Strawberry breeding program and new varieties from UPM Ancona (IT)

CRISTINA AND ROMINA: TWO NEW STRAWBERRY VARIETIES

1. Brief history of Marche Polytecnic University strawberry breeding program.

The strawberry breeding program of Marche Polytechnic University (Ancona, IT) started in 1993 thanks to the contribution of the national project (Frutticoltura) supported by the Italian Ministry of Agriculture (MIPAAF).

In the last 18 years the aim of the strawberry breeding program of Marche Politechnic University was to obtain cultivars showing interesting agronomic characteristics associated with high adaptability to open field cultivation in climatic conditions from the mid Adriatic to the Center-North of Europe and with high tolerance to limestone and clayey soils as well as to the major strawberry diseases. Lastly great importance was given to the evaluation of fruit with improved nutritional quality (mainly total antioxidant capacity, Poliphenols, Anthocyanin, Vitamin C and Folate contents). With this aim we developed a breeding program involving wild genotype (F. *virginiana* ssp. *glauca*) and its backcrosses because of the high nutritional quality of such specie. From this breeding program we have already licensed four strawberry variety with high sensorial and nutritional attribute, cv. Sveva (2003), Adria (2003), Cristina (2010) and Romina (2010). These varieties have been involved in experimental studies to validate their high nutritional quality.

Varieties and selections are evaluated at the "P. Rosati" Experimental Farm (43°31′N 13°36′E. 46 m altitude), on non-fumigated soil, having the following main characteristics: pH 7.9, active calcium 9% and texture composed at 40% clay, 25% sand and 35% silt. The experimental plots consisted of 10 plants growing in standard cultivation conditions with plastic film mulched double row (30 cm distance between plants, 1.5 m between rows), on raised bed, with drip irrigation.

Figure 1. Experimental strawberry field at "P. Rosati" experimental farm, Università Politecnica delle Marche, Ancona (IT).

2. Nutritional quality of strawberry fruits

A growing body of epidemiological studies suggests a consistent association between consumption of diets rich in fruits and vegetables and a lower incidence of chronic diseases, including cancer, cardiovascular and neurodegenerative diseases. In the last few decades, single subgroups of fruits or vegetables have been taken into consideration to facilitate the observation of specific health-promoting or -preventing actions. Furthermore, a detailed search of the plant compounds potentially expressing biological activities has been conducted.

Strawberry (*Fragaria* x *ananassa*, Duch.) is among the most popularly consumed berries. The relevant nutritional value of strawberry fruits has been exhaustively proven and correlated to the high content of micronutrients such as minerals, vitamin C and folate which are essential for health, and, more recently, to the high levels and variety of phytochemical constituents.

A huge number of studies show a relevant variation in the phytochemical content and the nutritional quality of the fruits depending on genotypes and several pre- and post-harvest factors.

The potential health benefits of phytochemicals found in strawberries have received ample attention in the recent literature, focusing especially on compounds with high antioxidant properties such as many phenolic compounds, together with the relevant contribution of vitamin C. The evaluation of the impact on human health has stimulated a detailed phytochemical investigation to identify the phenolics present in strawberries. Several subclasses of phenolics have been reported and well represented in strawberries. Flavonoids are the main phenolic representative class, and the most abundant compounds belonging to the subgroups of anthocyanins, flavonols quercetin and kaempferol, and flavanols. In addition, strawberries are extremely rich in phenolic acids such as hydroxycinnamic acid derivatives, and are among the popularly consumed foods with most relevant content of ellagic acid. Many studies carried on in vitro suggest that strawberry phenolics show a wide range of biological activities ranging from anticancer to anti-inflammatory, neurodegenerative and antioxidant effects. The anticancer effects of individual or whole strawberry phytochemicals seem to involve multiple synergistic mechanisms of action, including antioxidant protection of DNA from oxidative damage but also other effects beyond antioxidation.

Among strawberry micronutrients, the level of vitamin C is generally considered as a parameter of particular interest for the nutritional evaluation of strawberry varieties, and is often included in the pool of chemical measurements conducted for screening purposes and for the evaluation of the breeding strategies.

