



*Quali sono i geni responsabili della sintesi  
dei metaboliti di ortaggi e frutta  
dotati di effetti positivi per la salute  
e quindi  
del loro valore nutrizionale?*

*Qual è la struttura di tali geni?*

*Quali sono i fattori che regolano la loro  
espressione?*

Tre casi-studio: pomodoro, arancio, patata



Il frutto di pomodoro  
come fonte di  
metaboliti  
dall'elevato valore  
nutrizionale

**Chinoni  
(Vit K)**

**Glucosio,  
Fruttosio**



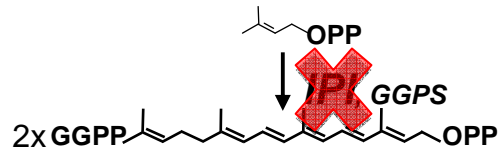
**Licopene  
 $\beta$ -carotene  
(proVit A)**

**Glutammato  
ed altri  
amminoacidi**

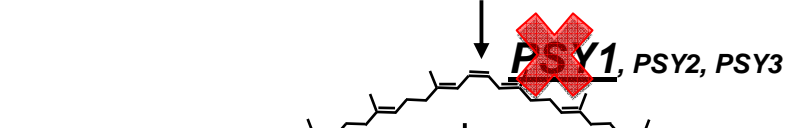
**Acido Ascorbico  
(Vit C)**

**Folati  
(Vit B)**

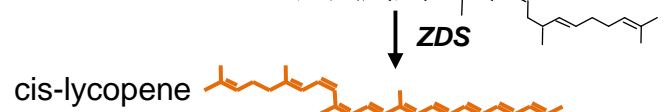
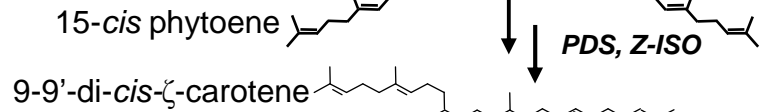
4x Isopentenyl diphosphate



Hirschberg, unpublished data



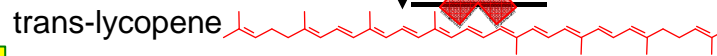
**yellow flesh, r**  
Fray et al., 1993



**tangerine, t**  
Isaacson et al., 2002



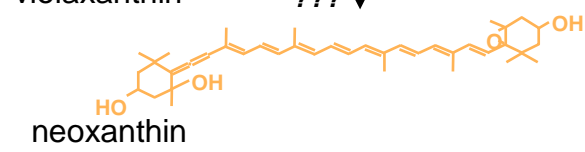
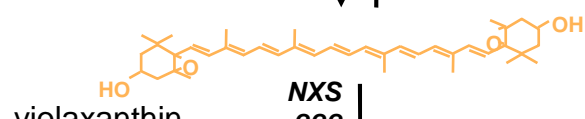
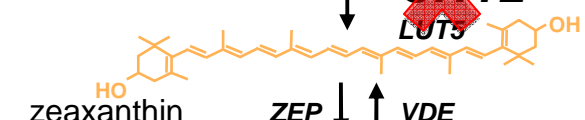
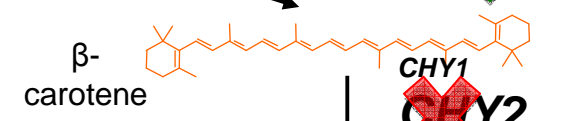
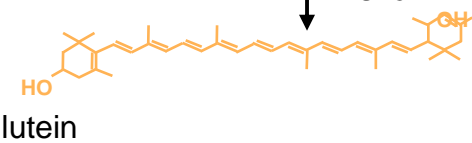
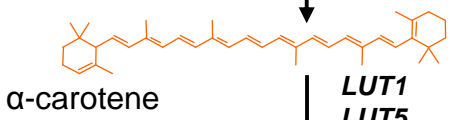
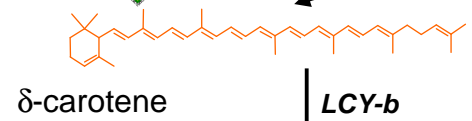
**Delta, Del**  
Ronen et al., 2002



