

**Research and Breeding
for strawberry quality traits:
the example of the antioxidants compounds**

Presented by:

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Breeding Strategy

Ciref is a professional association

Ciref is the breeding tool of the French growers,

Board composed of strawberry growers ->
Breeding strategy

Diseases resistance and tolerance to stresses

Sustainable production: profitability and return
on investments

Fruit quality: shelf life, consumer benefits



Breeding Strategy

Fruit taste: major goal since Ciref started its breeding program

INRA → 1976: Gariguette

80's new competition → new strategy / fruit quality

88: Grower decision to breed varieties for better fruit taste



CIREF in the Perigord area: SW of France: 1st area for strawberry production

DOUVILLE



Breeding Strategy

88 → 98 first release of new strawberry varieties with improved fruit quality



Ciflorette 1998

Cirafine 2001



Charlotte
2004



Breeding Strategy

The taste and yield antagonism

Difficulty to mix a high level of production and a high level of fruit quality for taste and flavour



Commercial
issue



Health benefit:

« Eat 5 fruits/vegetables
per day »

How can we integrate the health
benefit for the consumer in our
strategy of new cultivar release?

Nutritional quality
→ Antioxidant
compounds



What is an antioxidant?

respiration → production of Reactive Oxygen Species of (ROS)

oxidants (ROS)

- Free radical
- Oxygenated derivative of free radicals

oxydative stress

Cell damages



antioxidant defences

active

passive

enzymes

antioxidants

destruction of ROS

Trapping of ROS



What is an antioxidant?

antioxidant

=

compound able to neutralize the ROS
without being itself subject to conversion
to a harmful radical for the cell

Alhagdow, 2006





Health benefit of antioxidants for humans

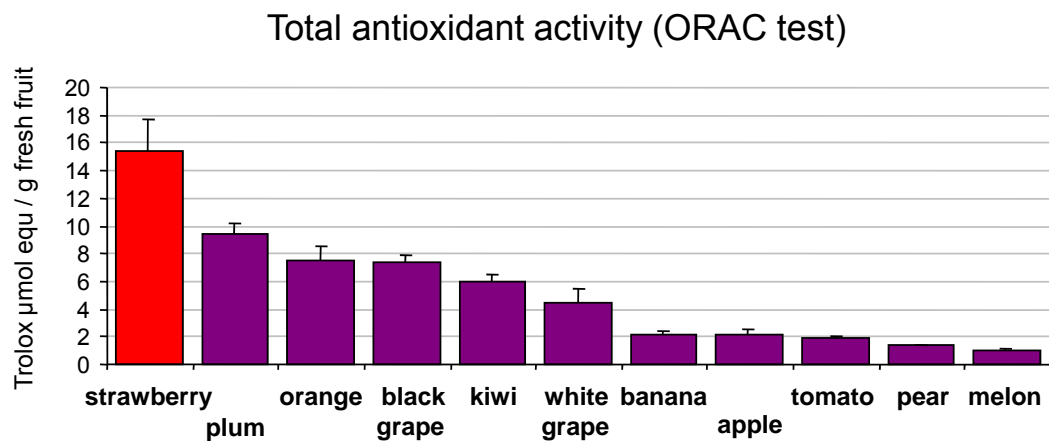
- To delay the ageing process of the cell
- Reduction of risks of:
 - cardiovascular diseases
 - degenerative diseases
 - cancers

