Seminar on Research Findings Assisted by
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Isolation and characterization of *Arabidopsis thaliana* genes to produce bacterium resistance plants

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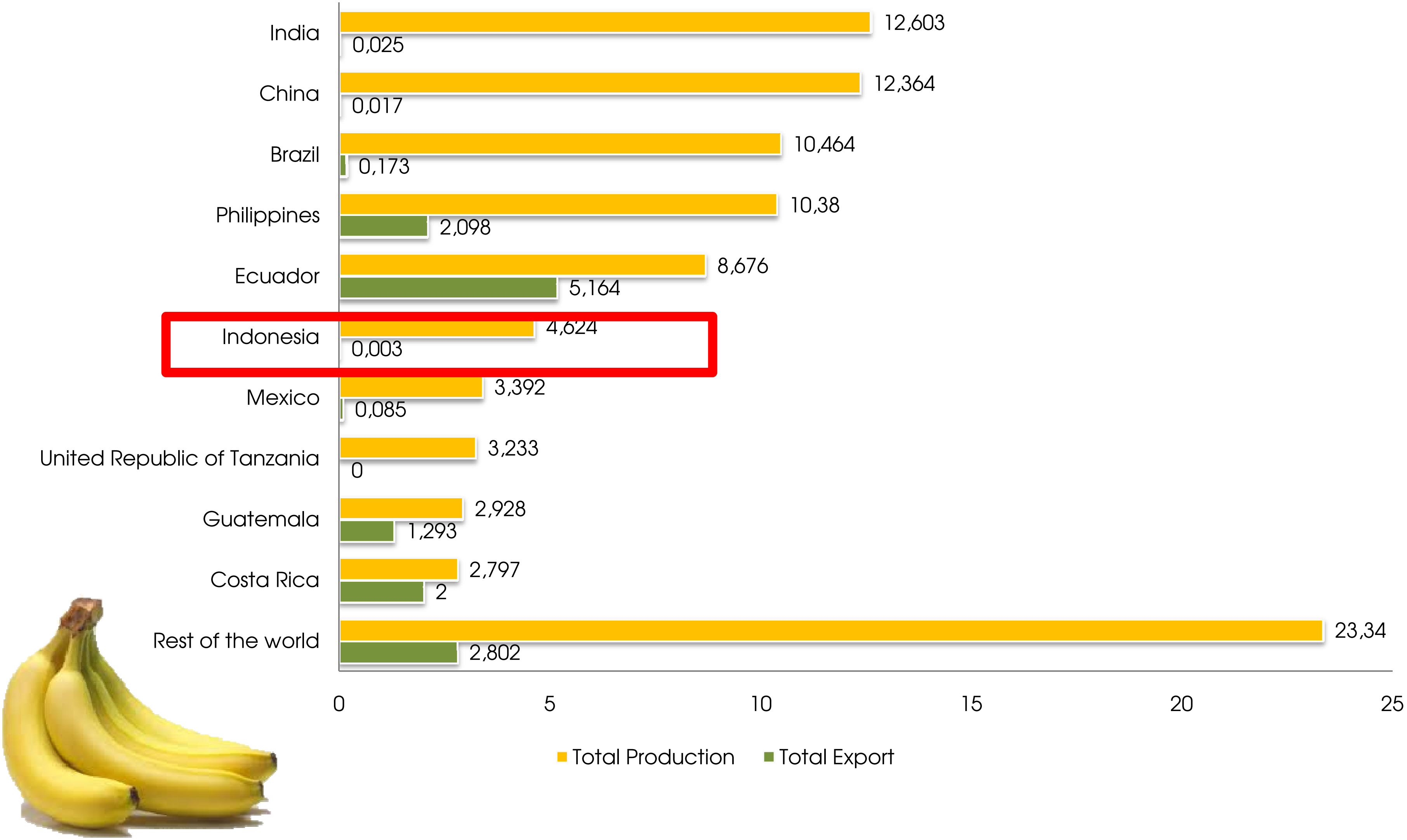
Background

Banana Production and Export Graphic in 2005-2010 (FAOSTAT, 2011)

