Instituto Andaluz de Investigación
y Formación Agraria, Pesquera, Alimentaria
y de la Producción Ecológica
Consejería de Agricultura, Ganadería,
Pesca y Desarrollo Sostenible

Breeding Value

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

Marker-assisted selection. A custom made low-density strawberry array.

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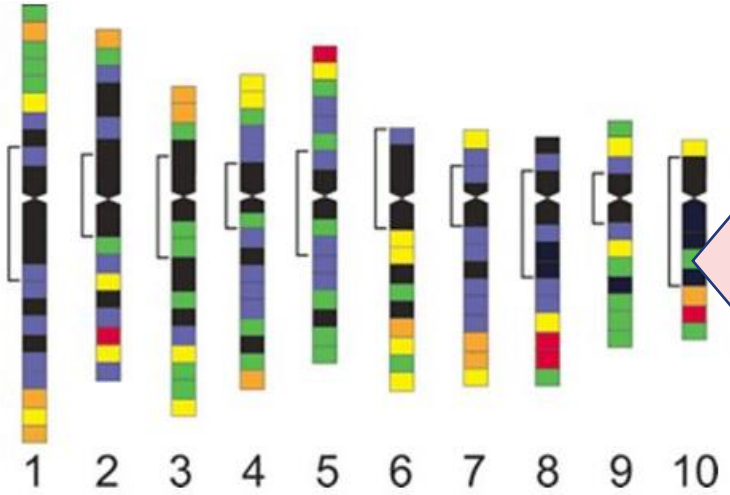
February 14th 2024

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

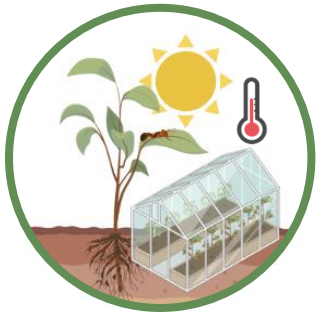
Modern plant breeders use molecular methods such as Marker Assisted Selection (MAS) as well as field studies



Genotype: sequence of all the genes in a genome

Selection using DNA markers is faster than selecting based in phenotype

Phenotype: physical expression of traits



Environment

Molecular Markers increase the efficiency of breeding programs

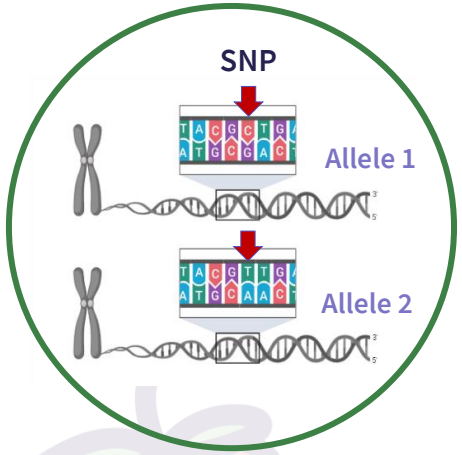
- Time
- Cost

→ Trait, Crop

Genotype is not affected by environmental factors

Development of Markers for MAS

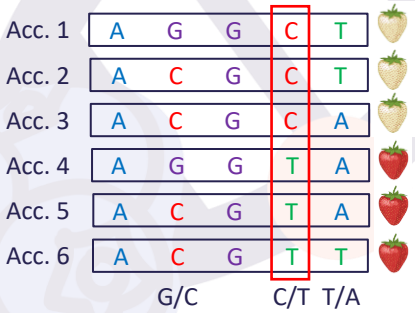
High-throughput markers



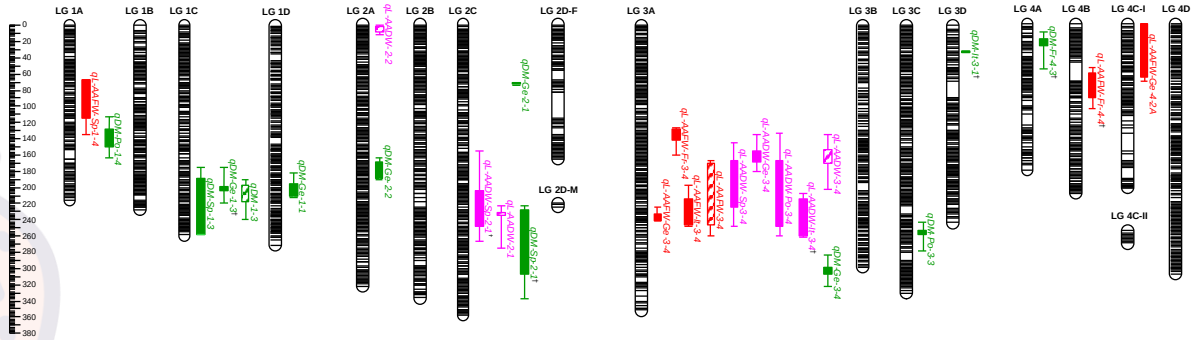
GENOTYPE

PHENOTYPE

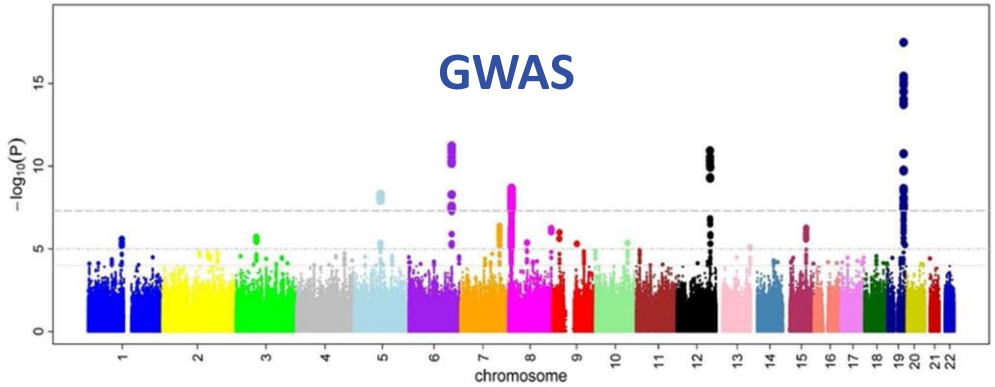
ASSOCIATION



QTL ANALYSIS



GWAS



1. DESIGN OF MARKER ASSAYS FOR LINKED SNP
2. VALIDATION

06.03.2024

Molecular Markers in Plant Breeding

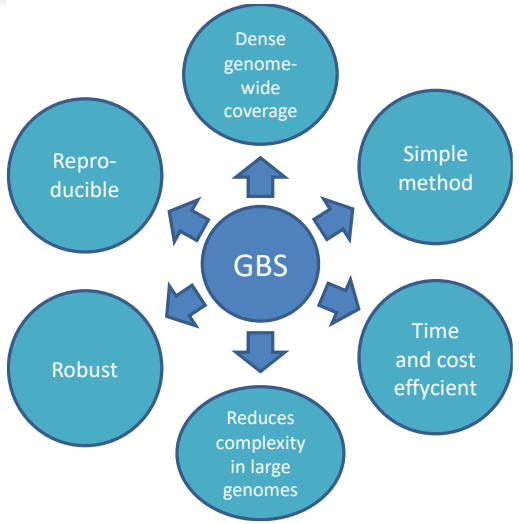
High-throughput markers

1. **High-density SNPs Arrays**
    1. **Illumina**
    2. **Affymetrix**
2. **Genotyping by Sequencing (GBS)**
3. … as costs of sequencing are going down, an alternative: **Whole Genome Sequencing**.

384-array

96-array

850K and 50K Strawberry Axiom SNP arrays: Hardigan *et al.* Genome Synteny Has Been Conserved Among the Octoploid Progenitors of Cultivated Strawberry Over Millions of Years of Evolution. ***Front. Plant Sci.* 10,** 1789 (2020).



Medium or Low-density arrays

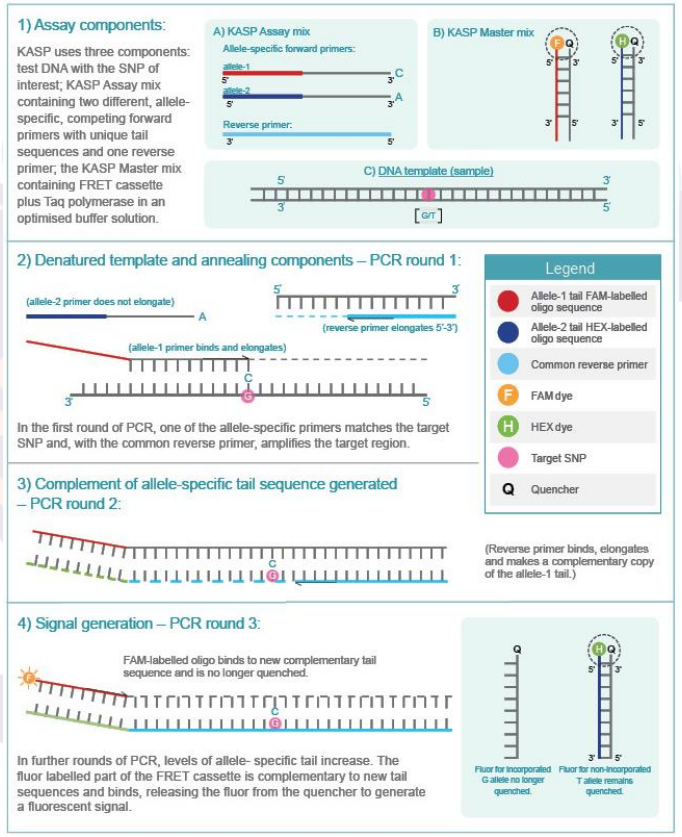
For Medium/low density Assays (< 200 SNPs): Fluidigm Arrays: 48 or 96 SNPs

Single SNP assays: High Resolution Melting (HRM) and Kompetitive Allele Specific PCR (KASP)

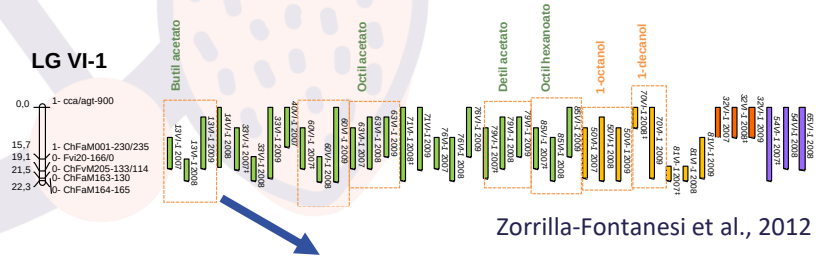
Kompetitive Allele Specific PCR (KASP) Assay

For Medium/low density Assays (< 200 SNPs): Allele specific PCR

1. Submit SNP and 50-100 bp flanking sequence (on either side) to service provider.
2. DNA: 10-100 ng/µl in 10-20 µl. QUALITY: 260/280= 1.7-2.0 y 260/230= 1.7-2.2
3. Marker development is more expensive: Develop marker in 96 samples.
4. Once successful assay, cheaper to genotype markers.

