008). Subcellular localization and *in vivo* interactions of the *Arabidopsis thaliana* ethylene receptor family members. *Molecular Plant* 1**,** 308-320. doi: doi:10.1093/mp/ssm015

Gunjate, R.T. (2009). Advances in mango culture in India. *Acta Hort.* 820**,** 69-78.

Guzman-Estrada, C. (1996). Fruit drop and yield of five mango cultivars in Southern Sinaloa. *Acta Hort.* 455**,** 459-464.

Hagemann, M.H., Winterhagen, P., M.G. Roemer, Hegele, M., and Wünsche, J.N. (in press). Proposed physiological mechanism of mango fruitlet abscission. *Acta Hort*.

Hall, B., Shakeel, S., and Schaller, G. (2007). Ethylene receptors: Ethylene perception and signal transduction. *J. Plant Growth Regul.* 26**,** 118-130. doi: 10.1007/s00344-007-9000-0

He, J.H., Ma, F.W., Chen, Y.Y., and Shu, H.R. (2012). Differentially expressed genes implicated in embryo abortion of mango identified by suppression subtractive hybridization. *Genet. Mol. Res.* 11**,** 3966-3974. doi: 10.4238/2012.November.14.4

Huong, P.T. (2004). Impacts of thinning and pre-harvest bagging on growth, yield and fruit's appearance of mango grown in Sap Vat commune, Yen Chau district, Son La province. *Tap chi KHKT Truong DHNNI Ha Noi, tap II* 5**,** 234-328.

Huong, P.T. (2010). Some measures for rehabilitation of neglected mango orchards in Yen Chau, Son La. *J. Sci. Dev.* 8**,** 69–75.

IFPRI (2002). *Fruits and vegetables in Vietnam: Adding value from farmer to consumer.* Washington D.C.: International Food Policy Research Institute (IFPRI).

Ish-Shalom, M., Dahan, Y., Maayan, I., and Irihimovitch, V. (2011). Cloning and molecular characterization of an ethylene receptor gene, *MiERS1*, expressed during mango fruitlet abscission and fruit ripening. *Plant Physiol. Biochem.* 49**,** 931–936. doi: 10.1016/j.plaphy.2011.05.010

Issarakraisila, M., and Considine, J.A. (1994). Effects of temperature on pollen viability in mango cv. 'Kensington'. *Ann. Bot.* 73**,** 231-240. doi: 10.1006/anbo.1994.1028

Iwahori, S., Tominaga, S., and Yamasaki, T. (1988). Stimulation of fruit growth of kiwifruit, *Actinidia chinensis* Planch., by N-(2-chloro-4-pyridyl)-N'-phenylurea, a diphenylurea-derivative cytokinin. *Sci. Hort.* 35**,** 109-115. doi: 10.1016/0304-4238(88)90042-8

John-Karuppiah, K.-J., and Burns, J.K. (2010). Expression of ethylene biosynthesis and signaling genes during differential abscission responses of sweet orange leaves and mature fruit. *J. Am. Soc. Hort. Sci.* 135**,** 456–464.

Ju, C., and Chang, C. (2012). Advances in ethylene signalling: protein complexes at the endoplasmic reticulum membrane. *AoB Plants* 2012. doi: 10.1093/aobpla/pls031

Krishna, H., and Singh, S.K. (2007). Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement — A review. *Biotech. Adv.* 25**,** 223-243. doi: 10.1016/j.biotechadv.2007.01.001

Lakshminarayana, S., and Aguilar, P.H. (1975). Effect of orchard heating in reducing parthenocarpic fruit in 'Haden' mango. *Proc. Fl. St. Hort. Soc.* 88**,** 502-505.

Lakso, A.N., and Robinson, T.L. (2015). Decision support for apple thinning based on carbon balance modeling. *Acta Hort.* 1068**,** 235-242.

Lewis, M.W., Leslie, M.E., and Liljegren, S.J. (2006). Plant separation: 50 ways to leave your mother. *Curr. Opin. Plant Biol.* 9**,** 59-65. doi: 10.1016/j.pbi.2005.11.009

Litz, R.E. (2009). *The mango: Botany, production and uses.* Oxon, UK: CAB International.

Lu, P., and Chacko, E.K. (2000). Effect of water stress on mango flowering in low latitude tropics of northern Australia. *Acta Hort.* 509**,** 283-290.

Luckwill, L.C. (1953a). Studies of fruit development in relation to plant hormones I. Hormone production by the developing apple seed in relation to fruit drop. *J. Hort. Sci. Biotech.* 28**,** 14-24.

Luckwill, L.C. (1953b). Studies of fruit development in relation to plant hormones ii. The effect of naphthalene acetic acid on fruit set and fruit development in apples. *J. Hort. Sci. Biotech.* 28**,** 25-40.

