

CHARACTERIZATION OF NPR1 ANKYRIN DOMAIN FROM CHILLI PEPPER (*Capsicum annuum* L.) var Lotanbar

JAMSARI

Faculty of Agriculture-Andalas University
Campus Limau Manis-25136-Padang, West Sumatera-Indonesia



**ANDALAS
UNIVERSITY**



Presented at International Conference of Life Science and Biotechnology (2nd ICOLIB)
August 7-8, 2017, Jember-East Java Indonesia

BACKGROUND



**ANDALAS
UNIVERSITY**



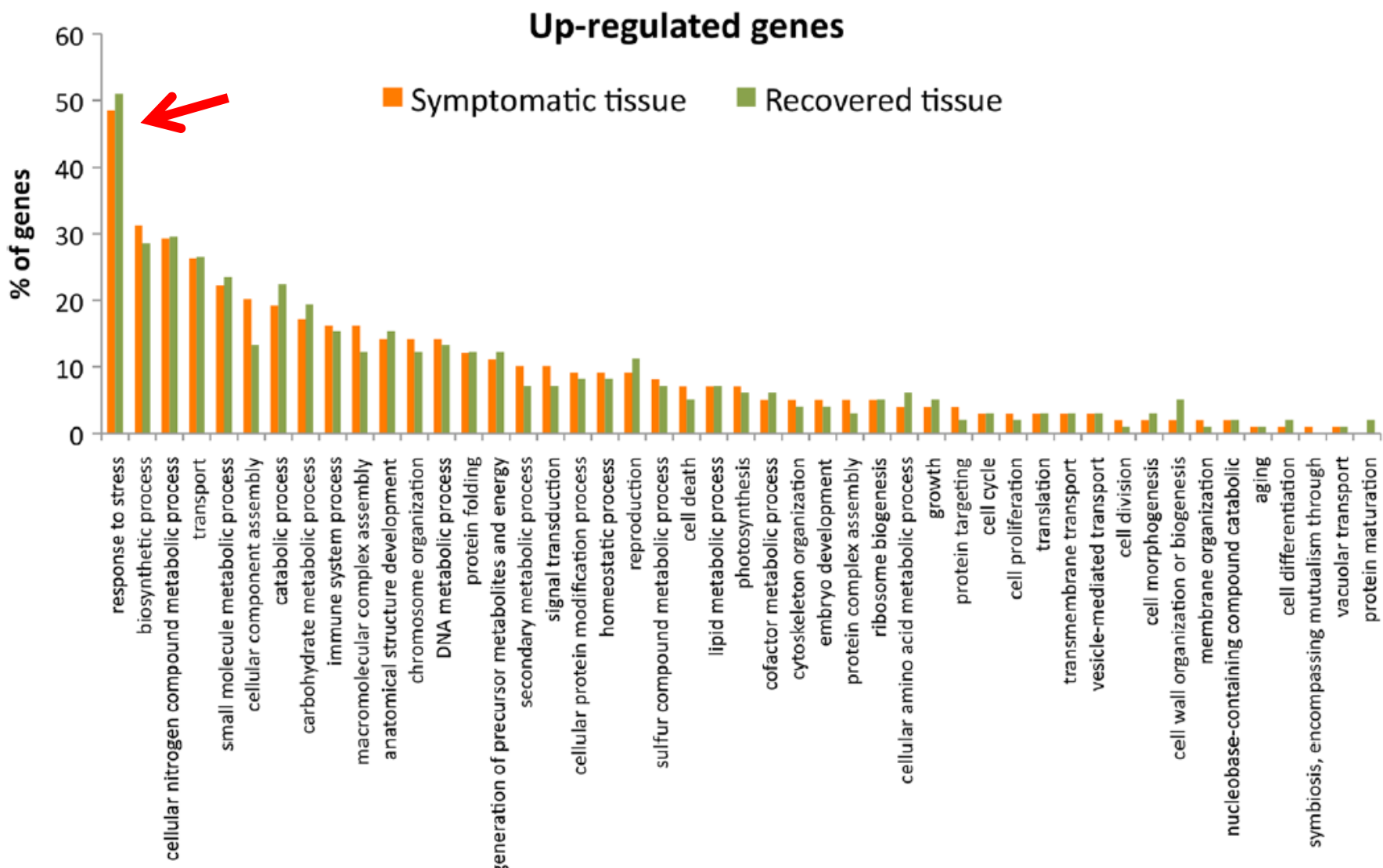
Pepper Yellow Leaf Curl Disease Caused by Gemini-Virus

- Decrease of yield can reach up to 100%
- No effective control so far.
- High genetic variation.
- Transmitted by Whitefly



Source: Rybicki, 2007

Genes Expressing During Gemini Virus Infection in Ca.



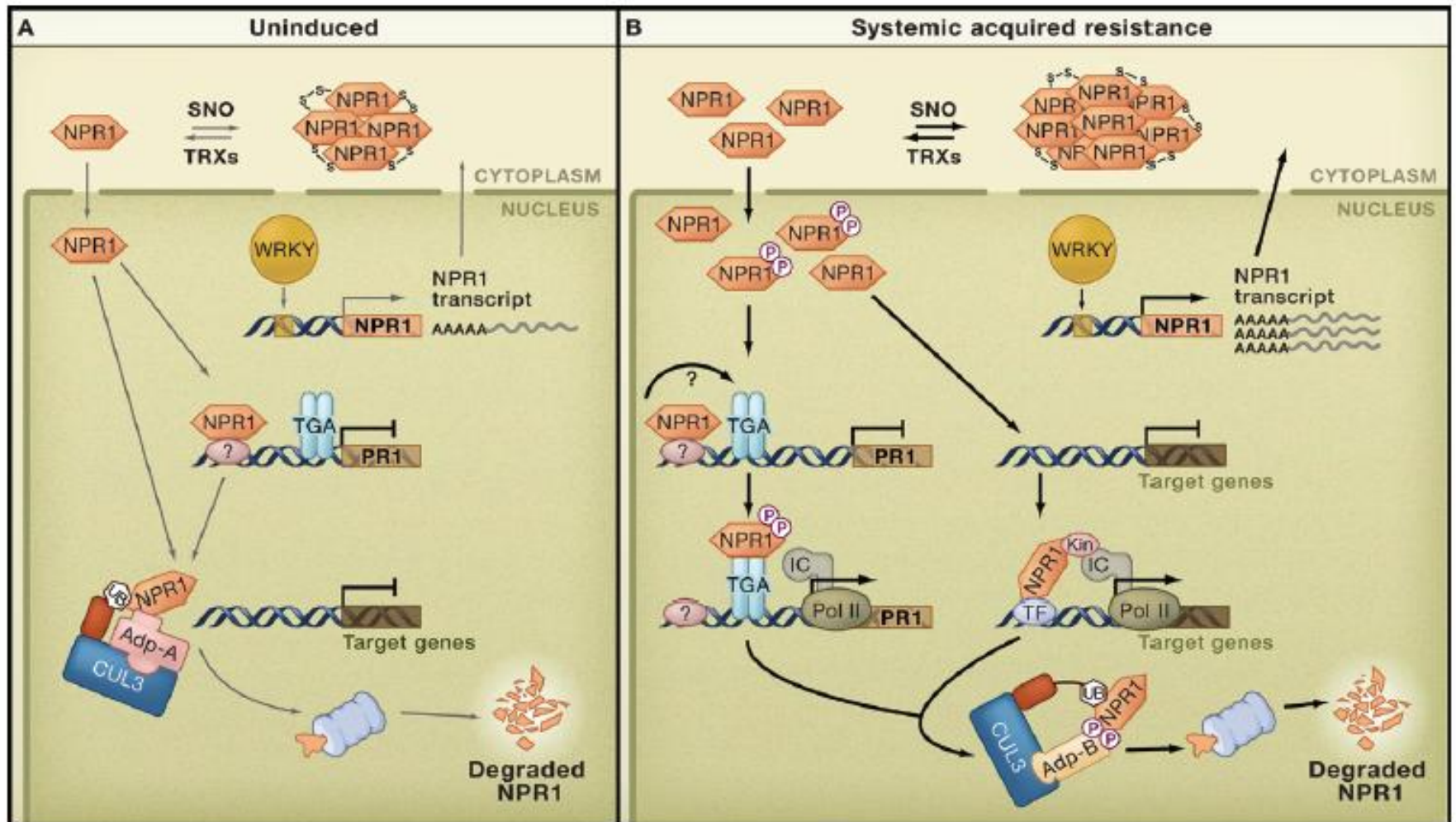
Source : Góngora-Castillo et al. (2012)

