



Trisaia Research Center

MT (Italy)

Comparative gene expression analysis between the chilean native strawberry, *Fragaria chiloensis*, and its world-wide cultivated relative, *Fragaria x ananassa*.

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Strategic Workshop
Sant Feliu de Guixols
23-24 May 2007

Aim of the work

Characterization and comparison of gene expression profiles during fruit development in *Fragaria x ananassa* and *Fragaria chiloensis* species

During the ripening:

- Modification of cell wall
- Carbohydrate metabolism
- Pigment synthesis
- Increase of volatile compounds

Variability

- Flavor-Aroma
- Firmness
- Pigmentation
- Shape
- Nutritional contents

Fragaria

F. vesca (2n)



F. chiloensis (8n)



F. virginiana (8n)



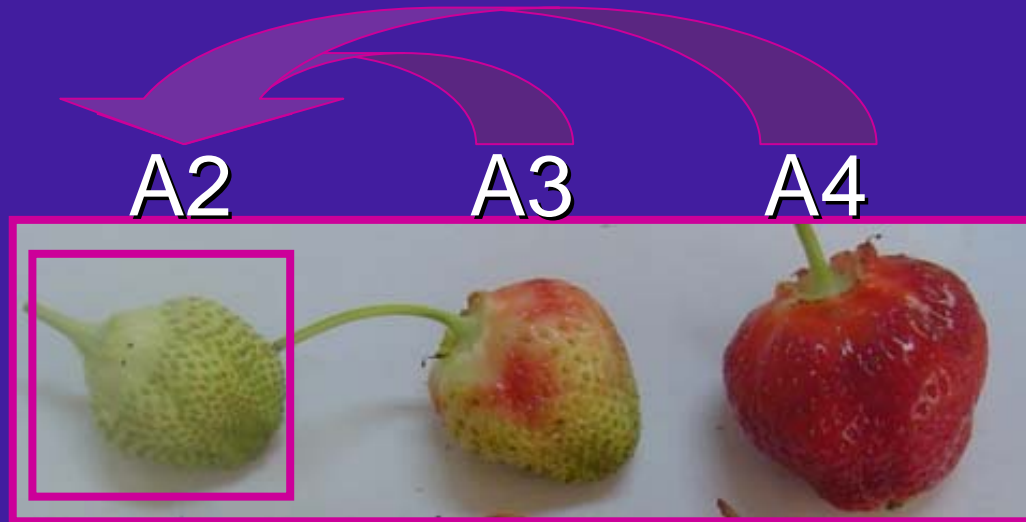
F. x vescana (10n)



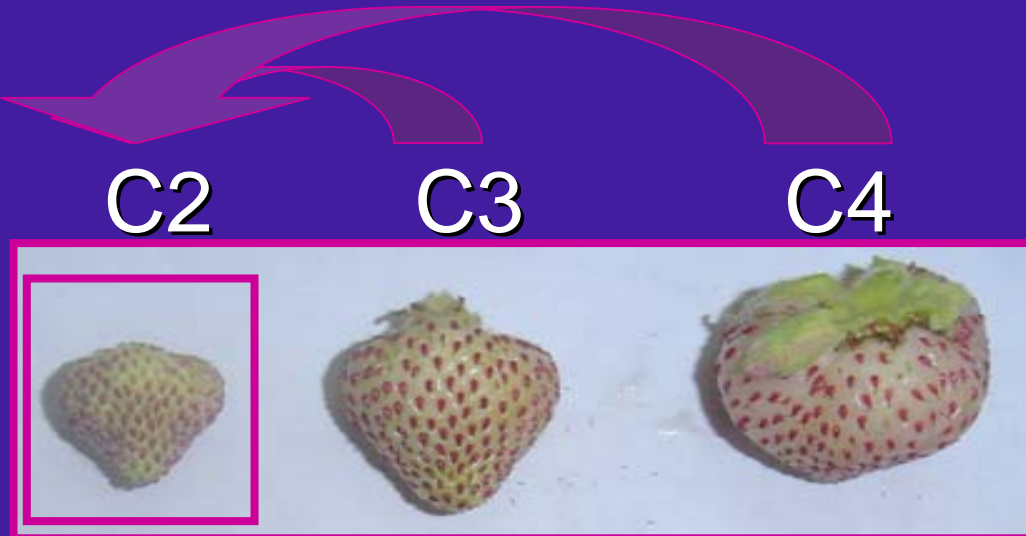
F. x ananassa (8n)