However, also folate plays a crucial role in emphasizing the nutritional value of strawberry, when considering that the fruit is among the richest natural food sources of folate. The term folate refers to a wide group of derivatives of the water-soluble B vitamin much more frequent in food and in the human body than the more stable synthetic form, folic acid, used as supplement and in food fortification.

However, most of these enthusiastic findings come from in vitro studies, and little is known on the health effects of strawberry phytochemicals once ingested together with the fruit matrix, absorbed and metabolized. Up to now, few published human studies investigated the metabolism and excretion of strawberry phenolics after ingestion of the whole strawberry fruits. Furthermore, even a future understanding of the mechanisms and entity of tissue distribution and accumulation of phenolics after berry consumption will not ascertain the direct role of these compounds in the health benefits correlated to the whole fruits.

The Strawberry fruit bioactive compounds show strong genetic variation which has stimulated our agronomic research to specifically define breeding programs, for obtaining and monitoring new selections with improved nutritional value. Interestingly, higher variation of content for all the bioactive compounds previously cited was observed among genotypes originated from our breeding program.

3. Strawberry Breeding to increase nutritional quality

The breeding approach can be successful if the variability and heritability of the bioactive compounds determining the Total Antioxidant Capacity (TAC) trait indicate the possibility of achieving a breeding progress. It is well know that the availability of genetic diversity within compatible species of any given crop will enhance the extent of improvement. However, the success of breeding approach is related to deep knowledge of the most useful wild and cultivated species genetic diversity.

The effect of genotypes in affecting the nutritional quality of strawberry is well known (Scalzo et al., 2005), the levels of antioxidants and antioxidant capacity in strawberry extracts from whole fruits vary considerably among genotypes (Scalzo et al., 2005) but few genotypes are well characterized for these important features. Furthermore, limited knowledge is available on the possibility of improving strawberry nutritional traits by breeding (Capocasa et al., 2008).

Accessions of the progenitor wild species are valued for their nutritional quality and have shown improvement in fruit nutritional quality in breeding material originated from Fragaria virginiana spp. glauca (FVG) inter-specific crosses (Scalzo, 2005 and Tulipani, 2008).

A breeding program based on the evaluation and comparison of different populations of F. x ananassa crosses and FVG inter-specific back-crosses has been started by the Università Politecnica delle Marche with the objective to generate new selections with increased fruit nutritional quality.

4. Description of the new varieties released by UPM Breeding Program

The cultivars released by UPM are characterized by high adaptability to not fumigated soil and to different culture conditions:

"Adria" ("Granda"x "Miss"), EU Plant Variety Rights - File number 2003/1797, exhibit a late harvesting season with a very high productivity of high fruit size and firmness, of red lucent colour and fair good taste.

Figure 2. Plant of Adria and its high production.

"Sveva" (EM 483 x 87.734.3), EU Plant Variety Rights - File number 2003/1796, performs with a very late production, about the same time of 'Florence', with a high productivity of conic long fruit with a good quality (Capocasa et al., 2003).

Figure 2. Sveva typical conic fruits.

Fruit of both these first varieties were also well characterized for their high content of anthocyanin and polyphenol (Sveva in particular) and their important benefits for consumer health (Tulipani et al., 2008; Alvarez-Suarez, 2011).

"Romina" AN99,78,51 (95.617.1 x Darselect), EU Plant Variety Rights - File number 2011/1275. Is a June bearing variety, with high adaptability to non fumigated soil. Very early ripening. Fruit conic or bi-conic shape. Good sweet taste (high sugar and low acidity). High firmness and shelf life. High fruit nutritional quality determined by high polyphenol, anthocyanin, vitamin C and Folate contents (Tulipani et al., 2008).

Figure 4. Romina plants can be quite vigouros, for this reason can be grown with low inputs.

Figure 5. Nice color and high firmness of Romina fruits.

"Cristina" (CN 95,602,8 x CN 95,419,4) EU Plant Variety Rights - File number 2011/1274. June bearing variety, with high adaptability to non fumigated soil. Very late ripening (Florence time). Very high productivity, large fruit (32 g FW) of conical shape, with good taste.

Figura 6. Late and high production of Cristina plants.