Heinonen *et al.*, 1998
De Ruvo C *et al.*, 2000
Johnsen *et al.*, 2003

Yao *et al.*, 2004
Zafra-Stone *et al.*, 2007
Rossi *et al.*, 2008



Strawberry is a fruit with high levels of antioxidants



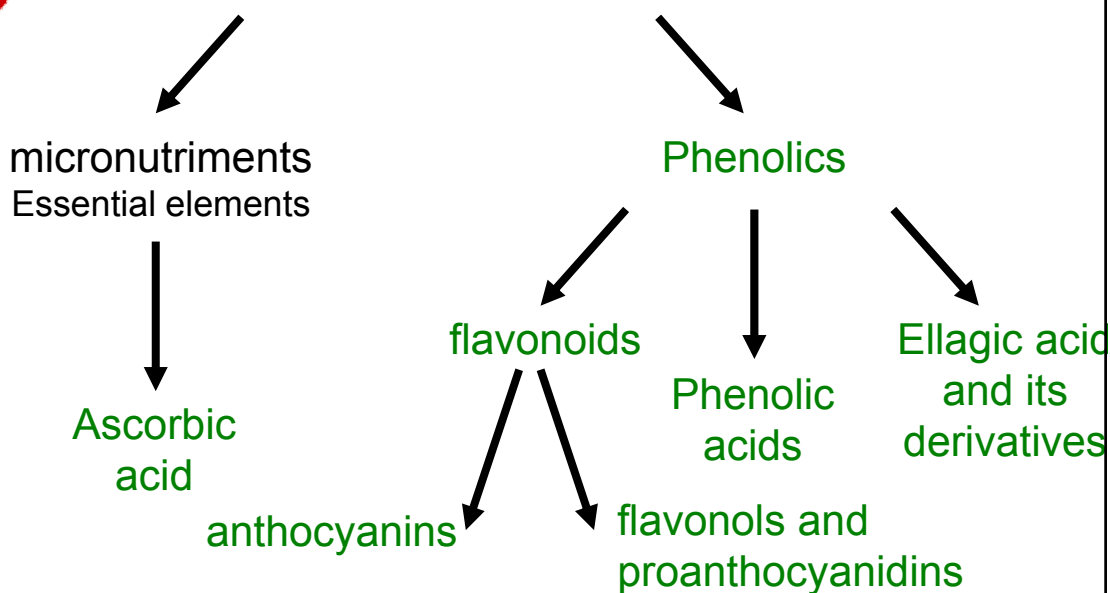
⇒ 2 to 11 times as many total antioxidant activity

⇒ **interest for research**

Wang *et al.*, 1996



The main strawberry antioxidants



Quantity in Strawberry:

Ascorbic acid > anthocyanins > flavonols



Factors influencing the antioxidants concentration in strawberry

■ Abiotic stresses

- Environment
 - High light intensity
 - Extreme temperatures
 - ...
- Cultural practices
 - Fertilizers
 - Soil / Soilless
 - ...
- Post-harvest
 - Storage
 - ...

■ Biotic stresses

- Pests
- Pathogens



■ Genetics

Capocasa *et al.*, 2008
Wang *et al.*, 2002
Olsson, 2004
Antonnen, 2006

Methods to quantify antioxidants

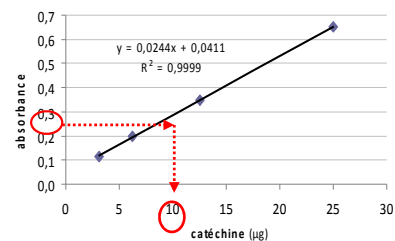
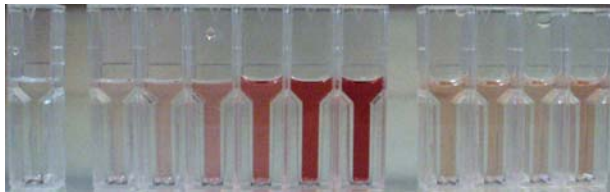
Fluorimetric: ORAC test

☞ Measurement of the fluorescence = light emission

From fractionation: HPLC, LC-ESI-MS

☞ Splitting and quantitative analysis of the compounds

Colorimetric ☞ Measurement of the colouring



- Lack of standardized methods
- Choice of the method according to the data use, the cost, the accuracy...