**LCY-e**



**LCY-b**



**Beta, B**  
Ronen et al., 2000

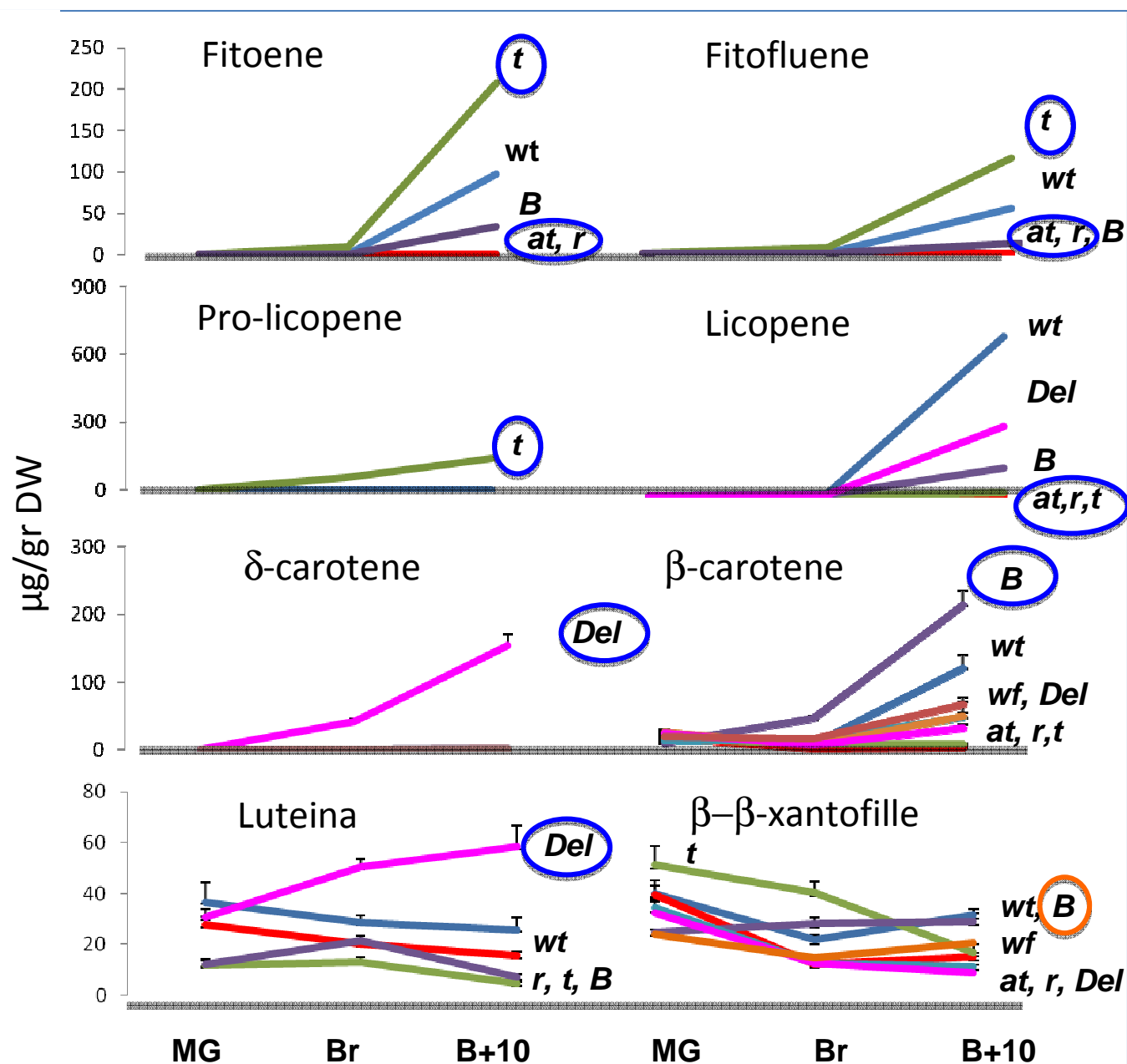
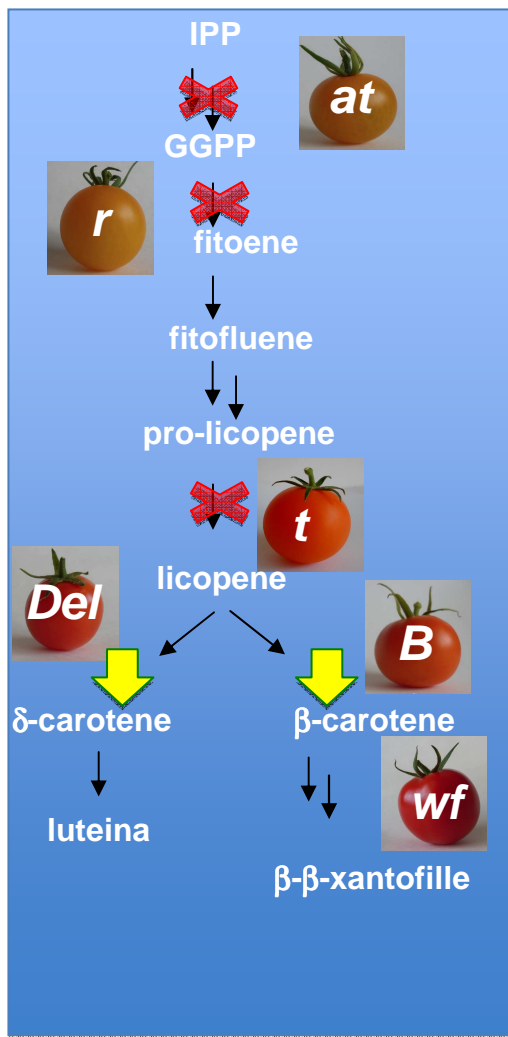