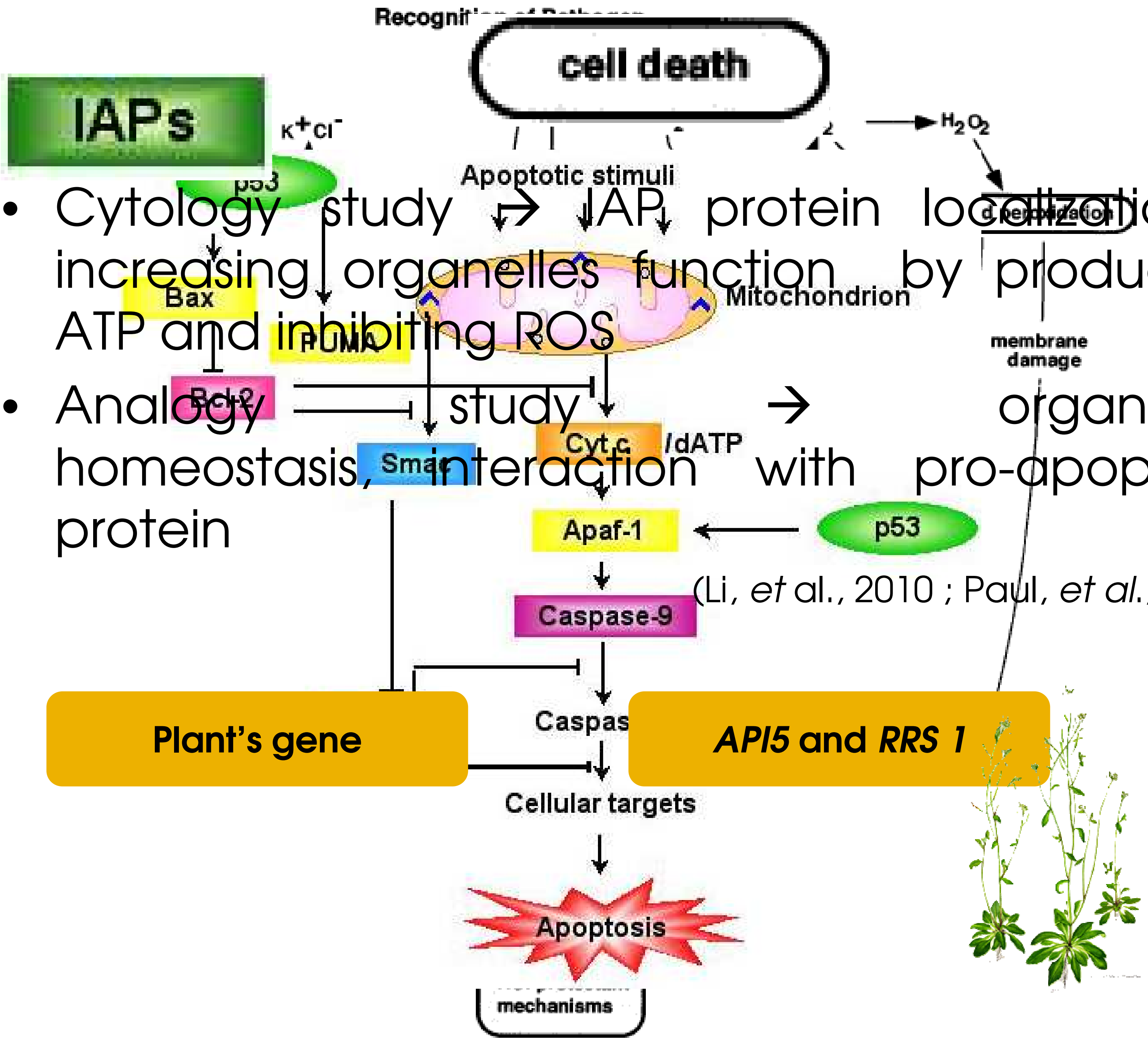


Banana Diseases

Figure 1. Banana plant with Panama disease (Vezina, 2012)

- Banana diseases as post harvest problems.
- One of major diseases caused by pathogenic fungi *Fusarium oxysporum cubense* (FOC) race 4.
- Causing banana wilt disease or Panama disease, a major loss for banana production in Indonesia.