Malik, A.U., Agrez, V., and Singh, Z. (2003). Fruitlet abscission of mango in relation to ethylene. *J. Hort. Sci. Biotech.* 78**,** 458–462.

Malik, A.U., and Singh, Z. (2006). Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *Sci. Hort.* 110**,** 167-174.

Mao, L., Begum, D., Chuang, H.-W., Budiman, M.A., Szymkowiak, E.J., Irish, E.E., and Wing, R.A. (2000). JOINTLESS is a MADS-box gene controlling tomato flower abscission zone development. *Nature* 406**,** 910-913. doi: 10.1038/35022611

Martínez, P.G., Gómez, R.L., and Gómez-Lim, M.A. (2001). Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. *J. Plant Physiol.* 158**,** 101–108. doi: 10.1078/01761610122258602

McAlister, D.F., and Krober, O.A. (1958). Response of soybeans to leaf and pod removal. *Agron. J.* 50**,** 674-677. doi: 10.2134/agronj1958.00021962005000110010x

Mehouachi, J., Serna, D., Zaragoza, S., Agusti, M., Talon, M., and Primo-Millo, E. (1995). Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107**,** 189-197. doi: 10.1016/0168-9452(95)04111-7

Mehrotra, R.C., Dilcher, D.L., and Awasthi, N. (1998). Palaeocene mangifera: like leaf fossil from India. *Phytomorpho.* 48**,** 191-100.

Mukherjee, S.K., and Litz, R.E. (2009). "Introduction: Botany and importance," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 1-18.

Mukherjee, S. (1972). Origin of mango (*Mangifera indica*). *Economic Botany* 26**,** 260-264. doi: 10.1007/BF02861039

Musvoto, C., and Campbell, B. (1995). Mango trees as components of agroforestry sytsems in Mangwende, Zimbabwe. *Agroforest. Sys.* 32**,** 247-260. doi: 10.1007/BF00711713

Naqvi, S.S.M., Alam, S.M., and Mumtaz, S. (1990). Effect of cobalt and silver ions and naphthaleneacetic acid on fruit retention in mango (*Mangifera indica* L.). *Aust. J. Exp. Agr.* 30**,** 433-435. doi: doi:10.1071/EA9900433

Notodimedjo, S. (2000). Effect of GA_3, NAA and CPPU on fruit retention, yield and quality of mango (cv. Arumanis) in East Java. *Acta Hort.* 509**,** 587-600.

Núñez-Elisea, R., and Davenport, T.L. (1983). Abscission and ethylene production in mango (*Mangifera indica* L.) fruit cv. Tommy Atkins. *Proc. Fla. Hort. Soc.* 96**,** 185-188.

Núñez-Elisea, R., and Davenport, T.L. (1986). Abscission of mango fruitlets as influenced by enhanced ethylene biosynthesis. *Plant Physiol.* 82**,** 991–994. doi: 10.1104/pp.82.4.991

Nzima, M.D.S., Martin, G.C., and Nishijima, C. (1999). Effect of fall defoliation and spring shading on shoot carbohydrate and growth parameters among individual branches of alternate bearing 'Kerman' pistacchio trees. *J. Am. Soc. Hort. Sci.* 124**,** 52-60.

Obeso, J.R. (1998). Effects of defoliation and girdling on fruit production in *Ilex aquifolium*. *Func. Ecol.* 12**,** 486-491. doi: 10.1046/j.1365-2435.1998.00216.x

Oosthuyse, S.A. (1993). Effect of spray application of KNO3, urea and growth regulators on the yield of 'Tommy Atkins' mango. *South African Mango Grow. Assn. Yrbk.* 13**,** 58-62.

Peña, J., Mohyuddin, A., and Wysoki, M. (1998). A review of the pest management situation in mango agroecosystems. *Phytoparasitica* 26**,** 129-148. doi: 10.1007/BF02980680

Ploetz, R.C., and Freeman, S. (2009). "Foliar, floral and soilborn diseases," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 231-302.

Prakash, S., and Ram, S. (1984). Naturally occurring auxins and inhibitor and their role in fruit growth and drop of mango Dashehari. *Scientia Horticulturae* 22**,** 241-248. doi: 10.1016/0304-4238(84)90057-8

Qu, X., Hall, B., Gao, Z., and Schaller, G.E. (2007). A strong constitutive ethylene-response phenotype conferred on arabidopsis plants containing null mutations in the ethylene receptors ETR1 and ERS1. *BMC Plant Biol.* 7**,** 3. doi: 10.1186/1471-2229-7-3

Racskó, J., Leite, G.B., Petri, J.L., Zhongfu, S., Wang, Y., Szabo, Z., Soltesz, M., and Nyeki, J. (2007). Fruit drop: the role of inner agents and environmental factors in the drop of flowers and fruits. *Int. J. Hortic. Sci.* 13**,** 13-23.

