



Pre-breeding strategies for obtaining new resilient and added value berries

Genome-wide association studies (GWAS) in a diverse strawberry collection from IFAPA unveil loci controlling agronomic and fruit quality traits

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y de la Producción Ecológica



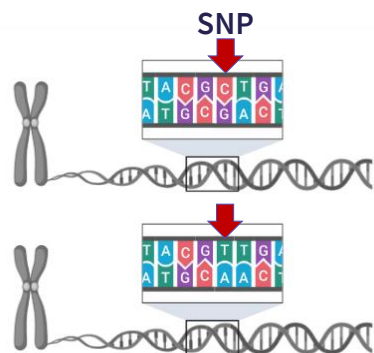
The BreedingValue project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000747.

GENOME-WIDE ASSOCIATION STUDIES IN BERRY PROGRAMS

Experimental population



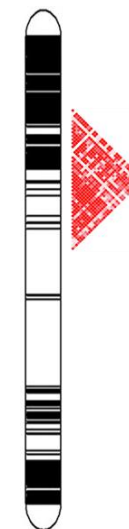
Genotyping



Phenotyping

Association studies (GWAS)

Accession 1	A	G	G	C	T	
Accession 2	A	C	G	C	T	
Accession 3	A	C	G	C	A	
Accession 4	A	G	G	T	A	
Accession 5	A	C	G	T	A	
Accession 6	A	C	G	T	T	
	G/C			C/T	T/A	



GENOME-WIDE ASSOCIATION STUDIES IN BERRY PROGRAMS



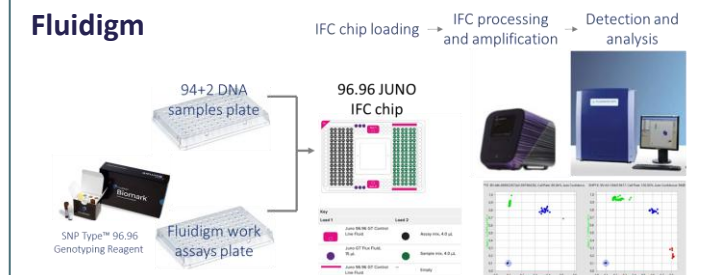
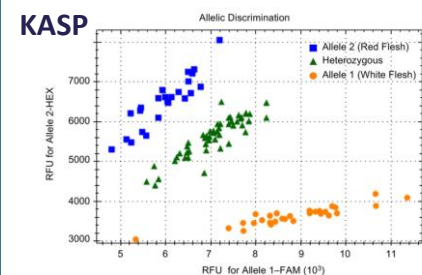
VALIDATION OF THE
SNP-TRAIT
ASSOCIATIONS

IDENTIFICATION OF THE
CAUSAL GENE AND
FUNCTIONAL
VALIDATION

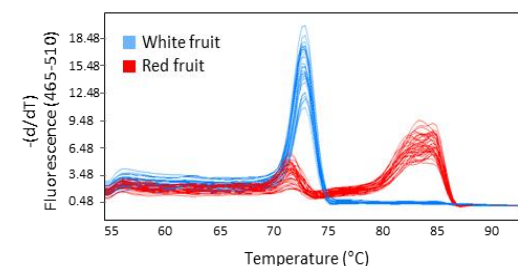
The discovered SNPs can be converted into valid markers for breeding programs:
DEVELOPMENT OF DIAGNOSTIC TESTS



Allele-specific PCR



High-resolution melting
(HRM) assays



Experimental population

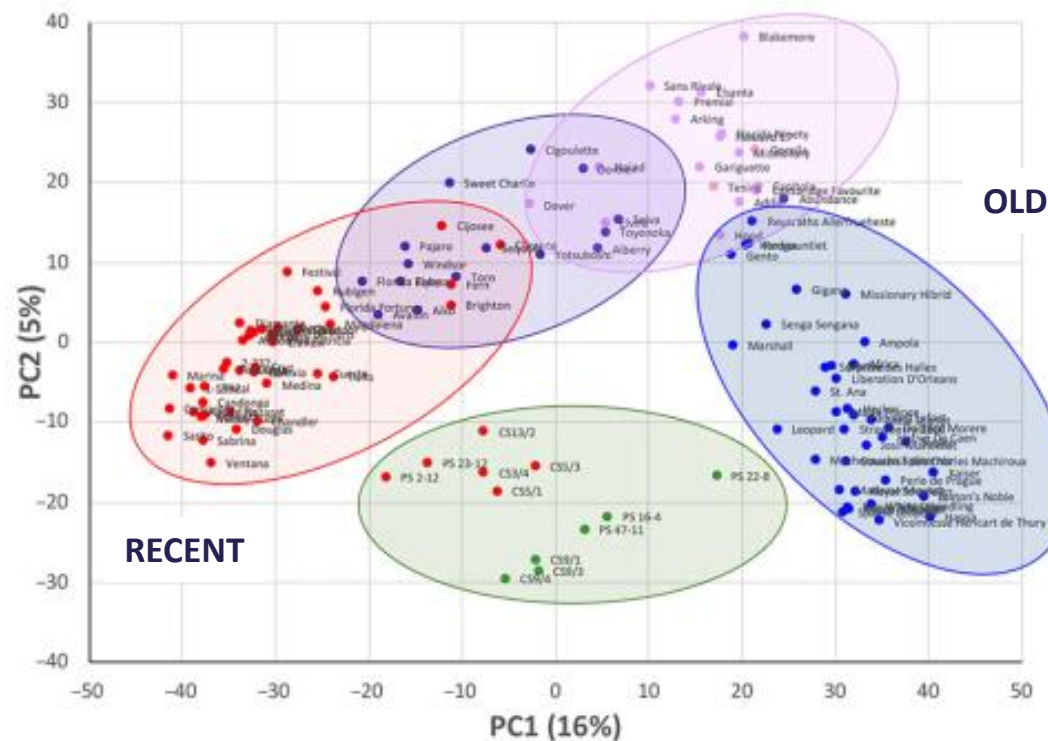


124 diverse accessions
from the **IFAPA *Fragaria* germplasm collection**

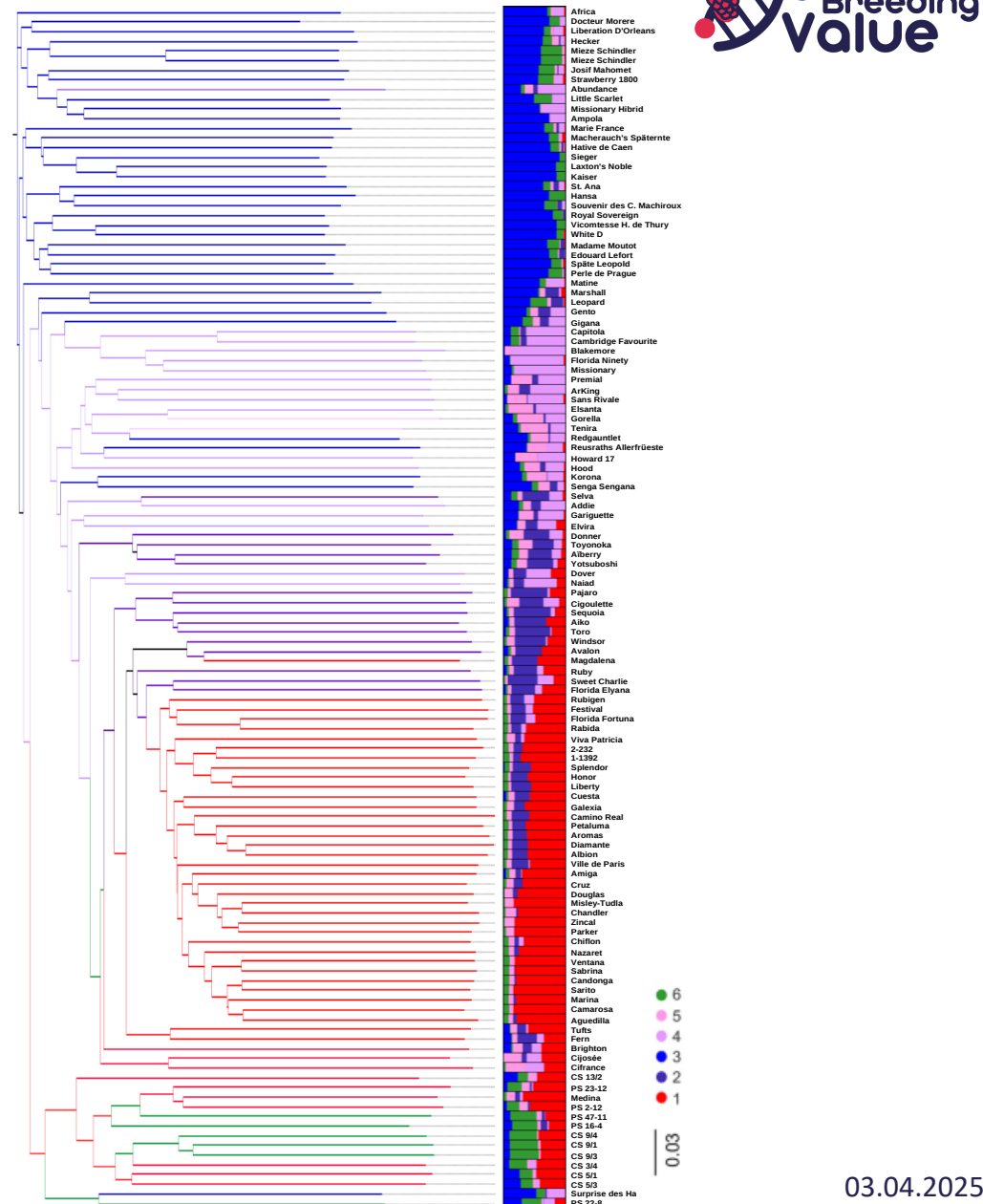
F. × ananassa and some hybrids with *F. chiloensis*

Genotyping: **50K Fana Axiom Array** = >40K SNPs

Genetic diversity and population structure of the experimental population



Software STRUCTURE: Six subpopulations but considerable admixture
 $F_{ST} = 0.13$: Limited Differentiation.



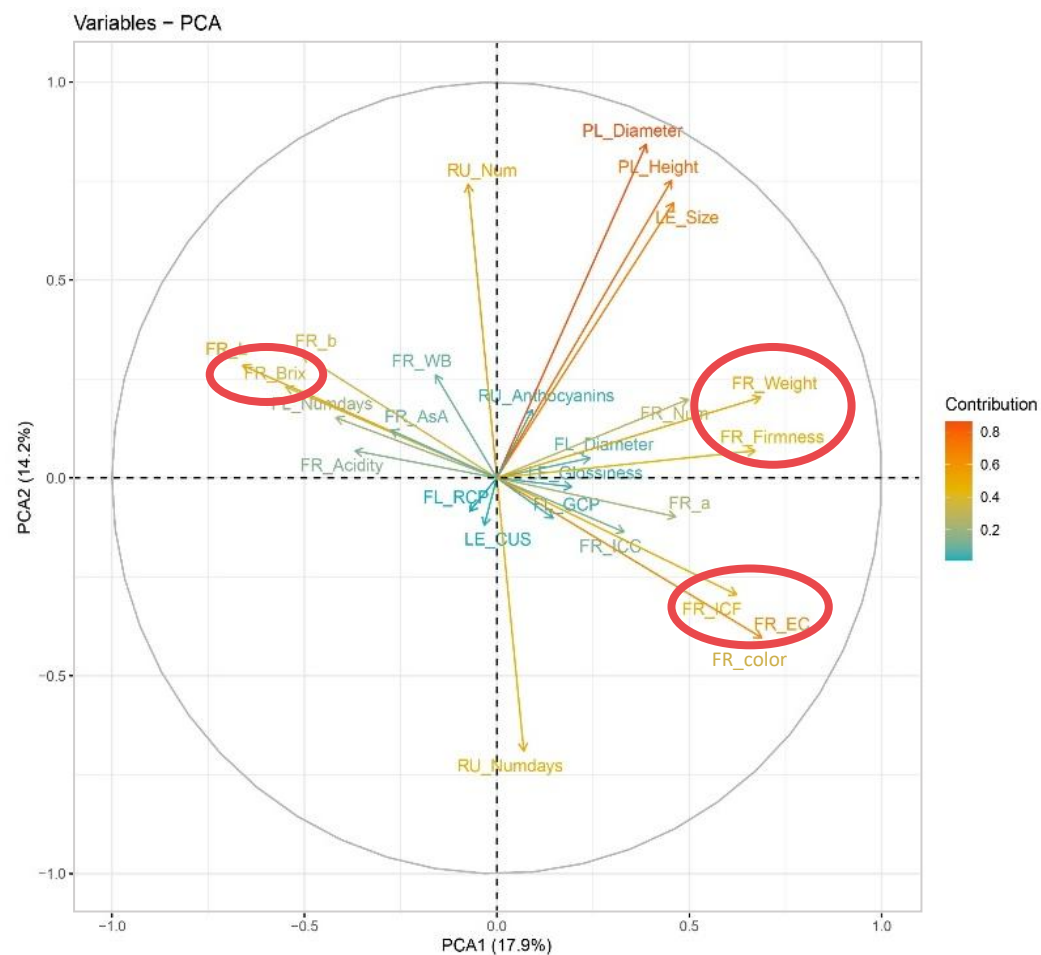


Experimental population phenotyping

Name	Trait		2020-2021		2019-2020		2018-2019		Correlations		
	Abbreviation	Units	Mean ($\pm$ sd)	Range	Mean ($\pm$ sd)	Range	Mean ($\pm$ sd)	Range	20-21 vs. 19-20	20-21 vs. 18-19	19-20 vs. 18-19
Flowering Time	FL_Days	d	111.95 $\pm$ 36.11	48 - 220	164.62 $\pm$ 38.63	58 - 237	111.56 $\pm$ 34.32	27 - 200	0.49*	0.34*	0.33*
Green Color in Petals	FL_GCP	2 - point scale	1.14 $\pm$ 0.35	1 - 2	1.02 $\pm$ 0.06	1 - 2	1.03 $\pm$ 0.12	1 - 2	0.14	0.15	0.15
Red Color in Petals	FL_RCP	3 - point scale	1.13 $\pm$ 0.34	1 - 3	1.03 $\pm$ 0.12	1 - 3	1.15 $\pm$ 0.37	1 - 3	0.50*	0.48*	0.41*
Flower Diameter	FL_Diameter	3 - point scale	5.00 $\pm$ 0.32	3 - 7	4.95 $\pm$ 0.60	3 - 7	4.80 $\pm$ 0.83	3 - 7	0.23*	0.13	0.44*
Leaf Color Upper Side	LE_CUS	5 - point scale	3.27 $\pm$ 0.68	1 - 5	3.17 $\pm$ 0.62	1 - 5	2.98 $\pm$ 0.33	1 - 5	0.57*	0.47*	0.35*
Leaf Glossiness	LE_Glossiness	3 - point scale	2.00 $\pm$ 0.47	1 - 3	2.13 $\pm$ 0.45	1 - 3	1.97 $\pm$ 0.35	1 - 3	0.40*	0.41*	0.49*
Leaf Size	LE_Size	3 - point scale	4.74 $\pm$ 0.99	3 - 7	4.94 $\pm$ 0.95	3 - 7	-	-	0.59*	-	-
Leaf Mildew	LE_Mildew	5 - point scale	-	-	-	-	0.34 $\pm$ 0.76	0 - 4	-	-	-
Plant Height	PL_Height	cm	19.01 $\pm$ 3.98	7.17 - 31.50	16.09 $\pm$ 3.77	6.40 - 27.83	14.38 $\pm$ 3.86	4 - 23.50	0.63*	0.61*	0.60*
Plant Diameter	PL_Diameter	cm	33.22 $\pm$ 8.35	11.50 - 60.67	28.60 $\pm$ 6.70	14.40 - 47.50	25.42 $\pm$ 5.63	14 - 38.00	0.65*	0.55*	0.59*
Runnening Time	RU_Days	d	223.99 $\pm$ 22.77	121 - 264	247.89 $\pm$ 22.81	202 - 303	256.17 $\pm$ 29.68	175 - 303	0.67*	0.82*	0.71*
Runner Number	RU_Num	n° of runners	5.83 $\pm$ 4.33	0 - 20	4.88 $\pm$ 3.60	0 - 19	3.14 $\pm$ 2.68	0 - 12	0.72*	0.66*	0.71*
Runner Anthocyanins	RU_Anthocyanins	5 - point scale	2.79 $\pm$ 0.87	1 - 5	2.33 $\pm$ 0.81	1 - 5	2.62 $\pm$ 0.97	1 - 5	0.53*	0.78*	0.73*
Fruit Number	FR_Num	n° of fruits	15.14 $\pm$ 6.10	4 - 32	9.03 $\pm$ 4.54	0 - 25	6.49 $\pm$ 3.36	0 - 14	0.60*	0.22*	0.15
Fruit Weight	FR_Weight	g	9.33 $\pm$ 5.29	0.67 - 23.44	8.04 $\pm$ 4.55	0.83 - 27.11	-	-	0.71*	-	-
Fruit Width of Band without achenes	FR_WB	5 - point scale	3.28 $\pm$ 1.07	1 - 9	3.12 $\pm$ 1.01	1 - 9	2.84 $\pm$ 0.97	1 - 9	0.53*	0.61*	0.59*
Fruit External Color	FR_EC	7 - point scale	4.67 $\pm$ 0.93	1 - 7	4.76 $\pm$ 0.80	1 - 7	-	-	0.64*	-	-
Fruit External Color: Lightness	FR_L*	black (0) - white (100)	30.18 $\pm$ 3.89	23.30 - 50.19	30.95 $\pm$ 3.86	23.23 - 52.38	-	-	0.77*	-	-
Fruit External Color: red-green	FR_a*	green (-a) - red (+a)	38.60 $\pm$ 4.40	9.85 - 49.40	41.27 $\pm$ 4.39	9.59 - 47.85	-	-	0.78*	-	-
Fruit External Color: yellow-blue	FR_b*	blue (-b) - yellow (+b)	20.89 $\pm$ 3.52	13.14 - 29.29	22.78 $\pm$ 3.66	14.63 - 32.29	-	-	0.57*	-	-
Fruit Internal Color: Flesh	FR_ICF	6 - point scale	4.59 $\pm$ 0.98	1 - 6	4.14 $\pm$ 1.31	1 - 6	-	-	0.44*	-	-
Fruit Internal Color: Core	FR_ICC	3 - point scale	2.09 $\pm$ 0.54	1 - 3	2.01 $\pm$ 0.66	1 - 3	-	-	0.36*	-	-
Fruit Firmness	FR_Firmness	g	170.91 $\pm$ 54.21	100 - 303.13	197.27 $\pm$ 67.24	101.25 - 340	211.36 $\pm$ 50.48	127.5 - 324.38	0.87*	0.27*	0.20*
Fruit Acidity	FR_Acidity	g kg ⁻¹	11.80 $\pm$ 2.50	4.70 - 19.50	11.30 $\pm$ 2.40	1.20 - 20.10	-	-	0.68*	-	-
Fruit Soluble solids content (SSC)	FR_Brix	%	7.61 $\pm$ 1.58	4.93 - 11.80	8.09 $\pm$ 1.13	5.87 - 10.90	-	-	0.65*	-	-
Fruit Ascorbic Acid	FR_AsA	mg kg ⁻¹	473.2 $\pm$ 116.6	161.1 - 834.9	478.6 $\pm$ 126.3	208.7 - 812.5	-	-	0.73*	-	-

Muñoz et al. (2024) *Genome-wide association studies in a diverse strawberry collection unveil loci controlling agronomic and fruit quality traits. Plant Genome*, **17**, e20509.

Modern accessions are characterized by larger plants with larger and firmer fruits but less sweet



Marker-trait associations

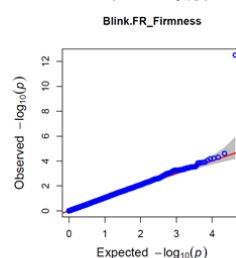
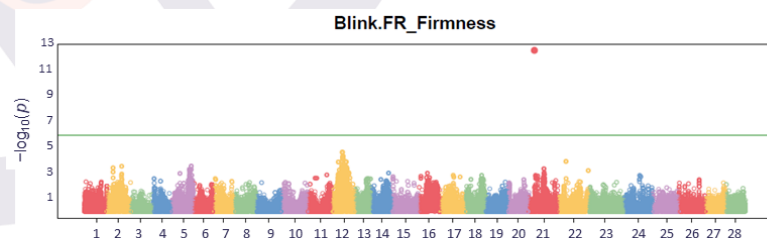
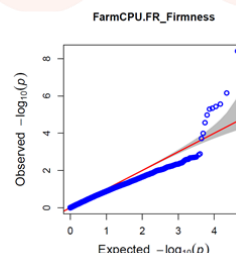
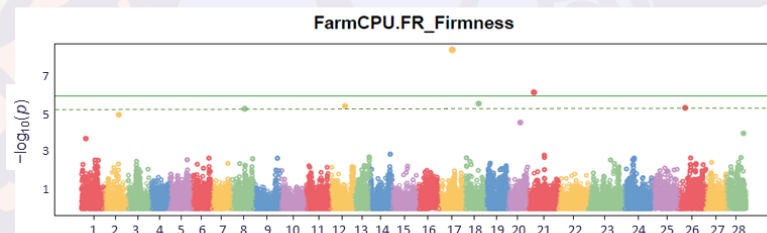
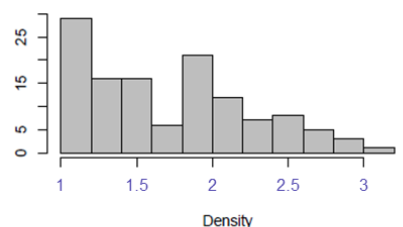
- 121 significant marker-trait associations detected in 19 of the 26 evaluated traits, distributed in 95 QTL, in the three seasons and with the four GWAS models (GLM, MLM, FarmCPU, BLINK).

Trait	Significant SNPs	Significant QTL
PL_Diameter	2	2
RU_Anthocyanins	1	1
RU_Days	10	8
RU_Num	10	8
LE_CUS	4	4
LE_Glossiness	1	1
LE_Mildew	1	1
FL_RCP	10	8
FL_Diameter	1	1
FR_Num	12	12
FR_Weight	4	4
FR_WB	1	1
FR_L*	6	5
FR_a*	13	12
FR_b*	4	4
FR_EC	4	4
FR_Acidity	2	2
FR_Brix	10	10
FR_Firmness	25	7
<i>Total</i>	<i>121</i>	<i>95</i>

GENOME-WIDE ASSOCIATION STUDIES IN BERRY PROGRAMS: FRUIT FIRMNESS

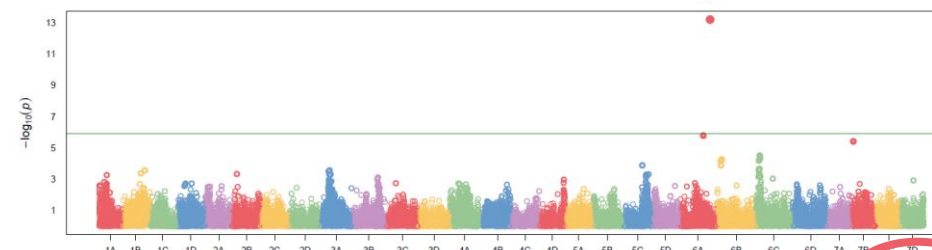
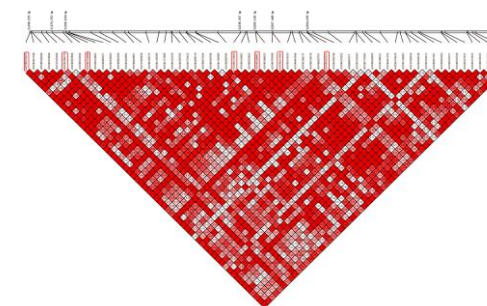
Six QTL detected for fruit firmness in 2020-2021

2020-2021



Chr. 6A

This haploblock is 891 kb long and
encloses 177 genes
5 candidate genes but only one
expressed: *FaPG1*



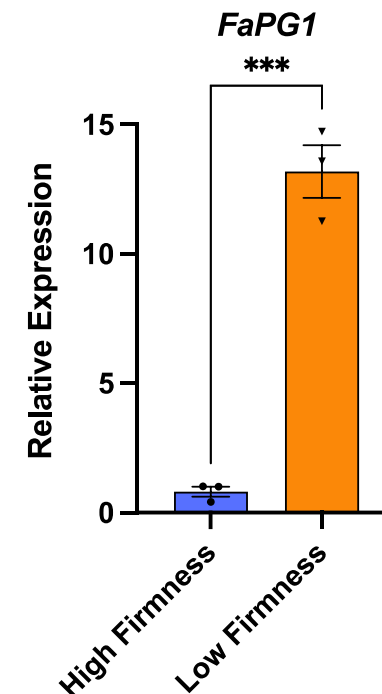
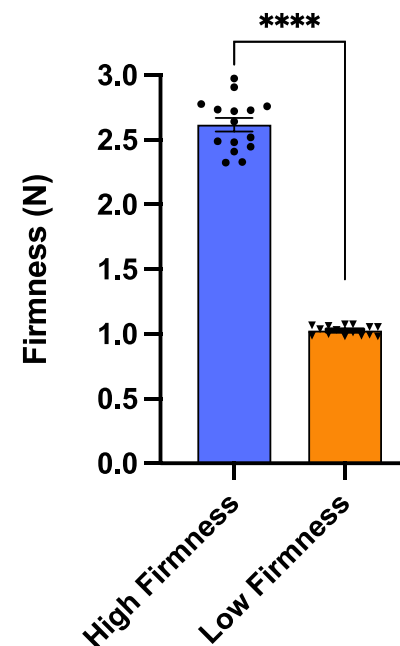
SNP	Chr	Position	P.value	MAF	H&B.P.Value	Effect	PVE (%)
AX-184210669	6A	28,017,174	6.19E-14	0.42	2.53E-09	-43.39	43.97

FaPG1 expression is downregulated in firmer fruits

HIGH FIRMNESS POOL

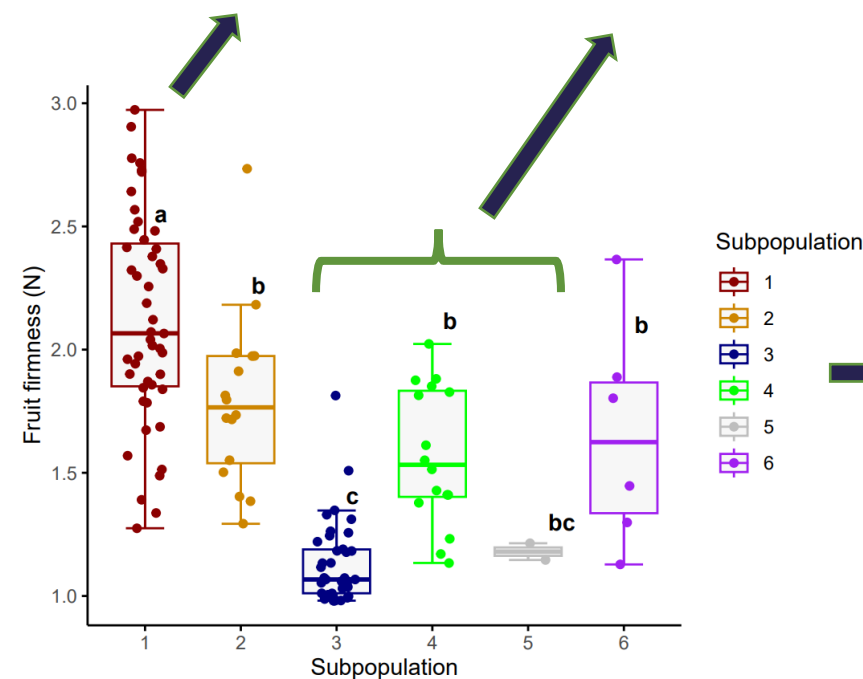
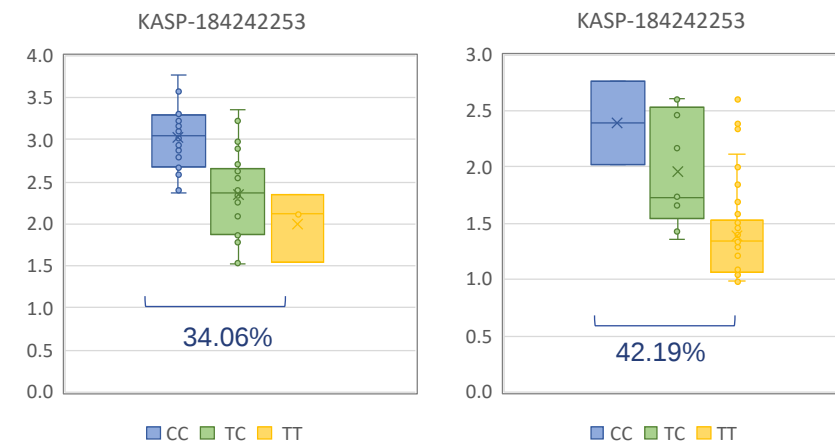
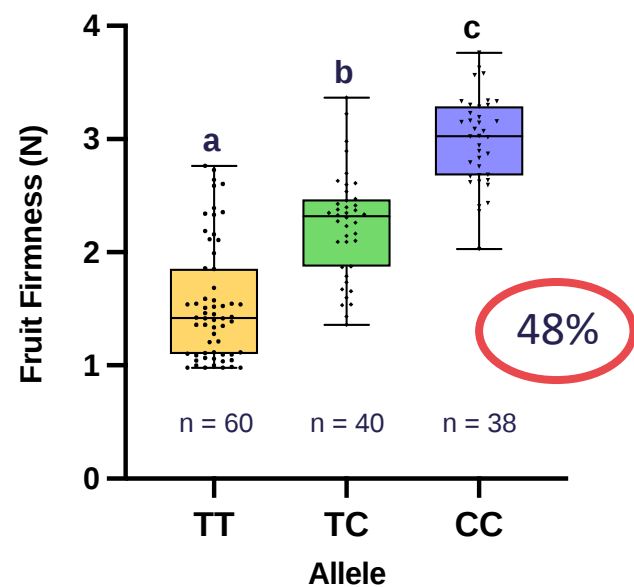
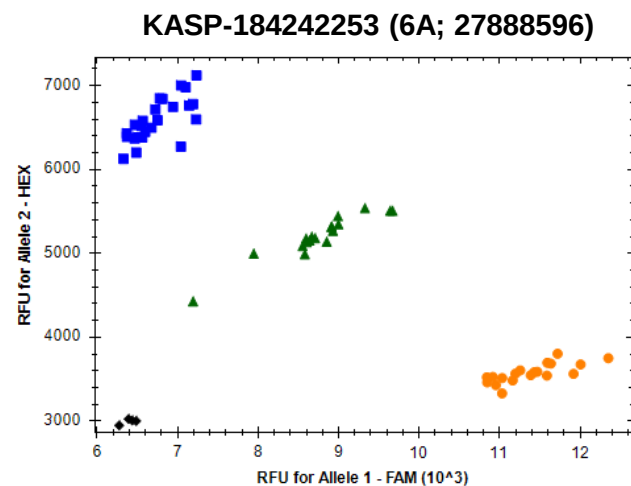
LOW FIRMNESS POOL

High_Firmness Pool			Low-Firmness Pool		
ACC ID	Name	Firmness (N)	ACC ID	Name	Firmness (N)
940	Sarito	2.973	251	Vicomtesse Hericart de Thury	0.981
733	Galexia	2.905	845	Strawberry 1800	0.981
732	Florida Festival	2.777	204	Macherauchs Späternte	0.987
870	Florida Fortuna	2.758	836	Matine	0.987
868	Florida Elyana	2.734	837	Ampola	0.993
965	Sabrina	2.728	177	Hansa	0.999
480	Amiga	2.721	262	Marie France	1.011
986	Rabida	2.642	830	Kaiser	1.030
715	Candongga	2.519	179	Josif Mahomet	1.036
839	Rubigen	2.489	311	Liberation D'Orleans	1.054
795	Sel. 1392	2.482	833	St. Ana	1.054
674	Chiflon	2.446	307	Mieze Schindler	1.060
953	Viva Patricia	2.409	320	Sieger	1.067
1016	Nazaret	2.329	213	Mara Des Bois	1.073
72	Camarosa	2.323	725	Missionary Hibrid	1.073



Together with previous knockdown experiments (Garcia-Gago et al., 2009; Quesada et al., 2009; Posé et al., 2013; López-Casado et al., 2023), our results indicate that *FaPG1* is the main contributor to natural variation in strawberry fruit firmness.

Firmness diagnostic marker

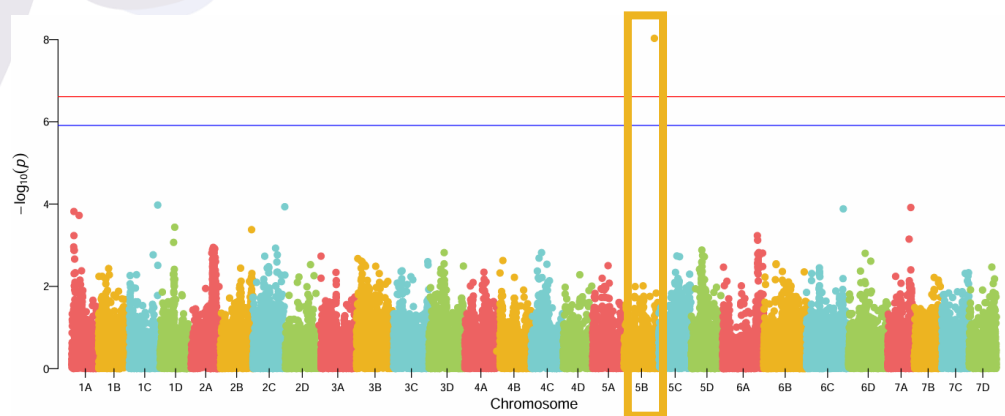
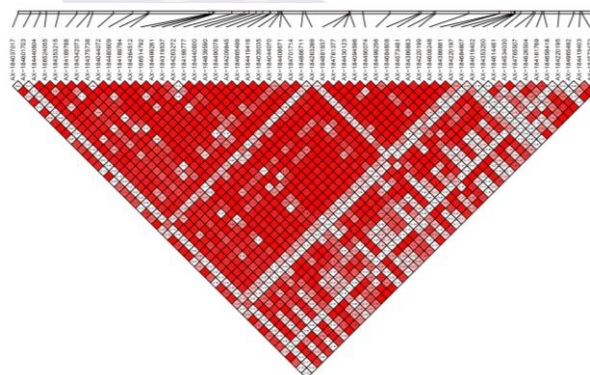


Genomic signatures of domestication

Hardigan et al., 2021;
Fan and Whitaker, 2023

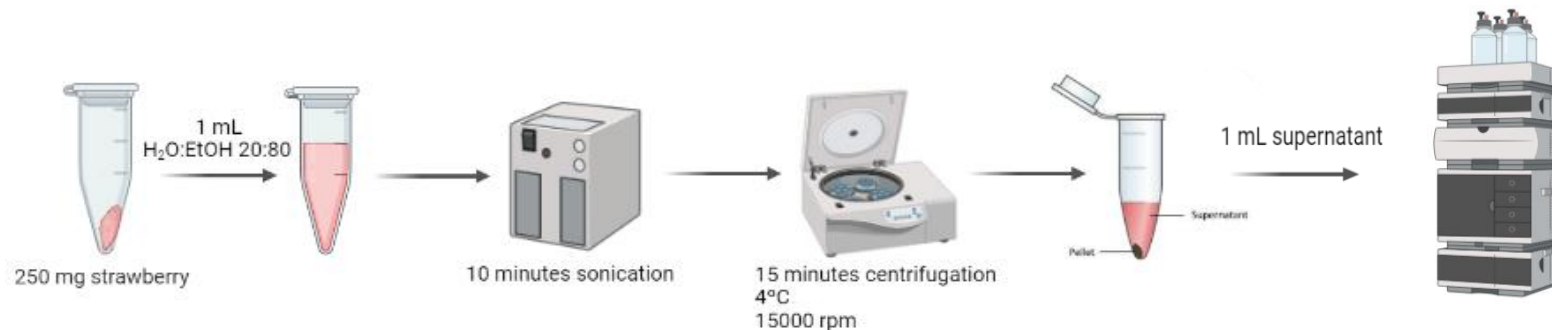
GENOME-WIDE ASSOCIATION STUDIES IN BERRY PROGRAMS: SUGAR CONTENT

1.3 Mb long
Chr_5B: 8349464-9659261
176 genes

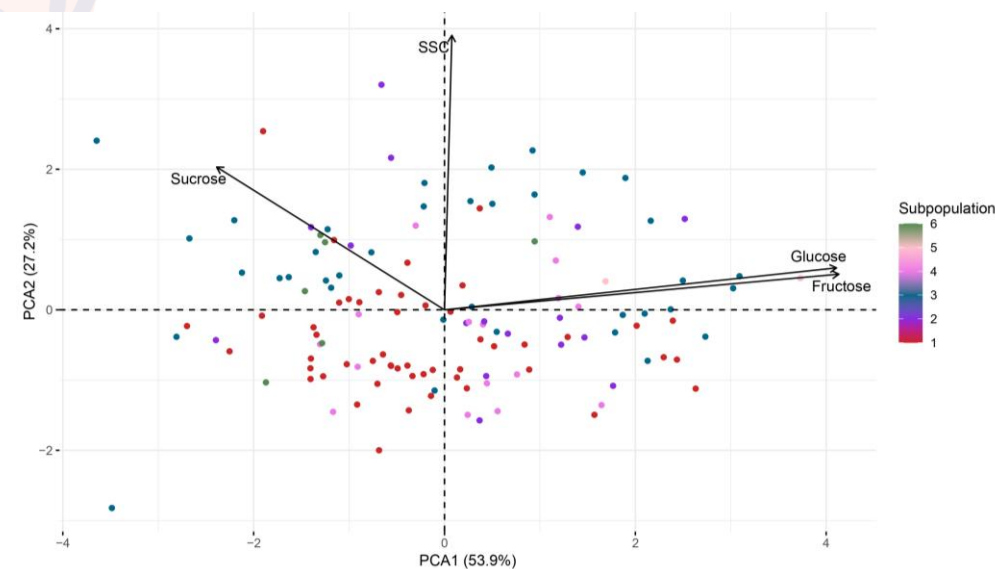
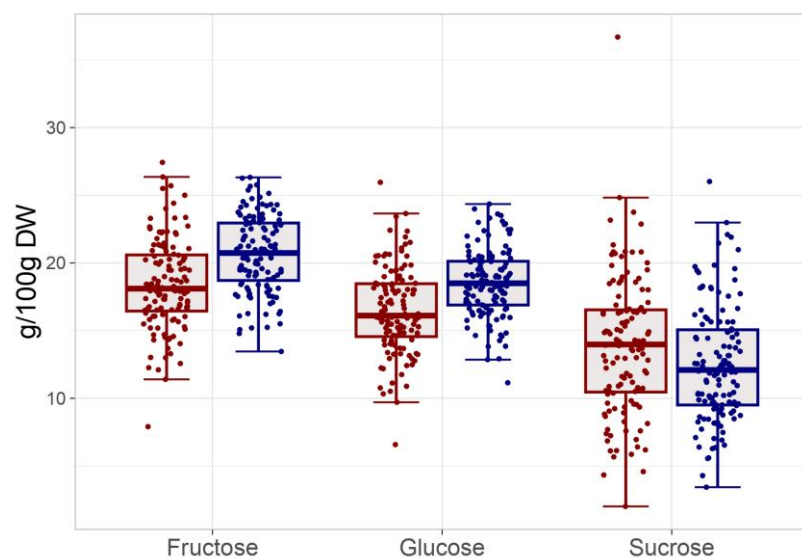


Quantification of sucrose, fructose and glucose

Javier Roldán



In coloboration with Dr. José Manuel Moreno Rojas & Dr. José Luis Ordóñez Díaz (IFAPA Córdoba. Spain)

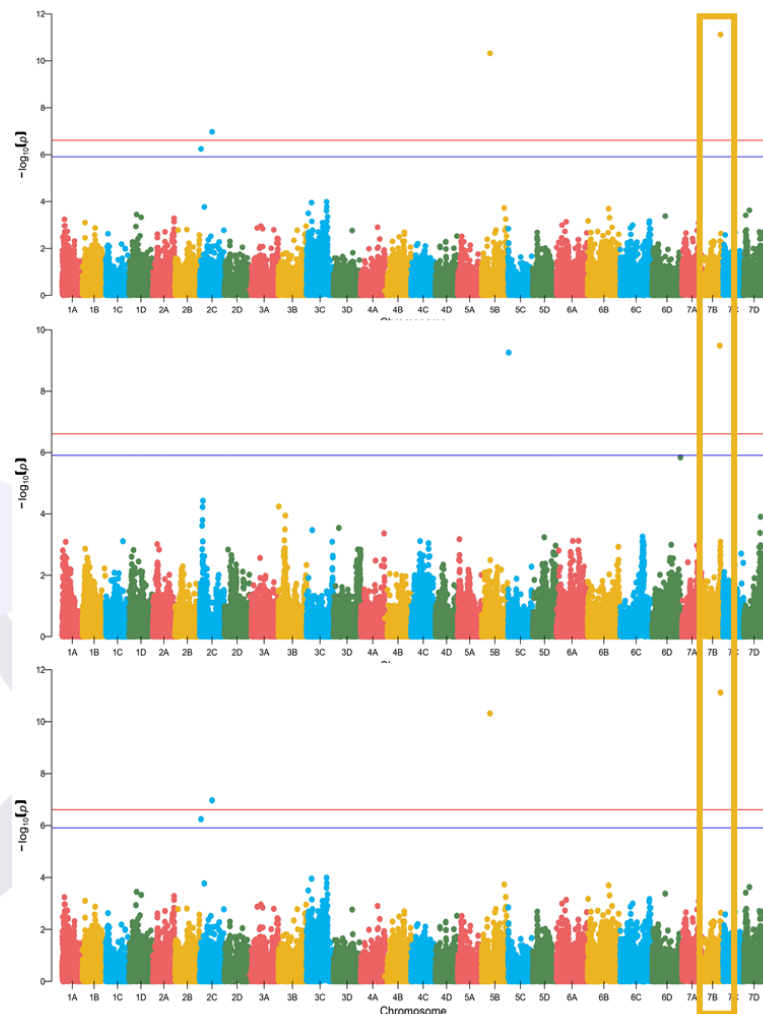


GWAS for sugar content

Sucrose both seasons (BLINK)

Fructose both seasons (BLINK)

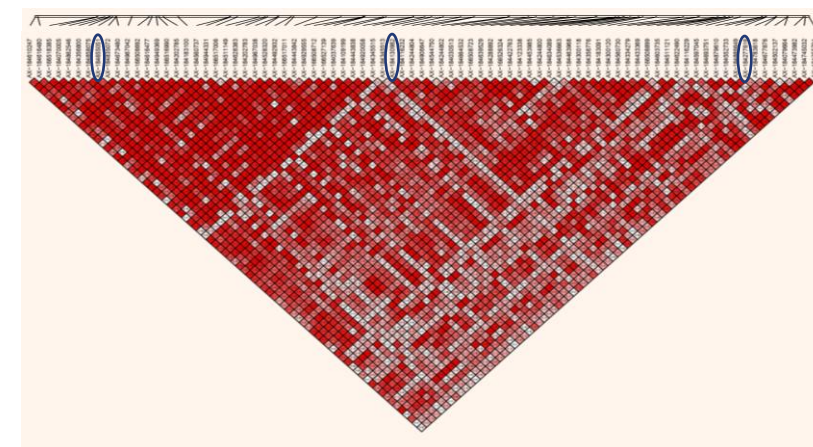
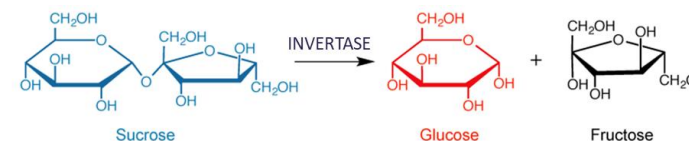
Glucose both seasons (BLINK)



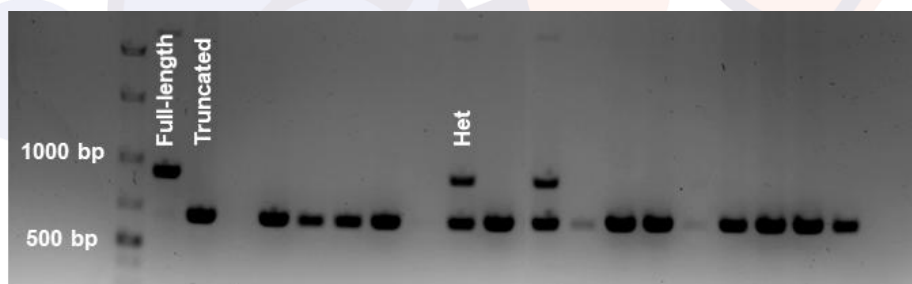
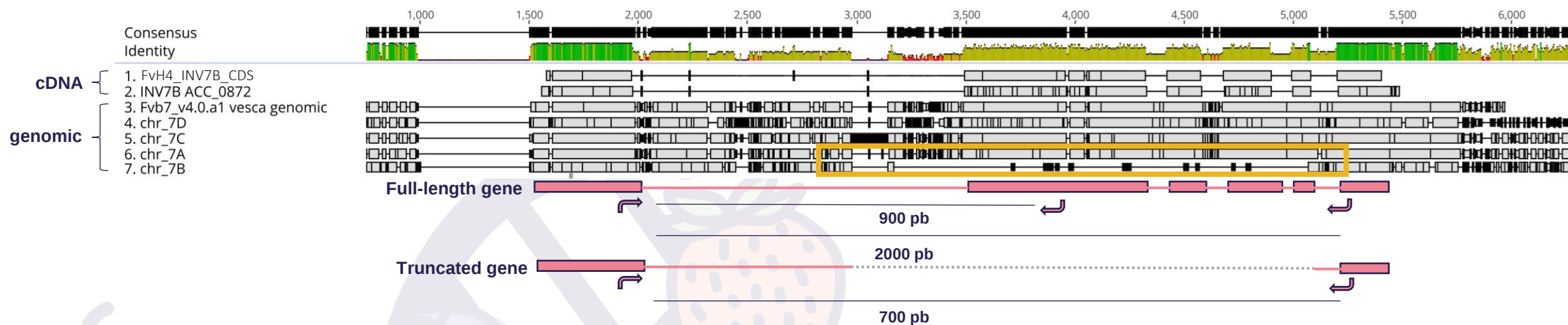
AX-184277616
PVE: 26.31%

AX-184655051
PVE: 35.64%

AX-184655051
PVE: 19.74%

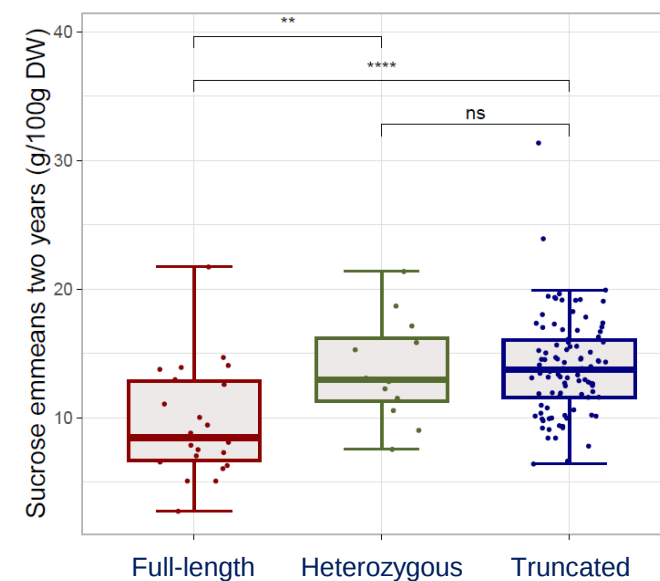


- 1 Mb long: Chr_7B: 19480943-20512360
- Enclosing 201 genes ('Royal Royce' annotation)



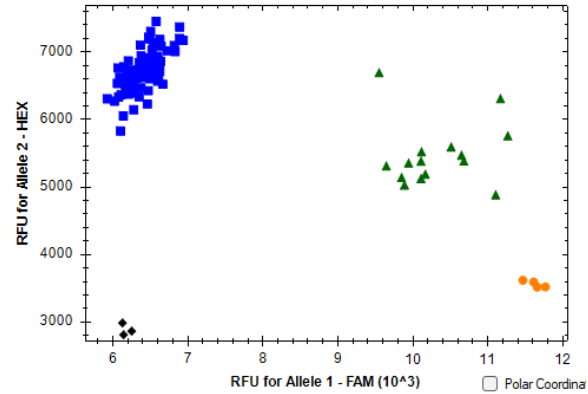
The truncated version of the *INV(7B)* gene correlates with higher sucrose content

SUCROSE CONTENT

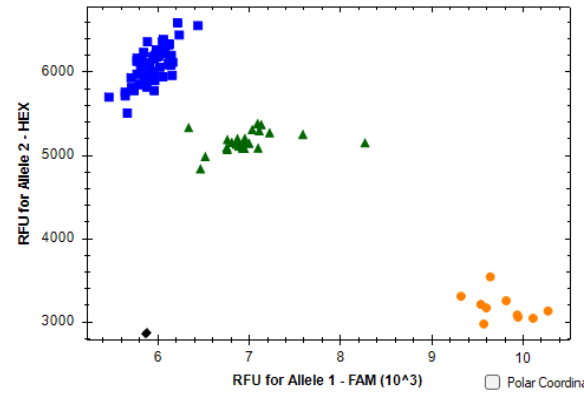


Sugar content diagnostic markers

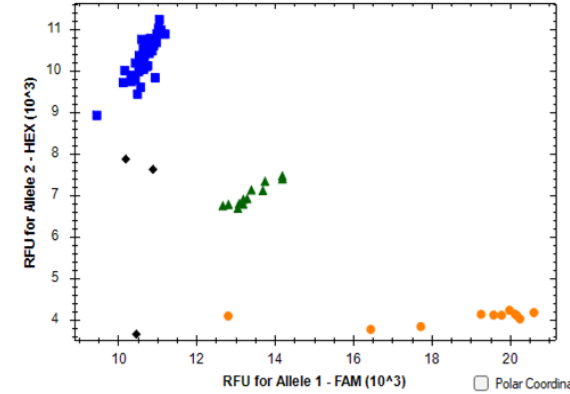
KASP SSC-5B SNP1



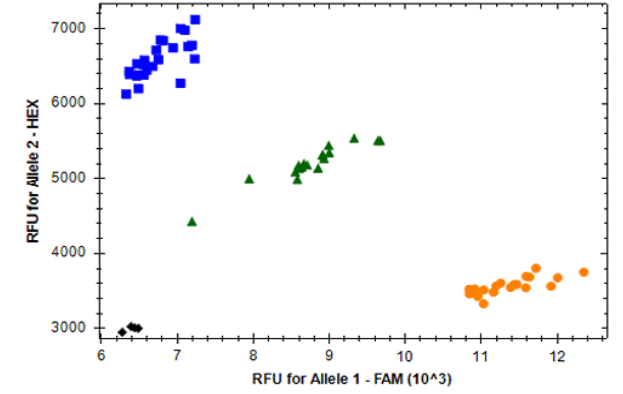
KASP SSC-5B SNP2



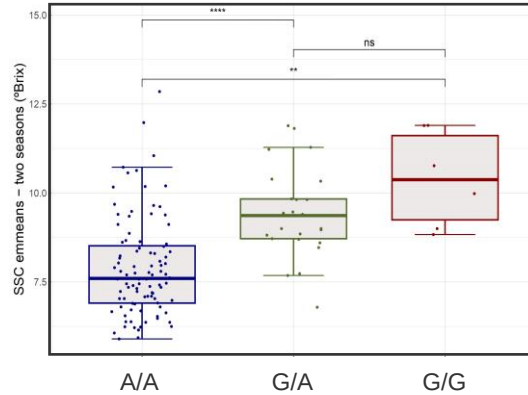
KASP Suc/Fru/Glu-7B



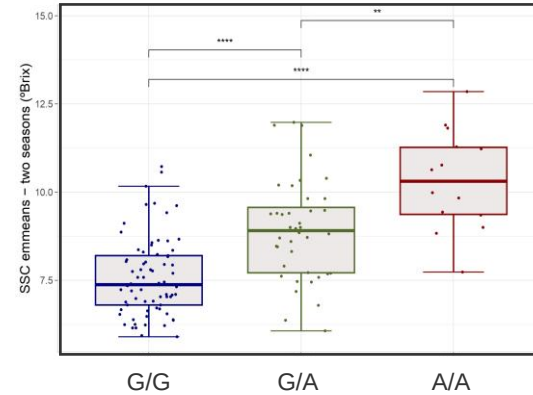
KASP Firmness-6A



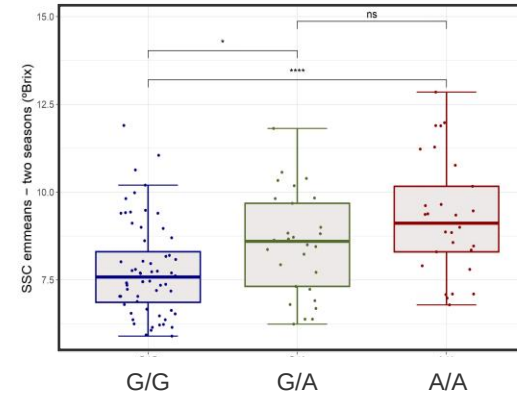
KASP SSC5B SNP1



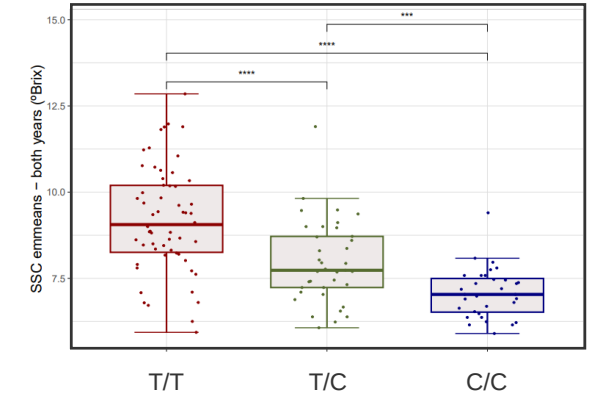
KASP SSC5B SNP2

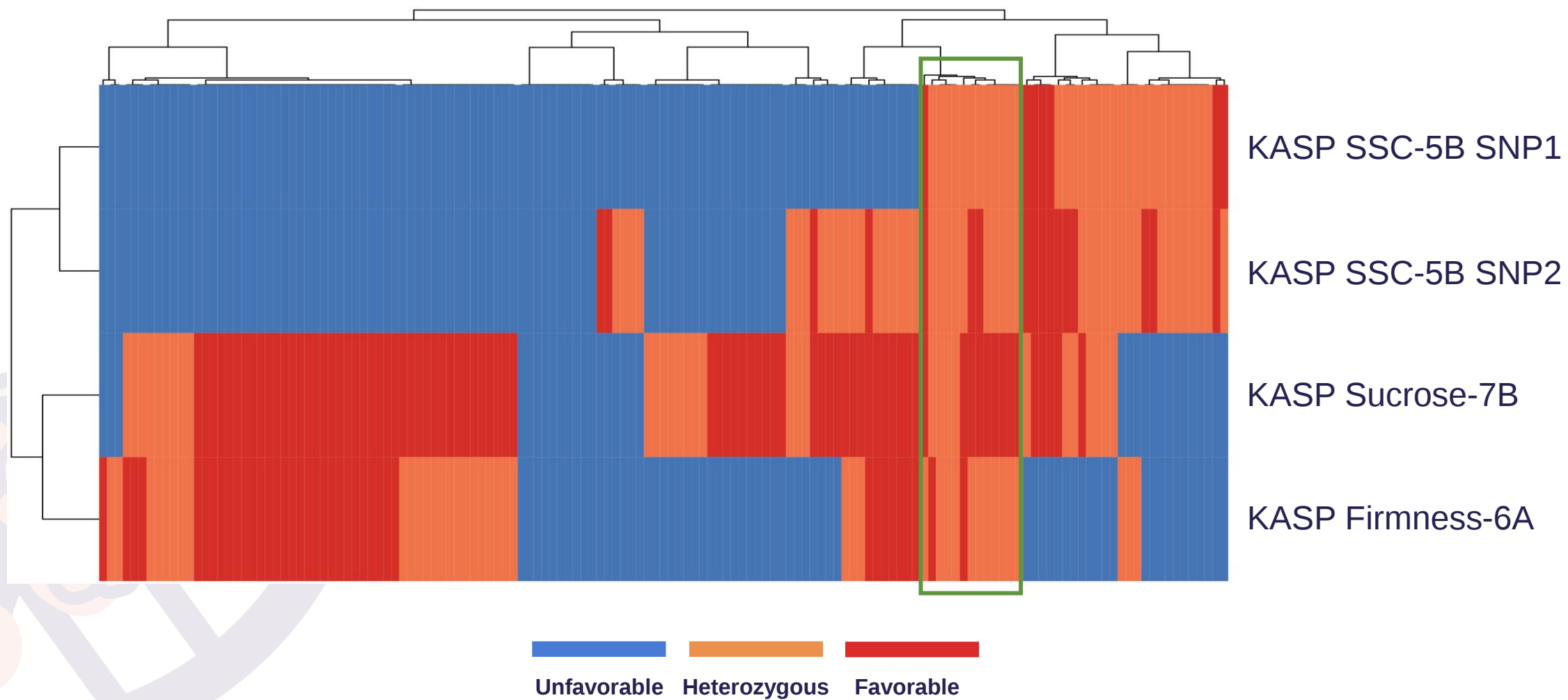


KASP Sucrose 7B

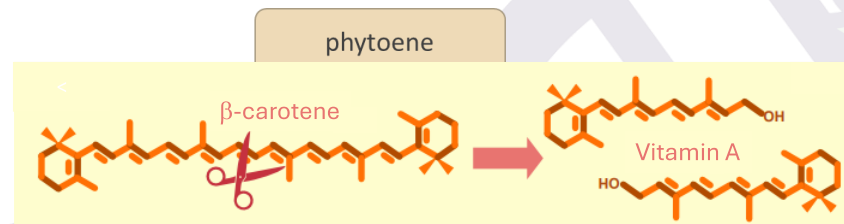


KASP AX-184242253 6A





GENOME-WIDE ASSOCIATION STUDIES IN BERRY PROGRAMS: CAROTENOID CONTENT



β -carotene

α -carotene

CYP97A

CYP97C

zeinoxanthin

α -cryptoxanthin

CYP97C

CYP97A

lutein

lutein epoxide

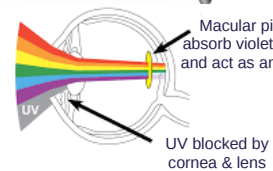
VDE

ZEP

Macular pigments protect the retina



- 90% Lutein
- Zeaxanthin
- Meso-Zeaxanthin

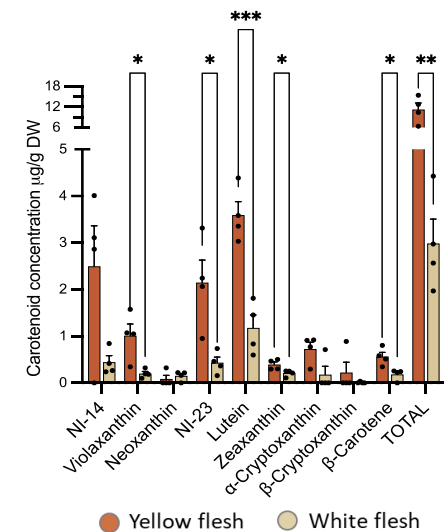
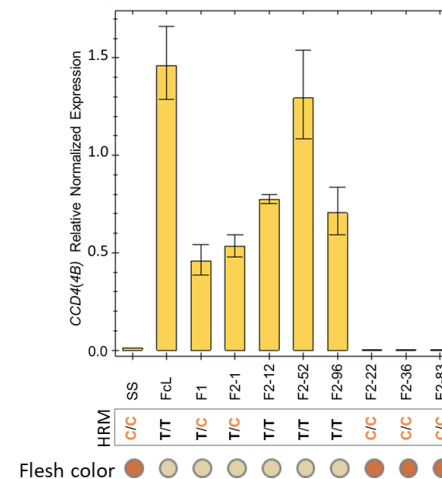


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Differential expression of *CCD4(4B)* drives natural variation in fruit carotenoid content in strawberry (*Fragaria* spp.)

Iraida Amaya^{1,2,*}, F. Javier Roldán-Guerra¹, José L. Ordóñez-Díaz³, Rocio Torreblanca¹, Henning Wagner^{4,5}, Veronika Waurich^{4,5}, Klaus Olbricht⁴, José M. Moreno-Rojas³, José F. Sánchez-Sevilla^{1,2} and Cristina Castillejo^{1,*}

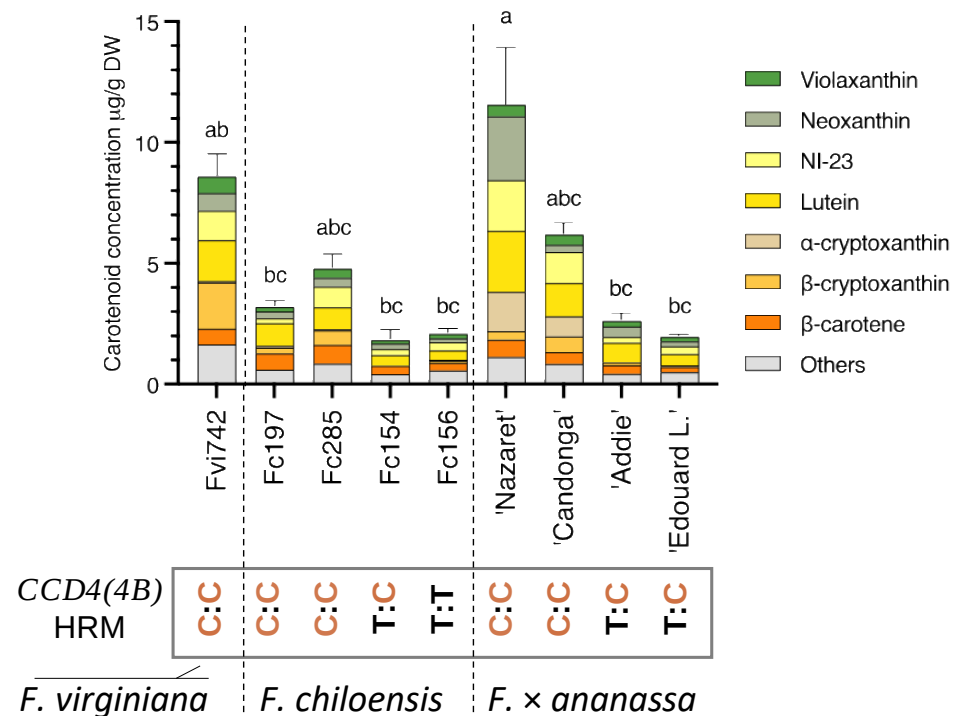


GWAS FOR CAROTENOID CONTENT

Carotenoids quantification-GWAS

In collaboration with Dr. J.M.Moreno Rojas & Dr. J.L.Ordóñez Díaz (IFAPA Córdoba. Spain)

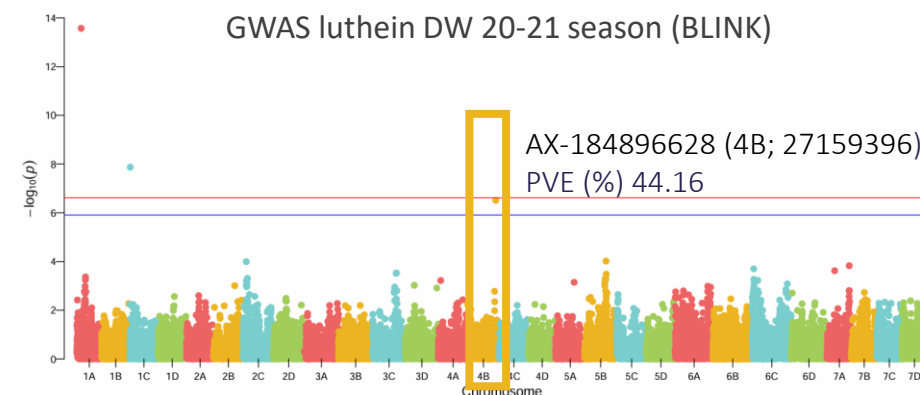
Fruit carotenoid content



Javier Roldán



GWAS lutein DW 20-21 season (BLINK)

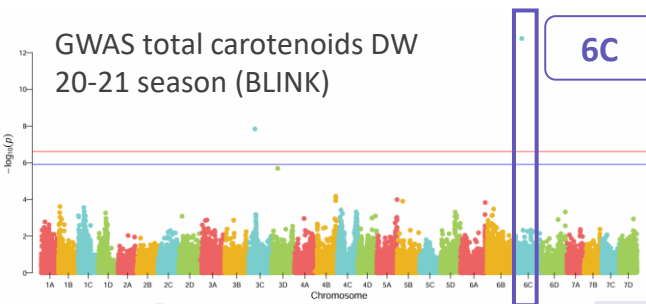


CCD4(4B) chr_4B:29119490..29122619

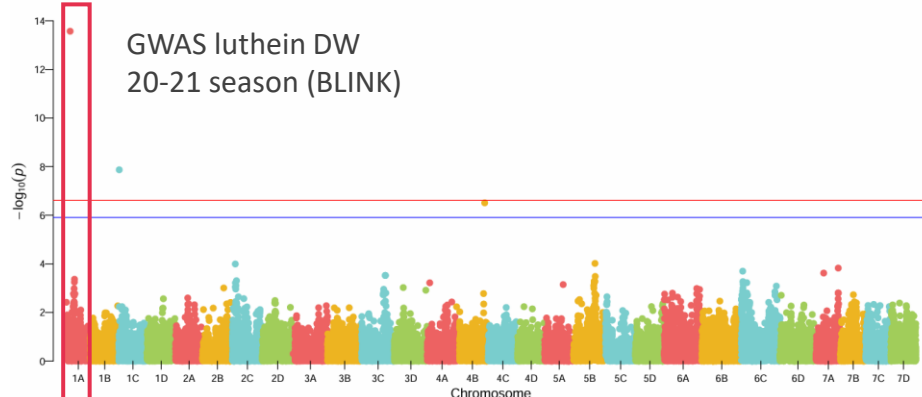
TOTAL CAROTENOIDS

PVE: 40.31 %

6C



1A

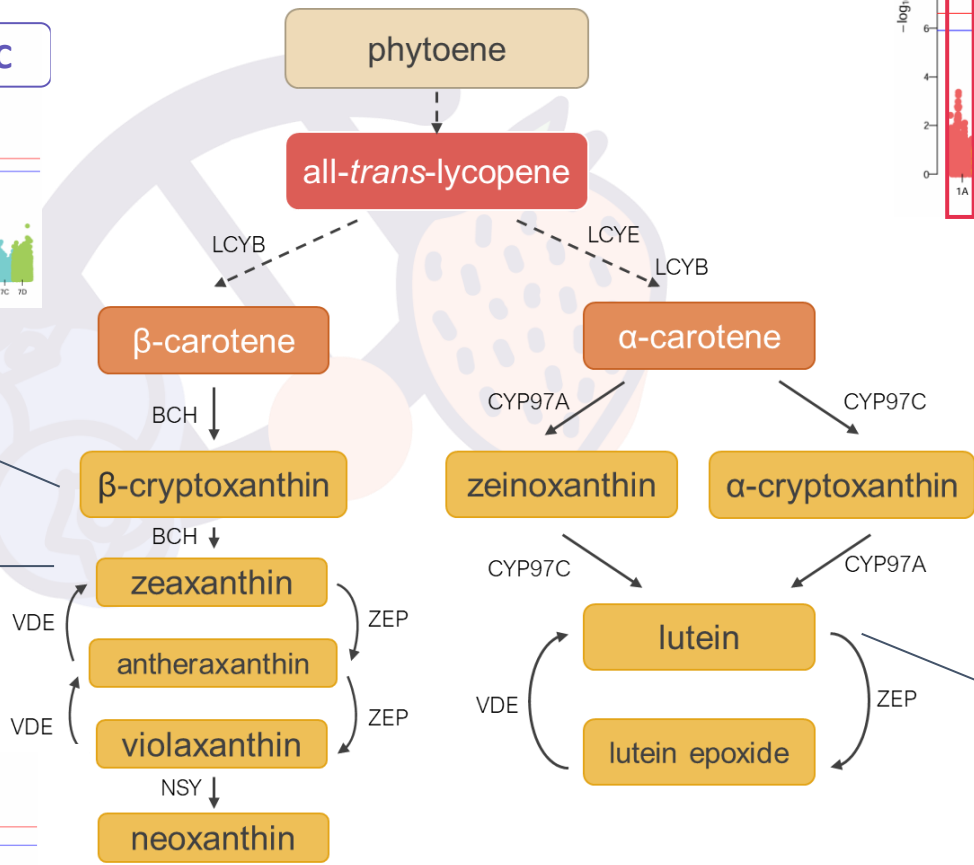
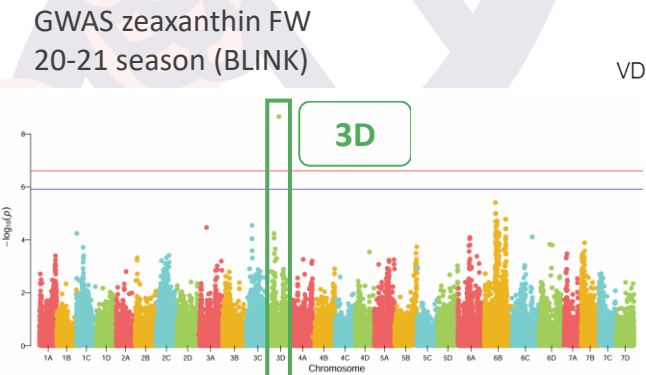


PVE: 41.47 %

3D

PVE: 33.16 %

3D



CCD4(4B)

4B

LCYE

1A

PVE: 30.58-64.84 %

CCD4(4B)

4B

LCYE

1A

PVE: 27.46-30.31 %

Not Identified (NI)-22

6C

PVE: 55.48 %

Strawberry Breeding and Biotechnology Lab

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Formación Agraria
y Pesquera



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- Junta de Andalucía and FEDER (P18-RT-4856)
- EU H2020 BreedingValue Project (No 101000747)
- *Fragaria* Germplasm collection at IFAPA: IFAPA PR.CRF.CRF202200.002, Programa de Desarrollo Rural de Andalucía 2014-2022.