Valorisation of typical products through characterising and promoting actions: morpho-biometric traits, sensory analysis and flavonol content in *Cipolla di Giarratana*

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Abstract

The performances of 9 accessions of *Cipolla di Giarratana* landrace were evaluated at 5 different sites located 2 in the area where this landrace is traditionally grown (South-Eastern Sicily, 550 m asl), 2 in the neighbor areas with similar average altitude, and one on the South East coast of Sicily (sea level). Biometric and morphological traits, flavonol content and sensory profile were all considered in the valorisation of a typical product of a marginal area of Sicily. Data on the actual yield and crop management at farm scale were also obtained involving more than half of the growers actually cultivating *Cipolla di Giarratana*, and measuring yield, plant density, and bulb weight directly on the farms. The *Cipolla di Giarratana* landrace is characterised by a large and heavy bulb (535 g), strongly flattened at the poles, with the maximum diameter at the central section, and white in colour. Significant differences in bulb weight and diameter were found among accessions and locations, but there were no interactions between them and one accession reported the highest bulb weight value in all of the locations studied. With regards to bulb weight, farm scale data confirm the results we obtained at the experimental locations. Fifty percent of registered yield at farm scale ranged between 86 and 152 t ha^{-1} with a median of 119 t ha^{-1}, and measured plant density revealed that 50% of the farmers planted 20-27 plants per square meter.

Analysis of the UV-vis and mass spectra showed the presence of 10 different flavonols with Quercetin as the most represented flavonol. The sensory profile of *Cipolla di Giarratana* onion showed that this landrace is characterised by a high intensity of sweet, typical fresh flavour and texture perception.

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Introduction

Many Italian food products, generated by specific crops, environments and managements (including growing and processing) could be considered important feedstock of the *Italian Style* industry. Given this, the preservation and valorisation of this asset should be considered a primary goal in terms of economy also affecting the sustainability of agronomical management of the environment.

In fact, this approach has meant that Italian products and foodstuffs are the most represented (289 products *vs* a total amount of 1403) among products reported in the specific European Commission quality schemes guaranteeing quality for designation (European Commission, 2013).

One of the first steps towards obtaining the requested certification based on European standards (European Commission, 2006) requires a clear demonstration of the characteristics that differentiate between products and the relationship between the product and its geographical region of origin.

In order to objectively delimit geographical origin, it is fundamental to know the importance of genetic and environmental factors in the phenotypes of the products that reach consumers (Florez *et al.,* 2009), and to evaluate performance at a number of different locations (Annicchiarico, 1997). Furthermore, traditional crop varieties, generally known as landraces (Harlan, 1992), represent the basis of traditional foods. These landraces, therefore, need to be preserved for the production of certified products indirectly affecting the conservation of biodiversity. In this context, crop characterisation has become an important field of multidisciplinary expertise (Avise, 1995; Avola *et al.*, 2009; Pérez-Gregorio *et al.*, 2010; Riggi *et al.*, 2013).

In this scenario, a Sicilian traditional onion landrace, *Cipolla di Giarratana*, characterised by large white bulbs and a sweet taste, and registered as Slow Food Presidium, merits the attention of researchers in order to avoid the risk of extinction and to promote economic activity in a marginal area. In fact, this population, like many other niche products cultivated in restricted areas, is at risk. Thus, with this in mind, morpho-biometrical, chemical, and sensory approaches were used to characterise the *Cipolla di Giarratana* landrace and to compare its performance between different experimental sites.

Materials and methods

Field evaluation

Nine accessions of *Cipolla di Giarratana* landraces have been grown in 5 locations: 2 in the area where this population is traditionally cultivated ('A' and 'B', both in Giarratana, South-Eastern Sicily, 550 m asl), one in the South East coast of Sicily ('C' in Sicily, 5 m asl), and the remaining 2 in the neighbor areas with similar average altitude ('D', in Chiaramonte Gulfi and 'E', in Palazzolo Acreide, SE Sicily, 450 and 650 m asl, respectively).