PLANT MATERIAL



Fragaria x ananassa, cv Chandler



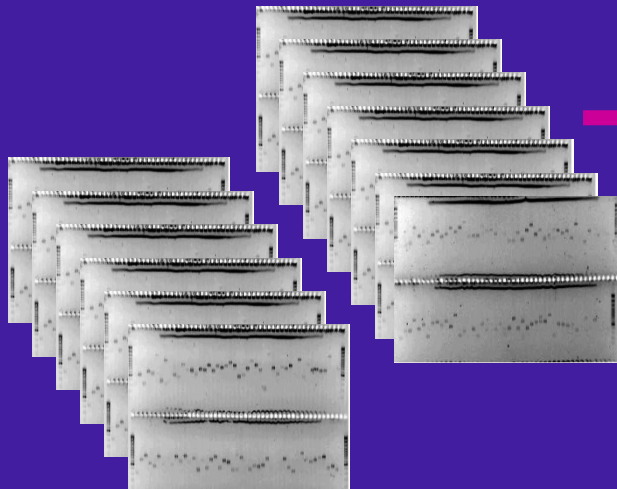
Fragaria chiloensis

PRODUCTION MICROARRAY

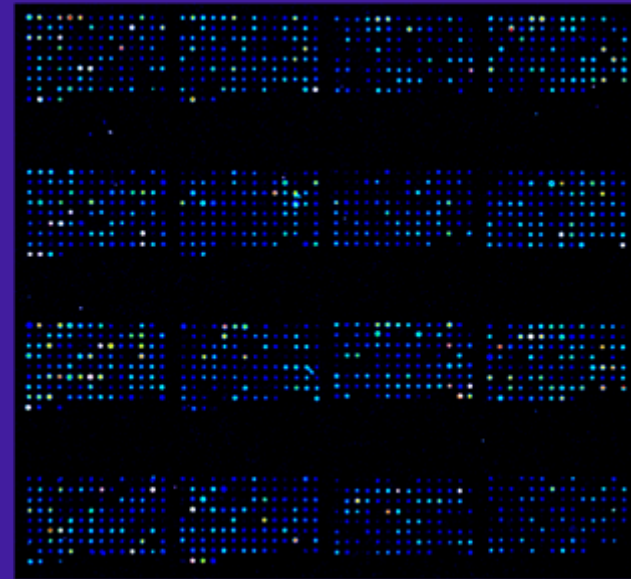
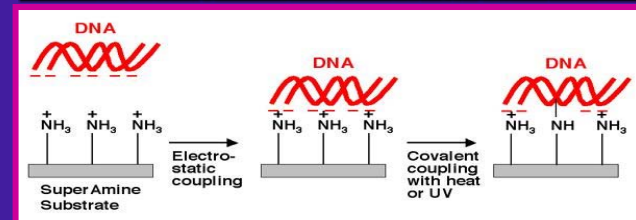
1811 expressed sequence tags (EST) were selected from an annotated database available at <http://fragariaest.trisaia.enea.it>

Production of a double strand cDNA microarray

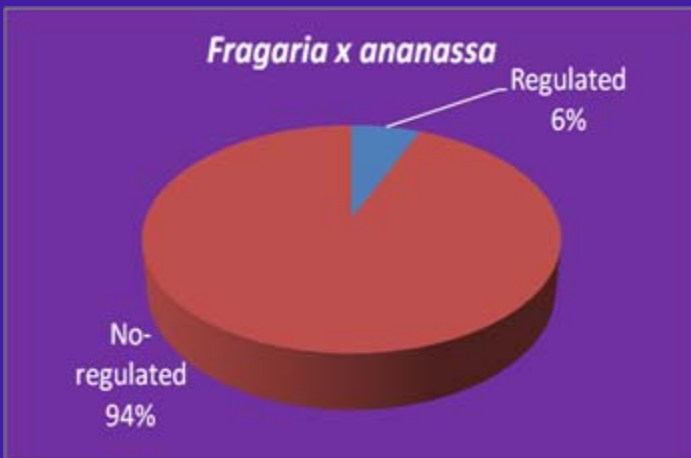
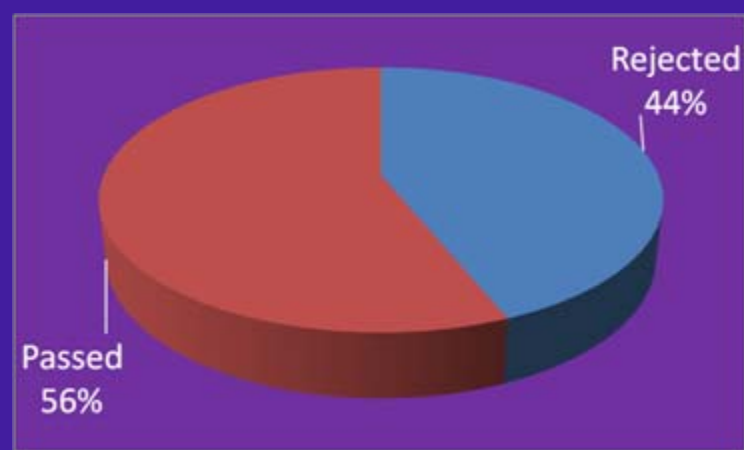
PCR of 1811 ESTs with universal primers T3/T7