The PR genes Are More Expressed

ID	GENE NAME	Fold-change		
		R / S	S / M	R / M
Pepper28784	CAT	0.94	3.67	3.48
Pepper25924	ANN4	1.07	7.42	7.98
Pepper30410	ANN1	1.11	4.26	4.74
Pepper28222	GST1	1.08	4.82	5.24
Pathogenesis-related protein				
Pepper00302	PR-5	0.97	25.05	24.37
Pepper27140	PR-1	1.25	14.22	17.89
Pepper31625	PR-1	1.11	16.08	17.89
Ethylene and jasmonic acid				
Pepper01276	ACC	0.98	2.02	1.99
Pepper00009	EIN3	1.18	0.48	0.57
Pepper26071	OPR1	0.90	2.76	2.51
Pepper31749	LOX1	0.99	3.48	3.47
Pepper32274	HEL	1.08	2.25	2.43
Pepper32368	b-Chi	1.04	39.59	41.35
Others				
Pepper05849	RRP1	0.96	69.59	66.96

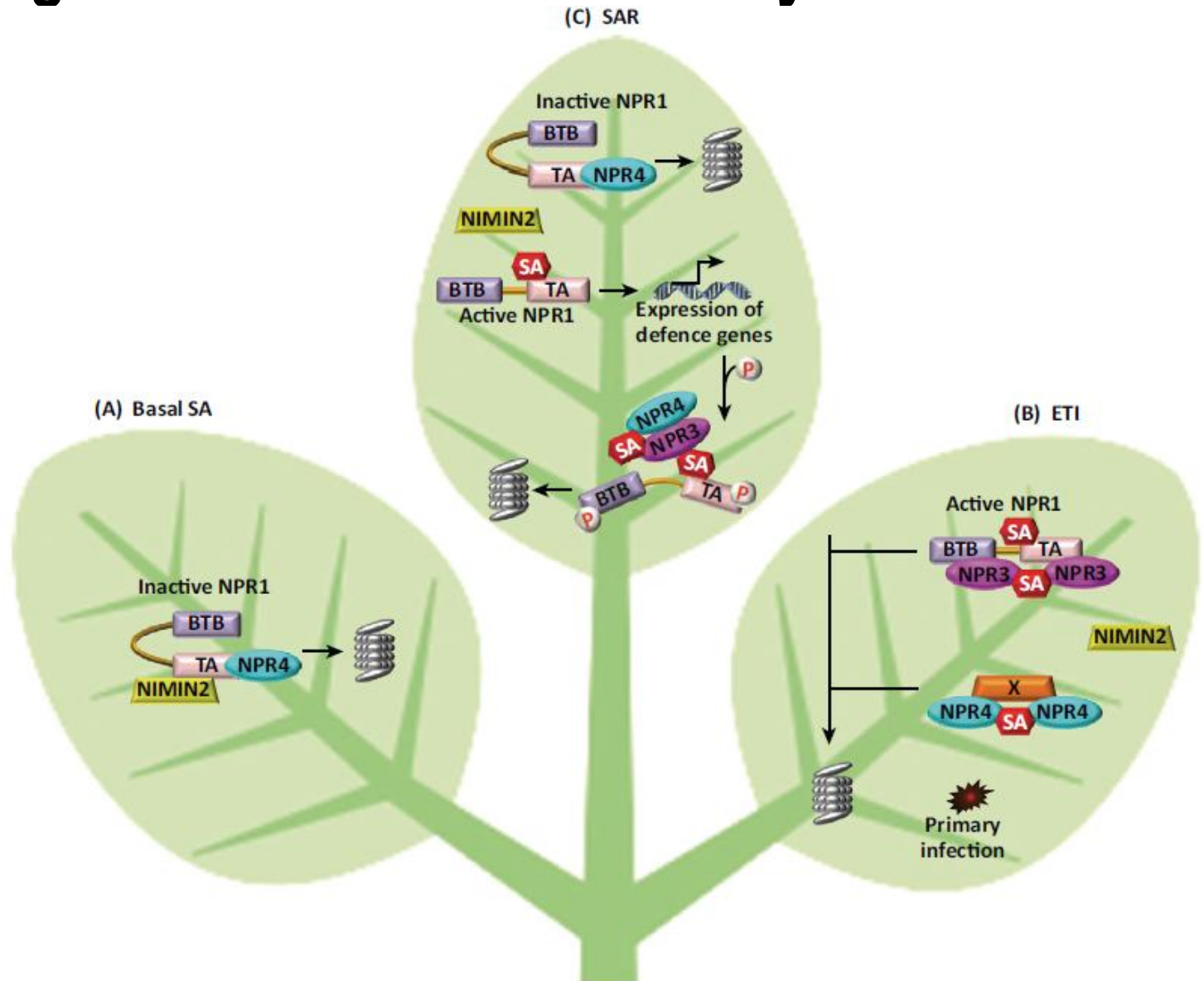
Further analyzed

Why NPR1?



Source: Mukhtar, et al. (2009)