Ram, S., Sirohi, S.C., and Rathore, V.S. (1983). Naturally occurring cytokinins in mango (*Mangifera indica* L.) fruit. *Aust. J. Plant Physiol.* 10**,** 65-73. doi: 10.1071/PP9830065.

Ram, S. (1992). Naturally occurring hormones of mango and their role in growth and drop of the fruit. *Acta Hort.* 321**,** 400-411.

Rasori, A., Ruperti, B., Bonghi, C., Tonutti, P., and Ramina, A. (2002). Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. *J. Exp. Bot.* 53**,** 2333–2339. doi: 10.1093/jxb/erf097

Reid, M.S. (1985). Ethylene and abscission. *HortSci.* 20**,** 45-50.

Roberts, J.A., Elliott, K.A., and Gonzalez-Carranza, Z.H. (2002). Abscission, dehiscence, and other cell separation processes. *Annu. Rev. Plant Biol.* 53**,** 131–158. doi: 10.1146/annurev.arplant.53.092701.180236

Roberts-Nkrumah, L.B. (2000). The establishment and growth of young mango trees in on-farm hillside trials in Trinidad, W.I. *Acta Hort.* 509**,** 705-712.

Roberts-Nkrumah, L.B. (2004). Evaluation of young fruit tree performance in hillside trials in Trinidad and Tobago. *Acta Hort.* 638**,** 459-464.

Roemer, M.G., Hegele, M., Wünsche, J.N., and Huong, P.T. (2011). Possible physiological mechanisms of premature fruit drop in mango (*Mangifera indica* L.) in Northern Vietnam. *Acta Hort.* 903**,** 999–1006.

Ruiz, R., García-Luis, A., Monerri, C., and Guardiola, J.L. (2001). Carbohydrate availability in relation to fruitlet abscission in citrus. *Ann. Bot.* 87**,** 805-812. doi: 10.1006/anbo.2001.1415

Satoh, S., Watanabe, M., Chisaka, K., and Narumi, T. (2008). Suppressed leaf senescence in Chrysanthemum transformed with a mutated ethylene receptor gene *mDG-ERS1*(*etr1-4*). *J. Plant Biol.* 51**,** 424-427. doi: 10.1007/BF03036064

Sato-Nara, K., Yuhashi, K.-I., Higashi, K., Hosoya, K., Kubota, M., and Ezura, H. (1999). Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiol.* 199**,** 321-329. doi: 10.1104/pp.120.1.321

Sawangchote, P., Grote, P.J., and Dilcher, D.L. (2009). Tertiary leaf fossils of *Mangifera* (Anacardiaceae) from Li Basin, Thailand as examples of the utility of leaf marginal venation characters. *Am. J. Bot.* 96**,** 2048-2061. doi: 10.3732/ajb.0900086

Schaffer, B., and Gaye, G.O. (1989). Effects of pruning on light interception, specific leaf density and leaf chlorophyll content of mango. *Sci. Hort.* 41**,** 55-61. doi: 10.1016/0304-4238(89)90049-6

Schaffer, B., Urban, L., Lu, P., and Whiley, A.W. (2009). "Ecophysiology," in *The mango: Botany, production and uses,* ed. R.E. Litz. (Wallingford, UK: CAB International), 170-209.

Schoeman, M., H., Manicom, B., Q., and Wingfield, M., J. (1995). Epidemiology of powdery mildew on mango blossoms. *Plant Dis.* 79, 524-528.

Schulze, K., Spreer, W., Keil, A., Ongprasert, S., and Müller, J. (2013). Mango (*Mangifera indica* L. cv. Nam Dokmai) production in Northern Thailand—Costs and returns under extreme weather conditions and different irrigation treatments. *Agr. Water Manage.* 126**,** 46-55. doi: 10.1016/j.agwat.2013.04.010

Sexton, R., Jamieson, G.G.C., and Allan, M.H.I.L. (1977). An ultrastructural study of abscission zone cells with special reference to the mechanism of enzyme secretion. *Protoplasma* 91**,** 369-387. doi: 10.1007/BF01291927.

Sexton, R., and Roberts, J.A. (1982). Cell biology of abscission. *Annu. Rev. Plant Physiol.* 33**,** 133–162. doi: 10.1146/annurev.pp.33.060182.001025

Shakeel, S.N., Wang, X., Binder, B.M., and Schaller, G.E. (2013). Mechanisms of signal transduction by ethylene: overlapping and non-overlapping signalling roles in a receptor family. *AoB Plants* 5. doi: 10.1093/aobpla/plt010

Singh, G. (1997). Pollination, pollinators and fruit setting in mango. *Acta Hort.* 455**,** 116-123.