**white flower, wf**  
Galpaz et al., 2006

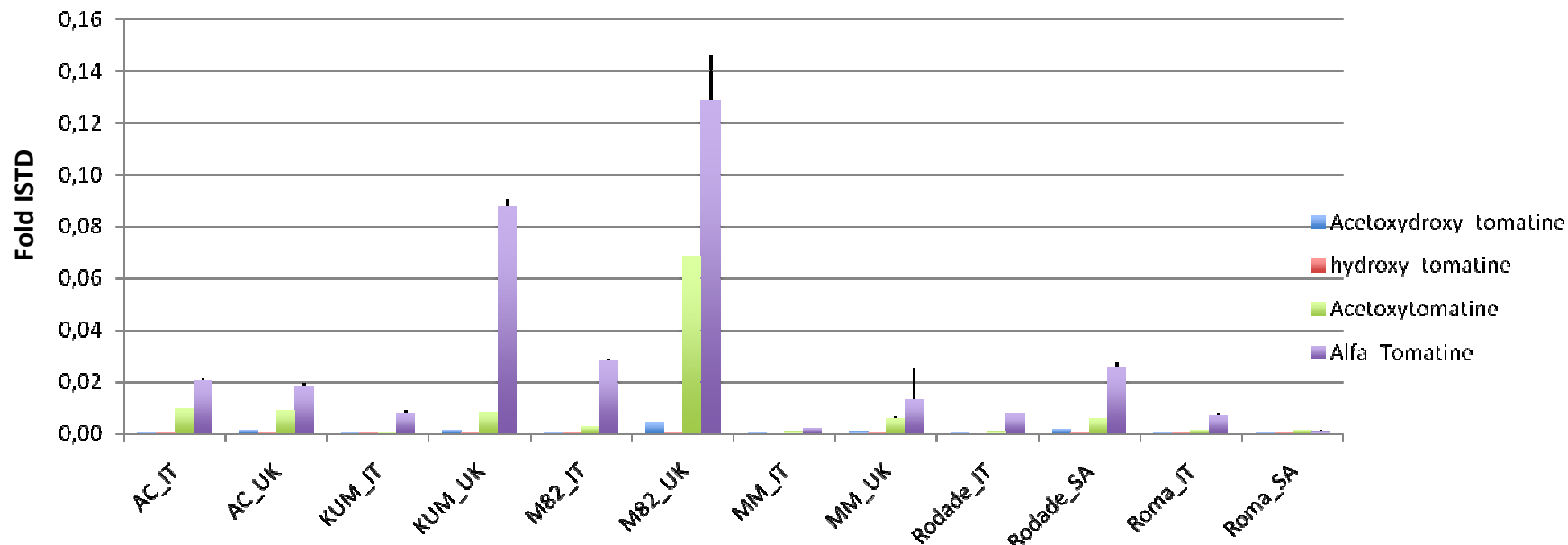


**Perdita di Funzione**  
**Guadagno di Funzione**

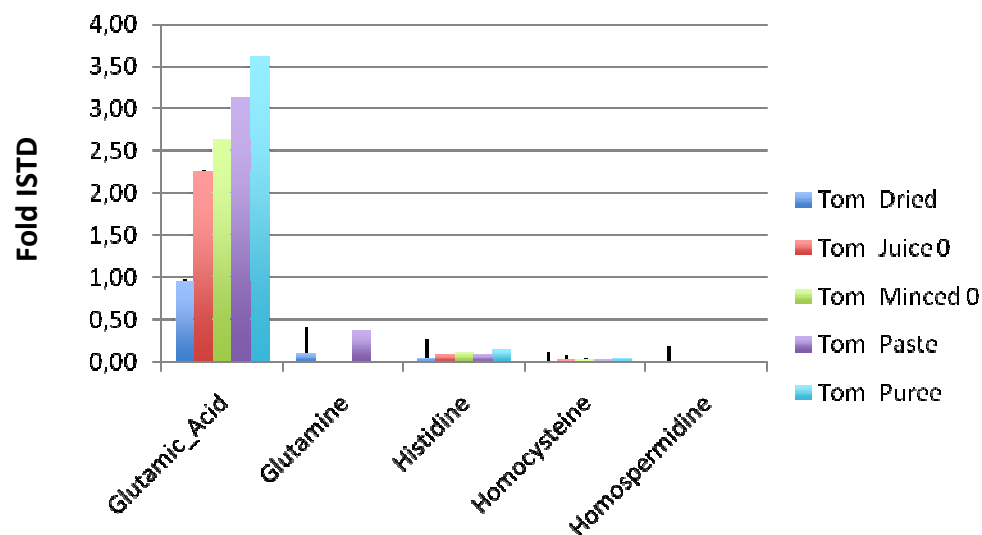
# Analisi LC-DAD-MS dei carotenoidi nella collezione di mutanti ENEA



### Livello di alcaloidi in varietà di pomodoro cresciute in condizioni pedoclimatiche differenti



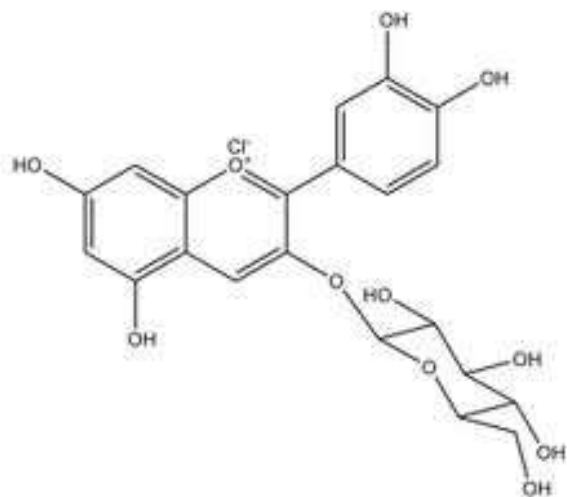
### Livello di amminoacidi in varietà di pomodoro sottoposte a processing



Analisi LC-ESI-MS di metaboliti polari in varietà di pomodoro cresciute in condizioni pedoclimatiche diverse o sottoposte a processamento (Develonutri, FP6)



## 2. Arancio





## ORIGINAL ARTICLE

### Blood orange juice inhibits fat accumulation in mice

L Titta<sup>1,2</sup>, M Trinei<sup>3</sup>, M Stendardo<sup>1</sup>, I Berniakovich<sup>1</sup>, K Petroni<sup>4</sup>, C Tonelli<sup>4</sup>, P Riso<sup>2</sup>, M Porrini<sup>2</sup>, S Minucci<sup>1,4</sup>, PG Pelicci<sup>1,4</sup>, P Rapisarda<sup>5</sup>, G Reforgiato Recupero<sup>5</sup> and M Giorgio<sup>1</sup>

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**Objective:** To analyze the effect of the juice obtained from two varieties of sweet orange (*Citrus sinensis* L. Osbeck), *Moro* (a blood orange) and *Navelina* (a blond orange), on fat accumulation in mice fed a standard or a high-fat diet (HFD).

**Methods:** Obesity was induced in male C57/Bl6 mice by feeding a HFD. *Moro* and *Navelina* juices were provided instead of water. The effect of an anthocyanin-enriched extract from *Moro* oranges or purified cyanidin-3-glucoside (C3G) was also analyzed. Body weight and food intake were measured regularly over a 12-week period. The adipose pads were weighted and analyzed histologically; total RNA was also isolated for microarray analysis.

**Results:** Dietary supplementation of *Moro* juice, but not *Navelina* juice significantly reduced body weight gain and fat accumulation regardless of the increased energy intake because of sugar content. Furthermore, mice drinking *Moro* juice were resistant to HFD-induced obesity with no alterations in food intake. Only the anthocyanin extract, but not the purified C3G, slightly affected fat accumulation. High-throughput gene expression analysis of fat tissues confirmed that *Moro* juice could entirely rescue the high fat-induced transcriptional reprogramming.

**Conclusion:** *Moro* juice anti-obesity effect on fat accumulation cannot be explained only by its anthocyanin content. Our findings suggest that multiple components present in the *Moro* orange juice might act synergistically to inhibit fat accumulation. *International Journal of Obesity* (2010) 34, 578–588; doi:10.1038/ijo.2009.266; published online 22 December 2009

**Keywords:** blood orange; orange juice; fat accumulation; anti-obesity; anthocyanins; cyanidin-3-glucoside.



Le antocianine determinano particolari qualità attrattive del frutto per il loro colore rosso brillante, ma esercitano un ruolo ancora più importante per le loro proprietà farmacologiche e antiossidanti.