NPR1 Regulation in Plant Defense System

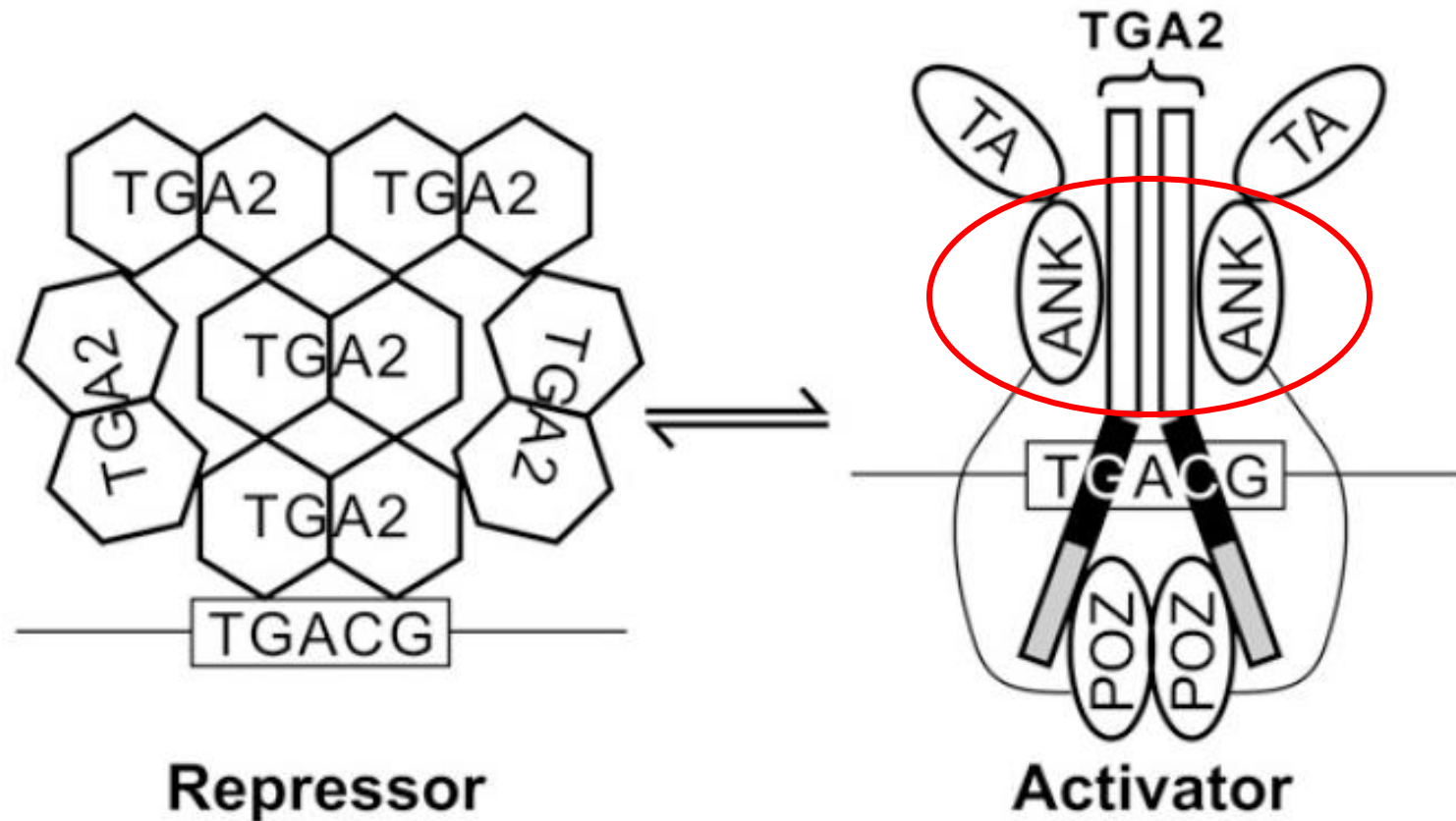


Source: Mukhtar, et al., (2013)

Protein-Protein Interaction During GV Infection

Identity	Function	Selection criteria	Reference
Group B (promote earlier infection)			
Bearskin 2 (<i>BRN2</i>)	Transcription	Phloem over-expression	[89]
Importin alpha isoform 4 (<i>IMPA-4</i>)	Transport	CP interaction	[71]
Lactoylglutathione lyase (<i>GLO1</i>)	Stress	C3 Interaction	Hericourt <i>et al.</i> (in preparation)
Replication protein A32 (<i>RPA32/RPA2</i>)	DNA metabolism	Rep interaction	[32]
Dehydration responsive 21 (<i>RD21</i>)	Stress	V2 interaction	Hericourt <i>et al.</i> (in preparation)
RING-type E3 ubiquitin ligase (<i>RHF2A</i>)	Protein modification	Transacted by TrAP/C2	[82]
Ubiquitin activating enzyme (<i>UBA1</i>)	Protein modification	TrAP/C2 Interaction	Hericourt <i>et al.</i> (in preparation)
Group C (delay, reduce or prevent the infection)			
4-coumarate:CoA ligase (<i>AT4CL1</i>)	Metabolism	Phloem over-expression	[89]
Allene oxide cyclase (<i>AOC1</i>)	Metabolism	Phloem over-expression	[82]
Barely any meristem 1 (<i>BAM1</i>)	Protein modification	C4 interaction	Hericourt <i>et al.</i> (in preparation)
Coatomer delta subunit (<i>deltaCOP</i>)	Protein transport	C3 interaction	Hericourt <i>et al.</i> (in preparation)
COP9 signalosome subunit 3 (<i>CSN3</i>)	Protein modification	Cellular process	[90]
Geminivirus Rep A-binding (<i>GRAB2</i>)	Transcription	Rep interaction	[29]
Heat shock protein cognate 70 (<i>HSC70</i>)	Protein modification	Phloem over-expression	[82]
Nuclear acetyltransferase (<i>NSI</i>)	Signal transduction	NSP Interaction	[31]
Patatin-like protein 2 (<i>PLP2</i>)	Stress	Phloem over-expression	[82]
Shaggy-related kinase kappa (<i>SK4-1/SKK</i>)	Protein modification	C4 interaction	Hericourt <i>et al.</i> (in preparation)
SKP1-like 2 (<i>ASK2</i>)	Protein modification	Transacted by TrAP/C2	[9]