(Dimyati *et al.*, 2001)



- Cytology study → IAP protein localization→ increasing organelles function by producing ATP and inhibiting ROS
- Analogy study → organelles homeostasis, interaction with pro-apoptosis protein

(Li, *et* al., 2010 ; Paul, *et al.*, 2009)

Plant’s gene

API5* and *RRS 1

Objective and Hypothesis

Objective

- Isolate and characterize resistance genes from *A. thaliana*^{WT} through PCR method and in silico study

Hypothesis

- Resistance gene can be isolated from *A.thaliana* genome and cDNA through PCR methods and can be characterized using in silico study

Research Method

Characterization of Genes Sequences from GenBank and primer design

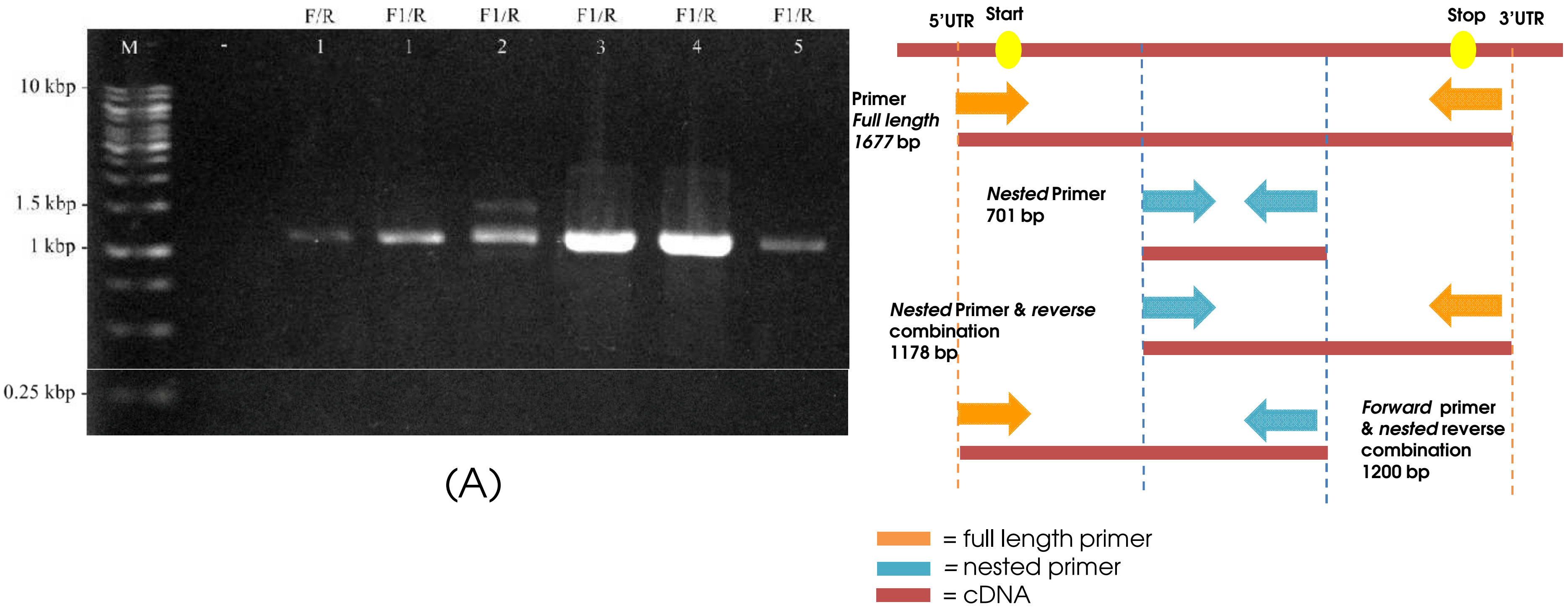
A. thaliana^{WT} total RNA isolation and cDNA synthesis

Gene fragment amplification using touchdown PCR based method

Cloning into pGEM-T Easy vector and transfer into *E. coli*

Sequencing of positive plasmid and sequence analysis

Gene Amplification



(A)

(B)

Figure 2. Electropherogram of *API5* gene amplification using touchdown PCR method at 55-60° C (A) and Illustration of primer design for *API5* amplification (B). M = ladder, F/R = sample with primer forward (F)/ reverse (R), F1/R = sample with nested forward primer and reverse primer

Sequence Analysis

- BLAST Nucleotide analysis (BLASTn)
- BLAST Protein analysis (BLASTx)
- Motif analysis
- Phylogenetic Tree

BLAST Nucleotide (BLASTn) Analysis

Table 1. Alignment score result of BLASTn analysis from putative *API5* nucleotide sequence