In September 2010, seeds of all 9 accessions of *Cipolla di Giarratana* landraces were collected, sown in a cold greenhouse, and grown till the 2-3 leaf stage to be transplanted in 2011. Before transplanting 150, 120 and 250 kg ha^{-1} of N (as ammonium sulphate), P_2O_5 (as mineral perphosphate) and K_2O (as potassium sulphate), respectively, were distributed. A further 50 kg ha^{-1} of N (as ammonium nitrate) was supplied as top dressing in May. A randomised blocks design, replicated 3 times, was adopted, with 6 m^2 plots (3×2 m) with a plant density of 25 plants *per* m^2. In July, when 90% of foliage had fallen, 15 bulbs per plot were randomly selected and harvested. Biometric (bulb weight and maximum diameter) and morphological traits (position of the maximum diameter, width of the neck, shape of the whole bulb, of the stem end, and of the root end), according to the Community Plant Variety Office (CPVO, 2009) were measured in each harvested bulb.

Flavonol content

Three bulbs for each replicate were washed with tap water to remove dirt and dried thoroughly with absorbent paper. Each bulb was cut longitudinally into quarters and a representative sample of each quarter (50 g) was immediately finely ground with an electric blender to a homogeneous whitish puree. Aliquots (3.5 g) of the puree were put in an 8 mL amber sample vial and 3 mL of 5% formic acid in methanol were added. The samples were then maintained at room temperature (20°C) overnight, in the dark and under vigorous shaking (350 rpm). The resulting heterogeneous mixtures were then centrifuged (20 min at 4000 rpm at room temperature), the resulting supernatant was filtered (PTFE filters, 15 mm diameter, 0.45 µm pore size, Chemtek Analytica, Bologna Italy), thus obtaining 1.4-1.7 mL of clear yellowish solutions that were put in 2 mL auto-sampler amber vials and analysed.

High performance chromatographic analyses were carried out on a Dionex (Dionex Corp., Sunnyvale, CA, USA) instrument equipped with a P580 binary high-pressure pump, a PDA-100 Photodiode Array detector, a TCC-100 Thermostated Column Compartment and an ASI-100 Automated Sample Injector. Collected data were processed through a Chromeleon Chromatography Information Management System version 6.70 (Dionex). Chromatographic runs were performed using a reverse-phase column (Ascentis C_{18}, 250×4.6 mm, 5 µm particle size, Supelco/Sigma Aldrich, St. Louis, MO, USA). Elution conditions were performed according to Riggi *et al.* (2013). Quantification was carried out at 350 nm for the majority of glycosilated flavonols using a calibration curve established with rutin (quercetin 3-*O*-rutinoside, R^2=0.999) and 370 and 364 nm for Aglycones (quercetin, isorhamnetin and kaempferol) using quercetin as reference standard (R^2=0.999). All standards were purchased from Fluka (Sigma-Aldrich), and submitted to exhaustive LC/UV-vis-DAD/MS analysis prior to use to verify their purity. All analyses were carried out in triplicate, reporting results in milligram of polyphenols per kilogram of vegetable material fresh weight.

Sensory evaluation

The sensory analysis applied followed the International Standard no. 13299 (ISO, 2003) on the general guidance for establishing a sensory profile.

Eight assessors were trained to evaluate samples using a 9-point hedonic scale ranging from 1 (minimal) to 9 (maximal) intensity of descriptors. For odour evaluation, the following descriptors were chosen: vegetal odour, onion odour, off-odour. For taste evaluation, the following descriptors were chosen: sweetness, bitterness, pungency, sapidness, texture perception, vegetal flavour, onion flavour, off flavour.

The sensory evaluation of a matrix characterised by strong flavour and odours makes it difficult to conduct a test involving a large sample number. For this reason, we selected only one accession (accession '12') from one environment at location 'B' (Giarratana area). The bulbs were chopped into approximately 0.5-cm long slices and placed in plastic portion control cups with lids.

