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Characterization of the rescued 'Voghera' sweet pepper landrace grown in northern Italy

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Abstract

A traditional Italian sweet pepper landrace, 'Peperone di Voghera', which faced the risk of extinction, was analyzed for its genetic, phenological, morphological, agronomic and biochemical traits. An extant population was compared with cultivars 'Quadrato d'Asti', 'Cuneo', and 'Giallo d'Asti', cultivated in the same area, in order to evaluate the chance of the landrace recovery. Amplified fragment length polymorphism (AFLP) analysis demonstrated that Voghera landrace is distinct with respect to reference cultivars, thus excluding extensive genetic contamination and providing a molecular basis of both phenological and biochemical differences. Leaf chlorophyll content is lower, fruits start ripening earlier than controls, and yield (1,100-1,300 g plant^{-1}) does not significantly differ. Sensitivity to root pathogens, the main factor that led to the decline in the past, does not seem to compromise the future chance of recovery. Vitamin C concentration is high (200-240 mg/100 g) and preserved by cold storage; more than 25% of vitamin C is also kept in pickled fruits. 'Voghera' landrace has nutritional and gastronomic properties that can be appreciated by consumers. The high internal genetic variability shown by AFLP analysis indicates that future selection work is necessary to fully maintain the original traits of the landrace and to improve it.

Additional key words: AFLP; biodiversity; *Capsicum annuum*; extinction; nutraceutical properties; root mycosis.

Resumen

Caracterización de la variedad de pimiento tradicional 'Voghera', rescatada en el norte de Italia

En este trabajo se han analizado las características genéticas, morfológicas, fenológicas, agronómicas y bioquímicas de una variedad tradicional italiana de pimiento dulce, 'Peperone di Voghera', en peligro de extinción. Se comparó una población existente con cultivares de la misma zona, 'Quadrato d'Asti', 'Cuneo', y 'Giallo d'Asti', a fin de evaluar la posibilidad de recuperarla. Análisis AFLPs (polimorfismos en la longitud de fragmentos amplificados) demostraron que 'Voghera' es distinta con respecto a los cultivares de referencia, lo que excluye que haya una amplia contaminación genética y proporciona una base molecular de las diferencias, tanto fenológicas como bioquímicas. Su contenido en clorofila es menor y los frutos comienzan a madurar antes que los controles, pero el rendimiento (1.100-1.300 g planta^{-1}) no es significativamente diferente. Su sensibilidad a patógenos de la raíz, el principal factor que llevó a su declive en el pasado, no parece poner en peligro la posibilidad de una futura recuperación. La concentración en vitamina C es alta (200-240 mg/100 g) y se preserva en el fruto conservado en cámara frigorífica; también se detecta una buena cantidad (más del 25%) de vitamina C en las frutas encurtidas. 'Voghera' tiene propiedades nutritivas y gastronómicas que pueden ser apreciadas por los consumidores. La alta variabilidad genética interna detectada en los análisis AFLP indica que el trabajo de selección es necesario para mantener plenamente los caracteres originales de la variedad y mejorarla.

Palabras clave adicionales: AFLP; biodiversidad; *Capsicum annuum*; extinción; micosis de la raíz; propiedades nutracéuticas.

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Introduction

Genetic variability coming from landraces is important for both the conservation of biodiversity and the improvement of commercial cultivars (Oyama *et al.,* 2006; Fideghelli & Engel, 2009). Wild relatives of domesticated plants and cultivars of minor commercial importance can be used for introgression of characters related to yield, quality and resistance to pests or environmental stresses (Hawkes, 1983; Burdon & Jarosz, 1989).

Genetic diversity is strictly related to specific different cultivation methods and local traditions (Frankel *et al*., 1995) which are part of the heritage of history and culture (Sereni, 1979; Santiago *et al.*, 2008). The major cause of genetic variability among cultivars is the diversity of environments in which extensive and prolonged breeders selection takes place (Pickersgill, 1997). Due to isolation, genetic drift also tends to introduce a certain variability among different populations of the same landrace.

In Italy the variety of climatic, environmental and cultural conditions favoured the selection of a large number of local crops. In particular, selection, genetic isolation, breeding and a widespread diffusion favoured the production of a large amount of Italian bell pepper (*Capsicum annuum*) cultivars and local landraces (Garcia *et al.*, 2002; Bonelli *et al.*, 2006).

An additional source of variability comes from cross-pollination: up to 30% of pepper flowers, usually self-pollinating, can be exposed to cross-pollination in presence of wind or pollinators, allowing hybridization when different landraces plots are next to each other (Csillery *et al.*, 1986).