Accesion Number	Species	Total Score	Query Coverage (%)	E-Value	Homology (%)
NM_128955.4	*Arabidopsis thaliana* Apoptosis Inhibitory protein (API5) (AT2G34040) mRNA	2050	88	0	98
AY_085219.1	*Arabidopsis thaliana* clone 13928 mRNA, complete sequence	2050	88	0	98
XM_002889499	*Arabidopsis lyrata* subsp. *Lyrata* hypotethical protein, mRNA	[illegible]	[illegible]	[illegible]	85
CP002685.1	*Arabidopsis thaliana* choromosom 2, complete sequence	[illegible]	[illegible]	[illegible]	100

Putative *API5* gene considered homologous with other *API5* gene in GenBank database

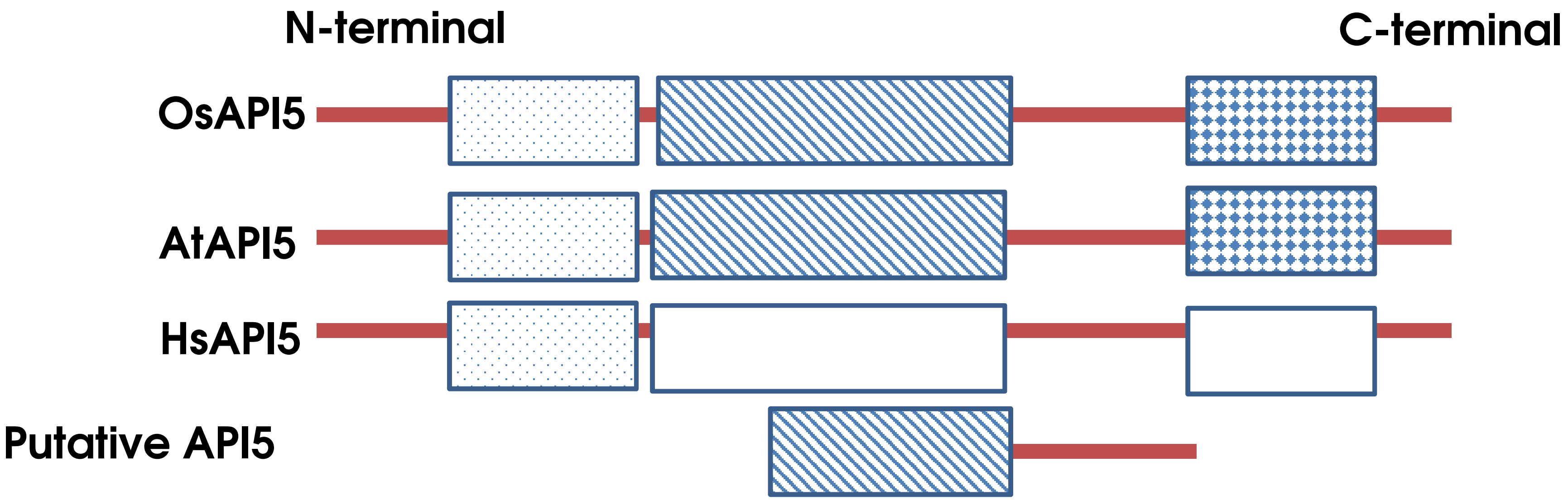
BLAST Protein (BLASTx) Analysis

Table 2. Alignment score result of BLASTx analysis from putative *API5* nucleotide sequence

Accession Number	Species	Query Coverage	E-value	Homology
NP_565777.1	Apoptosis inhibitory protein 5 (API5) (*Arabidopsis thaliana*)	74%	0	96%
XP_002893545.1	Hypothetical protein ARALYDRAFT_473107 (*Arabidopsis lyrata* subsp. lyrata)	66%	1e-174	82%
XP_002331951.1	Predicted protein (*Populus trichocar*			
EAY85554.1	hypothetical protein OsI_06927 (*Oryz* *sativa* Indica Group)			
AAB86528.1	Aac11 (Homo sapiens)	67%	9e-24	29%

Putative *API5* gene considered homologous with other *API5* protein in GenBank database

Motif Analysis of API5



Explanation :

▬ = amino acid sequence

⬚ = LXXLL motif

▨ = putative transactivation domain

▦ = nuclear localization sequence

Figure 3. Illustration of motif alignment between putative API5 and another API5 protein from different species.