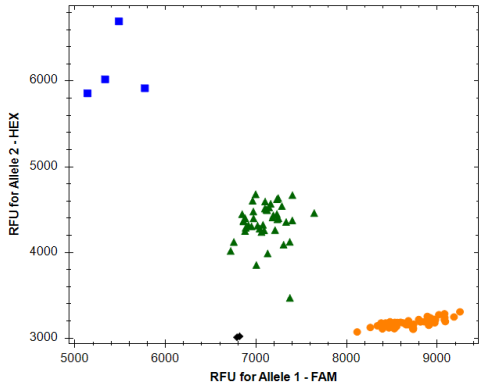


QTL FOR ESTER CONTENT



Zorrilla-Fontanesi et al., 2012

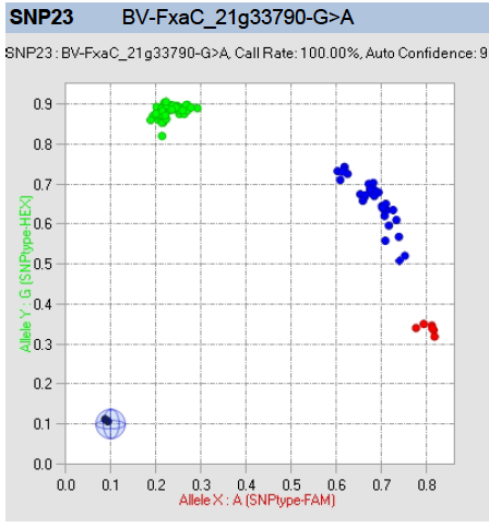
SNP in Candidate gene



KASP ASSAY FOR ESTER CONTENT

Fluidigm Array with SNP Type Assays:

1. Allele Specific Primers:
   - **ASP1-FAM**
   - **ASP2-HEX**
2. Pre-amplification Primer
3. Locus-Specific Primer (**LSP**)



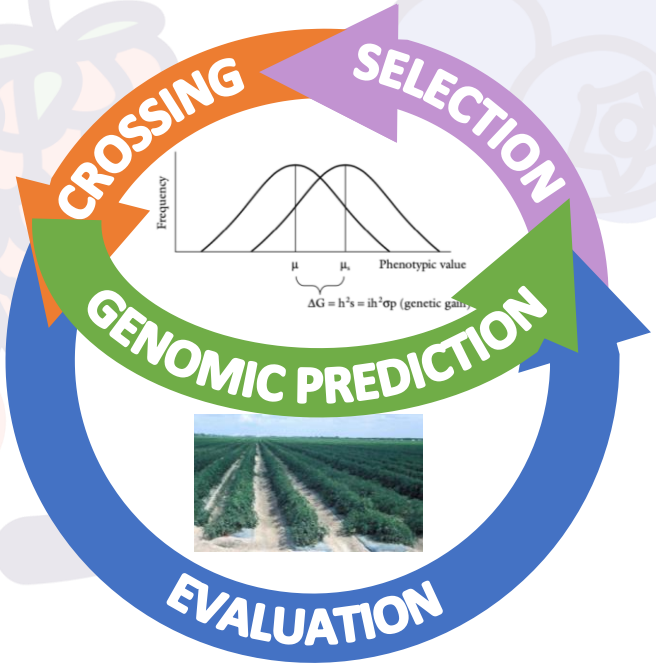
BREEDINGVALUE FLUIDIGM ASSAY FOR ESTER CONTENT

Marker Assisted Selection (MAS)

Traditional MAS is straightforward when a trait is controlled by one or few loci with large effects. However, many traits are controlled by dozens or perhaps even hundreds of loci, each with small effects that are individually difficult to detect.

Genomic Selection (GS)

Genomic Selection is not interested in determining which QTL has significant effects on a trait. GS is only concerned with predicting the performance of individuals.



Training Population

Genotyped and Phenotyped

Validation Population

Genotyped and Phenotyped

Breeding Program

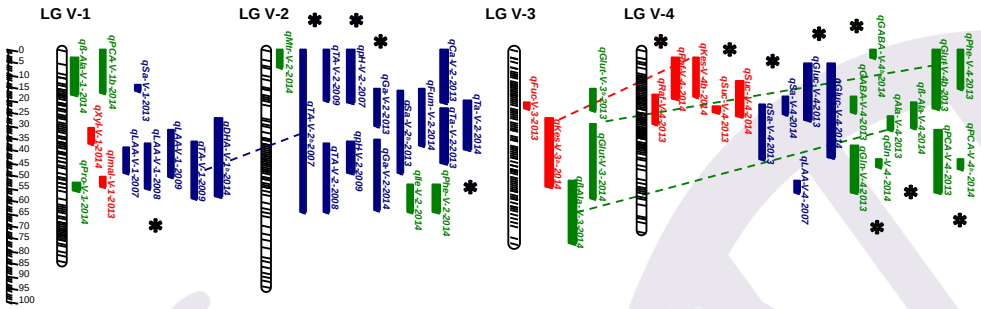
Genotyped. Seedling selection based in prediction model

Whole genome is densely blanketed with markers (HIGH-THROUGHPUT GENOTYPING), and effects are assigned to each marker using a statistical model.

Genetic Control of Fruit Quality traits in Strawberry

Zorrilla-Fontanesi, *et al.* (2011) *Theor. Appl. Genet.* **123,** 755–778

Sugar/acid balance

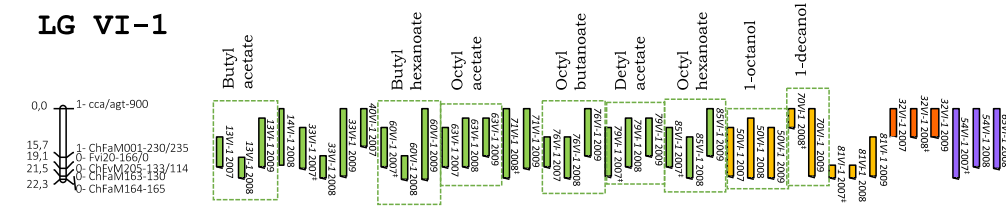


Vallarino, J. G., and Pott, et al. (2019). *Hortic Res* **6**, 4.

Fruit Firmness

Volatile organic compounds

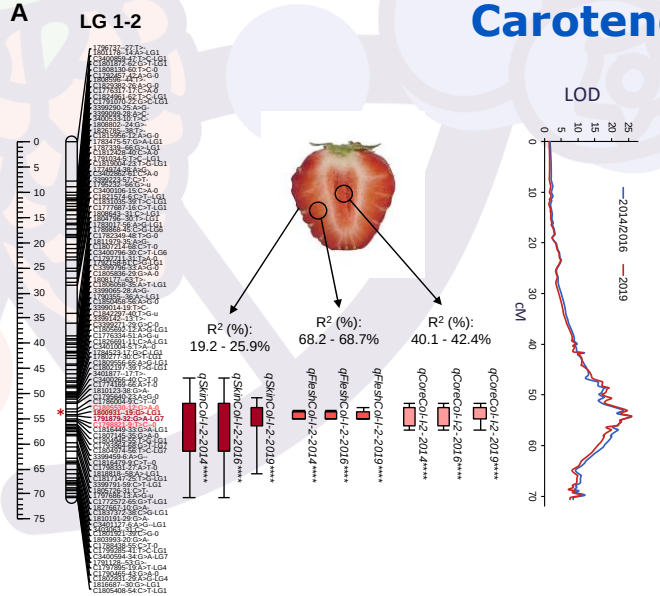
Gene	Chrom.	Position (bp)	Sequence Description	Fold Change	T statistic	P-value
gene15845-v1.0-hybrid	LG6	22474708	cinnamoyl reductase	-11.79	-24.13	0.0002
gene15923-v1.0-hybrid	LG6	22126331	solute carrier family 22 member 15-like protein	-10.64	-10.06	0.0021
gene28918-v1.0-hybrid	LG4	749522	hat family dimerization domain protein	-6.09	-7.23	0.0018
gene16004-v1.0-hybrid	LG5	14899903	---NA---	-5.32	-7.64	0.0046
Gene 131	LG3	1242288	---NA---	-3.99	-15.64	0.0003
Gene 418	LG6	20605123	---NA---	-3.92	-18.20	0.0001



Zorrilla-Fontanesi, *et al.* (2012) *Plant Physiology* **159,** 851–870.

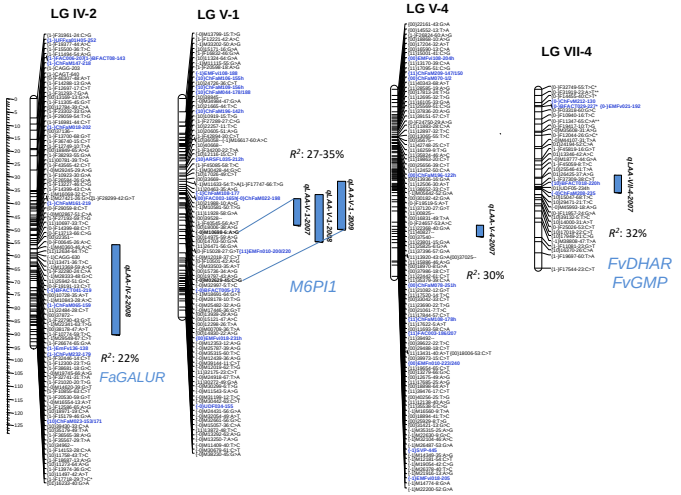
Sánchez-Sevilla, et al. (2014) *BMC Genomics*, **15**, 218.

Anthocyanins Carotenoids / Color



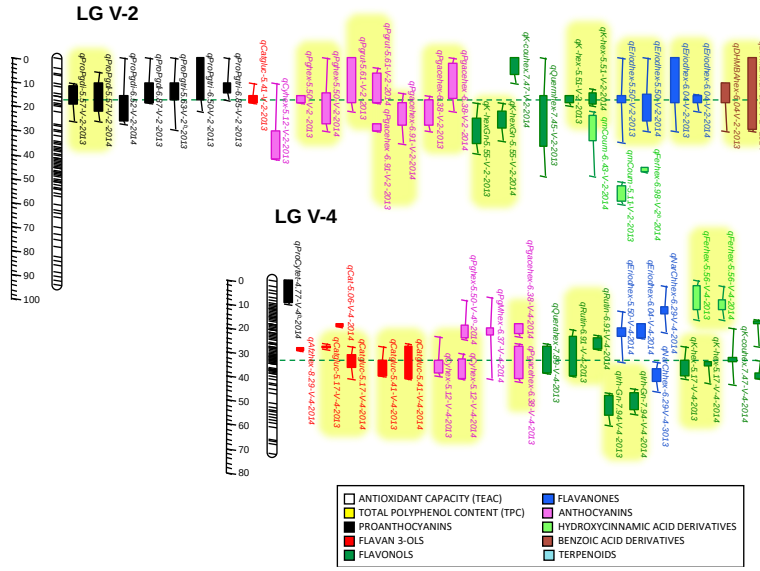
Castillejo et al. (2020). The Plant Cell **32**, 3723-3749.

Ascorbic Acid



Muñoz del Río., et al. (2023). *Hortic Res*, **10**, uhad006.

