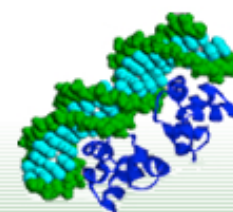




Evolutionary and structural aspects of Solanaceae RNases T2

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INTRODUCTION

◆ Solanaceae Botanical family:

- economically important family of flowering plants
- includes several important agricultural crops, medicinal plants, spices, ornamentals
- several members contain potent alkaloids - some highly toxic
- *Solanum* - the most economically important genus of the family



Petunia



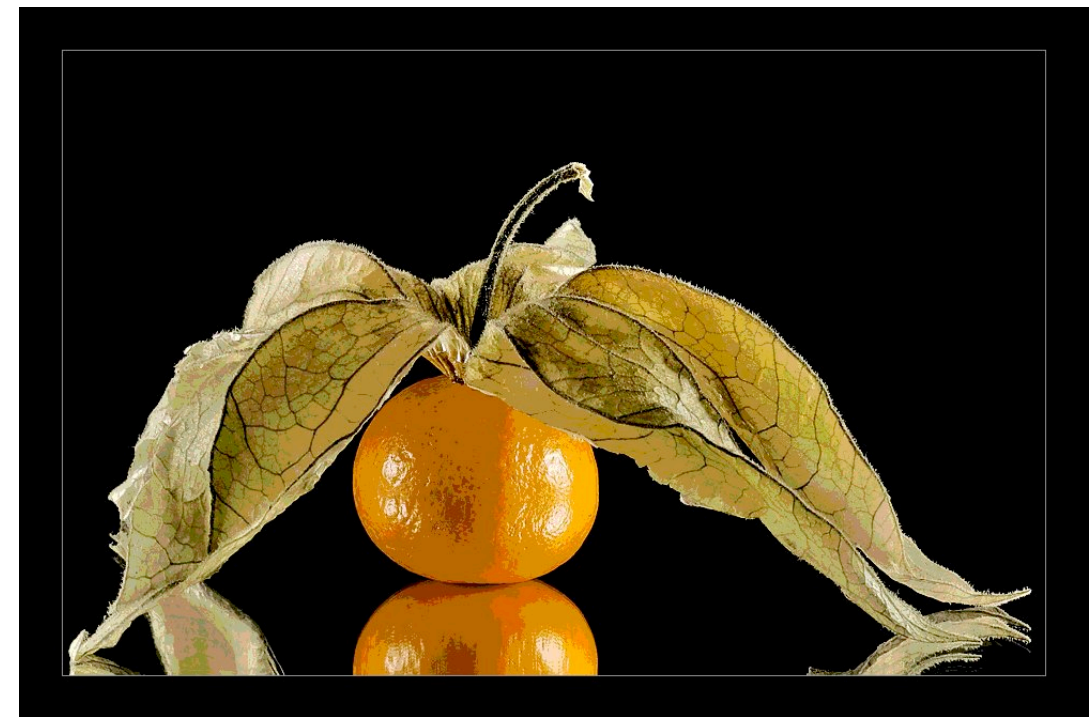
Capsicum



Nicotiana tabacum



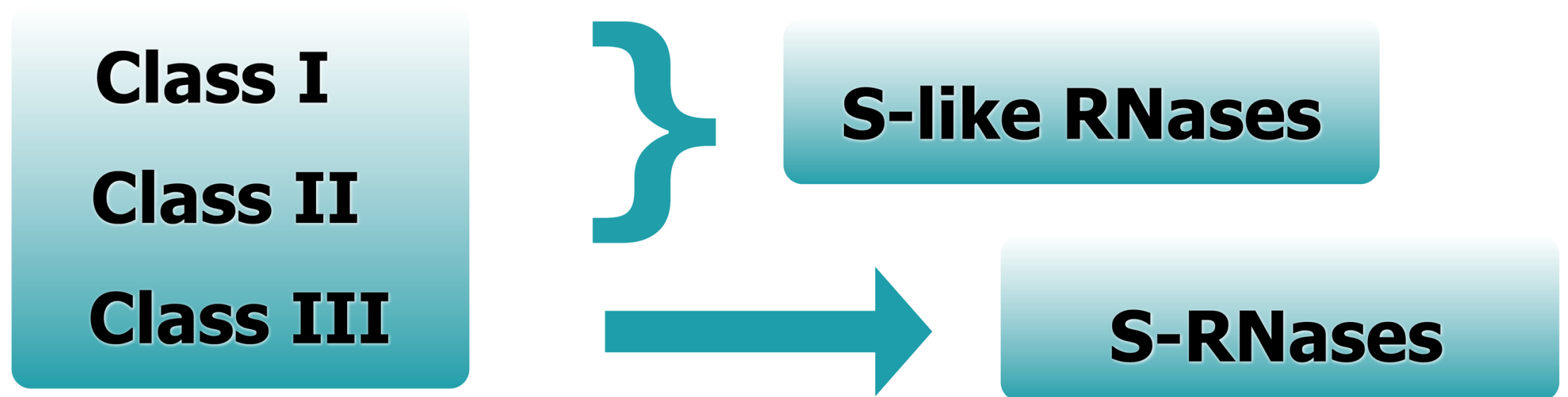
Tomato (Lycopersicum esculentum)
Potato (S. tuberosum)



Physalis

INTRODUCTION

- ◆ The plant RNases T2 are classified in three main classes:



