Expression Profile of Genes Involved in the Interaction of Jasmonates & Ethylene signal pathway in *H. brasiliensis*

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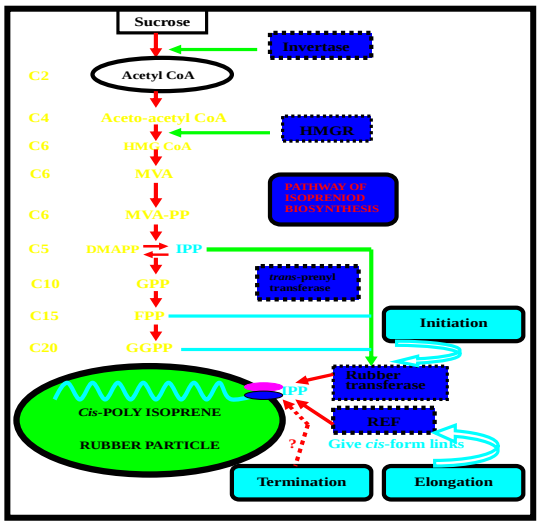
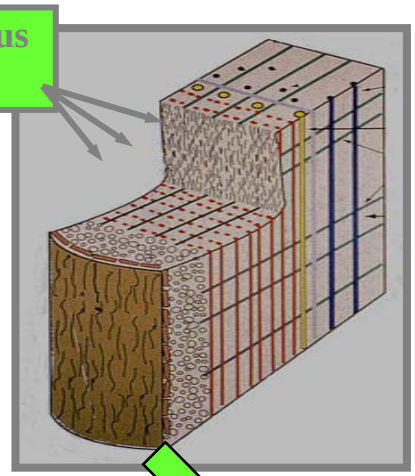
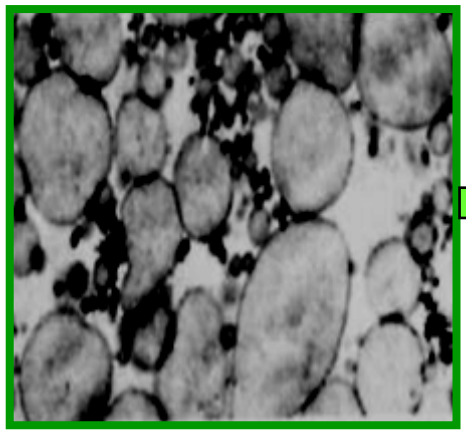
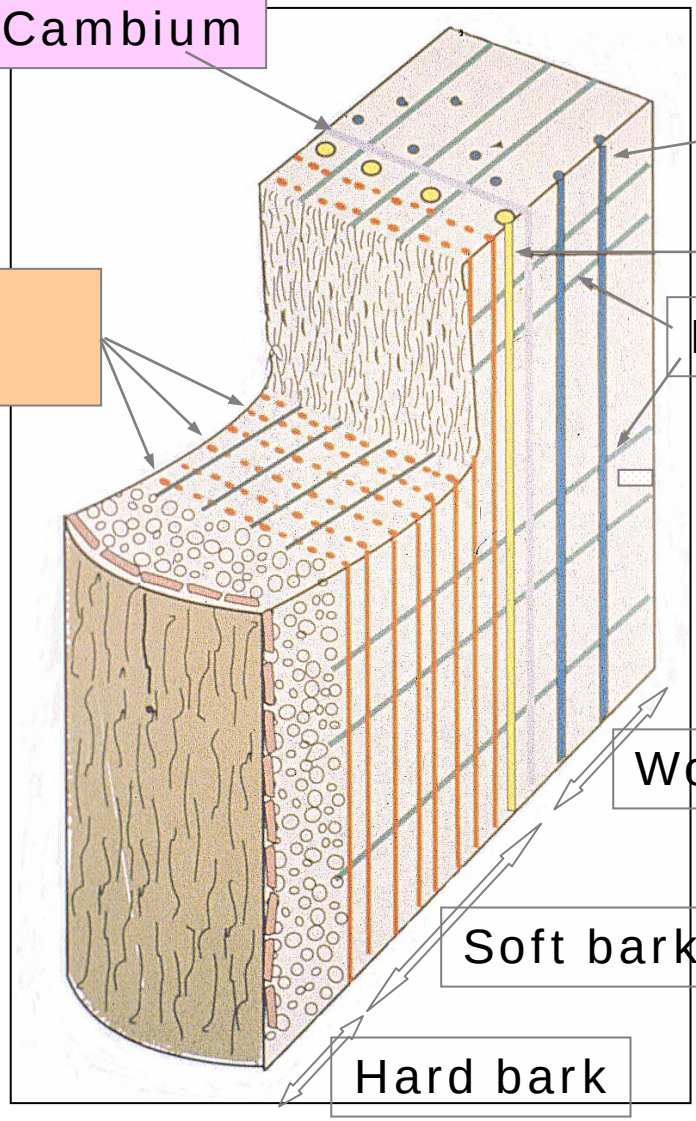
Natural Rubber Production







Anatomy of bark at the tapping cut



Key Elements in Natural Rubber Production

- **Latex Cell Metabolism (Natural Rubber Biosynthesis, ...)**
- **Latex Vessels Number**
- **Tapping /Wounding /Exploitation** Techniques
  - **Stimulants - Ethylene Application** etc.