Antioxidant Compounds



Pott, *et al.* (2020) *Sci. Rep.* **10,** 20197–15.

Strawberry aroma

- More than 360 volatile compounds identifyed in strawberry.
- About 15-20 are key for aroma.

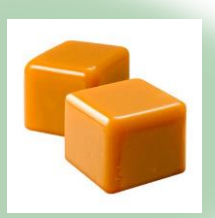
- **Esters**

methyl and ethyl hexanoates
methyl and ethyl butanoates
ethyl-2-methyl butanoate
hexyl and (E)-2-hexenil acetate

Methyl anthranilate
Characteristic of *F. vesca*

- **Furanones**

2,5-di-metil-4-hidroxi-3(2H)-furanone (HDMF, **Furaneol**)
2,5-di-metil-4-metoxi-3-(2H)-furanone (DMMF, **Mesifurane**)



- **Terpenes**

Linalool, terpineol,
Nerolidol…

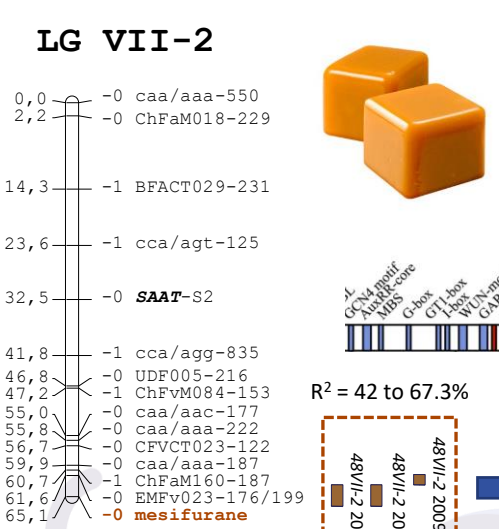
Aldehydes

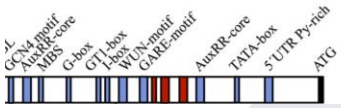
hexanal
(Z)-3-hexenal

- **Ketones**

2-heptanone, **γ–decalactone**

QTL controlling strawberry aroma



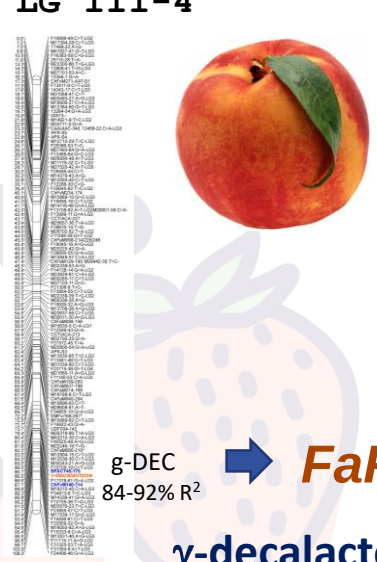


R^2 = 42 to 67.3%

FaOMT

Mesifurane

Zorrilla-Fontanesi, et al. (2012). *Plant Physiol.* 159, 851–870.

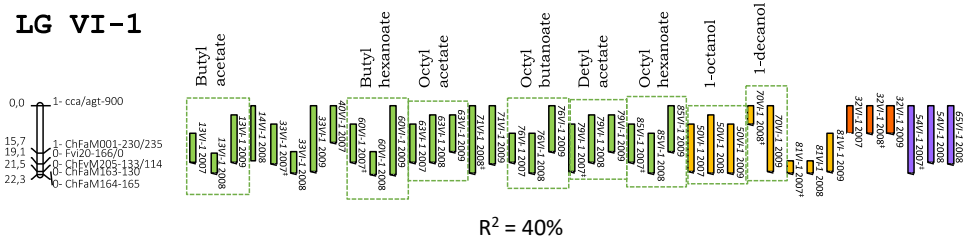


g-DEC
84-92% R^2

FaFAD1

γ-decalactone

Sánchez-Sevilla, et al. (2014) *BMC Genomics*, **15**, 218.
Codominant marker in Bassil et al., 2021.



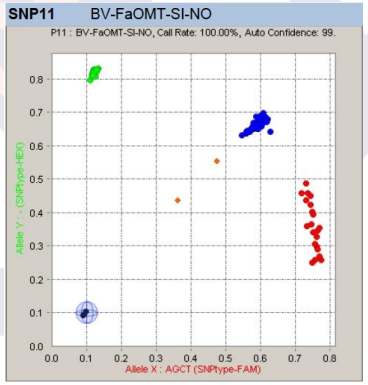
R^2 = 40%

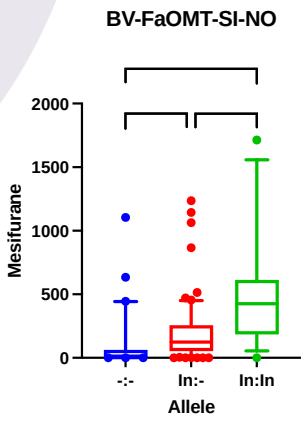
Two candidate genes

Medium chain Esters

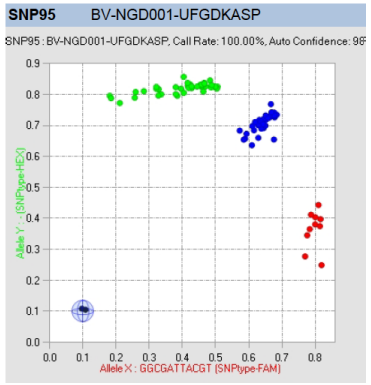
Zorrilla-Fontanesi, *et al.* (2012) *Plant Physiology* **159,** 851–870.
Roldan-Guerra et al., unpublished

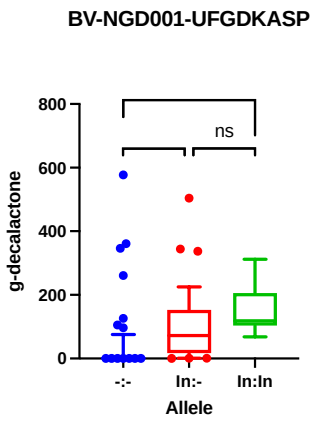
Validation using the Fluidigm MAS Array within the BreedingValue EU Project



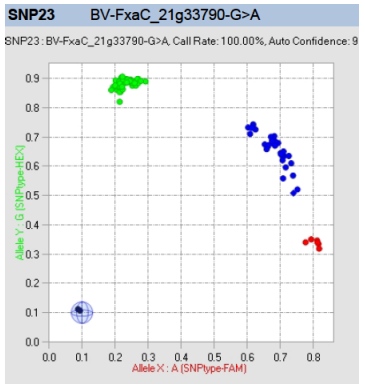


Zorrilla-Fontanesi et al., 2012
4-bp indel BVe new assay

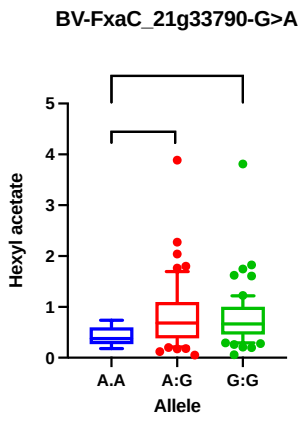




Bassil et al., 2021
11-bp indel BV new assay



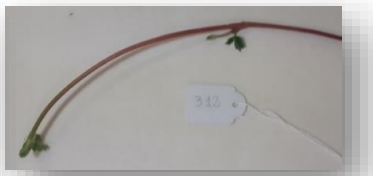
Candidate gene
SNP BV new assay



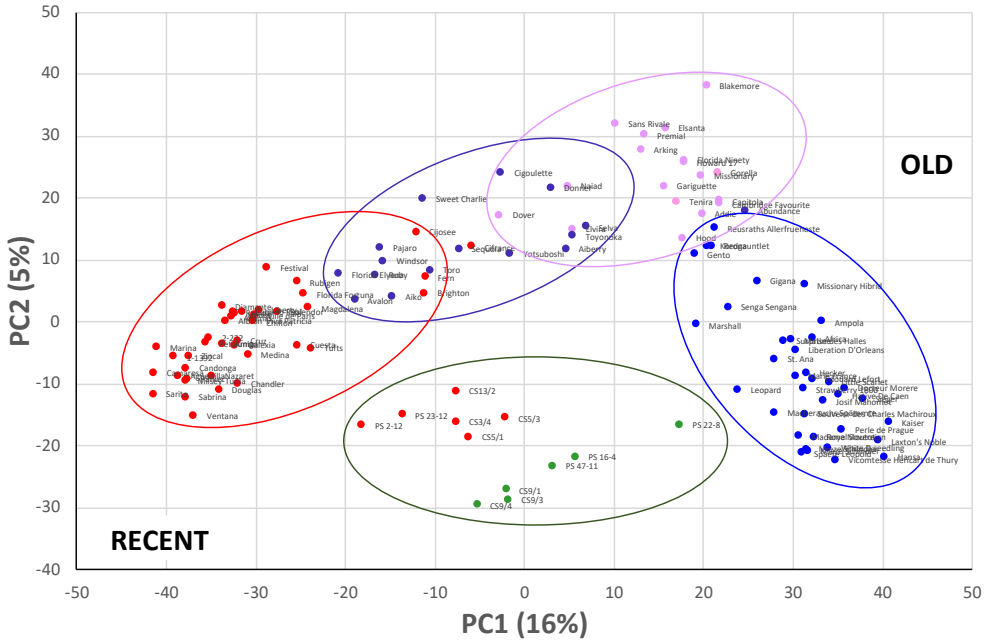
GWAS for Agronomic and Fruit Quality traits

- Experimental population: 138 diverse accessions of *F. x ananassa* and some hybrids with *F. chiloensis*
- Genotype: **50K Fana Axiom** Array = >40K SNPs