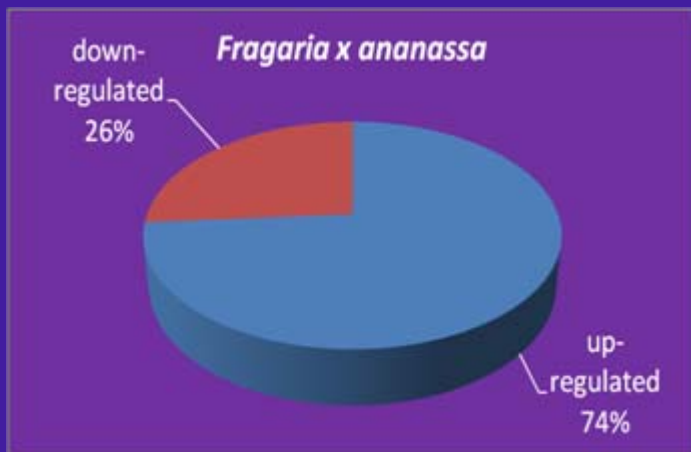
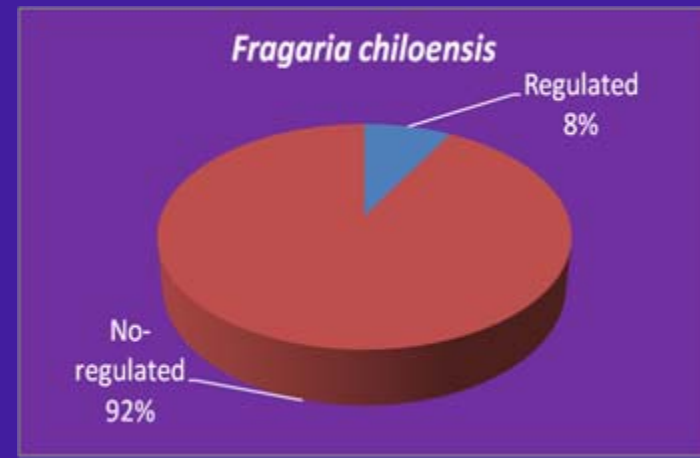
SpotArray® 24 Microarray Printing System



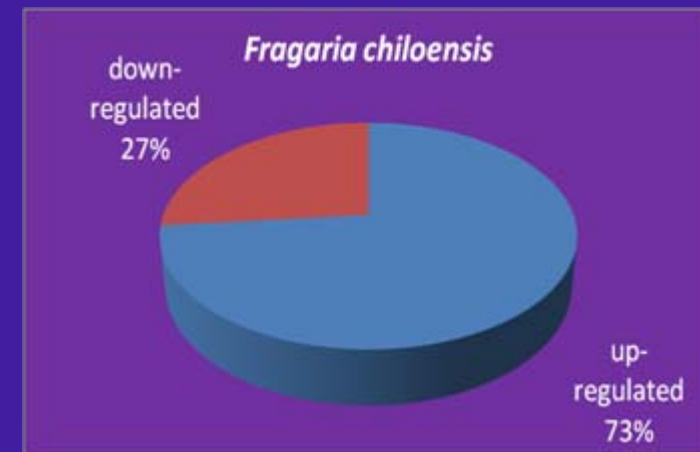
**Quality Filters:
1017 passed spots
of 1811**