Statistical analysis

Morphological data were graphically reported as box plots to represent the distribution of the values measured in the whole studied batch (9 accessions × 5 locations × 45 measurements = 2025 measures). In the box plots, the lowest and highest boundaries indicate the 25^{th} and 75^{th} percentile, respectively, and the black line within the box marks the median. Whiskers above and below the box indicate the 90^{th} and 10^{th} percentiles, respectively. Dark circles represent the 95^{th} and the 5^{th} percentiles.

The data referring to biometric and chemical traits were subjected to combined analyses of variance treating accessions and locations as fixed factors. When significant effects were observed, means were separated by Tukey's honestly significant difference (HSD) test. When significant interactions emerged, principal component analysis (PCA) was used to test the principal effects of locations and accessions and their combination, adopting a covariance matrix. Components with eigenvalues greater than one were used. For the PCA, the statistical package StatistiXL1.5 (StatistiXL Ltd., Broadway, Western Australia) was used.

The approach of Eberhart and Russell (1966) was applied to characterise potential productivity of different accessions in terms of bulb weight. According to this approach, within each environment, the mean value obtained combining all the studied genotypes is called *Environmental Index*. The regression coefficient (b) obtained regressing the different environmental indices *versus* the related accession means measures the linear response of the accession to the studied environments. When this value is higher than 1, it suggests a better response of the accession if compared to the general mean of the whole studied batch. For each accession we also calculated the bulb weight variation (percentage, %) with respect to the general mean value within the same location, and we graphically represented these variations by means of box plots.

Data of sensory profile were graphically represented as a net plot.

Results and discussion

Biometric and morphological traits

Cipolla di Giarratana landraces is characterised by a large and heavy bulb, strongly flattened at the poles, with the maximum diameter at the central section (Figure 1); it is white in colour. The bulb weight, diameter and height distribution, as registered in the whole studied batch, are shown in Figure 2. Bulb weight showed a wide variation as indicat-

ed by both the interquartile values (ranging from 338 and 535 g) and the end of the whiskers showing the smallest (10^{th}=280 g) and the largest (90^{th}=570 g) non-outlier observation, respectively. Fifty per cent of bulbs had a diameter ranging from 10.2 to 11.9 cm (median value 11.0 cm).

Significant differences in bulb weight and diameter were found among accessions and locations, but no significant interactions occurred between them (Table 1). This suggests that accessions responded similarly across environment sites for all the studied characteristics. Bulb weight (436 g for all accessions combined) varied among accessions in the average of all the locations, ranging from 394 g of accession '1' and '4' to 489 g for accession '12'; since bulbs with greater bulb weight also tended to have greater bulb diameter, accession '12' showed the highest value (114 mm), and accession '1' the lowest (103 mm).

In the experimental site 'B', much heavier bulbs have been reported (536 g all accessions combined) compared to those observed in the other locations, with 'C' as the lowest (290 g).

The regression coefficients of each accession for bulb weight ranged from 0.764 to 1.209 (accession '2' and '7', respectively). The regression coefficients over 1 (obtained by accessions '12', '7', '13', '9', and '6')

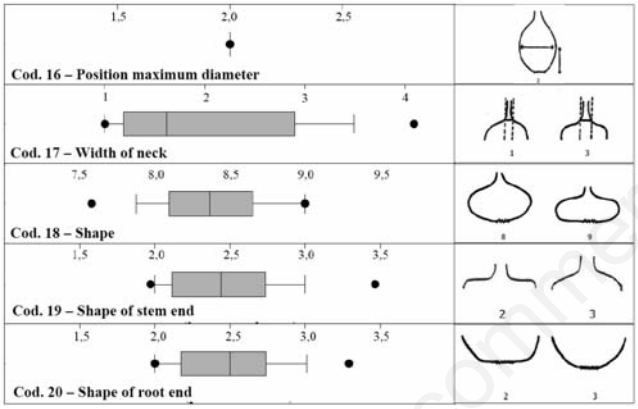

Figure 1. Main morphological traits of bulb obtained combining all the experimental data (n=2025) following the Community Plant Variety Office characteristics.