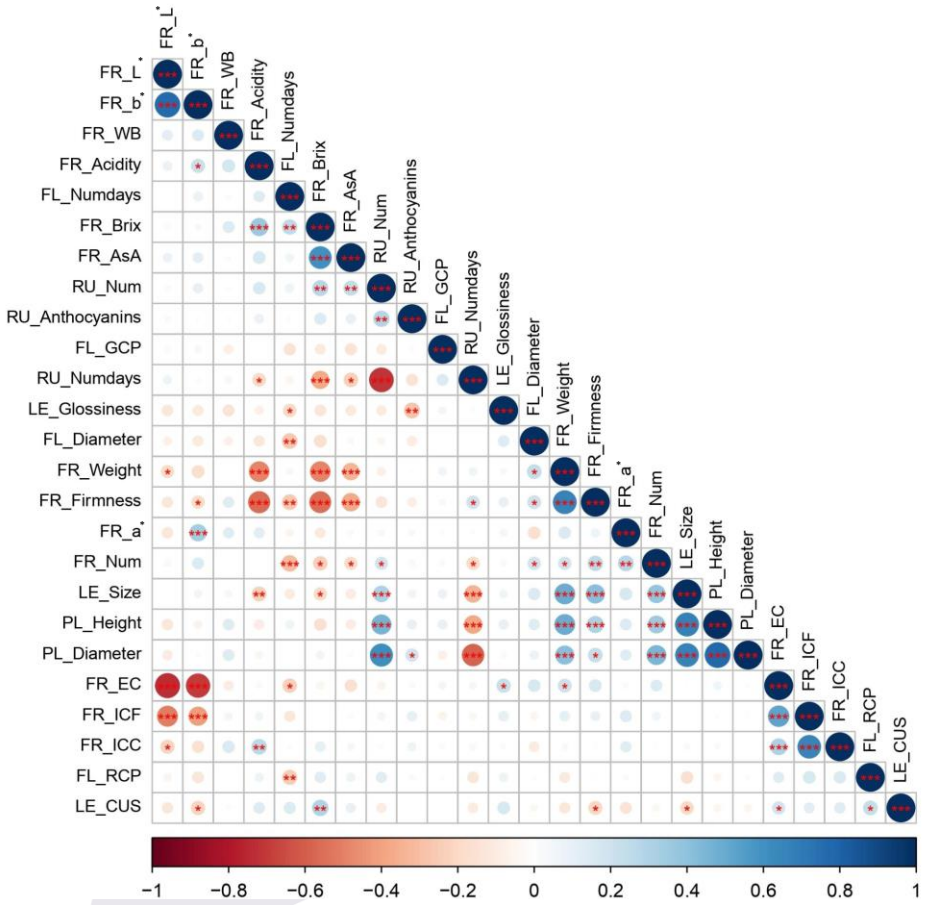

Name	Abreviation	Units	2020-2021 Mean (±sd)	2020-2021 Range	2019-2020 Mean (±sd)	2019-2020 Range	2018-2019 Mean (±sd)	2018-2019 Range
Flowering Time	FL_Days	nº of days	111.95 ± 36.11	48 - 220	164.62 ± 38.63	58 - 237	111.56 ± 34.32	27 - 200
Green Color in Petals	FL_GCP	2 - point scale	1.14 ± 0.35	1 - 2	1.02 ± 0.06	1 - 2	1.03 ± 0.12	1 - 2
Red Color in Petals	FL_RCP	3 - point scale	1.13 ± 0.34	1 - 3	1.03 ± 0.12	1 - 3	1.15 ± 0.37	1 - 3
Flower Diameter	FL_Diameter	3 - point scale	5.00 ± 0.32	3 - 7	4.95 ± 0.60	3 - 7	4.80 ± 0.83	3 - 7
Leaf Color Upper Side	LE_CUS	5 - point scale	3.27 ± 0.68	1 - 5	3.17 ± 0.62	1 - 5	2.98 ± 0.33	1 - 5
Leaf Glossiness	LE_Glossiness	3 - point scale	2.00 ± 0.47	1 - 3	2.13 ± 0.45	1 - 3	1.97 ± 0.35	1 - 3
Leaf Size	LE_Size	3 - point scale	4.74 ± 0.99	3 - 7	4.94 ± 0.95	3 - 7	-	-
Leaf Mildew	LE_Mildew	5 - point scale	-	-	-	-	0.34 ± 0.76	0 - 4
Plant Height	PL_Height	cm	19.01 ± 3.98	7.17 - 31.50	16.09 ± 3.77	6.40 - 27.83	14.38 ± 3.86	4.00 - 23.50
Plant Diameter	PL_Diameter	cm	33.22 ± 8.35	11.50 - 60.67	28.60 ± 6.70	14.40 - 47.50	25.42 ± 5.63	14.00 - 38.00
Runnering Time	RU_Days	nº of days	223.99 ± 22.77	121 - 264	247.89 ± 22.81	202 - 303	256.17 ± 29.68	175 - 303
Runner Number	RU_Num	nº of runners	5.83 ± 4.33	0 - 20	4.88 ± 3.60	0 - 19	3.14 ± 2.68	0 - 12
Runner Anthocyanins	RU_Anthocyanins	5 - point scale	2.79 ± 0.87	1 - 5	2.33 ± 0.81	1 - 5	2.62 ± 0.97	1 - 5
Fruit Number	FR_Num	nº of fruits	15.14 ± 6.10	4 - 32	9.03 ± 4.54	0 - 25	6.49 ± 3.36	0 - 14
Fruit Weight	FR_Weight	g	9.33 ± 5.29	0.67 - 23.44	8.04 ± 4.55	0.83 - 27.11	-	-
Fruit Band without achenes	FR_WB	5 - point scale	3.28 ± 1.07	1 - 9	3.12 ± 1.01	1 - 9	2.84 ± 0.97	1 - 9
Fruit External Color	FR_EC	7 - point scale	4.67 ± 0.93	1 - 7	4.76 ± 0.80	1 - 7	-	-
Fruit External Color: Lightness	FR_L*	black (0) - white (100)	30.18 ± 3.89	23.30 - 50.19	30.95 ± 3.86	23.23 - 52.38	-	-
Fruit External Color: red-green	FR_a*	green (*-a*) - red (*+a*)	38.60 ± 4.40	9.85 - 49.40	41.27 ± 4.39	9.59 - 47.85	-	-
Fruit External Color: yellow-blue	FR_b*	blue (*-b*) - yelow (*+b*)	20.89 ± 3.52	13.14 - 29.29	22.78 ± 3.66	14.63 - 32.29	-	-
Fruit Internal Color: Flesh	FR_ICF	6 - point scale	4.59 ± 0.98	1 - 6	4.14 ± 1.31	1 - 6	-	-
Fruit Internal Color: Core	FR_ICC	3 - point scale	2.09 ± 0.54	1 - 3	2.01 ± 0.66	1 - 3	-	-
Fruit Firmness	FR_Firmness	g	170.91 ± 54.21	100.00 - 303.13	197.27 ± 67.24	101.25 - 340.00	211.36 ± 50.48	127.5 - 324.38
Fruit Acidity	FR_Acidity	g citric acid/100g FW	1.18 ± 0.25	0.47 - 1.95	1.13 ± 0.24	0.12 - 2.01	-	-
Fruit Brix	FR_Brix	°Brix	7.61 ± 1.58	4.93 - 11.80	8.09 ± 1.13	5.87 - 10.90	-	-
Fruit Ascorbic Acid	FR_AsA	mg AsA/100 g FW	47.32 ± 11.66	16.11 - 83.49	47.86 ± 12.63	20.87 - 81.25	-	-



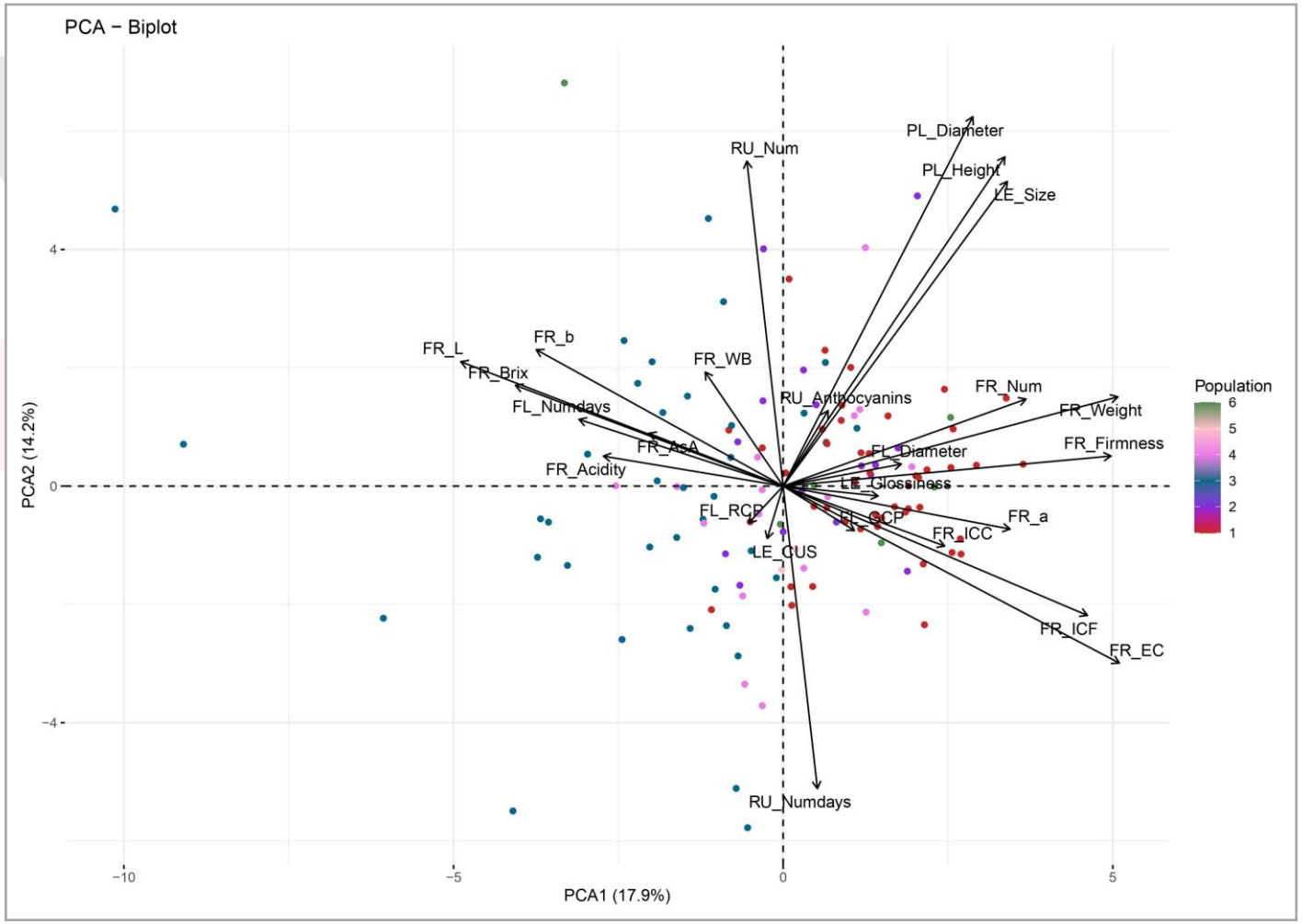
This work is funded by Agencia Estatal de Investigación (PID2019-111496RR-I00 / AEI / 10.13039/501100011033 and PID2022-138290OR-I00/ MCIN / AEI / 10.13039/501100011033 / FEDER, UE) and Junta de Andalucía, FEDER (P18-RT-4856)

Agronomic and Fruit Quality traits during breeding

Trait correlations



Modern accessions are characterized by larger plants with larger and firmer fruits



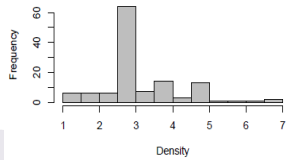
Muñoz et al., unpublished

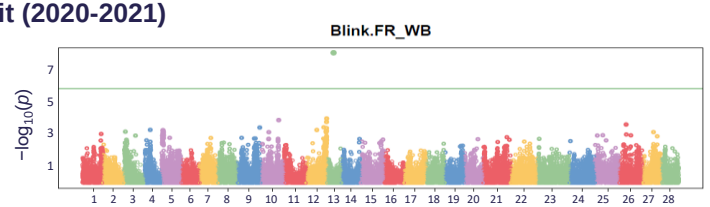
GWAS for Agronomic and Fruit Quality traits