DIVERSE FUNCTIONS

protection against
pathogens

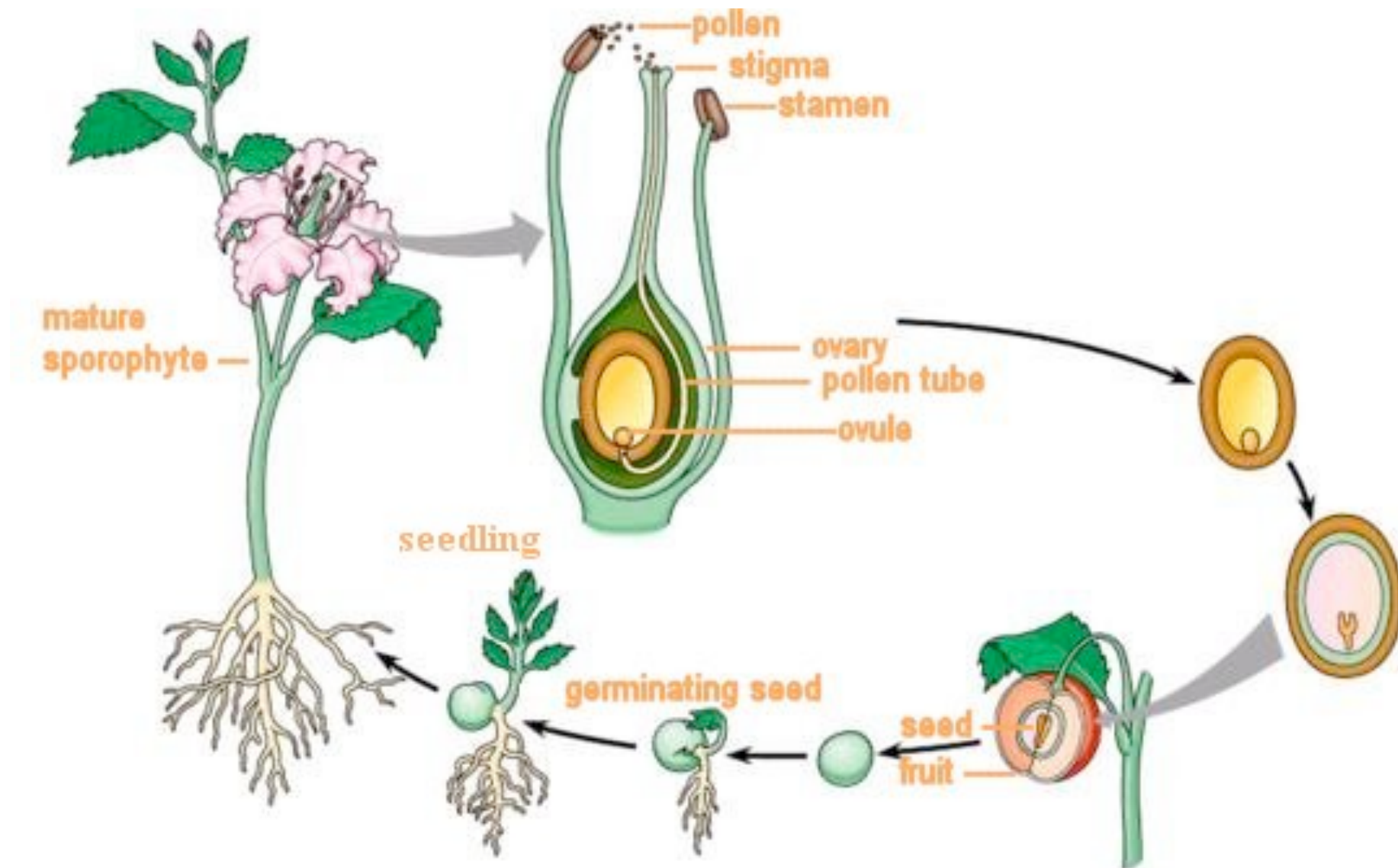
extracellular digestion of
polyribonucleotides