On the other hand, farm-based selection results in genetic erosion within the single farm population, particularly when seeds are harvested from a low number of plants (Lanteri *et al.*, 2003; Portis *et al.*, 2004). Altogether, hybridization, isolation and genetic pool reduction can lead to the loss of the original biological traits of a landrace. As the number of farmers involved in a local landraces cultivation tends to decrease, the risk of extinction tends to raise, as in the case of the 'Peperone di Voghera' or 'Voghera', which is the northern Italy landrace of sweet bell pepper reported in the present study.

In the past 'Voghera' landrace cultivation was widespread in a horticulture district of about 300 km^2 around the town (Voghera) that inspired the landrace name. The crop was consumed also outside of its cultivation range and was well known across the nation.

The decline of 'Voghera' landrace began in the late forties, when epidemic attacks by root mycosis (*Fusarium* spp.*, Phytophthora* spp.), caused severe damage to cultivations. Other factors, first of all the changes in consumer tastes and vegetable market dynamics, together with the availability of new cultivars improved by breeders, made the crisis worse. At the moment this research started, only three farmers were still involved in the cultivation of 'Voghera' pepper.

A renewed interest in the recovery of this landrace sparked by farmers, consumers and greengrocers was recently recorded.

The aim of this work was to estimate the chance of success of the landrace's rescue by evaluating its own traits and properties in comparison with those of other widespread cultivars. As regards genetic traits, the molecular marker analysis, based on genomic DNA sequences, is not affected by environmental conditions (Bachmann, 1994), epistacy or pleiotropic effects. More specifically, AFLP analysis was chosen because of its high reproducibility and ability to produce a rich band pattern as result of a single assay (Vos *et al.*, 1995). Furthermore, this method does not rely on prior knowledge of genome sequences.

Moreover, an exahustive characterization has been performed by the report of the results of analyses carried out on phenology, morphology, sensitivity to root mycosis, and biochemical properties of 'Peperone di Voghera' available population.

Data can be used to obtain financial support for landrace's rescue, as well as a knowledge basis to define a "gold standard" of the landrace and to assign priorities to future improvements to be achieved by breeding and horticultural techniques. In addition, results can reveal the peculiarities of the landrace and its nutraceutical properties, giving a contribution to its promotion.

Material and methods

Plant material

Three different seed stocks of 'Peperone di Voghera' landrace, from the three last land farmers who preserved the landrace, were retrieved and mixed.

Abbreviations used: AA (ascorbic acid); AFLP (amplified fragment length polymorphism); DHA (dehydroascorbic acid); DM (dry matter); DTT (dithiothreitol); SSC (soluble solids content); TTA (total titratable acidity); UPGMA (unweighted pair group method with arithmetic mean).

For comparison, four reference cultivars 'Quadrato d'Asti rosso', 'Quadrato d'Asti giallo', 'Cuneo' and 'Giallo d'Asti', typically grown in the western Po floodplain, an area next to the one where 'Voghera' landrace is cultivated, were used. Selected reference seeds came from the certified stocks preserved by INRAN (ex ENSE), the Italian National Institute for seeds certification.

For genetic analysis, 'Voghera' seedlings were compared to the seedlings of all reference cultivars**.** Seedlings were sown in plastic plates on wet paper and grown for 15 days with 16 hours of daylight and 8 hours of dark at 23°C constant temperature, then harvested and immediately frozen with liquid nitrogen.

For phenological, morphological and agronomic traits and physico-chemical characterization of plants and fruits, 'Voghera' landrace and two reference cultivars ('Quadrato d'Asti rosso' and 'Cuneo') were grown in field in the experimental farm of Istituto Tecnico Agrario Statale "Carlo Gallini" in Voghera (44° 98' 94" N; 8° 99' 84" E, 96 m a.s.l.) in 2006 and 2007. This area is characterized by a sub-continental climate zone; during summer the average temperature was 21.9°C in 2006 and 21.6°C in 2007, while total rainfall was 308 mm and 357 mm, respectively. Seeds were sown on March and plants were transplanted into open field in June; plants were tied to a stake in order to prevent breakage caused by strong winds. Plots were established on a clay loam and slightly alkaline soil (clay 31.3%, silt 26.6%, sand 42.1%; pH 7.8). Soil was fertilized with a commercial fertilizer ("Combislow": N 18%, P_2O_5 14%, K_2O 18%, MgO 2%) and well water was used for irrigation during drier summer periods. Twelve experimental plots (4 plots/genotype) were established, with 30 plants for each plot, at a distance of 40 cm × 50 cm.

Genetic analysis

The amplified fragment length polymorphism (AFLP) analysis was used in order to describe genetic variability of 'Voghera' landrace and compare it to reference cultivars.

A random sample of 32 15-d-old seedlings belonging to 'Voghera' landrace was analyzed. For comparison, six pools of 20 seedlings each, belonging to reference cultivars (1 line of 'Quadrato d'Asti rosso', 2 lines of 'Quadrato d'Asti giallo', 1 line of 'Cuneo' and 2 lines of 'Giallo d'Asti') were used.