Figure 7. Large size and sweet Cristina fruits.

Productive and quality data of these varieties are available in the annex Table 3.

5. Health related bioactive compounds of the new varieties

From studies carried out on strawberry genotypes, originated by our breeding program, now including also F. virginiana ssp glauca as inter-specific parent, an increase in their fruit nutritional quality, expressed as total antioxidant capacity, phenol, anthocyanin, vitamin c and folate content has been observed (Figure 8, 9 and 10) (Tulipani et al., 2008).

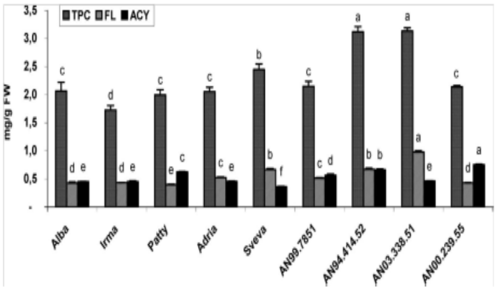

Figure 8. Phenol (tpc), anthocyanin (acy) and flavonoid (fl) content strawberry from UPM breeding program. Sel AN99,78,51 is the former codification for Romina; sel AN94,414,52 is f1 of *F. virginiana glauca* x *F. X ananassa*; sel AN00,239,55 is a first back cross of AN94,414,52 x *F. x ananassa.*

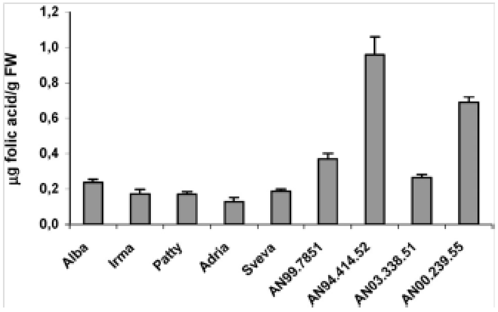

Figure 9. Folic acid content of strawberry from UPM breeding program. Sel AN99,78,51, now named Romina; sel AN94,414,52 is f1 of *F. virginiana glauca* x *F. x ananassa*; sel AN00,239,55 is a first back cross of an94,414,52 x F. x *ananassa.*

Folate content among our varieties and selections shows a high variability, in fact nearly a 4-fold difference was measured between fruit with the lowest (Adria, 19 µg/100g FW) and the highest value (AN94.414.52, 96 µg/100g FW) (Tulipani et al., 2008; Figure 9). From these date it is clear that depending from the fruit content of Folic acid, 250–350 g of strawberries can easily supply at least the 30–40% (125mg folate on average) of the daily human demand, this considering the US recommendation of 400 mg/d as average for adult consumer (http://ods.od.nih.gov/).

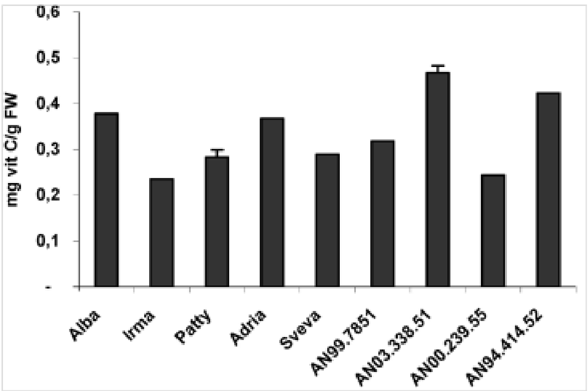

Figure 10. Ascorbic acid content of strawberry from UPM breeding program. Sel AN99,78,51, now named Romina; sel AN94,414,52 is f1 of *F. Virginiana glauca* x *F. X ananassa*; sel AN00,239,55 is a first back cross of an94,414,52 x *F. x ananasa.*

The evaluation of folic acid and vitamin C content for the strawberry varieties released by the UPM, confirm the improvement of the nutritional quality of fruit. Romina fruit differ for the highest content of folates and vitamin C, in fact about 300g of Romina fruits can supply the 30% of folates and the 100% of vitamin C requested for human daily intake. About 300g of Sveva fruit can contribute to the 15% of folate and 100% of vitamin C requested human daily intake. For the higher content of vitamin C can be enough 250g of fresh Adria fruit to satisfy the daily demand of this important vitamin and also the 15% of folate demand (Table 1). Fruits of Adria, Sveva and Romina have been characterized also for the high content of other bioactive compounds, such as polyphenols and anthocyanin.