remobilization of Pi from RNA
under phosphate deficiency

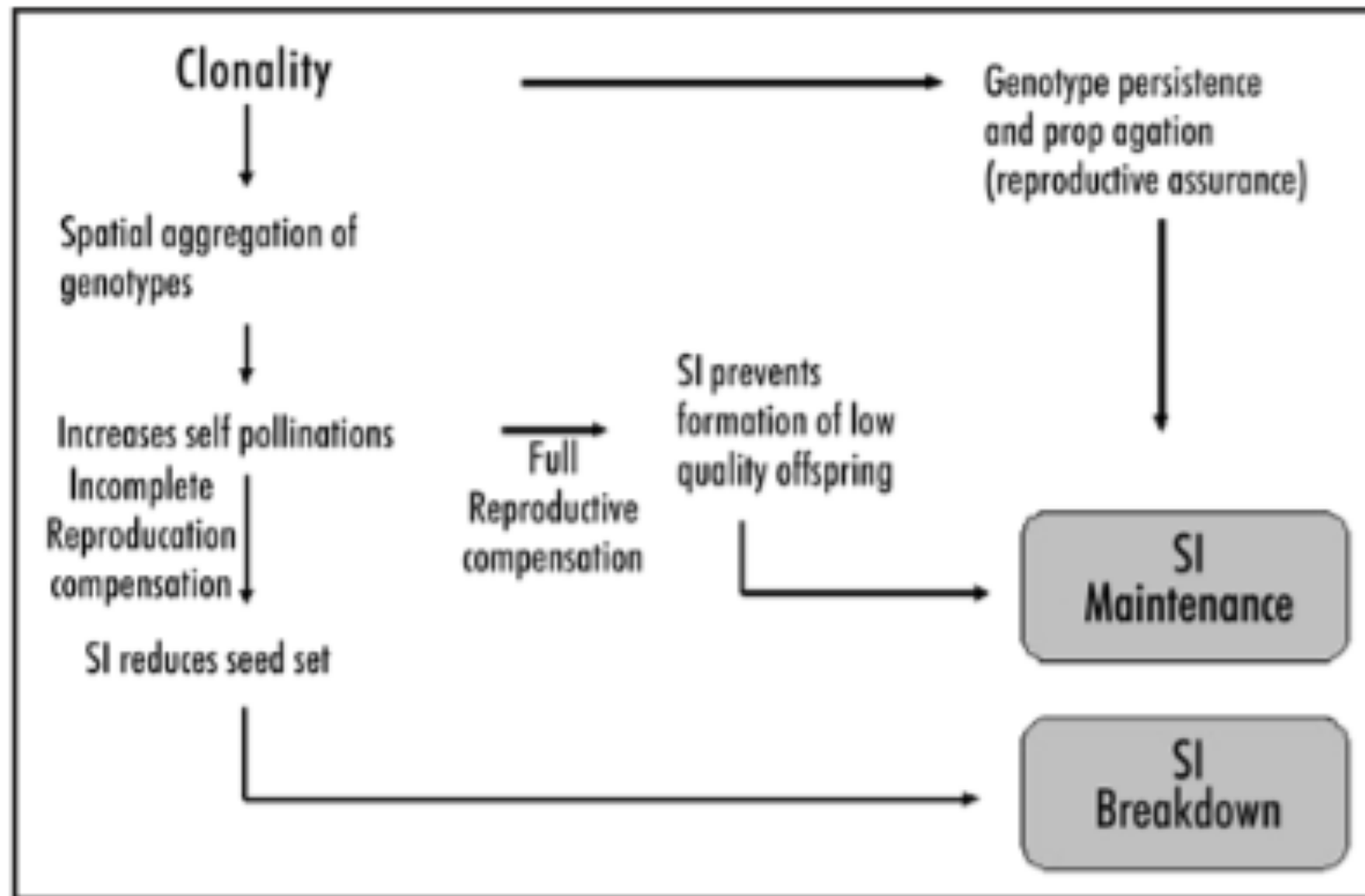
modulation of immune
responses

prevention of self-fertilization

PLANT REPRODUCTIVE SYSTEM



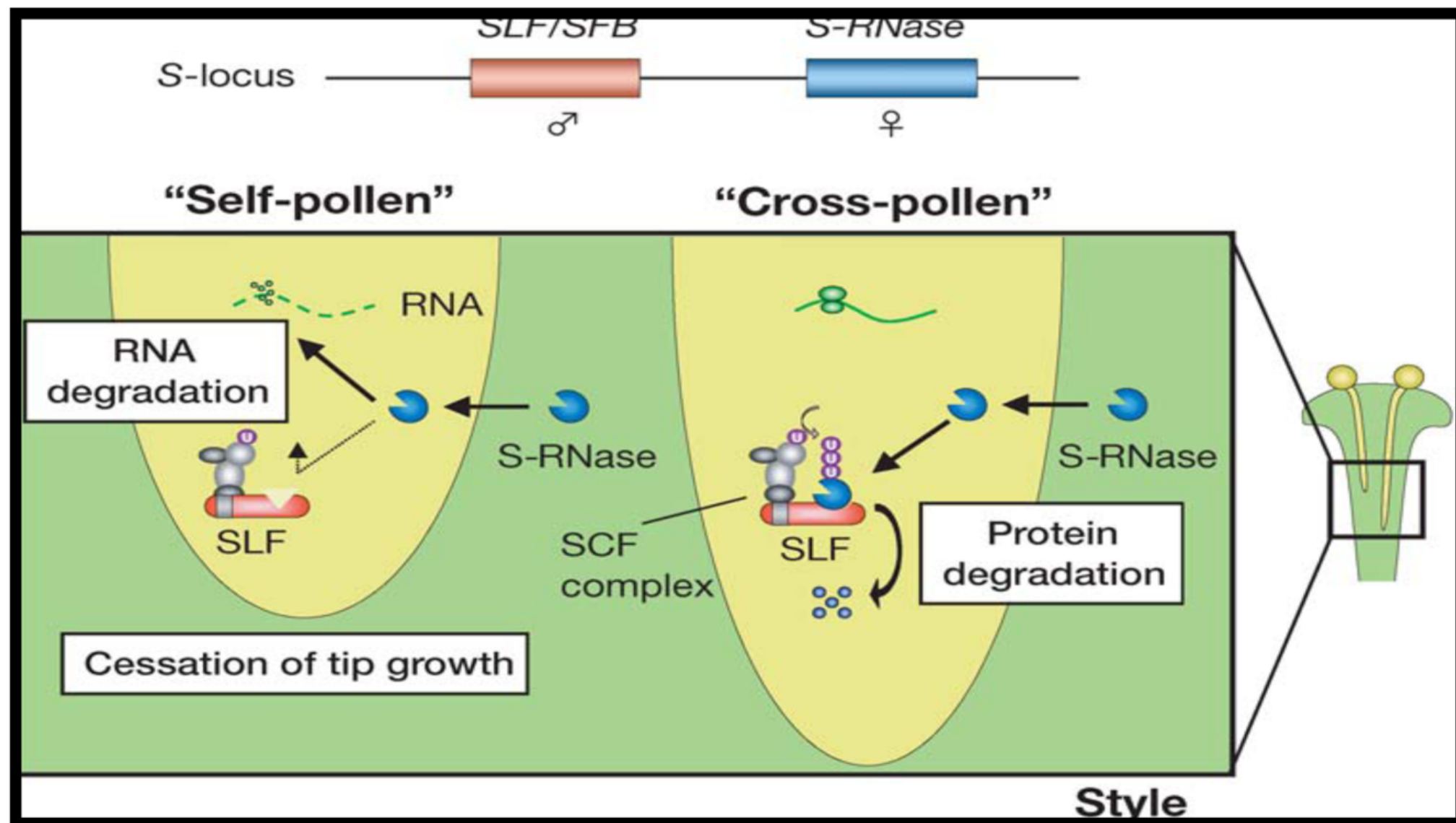