The seedlings were finely chopped and ground with mortar and pestle in liquid nitrogen. DNA was extracted according to a CTAB method (Doyle & Doyle, 1990).

The AFLP protocol was essentially the one described by Vos *et al.* (1995), with some modifications: 250 ng of DNA were restricted with 2.5 units of *Eco*RI and *Mse*I, each in a final volume of 25 µL (50 mM NaCl, 100 mM Tris-HCl, 10 mM $MgCl_2$, 0.025% TritonX-100, pH 7.5; 1X BSA). Ten pmol of EcoRI adapters, 25 pmol of *Mse*I adapters and 1 unit T4 DNA Ligase (NEB) were added to the digested DNA in a final volume of 30 µL. The adapter-ligated DNA was pre-amplified using a single base selective AFLP primer. The pre-amplified product was diluted (1:10) and selective amplifications were performed with primers having three selective nucleotides. Polymerase chain reaction (PCR) products were separated in a 5% polyacrylamide gel (7 M urea and 1X TBE). Patterns were visualised by means of a silver-staining method (Creste *et al.*, 2001). Seven *Eco*RI:*Mse*I primer pairs were initially screened on five randomly selected samples in order to assess their informativeness in detecting molecular variation (Geleta *et al.*, 2005; Barchi *et al.*, 2007). The four most informative pairs were selected for AFLP analysis (Table 1).

The 32 'Voghera' landrace individuals and reference varieties were examined using four primer combinations (Table 1) to evaluate their distinctness and homogeneity. For each AFLP gel from a primer pair, the total number of AFLP bands (monomorphic and polymorphic) was counted. DNA fragments were scored as present (1) or absent (0) and a binary matrix was built. A cluster analysis was then performed by using the pc package PHYLIP (the *PHYL*ogeny *I*nference *P*ackage) (Felsenstein, 1989). Genetic distance among samples was estimated using Nei and Li coefficients (Nei & Li, 1979). A genetic similarity matrix was used to construct the UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrograms. The robustness of phylogenetic tree branches was tested by calculating Boostrap values. Boostrapping of the resulting dendrogram was performed with 100 permutations.

Phenological, morphological and agronomic traits

Chlorophyll content, number of nodes and fruit volume were measured in 2006. All other data were measured in both 2006 and 2007.

Table 1. Name, sequence and bibliographic reference of AFLP primers chosen for genetic analysis of 'Voghera' pepper landrace and controls. The sequences in bold are three selective nucleotides

Primer combination	Sequence	Reference
E35/M48	5'--GACTGCGTACCAATTC **ACA**--3' 5'--GATGAGTCCTGAGTAA **CAC**--3'	Portis *et al.* (2004)
E32/M61	5'--GACTGCGTACCAATTC **AAC**--3' 5'--GATGAGTCCTGAGTAA **CTG**--3'	Geleta *et al.* (2005)
E37/M49	5'--GACTGCGTACCAATTC **ACG**--3' 5'--GATGAGTCCTGAGTAA **CAG**--3'	Paran *et al.* (1998)
E32/M47	5'--GACTGCGTACCAATTC **AAC**--3' 5'--GATGAGTCCTGAGTAA **CAA**--3'	Geleta *et al.* (2005)

Chlorophyll content in leaves was measured by means of a Minolta SPAD-502 chlorophyll meter: 20 measurements were taken from 20 plants just before transplantation to open field. A leaf attached to the third node was randomly sampled from each plant. Values are reported as indexed chlorophyll content reading SPAD-502 (arbitrary SPAD units).

Number of nodes was manually scored on 'Voghera' landrace and reference cultivars. The number of nodes is an important morphological character because it is related to the number of fruits that can be produced, thus affecting both plant growth and yield.

Fruits of six marked plants per plot (24 plants/genotype) were harvested and weighed for yield estimation. The number of early fruits harvested during the second half of July was recorded and compared.

Shape and size were studied on a sub sample of mature fruits; diameter and height were measured by means of a calliper, while the volume of fruits was measured by immersion in a glass transparent graduated cylinder (18 cm diameter) containing water.

Symptoms of damage caused to fruits by sunscald were recorded on fruits coming from marked plants.

Sensitivity to root mycosis was studied on the whole amount of 360 plants (120 plants/genotype) of the plots; the number of wilt affected plants was periodically recorded and the percentage of dead plant was then calculated for each one of compared genotypes.

Data were processed according to both non parametric and parametric tests by mean of "Biostat" software. Data in form of frequencies (*i.e.* early fruits, wilt affected plants) were analyzed by means of the chi-square test, while other data were subjected to ANOVA (*i.e.* yield, fruit dimensions) or a non-parametric test (Kruskall Wallis test) when data were not normally distributed (*i.e.* sunscald damage); differences were considered statistically significant when the p value was < 0.05.