Protein-Protein Interaction In Plant



MATERIALS AND METHODS



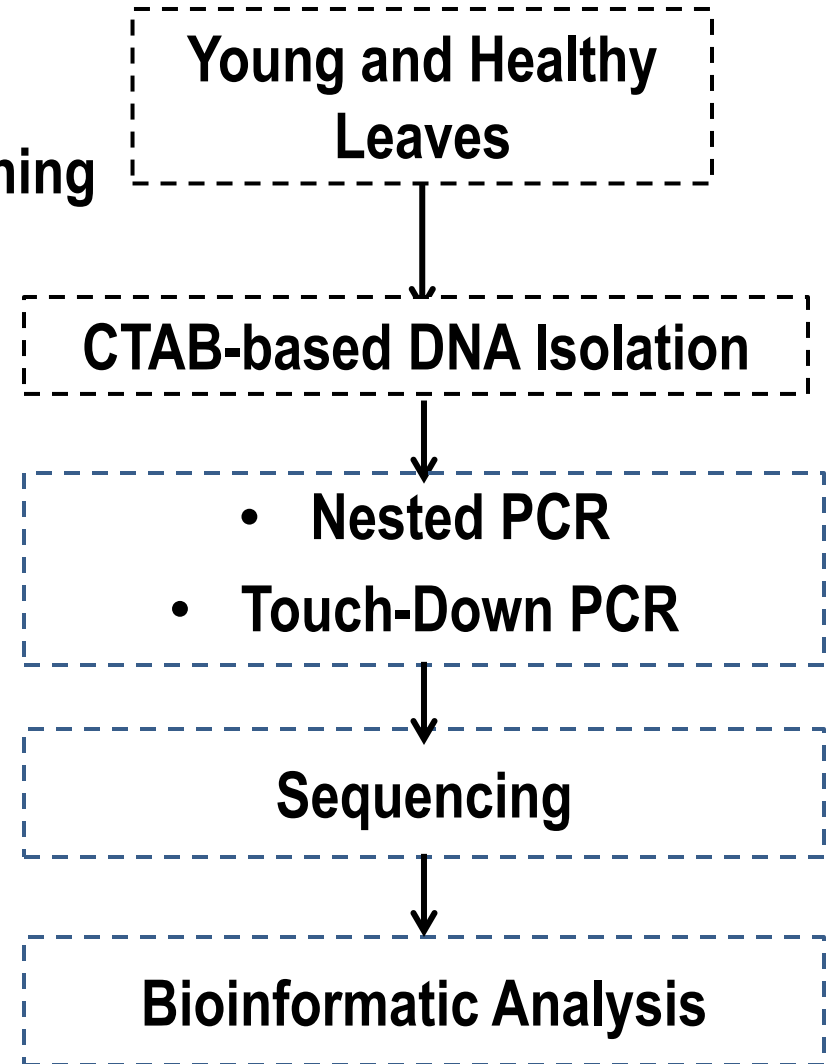
**ANDALAS
UNIVERSITY**



Materials and Methods

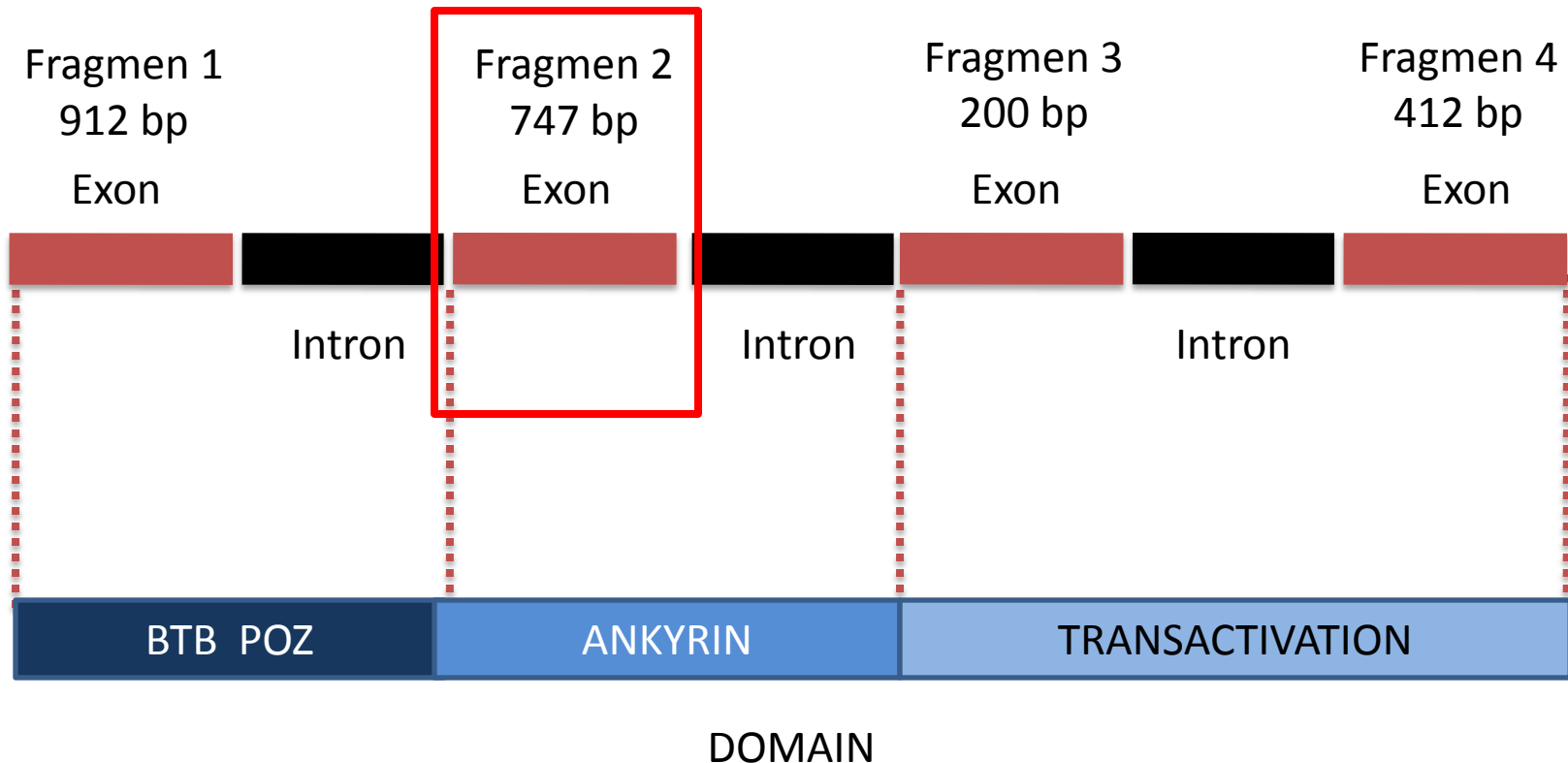


PCR –based Cloning



Domain Structure of NPR1

Capsicum annum var. Zunla



NPR1 Structure of *Capsicum annum* var. Zunla

NPR1	
Access No.	DQ648785.1
Size of NPR1 mRNA	2,191 bp
Start Location in Chromosome 7	112,610,515
End Location in Chromosome 7	112,636,367
Size of NPR1 Genome	25,852 bp
Exon Fragment 1	112,610,515 ... 112,611,427 (912 bp)
Exon Fragment 2	112,632,267 ... 112,633,014 (747 bp)
Exon Fragment 3	112,634,512 ... 112,634,712 (200 bp)
Exon Fragment 4	112,635,955 ... 112,636,367 (412 bp)

Specific Primers for NPR1 Gene

Primer ID	Sequence (5' - 3')	Length (nt)	Size of PCR product (bp)
Outer Forward	TTCCCCCTTTGCAGAGACAC	20	2626
Outer Reverse	CCTGCACCCACTTTGAGCTT	20	
Inner Forward	AACAAAGCTGCAGCAGACGA	20	687
Inner Reverse	CGCCTGCCATAGCAAGAGAT	20	