CLONALITY x SELF-INCOMPATIBILITY SYSTEM

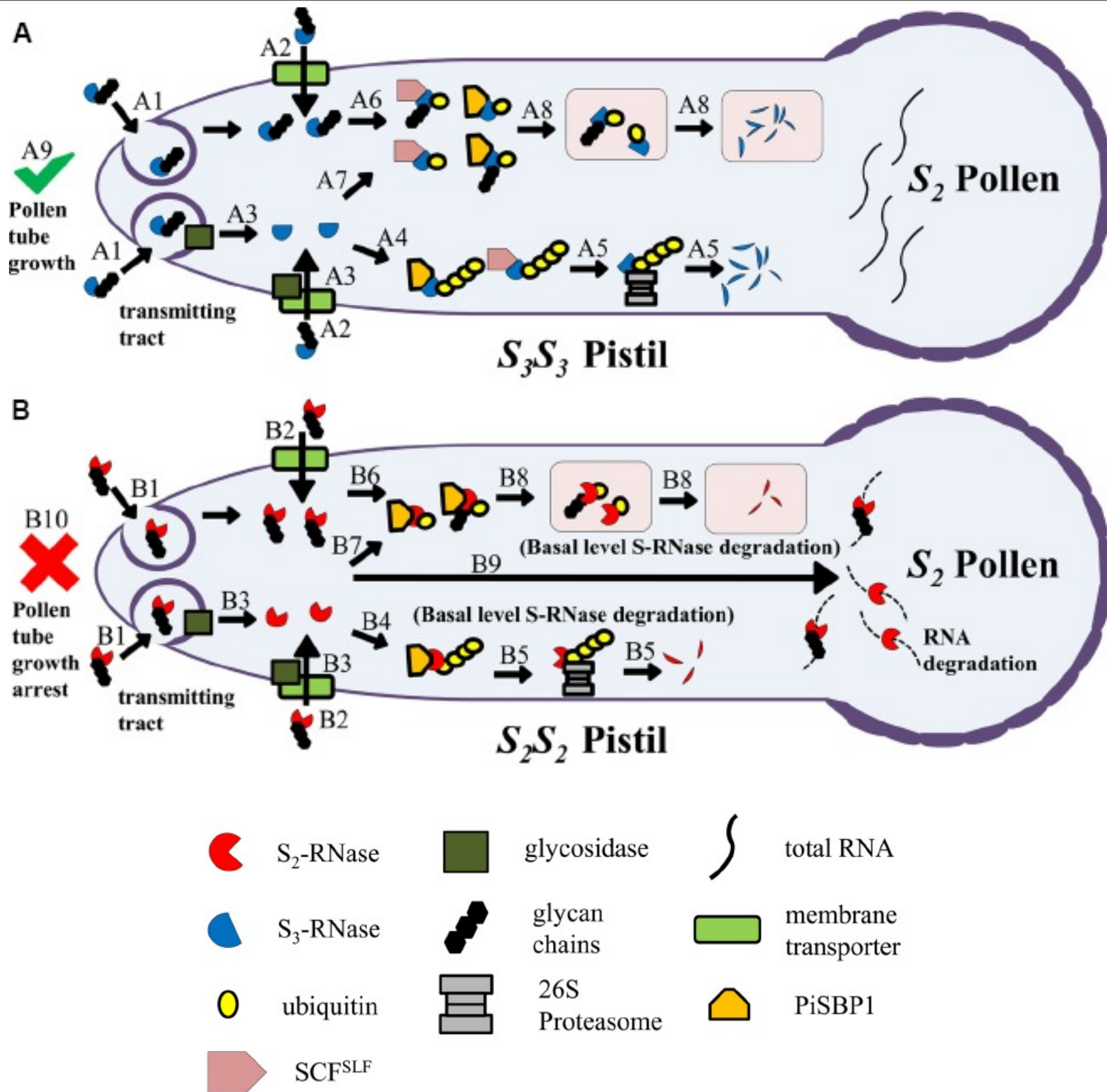


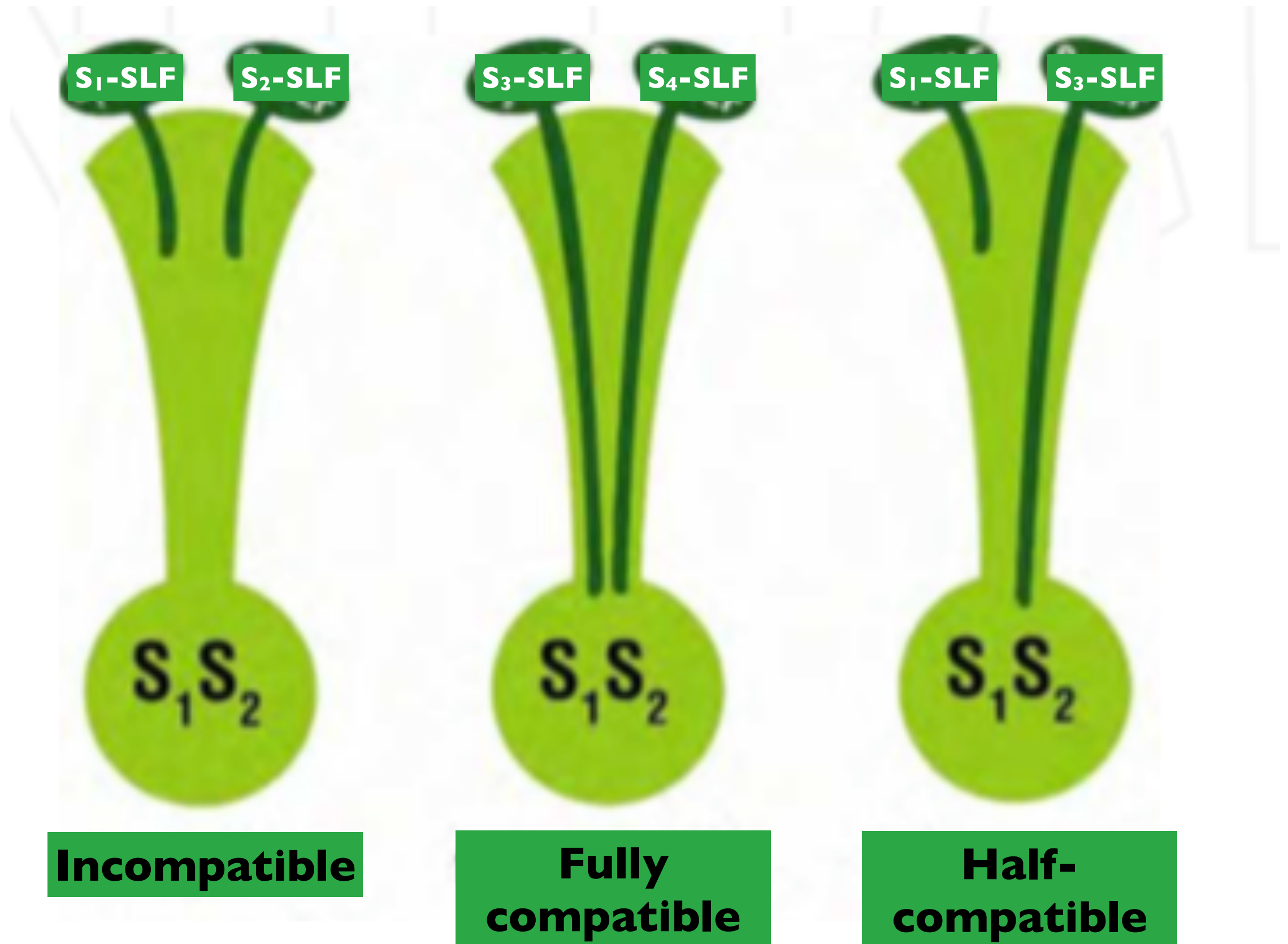
- **Clonality - decrease genetic variability**
- **However, it can be advantageous in some habitats**

