



# Identification and Characterization of Two Genes Associated to Postharvest Disorders of 'Hass' Avocados (*Persea americana* Mill.)

Mauricio González-Agüero<sup>1,2</sup>, Miguel García<sup>3</sup>, Reinaldo Campos-Vargas<sup>2,3</sup>, Orianne Gudenschwager<sup>1</sup>, Daniel Manríquez<sup>1</sup> and Bruno G. Defilippi<sup>1,2</sup>.

<sup>1</sup> Institute of Agricultural Research (INIA-La Platina). P.O. Box 439-3, Santiago, Chile.

<sup>2</sup> The Plant Cell Biotechnology Millennium Nucleus (PCB-MN)

<sup>3</sup> Andrés Bello University, República 217, Santiago, Chile.

Email: [maugonzalez@inia.cl](mailto:maugonzalez@inia.cl)

## Abstract

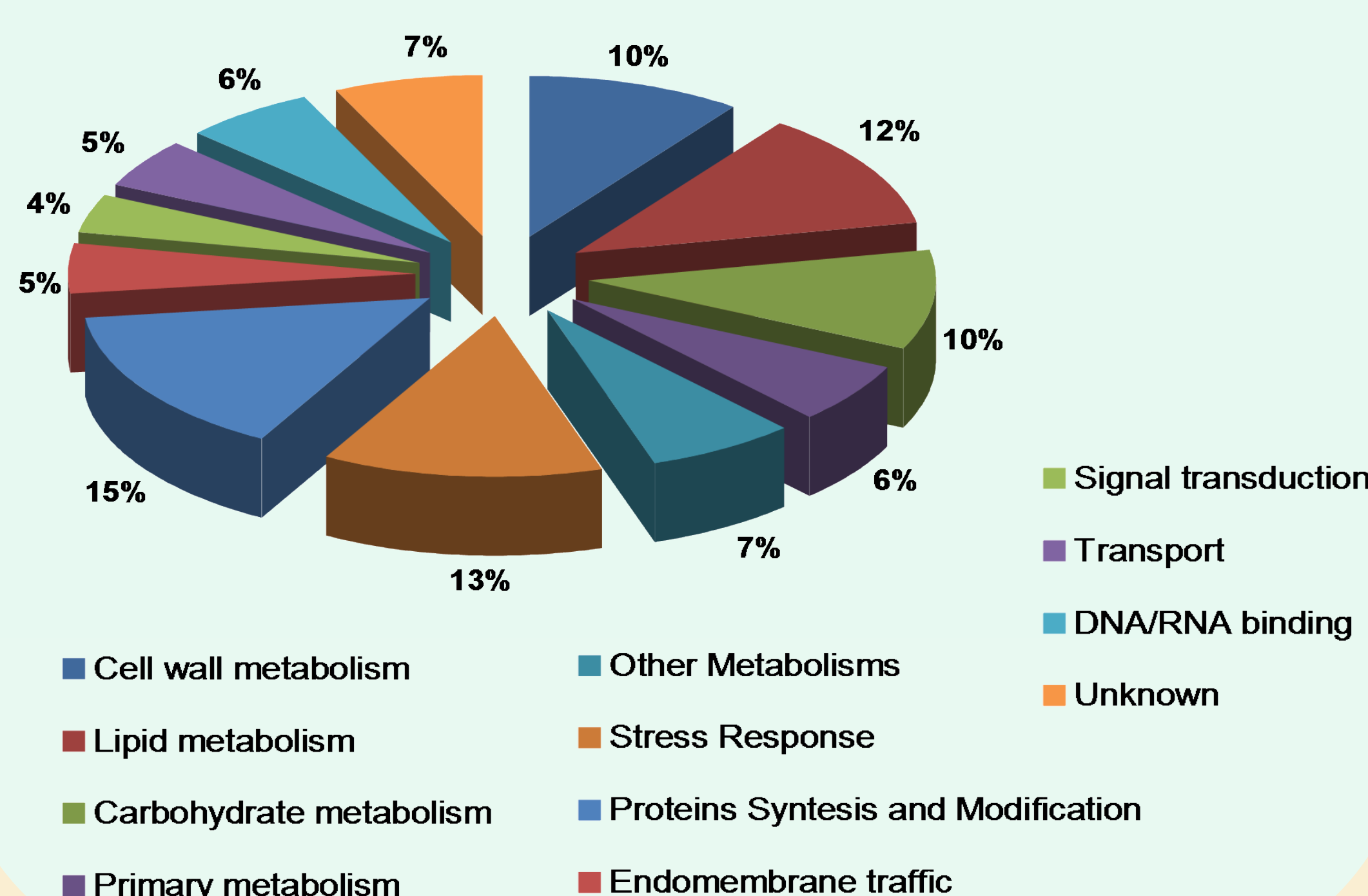
The most important avocado variety cultivated worldwide is 'Hass' (*Persea americana* Mill.). This variety is grown and exported from Chile to distant markets, especially EE.UU and Europe. Avocado fruit quality during cold storage and shipment is affected by several processes, including internal browning, softening and water loss. Internal browning development is caused by the interaction of maturity stage, senescence and temperature during postharvest life. Despite the importance of this disorder, the biochemical processes or mechanisms remain unknown. Therefore, in order to understand internal browning development, a forward suppression subtractive hybridization (SSH) cDNA library was constructed to study this problem. To date, we have identified near to 300 differentially expressed genes that codify for key enzymes involved in different biochemical pathways. From this group of genes, two of them showed an expression pattern related to internal browning. We cloned and characterized these genes encoding for enzymes related to lipid metabolism, i.e. acetyl-CoA carboxylase (ACCase) and stearoyl-ACP desaturase (SAD). Rapid amplification of cDNA ends (RACE-PCRs) were conducted to obtain full length cDNAs, and real-time quantitative PCR were performed to analyze transcription profiles. Experiments designed to localize the cloned genes by transient plant expression are on development.