Physico-chemical characterization

A first evaluation of peppers was performed in 2006 season on a representative fruit sample just after harvest. In the 2007 season the analyses were carried out on groups of 9 fruits, both after harvest and after 11 days of cold storage at 8°C (80% relative humidity).

Besides, a sample of home-made pickled 'Voghera' pepper was also examined. According to a traditional recipe, whole fruits were put into a glass jar and preserved in a water solution including vinegar (3% acetic acid) and salt (3.8%). Dipped peppers were analysed after 90 days of storage at room temperature.

Physical analyses were performed on single fresh fruits, then the central portions of 3 fruits were put together to make 3 sub-samples per group, and freeze-dried for chemical analyses.

General quality parameters

Weight loss was expressed as percentage after cold storage. Thickness was measured at about 1/3 fruit length and expressed in millimetres. Firmness was measured by cutting from internal to external side a strip taken from the central part of fruits; an Instron Universal Testing Machine equipped with a 3 mm blade (speed 200 mm min^{-1}) was used and results were expressed in kg cm^{-1} (Testoni *et al.*, 1983).

The freeze-drying yield was expressed as dry matter (DM, %). Soluble solids content (SSC, expressed as °Brix) and total titratable acidity (TTA, expressed in mEq/100 g) were measured on a mixture from a conveniently rehydrated freeze-dried powder, by means of a digital refractometer and an automatic potentiometric titration (0.1 N NaOH; pH 8.2).

Nutraceutical characterization

The nutraceutical profile was carried out by the measurement of the two main classes of healthy compounds that characterize sweet pepper fruits: carotenoids and vitamin C.

Carotenoids were analyzed as follows: 50 mg of freeze-dried powder was extracted in 0.8 mL isoctane, 0.1 mL acetone and 0.1 mL BHT solution (1% butylated hydroxytoluene in ethanol), by vortexing for 30 s. Samples were then centrifuged at 10000×g for 5 min. The pellet was extracted twice. Extract absorbance at 450 nm was measured in a 1 cm-path length cuvette (UNICAM UV/Vis spectrometer), and total carotenoids were expressed as β-carotene equivalents at 450 nm (mg β-car. eq./100 g fw) by using the calculated absorption coefficient ($A^{1\%}$ = 2786.4) of a pure β-carotene commercial standard solution, according to the Beer-Lambert law.

Extractions and quantification of ascorbic acid (AA) and its oxidized form (dehydroascorbic acid, DHA) were performed according to Davey *et al.* (2003), with minor modifications. Freeze-dried powder (50 mg) was extracted in 1.5 mL of 6 % metaphosphoric acid, by vortexing for 30 sec; then samples were centrifuged at 10000×g for 10 min. AA level was determined by HPLC on aliquot of the extracts 10 folds diluted in cold 0.02 M H_3PO_4. The AA+DHA level was assessed by HPLC after a reduction of the DHA to AA using dithiothreitol (DTT): 50 µL extract were added to 50 µL of a solution of 0.2 M DTT in 0.4 M Tris base. The reaction was then stopped after 15 min at room temperature by diluting 1:10 in 0.02 M H_3PO_4 and immediately analyzed by HPLC. DHA was determined as the difference between AA + DHA level and AA level. HPLC conditions were as follows: column Inertsil ODS-3, 4.6 mm × 250 mm, 40°C, mobile phase 0.02 M H_3PO_4, flow rate 0.6 mL min^{-1}. The AA (retention time 7.8 min) was measured at 254 nm (Jasco MD2010plus Multiwavelenght detector) and quantified by comparison to a calibration curve of authentic standard solutions. Vitamin C was expressed as AA + DHA level (mg/100g fw); DHA was expressed as percentage of the total vitamin C.

Experimental data were submitted to statistical analysis by ANOVA and Tukey test. Weight loss, thickness, and firmness data are the means of 9 measures per group; data concerning DM, SSC, TTA, carotenoids and vitamin C are the means of 3 measures per group.

Results

Genetic analysis

The four AFLP primer pairs produced 326 bands; 110 of those bands (33.7%) were polymorphic among all samples (Table 2). In particular, three fragments were present only in 'Voghera' sample. Cluster analysis of 'Voghera' AFLP data confirms a high level of variability among the samples. The dendrogram (Fig. 1) derived from distance matrix, shows two main clusters: the first one includes the whole 'Voghera' population, and a second one was composed by reference cultivars. Despite the genetic distance is very low, reference genotypes can be distinguished as separate branches according to their fruit shape and colour. High level of boostrapping confirms the robustness of the dendrogram.