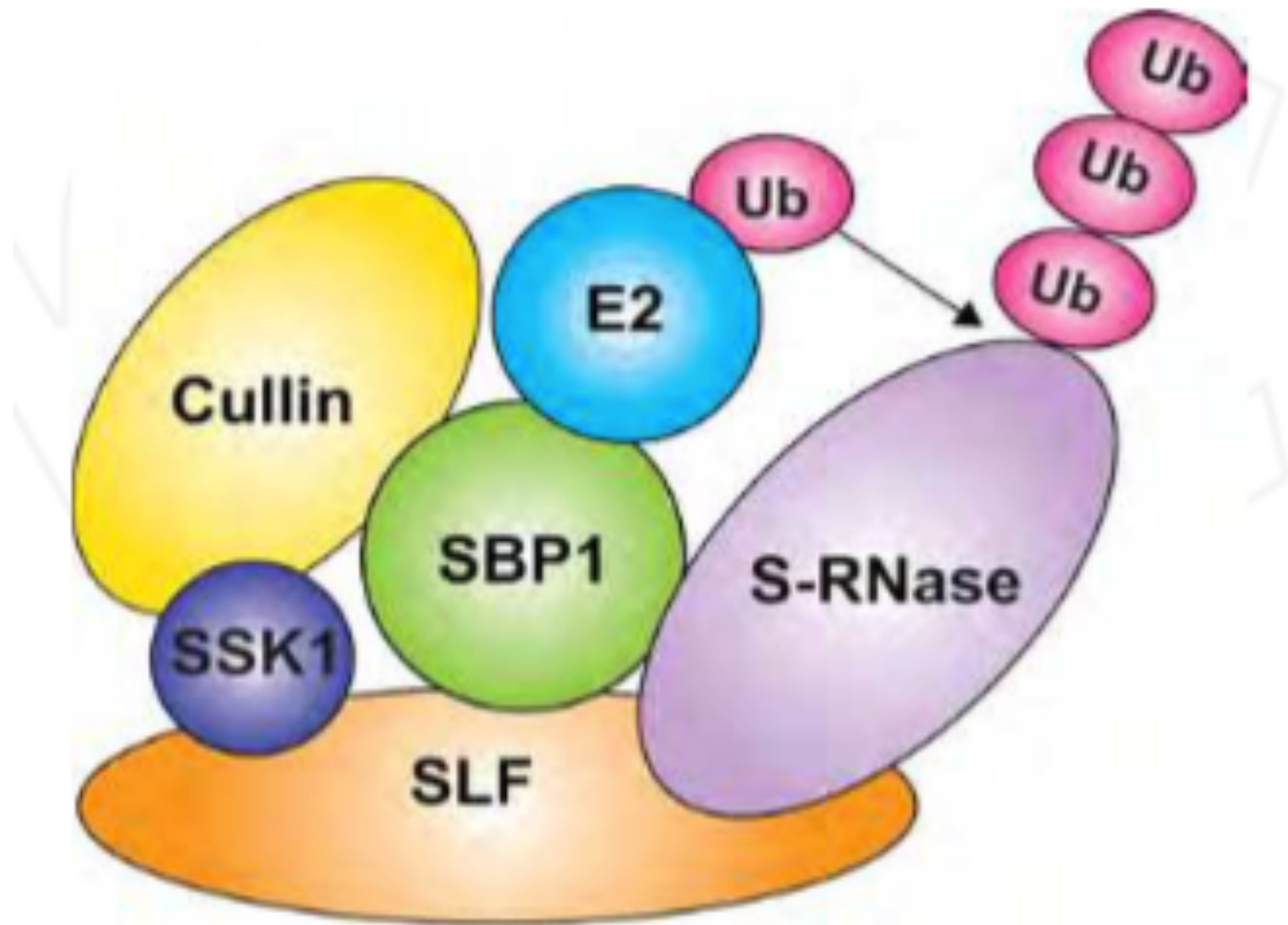
Plant Signaling & Behavior 2:4, 265-266;
July/August 2007.

SELF-INCOMPATIBILITY SYSTEM





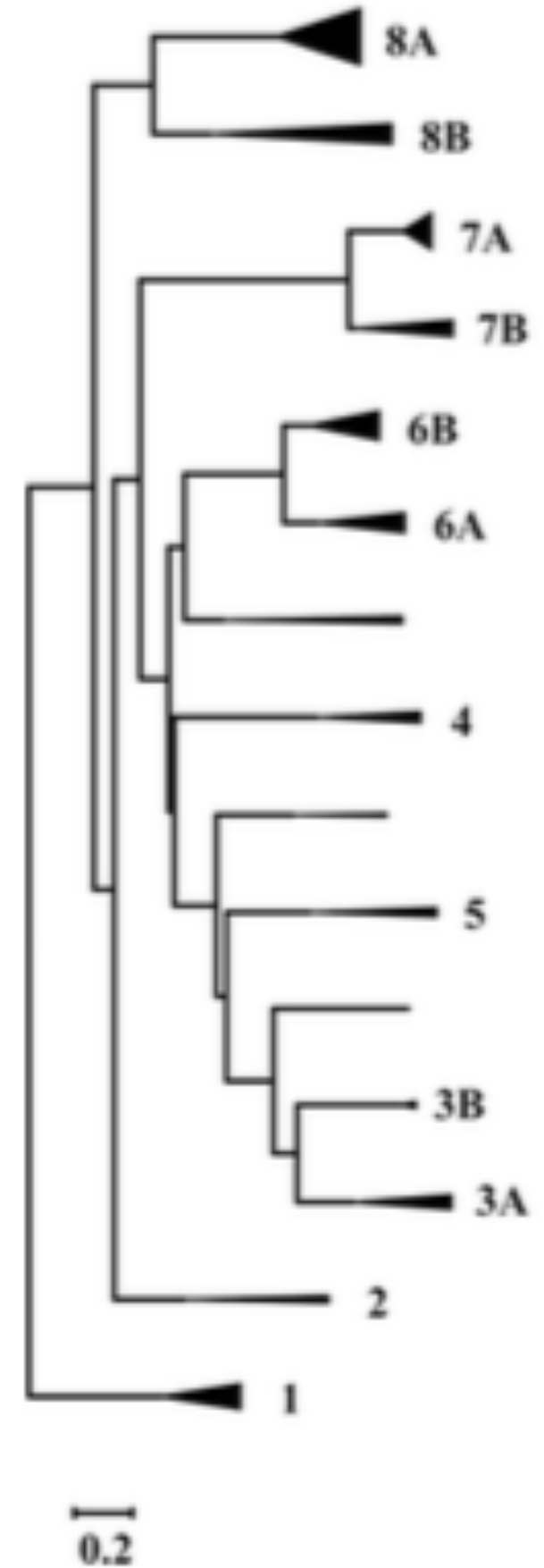




Solanum S- RNases

1. **eight** distinct monophyletic groups
2. the most complete phylogenetic analysis of *Solanum* S-RNases up to date
3. self-incompatible species must maintain S-RNase variability to preserve the SI function
4. HV regions are more conserved in sequences of the same cluster