**Regulated
Transcripts:
65 in *F.x ananassa*
and 85 in
*F.chiloensis***



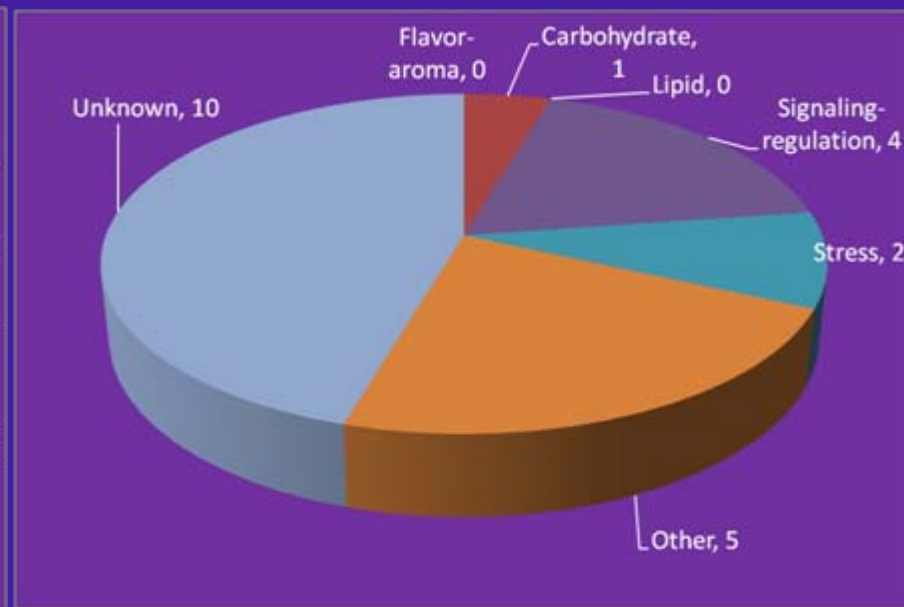
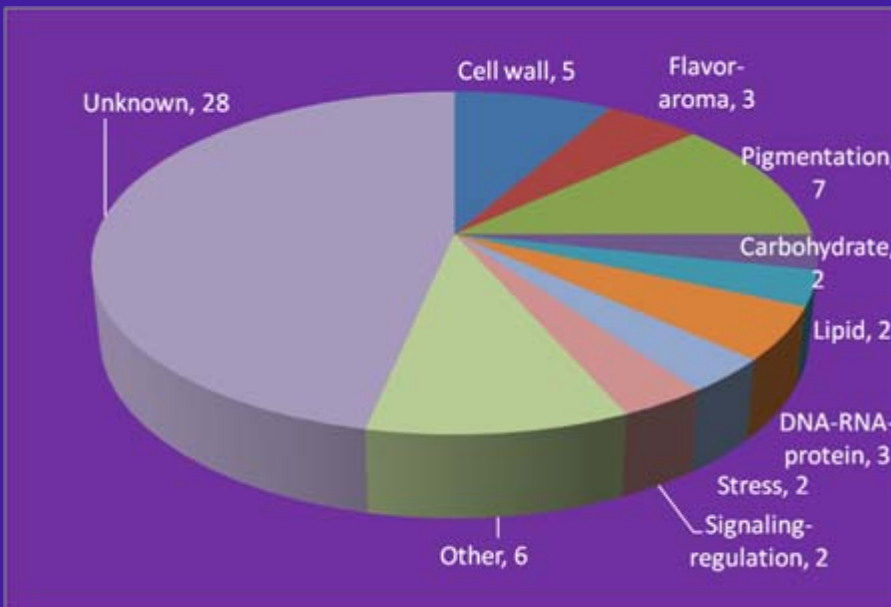
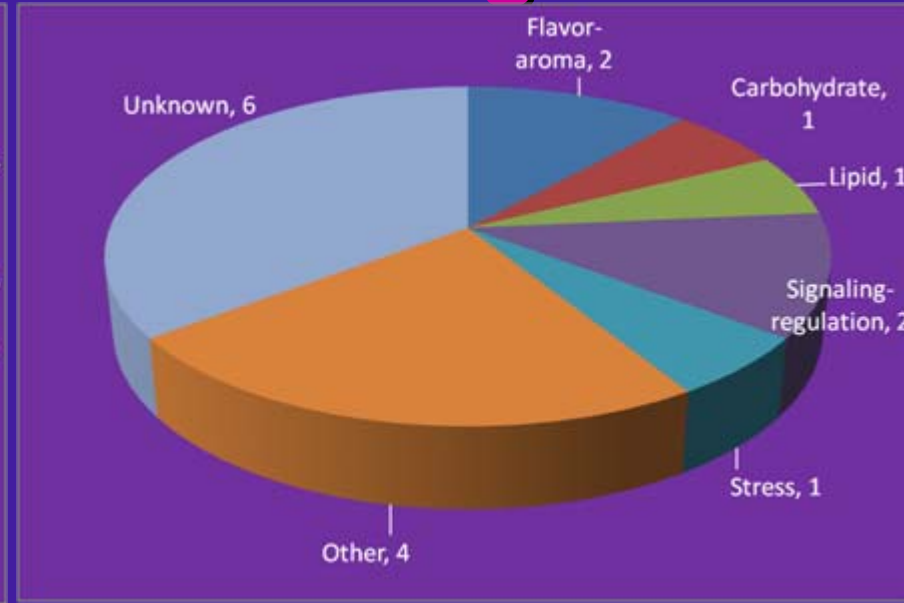
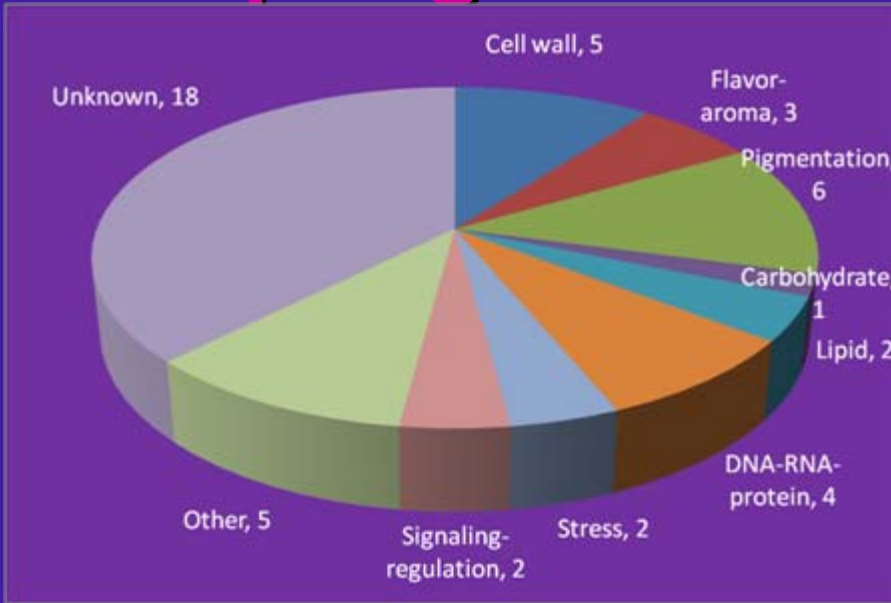
**Up-regulated
transcripts
represented the
majority :
48 in *F.x ananassa*
and 61 in
*F.chiloensis***