Interests of the Interaction between JA & ET in *Hevea*

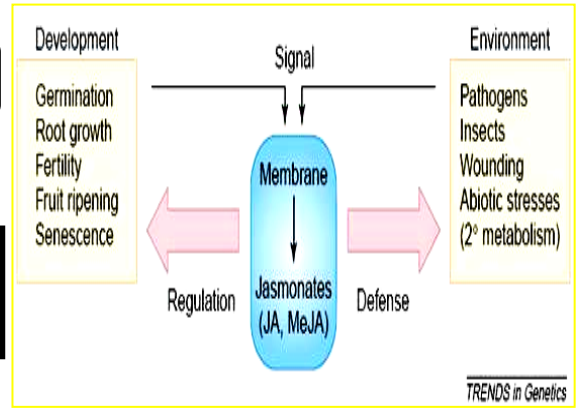
Stimulant

ET

TPD

Tapping/Wounding

JA



Latex Flow Duration
Latex Regeneration

Laticifer Differentiation
Latex Vessels Increase

Latex production

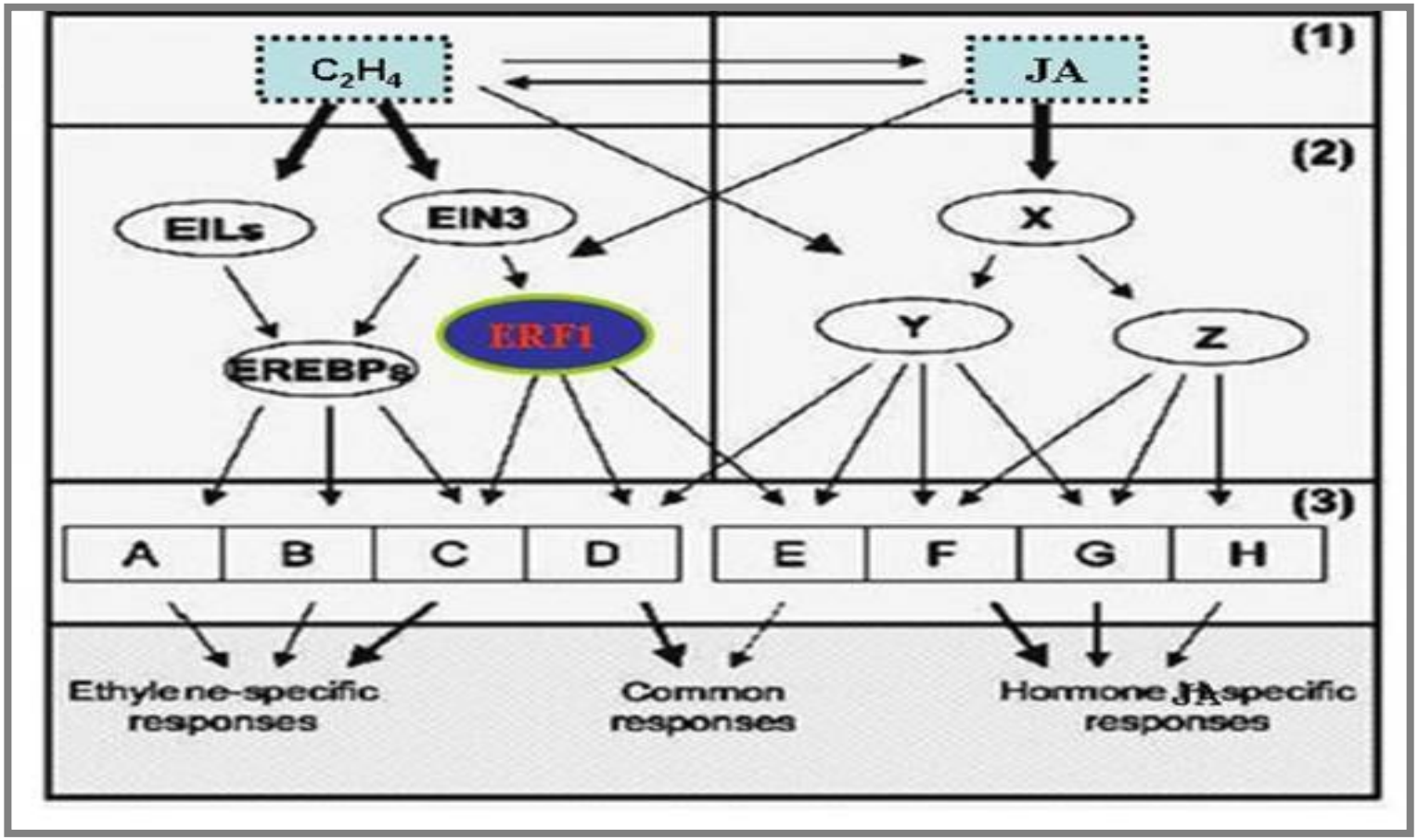
ET + JA

ET ⟷ JA

demands

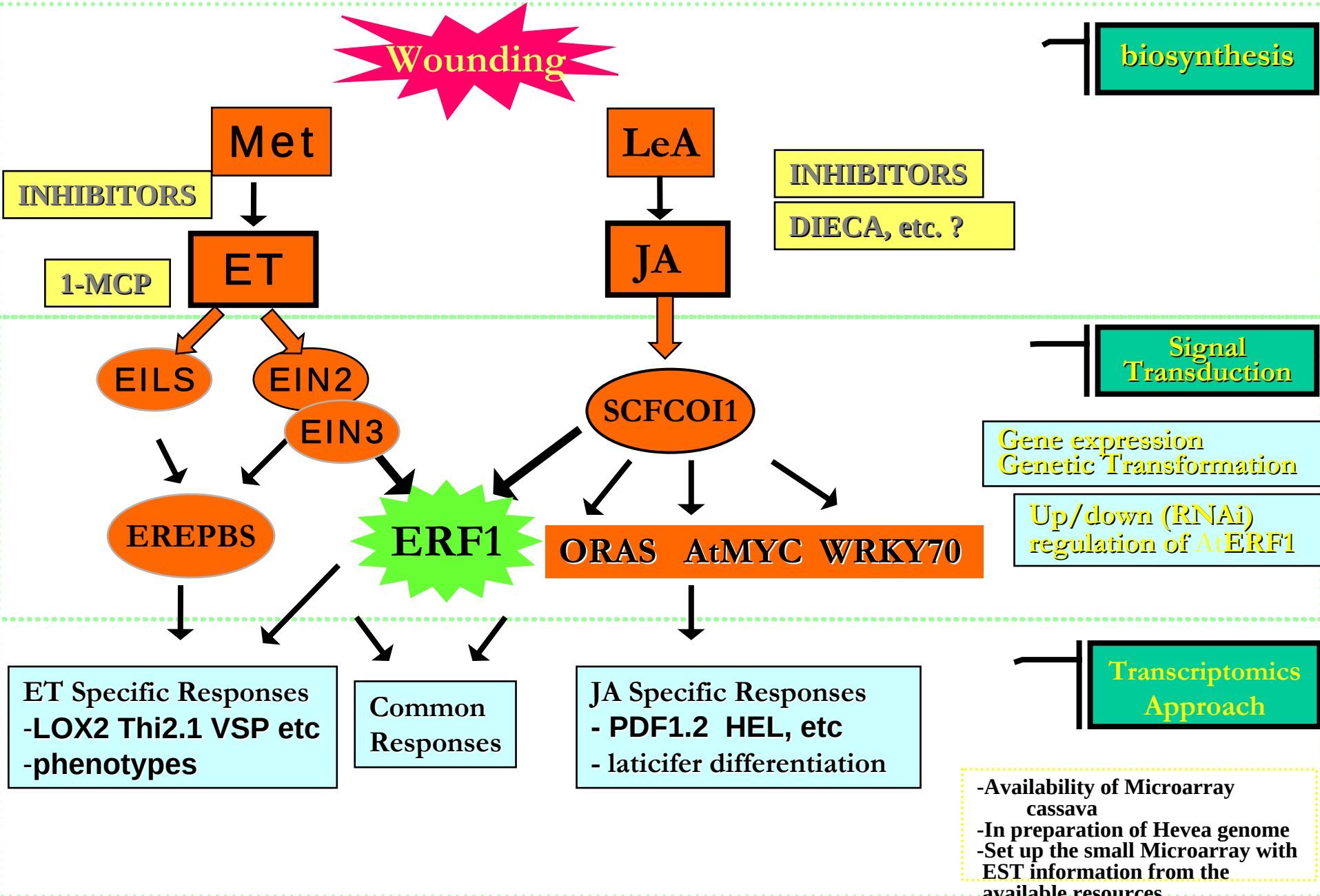
Latex production

The simplified diagram of Interaction between JA &ET



Three basic levels of interaction: (1) the synthesis of each other through the regulation of key biosynthetic genes;(2) crosstalk through the common components of the signal transduction examplified by ERF1 in the interaction of JA&ET; (3)Signals may converge on the regulation of common target genes in the interaction of JA &ET (M.Benovent,MBS,2006)