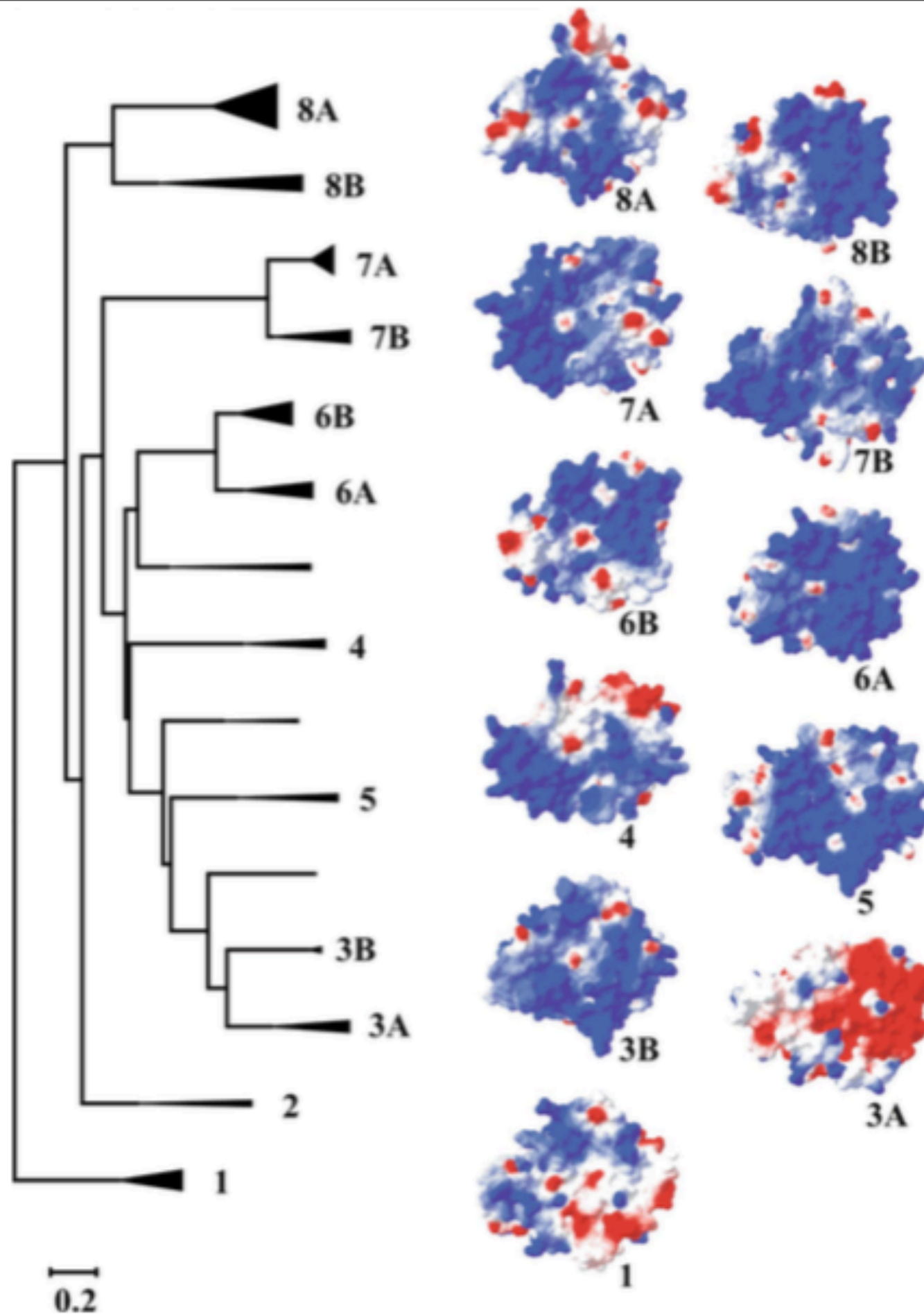
Brisolara et al. (2015) Mol. Genet Genomics



Positive Selection

Nested model pairs	l	$2\Delta l$ (df , p value)	d_N/d_S^b	Parameter estimates ^c	PSS ^d BEB/NEB
M0: one-ratio (1) ^a	-32538.66		0.55	$\omega = 0.5495$	None
M3: discrete (5)	-30415.84	4,245.64 (4, <0.0001)	0.14	$p_0 = 0.8538, p_1 = 0.1069,$ $(p_2 = 0.0393) \omega_0 = 0.0307,$ $\omega_1 = 0.4973, \omega_2 = 1.5723$	NO/60 (50)
M1a: nearly neutral (2)	-30602.19		0.12	$p_0 = 0.9288, (p_1 = 0.0712)$ $\omega_0 = 0.0586, (\omega_1 = 1)$	Not allowed
M2a: positive selection (4)	-30445.56	313.26 (2, <0.0001)	0.19	$p_0 = 0.9032, p_1 = 0.0799,$ $(p_2 = 0.0168) \omega_0 = 0.0796,$ $\omega_1 = 1, \omega_2 = 2.2554$	28 (19)/21 (17)
M7: β (2)	-30497.29		0.12	$p = 0.0301, q = 0.2100$	Not allowed
M8: β and $\omega > 1$ (4)	-30359.62	275.34 (2, <0.0001)	0.14	$p_0 = 0.9831, (p_1 = 0.0169)$ $p = 0.0709, q = 0.5463,$ $\omega = 1.9599$	23 (17)/25 (19)