'Voghera' landrace genetic traits are not uniform across the populations that survived to extinction, but

Table 2. Total and polymorphic bands generated with each primer combination in 'Voghera' pepper landrace and controls

Primer combination	Bands generated	Polymorphic bands
E35/M48	105	51
E32/M61	82	27
E37/M49	50	15
E32/M47	89	17
Total	326	110

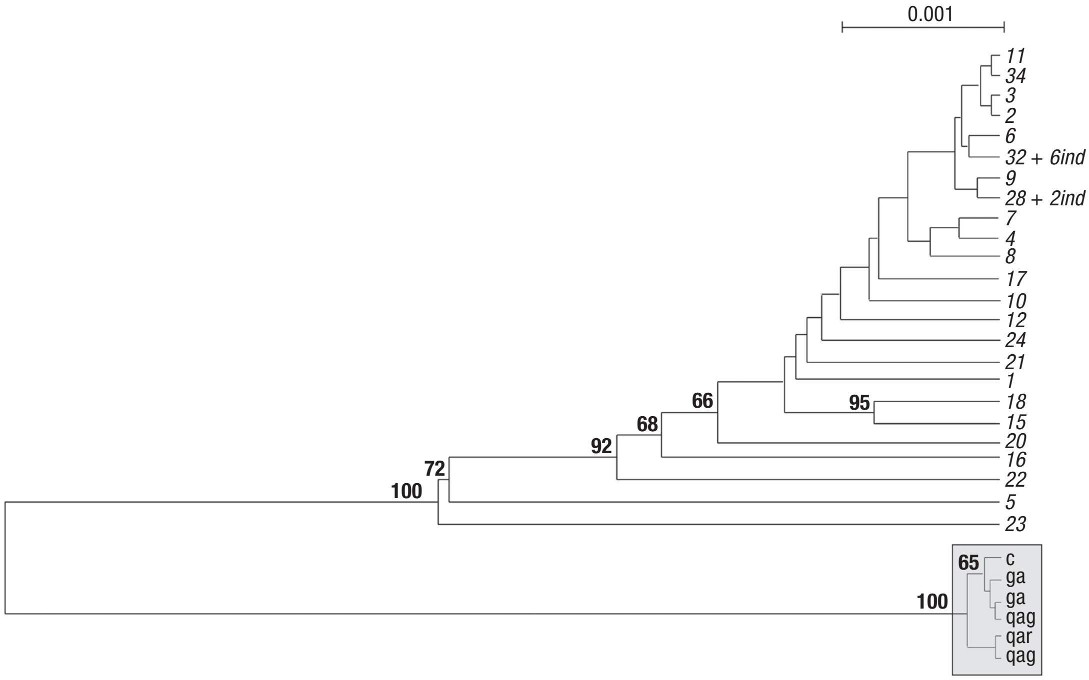

Figure 1. Dendrogram from AFLP analysis (UPGMA algorithm). Italic numbers indicate different individuals of 'Voghera' population. Individual 32 shared the same profile with other six individuals (32 + 6ind); individual 28 shared the same profile with other two individuals (28 + 2ind). The square indicates individuals belonging to reference cultivars (c: 'Cuneo'; ga: 'Giallo d'Asti'; qag: 'Quadrato d'Asti giallo'; qar: 'Quadrato d'Asti rosso'. Bold numbers indicate boostrap values.

the genetic pool can be clearly distinguished from the one of reference cultivars.

Phenological, morphological and agronomic traits

The chlorophyll content range in 'Voghera' leaves was significantly lower (30.2 ± 3.9 SPAD units) and never reached 'Cuneo' (38.8 ± 1.3) and 'Asti' (39.9 ± 0.7) chlorophyll concentration. As consequence, the green colour of leaves is more pale than reference genotypes and this is a distinctive morphological trait of 'Voghera' landrace that can be observed during the whole plant life cycle.

The number of nodes in developed sweet pepper plants ranged from 12 to 16 and no significant differences were observed among compared genotypes.

'Voghera' fruits tend to ripen earlier in the summer respect to compared cultivars, the number of fruits harvested in the second half of July was significantly different (Table 3). The difference between 'Voghera' and reference landraces was significant both in 2006 ($\chi^2 = 8.77$, $p < 0.05$) and in 2007 ($\chi^2 = 8.77$, $p < 0.05$). 'Voghera' landrace yield was higher both in 2006 and in 2007 (Table 3), but ANOVA did not reveal a statistically significant difference ($p > 0.05$).

Data on fruit weight and morphology are summarized in Table 3. The dimensions of fruits were significantly different both in 2006 and 2007. Weight of 'Voghera' fruit was on average lower than control cultivars ($p < 0.001$). Height and diameter of the fruit were significantly different, too ($p < 0.001$). 'Voghera' fruit is cubic shaped, while 'Asti' fruit tend to be elongated. 'Cuneo' fruit is heart shaped and diameter tends to prevail on height.