## Contenuto dei parametri salutistici nel germoplasma pigmentato e biondo

	Acido Ascorbico (mg/100mL)	Unità ORAC ( $\mu$ molTE/100m)	Polifenoli totali (mg/L)	Antocianine totali (mg/L)
Tarocco	61,4 A	1734,5 C	771,2 BC	16,7 C
Moro	46,2 BC	2839,0 B	817,8 BC	127,3 B
Sanguinello e Doppio Sanguigno	61,5 A	1973,3 C	912,0 B	18,9 C
Ibridi pigmentati di mandarino	32,2 C	6726,1 A	1504,2 A	545,5 A
Navel	51,7 B	1494,6 C	775,5 BC	-
Valencia	57,8 AB	1384,7 C	739,9 BC	-
Vaniglia	29,5 C	1117,8 C	564,8 C	-
Germoplasma pigmentato *	59,4 A	2033,9 A	823,0 a	44,8
Germoplasma biondo **	50,5 B	1428,3 B	744,1 b	-

## Geni Strutturali

*chs* (calcone sintasi)

*dfr* (diidroflavonol 4-reduttasi)

*ans* (antocianidina sintasi)

*ufgt* (UDP-glucose:flavonoid 3-O-glucosiltransferasi)

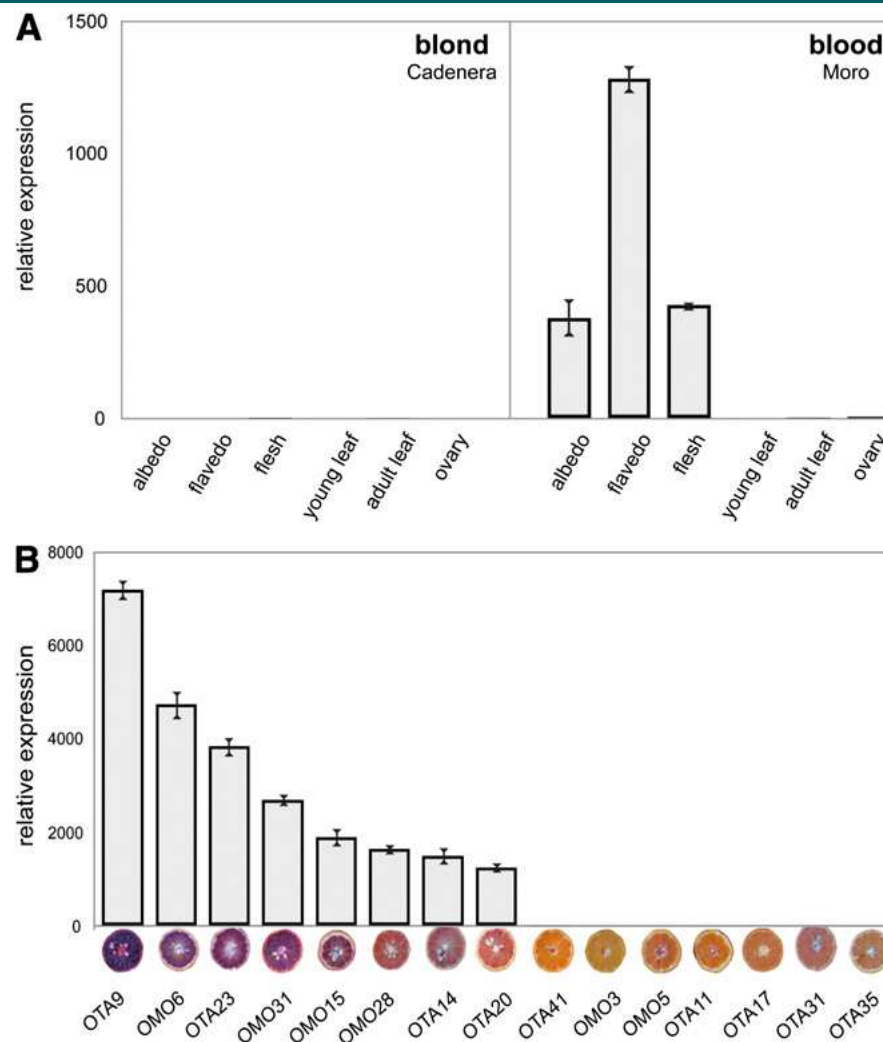
*gst* (glutathione S-transferasi).

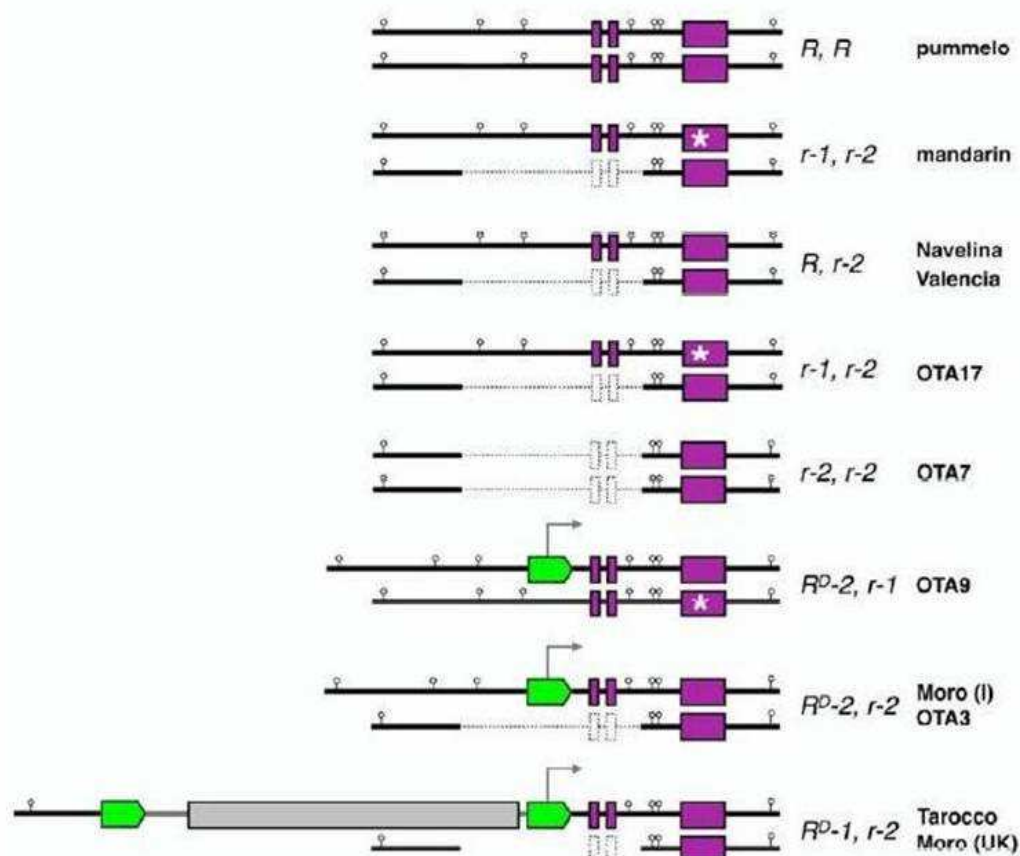


## Geni Regolatori

combinazione di due diverse famiglie di fattori trascrizionali, *c-myb* e *bHLH*; l'interazione tra MYB e bHLH e il coinvolgimento di proteine regolatrici con dominio WD40 è il meccanismo attraverso cui si sviluppa la regolazione della biosintesi delle antocianine e delle proantocianidine