Table 1. Folic acid and vitamin C content of UPM varieties and relative RDA for adults (men/women).

Genotype	Folic Acid (µg/100 g FW)	RDA* Folic acid	Vitamin C (mg/100g FW)	RDA* Vitamin C
SVEVA	21	5%	30	33%
ADRIA	19	5%	38	42%
ROMINA	40	10%	31	33%

***Office of dietary supplements, National institute of health, (http://ods.od.nih.gov/)**

6. Health validation of the UPM new varieties

Working in collaboration with colleagues of UPM Faculty of Medicine, Prof. Maurizio Battino group, have been developed differed studies finalized to have a first validation of some specific health benefits that can derive from the consumption our strawberry varieties fruit. A recent study (Giampieri et al., 2012) demonstrated that strawberry anthocyanin plays an active role in defense epithelial cells against UVA ray exposure. Such a study has demonstrated that anthocyanin extracts, from fruits of Sveva variety, reduce the DNA damage and viability of hude fibroblast in vitro culture (Figure 11). Overall, these data show that Sveva strawberry fruit contains compounds that confer photoprotective activity in human cell lines and may protect skin against the adverse effect of UV-A radiation.

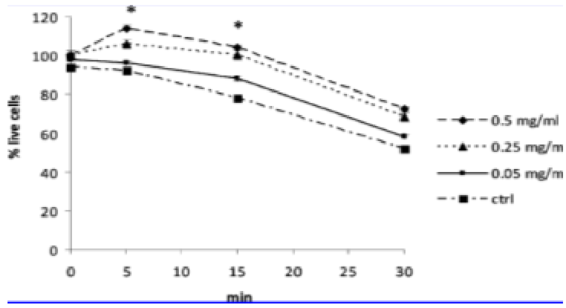

Figure 11. Viability of hude fibroblast after exposure to UV-A. Control cells and cells preincubated with strawberry extracts were exposed to uv-a and analyzed to determine the percentage of viability. Cells preincubated with 0.5 mg/ml extract showed a increase in viability after exposure between 5 and 15 min than the controls.

Table 2. Average change in plasma Total Antioxidant Capacity (measured by FRAP and TEAC) assays and in serum levels of ascorbic uric acids among all the subjects, after consumption of a single dose (1kg) of strawberry fruits. Data are presented as means ± SEM. Values in brackets indicate the % variation from baselines.

	FRAP (μmol TE/l)	TEAC (mmol TE/l)	Ascorbic acid (μm)	Uric acid (μm)
Baseline	34.8 ± 3	3.2 ± 0.1	28.8 ± 1	124 ± 13
After 1 h	40.0 ± 2* (+18.1%)	3.2 ± 0.1 (+0.1%)	49.5 ± 1* (+73.7%)	125 ± 13 (+0.8%)
After 2 h	42.9 ± 3* (+22.8%)	3.2 ± 0.1 (+0.1%)	55.9 ± 2* (+96.0%)	125 ± 14 (0.0%)
After 3 h	42.0 ± 3* (+22.0%)	3.2 ± 0.1 (+0.6%)	55.8 ± 2* (+95.4%)	125 ± 14 (- 0.4%)

2009) with volunteers to evaluate the possible acute plasma effects resulting from a high single dose intake of strawberry fruit, this always using strawberry generated by UPM breeding program, compared to other commercial varieties. The result of this study showed a significant acute increase in plasma total antioxidant capacity (TAC) already 1 h after fruit intake (18.1% increase) and this persisted during the following 2 h (22–23% increase). Together with the rise in total antioxidant capacity (FRAP values) was also observed a highly significant increase in the serum concentration of ascorbate (74% increase after 1 h from the strawberry intake and up to 96% increase during the following 2 h) (Table 2).