Up-regulated

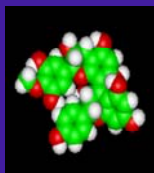
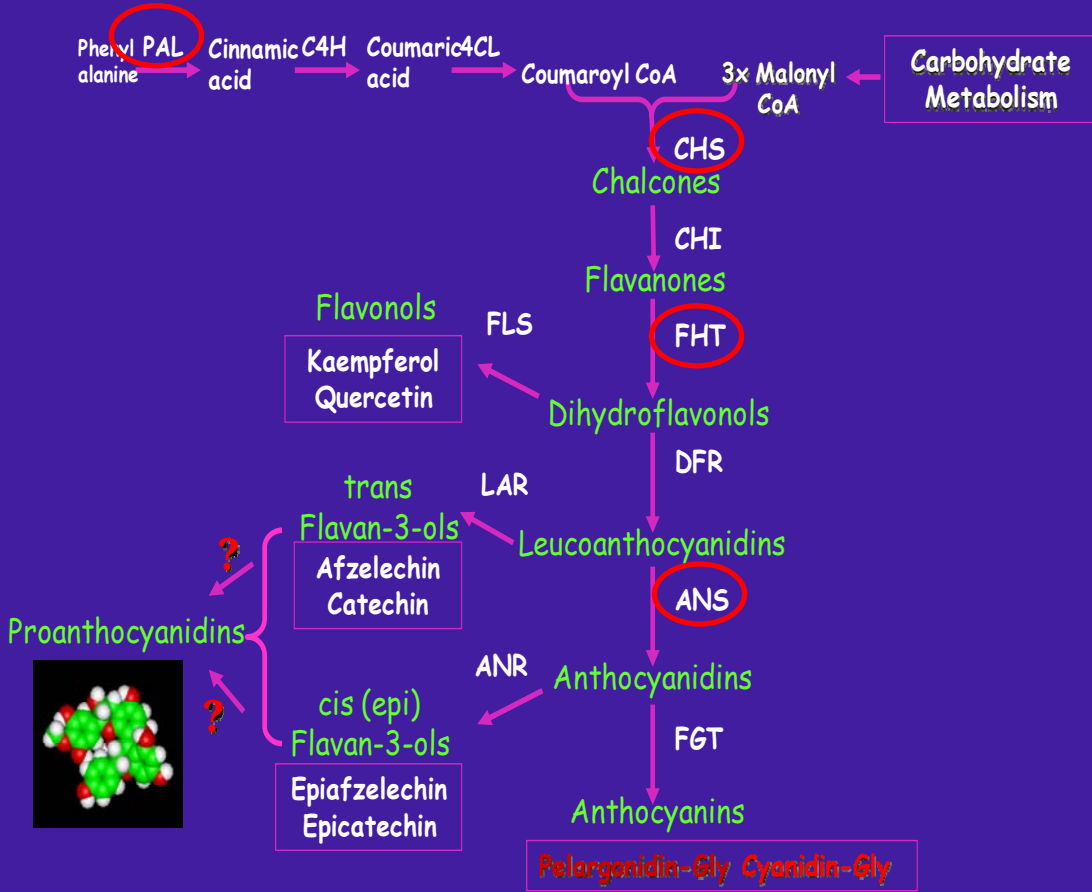
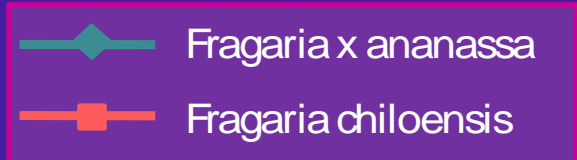
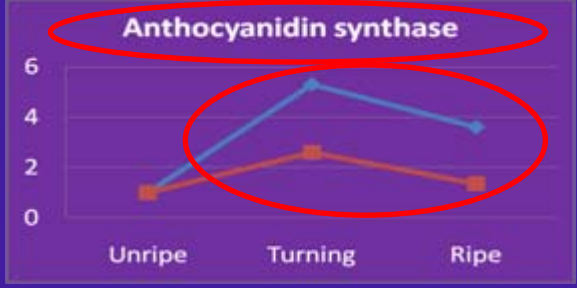
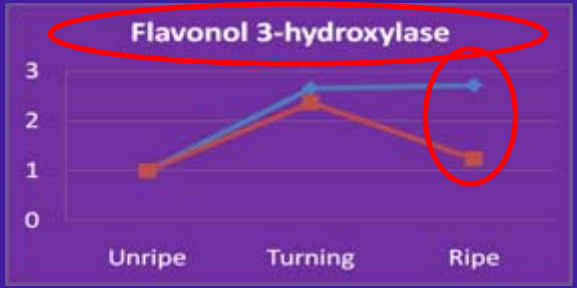
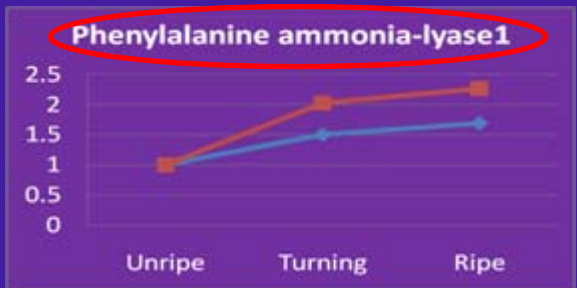
Down-regulated

F. x ananassa
F. chiloensis



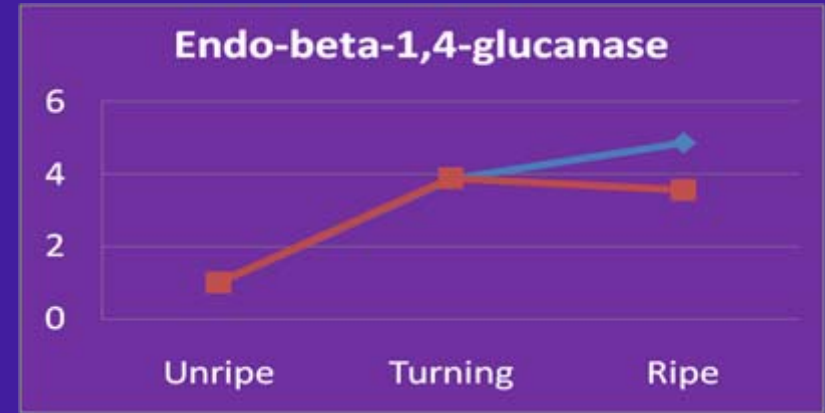
Phenylpropanoid and flavonoid transcripts regulated during the ripening