Several amino acid residues submitted to positive selection

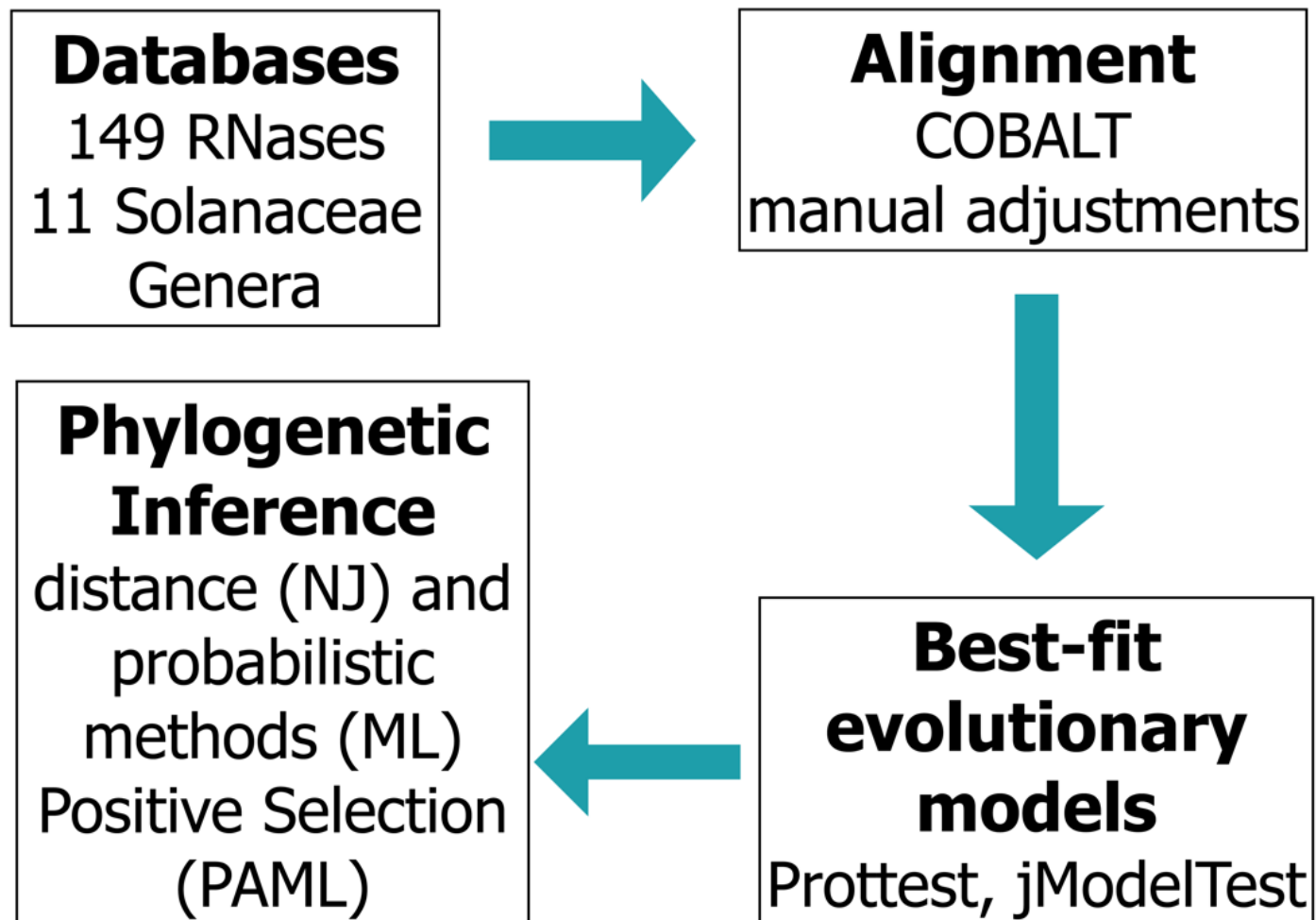


◆ main changes related to regions submitted to positive selection

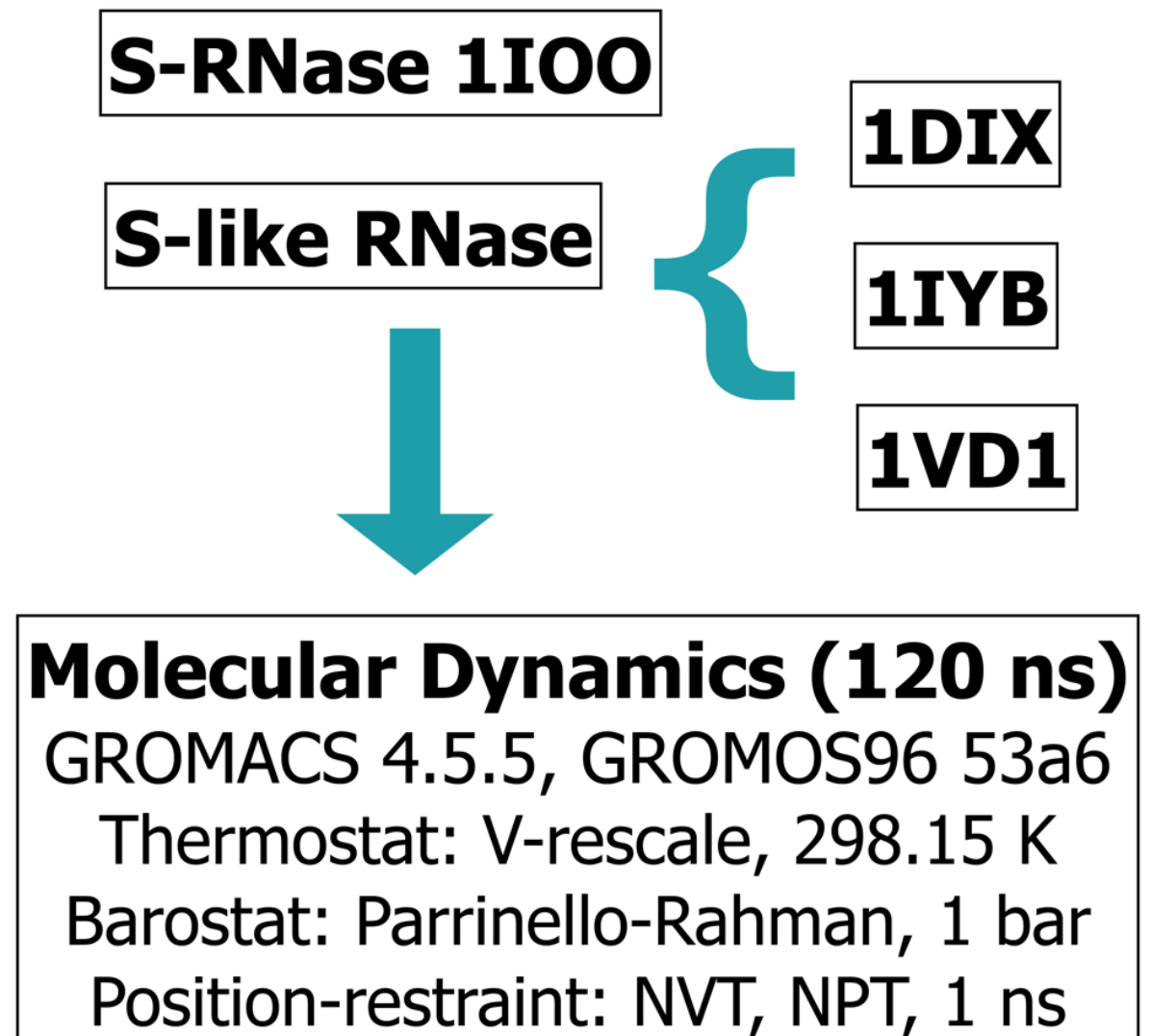
◆ different clades showed differences in the charges distribution