Phylogenetic Tree

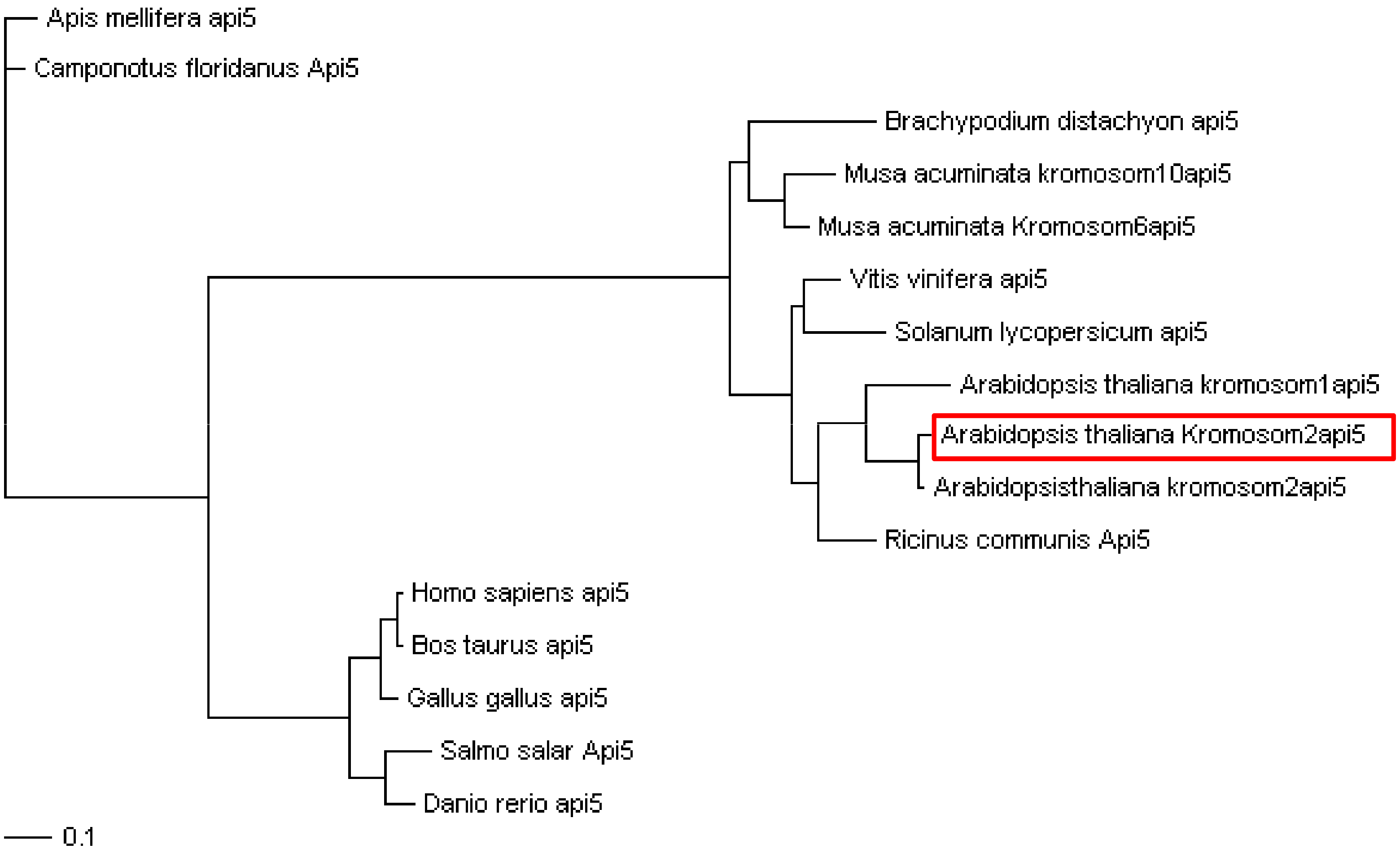


Figure 4. Phylogenetic tree of *API5* gene generated by Bayesian Method

Conclusion and Suggestion

Conclusion

- Partial *API5* have been successfully isolated from cDNA of *A. thaliana*^{WT} with combination of primers using touchdown PCR method. Putative *API5* gene have trans activation domain which is one of characteristic of anti-apoptosis gene.

Suggestion

- Further gene isolation and characterization need to be done to get a whole resistance gene and its protein function.

References

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THANK YOU