Research on the Interaction between JA & ET in *Hevea*



Materials and Methods

- **Selection of genes and EST cloning**

  Combining available molecular resources (CATAS, CIRAD, EUSC, IRD)

- **Setting-up the MeJA stimulation system in *Hevea***

- Real Time Quantitative PCR Analysis

FUNCTIONAL CLARIFICATION OF GENES INVESTIGATED

Total genes number: 30

Latex cell metabolism	*Hb cn919 Aquaporin (major intrinsic protein family*	**HbAquaporin**
	Hb Y01D02 plasma membrane aquaporin	**HbAquaporin2**
	HbLatexY05B05 small GTP-binding proteinrab-type	**HbsGProtein**
	HbAY207389 latex beta-cyanoalanine synthase mRNA	**HbLBCS**
	HbAY207388 phloem beta-cyanoalanine synthase mRNA	**HbPBCS**
Ethylene signaling	*HbCn1093ethylene insensitive 3 like 1 protein or EIN 3*	**HbEIN3**
	HbEthylene receptor 5END	**HbETR**
	HbKN-1048-02_B16 ethylene receptor 2.	**HbETR2**
	HbA221 ethylene insensitive protein 2	**HbEIN2**
ET & JA	*HbEthylene responsive factors*	**HbERF**
Jasmonate signaling	*Hb Y69G04 Mitogen-activated protein kinase*	**HbMAPK**
	BTF3b-like transcription factor [Musa acuminata]	**HbBFT3**
	Hb cn261 Auxin responsive SAUR protein	**HbSAUR**
	Hbcn567DNA-binding WRKY	**HbWRKY**
	HbLOX LIPOXYGENASE	**HbLOX**
	HbY21F01coronatine insensitive protein 1/auxin-responsive factor	**HbCOI1**

FUNCTIONAL CLARIFICATION OF GENES INVESTIGATED

Natural rubber biosynthesis	***HbCL11Contig1G protein beta subunit-like***	**HbGP**
	HBKN-1048-08_A03acyl-CoA-binding protein	**HbACBP**
	Lipid transfer precursor protein	**HbLTPP**
	HbAY461413 acyl CoA reductase mRNA, complete cds.	**HbACR**
	HbAF003197 glutamine synthetase mRNA, complete	**HbGS**
Defence genes	***HbKN-1048-04_E06CBL-interacting protein kinase.***	**HbCBLK**
	HbKN-1048-07_N14hypersensitive-induced response protein	**HbHIRP**
	Hb Y21E08 Transcription factor, Myb superfamily.	**HbMYB**
	Hb AY221985 protease inhibitor protein 1 (PI1) mRNA	**HbPI1**
	Hb AJ010397 chitinase	**HbCHI**
	Hb AY275680 Ubiquitin	**HbUBI**
Senescence	***HbCL36Contig1QM family protein***	**HbQM**
Programed Cell Death inhibitor	***HB24_0Q2_01-P07-T7-384defensin precursor***	**HbDefensinP**
	HbKN-1048-04_defender against apoptotic cell death	**Hbdefcelldeath**
	Lipid transfer precursor protein	**HbLTPP**
Other signaling	***HbCL60Contig2calmodulin***	**Hbcalmodulin**

GENES EST CLONING

Function	Genes	Length	Function	Genes	Length
Latex cell metabolism	HbAQ1	195bp	Jasmonates signaling	HbMAPK	194bp
	HbAQ2	200bp		HbBFT	207bp
	HbCalmodulin	200bp		HbSAUR	200bp
	HbPAPD	197bp		HbWRKY	200bp
	HbLBCS	194bp			
	HbGP	183bp		HbCOI1	200bp
Ethylene signaling	HbETR2	208bp	Defence genes	HbUBI	214bp
	HbEIN3	194bp		HbPI1	200bp
	HbEIN2	210bp		HbMYB	183bp
N. RUBBER biosynthesis	HbCBLK	188bp		HbCHI1	214bp
	HbACBP	180bp	Programed cell death inhibitor	HbDefensin	212bp
	HbLTPP	180bp			
	HbACR	186bp	Senescence	HbQMR	214bp
	HbGS2	195bp	JA BIOSYNTHEIS	HbLOX	220BP

The Establishment of Methyl Jasmonate Treatment System

MeJA Application in Various System

Species / tissue	Methods	Concentration	References
Rice,seedlings	spray for 3days	100 µM	Randeep Rakwal, Molecular Biology Reports , 2004.
Cotton wicks	MeJA vapors,in 2-gallon sealed plastic bags for 12 h	7 µ l solved in 100 µ l of ethanol	Pramod Kaitheri Kandoth,plant biology,2007
Arabidopsis thaliana	sprayed with MeJA	0.2 UM	Ricarda Jost, Photosynthesis Research,2005
Tomato	5h gas	1ul 5mg/L in 25ul absolute ethanol	Julie L.,Journal of experimental botany, 2004
Spruce	MeJA was sprayed	100 mM MeJA	Nadir Erbilgin, Oecologia (2006)
Tobacco	The MeJA was in sealed plastic bags	50mM jasmonic acid methyl ester	Mi-Hyun Lee,2007 *Plant Biotechnology*

Application of Exogenous MeJA in *H. brasiliensis*

MeJA, 0.3µM GAS

PB260 Bark&Leaf

CLONE	TISSUE	TIME	SPRAY	GAS	APPLICATION
PB260	B ARK	8H	10µM	5µM	MeJA DILUTED IN ETHANOL
	B ARK	8H	10µM	10µM	
	LEAF	8H	10µM	5µM	
	LEAF	8H	10µM	10µM	
PB217	B ARK	8H	10mM		CONTROL ONLY ETHANOL
	B ARK	8H	50mM		
	B ARK	8H	100mM		

TESTS ON MeJA APPLICATION

RESULTS

Gene Expression Analysis

1 The Dose Effects of MeJA

2 Kinetics of expression of genes

3 Effect of wounding

4 Effect of MeJA on the gene expression

5 Effect of ethylene on the gene expression

6 Synergistic effects of MeJA & ET signals in *H.*

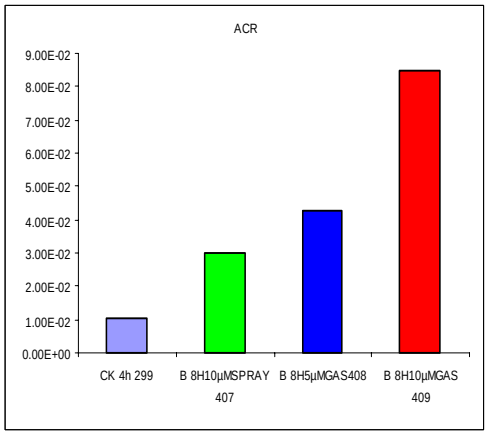
7 Synergistic effects of MeJA & wounding in H.