Singh, Z. (2009). Gibberellin type and time of application influence fruit set and retention in mango. *Acta Hort.* 820**,** 407-412.

Singh, Z., and Agrez, V. (2002). Fruit set, retention and yield of mango in relation to ethylene. *Acta Hort.* 575**,** 805-811.

Singh, Z., and Dhillon, B.S. (1987). Effect of foliar application of boron on vegetative and panicle growth, sex expression, fruit retention and physicochemical characters of fruits of mango (*Mangifera indica* L.) cv. Dusehri. *Trop. agr.* 64**,** 305-308.

Singh, Z., Malik, A., and Davenport, T.L. (2005). Fruit drop in mango. *Hort. Rev.* 31:111–153. doi: 10.1002/9780470650882.ch3

Singh, R.N. (1954). Studies on the floral biology and subsequent developments of fruits in the mango (*Mangifera indica* L.) varieties of Dashehari and Langra. *Indian J. Hort.* 11**,** 69-88.

Singh, R.N., and Arora, K.S. (1965). Some factors affecting fruit drop in mango (*Mangifera indica* L.). *Indian J. Agric. Sci.* 35**,** 196–205.

Singh, U.R. (1961). Studies in the fruit drop of mango. IV Embryo development, its degeneration and studies on fruit-pedicel and abscission zone. *Hort. Adv.* 5**,** 218–227.

Singh, V.K., Anurag Singh, Kumar Soni, M., Singh, K., and Singh, A. (2015). Increasing profitability of mango (*Mangifera indica* L.) orchard through intercropping. *Acta Hort.* 1066**,** 151-157.

Sivakumar, D., Jiang, Y., and Yahia, E.M. (2011). Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. *Food Res. Int.* 44**,** 1254-1263. doi: 10.1016/j.foodres.2010.11.022

Spreer, W., Nagle, M., Neidhart, S., Carle, R., Ongprasert, S., and Müller, J. (2007). Effect of regulated deficit irrigation and partial rootzone drying on the quality of mango fruits (*Mangifera indica* L., cv. 'Chok Anan'). *Agr. Water Manage.* 88**,** 173-180. doi: 10.1016/j.agwat.2006.10.012

Spreer, W., Ongprasert, S., Hegele, M., Wünsche, J.N., and Müller, J. (2009). Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes. *Agr. Water Manage.* 96**,** 574-584. doi: 10.1016/j.agwat.2008.09.020

Stepanova, A.N., and Alonso, J.M. (2009). Ethylene signaling and response: where different regulatory modules meet. *Curr. Opin. Plant Biol.* 12**,** 548–555. doi: 10.1016/j.pbi.2009.07.009

Stephenson, A.G. (1980). Fruit set, herbivory, fruit reduction, and the fruiting strategy of *Catalpa speciosa* (Bignoniaceae). *Ecology* 61**,** 57-64.

Stephenson, A.G. (1981). Flower and fruit abortion: proximate causes and ultimate functions. *Annu. Rev. Ecol. Syst.* 12**,** 253-279.

Stino, R.G., El-Wahab, S.A.M.A., Habashy, S.A., and Kelani, R.A. (2011). Productivity and fruit quality of three mango cultivars in relation to foliar sprays of calcium, zinc, boron or potassium. *J. Hort. Sci. Ornamental Plants* 3**,** 91-98.

Stover, E., Fargione, M., Risio, R., Yang, X., and Robinson, T. (2001). Fruit weight, cropload, and return bloom of 'Empire' apple following thinning with 6-benzyladenine and NAA at several phenological stages. *HortSci.* 36**,** 1077-1081.

Sukhvibul, N., Whiley, A.W., Smith, M.K., and Hetherington, S.E. (2000). Susceptibility of mango (*Mangifera indica* L.) to cold-induced photoinhibition and recovery at different temperatures. *Aust. J. Agric. Res* 51**,** 503-513. doi: 10.1071/AR99076

Sukhvibul, N., Whiley, A.W., Smith, M.K., Hetherington, S.E., and Vithanage, V. (1999a). Effect of temperature on inflorescence and floral development in four mango (*Mangifera indica* L.) cultivars. *Sci. Hort.* 82**,** 67-84.

Sukhvibul, N., Whiley, A.W., Smith, M.K., Hetherington, S.E., and Vithanage, V. (1999b). Effect of temperature on inflorescence development and sex expression of mono- and poly-embryonic mango (*Mangifera indica* L.) cultivars. *J. Hort. Sci. Biotech.* 74**,** 64-68.

Sun, L., John Bukovac, M., Forsline, P.L., and Van Nocker, S. (2009). Natural variation in fruit abscission-related traits in apple (*Malus*). *Euphytica* 165**,** 55-67. doi: 10.1007/s10681-008-9754-x

Syamal, M.M., and Mishra, K.A. (1989). Effect of NPK on growth, flowering, fruiting and quality of mango. *Acta Hort.* 231**,** 276-281.