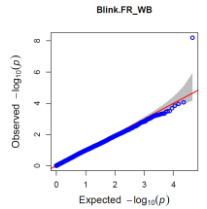
Trait	Significant SNPs	Significant QTLs
PL_Diameter	2	2
RU_Anthocyanins	1	1
RU_Days	10	8
RU_Num	10	8
LE_CUS	4	4
LE_Glossiness	1	1
LE_Mildew	1	1
FL_RCP	10	8
FL_Diameter	1	1
FR_Num	12	12
FR_Weight	4	4
FR_WB	1	1
FR_L*	6	5
FR_a*	13	12
FR_b*	4	4
FR_EC	4	4
FR_Acidity	2	2
FR_Brix	10	10
FR_Firmness	25	7
Total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

Model	2020-2021	2019-2020	2018-2019	*Total SNPs /model*
GLM	29	6	1	*36*
MLM	6	1	0	*7*
FarmCPU	31	17	3	*51*
BLINK	39	12	5	*56*
Total SNPs /season	*105*	*36*	*9*	*150*

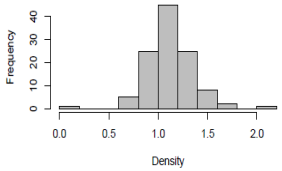
- **Achene-free band on the fruit (2020-2021)**

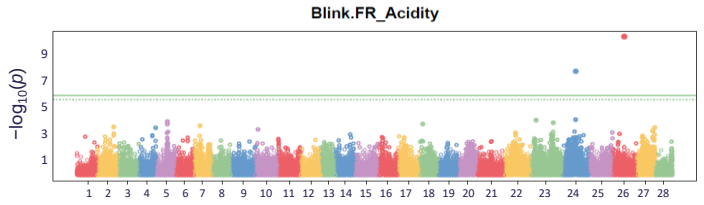


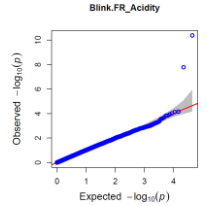




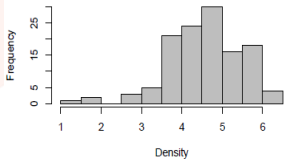
- **Fruit acidity (2019-2020)**

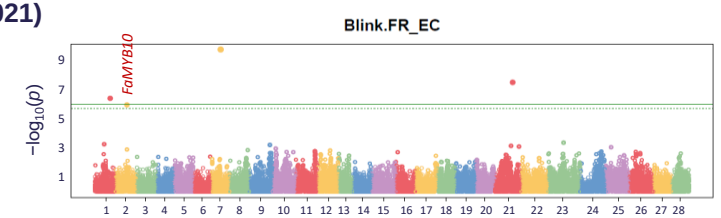


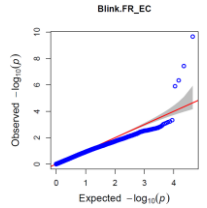




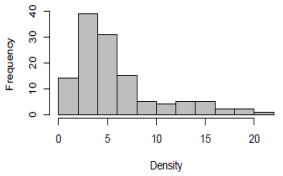
- **External fruit color (2020-2021)**

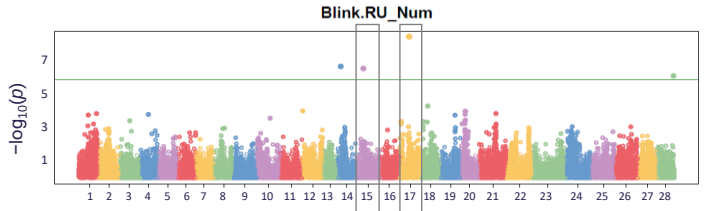


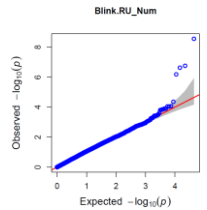




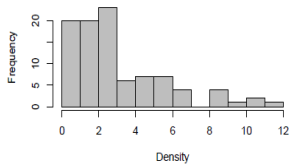
- **Runner number (2020-2021)**

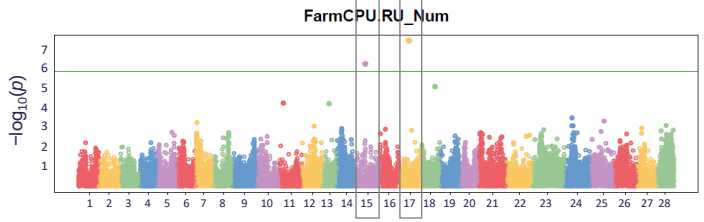


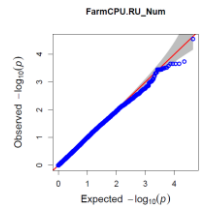




- **Runner number (2019-2020)**



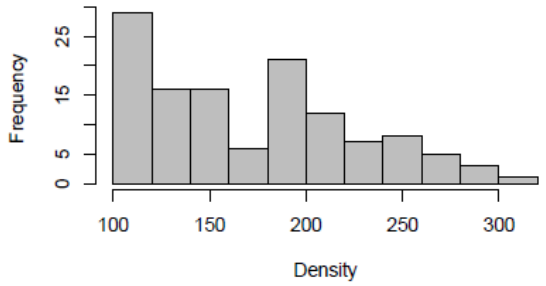


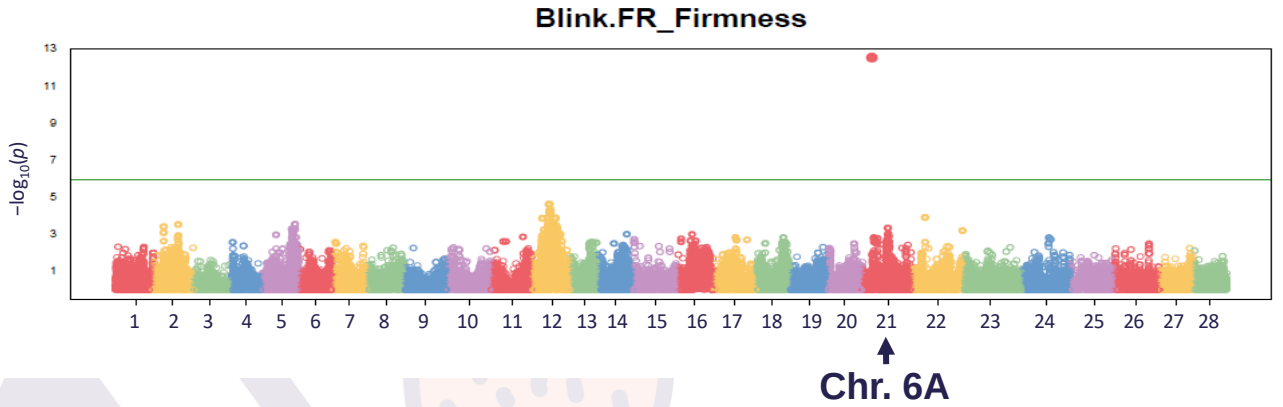


Muñoz et al., unpublished

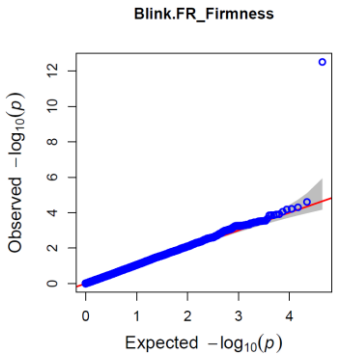
GWAS for Fruit Firmness

2020-2021

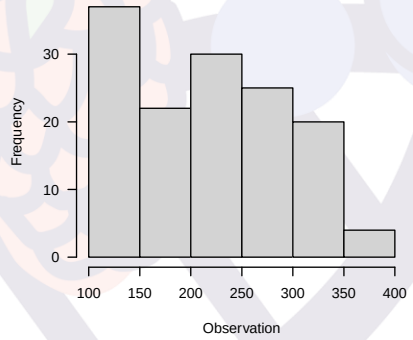


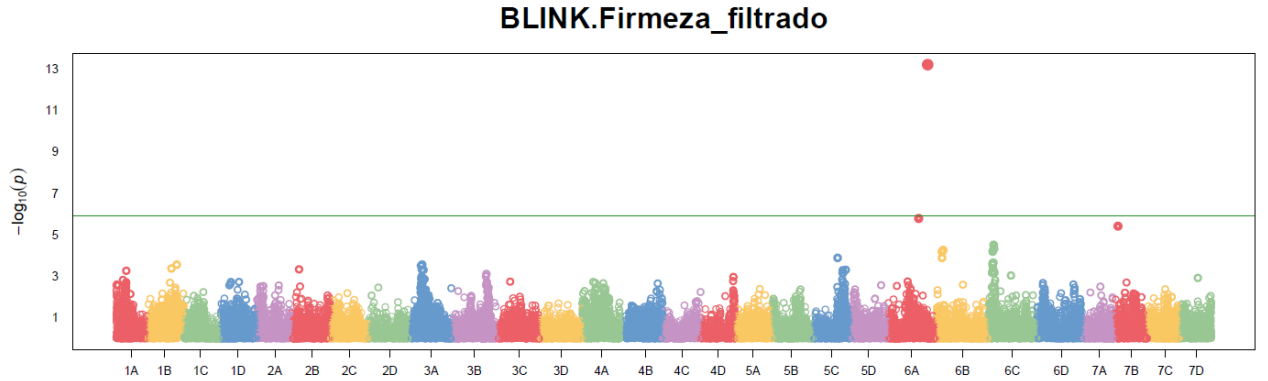


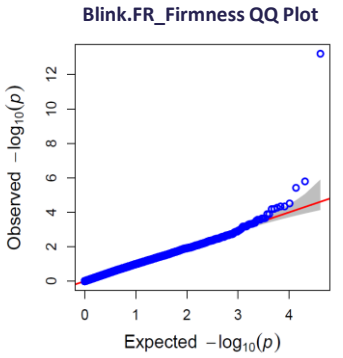
Chr. 6A



2022-2023







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

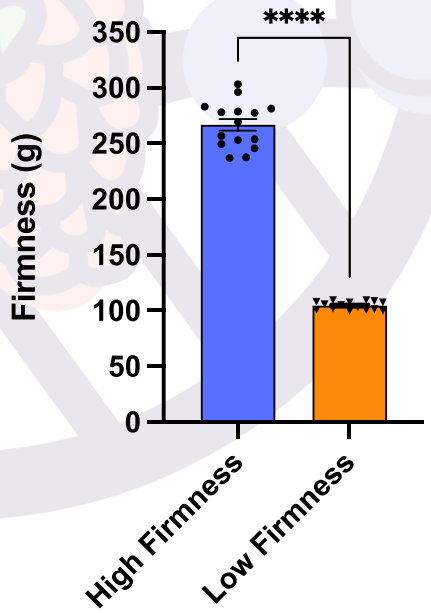
5 candidate genes: Only one expressed during ripening: *FaPG1*

Muñoz et al., unpublished

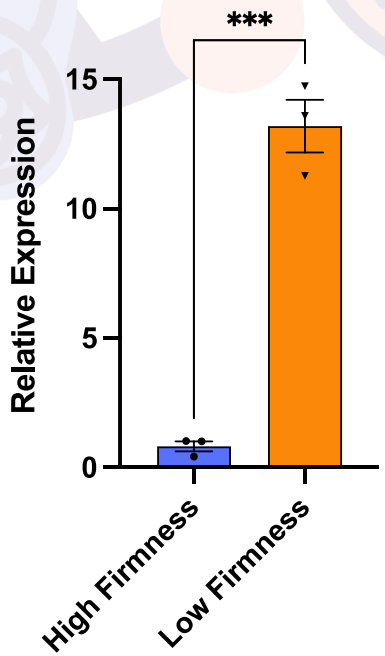
GWAS for Fruit Firmness

High_Firmness Pool			Low-Firmness Pool		
ACC ID	Name	Firmness (g)	ACC ID	Name	Firmness (g)
940	Sarito	303.13	251	Vicomtesse Hericart de Thury	100.00
733	Galexia	296.25	845	PS 2-12 (1-187 × Medina)	100.00
732	Florida Festival	283.13	204	Macherauchs Späternte	100.63
870	Florida Fortuna	281.25	836	Matine	100.63
868	Florida Elyana	278.75	837	Ampola	101.25
965	Sabrina	278.13	177	Hansa	101.88
480	Amiga	277.50	262	Marie France	103.13
986	Rabida	269.38	830	Kaiser	105.00
715	Candonga	256.88	179	Josif Mahomet	105.63
839	Rubigen	253.75	311	Liberation D'Orleans	107.50
795	Sel. 1392	253.13	833	St. Ana	107.50
674	Chiflon	249.38	307	Mieze Schindler	108.13
953	Viva Patricia	245.63	320	Sieger	108.75
1016	Nazaret	237.50	213	Mara Des Bois	109.38
72	Camarosa	236.88	725	Missionary Hibrid	109.38

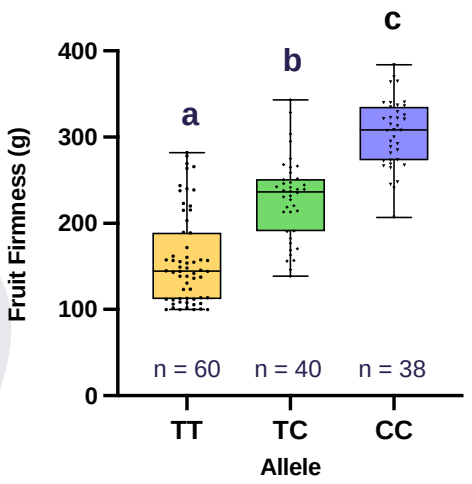
Fruit Firmness

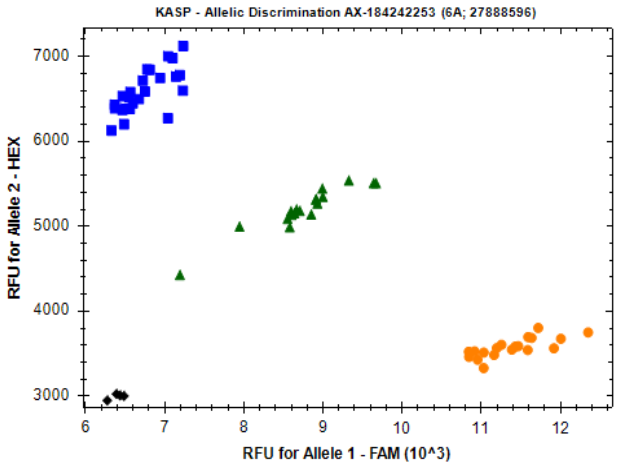


FaPG1

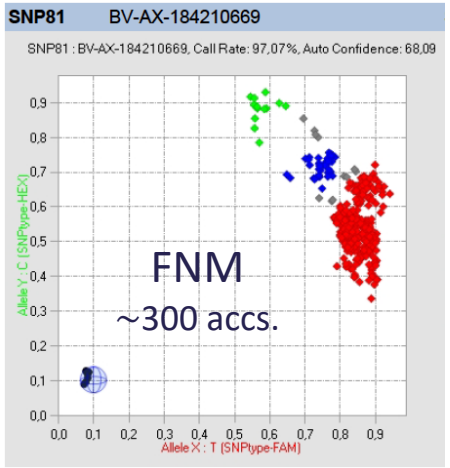


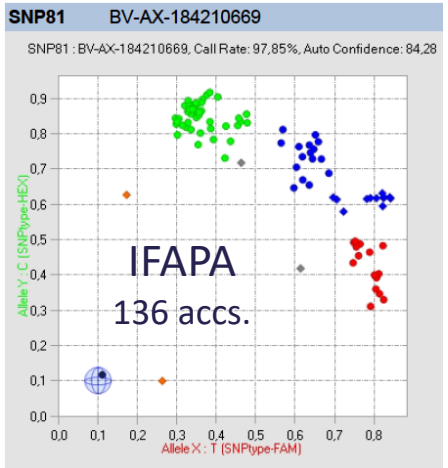
KASP-184242253





BreedingValue MAS Array





Junta de Andalucía
Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible
INSTITUTO DE INVESTIGACIÓN Y FORMACIÓN AGRARIA Y PESQUERA

MAS at FNM Spain

fnm Fresas Nuevos Materiales

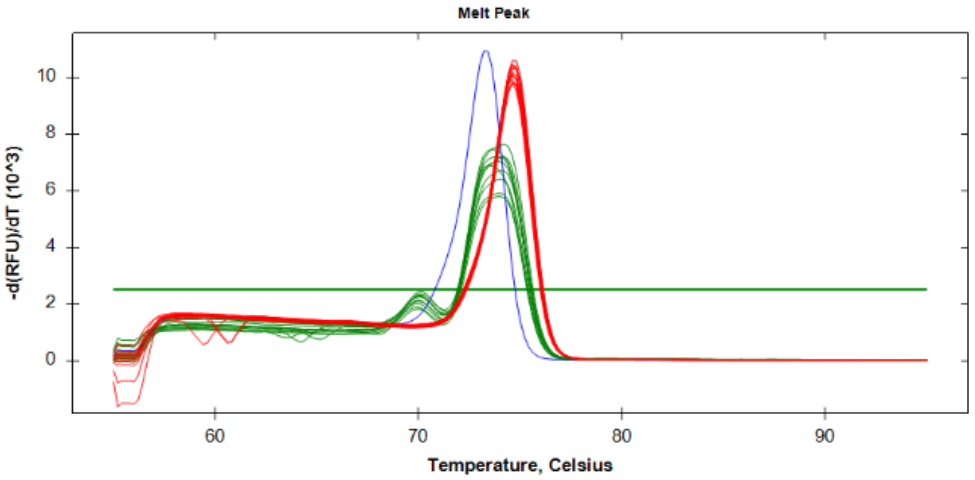
Breeding Value

Agreement IFAPA-FNM for Marker-Assisted Selection

FNM main interests are Resistance to different pathogens and SF/DN

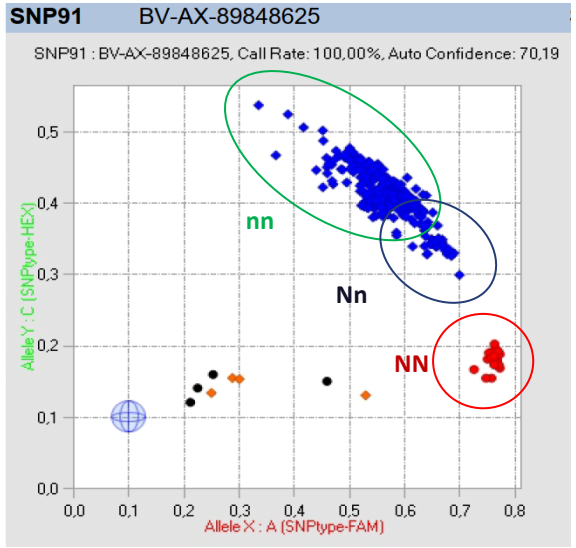
High Resolution Melting (HRM) Marker for Day neutrality (*FaPFRU* locus)

SNP AX-89848625 (Verma et al., 2017; >90% accuracy)



IFAPADNHRM02_4-4 (AX-89848625): Highly predictive

BV MAS Array

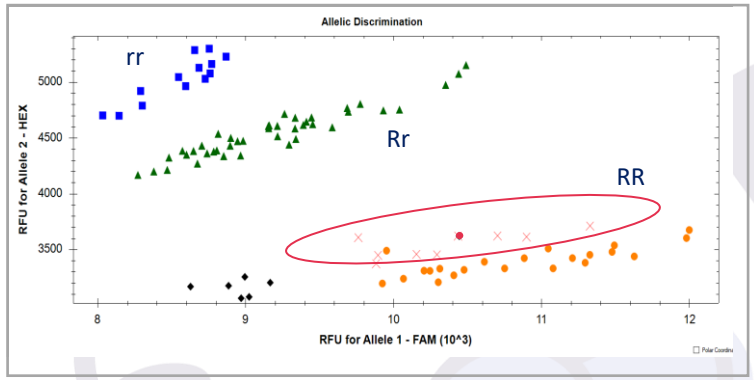


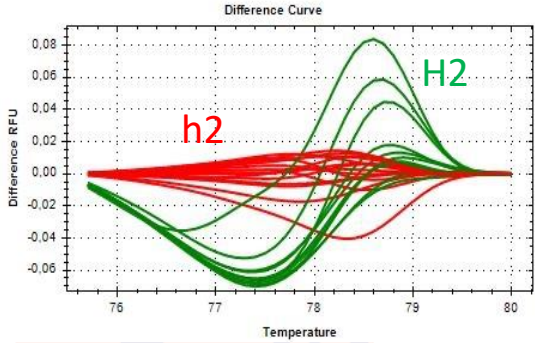
Comparison with 260 FNM accessions

NOT PREDICTIVE

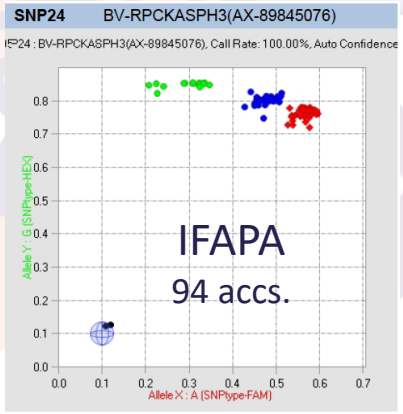
Fluidigm Markers for *Phytophthora cactorum and Colletotrichum acutatum*

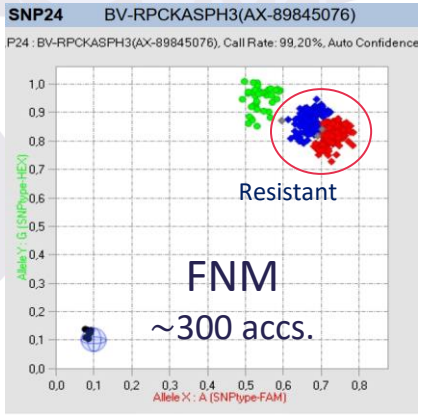
Rce. to *Phytophthora cactorum*





KASP for *FaRPc2* H3 described in (Noh et al 2018)