A sharp difference appears from volume comparison, too. Data confirm that 'Voghera' fruits were on average significantly smaller ($p < 0.001$); this tendency is confirmed by the comparison of the percentages of fruits weighing less than 100 g (Table 3).

Cubic shaped fruits, such as the ones produced by 'Asti' and 'Voghera' varieties, usually featured 4 lobes, less commonly 3 lobes. The majority of 'Quadrato d'Asti' (81%) and of 'Voghera' fruits (80%) coming from experimental samples featured the typical 4 lobes.

Table 3. Phenological, morphological and agronomic traits of 'Quadrato d'Asti rosso', 'Cuneo' and 'Voghera' pepper landraces

	Quadrato d'Asti rosso		Cuneo		Voghera	
	2006	2007	2006	2007	2006	2007
No. of early fruits plant^{-1}	5	5	14	5	20	14
Yield (g plant^{-1})[a]	1,028 ± 376	1,100 ± 643	997 ± 285	1,219 ± 566	1,111 ± 471	1,311 ± 449
% of fruits < 100 g	16.9	0.8	5.2	2.9	31.8	5.6
No. of plants	*22*	*24*	*23*	*24*	*23*	*23*
Diameter (cm)[a]	7.02 ± 0.94	8.63 ± 0.91	8.72 ± 1.32	9.36 ± 0.86	7.77 ± 0.92	8.45 ± 0.81
Height (cm)[a]	8.44 ± 1.71	9.88 ± 1.62	7.28 ± 1.82	8.55 ± 0.93	7.22 ± 1.11	8.47 ± 1.12
Height/diameter[a]	1.21 ± 0.23	1.15 ± 0.21	0.84 ± 0.22	0.92 ± 0.14	0.94 ± 0.16	1.00 ± 0.15
Weight (g)[a]	145.3 ± 39.3	242.0 ± 42.0	189.0 ± 77.9	240.6 ± 43.9	136.3 ± 36.3	169.0 ± 41.4
Volume (cm^3)[a]	441.5 ± 124.9	–	515.5 ± 195.1	–	428.0 ± 129.8	–
Density (g cm^{-3})[a]	0.33 ± 0.04	–	0.35 ± 0.04	–	0.33 ± 0.12	–
No. of fruits	*96*	*31*	*73*	*35*	*89*	*43*
Sunscald damage (% on weight)	4.1	3.3	10.0	9.7	0.6	0.8
No. of plants	*22*	*24*	*23*	*24*	*23*	*23*
Wilt affected plants (%)	15.0	1.7	11.7	1.7	7.5	4.2
No. of plants	*120*	*120*	*120*	*120*	*120*	*120*

[a] mean ± sd.

The occurrence of 5 or more lobes was 5% in the 'Voghera' landrace and 8% in the 'Asti' cultivar.

Sunscald damage was negligible for 'Voghera' fruits: less than 1% of production was compromised by sunscald (Table 3). 'Cuneo' and 'Asti' fruits were significantly more sensitive to injuries coming from sunlight both in 2006 and in 2007 ($p < 0.01$, Kruskall Wallis test).

Root mycosis attacks tend to wilt plants and symptoms start appearing in the second half of August; the number of wilt affected plants tends to raise in second half of September, near the end of plants life cycle. The incidence of root mycosis in 2006 was higher than the one recorded in 2007 (Table 3): the difference between compared landraces was not significant both in 2006 and 2007 ($p < 0.05$); 'Voghera' plants did not appear to be more sensitive than the reference landraces.

Physico-chemical characterization

Indications coming from data of 2006 samples at harvest (data not shown) were confirmed by the results of the experiment performed in 2007, on fruits at harvest, after cold storage and preserved in vinegar, as shown in Table 4.

'Asti' and 'Cuneo' fruits showed a thicker flesh, but they are less resistant to cut than 'Voghera' fruits. In spite of the fact that they had a thinner wall, 'Voghera' fruits showed to be more resistant to cut than compared cultivars. Such a resistance is probably due to the higher content of organic matter, resulting from the measure of DM and SSC in 2007.

The SSC content of the 'Voghera' pepper landrace was on average 7.7%. The TTA showed no significant differences between the three assayed varieties.

The amount of total carotenoids was conditioned by fruit colour, being much higher in the red fruits: in 2006 'Voghera' and 'Cuneo' yellow fruits showed the lowest content of 2.9 mg/100 g fw. These values are very considerable compared to nutritional data (USDA, 2011) that reported 2.2 mg/100 g fw for red sweet pepper. In 2007 (Table 4) total carotenoids concentration in 'Voghera' yellow fruits was higher and intermediate between the yellow fruits of 'Cuneo' and the red fruits of 'Asti' both at harvest and after cold storage.