L'espressione del gene  
regolatore *Ruby* determina  
l'accumulo di antocianine nei  
frutti





Variazioni alleliche del gene regolatore *Ruby* (in fucsia), del suo retrotrasposone (grigio) e delle LTR (verde) in differenti specie di agrumi, in varie accessioni di arancio e suoi ibridi





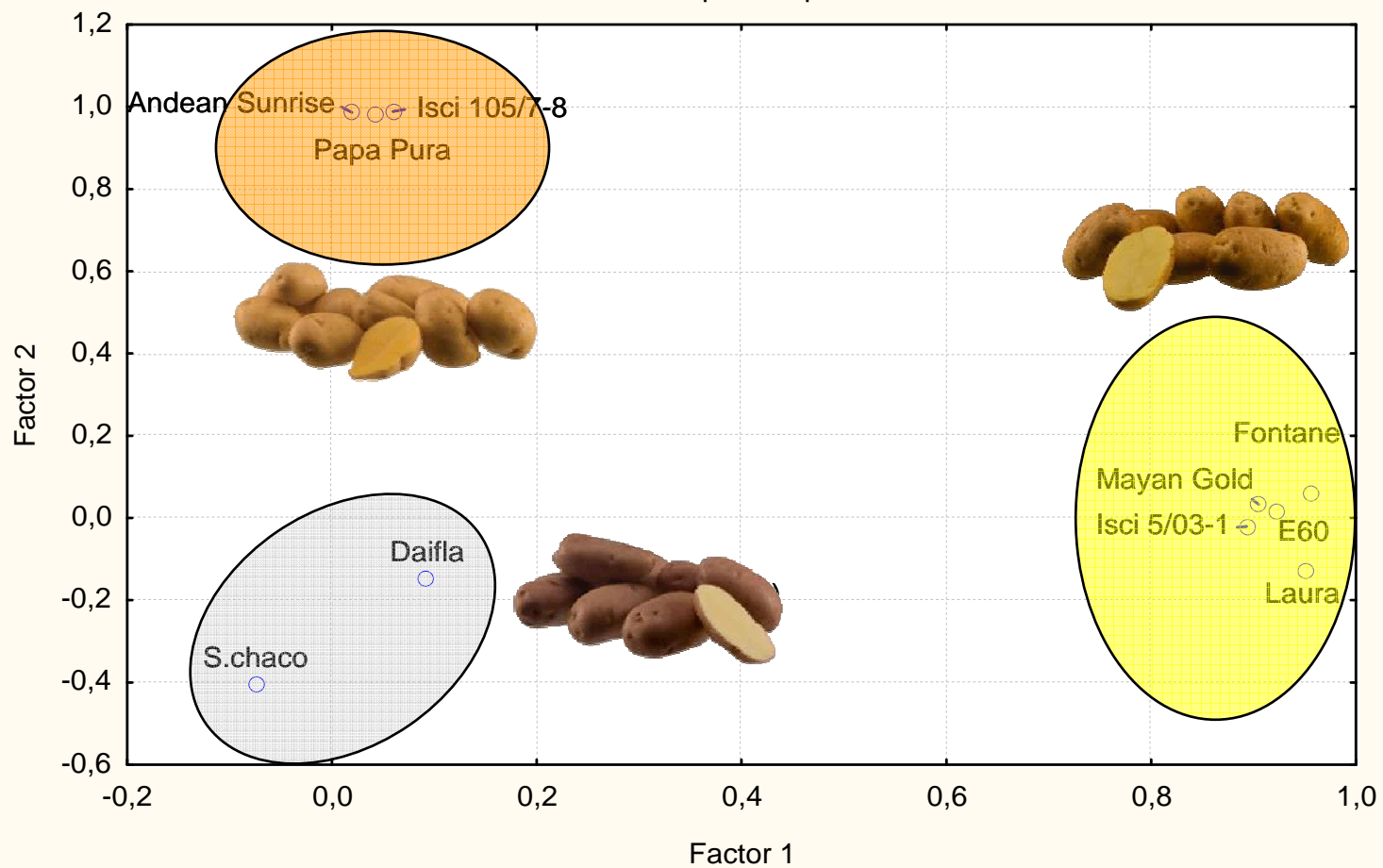




Factor Loadings, Factor 1 vs. Factor 2

Rotation: Unrotated

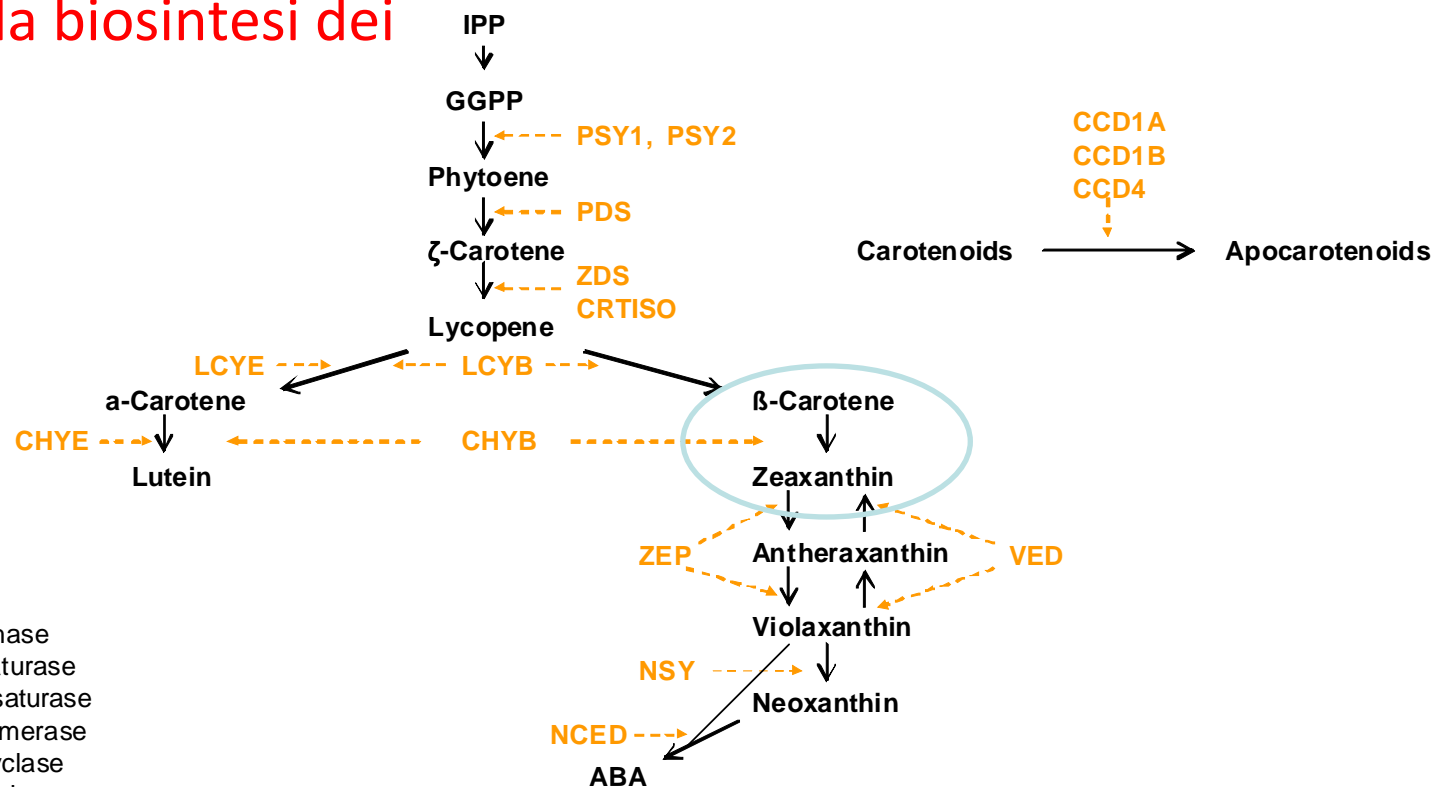
Extraction: Principal components



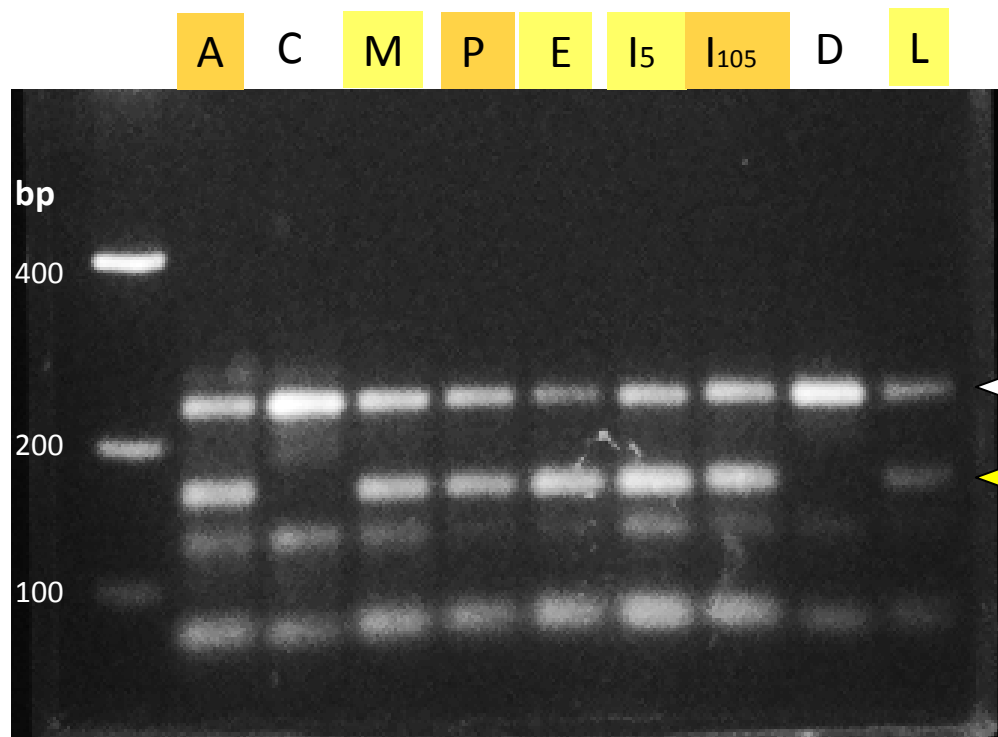
Genotipo	Specie	Ploidia	Contenuto di carotenoidi (µg/g DW)
Yema de Huevo	<i>S. phureja</i>	2 n	n.d.
Andean Sunrise	„	„	21.26 ± 1.84
Papa Pura	„	„	29.68 ± 2.21
ISCI 105/7-8	„	„	17.06 ± 1.29
Mayan Gold	„	„	35.25 ± 2.67
E60	<i>S. tuberosum</i>	4 n	16.24 ± 1.81
Fontane	„	„	15.50 ± 2.34
Laura	„	„	30.12 ± 1.98
ISCI 5/03-1	„	„	25.06 ± 1.52
Daifla	„	„	4.74 ± 0.36
<i>S. chacoense</i>	<i>S. chacoense</i>	2 n	1.74 ± 1.07



## Il pathway della biosintesi dei carotenoidi



- PSY** Phytoene synthase
- PDS** Phytoene desaturase
- ZDS** ζ-Carotene desaturase
- CRTISO** Carotenoid isomerase
- LCYB** Lycopene β-cyclase
- LCYE** Lycopene ε-cyclase
- CHYB** β-carotene hydroxylase
- CHYE** ε-carotene hydroxylase
- ZEP** Zeaxanthin epoxidase
- VDE** Violaxanthin de-epoxidase
- NSY** Neoxanthin synthase
- NCED** 9-cis-epoxycarotenoid dioxygenase
- CCD** Carotenoid cleavage dioxygenase



Frammento di 302 bp amplificato con i primer  
CHY2ex4F+ Beta-R822  
2 siti Alu I nell'allele 3  
1 sito Alu I in tutti gli altri alleli

237 : altri alleli *chy2* (recessivi)