Funded by  
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11080236

## Results

**1. Construction and characterization of a cDNA avocado library under cold storage.** A current summary of the characterized forward subtracted library and a partial classification of near to 500 genes differentially expressed by their putative function is shown.

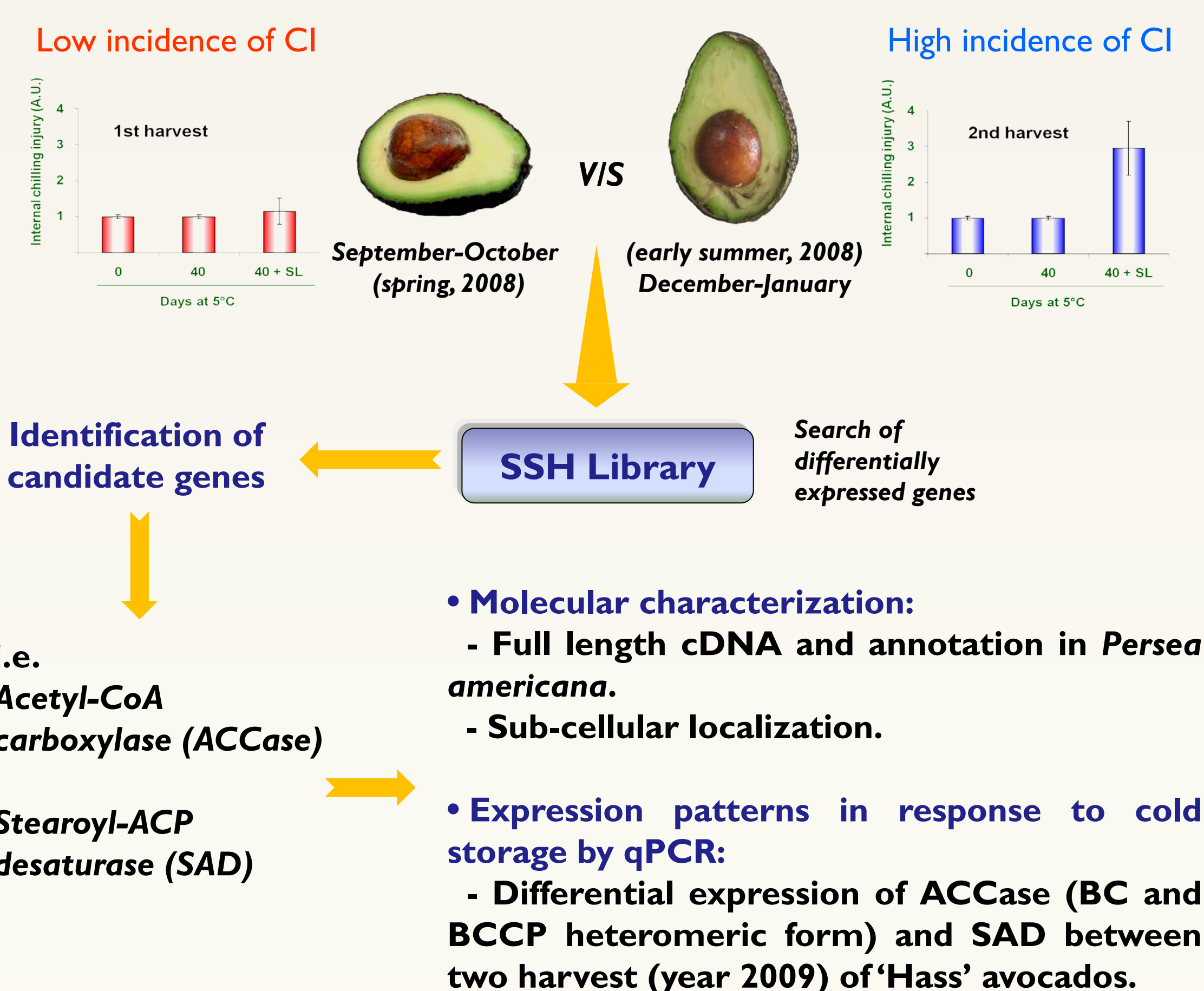
Summary of subtracted library:			
- Positive clones	~ 800 clones	- Redundancy of seqs.	40%
- Sequenced	~ 650	- Analyzed sequences	~ 500
- Average insert size	600 bp		



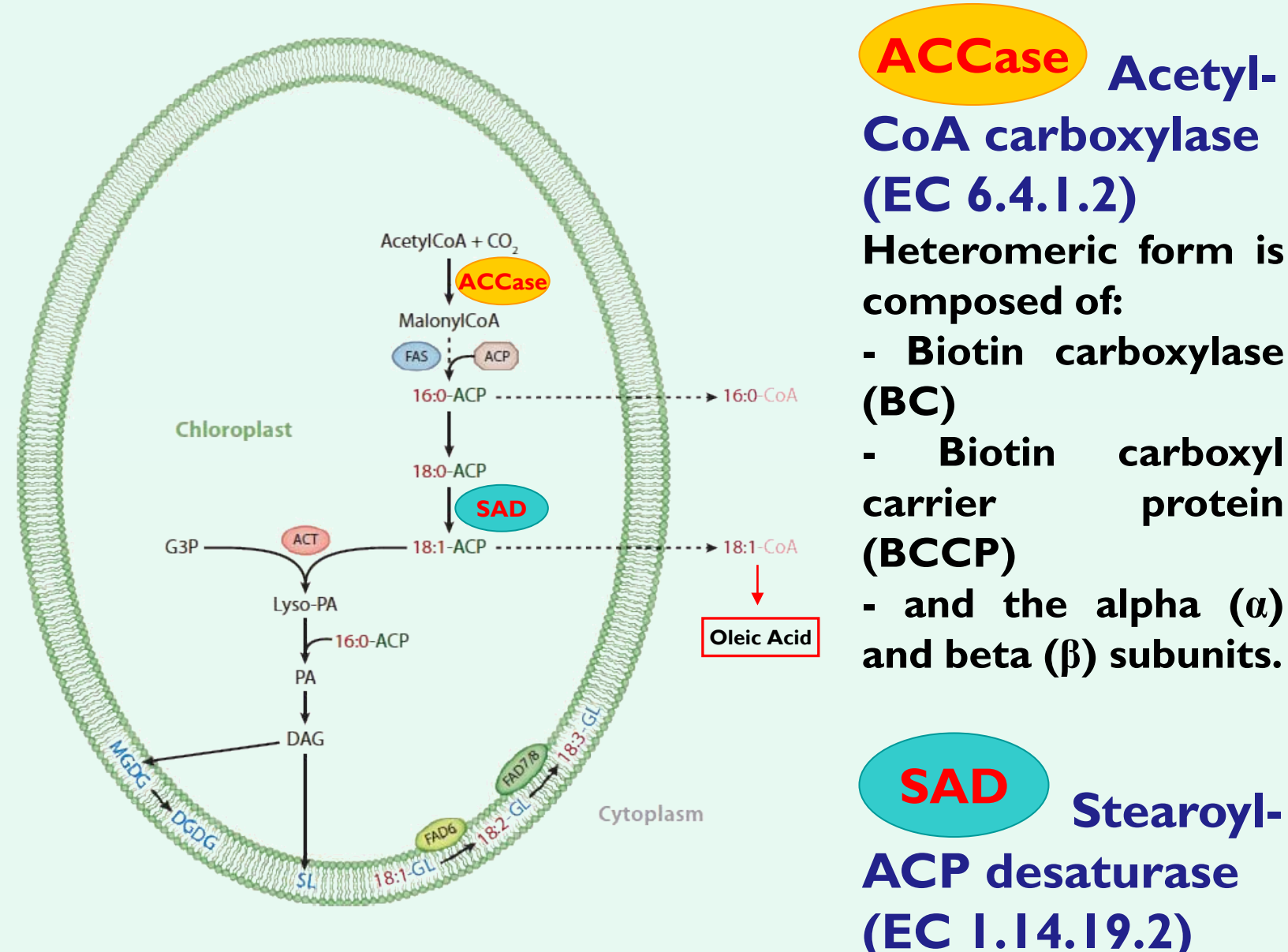
## Conclusions

- A higher internal browning incidence in fruit from 2<sup>nd</sup> harvest and storage at 0°C (chilling injury conditions). Under this circumstances we observed a decrease in the expression of ACCase subunits (*PamBC* and *PamBCCP*) during avocado ripening (40 days + SL).
- *PamSAD* has a different response to the other analyzed genes, since it remains suppressed during storage at 0°C in the second harvest.
- We have identified new genes related to internal browning in avocado, one of them (*PamBCCP*) being specifically expressed in fruit. This is a very important feature for using them as possible markers or as candidate genes for breeding.

## Experimental design

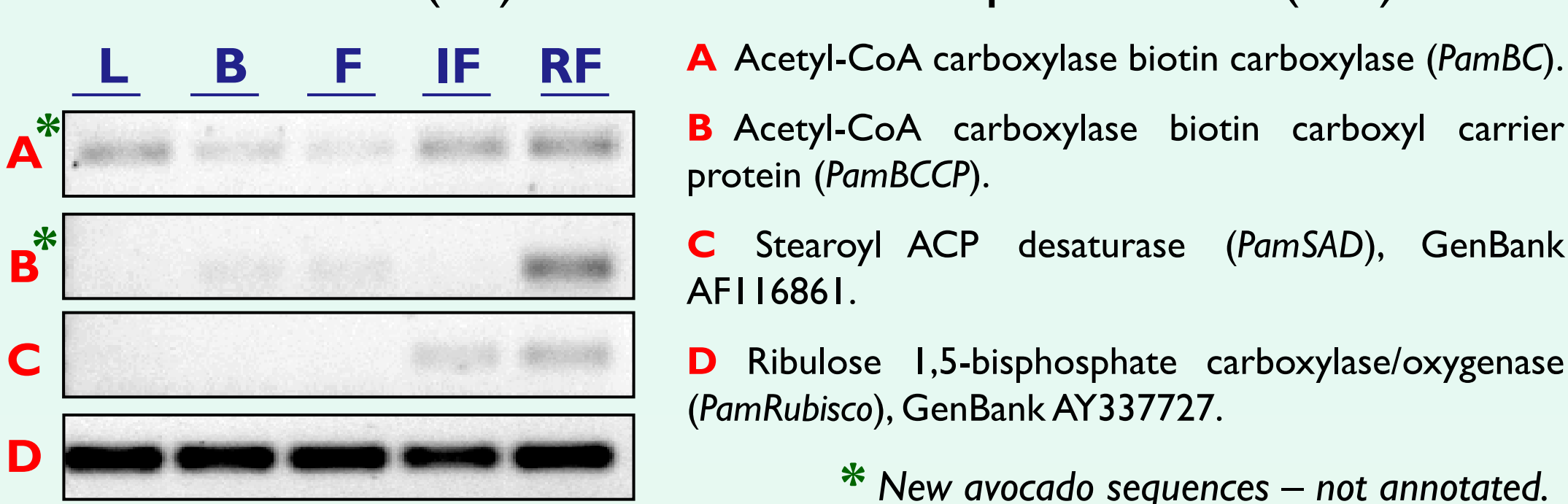


**2. Schematic representation of role ACCase and Sad during fatty acid biosynthesis.** Modified from Kachroo and Kachroo. *Annu. Rev. Phytopathol.* 2009. 47:153–176.



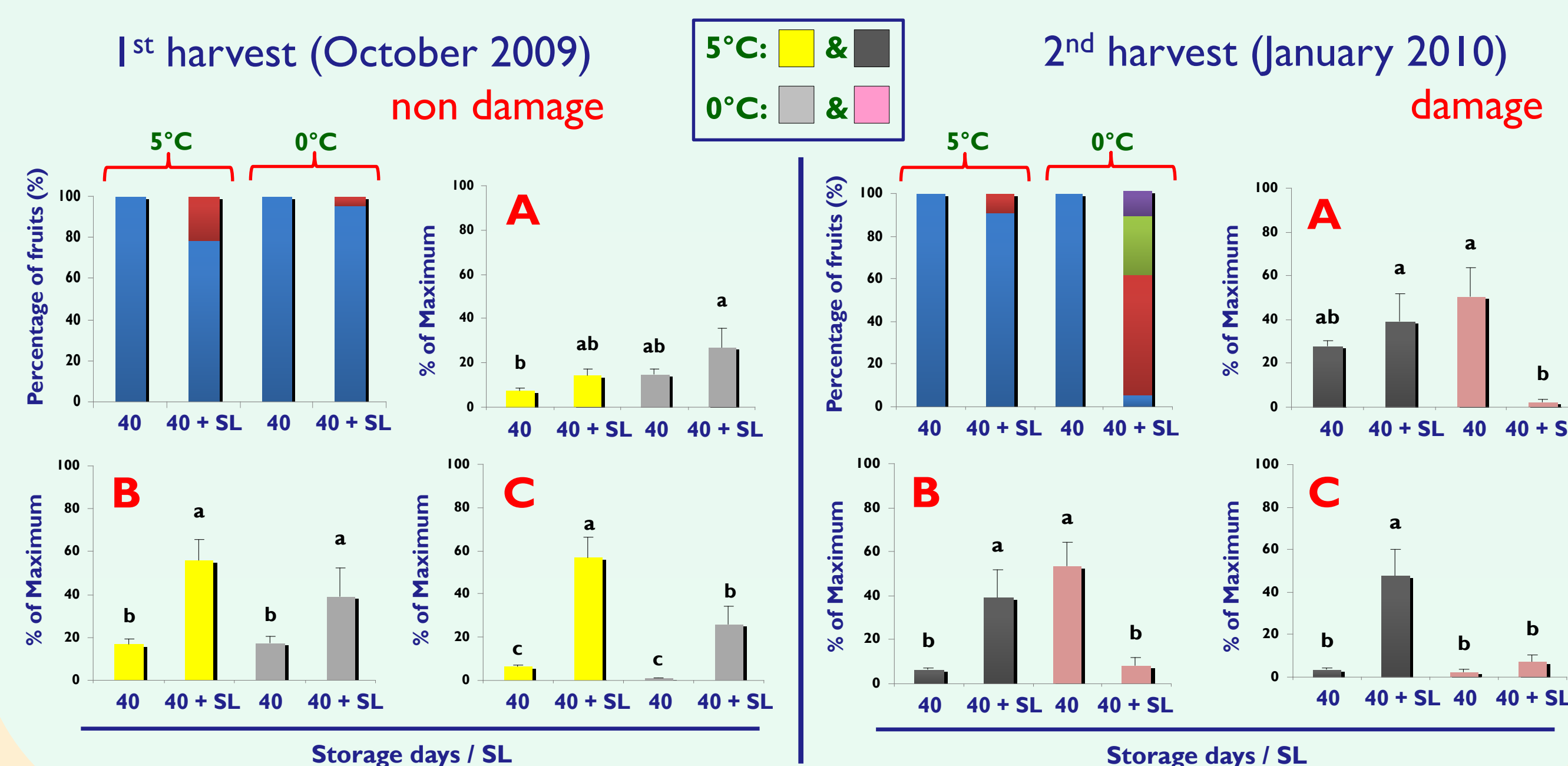
Oleic acid (18:1 cis 9) is the major fatty acid constituent (near to 70%) in 'Hass' avocado

**3. Identification and characterization of two subunits of ACCase heteromeric form (BC and BCCP) and SAD genes.** Expression in cDNAs from different avocado tissues: leaves (L), buds (B), flowers (F), immature fruit (IF) and mature and ripened fruit (RF).

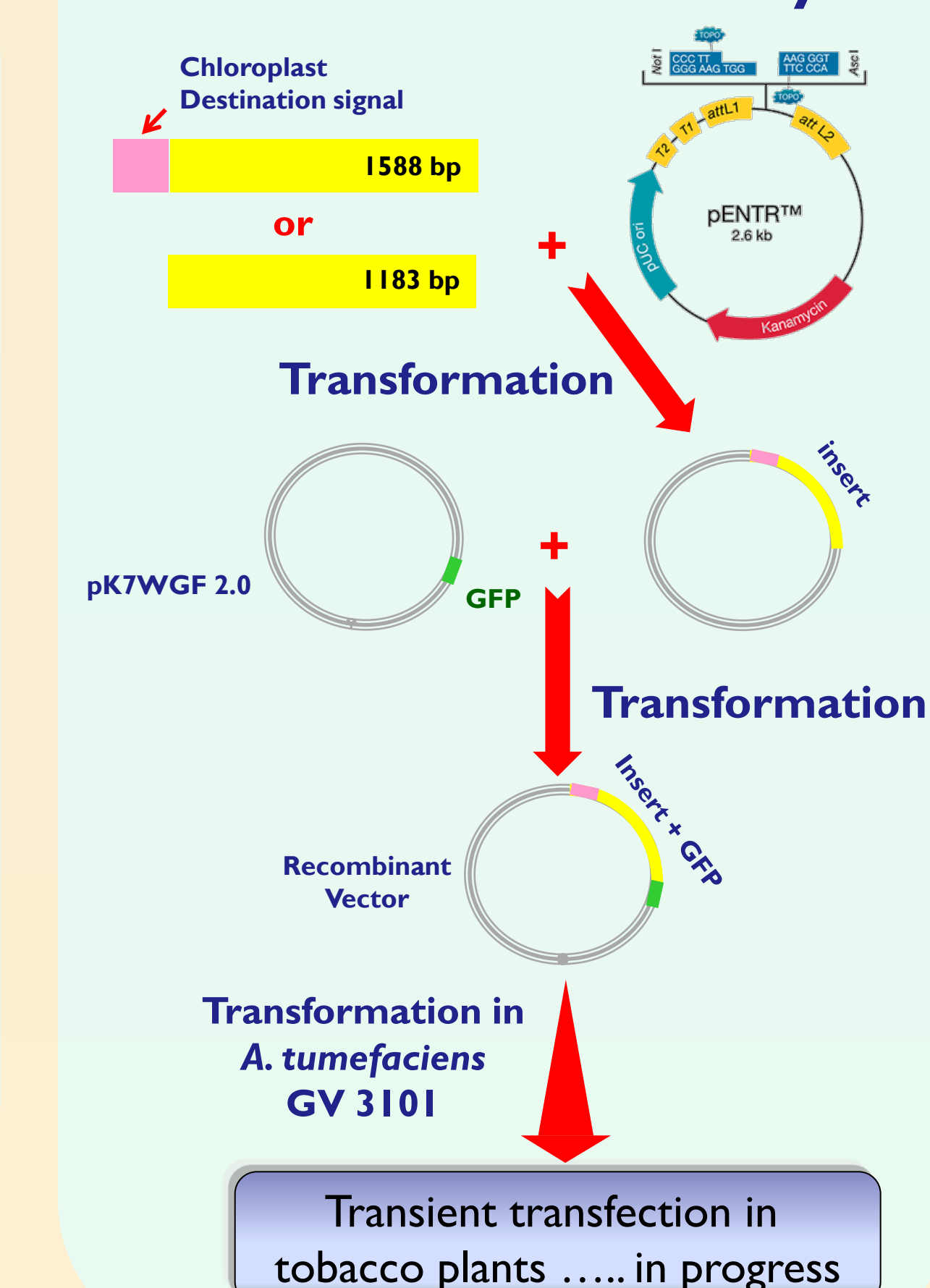


\* i.e. cDNA sequence and putative amino acid sequence of *P. americana* Acetyl-CoA carboxylase biotin carboxylase (*PamBC*). (Annotation in progress)

**4. Expression profiles for *PamBC* (A), *PamBCCP* (B) and *PamSAD* (C) in two harvest with differential occurrence of internal browning at two storage temperatures (5° and 0°C).** qPCR assays were performed in 6 fruits per treatment, three times each. Expression was normalized considering a house-keeping gene (*PamRubisco*), and expressed as a percentage of the highest value of relative abundance.



**5. *PamBC* & *PamBCCP* sub-cellular localization assays.**



\* Different letters represent significant differences at  $P < 0.05$  by LSD test.