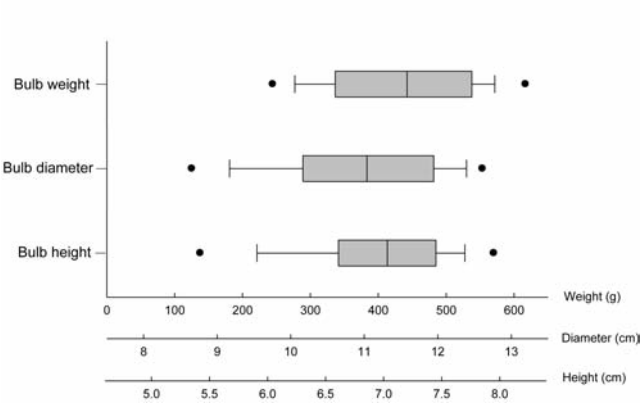

Figure 2. Distribution of the bulb dimensions obtained combining all the experimental data (n=2025).

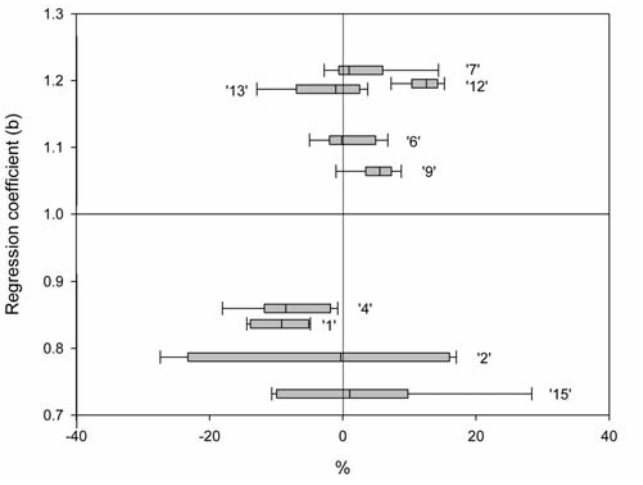

Figure 3. Regression coefficient (b) for the studied accessions each represented in terms of boxes plot. The box shows the distribution of 5 data reporting the percentage variation in bulb weight with respect to the general mean value of each location.

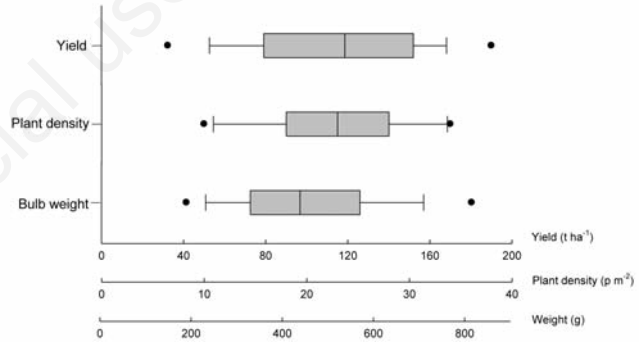

Figure 4. Distribution of the yield, plant density and bulb weight obtained combining all data at farm scale (n=25 farms×45 measurements=1125 data).