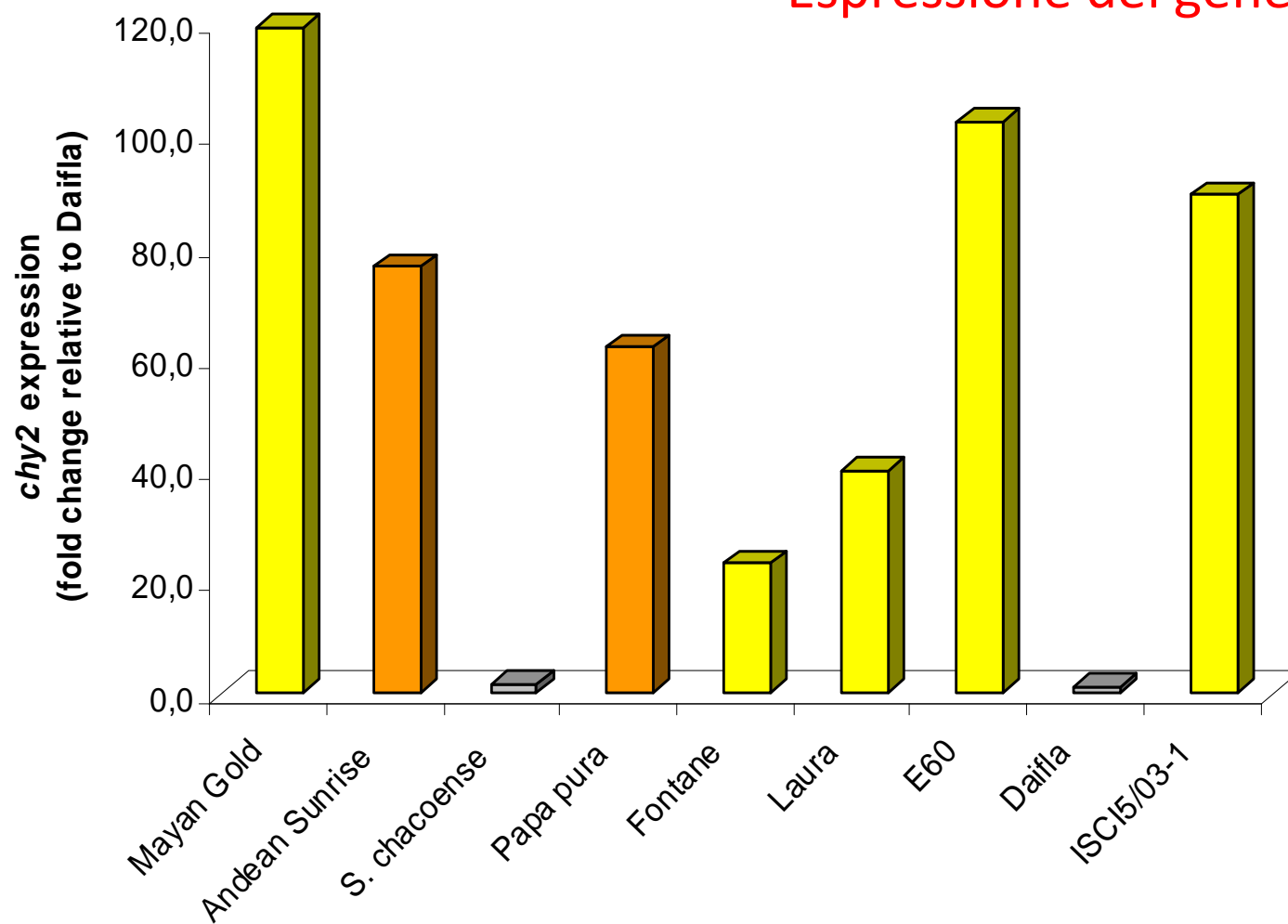
163 : *Chy2* allele 3 (dominante)

A – Andean Sunrise  
C – S. chacoense  
M – Mayan Gold  
P – Papa Pura  
E – E60  
I<sub>5</sub> – ISCI5/03-1  
I<sub>105</sub> – ISCI105/7-8  
D – Daifla  
L - Laura

**Variabilità al locus *chy2***

Identificazione dell'allele 3 mediante  
marcatore CAPS

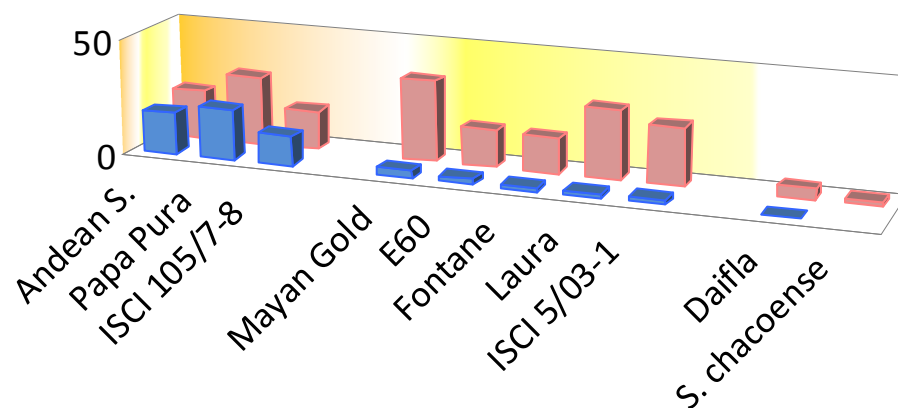
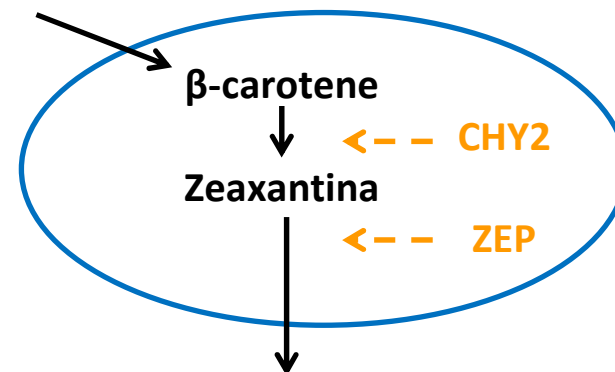
## Espressione del gene *chy*