Cao et Prior, 1999





Heavy applied research
Teamwork with partners
3 collaboration projects

European DG-Agri : '**GenBerry**'



European FEDER and regional : '**SantéFraise**'



PLANT-KBBE : '**FraGenomics**'



1st results at Ciref

Screening of genetic resources

QTL detection for level of antioxidants

Choice of the parents with the highest level of antioxidants

Marker-Assisted Selection (MAS)





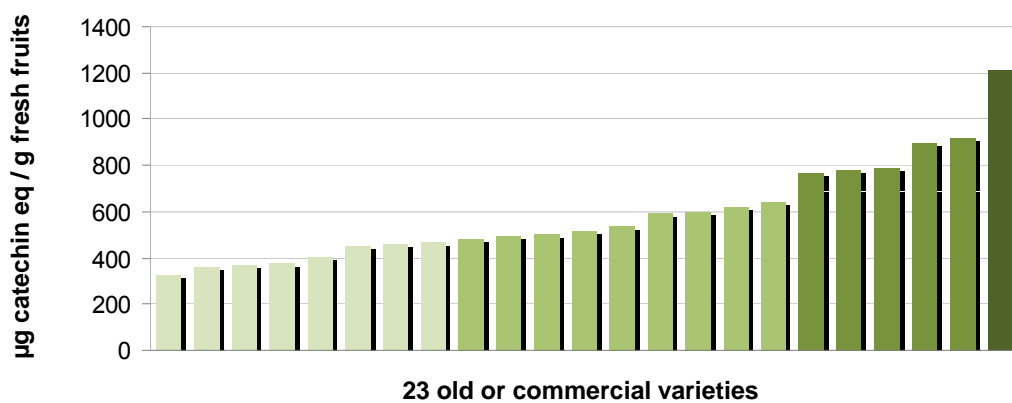
Methods to quantify antioxidants at Ciref

- Strawberries harvest: 2nd to 4th commercial production, without primary fruits, and fast frozen
- Grinding and hydrophilic extraction
- 5 colorimetric analyses of the antioxidants content
 - Total antioxidant activity : FRAP + TEAC ⇒ Trolox
 - Total anthocyanins content ⇒ pelargonidin-0-β-glucopyranosid
 - Total flavonoids content ⇒ catechine
 - Total phenolics content ⇒ gallic acid



Genetic diversity of the antioxidants content

Quantity of total flavonoids Douville 2009



- ⇒ Significant variation, factor of variation x3
- ⇒ **Choice of parents for the creation of new varieties**



QTL detection

Segregating Population

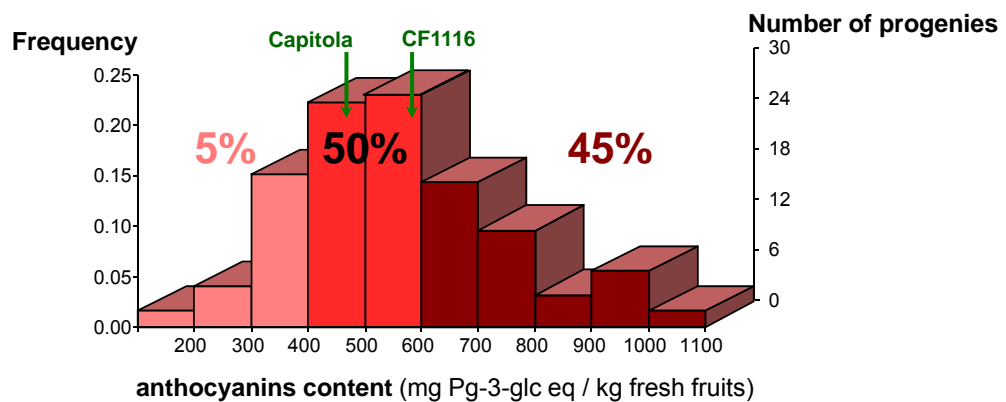


Soilless culture under plastic tunnel
Douville 2009



Introgression of high levels of antioxidants

Distribution of the hybrids of the population « Capitola x CF1116 » according to their content in anthocyanins



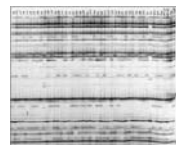
⇒ Possibility to double the antioxidants content by selection

⇒ **Marker-Assisted Selection (MAS)**

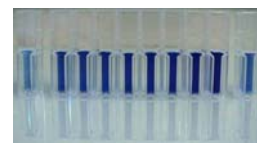
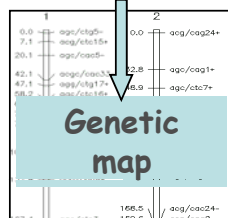