The percentage of variation, from baseline, of the analyzed parameters according to the genotype of strawberries consumed by the subjects revealed that the highest increases in plasma TAC were clearly observed, in correspondence with the intake of Sveva > Adria > Romina (AN99.78.51) > Alba while, in keeping with their poorest Irma and Patty was associated with the lowest acute increases in plasma TAC (Fig. 12).

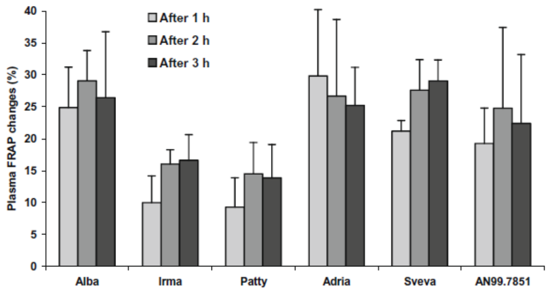

Figure 12. Plasma % variation of total antioxidant capacity following acute intake of strawberries from different genotypes, when measured by FRAP assay. Data are presented as. mean% changes from baseline value ± SEM.

While a 2-fold higher concentration of serum ascorbate was observed after the consumption of Sveva and Romina (former AN99.78.51 selection) fruits (Figure 13).

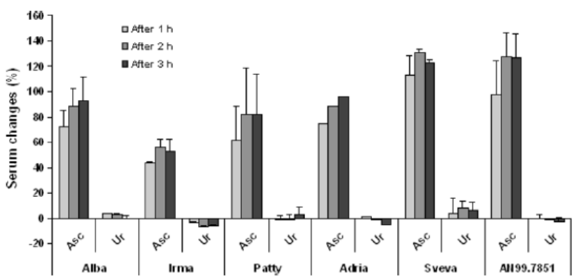

Figure 13. Serum% variation in the levels of vitamin C ("Asc" columns) and uric acid ("Ur" columns) following acute intake of strawberries from different genotypes.

Another study of validation was developed by Alvarez-Suarez (2012), by using rats, as a model animal study, feed with strawberry extracts of Adria and Sveva, both rich in anthocyanin compounds (mainly pelargonidin -3-glucoside) (Figure 14), and then treated with ethanol. In this study, strawberry extracts prevented exogenous ethanol-induced damage to rats' gastric mucosa. These effects seem to be associated with the antioxidant activity and phenolic content in the extract as well as with the capacity of promoting the action of antioxidant enzymes.

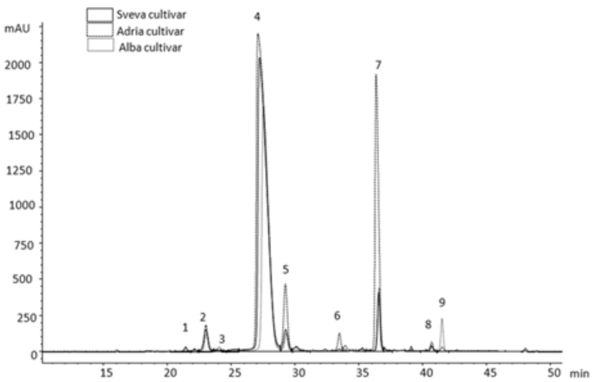

Figure 14. Representative chromatograms showing the anthocyanin profiles of the strawberry samples. Adria, Alba and Sveva cultivars. Peaks: 1, (Epi)afzelechin-(4→8)Pg 3-glucoside; 2, Cy-3-glucoside; 3, Pg 3,5-diglucoside; 4, Pg 3-glucoside; 5, Pg 3-rutinoside; 6, Cy 3-malonylglucoside; 7, Pg-3-malonylglucoside; 8, Pg-3-acetylglucoside; 9, Pg-3-succinylglucoside.

Adria strawberry extracts presented the same protective effects as quercetin in suppressing the area of gastric mucosal damage (figure 15) and confirming the importance of anthocyanin compounds. A diet rich in such strawberries might exert a beneficial effect in the prevention of gastric diseases related to generation of reactive oxygen species.