MATERIAL AND METHODS

Evolution



Structure



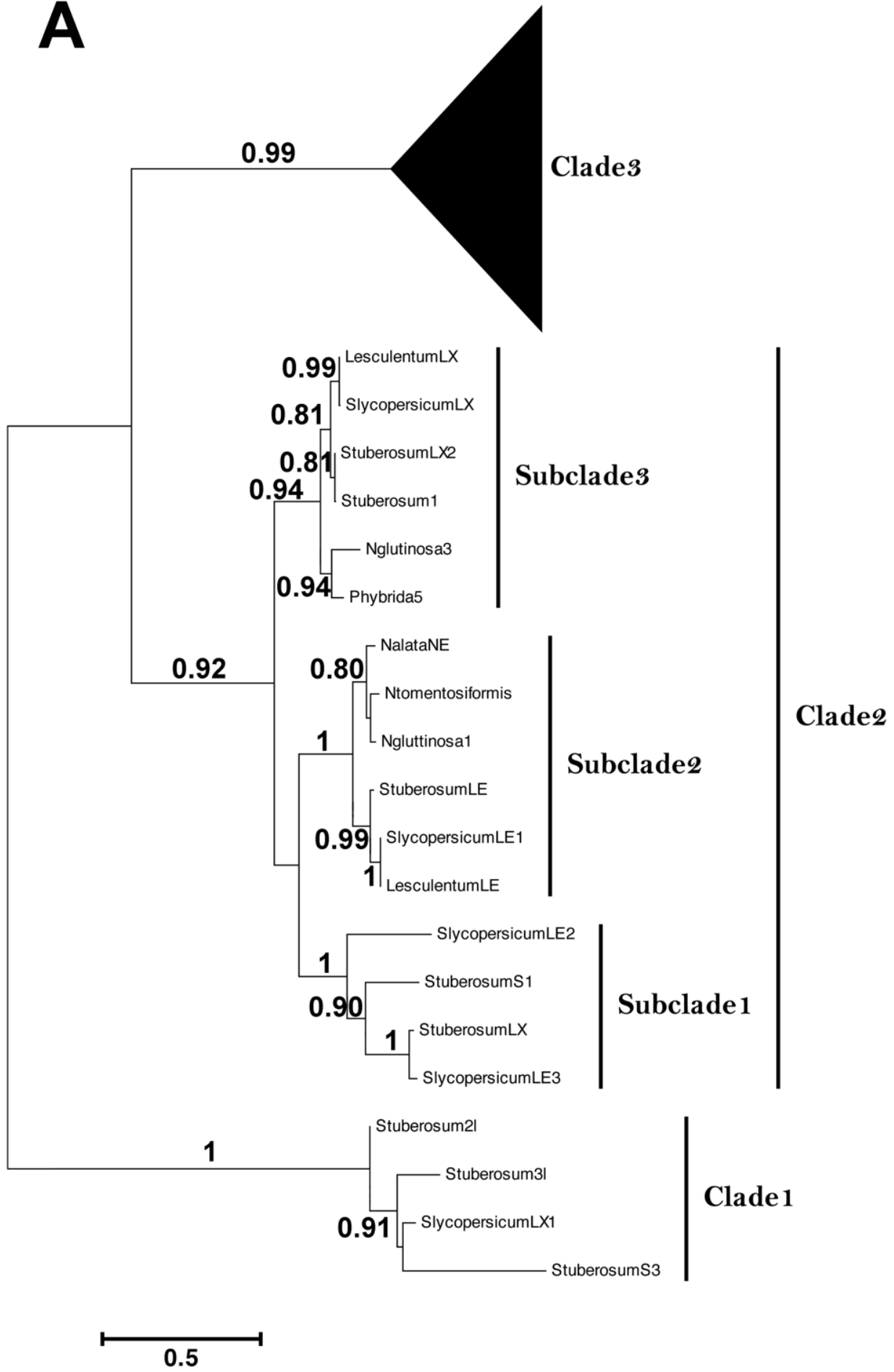
RESULTS

Positive Selection

Site-specific models	2 Δ l (df, P-value)	dN/dS	Parameter estimates	PSS ^d BEB
M1a: nearly neutral		0.13	$p_0=0.9428$, ($p_1=0.0572$) $\omega_0=0.0796$, ($\omega_1=1.0000$)	Not allowed
M2a: positive selection	494.06 (2, <0.0001)	0.20	$p_0=0.9184$, $p_1=0.0663$, ($p_2=0.0153$) $\omega_0=0.1077$, $\omega_1=1.0000$, $\omega_2=2.2078$	31 (27)
M7: β		0.12	$p=0.0291$, $q=0.2012$	Not allowed
M8: β & $\omega > 1$	447.98 (2, <0.0001)	0.14	$p_0=0.9823$, ($p_1=0.0177$) $p=0.0942$, $q=0.7463$, $\omega=1.7906$	26 (33)

RESULTS

Evolutionary history of plant RNases T2 based on DNA sequences, obtained by maximum likelihood (ML). Numbers indicate the branch support.



Clade 2
Subclade 2

extracellular phosphate-starvation-
induced ribonuclease from tomato
(*Lycopersicon esculentum*)



wound-inducible in tobacco (*N. glutinosa*) leaves



Clade 2 Subclade 2

Clade 3

self-incompatibility system
in ornamental tobacco
(*Nicotiana glauca*)



induced upon TMV
(tobacco mosaic virus,
single-stranded RNA)-
infection in tobacco (*N.*
glutiosa)



Clade 2
Subclade 3

Clade 3

PPS in very rigid regions

Allelic identity



HVa

HVb

TYR-88

LEU-7

TRP-11

PRO-12

A

B1, B2 substrate binding sites

the base recognition non-specific is mainly dependent of the hydrophobic pocket residues

S-RNase

self-incompatibility system in ornamental tobacco (*Nicotiana glauca*)

Clade 2 Subclade 3

PPS in flexible regions

LEU 60

GLN 52

TYR 156

ASN-134

B

protein surface

PPI ?

S-like RNase

induced upon TMV (tobacco mosaic virus, single-stranded RNA)-infection in tobacco (*N. glutinosa*)

CONCLUSIONS

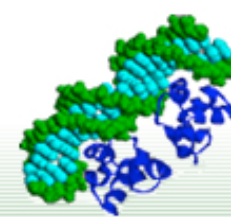
- hypervariable regions submitted to selective pressures leading to increase in the amino acids variability
- hypervariable regions - responsible for allelic identity; interaction with SLF complex
- S-RNase: rigid regions near the active site, B1 and B2 substrate binding sites - role in the RNA degradation
- S-like RNases: flexible sites in the protein surface - possibly important to some PPI

PERSPECTIVES

- to investigate the evolutionary history of the SLF complex
- to study the *Nicotiana alata* thioredoxin (Trx) that interacts with S-RNase (may inhibit or stabilize it) - evolutionary and structural history.
- NaTrx interacts *in vitro* with S-RNase and co-localizes with it in the extracellular matrix of the stylar transmitting tissue (BMC Plant Biol 14:147, 2014)

MOLECULAR DYNAMICS

- energy minimization: LBFGS algorithm
- NVT, NPT - 0.5 ns
- MD 119 ns in NPT ensemble
- Barostat: Parrinello-Rahman pressure-coupling scheme, 1 bar
- Thermostat: V-rescale, 298.15K
- all protein bonds and water molecules were constrained using LINCS and SETTLE algorithms
- long-range electrostatic interactions: SPME
- time-step 2fs
- Coulomb and van der Waals interactions were computed within spherical cutoff radii of 1.25nm



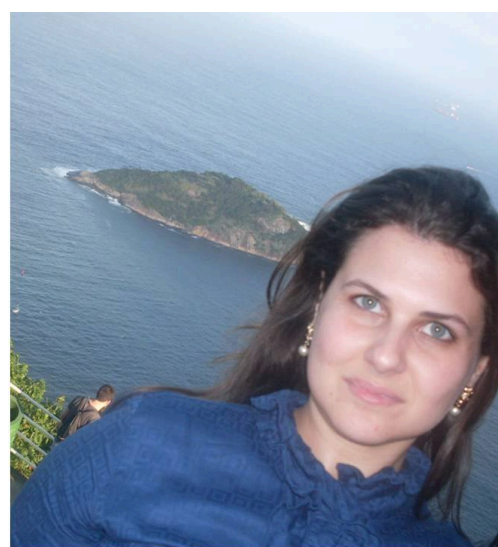
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