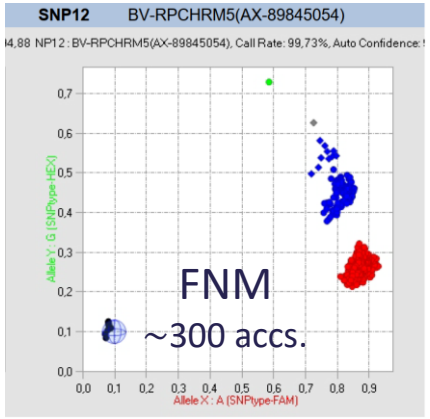




BreedingValue Fluidigm Array

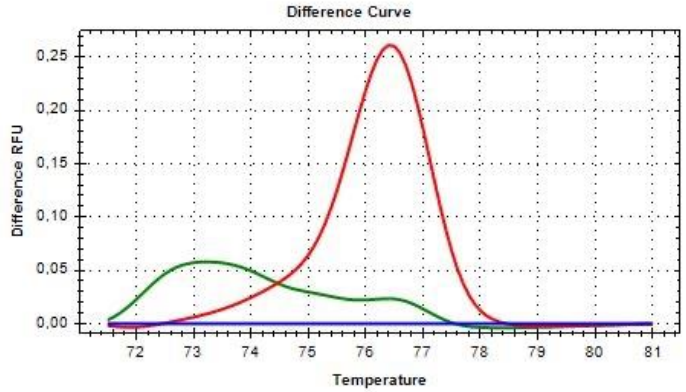
16 -2.7% Error

HRM for *FaRPc2* H2 described in (Noh et al 2018)



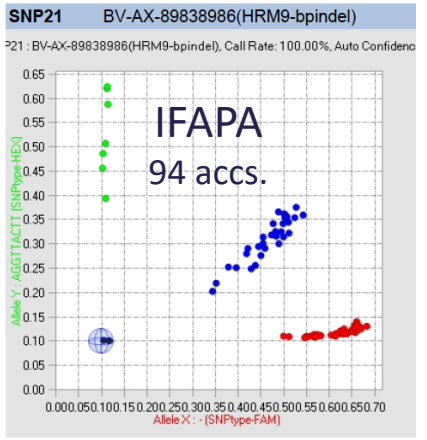
2.3% Error

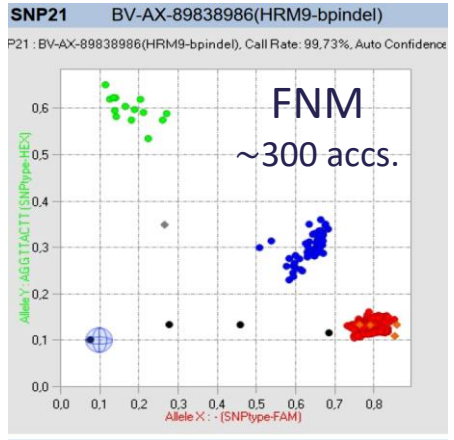
Rce. to *Colletotrichum acutatum*



HRM **ID3** (SNP AX-89838986) for *FaRCa1* (Salinas et al 2020)

1.2% Error





BreedingValue Fluidigm Array

Task 2.2: Investigate the applicability of known molecular markers in breeding (Strawberry).

- A comprehensive list of molecular markers associated with important monogenic traits or major QTLs will be available: Using information (i) available in publications and (ii) confidential data from different partners.
- The targeted traits include Resistances to pathogens, Flowering-related, Production-related and Fruit quality.
- 96 assays are included in the **Fluidigm 96.96 JUNO chip** format.
- Interested partners including private and public breeders will provide **phenotype data** from breeding material in their respective programs.
- We will investigate whether reported marker/trait associations can be used to accelerate breeding programs and cultivar development by MAS. Statistical tests for single marker or array-wide association studies will be performed.

Task 2.2: Investigate the applicability of known molecular markers in breeding (Strawberry).

RESISTANCES TO PESTS	Reported markers	SNPs in BV_v3 array	Final Number of loci
Rce to *Pyhtophtora Cactorum*	6	5	4
Rce to *Coletotrichum acutatum*	1	1	1
Rce to *Fusarium oxysporum* f. sp.	4	4	3
Rce to *Colletotrichum gloesporioides*	4	3	1
Resistance to *Verticillium dahliae*	2	2	2
Resistance to *Xanthomonas fragariae*	2	2	1
Resistance to Powdery mildew (*Podosphaera aphanis*)	10	5	5
Resistance to *Macrophomina phaseolina*	5	3	2
Resistance to *Tetranychus urticae*	2	2	1
Resistance to *Botritis cinerea*	1	-	-
VEGETATIVE TRAITS	**Reported markers**	**SNPs in BV_v3 array**	**Final Number of loci**
Day neutrality, Everbearing	5	3	1
Flowering time	5	3	3
PRODUCTION TRAITS			
Fruit weight (FW)	3	3	3
Total Yield	2	1	1
Class one yield specific	1	1	1
Fruit number	5	4	3
Total Fruit Number & Marketable Number	5	4	4
yield related	5	2	2
FRUIT QUALITY			
Fruit firmness	4	5	4
Vitamin C	5	4	4
SSC, Brix	8	5	5
Sucrose, raffinose, SSC and succinate	3	2	2

FRUIT QUALITY	Reported markers	SNPs in BV_v3 array	Final Number of loci
pH	1	1	1
pH / acidity perception	1	1	1
Malic acid	2	1	1
Internal fruit color (qualitative)	1	1	1
Fruit color (internal and external; qualitative)	1	1	1
Fruit color (quantitative), pelargonidins and Eriodictyol	1	1	1
Yellow flesh color and carotenoids	1	1	1
Total anthocyanins	2	2	1
Total anthocyanins + Pelargonidin-3-Glucoside	2	2	1
epicatechin glucuronide isomer 1 and 2, kaempferol hexose 1, cyanidin hexose and rutin 2	2	1	1
propelargonidin dimer 2 and kaempferol hexose 2	2	2	2
pelargonidin-3-O-malonylglucoside	2	1	1
ellagic acid deoxyhexoside	1	1	1
Ellagic acid hexose	2	1	1
cinnamoyl glucoside	2	2	2
Galloyl-bis(HHDP)-glucose	2	1	1
Caramel aroma (Mesifurane)	5	3	2
Peach aroma (γ-decalactone)	1	1	1
grape aroma (Methyl anthranilate)	3	2	2
decyl, hexyl, octyl and nonyl acetates, octyl butanoate	1	1	1
Butyl, hexyl, octyl, nonyl, decyl, cinnamyl acetates, butyl and octyl butanoate, butyl and octyl hexanoate	3	2	1
terpenes	2	2	1
Bostwick consistency	1	1	1
TOTAL	**140**	**96**	**80**

Low-density SNP array for Marker-Assisted Selection (MAS)

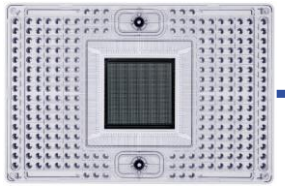
- The array includes 96 SNPs associated with different traits and can be interrogated with 94 samples (+ 2 internal Controls) at a time.

What each partner provide:

94 DNA **SAMPLES** PLATE

Fluidigm SNPtype **Reagents**

FLUIDIGM WORK **ASSAYS** PLATE



IFC 96.96 JUNO

Each IFC enables 9,216 reactions using 96 samples and assays.

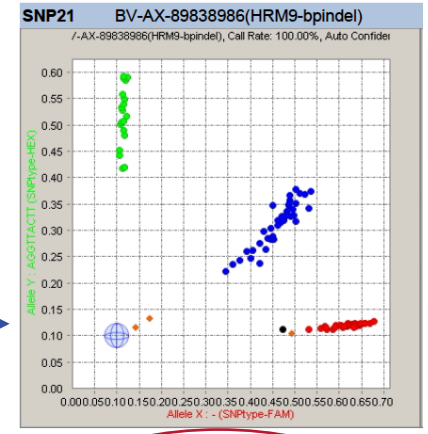
JUNO

1. Load the IFC
2. Pre-amplification: STA
3. qPCR at end point fluorescence detection.

BIOMARK HD

1. Chip Reading and visualization.
2. Analysis of results (SNP Genotyping Analysis Software)

EXAMPLE OF ONE PLOT: RESISTANCE TO ANTHRACNOSIS (*FaRca1*)



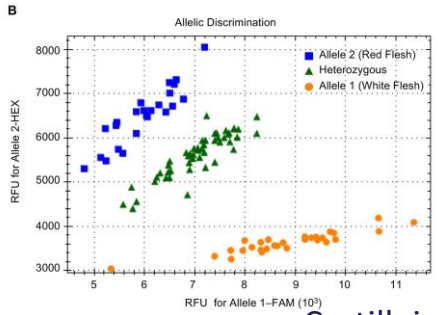
96 PLOTS (and excel file), each with genotypes for 94 strawberry samples

What we get:

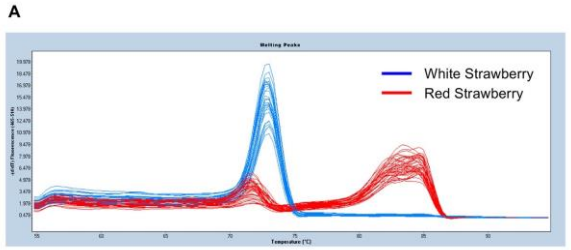
Low-density SNP array for Marker Assisted Selection (MAS)

More Examples of the 96.96 JUNO FLUDIGM BreedingValue Array:

IFC-2 KASP marker for flesh color



WS_CID_01 HRM marker for White strawberries



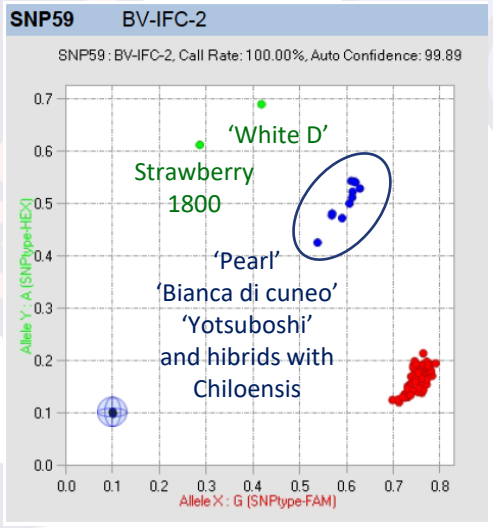
Castillejo et al., 2020. The Plant Cell

17-24% of variation

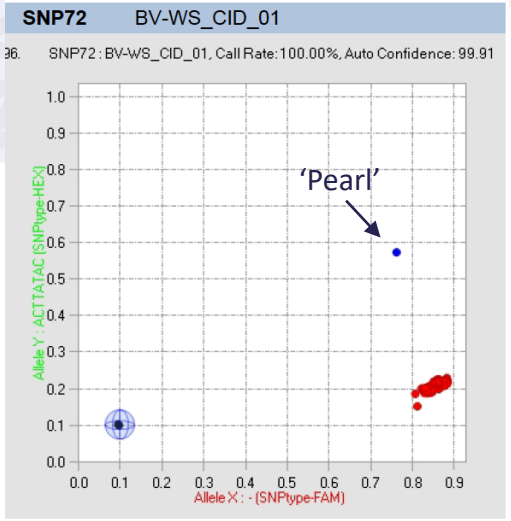
Zorrilla-Fontanesi et al., 2011

Published Marker explains only 18% of variation

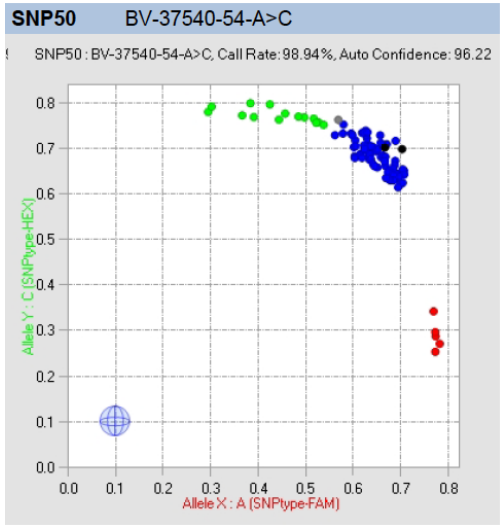
Antanaviciute et al., 2015; Cockerton et al., 2019