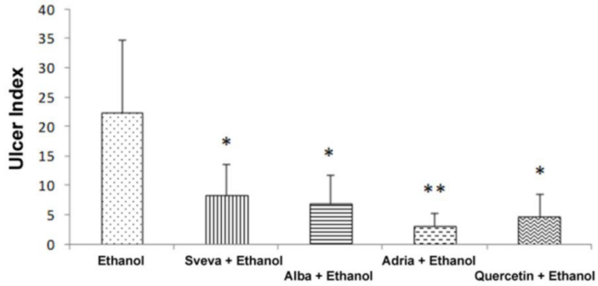

Figure 15. Effect of intragastric pretreatment with the different strawberry extract and quercetin on the ulcer index induced by ethanol.

ANNEX – Table 3. Production and quality of the 2 new varieties and 2 best selections in comparison with standard cultivars (mean data of two production seasons: 2010 and 2011). IP: precocity index. FW: fresh weight. TP: total production (g/plant). M%T: marketable fruit over total production (%). SS: soluble solids (°Brix). TA: total acidity (mEq NaOH/100g). F: firmness (g). L*: brightness. Chroma: Chroma index. TAC: Total antioxidant capacity (mmolTE/kgFw); TPH: Total Phenol content (GA/kg FW); ACY: Antocyanin content (Pel-3-Glu mg/Kg). Significant difference among means was calculated by SNK test ($p \leq 0.05$).

Genotype	IP	FW	TP	M%T	SS	TA	F	L*	Chroma	TAC	TPH	ACY
AN01,243,54	134 f	22.5 de	645 f	73.9 bcd	7.9 ab	12.3 ab	433 ab	36.8 a	47.1 a	12.4 bc	1303 e	296 c
ALBA	137 e	23.9 cd	1109 c	81.0 ab	6.6 de	13.1 a	425 ab	37.4 a	47.3 a	11.4 cd	1409 d	264 de
ROMINA*	137 e	19.1 g	895 d	77.3 bc	7.5 bc	10.5 b	483 a	35.2 ab	45.3 ab	12.6 bc	1420 d	404 a
CLERY	140 d	21.7 ef	805 de	75.9 bcd	8.2 a	11.7 ab	387 b	37.4 a	47.3 a	14.4 b	1649 b	320 b
AN02,119,53	144 c	19.9 fg	540 f	72.9 cd	8.4 a	12.0 ab	490 a	32.8 c	42.4 bc	12.9 bc	1530 c	243 e
ADRIA	150 b	28.8 b	1546 a	68.9 d	6.3 e	12.4 ab	460 a	35.7 ab	41.1 c	13.7 bc	1536 c	247 e
SVEVA	151 b	24.9 c	645 ef	68.7 d	6.7 e	12.1 ab	391 b	33.9 bc	42.5 bc	21.4 a	1973 a	396 a
CRISTINA*	153 a	31.9 a	1300 b	84.2 a	7.4 cd	11.8 ab	335 c	37.2 a	45.9 a	10.1 d	1225 e	273 d
AN01,188,53 White strawberry										35,5	4911	11,5

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WEB LINK:

- www.euberry.univpm.it/
- http://www.fragoladriatica.it/
- http://www.newsfood.com/q/6ebda50d/fragole-buona-prevenzione-contro-cancro-diabete-e-malattie-cardiovascolari/
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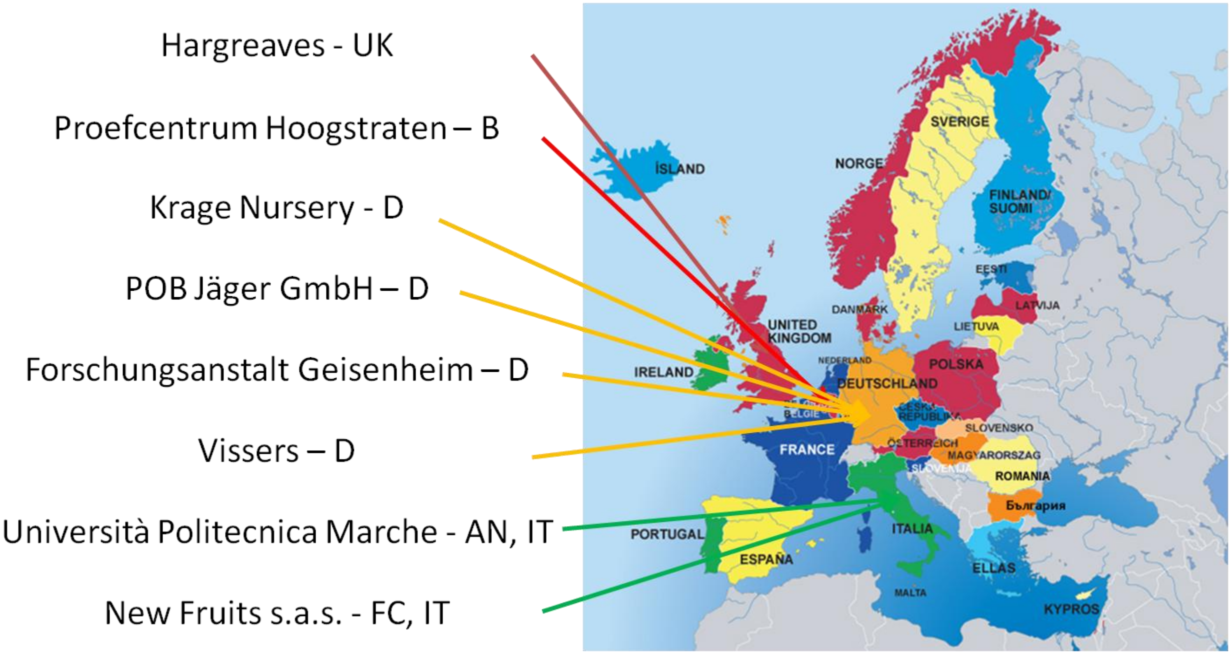
- http://pubs.acs.org/doi/abs/10.1021/jf205065x?prevSearch=%255BContrib%253A%2Btulipani%255D&searchHistoryKey=
- http://www.sciencedirect.com/science/article/pii/S0308814609004178

Cristina and Romina are been evaluated by the following European nurseries and research centers



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LOCATIONS, HARVESTING TIME AND VISIT DATES FOR 2012 HARVESTING PERIOD

Institute / Farm	Country	Address	Contact	Telephone	e-mail	Visit date Agreed	Estimated Harvest period for cv ROMINA	Estimated Harvest period for cv CRISTINA – ADRIA – SVEVA
Università Politecnica delle Marche D3A	It	Via Brecce Bianche, 60131 Ancona	Bruno Mezzetti	+390712204933	b.mezzetti@univpm.it	May 11 early, May 25 late production	10 May – 20 May	25 May – 05 June
New Fruits s.a.s.	It	V. Cerchia di S. Egidio 3000, Cesena (FC)	Danilo Bernardini		info@newfruits.it		10 May – 20 May	25 May – 05 June
POB Jäger GmbH	D	Hundweg 10, D - Ortenberg	Wolfgang Jäger	+49 7819400640	info@pob-jaeger.de	6 June	yes	yes
Forschungsanstalt Geisenheim	D	Von-Lade-Straße 1, D-Geisenheim	Krüger Erika	+49 6722 502-563	erika.krueger-steden@fa-gm.de	1 June	15 May – 25 May	30 June – 10 June Cristiana and Adria
Proefcentrum Hoogstraten	B	Voort 71, Meerle	Tom Van Delm	+32 33157052	tom.vandelm@proefcentrum.be	Estimated 15 June	_	10 June-20 June
Vissers	D	Herr Joachim Huber, Spitzenweg 1, in Ifferzheim BRD	Hans Obers -	+31 (0)77-4648100	hans@vissers.com		Yes	no
Hargreaves	UK	Hargreaves Plants, Cowpers Gate, LS PE12 9BS UK	Rupert Hargreaves	+44 (0)1406 366300	rupert@hargreavesplants.com	31/05	Yes	Yes
Krage Nursery	D	Blanke Delsener Heide 30, Telgte	Stefan Krage	+491713418134	rueter@kraege.de	June 8	NO	Sveva – June 7th

NOTES: