

Ripening process study in persimmon (*D. kaki*) fruits on tree focused on ethylene

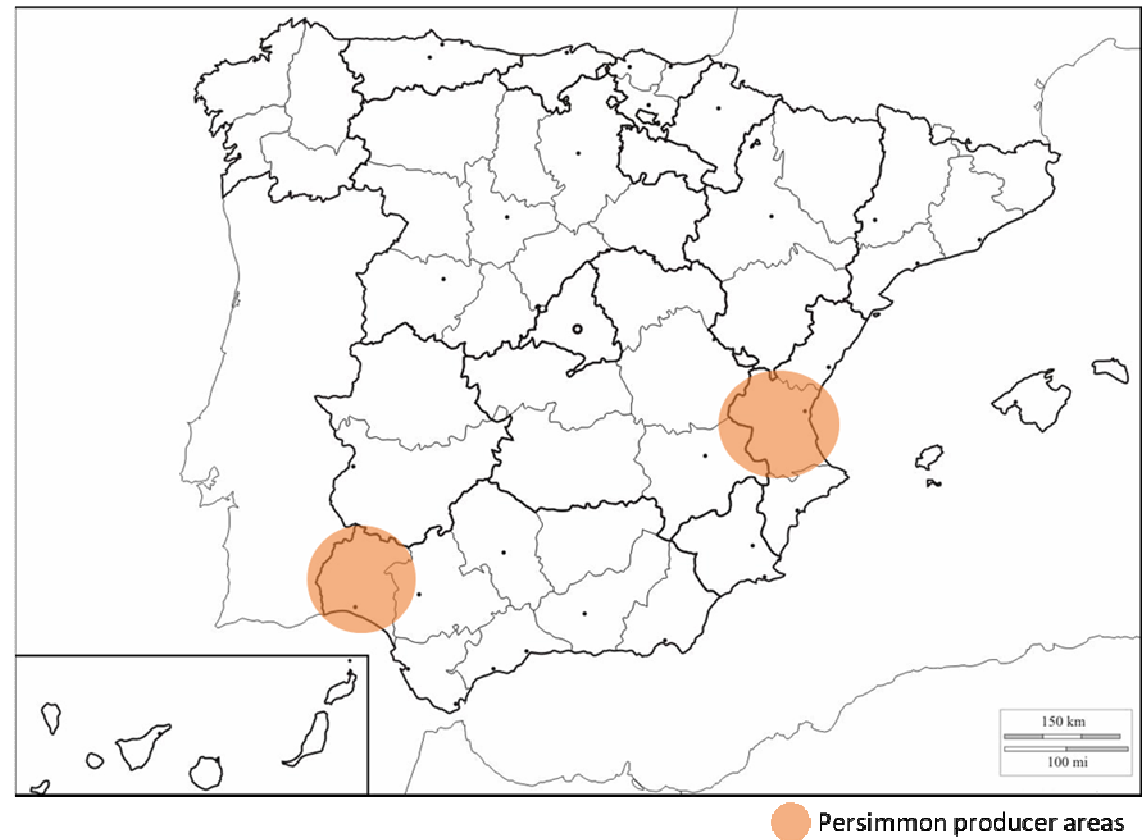
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INTRODUCTION

- **Spanish persimmon data:**
 - Production: 450.000 t
 - Cultivated area: 813.536 ha
- **Varietal distribution:**
 - Var. Rojo Brillante (83%)
 - Var. Sharon (15%)



Var. Rojo Brillante represents the 96% of the total persimmon production in Valencia

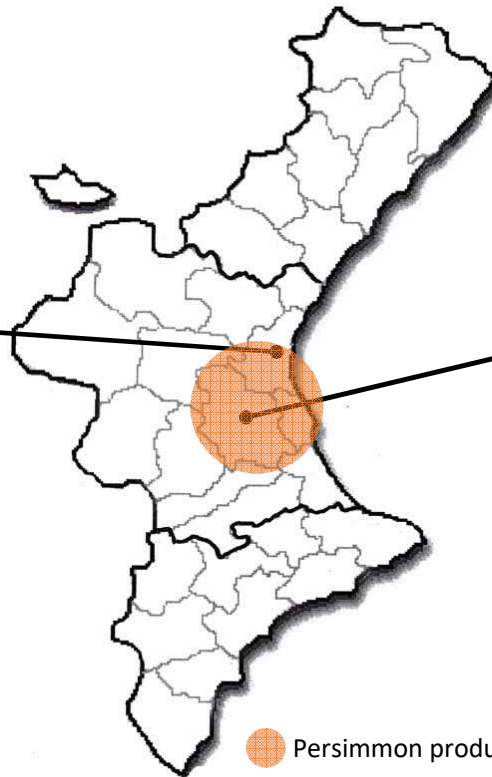
INTRODUCTION




- The 'Instituto Valenciano de Investigaciones Agrarias' (IVIA) and the grower association CANSO started a persimmon breeding program focused on expanding the varietal range developing new varieties with different commercial ripening dates.



IVIA (Valencia, Spain)
 $39^{\circ}35'22.6''\text{N } 0^{\circ}23'41.0''\text{W}$



 Persimmon producer area



CANSO (L'Alcudia, Spain)
 $39^{\circ}11'33.1''\text{N } 0^{\circ}30'41.1''\text{W}$

MATERIAL & METHODS

- Fruit samples were collected from four accessions from the IVIA's persimmon germplasm collection.

Varieties	Commercial Ripening*	Physiological Ripening*	Astringency Type
Agakaki	September, 5th	November, 8th	PVNA
Isahaya	November, 12th	January, 28th	PCNA
Rojo Brillante	October, 29th	January, 4th	PVA
Tone Wase	September, 23rd	November, 8th	PVA

*Ripening dates are the mean of 4 years obtained from the IVIA persimmon germplasm bank database.

- Six fruits of each accession were collected every 15 days from August 8th until physiological ripening.

Sample	Date (m/d/y)	Sample	Date (m/d/y)	Sample	Date (m/d/y)	Sample	Date (m/d/y)
S1	08/06/2019	S5	09/30/2019	S9	11/25/2019	S13	01/23/2020
S2	08/19/2019	S6	10/14/2019	S10	12/08/2019		
S3	09/02/2019	S7	10/28/2019	S11	12/23/2019		
S4	09/16/2019	S8	11/11/2019	S12	01/08/2020		

MATERIAL & METHODS



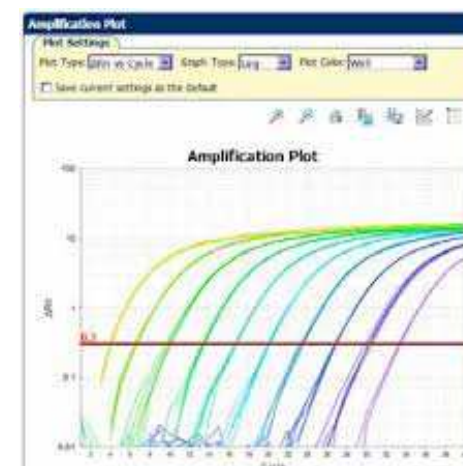
- **Morphological parameters:**

- Weight
- Color index (CI)