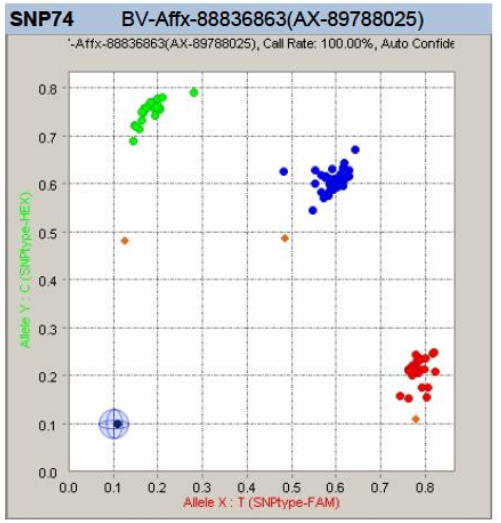
Internal fruit color: Most of the accessions have red Flesh.



Fruit color: Only one accession in all assayed plates.

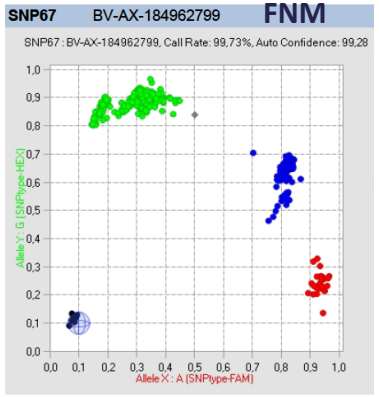


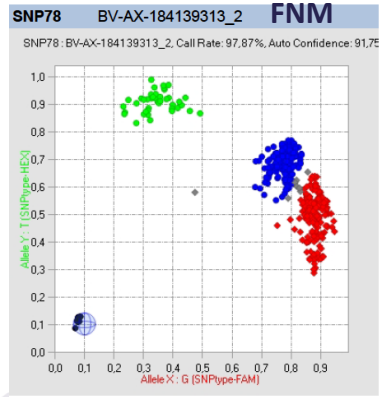
QTL for Fruit number: Not good cluster separation.

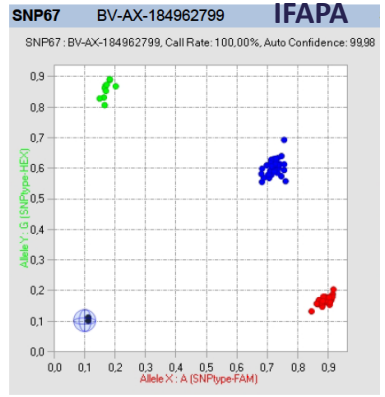


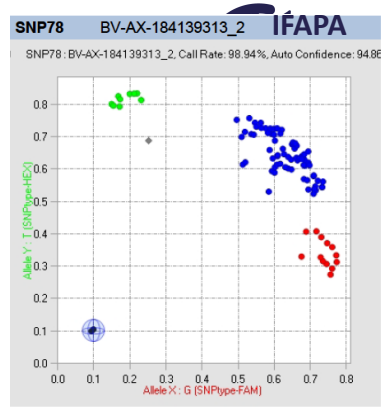
Rce. to Verticillium: Nice clusters. Not validated

RESISTANCES TO PESTS	Reported markers	SNPs in BV_v3 array	Final Number of loci
Rce to *Pyhtophtora Cactorum*	6	5	4
Rce to *Coletotrichum acutatum*	1	1	1
Rce to *Fusarium oxysporum* f. sp.	4	4	3
Rce to *Colletotrichum gloesporioides*	4	3	1
Resistance to *Verticillium dahliae*	2	2	2
Resistance to *Xanthomonas fragariae*	2	2	1
Resistance to Powdery mildew (*Podosphaera aphanis*)	10	5	5
Resistance to *Macrophomina phaseolina*	5	3	2
Resistance to *Tetranychus urticae*	2	2	1
Resistance to *Botritis cinerea*	1	-	-
VEGETATIVE TRAITS			
Day neutrality, Everbearing	5	3	1
Flowering time	5	3	3
PRODUCTION TRAITS			
Fruit weight (FW)	3	3	3
Total Yield	2	1	1
Class one yield specific	1	1	1
Fruit number	5	4	3
Total Fruit Number & Marketable Number	5	4	4
yield related	5	2	2
FRUIT QUALITY			
Fruit firmness	4	5	4
Vitamin C	5	4	4
SSC, Brix	8	5	5
Sucrose, raffinose, SSC and succinate	3	2	2
pH	1	1	1
pH / acidity perception	1	1	1
Malic acid	2	1	1
Internal fruit color (qualitative)	1	1	1
Fruit color (internal and external; qualitative)	1	1	1
Fruit color (quantitative), pelargonidins and Eriodictyol	1	1	1
Yellow flesh color and carotenoids	1	1	1
Total anthocyanins	2	2	1
Total anthocyanins + Pelargonidin-3-Glucoside	2	2	1
epicatechin glucuronide isomer 1 and 2, kaempferol hexose 1, cyanidin hexose and rutin 2	2	1	1
propelargonidin dimer 2 and kaempferol hexose 2	2	2	2
pelargonidin-3-O-malonylglucoside	2	1	1
ellagic acid deoxyhexoside	1	1	1
Ellagic acid hexose	2	1	1
cinnamoyl glucoside	2	2	2
Galloyl-bis(HHDP)-glucose	2	1	1
Caramel aroma (Mesifurane)	5	3	2
Peach aroma (γ-decalactone)	1	1	1
grape aroma (Methyl anthranilate)	3	2	2
decyl, hexyl, octyl and nonyl acetates, octyl butanoate	1	1	1
Butyl, hexyl, octyl, nonyl, decyl, cinnamyl acetates, butyl and octyl butanoate, butyl and octyl hexanoate	3	2	1
terpenes	2	2	1
Bostwick consistency	1	1	1
TOTAL	140	96	80

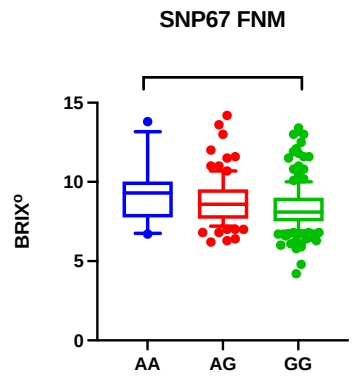


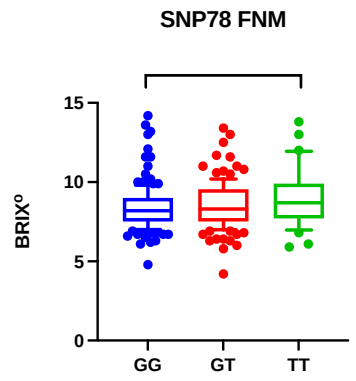


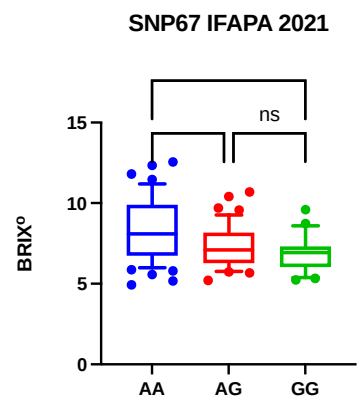


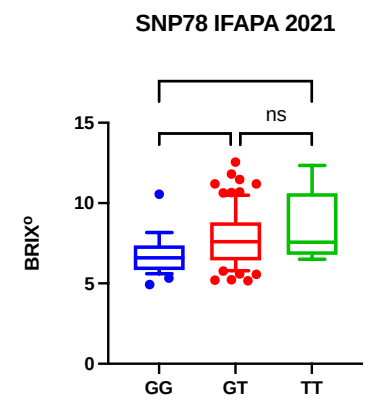


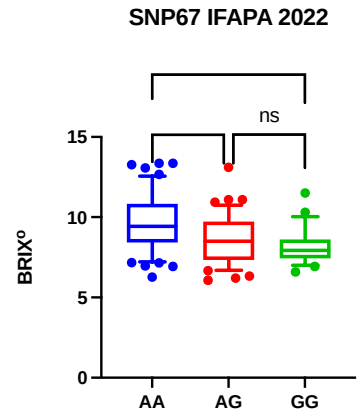
ID_v3	SNP Assay	Chrom.	Reference	SSC FNM (345 acc.)	SSC IFAPA (128 acc.)	Sucrose IFAPA (128 acc.)
SNP31	BV-AX-184380255	Fxa 1-3	INRAE, unpublished			
SNP34	BV-F19519-5-A>T-LG5	Fxa 5-3	Zorrilla-Fontanesi et a., 2011; Vallarino and Pott et al., 2019			
SNP36	BV-UDP-Glu4-epi(5-3)	Fxa 5-3	Zorrilla-Fontanesi et al., 2011; Vallarino and Pott et al., 2019			p=0.0004 p=0.008
SNP60	BV-UDP-Glu4-epi(5-4)	Fxa 5-4	Natarajan et al., 2020			
SNP66	BV-AX-184103725	Fxa 2-1	INRAE, unpublished		p=0.016 p=0.017	
SNP67	BV-AX-184962799	Fxa 4-1	INRAE, unpublished	p=0.031	p=0.001 p=0.0004 p=0.0001	p=0.029
SNP78	BV-AX-184139313_2	Fxa 1-1	INRAE, unpublished	p=0.016	p=0.004 p=0.035 p=0.007	

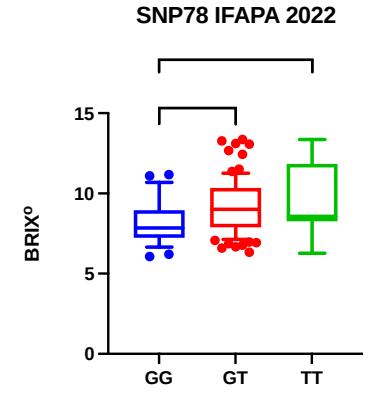












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