RESULTS AND DISCUSSION

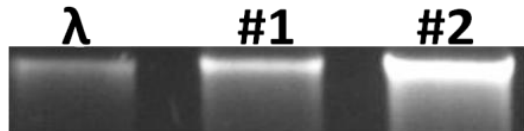


**ANDALAS
UNIVERSITY**

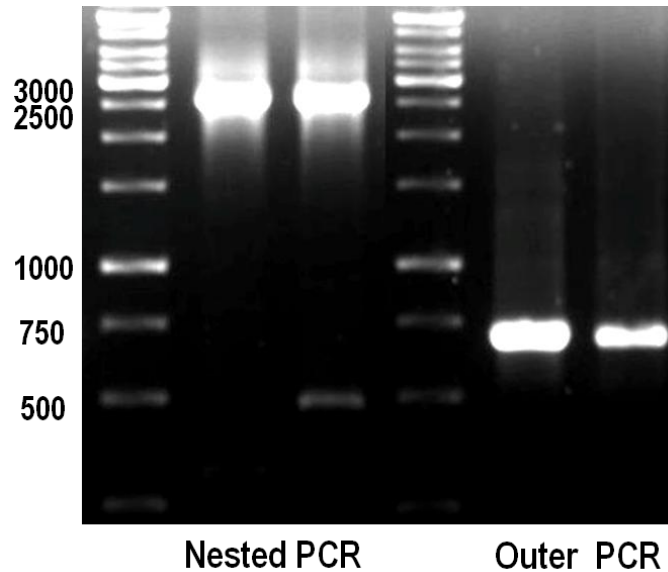


Cloning of NPR1 Fragment

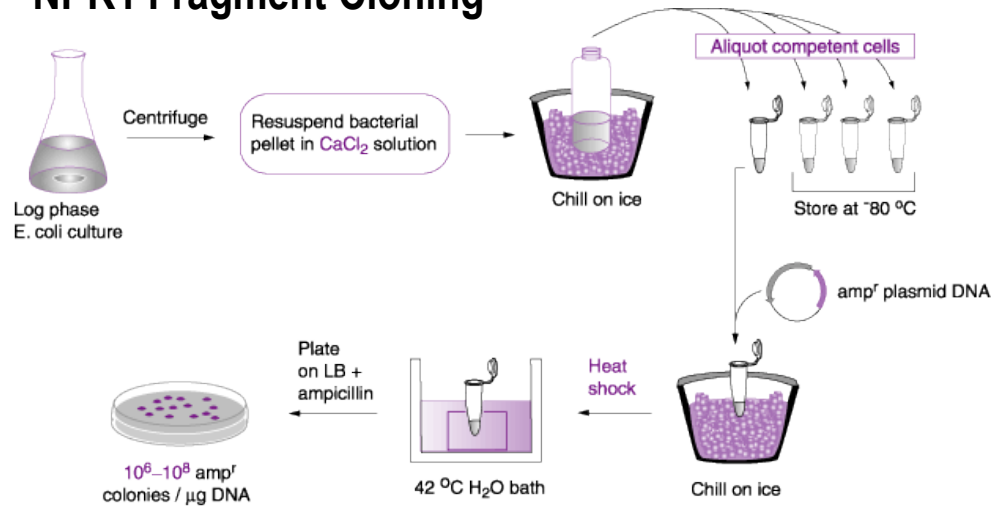
DNA Isolation



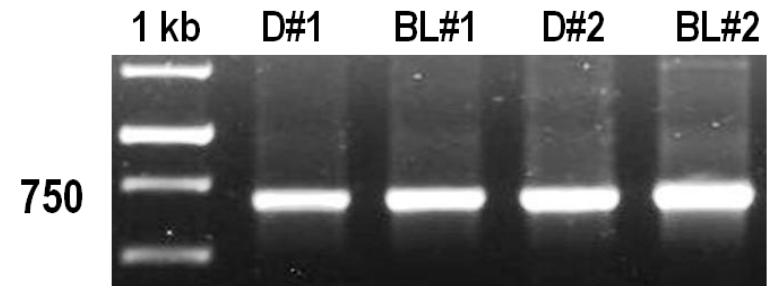
NPR1 Fragment Isolation



NPR1 Fragment Cloning



Colony PCR of NPR1



Sequence Analysis

[Download](#) [GenBank Graphics](#)

Capsicum annuum cultivar Zunla-1 chromosome 7, Pepper Zunla 1 Ref_v1.0, whole genome shotgun sequence
Sequence ID: [NC_029983.1](#) Length: 222112641 Number of Matches: 1

Range 1: 112632325 to 112632969 [GenBank Graphics](#) [Next Match](#) [Previous Match](#)

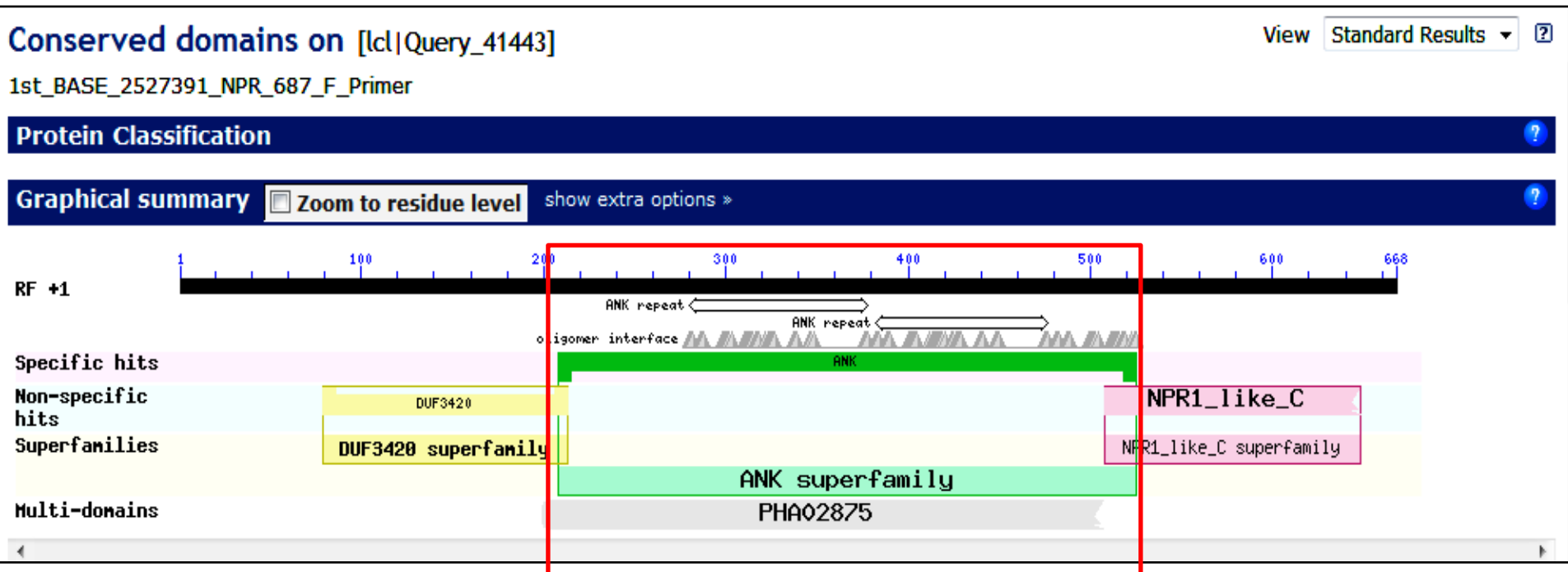
Score	Expect	Identities	Gaps	Strand
1166 bits(631)	0.0	641/645(99%)	4/645(0%)	Plus/Plus

Feature: [regulatory protein NPR1](#)

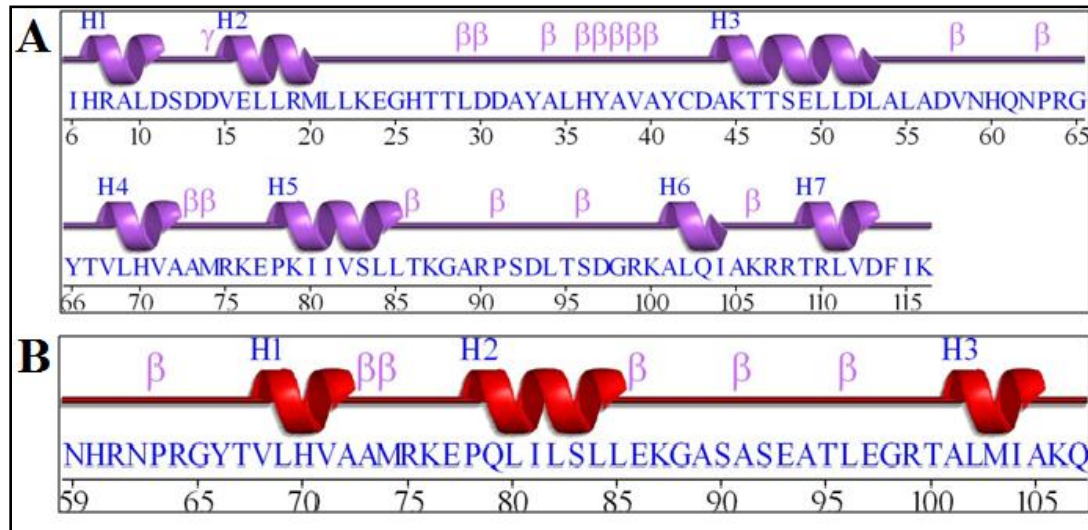
Query	11	CTGTTGC--ACATTTGTGGT-AAGCATGTGAGAGATTGCTTTCAAGCTGCATTGAGATTA	67
Sbjct	112632325	CTGTTGCAAACATTTGTGGTAAAGCATGTGAGAGATTGCTTTCAAGCTGCATTGAGATTA	112632384
Query	68	TTGTCAAGTCTAATGTTGATATTATAACCCTTGATAAGGCTTTGCCTAATGACATCGTAA	127
Sbjct	112632385	TTGTCAAGTCTAATGTTGATATTATAACCCTTGATAAGGCTTTGCCTAATGACATCGTAA	112632444
Query	128	AACAAATTACTGATTCACGCACTGAACTTGATCTACAAGGGCCTGTAAACCATGGTTTTTC	187
Sbjct	112632445	AACAAATTACTGATTCACGCACTGAACTTGATCTACAAGGGCCTGTAAACCATGGTTTTTC	112632504
Query	188	CTGATAAACATGTTAAGAGGATACATAGGGCATTAGACTCTGATGATGTTGAATTACTAA	247
Sbjct	112632505	CTGATAAACATGTTAAGAGGATACATAGGGCATTAGACTCTGATGATGTTGAATTACTAA	112632564
Query	248	GGATGTTGCTTAAAGAGGGGCATACTACTCTAGATGATGCGTATGCTCTCCACTATGCTG	307
Sbjct	112632565	GGATGTTGCTTAAAGAGGGGCATACTACTCTAGATGATGCGTATGCTCTCCACTATGCTG	112632624
Query	308	TAGCATATTGCGATGCAAAGACTACATCAGAACTTTAGATCTTGCACTTGCTGATGTTA	367
Sbjct	112632625	TAGCATATTGCGATGCAAAGACTACATCAGAACTTTAGATCTTGCACTTGCTGATGTTA	112632684
Query	368	ATCACCAAATCCTAGAGGATACACGGTGCTTCATGTTGCTGCCATGAGAAAAGAGCCTA	427
Sbjct	112632685	ATCACCAAATCCTAGAGGATACACGGTGCTTCATGTTGCTGCCATGAGAAAAGAGCCTA	112632744
Query	428	AAATTATAGTGTCCCTTTTAACCAAAGGAGCTAGACCTTCTGACCTGACATCCGATGGCA	487
Sbjct	112632745	AAATTATAGTGTCCCTTTTAACCAAAGGAGCTAGACCTTCTGACCTGACATCCGATGGCA	112632804
Query	488	GAAAAGCACTTCAAATGCAAAGAGGGCGCACTAGGCTTGTGGATTTTATTAAGTCTACAG	547
Sbjct	112632805	GAAAAGCACTTCAAATGCAAAGAGGGCGCACTAGGCTTGTGGATTTTATTAAGTCTACAG	112632864
Query	548	AGGAAGGAAAATCTGCTCCAAAGGATCGGTTATGCATTGAGATTCTAGAGCAAGCAGAAA	607
Sbjct	112632865	AGGAAGGAAAATCTGCTCCAAAGGATCGGTTATGCATTGAGATTCTAGAGCAAGCAGAAA	112632924
Query	608	GAAGAGATCCACTACTTGGAGAAGCTTCAGTATCTCTTGC-ATGG	651
Sbjct	112632925	GAAGAGATCCACTACTTGGAGAAGCTTCAGTATCTCTTGCATGG	112632969