8 Synergistic effects of Ethylene & wounding in H.

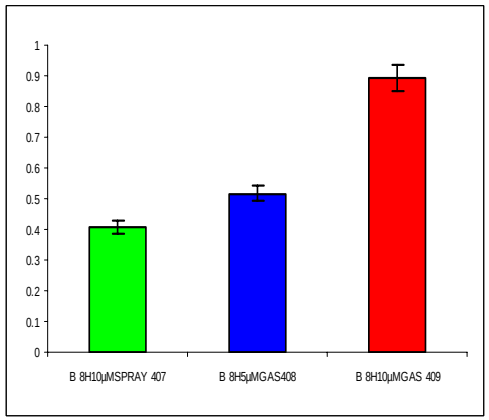
Effects of MeJA Application (spray or gas) & Concentration on Gene Expression in clone PB 260

BARK

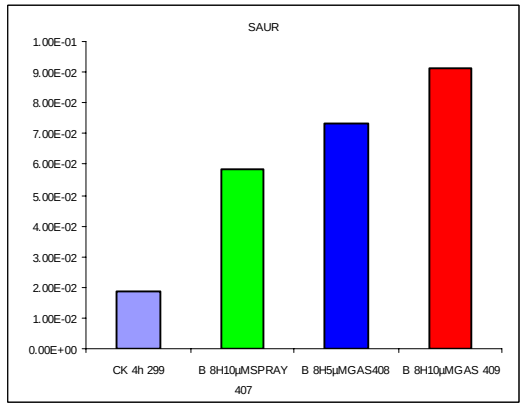
ACR



CBLK

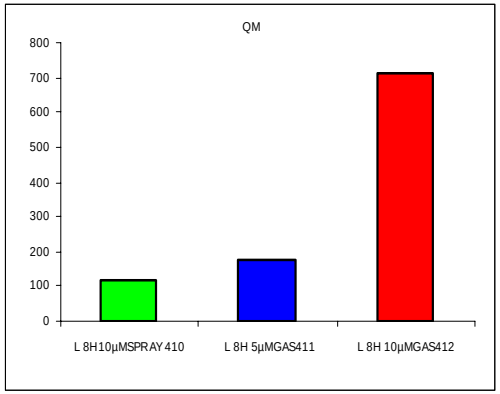


SAUR



LEAF

QM



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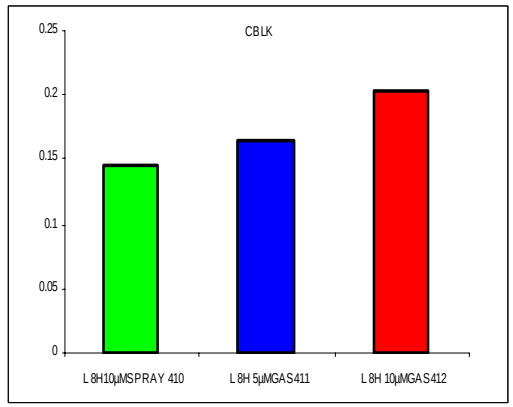
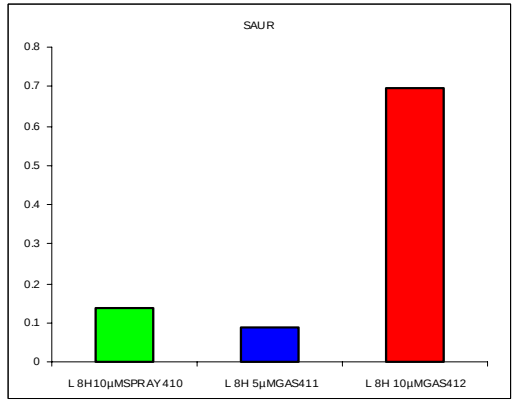


SAUR



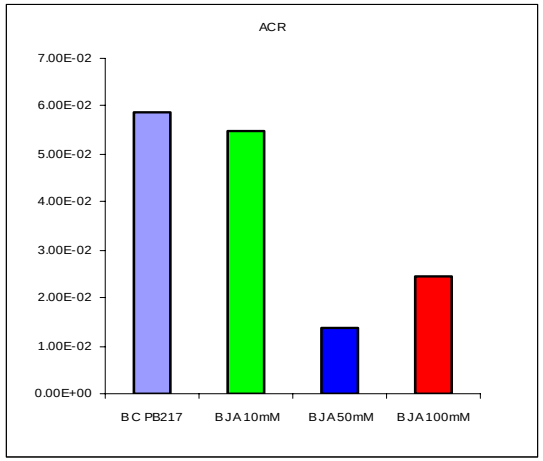
8h10µMSPRAY

8h5µMGAS

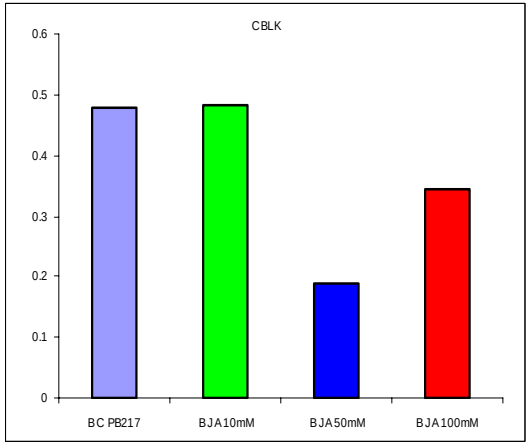
8h10µMGAS

MeJA ON PB217 BARK /SPRAY

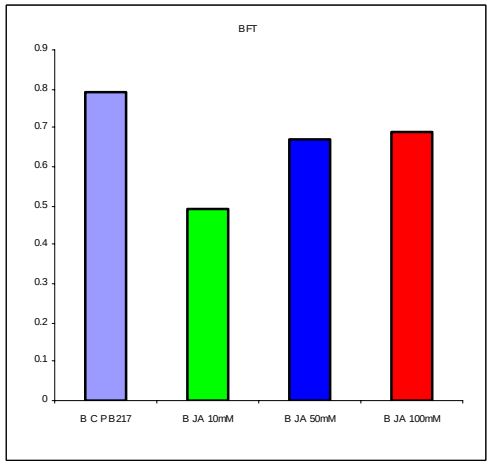
ACR



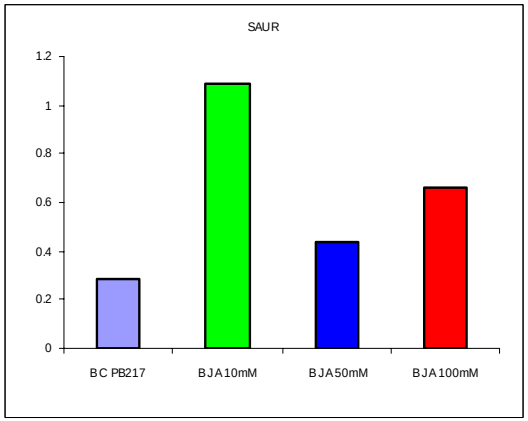
CBLK



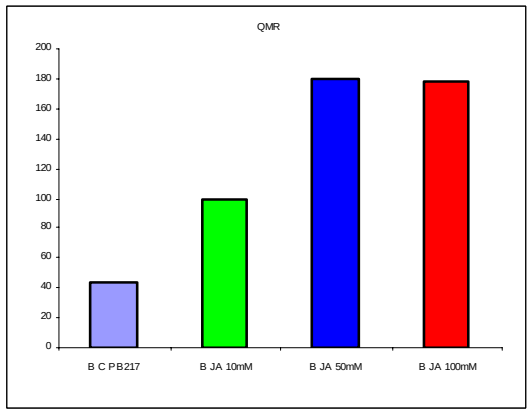
BFT



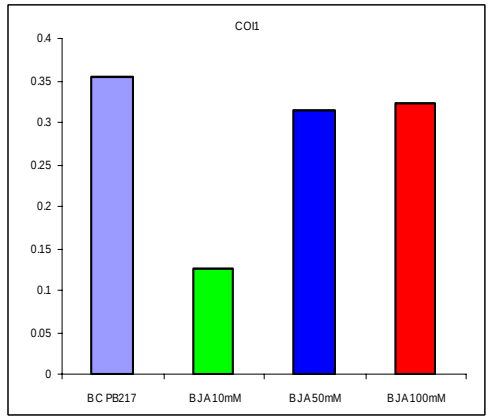
SAUR



QMR



COI1



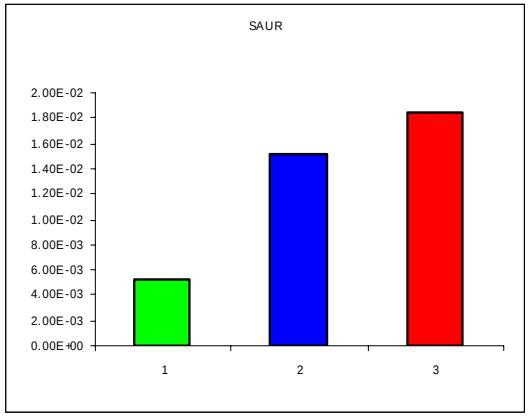
10mM

50mM

100mM

Kinetics Effects of Gene Expression

SAUR



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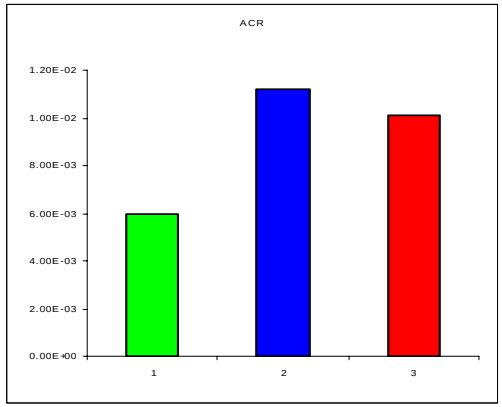
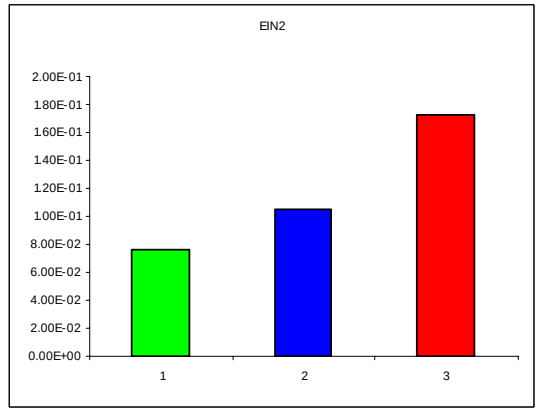
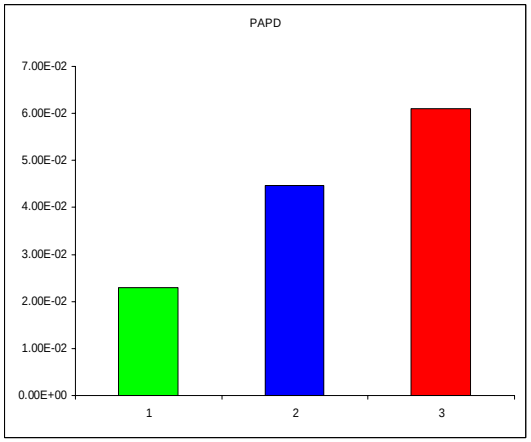


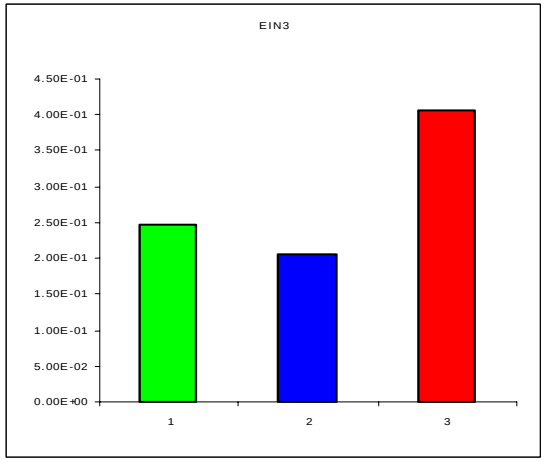
EIN2



PAPD



EIN3



8:00AM

12:00AM

16:00PM

Gene Expression Analysis in Response to Wounding & MeJA and ET

Treatment	Wounding15min		Wounding4h		Ethylene4h		MCP+ET4h		Ethylene24h		MCP+ET24h		MeJA1h		MeJA4h	
PB 260	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value	**ratio**	p-value
WRKY	**1.435**	0.283	**1.162**	0.669	**0.938**	0.653		0.423	**1.030**	0.925	0.343	0.475	**0.954**	0.859	**1.360**	0.271
MAPK	**0.298**	0.010	**0.607**	0.182	**1.281**	0.181	0.136	0.478	**0.367**	0.412	3.308	0.388	**0.684**	0.337	**0.680**	0.291
ETR	**1.969**	0.547	**0.572**	0.370	**1.736**	0.064	0.075	0.447	**1.770**	0.634	0.546	0.598	**1.232**	0.753	**0.138**	0.340
ACR	**0.436**	0.333	**4.417**	0.010	**2.397**	0.046	0.066	0.449	**1.802**	0.571	0.748	0.742	**0.823**	0.599	**3.854**	0.003
PAPD	**0.479**	0.008	**0.252**	0.005	**0.739**	0.266	0.032	0.435	**0.339**	0.428	3.151	0.485	**0.695**	0.345	**0.582**	0.262
PBCS	**0.950**	0.863	**2.110**	0.300	**2.127**	0.157	0.059	0.445	**1.596**	0.658	0.696	0.713	**2.477**	0.030	**3.198**	0.009
EIN2	**0.843**	0.769	**1.180**	0.478	**0.912**	0.534		0.423	**0.000**	0.130	0.902	0.381	**0.843**	0.190	**1.513**	0.177
GS	**1.048**	0.892	**1.177**	0.574	**1.737**	0.119	5.824	0.395	**0.000**	0.423	0.188	0.068	**0.584**	0.063	**1.162**	0.575
COI1	**1.106**	0.666	**0.672**	0.028	**1.219**	0.205	0.899	0.587	**1.025**	0.895	0.972	0.893	**0.566**	0.407	**0.492**	0.173
SAUR	**1.342**	0.418	**5.608**	0.004	**1.486**	0.078		0.435	**0.322**	0.467	0.120	0.130	**1.286**	0.503	**7.668**	0.007
LBCS	**0.331**	0.126	**1.048**	0.794	**2.403**	0.219	0.626	0.396	**1.070**	0.883	0.845	0.653	**0.841**	0.611	**1.145**	0.323
CAL	**0.670**	0.529	**0.616**	0.103	**1.173**	0.508		0.423	**0.000**	0.080	0.890	0.637	**1.193**	0.281	**0.936**	0.695
AQ	**0.667**	0.226	**0.583**	0.452	**1.032**	0.903	7.653	0.452	**1.193**	0.420	2.477	0.106				
EIN3	**1.409**	0.480	**2.382**	0.121	**1.166**	0.421		0.423	**0.000**	0.138	0.439	0.019	**1.066**	0.871	**0.698**	0.711
CBLK	**1.131**	0.865	**1.615**	0.426	**0.821**	0.642	0.686	0.588	**0.920**	0.644	0.964	0.870	**0.826**	0.083	**2.221**	0.097
AQ2	**0.769**	0.143	**1.099**	0.568	**1.934**	0.166	0.655	0.053	**0.940**	0.645	0.698	0.177	**0.995**	0.987	**1.048**	
ACBP	**0.236**	0.343	**1.365**	0.688	**3.676**	0.100	1.148	0.605	**0.847**	0.796	0.916	0.717	**1.023**	0.947	**0.970**	0.520
BFT	**0.435**	0.164	**1.039**	0.823	**1.445**	0.461	0.886	0.596	**0.690**	0.497	0.770	0.182	**0.783**	0.532	**1.022**	0.497
UBI	**1.366**	0.637	**0.785**	0.278	**2.410**	0.420	0.421	0.357	**0.416**	0.140	2.963	0.108	**1.080**	0.674	**1.448**	0.120
ETR2	**2.256**	0.401	**1.047**	0.929	**4.260**	0.008	0.135	0.010	**8.087**	0.018	0.110	0.038	**1.498**	0.727	**1.352**	0.412
MYB	**0.220**	0.400	**5.817**	0.185	**1.119**	0.483	0.875	0.634	**1.457**	0.421	1.151	0.678	**0.508**	0.012	**1.131**	0.917
CHI	**0.000**	0.261	**6.759**	0.420	**0.000**	0.421		0.422	**1.052**	0.901	0.684	0.693	**0.316**	0.045	**0.004**	0.423
QMR	**0.789**	0.069	**2.300**	0.161	**1.770**	0.038	0.674	0.442	**1.274**	0.710	0.994	0.991	**1.537**	0.077	**1.562**	0.075
LTPP	**0.496**	0.280	**0.811**	0.697	**1.136**	0.527	0.966	0.899	**0.957**	0.916	1.090	0.818	**0.969**	0.923	**0.622**	0.371
DEFENSIN	**0.165**	0.316	**0.379**	0.018	**2.252**	0.072	0.919	0.744	**1.219**	0.425	1.145	0.527	**0.600**	0.610	**0.476**	0.097
GP	**0.995**	0.983	**1.734**	0.011	**2.394**	0.004	0.511	0.073	**1.111**	0.458	0.990	0.965	**2.010**	0.033	**2.148**	0.115

Treatment	W 15min	W 4h	ET 4h	MCP+ET4h	ET24h	MCP+ET24h	MeJA01h	MeJA4h
LTPP	↓							
CAL		↓						
PAPD	↓	↓ ↓						
CBLK							↓	+
EIN2						↓ ↓ ↓	↓	+
ETR2			++	↓ ↓ ↓	++++	↓		
AQ2			+	↓				
ACBP			++					
ETR			+					
MAPK	↓ ↓	↓	+					
LBCS	↓ ↓		+					
EIN3		+				↓		
UBI					↓			+
MYB		++++					↓	
CHI		++++					↓ ↓	
SAUR		++++	+			↓		++++
ACR		++	+					++
PBCS		+	+				+	++
QMR	↓ ↓	+	+				+	+
DEFENSIN	↓	↓	+			↓ ↓ ↓		↓
GS			+			↓	↓	
COI1		↓	+					↓
GP		+	+	↓			+	+

PB 260	+ up- regulated 1-3 folds; ++ up- regulated 3-5 folds; ++++ up- regulated >5 folds; ↓ down- regulated 1-3 folds; ↓ ↓ down- regulated 3-5 folds; ↓ ↓ ↓ ↓ down- regulated >5 folds

WOUNDING EFFECTS

Treatment	W 15min	W 4h
SAUR		+ + + +
MYB		+ + + +
CHI		+ + + +
ACR		+ +
PBCS		+
EIN3		+
GP		+
QMR	羊	+
DEFENSIN	羊 羊 羊 羊	羊
MAPK	羊 羊	羊
LBCS	羊 羊	
PAPD	羊	羊 羊
BFT	羊	
LTPP	羊	
CAL		羊
COI1		羊

GENES NOT RESPONSIVE	ETR2	ACBP	ETR	UBI	GS	CBLK	EIN2	WR KY	AQ	AQ2

Notes: + up- regulated 1-3 folds; ++ up- regulated 3-5 folds; ++++ up- regulated >5 folds; 羊 down- regulated 1-3 folds; 羊 羊 down- regulated 3-5 folds; 羊 羊 羊 羊 down- regulated >5 folds

MeJA Effects

Treatment	MeJA1h	MeJA4h
PBCS	+	+ +
QMR	+	+
GP	+	+
SAUR		+ + + +
ACR		+ +
UBI		+
CBLK	羊	+
EIN2	羊	+
CHI	羊 羊	
GS	羊	
MYB	羊	
COI1		羊
DEFENSIN		羊

GENES NOT RESPONSIVE							
	PAPD	LBCS	MAPK	CAL	ACBP	EIN3	AQ2
	BFT	ETR2	WRKY	AQ	LTPP	ETR	

Notes: + up- regulated 1-3 folds; ++ up- regulated 3-5 folds; ++++ up- regulated >5 folds;
羊 down- regulated 1-3 folds; 羊 羊 down- regulated 3-5 folds; 羊 羊 羊 羊 down- regulated >5 folds

ETHYLENE EFFECTS

Treatment	ET 4h	MCP+ET4h	ET24h	MCP+ET24h
ETR2	+ +	↓ ↓ ↓ ↓	+ + + +	↓ ↓ ↓ ↓
ACBP	+ +			
GS	+			↓ ↓ ↓ ↓
SAUR	+			↓ ↓ ↓ ↓
AQ2	+	↓		
GP	+	↓		
MAPK	+			
ETR	+			
ACR	+			
PBCS	+			
COI1	+			
LBCS	+			
QMR	+			
DEFENSIN	+			
EIN3				↓
UBI			↓	

GENES NOT RESPONSIVE						
	WRKY	CBLK	LTPP	BFT	CAL	MYB	AQ
	PAPD	EIN2	CHI				

Notes: + up- regulated 1-3 folds; ++ up- regulated 3-5 folds; ++++ up- regulated >5 folds;
↓ down- regulated 1-3 folds; ↓ ↓ down- regulated 3-5 folds; ↓ ↓ ↓ ↓ down- regulated >5 folds