MAS: detection of QTL

QTL: region of the chromosome closely linked to the gene controlling the quantitative variation of a trait



Analysis of the markers of the progenies



Quantitative analysis

Quantitative datas

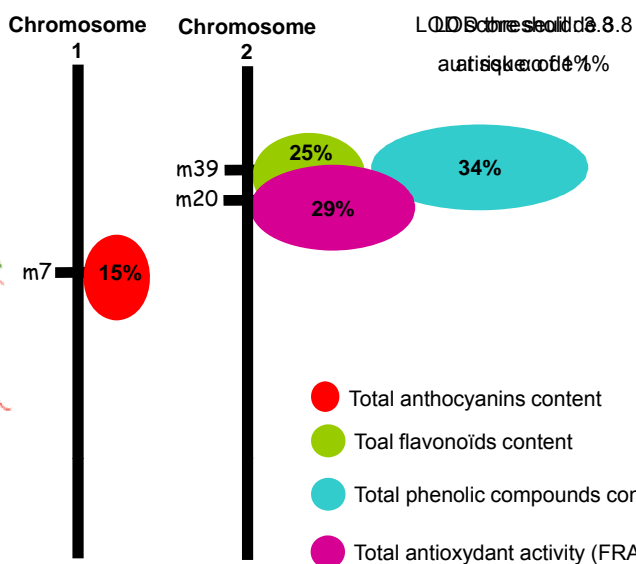
Sample	Value
1	100.0
2	100.0
3	100.0
4	100.0
5	100.0
6	100.0
7	100.0
8	100.0
9	100.0
10	100.0
11	100.0
12	100.0
13	100.0
14	100.0
15	100.0
16	100.0
17	100.0
18	100.0
19	100.0
20	100.0
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31	100.0
32	100.0
33	100.0
34	100.0
35	100.0
36	100.0
37	100.0
38	100.0
39	100.0
40	100.0
41	100.0
42	100.0
43	100.0
44	100.0
45	100.0
46	100.0
47	100.0
48	100.0
49	100.0
50	100.0

QTL detection

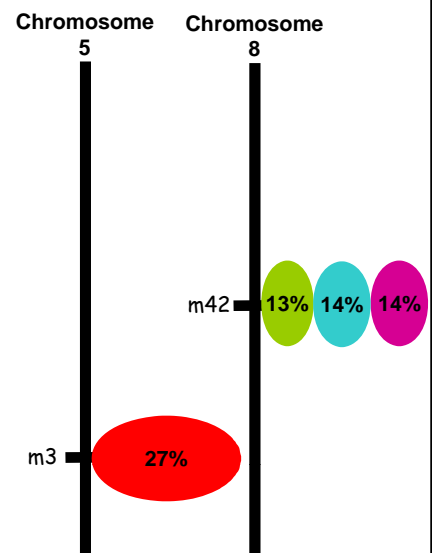


Detection of QTL related to the content of antioxidants

Female genetic map



Male genetic map



- Total anthocyanins content
- Total flavonoids content
- Total phenolic compounds content
- Total antioxidant activity (FRAP)

m = molecular marker

The size of the circle is proportional to the % of variability explained by the QTL
 Name of chromosomes and markers not contractual



Conclusion

- Strawberries are rich in antioxidants
- Variability among varieties
- Genetic improvement is possible
- Many international collaborations for the genetic study
- 1st results show great hope
- Next analysis: ascorbic acid content



Conclusion

- Importance of antioxidants for human health
- But it is not enough
- Improvement for health benefit must be done on the same basis than:
 - consumer acceptance: fruit taste,
 - grower benefit to extend the profitability of their production and the sustainability of their activity



Aknowledgements

- Ciref team: Pierre Gaillard and Aurélie Petit (Ciref - Villenave d'Ornon)



- INRA: Béatrice Denoyes-Rothan



- The team of INVENIO (ex HORTIS AQUITAINE) of Douville



- Aquitaine Region and Europe



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