Taylor, J.E., and Whitelaw, C.A. (2001). Tansley Review No. 127. Signals in abscission. *New Phytol.* 151**,** 323-339.

Torre, F.D.L., Rodríguez-Gacio, M.D.C., and Matilla, A.J. (2006). How ethylene works in the reproductive organs of higher plants. *Plant Signal. Behav.* 1**,** 231-242.

Trinh, L.N., Watson, J.W., Hue, N.N., De, N.N., Minh, N.V., Chu, P., Sthapit, B.R., and Eyzaguirre, P.B. (2003). Agrobiodiversity conservation and development in Vietnamese home gardens. *Agr. Ecosyst. Environ.* 97**,** 317-344. doi: 10.1016/S0167-8809(02)00228-1

Van Doorn, W.G., and Stead, A.D. (1997). Abscission of flowers and floral parts. *J. Exp. Bot.* 48**,** 821-837. doi: 10.1093/jxb/48.4.821

Vandenbussche, F., Vaseva, I., Vissenberg, K., and Van Der Straeten, D. (2012). Ethylene in vegetative development: a tale with a riddle. *New Phytol.* 194**,** 895-909. doi: 10.1111/j.1469-8137.2012.04100.x

Varoquaux, F., Blanvillain, R., Delseny, M., and Gallois, P. (2000). Less is better: new approaches for seedless fruit production. *Trends biotechnol.* 18**,** 233-242. doi: 10.1016/S0167-7799(00)01448-7

Verghese, A., Nagaraju, D.K., Kamala Jayanthi, P.D., and Madhura, H.S. (2005). Association of mango stone weevil, *Sternochetus mangiferae* (Fabricius) (Coleoptera: Curculionidae) with fruit drop in mango. *Crop Prot.* 24**,** 479-481. doi: 10.1016/j.cropro.2004.07.010

Villalobos-Acuña, M.G., Biasi, W.V., Flores, S., Mitcham, E.J., Elkins, R.B., and Willits, N.H. (2010). Preharvest application of 1-methylcyclopropene influences fruit drop and storage potential of 'Bartlett' pears. *HortSci.* 45**,** 610-616.

von Mohl, H. (1860). Über den Ablösungsprozess saftiger Pflanzenorgane. *Bot. Zeit.* 18**,** 273-277.

Wardlaw, I. (1968). The control and pattern of movement of carbohydrates in plants. *Bot. Rev.* 34**,** 79-105. doi: 10.1007/BF02858622

Whiley, A.W., Rasmussan, T.S., Saranah, J.B., and Wolstenholme, B.N. (1989). Effect of temperature on growth, dry matter production and starch accumulation in ten mango (*Mangifera indica* L.) cultivars. *J. Hort. Sci.* 64**,** 753–765.

Xie, R.J., Deng, L., Jing, L., He, S.L., Ma, Y.T., Yi, S.L., Zheng, Y.Q., and Zheng, L. (2013). Recent advances in molecular events of fruit abscission. Biol. Plantarum 57, 201–209. doi: 10.1007/s10535-012-0282-0

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. *Ann. rev. plant biol.* 59**,** 225-251. doi: 10.1146/annurev.arplant.59.032607.092804

Young, A. (1989). *Agroforestry for soil conservation.* Wallingford, UK.: CAB International. .

Yuan, R., and Huang, H. (1988). Litchi fruit abscission: its patterns, effect of shading and relation to endogenous abscisic acid. *Sci. Hort.* 36**,** 281-292.

Yuan, R., and Li, J. (2008). Effect of sprayable 1-MCP, AVG, and NAA on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Delicious' apples. *Hort. Sci.* 43**,** 1454-1460.

Zahara, M.B., and Scheuerman, R.W. (1988). Hand-harvesting jointless vs. jointed-stem tomatoes. *Calif. Agr.* 42**,** 14.

Zhang, J., Yu, J., Wen, C.K. (2014) An alternate route of ethylene receptor signaling. *Front. Plant Sci.* 5, article 648. doi: 10.3389/fpls.2014.00648

Zucconi, F., Monselise, S.P., and Goren, R. (1978). Growth abscission relationships in developing orange fruit. *Sci. Hort.* 9**,** 137-146.

8. Summary

Compared to the typical high initial fruit set of mango (*Mangifera indica* L.), only a small share of those fruits reach harvest-maturity. This extensive fruitlet drop is a major yield-limiting factor, leading to substantial economic losses for mango growers world-wide. The numerous causes of fruitlet drop include infections with pests or diseases and unsuitable environmental or crop management conditions. Due to the high impact of fruitlet drop for mango production, the overall objective of this study was to further the understanding of the underlying mechanisms and to develop strategies for reducing fruitlet drop in mango.