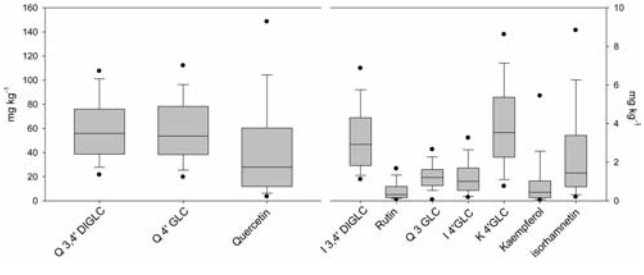

Figure 5. Distribution of the 10 measured flavonol contents obtained combining all the experimental data (n=135). Q 3,4' DIGLC, quercetin 3,4' di-*O*-glucoside; Q 4'GLC, quercetin 4'-*O*-glucoside; Q, quercetin; I 3,4' DIGLC, isorhamnetin 3,4' di-*O*-glucoside; RUT, rutin; Q 3GLC, quercetin 3-*O*-glucoside; I 4'GLC, isorhamnetin 4'-*O*-glucoside; K 4'GLC, kaempferol 4'-*O*-glucoside; K, kaempferol; I, isorhamnetin.

Table 1. Analysis of variance and mean values of biometrical and morphological traits in the different genotypes and locations.

Bulb weight (g)	Bulb Ø (cm)		Bulb characteristics 16	17	18	19	20
ANOVA results							
Location	***	***	-	-	-	-	-
Accession	**	**	-	-	-	-	-
Accession × location	ns	ns	-	-	-	-	-
Average comparison							
Accession							
1	389.3^{b}	10.3^{b}	2	2	8	3	2
2	414.1^{ab}	10.8^{ab}	2	2	8	3	3
4	398.2^{b}	10.8^{ab}	2	2	9	2	2
6	437.6^{ab}	11.0^{ab}	2	3	8	3	3
7	449.0^{ab}	11.1^{ab}	2	2	8	3	3
9	455.0^{ab}	11.4^{a}	2	2	8	2	2
12	488.5^{a}	11.4^{a}	2	2	8	2	2
13	424.2^{ab}	10.8^{ab}	2	2	8	2	3
15	437.9^{ab}	11.1^{ab}	2	2	8	2	3
Location							
A	360.0^{c}	10.5^{b}	2	2	9	2	2
B	536.5^{a}	11.8^{a}	2	3	8	3	3
C	290.1^{d}	9.4^{c}	2	3	8	3	2
D	503.0^{ab}	11.7^{a}	2	1	8	2	2
E	473.6^{b}	11.5^{a}	2	2	8	2	3
Average	435.5	11.0	2	2	8	2	3

CPVO (2009) code: 16, position of maximum diameter; 17, width of neck; 18, shape of the whole bulb; 19, shape of the stem end; 20, shape of the root end; ANOVA, analysis of variance; ns, not significant, **P<0.01; ***P<0.001; ^{a,b,c,d}within each experimental treatment, values reported in the column followed by different letters are significantly different (P<0.05 determined by Tukey honestly significant difference significant difference test).

describe a genotype with higher productivity compared to the mean value reported by the whole batch of studied accessions. But, as shown in Figure 3, only for '12' bulb weight report the highest value in all of the studied locations.

To characterise the crop production as actually cultivated in the area of Giarratana, we also involved 15 growers (representing more than half of the growers cultivating *Cipolla di Giarratana*) harvesting directly from representative areas (10 m^2) of their farms to obtain data on actual yield and crop management at farm scale. The bulbs harvested from the representative areas were weighed as a batch, to obtain real yield, and counted to obtain real plant density. Then 45 bulbs per area were sampled to measure biometrical traits as reported for experimental locations. The observations have been carried out in 2010 and 2011 thus collecting 25 data sets from actual farms concerning yield, plant density and bulb weight. None of these data are directed towards an experimental evaluation of the different accessions as in the previously described experiment, but they contributed to our knowledge about the crop and its environment, and helped stimulate interest in research into crop valorisation on the part of the growers.

The observation allowed us to synthesise the large variation in bulb yield (Figure 4) ranging between 27 and 195 t ha^{-1}. This wide variation is, obviously, related to the differences in terms of environment (mainly concerning soil type and altitude) and of the different crop managements practised by farmers (*e.g.* irrigation schedule, fertilisation practices). Nevertheless, 50% of registered yield ranged between 86 and 152 t ha^{-1} with a median of 119 t ha^{-1}.