Vitamin C (ascorbic + dehydroascorbic amount) content in fruits sampled in 2006 ranged from 150 to 200 mg/100 g fw. The greatest amount was found in 'Voghera' (203.3 mg/100 g fw). Vitamin C levels in 2007 samples were similar to the ones recorded in 2006: the highest amount (241.1 mg/100 g fw) was found in 'Voghera' fruits (Table 4). In addition, 'Voghera' showed the lowest proportion of dehydroascorbic acid at harvest in comparison to compared varieties. This fact points to a lower level of oxidative stress suffered by fruits during the growing season.

Table 4. Physico-chemical analysis performed in the season 2007, on fruit at harvest, after cold storage and pickled of 'Quadrato d'Asti rosso', 'Cuneo' and 'Voghera' pepper landraces

	Quadrato d'Asti rosso		Cuneo		Voghera		
	Harvest	Storage	Harvest	Storage	Harvest	Storage	Pickled[1]
Weight loss after storage (%)	–	3.8 a	–	3.2 a	–	3.6 a	–
Flesh thickness (mm)	5.6 a	5.7 a	5.7 a	5.5 a	4.9 ab	4.2 b	–
Resistance to cut (kg cm^{-1})	3.6 ab	3.3 b	3.1 b	3.1 b	4.1 a	3.9 a	–
Dry matter (DM, %)	9.0 bc	9.1 bc	8.0 c	9.6 abc	10.3 ab	11.4 a	12.5
Soluble solids content (SSC, °Bx)	6.8 ab	7.1 ab	6.4 b	7.6 ab	7.7 ab	8.2 a	10.6
Titratable acidity (TTA, mEq/100 g fw)	3.7 ab	3.8 ab	3.3 b	3.4 ab	3.9 ab	4.1 a	9.5
Total carotenoids at 450 nm (mg ß-car. eq./100 g fw)	8.9 ab	10.0 a	2.1 c	2.9 c	4.1 abc	3.7 bc	3.3
Vitamin C (mg/100 g fw)	155.7 b	177.7 ab	176.8 ab	216.6 ab	241.1 a	236.1 ab	65.6
Dehydroascorbic acid (DHA, % on total vitamin C)	8.5 a	6.0 a	12.9 a	3.3 a	5.7 a	4.8 a	28.6

[1] Pickled fruits were not included in the ANOVA analysis due to the completely different characteristic of the product. Different letters within each row indicate significant differences according to the Tukey test ($p < 0.05$).

Fruits were subjected to a 11-days cold storage: the main quality parameters were generally retained, in spite of a negligible index of chilling injuries. Weight loss was less than 4%, resulting in a slight increase of DM, SSC and TTA. No difference in weight loss between compared landraces was recorded.

Nutraceutical parameters profile was differently affected by cold storage: 'Voghera' showed a slight decrease in total carotenoids and vitamin C, while 'Asti' and 'Cuneo' showed an increase (Table 4). However, the highest level of vitamin C was found in samples of 'Voghera' both at harvest and after cold storage.

Besides, 'Voghera' sweet pepper was analyzed after three months of immersion of the whole fruits in vinegar according to a traditional recipe: quality parameters were affected by this treatment in terms of DM, SSC and TTA availability. Total carotenoids content slightly decreased; vitamin C was strongly depleted, but remained at a nutritionally significant level (109% recommended daily allowance) and a significant increase of its oxidized form was recorded.

Discussion

In the past 'Voghera' fruits precocity was conveniently exploited in order to produce the crop early in summer (Pezzullo, 1986). From plants sown at the beginning of March it was possible to harvest the crop early in July.

An additional advantage coming from the phenology of 'Voghera' landrace was the chance of double cropping: pepper seeding could be delayed from March to April and plants transplanted late in July, on plots where cereals had been reaped. Around October fruits from late transplanted plants were finally pickled. In such a way fresh fruits could be consumed during summer, while pickled fruits could be preserved until the spring of the following year.

This optimal exploitation pattern was compromised by several changes. Peppers pickling according to the traditional recipe is time consuming, but it was common among consumers until the sixties. In the past, before the diffusion of glasshouse crops, weather and seasons worked as limiting factors to open field vegetable cultivations; for this reason the availability of a crop that could be harvested early and late in the summer and preserved across winter was particularly appreciated by consumers. The achievement of new sweet pepper cultivars and the introduction of glass house cultivations, together with the diffusion of refrigerators and freezers, modified consumers feeding habits.

Fresh sweet peppers and pickled ones produced by food industry are cheaply available in the markets all year long today. Consumers tend to prefer thick, fleshy hybrid peppers, that are suitable for grilling, and do not appreciate spicy taste in peppers used in salads. 'Voghera' fruits are thin and quite spicy: for this reason they are less attractive than other varieties.