Relative transcript abundance



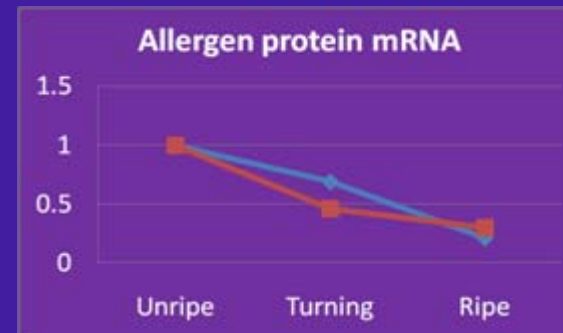
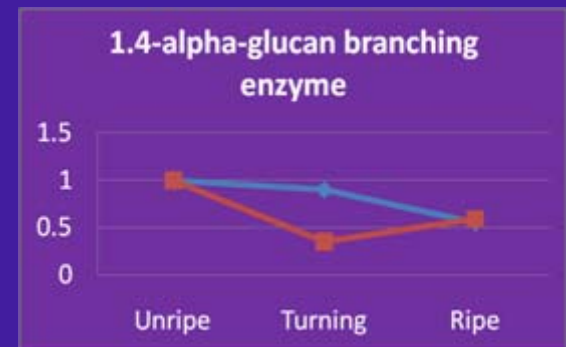
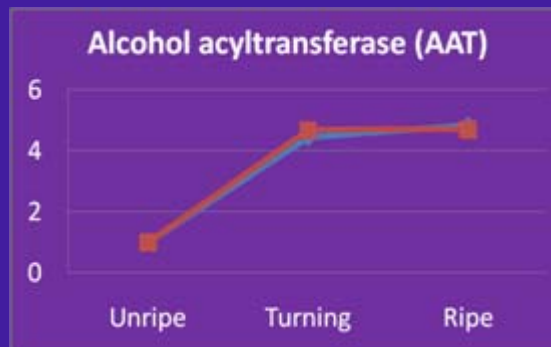
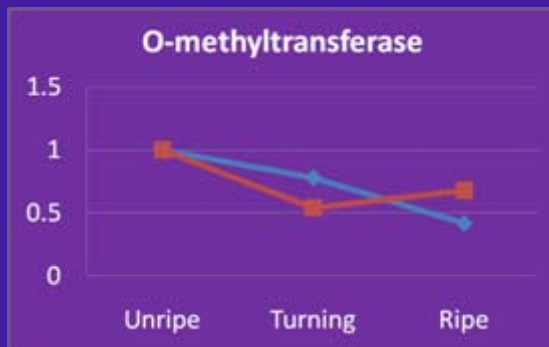
Some genes involved in fruit firmness and ripening - more expressed in *F. x ananassa*

Relative transcript abundance



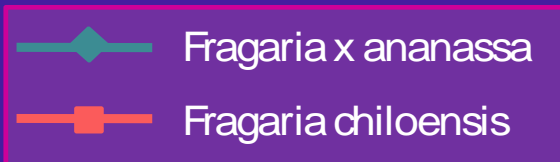
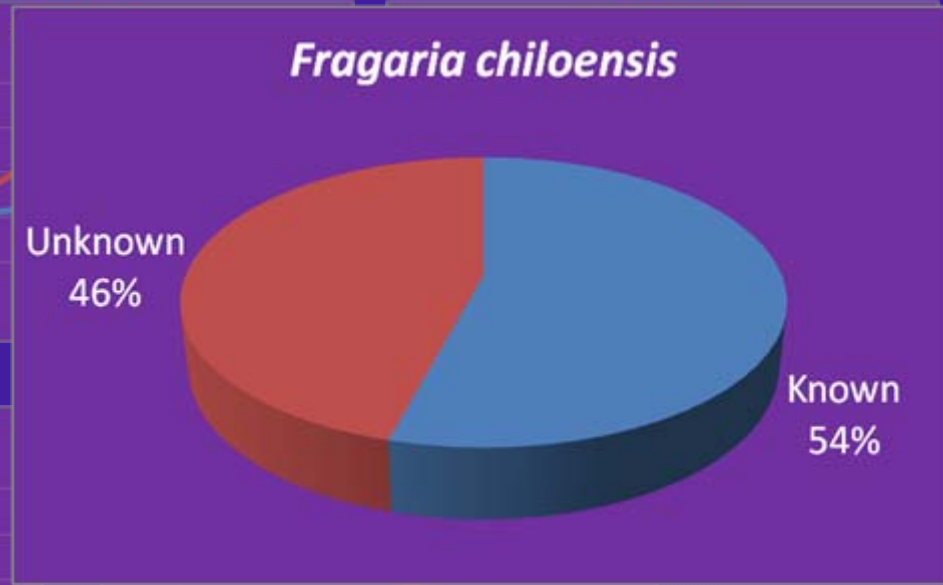
Genes involved in fruit flavor and development