Synergistic Effects of Signal Pathways on Genes Expression

Genes invovled in Wounding & Ethylene

Treatment	W 15min	W 4h	ET 4h	MCP+ET24h
MAPK	--	--	+	
LBCS	--		+	
EIN3		+		--

Genes invovled in MeJA & Wounding

Treatment	W 4h	MeJA1h
MYB	++++	--
CHI	++++	--

Genes invovled in MeJA &ET

Treatment	ET 24h	MeJA 4h
UBI	--	+

Synergistic effect of Wounding, MeJA &ET

GENES INVOLVED IN THE INTERACTION OF WOUNDING&MeJA , ET SIGNAL

Treatment	W 15min	W 4h	ET 4h	MCP+ET 4h	MCP+ET 24h	MeJA 1h	MeJA 4h
SAUR		++++	+		----		++++
ACR		++	+				++
PBCS		+	+			+	++
QMR	--	+	+			+	+
DEFENSIN	-- --	--	+				--
GS			+		----	--	
COI1		--	+				--
GP		+	+	--		+	+

Marker Genes with Specific Responses

Genes only Specific Responsive to ET

Treatment	ET 4h	MCP+ET4h	ET24h	MCP+ET24h
ETR2	+ +	羊 羊 羊 羊	+ + + +	羊 羊 羊 羊
ACBP	+ +			
AQ2	+	羊		
ETR	+			

Genes only Specific Responsive to Wounding

Treatment	W 15min	W 4h
PAPD	羊	羊 羊
LTPP	羊	
CAL		羊

Genes only Specific Responsive to MeJA

Treatment	MeJA1h	MeJA4h
CBLK	羊	+
EIN2	羊	+

CONCLUSION

INTERACTION OF JA , ET& CHARACTERIZED RESPONSED IN HEVEA

Tapping

Wounding

Ethephon

Hormone Biosynthesis

JA

MeJA

C_2H_4

SIGNALING TRANSDUCTION

Perception

SAUR COI1 WRKY BFT

Transduction

MAPK ETR ETR2 EIN2 EIN3

Transcription factors

Characterzsed responses in Hevea brasiliensis

AQ1 AQ2 GP CAL

QM, MYB

MOLECULAR RESPONSE

HEV, PI, CHI,UBI *etc.*

CAT, CuZnSOD, MnSOD, GCL cyt, GCL chl,

GS,PAPD ,LBCS etc

ACR ACBP CBLK LTPPetc,

Latex cell response

Regulation of coagulation

Detoxification of ROS

Metabolic activation

Secondary metabolism: rubber biosynthesis

Stability of lutoids

Response in planta

In plantaesponses

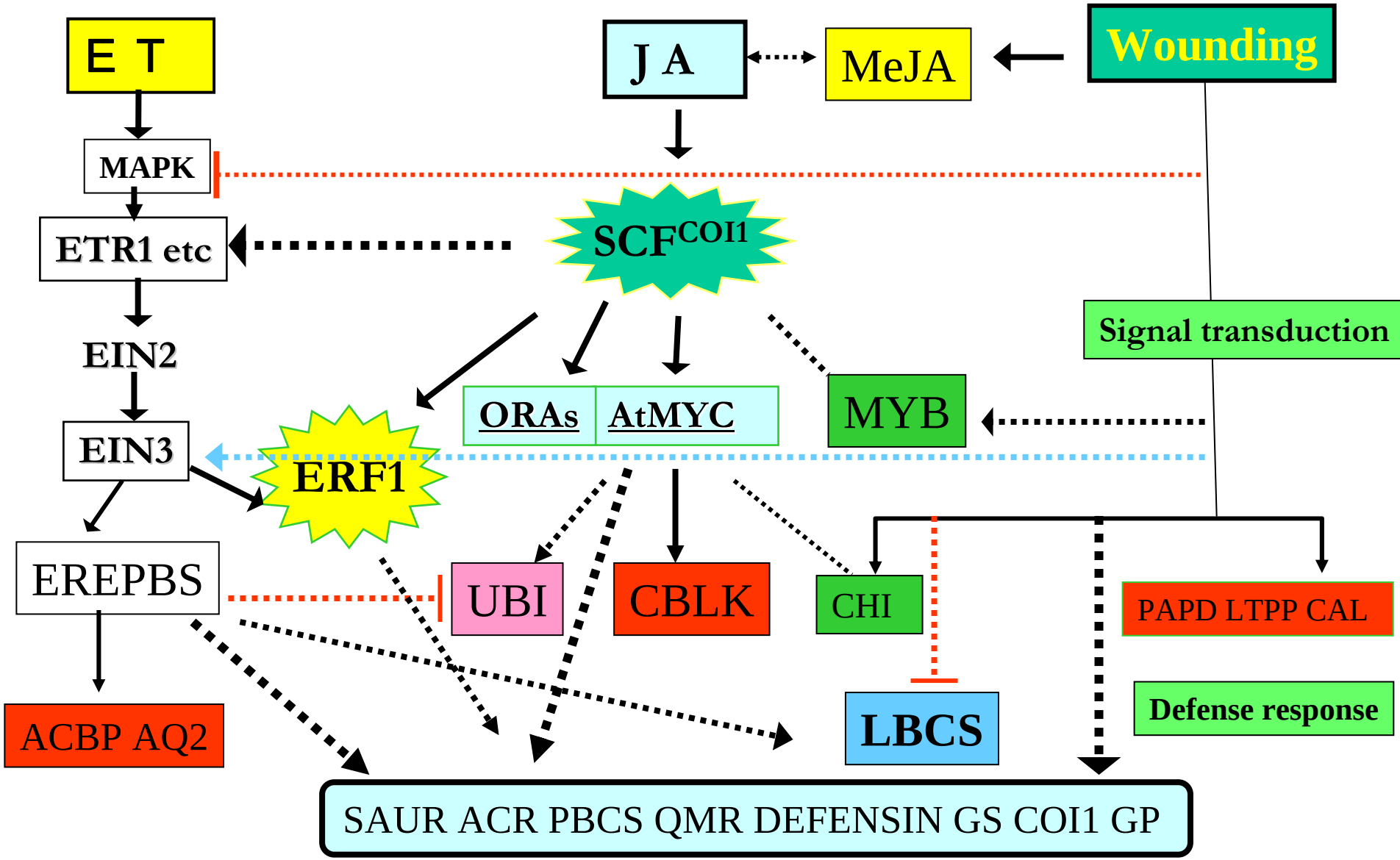
Latex flow

Latex regeneration

Production of latex

Senescence (TPD, leaf abscision, etc.)