BLAST :
99% homology with
NPR1 *Capsicum*
annuum

Domain Analysis of CbNPR1-Fragment.

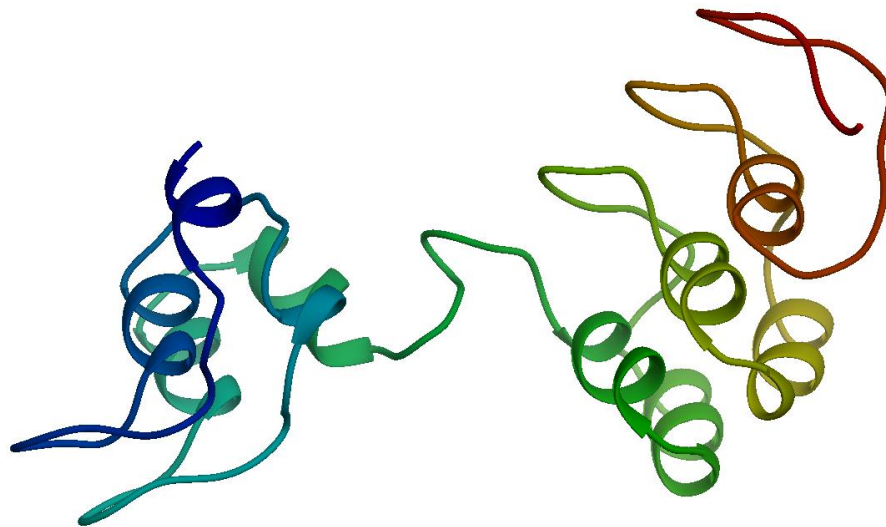


Secondary and Tertiary structure NPR1 Ankyrin Domain

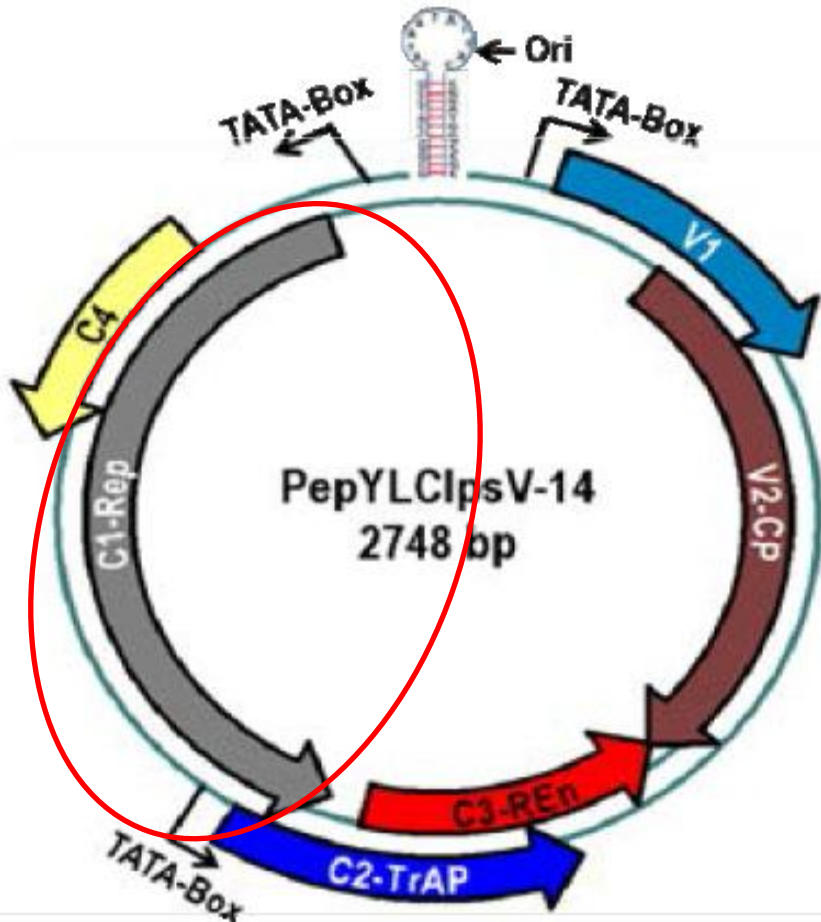


C. annuum var Lotanbar
(7 helices)

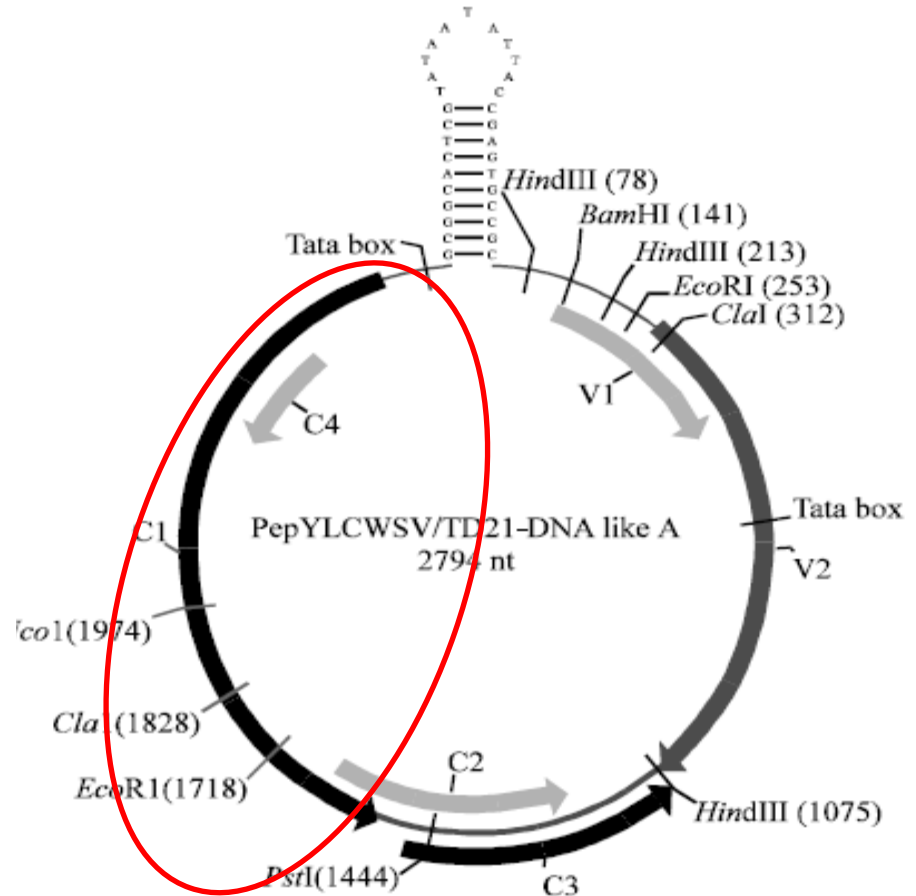
A. thaliana
(3 helices)



DNA-A like Genome of Pathogenic and Mild Isolate of GV

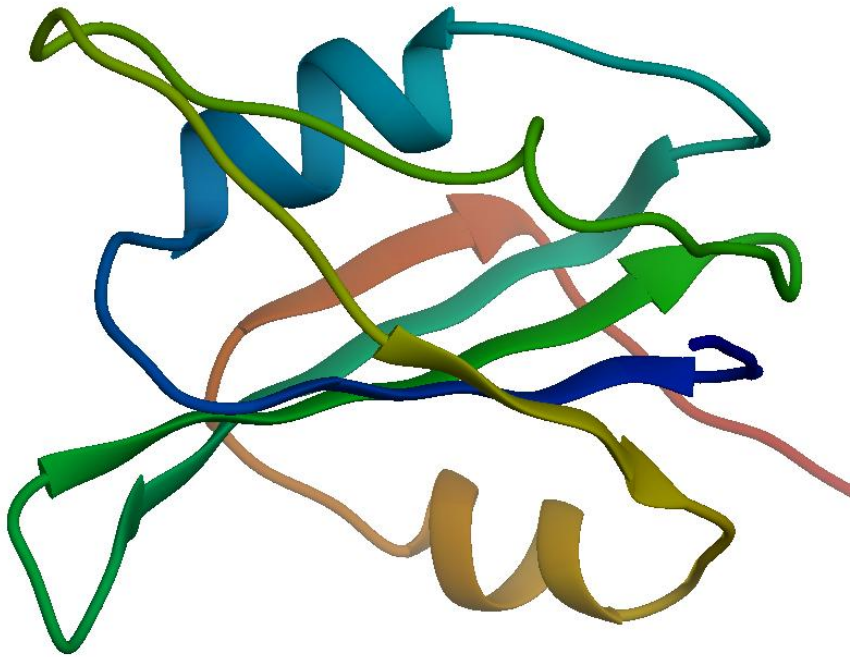


Jamsari, et al., (2016)

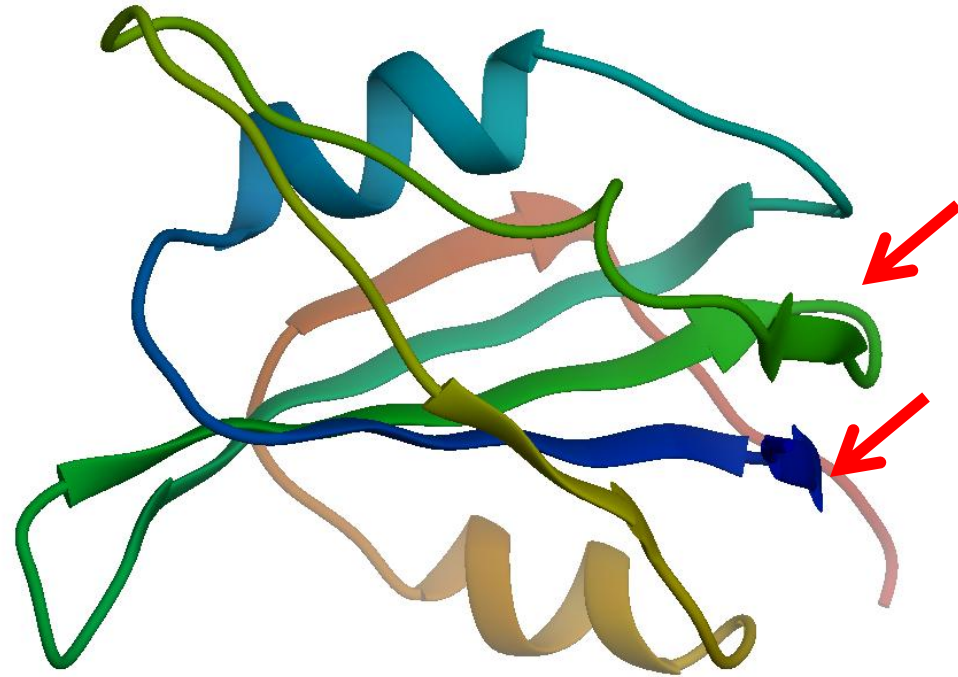


Jamsari, et al., (2013)