Relative transcript abundance



◆ *Fragaria x ananassa*
■ *Fragaria chiloensis*

Any regulated transcripts show no similarity entry in the database



Relative

Conclusions

- Current work reports a medium scale transcript analyses in two interesting *Fragaria* species: *F. x ananassa* is a popular hybrid fruit species and *F. chiloensis* is a rich repository of genetic resources for biotechnology and breeding
- 65 and 85 transcripts are regulated during fruit ripening in *F. x ananassa* (6%) and *F. chiloensis* (8%), respectively
- Of them, about 73% are up-regulated and about 27% are down-regulated in both species
- 37% and 46% of regulated transcripts showed no similarity entry in the database in *F. x ananassa* (6%) and *F. chiloensis* (8%), respectively
- The two strawberry species show several differences of gene expression which could account either for specie-specific divergences, either for their peculiar pigment accumulation pattern

Plant Material



MT99.163.14

VS



Camarosa

High Anthocyanin

VS



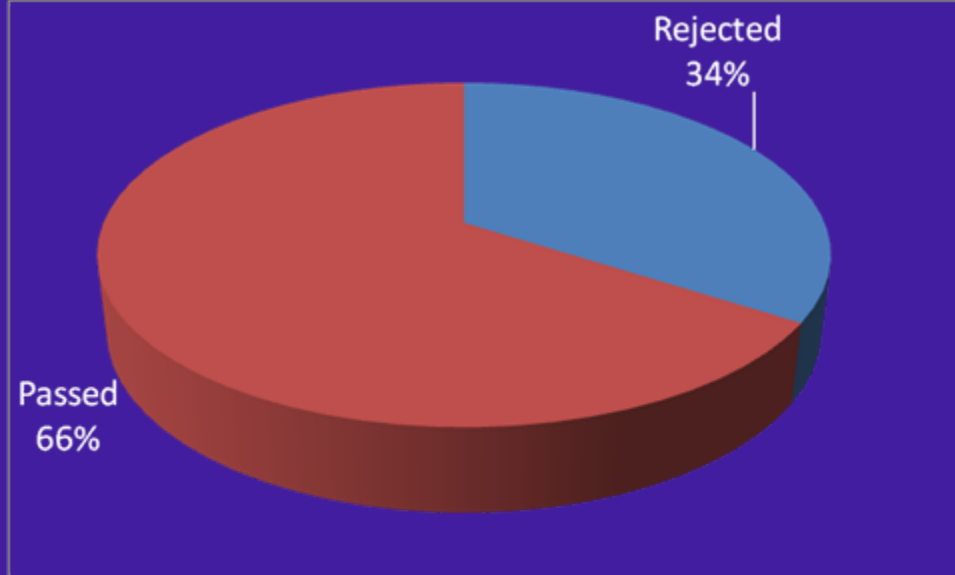
MT96.J24.2

Low Proanthocyanidin (Pas)

Low Anthocyanin

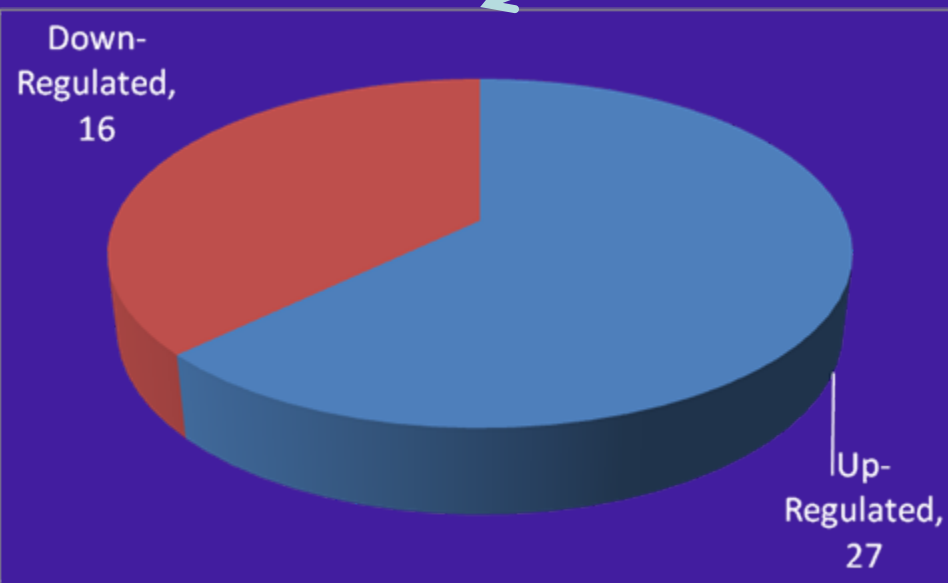
High Proanthocyanidin (Pas)
Medium anthocyanin

Genotype	firmness (g)	SSC (°Brix)	TA (meq/100g)
MT96.J24.2	400	6.4	10.7
MT99.163.14	451	7.3	9.9
Camarosa	505	7.0	11.6