Plant density was recorded to widen our knowledge of crop management and this showed that 50% of farmers planted 20-27 plants *per* m^2.

Regarding bulb weight, the reported data confirm the results we obtained in the experimental locations (Table 2), in particular in terms of median (430 *vs* 439 g combining all experimental and farm scale data, respectively) and 75th percentile (570 g for both data sets). This last observation could also be an important commercial consideration.

Table 2. Bulbs weight distribution as registered on experimental and farm scales.

	Obs. (no.)	Min	Percentiles 25th	50th	75th	Max
Experimental scale	2025	30	290	430	570	1450
Farm scale	1125	70	330	439	570	1350

Obs., observation.

Table 3. Analysis of variance on the 10 flavonols identified in *Cipolla di Giarratana* landrace.

Flavonols	Factors Blocks 2	Location 4	Genotype 8	G×L 32
Q 3,4' DIGLC	0.169 ns	<0.001***	0.001**	<0.001***
I 3,4' DIGLC	0.173 ns	<0.001***	0.056 ns	0.001***
RUT	0.263 ns	<0.001***	<0.001***	<0.001***
Q 3GLC	0.283 ns	0.141 ns	0.012*	0.001**
Q 4'GLC	0.334 ns	<0.001***	<0.001***	<0.001***
K 4'GLC	0.506 ns	<0.001***	<0.001***	<0.001***
I 4'GLC	0.757 ns	<0.001***	<0.016*	<0.001***
Q	0.829 ns	<0.001***	0.002**	<0.001***
K	0.128 ns	<0.001***	0.03*	<0.001***
I	0.132 ns	<0.001***	0.021*	<0.001***
Total flavonols	0.581 ns	<0.001***	<0.001***	<0.001***

ns, not significant, *P<0.05; **P<0.01; ***P< 0.001. Q 3,4' DIGLC, quercetin 3,4' di-*O*-glucoside; I 3,4' DIGLC, isorhamnetin 3,4' di-*O*-glucoside; RUT, rutin; Q 3GLC, quercetin 3-*O*-glucoside; Q 4'GLC, quercetin 4'-*O*-glucoside; K 4'GLC, kaempferol 4'-*O*-glucoside; I 4'GLC, isorhamnetin 4'-*O*-glucoside; Q, quercetin; K, kaempferol; I, isorhamnetin.

The large dimension of *Cipolla di Giarratana* bulbs represents a highly characterising trait traditionally preferred by the consumers for the preparation of typical dishes. However, this trait could limit its use in the modern daily diet in a social context characterised by small families and less time for household cooking than in previous generations. The large observed variation leads to an evaluation of bulb production for different potential uses. In particular, on both an experimental and a farm scale, a quarter of the total production ranged between 100 g and 300 g. Furthermore, the typical large dimension of the bulbs requested (exceeding 1000 g) was measured as 99th percentile.

Flavonols profile and content

The analysis of the UV-vis and mass spectra showed the presence of 10 different flavonols with Quercetin as the most represented flavonol (Figure 5). Notably, all of the extracts contained free quercetin (aglycon) in considerably high amounts, ranging from 4.1 to 177.8 mg kg^{-1} fresh weight. In fact, we found free quercetin as the third compound in order of abundance, right after quercetin, quercetin 3,4' di-*O*-glucoside (ranging from 19.8 to 129.3 mg kg^{-1} fresh weight) and quercetin 4'-*O*-glucoside (from 18.7 to 116.6 mg kg^{-1} fresh weight). Isoramnetin 3,4' di-glucoside, rutin, quercetin 3-*O*-glucoside, isorhamnetin 4'-*O*-glucoside, kaempferol 4'-*O*-glucoside, kaempferol and isoramnetin were detected in trace amounts (<10 ppm). As observed by Riggi and co-authors (2013), the large number of flavonols and the total flavonols content represent a particular trait of this landrace differentiating it from most white coloured onions (Marotti and Piccaglia, 2002; Simestad *et al.*, 2007).