Different experimental approaches have been applied to reduce mango fruitlet drop, however, almost as numerous methods have been used for data interpretation, which makes the comparison of data between studies difficult. Therefore a model was developed for defining the timely pattern of fruitlet drop more generally, thus allowing inter-study comparisons of results. The model was tested and validated by monitoring the fruitlet drop in different management systems: traditional monocropping orchard versus 1) intercropping; 2) irrigation; and 3) plant growth regulator applications, respectively. The timely pattern of fruitlet drop was best described with a sigmoid function, which also formed the basis for defining the post-bloom, the midseason and the pre-harvest fruitlet drop stage. Results of the crop management evaluation show that intercropping of maize with mango has no detrimental effect on fruitlet drop. Irrigation resulted in approximately three times higher fruit retention compared with the non-irrigated control. A single application of 40 ppm 1-naphthaleneacetic acid at the end of the post-bloom drop stage resulted consistently in the highest fruit retention. The developed model permits for example the evaluation the treatment efficacies during midseason drop or yield forecasting at the beginning of the pre-harvest stage.

It was suggested that especially during the midseason drop stage tree resources are limited, which results in inter-organ concurrence and subsequently induces fruitlet drop. This is supported by the current findings that during midseason drop mango trees show low rates of photosynthesis, which indicates drought stress. Such stress can induce ethylene-dependent fruitlet abscission. Therefore the ethylene releasing substance ethephon was used in order to study the onset and time-dependent course of fruitlet abscission. The results show that ethephon at a concentration of 7200 ppm (ET7200) is a reliable abscission inducer.

The experiment was extended using ethephon at an additional concentration of 600 ppm (ET600). Both ethephon treatments reduced significantly the capacity of polar auxin transport (PAT) in the pedicel at 1 day after treatment (DAT) and thereafter compared to untreated pedicels. The transcript levels of the ethylene receptor genes *MiETR1* and *MiERS1* were significantly upregulated already at 1 DAT in the ET7200 while only at 2 DAT in the ET600 when compared to the control fruitlets. Specifically, a significant increase of *MiETR1* in the pericarp at 2 DAT and of *MiERS1* in the pedicel at 2 and 3 DAT was induced by ET600. In contrast, both genes were significantly upregulated in both tissues, except *MiETR1* in the pedicel, at 1 DAT and thereafter by ET7200. The last parameter that significantly changed in response to the ethephon treatments was the concentration of sucrose in fruitlet pericarps, which was reduced at 2 DAT compared to control fruitlets. Based on these results, it is postulated that the ethephon-induced abscission process commences with a reduction of the PAT capacity in the pedicel, followed by an upregulation of ethylene receptors and finally a decrease of the sucrose concentration in the fruitlets.

Ethylene receptors are key elements of abscission and other processes of the plants life cycle. Therefore the ethylene receptors were further studied at the molecular level in mango. Additionally to the previously known receptors *MiETR1* and *MiERS,* two novel versions of the *MiERS1* were identified in mango. These receptor genes, *MiERS1m* and *MiERS1s*, translate into truncated proteins with deletions of functional domains and show different expression patterns compared to *MiERS1*. The receptors were further studied through transient expression of fluorescent fusion proteins in the leaves of the model plant tobacco. All receptors are localized at the endoplasmic reticulum. Specific dimerization assays via bi-molecular fluorescence complementation indicate, that MiERS1m can dimerize with itself and with MiERS1, but not with MiETR1. In contrast, no dimerization of MiERS1s with the other receptors could be detected.

In conclusion, the four chapters of the study provide 1) specific recommendations and a 2) model for fruitlet abscission that can be applied by mango growers and researchers, 3) further it could be shown that ethylene perception plays a prevailing role in mango fruitlet abscission and 4) that the ethylene receptors are more diverse than previously known, which suggests that higher levels of complexity are involved in the regulation of mango fruitlet abscission.

9. Zusammenfassung

Im Vergleich zum typischerweise hohen Fruchtansatz bei Mango (*Mangifera indica* L.) erreicht oft nur ein geringer Teil der Früchte die Erntereife. Dieser extensive Fruchtfall ist ein Hauptgrund für Ertragseinbußen und führt somit weltweit zu substantiellen ökonomischen Verlusten für Mangoanbauer. Die zahlreichen Ursachen des Fruchtfalls umfassen Schädlings – und Krankheitsbefall, ungeeignete Umweltbedingungen oder Kulturführungsmaßnahmen. Aufgrund der negativen Auswirkung des Fruchtfalls auf die Mangoproduktion ist das Ziel dieser Arbeit das Verständnis der zugrundeliegenden Mechanismen zu erweitern und Gegenmaßnahmen zu entwickeln.