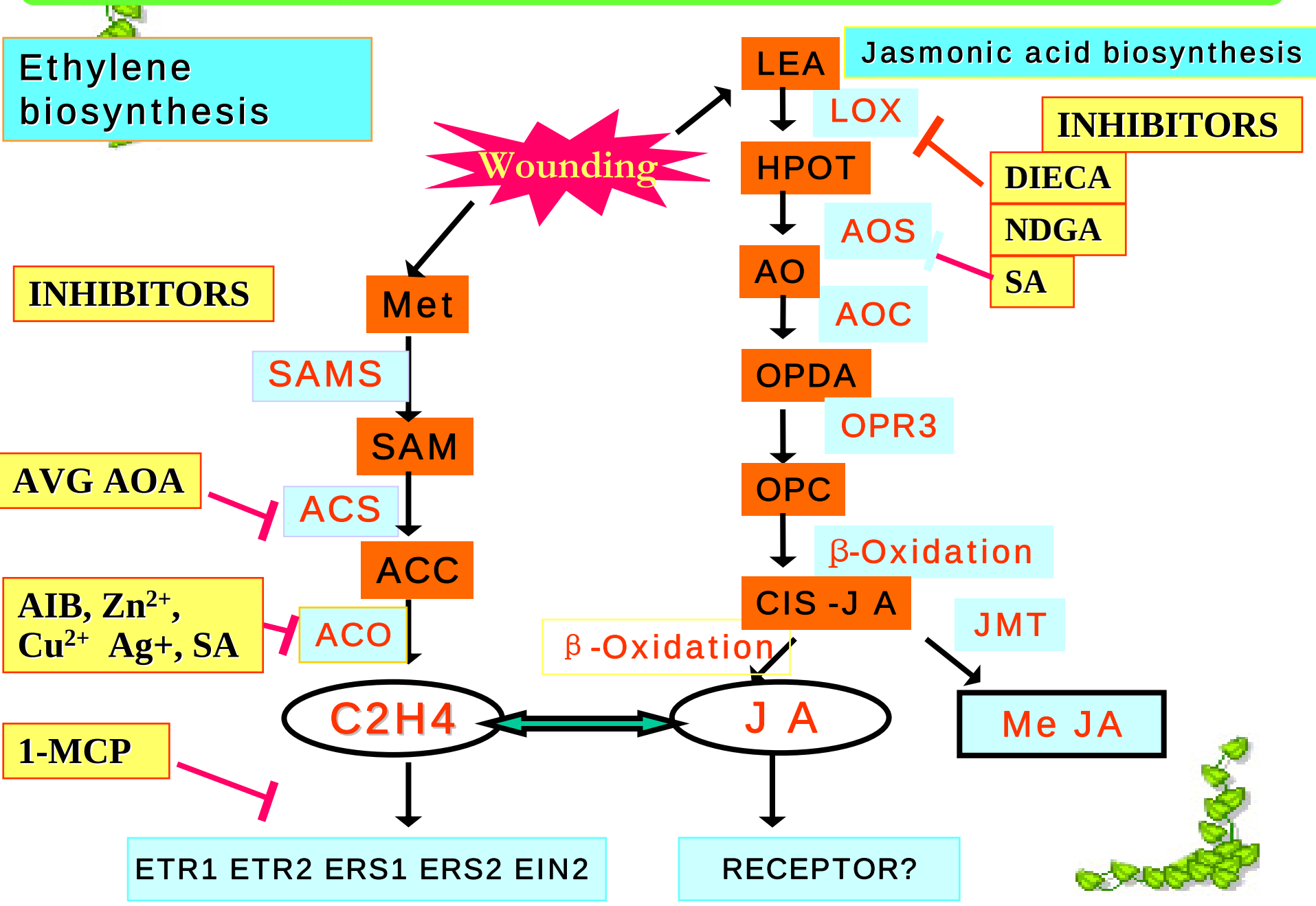
Proposed Model of ineraction of Wounding&JA & ET Signal in *Hevea*



Arrows indicate positive regulation, and blunt ends indicate negative regulation.

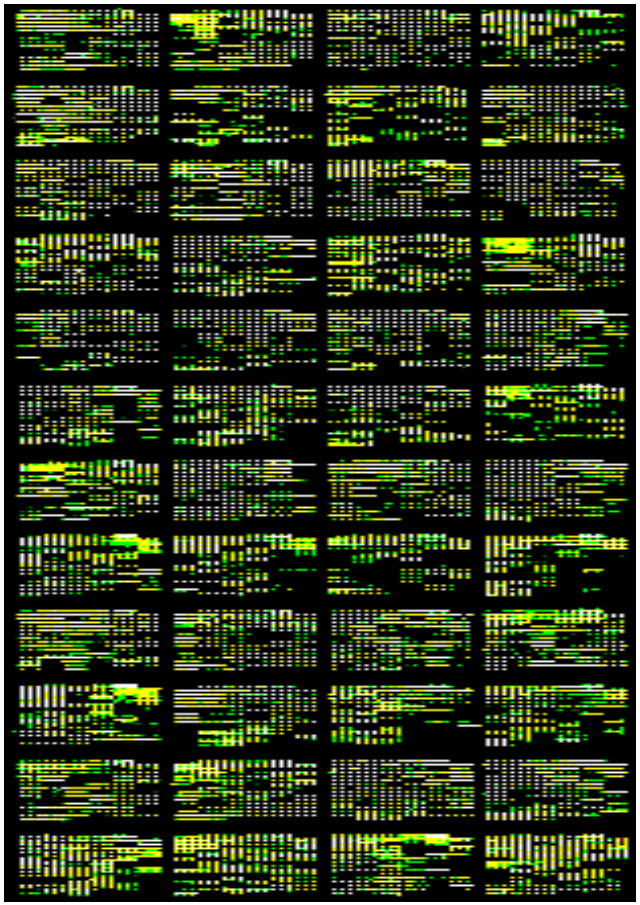
DISCUSSION & PROSPECTIVE

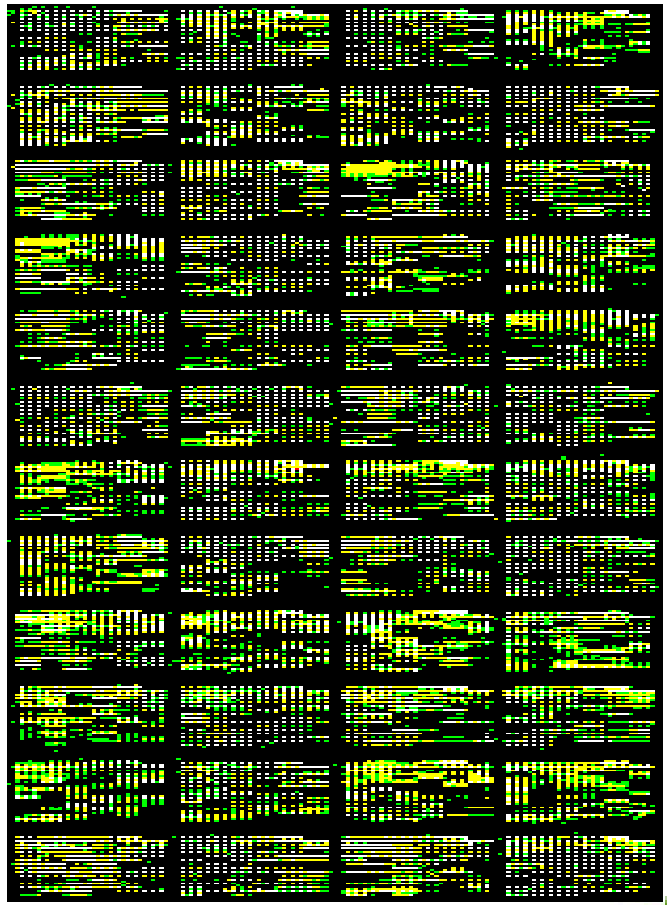
Pharmological Approach on JA & ET Biosynthesis in *Hevea*. Identification of conditions to use JA inhibitor



Transcriptomics Approach

According to the results, for the further research on the interaction of JA and ET signal pathway, it would be proposed to try Transcriptomics approach with the mix cDNA to set up the network of interaction among Wounding& JA and ET





Thank you !