The analyses of variance performed on the phenols data indicated a statistical difference between all the main and interaction effects (Table 3). The interactions that emerged stimulated our interest in performing a PCA for further evaluation of all the flavonols identified, relating flavonol content to a productive trait (bulb weight). It showed that the first two components explained 74% of total variance. The principal component 1 (PC1) explained 53.4% of total variance, and were strongly and positively correlated with all the identified flavonols except isorhamnetin 4'-*O*-glucoside, and strongly and negatively correlated with the bulb weight. The PCA analysis (Figure 6) showed sepa-

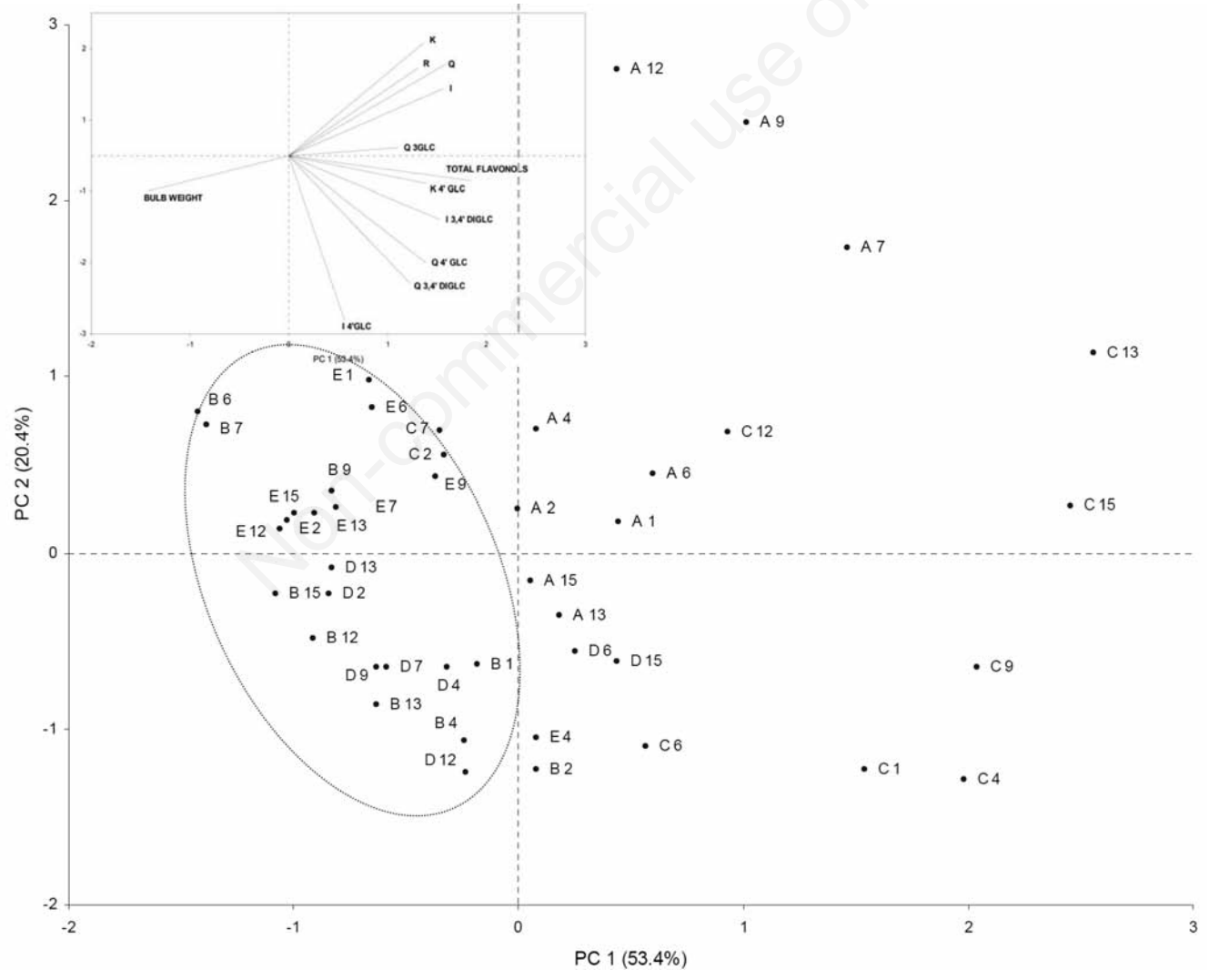

Figure 6. Principal component (PC) analysis based on the variation of flavonols content and bulb weight in the 9 accessions grown in the five locations. In the inner figure, each variable (flavonols and bulb weight) is represented by vectors (lines) drawn from the origin of the plot.

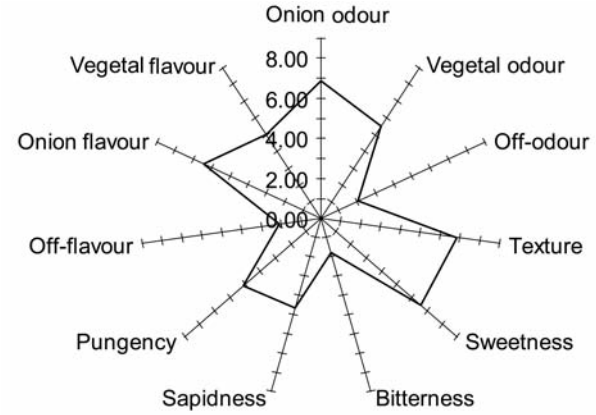

Figure 7. Sensory profile of the *Cipolla di Giarratana* landrace.

ration of plants grown at one of the Giarratana experimental sites and at sea level (locations 'A' and 'C', respectively) from those grown in the other locations in Giarratana ('B'), Chiaramonte Gulfi ('D'), and Palazzolo Acreide ('E'). In fact, most of the genotypes cultivated at the experimental sites 'B', 'D' and 'E' (23 of 27 cases) clustered in a cloud on the left of the ordination along PC1 (negative PC1 values) and were mainly characterised by higher bulb weight but lower flavonol content. On the other hand, the genotypes cultivated at locations 'A' and 'C' were spread on a larger cloud over the right of the ordination along PC1, mainly characterised by higher flavonol content, but with a much lower bulb weight. Also for flavonols content, the environment was found to explain the greater part of the total variation according to recent results obtained in traditional cultivars of white and red onion (Rodrigues *et al.*, 2011). In fact, different geographical locations formed separate clusters suggesting that the genotype was not the dominant source of variation.