Zur Verminderung des Fruchtfalls sind bereits verschiedene Ansätze getestet worden, jedoch wurden auch ebenso viele Auswertungsmethoden genutzt, was den Vergleich verschiedener Studien erschwert. Daher wurde ein Modell entwickelt das den Zeitverlauf des Fruchtfalls allgemeingültig definiert und somit den Vergleich verschiedener Studien untereinander ermöglicht. Das Model wurde anhand des Fruchtfalls im Kontext verschiedener Kulturführungsmaßnahmen getestet und validiert, wobei traditionelle Monokulturplantagen jeweils mit 1) Mischkultur; 2) Bewässerung; und 3) Applikation von Wachstumsregulatoren verglichen wurde. Der zeitliche Verlauf des Fruchtfalls konnte am besten mit einer Sigmoidfunktion angenähert werden, worauf basierend der Nachblüte-, der mittelsaisonale und der Vorerntefruchtfall unterschieden wurde. Die Ergebnisse der Evaluation der Kulturführungsmaßnahmen zeigte, dass der Anbau von Mais und Mango als Mischkultur keine negativen Effekte auf den Fruchtfall bei Mango hat. Die Bewässerung führte zu einem dreimal höheren Fruchtbehang im Vergleich zur unbewässerten Kontrolle. Die Einzelbehandlung mit 40 ppm 1-Naphthylessigsäure am Ende des Nachblütefruchtfalls führte zu durchgängig höherem Behang. Das entwickelte Model erlaubt unter anderem eine frühe Abschätzung der Behandlungswirksamkeit oder fundierte Ernteprognosen.

Die Ressourcen eines Baumes sind im Besonderen während des mittelsaisonalen Fruchtfalls limitiert, welches zur Konkurrenz zwischen den Pflanzenorganen führt und somit Fruchtfall induziert. Das Mangobäume während des mittelsaisonalen Fruchtfalls geringe Photosyntheseraten aufweisen die auf Trockenstress hindeuten, unterstützt diese Theorie. Da das Stresshormon Ethylen am Abszissionsprozess beteiligt ist, wurde die ethylenemittierende Substanz Ethephon genutzt um den zeitlichen Verlauf der Abszission zu studieren. Dabei zeigte sich, dass eine Behandlung mit 7200 ppm Ethephon (ET7200) zuverlässig die Fruchtabszission bei Mango induziert.

Das Experiment wurde um eine zusätzliche Ethephonkonzentration von 600 ppm (ET600) erweitert. Im Vergleich zur unbehandelten Kontrolle war die polare Auxintransportkapazität (PAT) des Pedizels infolge beider Ethephonbehandlungen bereits am Tag 1 nach Behandlung und an den Folgetagen signifikant reduziert. ET7200 führte im Vergleich zur Kontrolle bereits am Tag 1 nach Behandlung zur Hochregulierung der Transkriptlevel der Ethylenrezeptorgene *MiETR1* und *MiERS1*, wobei dies durch ET600 erst am Tag 2 nach Behandlung erreicht wurde. ET600 induzierte einen signifikanten Anstieg der Rezeptoren *MiETR1* im Perikarp am Tag 2 nach Behandlung und *MiERS1* im Pedizel am Tag 2 und 3 nach Behandlung. Im Gegensatz dazu waren, abgesehen von *MiETR1* im Pedizel, in Folge von ET7200 ab Tag 1 nach Behandlung beide Gene in beiden Geweben signifikant hochreguliert. Der Effekt von Ethephon war als letztes anhand der Saccharosekonzentration im Perikarp messbar, welche im Vergleich zur Kontrolle am Tag 2 nach Behandlung signifikant reduziert war. Basierend auf diesen Ergebnissen wurde postuliert, dass eine durch Ethephon induzierte Abszission mit der Reduzierung des PAT im Pedizel beginnt, gefolgt von der Hochregulierung der Ethylenrezeptoren und letztlich der Reduktion von Saccharose in den Früchten.

Ethylenrezeptoren sind Schlüsselelemente der Abszission und anderer Prozessen des Lebenszyklus der Pflanzen. Daher wurden diese Rezeptoren bei Mango tiefgehender auf molekularer Ebene untersucht. Zusätzlich zu den zuvor bekannten Rezeptoren *MiETR1* und *MiERS,* konnten zwei neue Versionen des *MiERS1* bei Mango identifiziert werden. Im Vergleich zu *MiERS1*, weisen diese Gene, *MiERS1m* und *MiERS1s,* andere Expressionsmuster auf und kodieren für Proteine denen bestimmte funktionale Domänen des MiERS1 fehlen. Die Rezeptoren wurden mittels transienter Expression von Fluoreszenzfusionsproteinen in der Modellpflanze Tabak untersucht. Alle Rezeptoren konnten am endoplasmatischen Retikulum lokalisiert werden. Experimente mit bi-molekularer Fluoreszenzkomplementierung zeigten, dass der MiERS1 mit sich selbst und mit dem MiERS1, aber nicht mit dem MiETR1 dimerisieren kann. Beim MiERS1s hingegen, wurde keine Dimerisation mit anderen Rezeptoren detektiert.