Root mycosis has been an historical problem for sweet pepper growers in the horticultural district where 'Voghera' landrace was selected, as reported by local farmers and previous literature (Montemartini, 1907; Pezzullo, 1986). Hence, damage coming from epidemic diffusion of root mycosis recorded around the fifties is usually identified as the main factor that led

'Voghera' landrace to the edge of extinction (Pezzullo, 1986). With no doubt this factor played an important role, but changes in consumers behaviours were so important as the threaten coming from mycosis.

Today farmers rely on a better knowledge of root mycosis; resistance improvement coming from selection and agronomic practices (*i.e.,* crop rotation) can help control this disease, as well as the exposure to summer sunlight that can cause sunscald injuries to fruits (Rabinovich *et al.*, 1983): results coming from this research state that 'Voghera' populations that survived to extinction are no more sensitive to wilt affections than control varieties and are more resistant to sunscald injuries.

Even productivity cannot be regarded as a weak point of 'Voghera' landrace: yield from experimental plots is similar to those of 'Quadrato d'Asti' and 'Cuneo' cultivars, that are landraces of commercial importance, broadly cultivated in northern Italy. 'Voghera' fruit size is smaller than reference varieties and this morphological trait implies a more time-consuming harvesting, but this weak point does not seem so critical to compromise a future rescue of this landrace.

Moreover, several strong distinctive points of 'Voghera' landrace can aid the rescue project: first of all, physical and biochemical properties agree with the preference for this landrace in traditional cooking, thanks to its better texture and firmness. Fruits are thinner, tougher and less rich in water than the compared varieties. It has to be emphasized that SSC data are higher than indication found in nutritional tables (USDA, 2011) that report an average of about 6 g of total carbohydrates per 100 g fw (comparable to SSC).

These traits are favourable to pickling and preservation by freezing and that these physical and chemical properties can be taken into account to explain the different sensitivity to sunscald injuries.

The results of the genetic molecular analysis, showing significant differences between 'Voghera' and compared landraces, confirm observations coming from phenology and biochemistry: 'Voghera' cultivar is characterized by peculiar biological traits. The results of AFLP analysis show that this landrace is located into a separate cluster within the dendrogram, with no interposition of genetic profiles. The level of polymorphism of 'Voghera' landrace appears to be high when compared to the one reported in a study using a similar crop (Portis *et al.*, 2004).

The presence of an internal variability is an optimal starting point for the rescue strategy and could be useful in order to identify introgression factors for genetic improvement and selection of new cultivars (Hawkes, 1983; Burdon & Jarosz, 1989; Oyama *et al.*, 2006).

One more strong peculiarity is given by nutraceutical properties: SSC, titratable acidity and vitamin C contents are higher than the reported nutritional values (USDA, 2011) and in good accordance with other from previous works (Howard *et al.*, 2000; Niklis *et al.*, 2002; Marìn *et al.*, 2004; Fox *et al.*, 2005). The average vitamin C content in 'Voghera' fruits ranged from 203 to 241 mg/100 g fw, a considerable concentration, compared to nutritional tables, which report an average reference concentration of 155 mg/100 g fw in yellow and red sweet pepper. This is four times higher than the recommended daily intake of this antioxidant compound that is so important for human health (Montecinos *et al.*, 2007; USDA, 2011). Vitamin C content is kept by cold storage and partially preserved by traditional storage in vinegar. Storage does not compromise the fruits biochemical outcome, so preserving the nutraceutical value of the crop (Navarro *et al.*, 2006; Raffo *et al.*, 2007).

The 'Voghera' pepper case study demonstrates that positive agronomic and biochemical characters of a landrace do not save it from the risk of extinction. Productive and commercial dynamics, market choices and customer's tastes can lead to the decline of a cultivar. Globalization of food market was an occasion for the widespread diffusion of some fruit and vegetable varieties, but at the same time a factor that caused the decline of many landraces. The importance given by consumers to appearance rather than to nutritional qualities and flavour deeply changed parameters of choice, but it was then recognized that the decline of crop biodiversity was not only a major cause of genetic erosion, but also of nutraceutical food quality loss (Davis, 2009).

The decline of 'Voghera' sweet pepper followed and emphasized social and cultural change of the land where the cultivar was selected: as a landrace, 'Voghera' pepper was in close relation to environmental conditions, cultural heritage and tradition. After some decades of abandon, local community is now trying to recover the historical importance of this crop: schools, researchers, greengrocers are discovering this cultivar and a farmers association was founded in order to join forces for the landrace full recovery.

Conservation and future economic role of this landrace need a breeding project: selection of positive genetic traits can improve productivity and resistance to root mycosis and reduce spicy taste. Moreover, an information work of customers about nutraceutical properties is needed, because the existence and the diffusion of a landrace crop can strongly depend on customer choices and their knowledge of nutritional qualities and gastronomic potential.

As a logical conclusion, the results of the present research can be a useful basis reference for a future selection work.

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