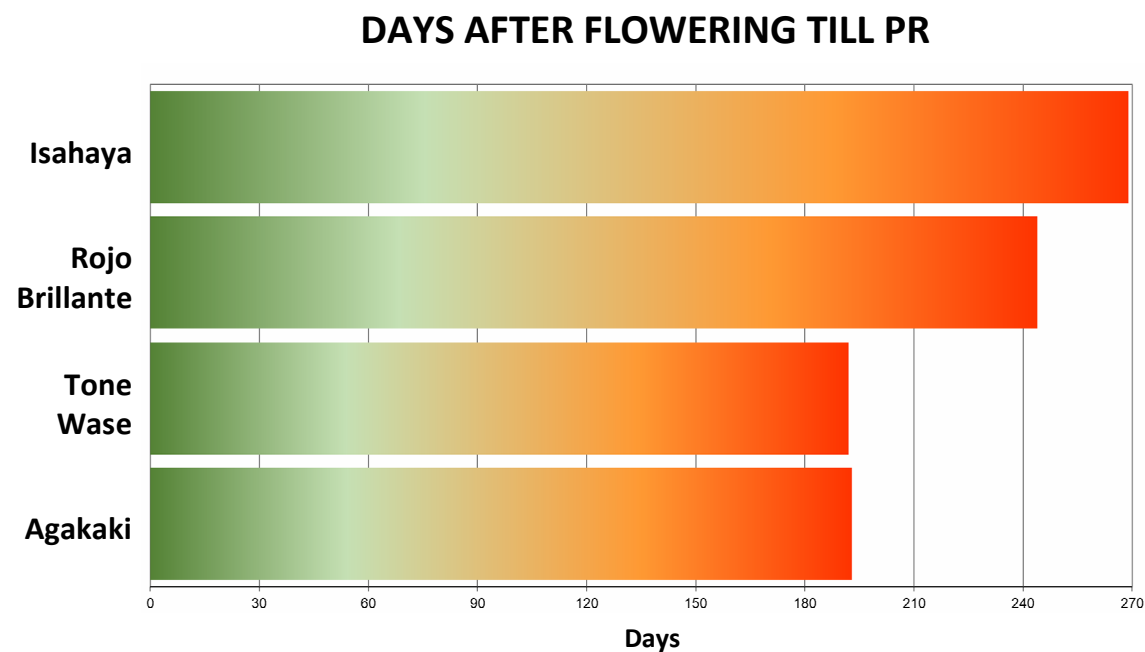
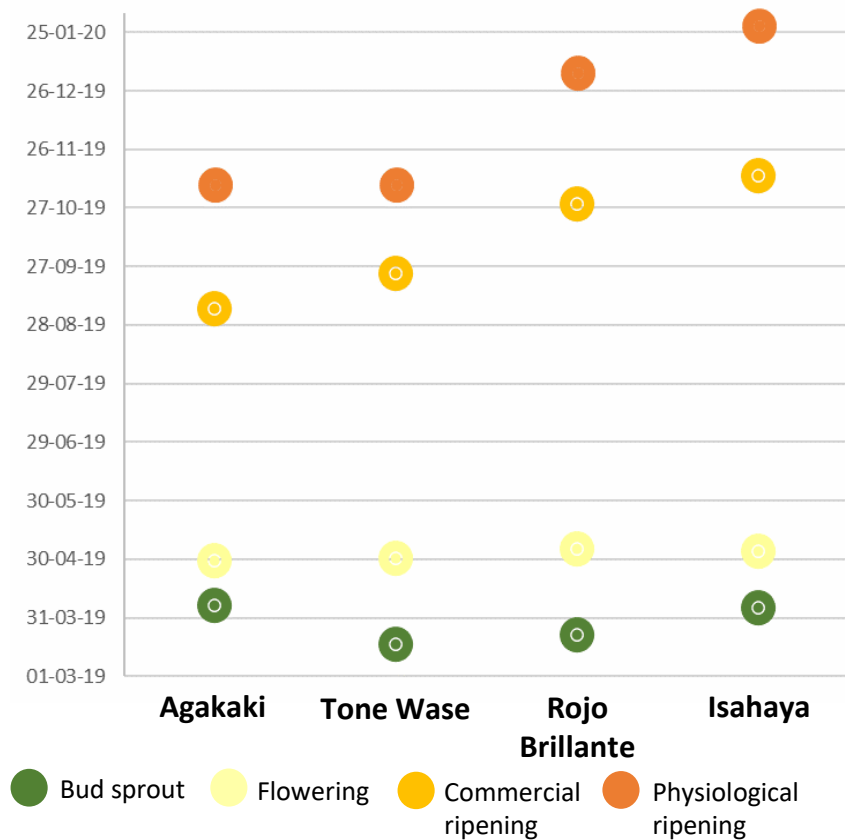
- **Genes studied:**

- DkACO-1 (Ortiz *et al.*, 2006)
- DkACO-2 (Ortiz *et al.*, 2006)
- DkERF-18 (Min *et al.*, 2014)
- DkACT (Akagi *et al.*, 2009)
- DkTUA (Wang *et al.*, 2017)



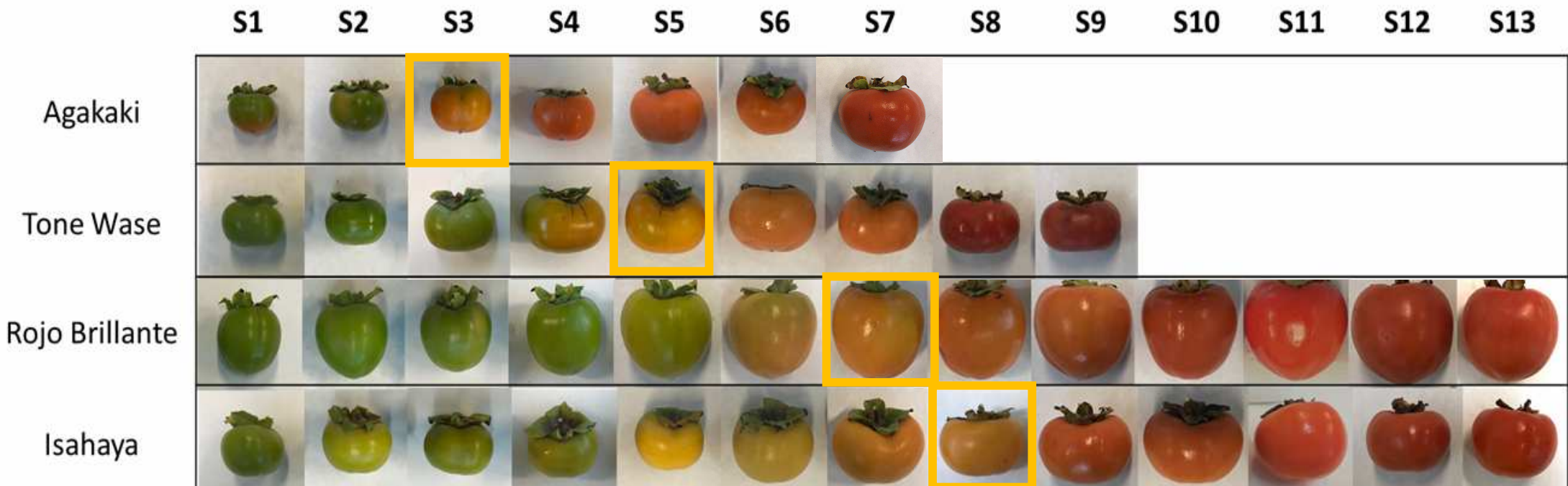


FRUIT RIPENING PROCESS DURATION





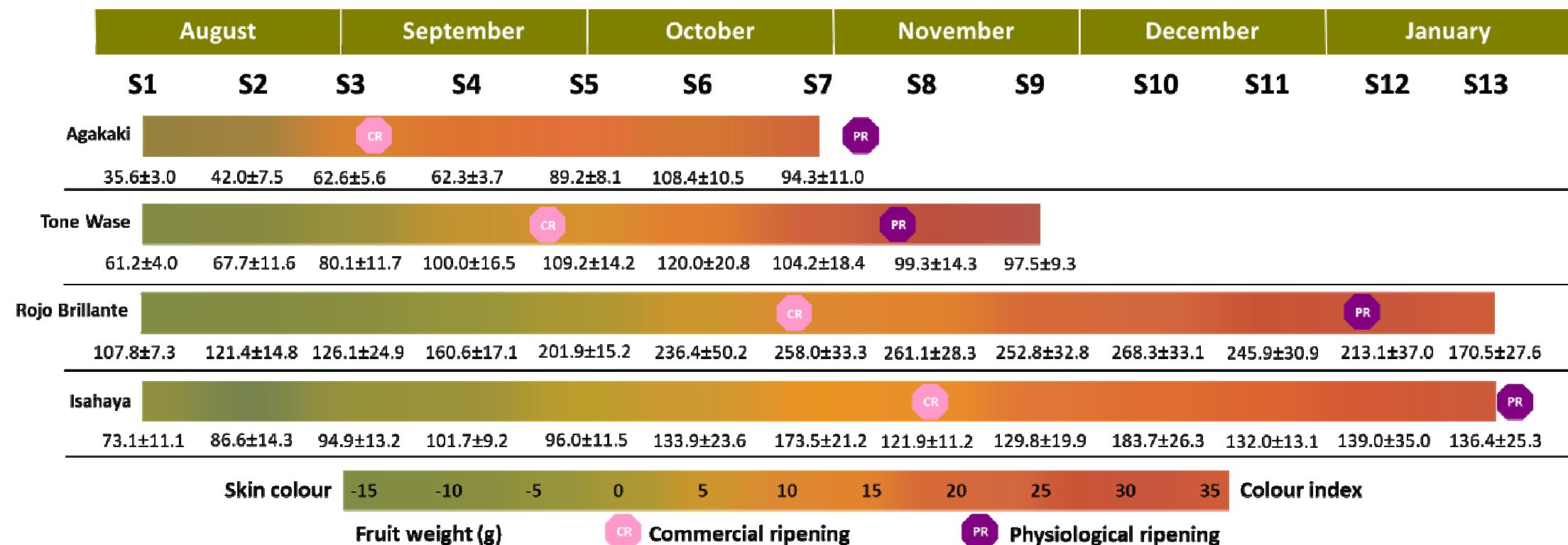
FRUIT RIPENING ALONG SAMPLING DATES



 Commercial ripening

Sample	Date (m/d/y)	Sample	Date (m/d/y)	Sample	Date (m/d/y)	Sample	Date (m/d/y)
S1	08/06/2019	S5	09/30/2019	S9	11/25/2019	S13	01/23/2020
S2	08/19/2019	S6	10/14/2019	S10	12/08/2019		
S3	09/02/2019	S7	10/28/2019	S11	12/23/2019		
S4	09/16/2019	S8	11/11/2019	S12	01/08/2020		

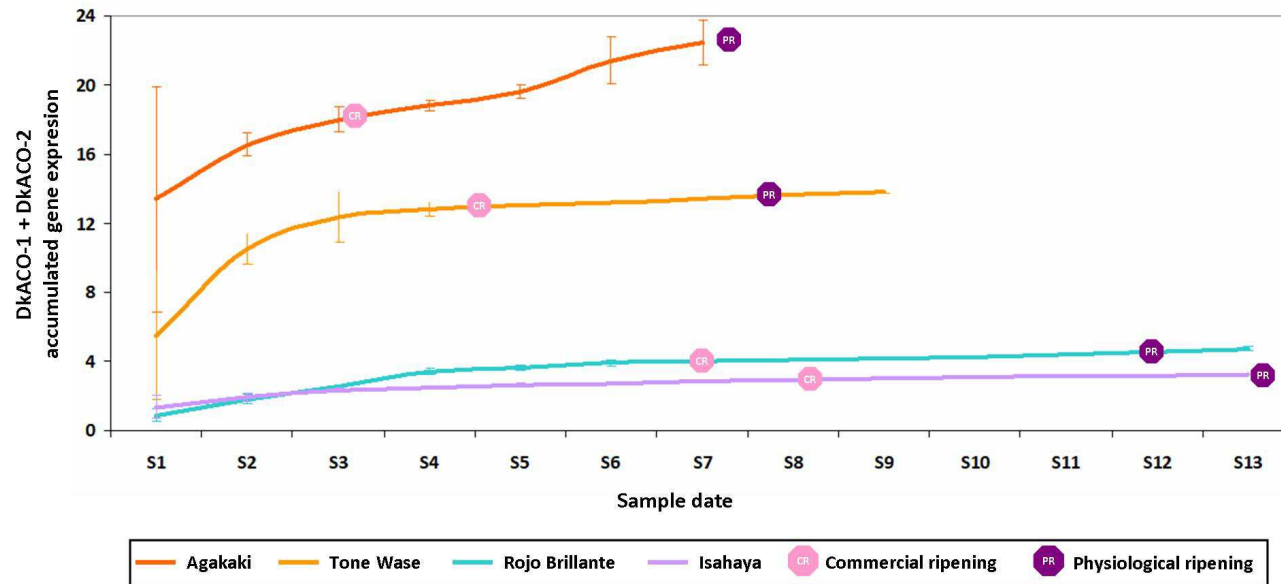
FRUIT RIPENING ALONG SAMPLING DATES



- Color variation is produced by the degradation of chlorophylls during the green stages until breaker, when synthesis and accumulation of carotenoid compounds are the main responsible of persimmon fruits color (Brossard and Mackinney, 1963).



ETHYLENE PRODUCTION

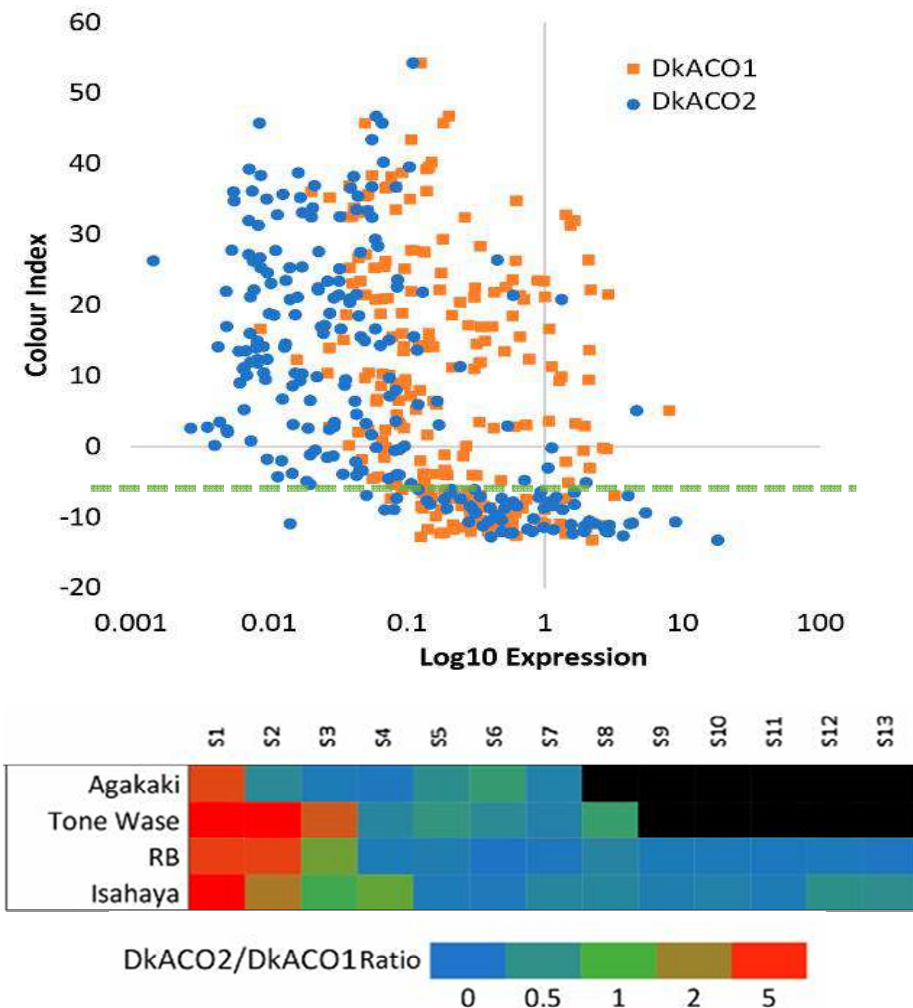


- Ethylene synthesis level in persimmon fruits is similar to those produced by non-climacteric ones (Nakano *et al.*, 2003).
- There is a strong evidence that ACO genes expression is positive correlated to the ethylene production rates (Ruduś *et al.*, 2013).

- Analysis of the results showed that combined DkACO-1 and DkACO-2 gene expression level profile was in agreement with varieties' precocity.
- Both ACO genes accumulated expression was consistent with commercial and physiological maturity of the analyzed varieties (Figure 2).

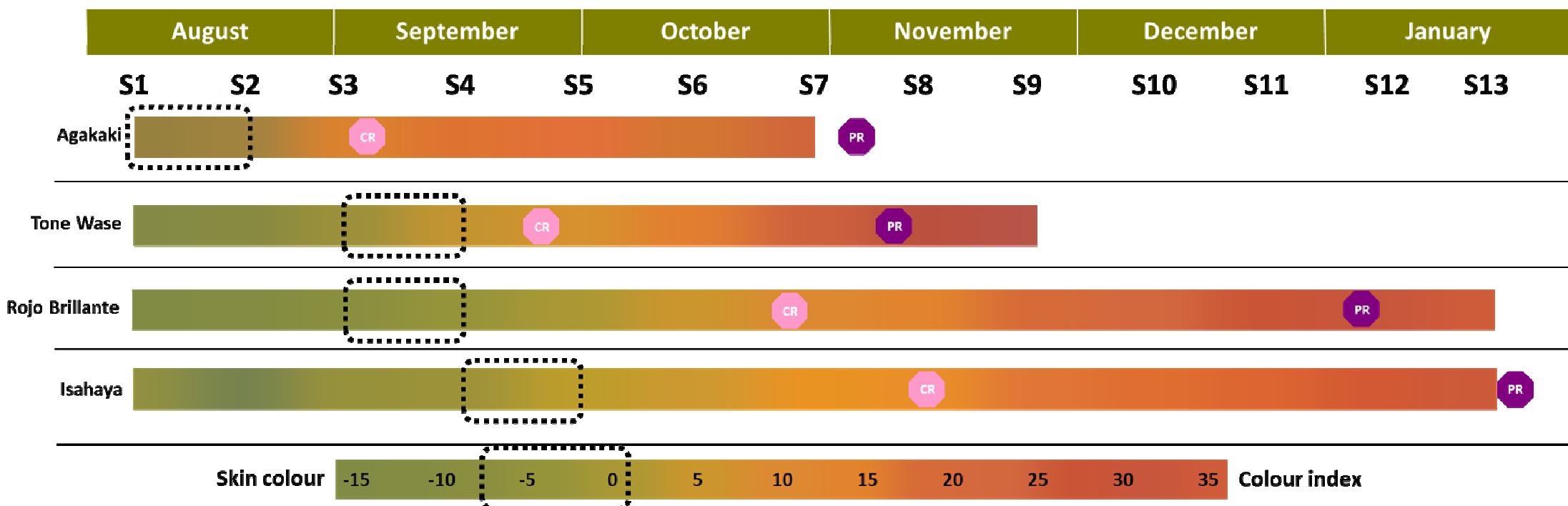


SYSTEM OF ETHYLENE PRODUCTION



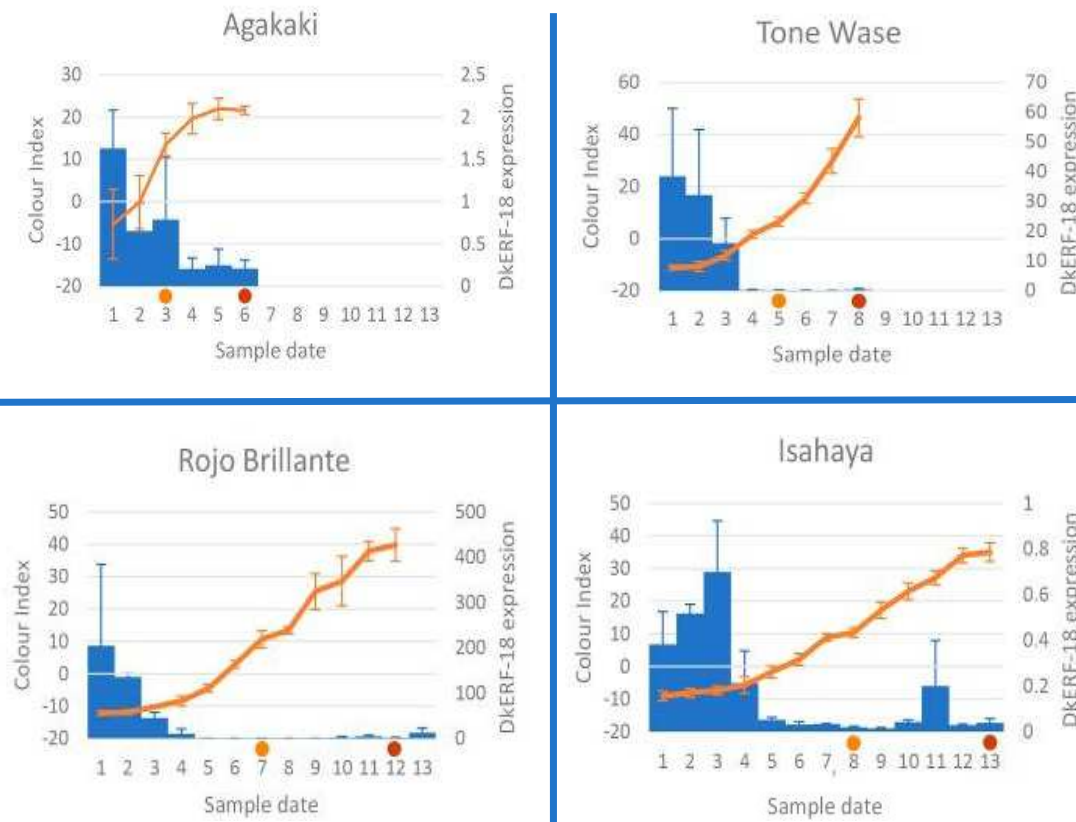
- Ethylene production in plants has been divided into System 1 and System 2 (McMurchie *et al.* 1972).
 - The physiological and molecular pathways that initiate the transition from System 1 to System 2 are unknown (Barry and Giovannoni 2007; Cara and Giovannoni 2008).
- In our experiment:
 - Immature green fruits
 $\uparrow \text{DkACO-2 expression} + \text{DkACO-2/DkACO-1} \geq 1$
 - CI ≥ -8
 $\downarrow \text{DkACO-2 expression} + \text{DkACO-2/DkACO-1} \leq 1$

SYSTEM OF ETHYLENE PRODUCTION



- System 1 type stage, as it is responsible for the basal level of ethylene production in vegetative tissues and unripe fruit (Paul *et al.*, 2012).
- Cumulative effects of System 1 ethylene emission reaches certain limit and induces System 2, that it operates during floral senescence and fruit ripening (Klee, 2004; Paul *et al.*, 2012).

ETHYLENE RESPONSE



- In our experiment:
 - Immature green fruits
↑ **DkERF-18 expression**
 - CI ≥ -8
↓ **DkERF-18 expression**
- He *et al.* (2020) demonstrated the involvement of DkERF-18 in the autocatalytic ethylene system through the activation of DkACO-2 gene via ACCGAC motif, present in *Diospyros oleifera* DkERF-8 putative orthologue.

CONCLUSIONS



- According to our results, and previous evidences, we propose:
 - DkACO-2 gene downregulation would determine the transition from System 1 to System 2.
 - System 2 is characterized by a DkACO-2/DkACO-1 ratio values below one.
- This information is of great interest for further analyses and to develop tools aimed to increase the effectiveness and efficiency of persimmon breeding programs.
- These results provide new knowledge to understand this important trait, of great interest for breeding new varieties and the most limiting factor on persimmon marketing and postharvest management.

Thanks for your attention!