Zusammenfassend liefern die vier Kapitel dieser Studie 1) spezifische Empfehlungen und 2) ein Modell des Verlaufs des Fruchtfalls bei Mango das von Anbauern und Wissenschaftlern genutzt werden kann. 3) Es konnte zudem gezeigt werden, dass die Ethylenperzeption entscheidend an der Regulierung der Fruchtabszission beteiligt ist und, 4) basierend auf den entdeckten Ethylenrezeptoren, ein höheres Komplexitätslevel für die Regulierung der Fruchtabszission bei Mango angenommen werden kann.

10. Author's declaration

I hereby declare that this doctoral thesis is a result of my own work and that no other than the indicated aids have been used for its completion. All quotations and statements that have been used are indicated. I did not accept the assistance from commercial agency or consulting companies. Furthermore, I assure that the work has not been used, neither completely or in parts, for achieving any other academic degrees.

_____

Michael Helmut Hagemann

Stuttgart, _____

11.Curriculum vitae

Personal details

Degree	diplom biologist
Date of birth:	16. November 1982
Place of birth:	Ibbenbüren

Studies

Today – 09/2009	PhD studies, University of Hohenheim, Stuttgart, Germany.
05/2009 – 07/2008	Diploma thesis at the Institute of Crop Science and Resource Conservation, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany. (grade excellent)
06/2008 – 10/2003	Study of biology at the Heinrich-Heine University, Düsseldorf, Germany.

Working experience

05/2014 – today	Project management, Life Science Center, University of Hohenheim, Stuttgart, Germany
05/2014 – 09/2013	Technical assistant at the Competence Centre for Fruit Production at Lake Constance, Ravensburg, Germany.
09/2013 – 08/2013	Certified scientific assistant at the University of Hohenheim, Stuttgart, Germany.
07/2013 – 10/2012	Technical assistant at the University of Hohenheim, Stuttgart, Germany.
08/2012 – 07/2012	Certified scientific assistant at the University of Hohenheim, Stuttgart, Germany.
06/2012 – 09/2009	Research associate at the University of Hohenheim, Stuttgart, Germany.

Stay abroad

2012 – 2010	(January until April) field work in Vietnam for the Sonderforschungsbereich564 (Deutsche Forschungsgemeinschaft)
03/2003 – 11/2002	Travel (Working-Holiday-Visa) through Southeast Asia and Australia.

Education

06/2002 – 08/2000	Johannes-Kepler Gymnasium, Ibbenbüren, Germany. (Graduation: General qualification for university entrance)
07/2000 – 08/1993	Städtische Realschule Hörstel, Germany.
07/1993 – 08/1989	alternately visited: Sünte-Rendel Grundschule, Riesenbeck, Germany. Grundschule Shilo, Manitoba, Canada.

Language skills

German native language, English business fluent

Scientific publication

01/2015	Hagemann, M.H., Winterhagen, P., Hegele, M. and Wünsche, J.N. 2015. Ethephon induced abscission of mango fruitlets − physiological fruit pedicel response. Acta Hort. 1106:109-116.
12/2014	Hagemann, M.H., Roemer, M.G., Kofler, J., Hegele M. and Wünsche J.N. 2014. A new approach for analyzing and interpreting data on fruit drop in mango. HortSci., 49(12):1498-1505.
06/2011	Menjivar, R.D., Hagemann, M.H., Kranz, J., Cabrera, J.A., Dababat, A.A., and Sikora, R.A. 2011. Biological control of *Meloidogyne incognita* on cucurbitaceous crops by the non-pathogenic endophytic fungus *Fusarium oxysporum* strain 162. Int. J. Pest Manage., 57(3): 249-253.

Scientific presentations (Selection)

03/2013 "Quantifizierung und Charakterisierung des Fruchtfalls bei Mango" presented at *48. Gartenbauwissenschaftliche Tagung*, Bonn, Germany.

04/2012 "Soil protective potential and economic viability of mango- maize intercropping on steep slopes in northern Vietnam" presented at the *International Scientific Conference on Sustainable Land Use and Rural Development in Mountain Areas*, University of Hohenheim, Stuttgart, Germany.

06/2011 "Ethephon Induced Abscission of Mango Fruitlets - Transcription Pattern of Ethylene Receptors in the Fruit Pedicel" presented at the *Global Conference on 'Augmenting Production and Utilization of Mango'* (ISHS), Lucknow, India.

_____

Michael Helmut Hagemann

Stuttgart, _____