Protein Modelling of C1 (Rep) Protein from PSS14 –TD21



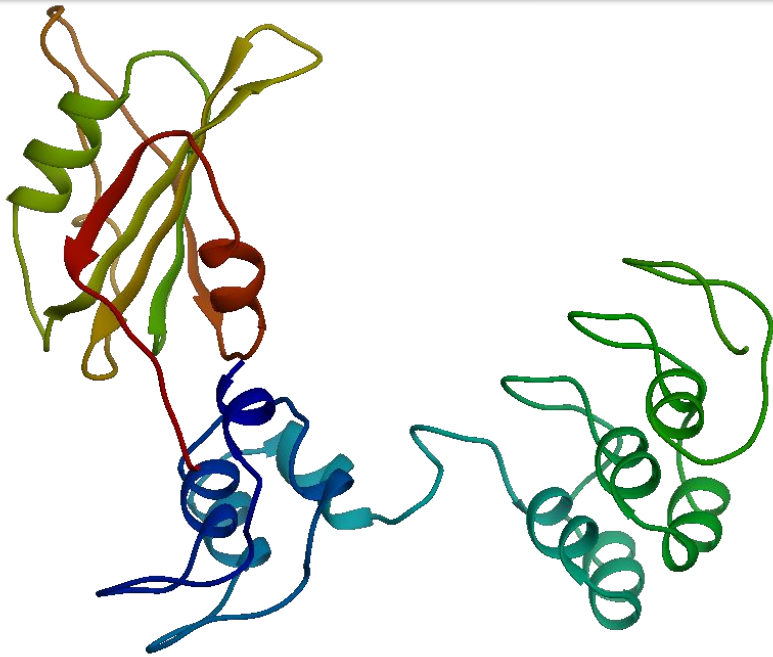
PepYLCIpsV-14
Pathogenic



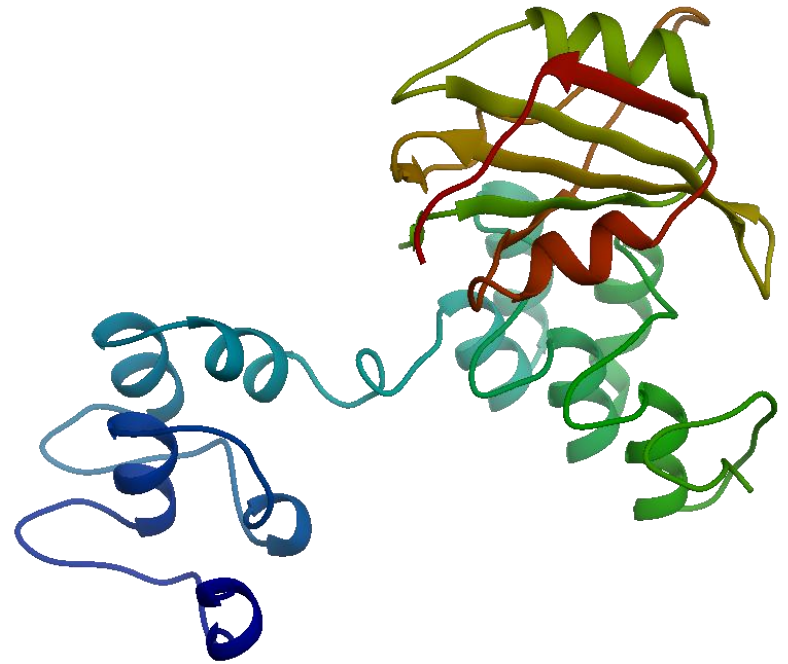
PepYLCWSV/TD21
Mild Isolate

Docking of Ankyrin with C1 PSSWS vs TDWS

PepYLCIpsV-14
Pathogenic

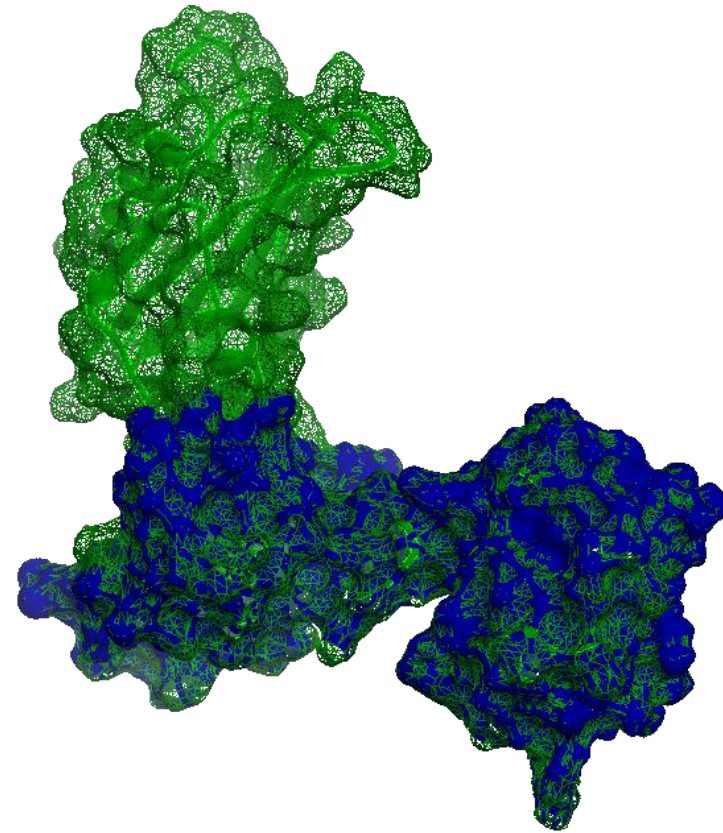


PepYLCWSV/TD21
Mild Isolate



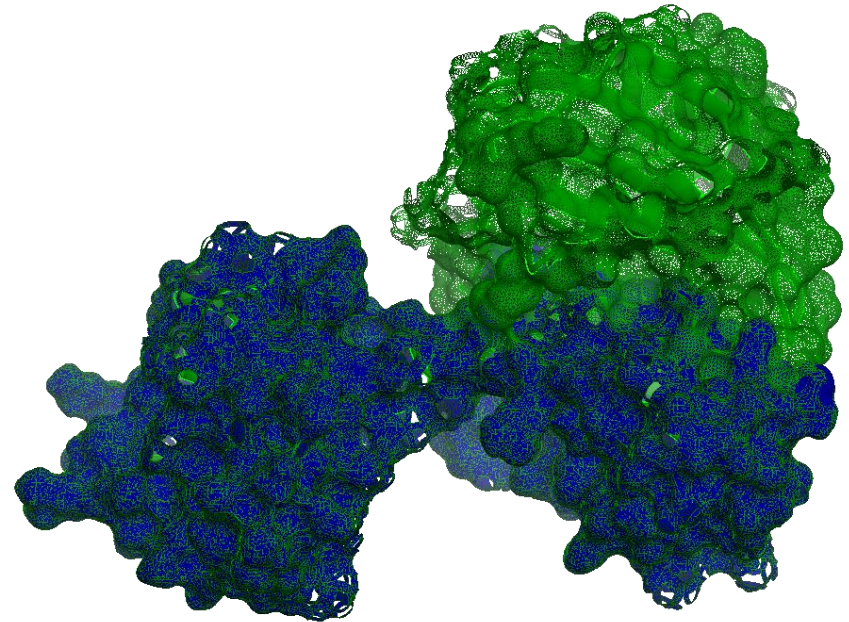
Predicted Docking Between Ankyrin with Pathogenic and Mild Replicase Protein

PepYLCIpsV-14
Pathogenic



Less Effective
< affinity

PepYLCWSV/TD21
Mild Isolate



More Effective
> affinity

CLOSING REMARKS



**ANDALAS
UNIVERSITY**



- 1. CaL-NPR1 ankyrin domain shares 78.1% similarity with AtNPR1, suggested that CaL-NPR1 gene is predicted to be homologous with Arabidopsis AtNPR1.**
- 2. The conserved residue of Cys216 and His334 are suggested that they may have similar in structural conformation and related with Pathogenesis Related Protein.**
- 3. Docking simulation with two different in pathogenic level indicated that Resistancy against GV might be related with interaction between GV-Rep protein with Ankyrin domain.**

ACKNOWLEDGEMENT

Funding:

Professorship Cluster Research Grants, the fiscal year 2016, Contract No.:.524/XIV/A/UNAND-2016 and 2017

Contributors:

Bastian Nova SSI, MSi,

Lily Syukrinai, SP., MP.

Dr. Elfi Asben

Thanks

Integrated Biological Sciences for Human Welfare

Dear Mr/Mrs Jamsari

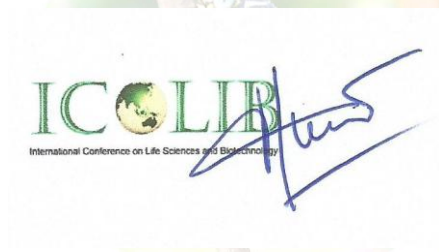
Jember, April 20th 2017

On behalf of The 2nd ICOLIB committee, it is our pleasure to inform you that your paper entitled: **“CHARACTERIZATION OF NPR1 ANKYRIN DOMAIN FROM CHILLI PEPPER (*Capsicum annuum* L.)”** has been accepted to be presented in Plenary Session of The 2nd ICOLIB 2017. You are requested to submit the full paper through “Online paper Submission” in the ICOLIB homepage by June 30 2017 and to arrange the payment (Invoice is attached). Please follow the guidelines as mentioned in our website: <http://icolib.unej.ac.id>

To those DAAD alumni, please fill the alumni DAAD form which can be downloaded at http://icolib.unej.ac.id/?page_id=466 *)

We look forward to having you participate in this upcoming seminar and present your work.

Sincerely,



Purwatiningsih, Ph.D

Chairwoman of The 2nd International Conference of Life Science and Biotechnology

*) Please disregard the invoice if you have already filled the form