Sensory analysis

The graphic diagrams of sensory profile of the *Cipolla di Giarratana* onion (Figure 7) showed that this landrace was distinguished mainly by a high intensity of sweet, typical fresh flavour of onion and its texture perception. All these characteristics are highly appreciated by consumers. On the contrary, the off-odours, the off-flavours and the pungency (characteristics associated with a negative perception of onion) showed the lower values.

Conclusions

Today, publicly funded research could play a strategic role in the promoting the *Made in Italy* image, strongly linked to the worldwide perception of Italian food as the basis for a kaleidoscopic of culinary art. Moreover, the need to protect both crop biodiversity and traditional production systems actually represents a challenging context for agronomic research. This is even more important for niche products for which low economic resources could be used to apply the valorisation strategies needed to maintain a traditional crop. In this context, the research aimed to characterise and promote the activities of a multidisciplinary group of the Italian National Research Council (CNR) and university researchers with agronomical, technological and chemical expertise. Furthermore, the adopted approach allowed us to involve many different stakeholders in the area of Giarratana (growers, local authority administrators, people involved in the food supply chain) representing in itself a strategic tool for the application of certifying rules based on international standards [protection of geographical indications (PGI) and products designations of origin (PDO)].

This research allowed us to collect a range of data on landrace characteristics and crop management both on an experimental and on a farm scale.

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