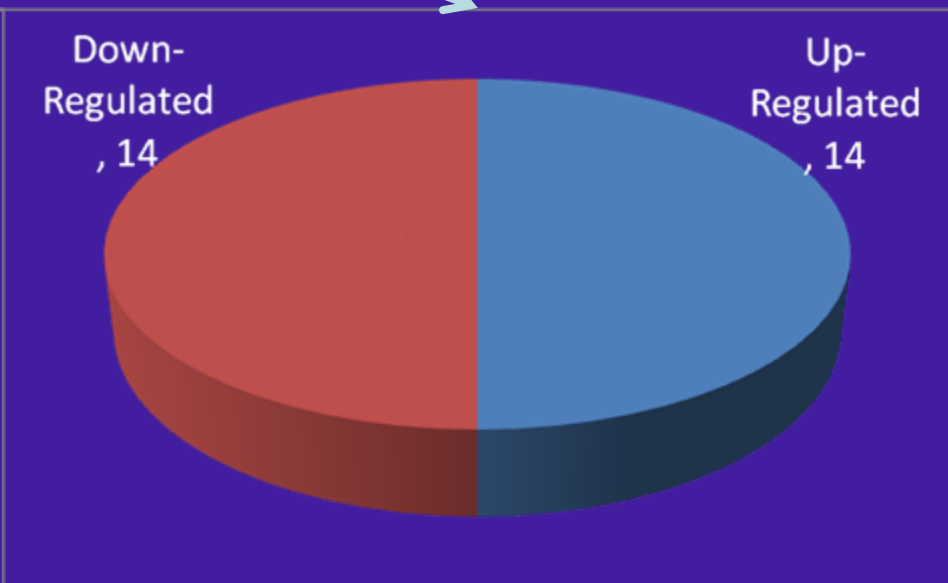


43 regulated transcripts

28 regulated transcripts



MT96, J24, 2 vs Camarosa



MT99, 163, 14 vs Camarosa

Microarray results

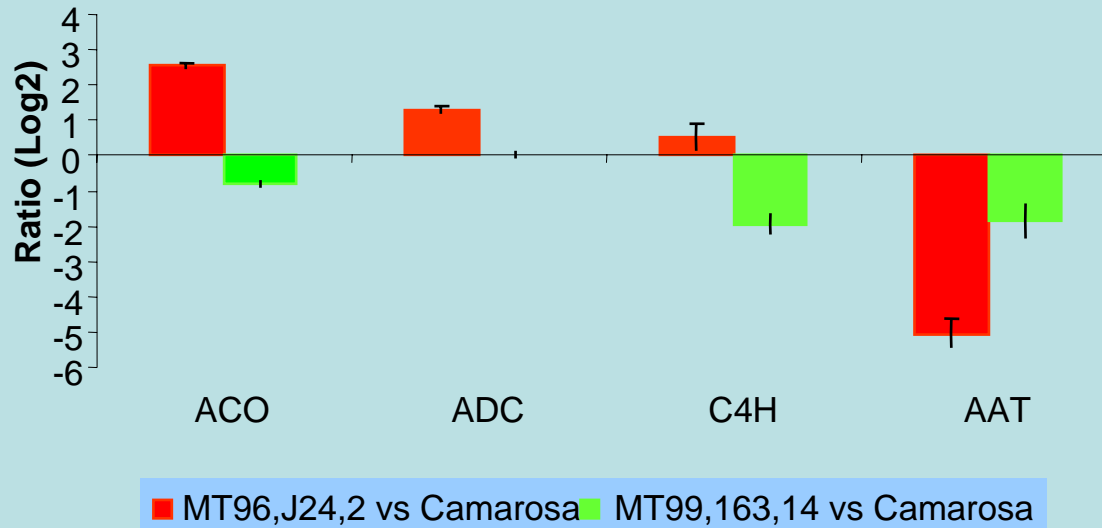
We didn't find considerable differences in **red ripe fruit** transcripts among genes putatively involved in flavonoid biosynthesis spotted on our microarray (**fht** AY691918, **fgt** AY695815, **dfr** AY695812, **anr** and **ifr-like**).

Only gene coding anthocyanidin synthase (**ans** AY695817) is up-regulated in MT96.J24.2 compared to Camarosa.



Real Time quantitative PCR (RT-qPCR)

Differentially Expressed Genes in Microarray



Real Time PCR

Genes	MT96,J24,2 vs Camarosa		MT99,163,14 vs Camarosa	
	average ratio	SD ratio	average ratio	SD ratio
1-aminocyclopropane-1-carboxylate oxidase (ACO)	5.83	± 0.37	0.57	± 0.04
S-adenosylmethionine decarboxylase (ADC)	2.44	± 0.19	1.02	± 0.09
cinnamic acid 4-hydroxylase (C4H)	1.49	± 0.40	0.26	± 0.05
alcohol acyltransferase (AAT)	0.03	± 0.01	0.29	± 0.09

Genes	MT96,J24,2 vs Camarosa			MT99,163,14 vs Camarosa		
	average ratio	SD ratio	cv	average ratio	SD ratio	cv
1-aminocyclopropane-1-carboxylate oxidase, 88.2	3,48	1,44	41,42	0,84	0,12	13,91
S-adenosylmethionine decarboxylase, 96.3	2,03	0,26	12,79	1,94	0,29	14,92
cinnamic acid 4-hydroxylase, 37	0,78	0,10	12,51	0,24	0,03	14,74
alcohol acyltransferase (AAT), 86	0,07	0,01	9,73	0,43	0,12	28,56

Microarray



**New generation
Strawberry
MICROARRAY?**