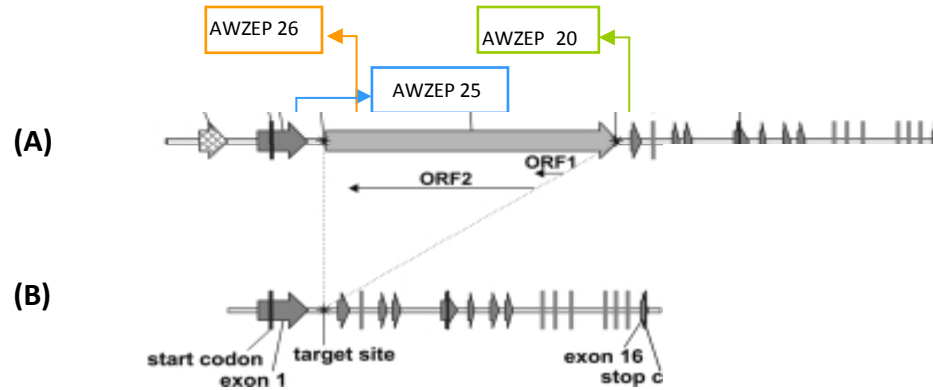
## Zeaxantina

- Valore nutrizionale
- Valore commerciale → colore della pasta del tubero



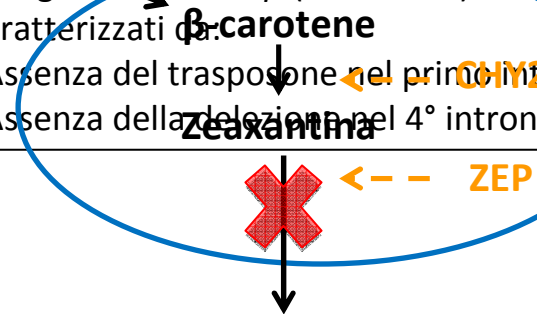
■ zeaxanthin    ■ total carotenoids

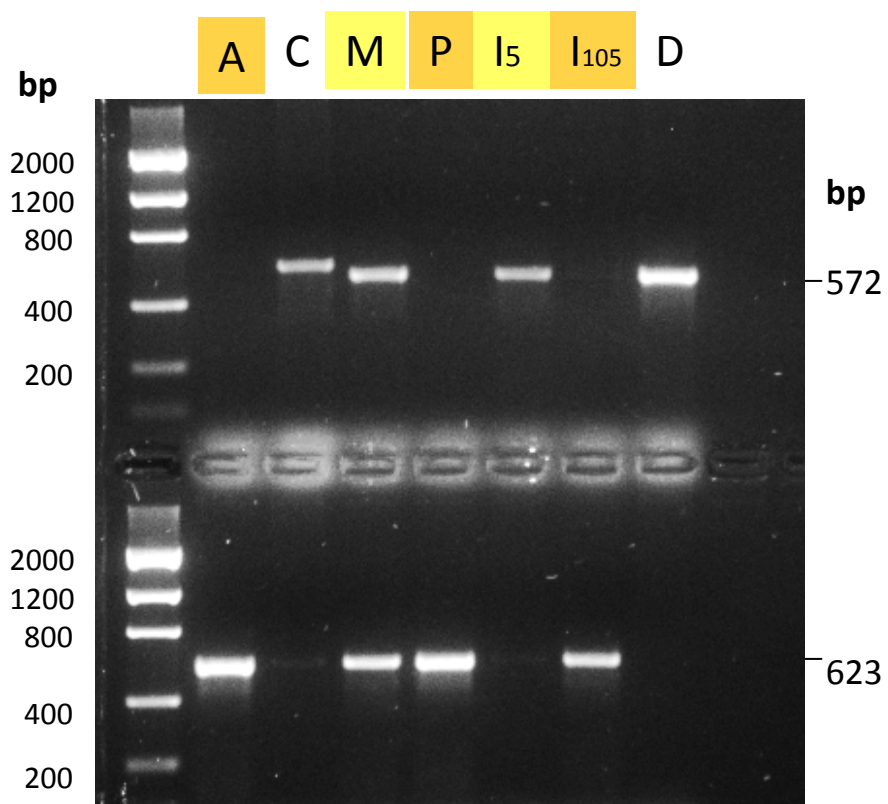
## Gli alleli al locus *zep*, che determinano l'accumulo o meno della zeaxantina



allele ***zep 1***, recessivo  
 Caratterizzato da:  
 - trasposone di 4102 bp nel primo introne  
 - delezione di 49 bp nel 4° introne

tutti gli altri alleli *Zep* (dominanti)  
 Caratterizzati da:  
 - Assenza del trasposone nel primo introne  
 - Assenza della delezione nel 4° introne





## Analisi della presenza del trasposone

Primers AWZEP25 / AWZEP20

Amplificazione degli alleli **Zep** dominanti (B)  
senza trasposone

Primers AWZEP25 / AWZEP26

Amplificazione dell'allele recessivo **zep 1** (A)  
con il trasposone

Genotipo	Ploidia	Zeaxantina ( $\mu\text{g/g DW}$ )	% carotenoidi totali	Aplotipo <i>Chy2 : chy2</i>	Aplotipo <i>Zep : zep</i>
Yema de Huevo	2 n	n.d.	n.d.	n.d.	n.d
Andean Sunrise	,,	18.23 + 1,62	85.8	1 : 1	0 : 2
Papa Pura	,,	22.23 + 1,74	74.9	1 : 1	0 : 2
ISCI 105/7-8	,,	13.02 + 0,83	76.3	1 : 1	0 : 2
Mayan Gold	,,	3.45 + 0,18	9.9	1 : 1	1* : 1
E60	4 n	2.38 + 0,12	14.7	3 : 1	4 : 0
Fontane	,,	1.52 + 0,23	9.8	2 : 2	4 : 0
Laura	,,	1.77 + 0,06	5.9	2 : 2	4 : 0
ISCI 5/03-1	,,	2.14 + 0,12	8.5	3 : 1	4 : 0
Daifla	,,	0.18 + 0,02	3.8	0 : 4	4 : 0
<i>S. chacoense</i>	2 n	0	0	0 : 2	2* : 0

### **CRA-ACM**

#### **Acireale**

Reforgiato Recupero G.

Recupero S.

Russo G.

Licciardello C.

Russo M.P.

Pietro Paolo D.

Rapisarda P.

Fabroni S.

Amenta M.

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***GRAZIE PER L'ATTENZIONE...***

*Giuseppe Mandelino*