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PHYSICOCHEMICAL CHARACTERISTICS AND PHENOTYPIC DIVERSITY OF SICILIAN WINTER PUMPKIN (*CUCURBITA MAXIMA*) POPULATIONS

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ABSTRACT

Six pumpkin genotypes (G1, G2, G3, G4, G5 and G6) were evaluated for their morphological, agronomical and physico-chemical properties under ecological conditions of Sicily (Italy). Considering the agronomical, morphological and physicochemical data including fiber, humidity, pH, total acidity, soluble solids, carotenoids, phenolic and ascorbic acid content, it was possible to differentiate among genotypes. The morphological analysis showed that the pumpkin genotypes did not have a homogenous morphology and present a large physico-chemical characteristic variability. Furthermore, high contents of carotenoids and dietary fiber, soluble solids, phenolic and ascorbic acid were observed with the maximum values in G1 and G5 landraces. The correlation between the thirty four parameters and the genotypes showed a wide range of variability in both positive and negative direction. The variability was statistically accumulated with the considered parameters following a significant characterization. In conclusion, we found a wide range of genotype variability among Sicilian winter pumpkin populations. This germplasm may represent a valuable genetic source for future breeding studies.

1. Introduction

Cucurbita maxima Duch. (winter pumpkin) belongs to the *Cucurbitaceae* family. The species, is originated in South America and was introduced early into Europe (Ferriol et al., 2003). It represents one of the most cultivated and economically important vegetable, characterized by high production standards (Taylor and Brant, 2002). It can be used for human consumption (Ferriol et al., 2003), as fodder for livestock, medicinal and cosmetic purposes, containers, or ornamental objects, and as rootstock (Lira-Saade, 1995). In addition, it is suitable to realize cross with the *Cucurbita moschata* species, that allows to obtain hybrid of *Cucurbita maxima* x *Cucurbita moschata* that confer tolerant or resistance of soilborne disease (Ekaterini et al., 2000).

Sicily is the largest Mediterranean island located in southern Italy. It is a cultural and a commercial port, and one of the most important centre of origin and differentiation of vegetables. During the centuries, the farmers obtained many genotypes for each species, adapting them to the pedoclimatic requirements, and don't caring them to the genetic purity. For this reason, it was estimated a presence of 2650 taxa (Raimondo et al., 1992) in Sicily on an extension of 26000 Km^2. This selection criteria allowed to

obtain an inter-specific variability that brought other genotypes perfectly integrated with the cultural environment and with positive effects on the qualitative and organoleptic characteristics (Schiavi et al., 1991). Breeding activity is always depended on the availability of genetic variability, and thanks to the selection criteria applied by the farmers the biodiversity was saved (Schippmann et al., 2002). The local populations are genotypes of high intrinsic value with a particular capacity of adaptability to their environment (D'Anna and Sabatino 2013; Sabatino et al., 2013; Sabatino et al., 2014; Sabatino et al., 2016). These characters might allow easier cultivation in Mediterranean basin compared to the varieties selected in different environments. The antioxidant capacity of different vegetables is one of the most features of food product (Dragsted et al., 2006). Plants contain high concentrations of numerous redox-active secondary metabolites (ie, antioxidants), such as polyphenols, carotenoids, ascorbic acid, and enzymes with antioxidant activity (Torunn et al., 2009), which help them from hazardous oxidative damage to plant cell components. It is well known that carotenoids are synthesized only by plants and micro-organisms, therefore, the interest on their content increased the attention on pumpkin fruits (Murkovic et al., 2002). This interest was justified by cancer prevention (Astorg, 1997), atherosclerosis, cataracts, age-related macular degeneration and an array of other degenerative diseases (Murkovic et al., 2002). Moreover, the quality and the quantity of antioxidants in fruits and vegetables, change by cultivar, environment, soil type, growing and storage conditions (Achouri et al., 2005; Antolovich et al., 2000; Griffith and Collison, 2001; Lee et al., 2004; Luthria, 2006; Naczk and Shahidi, 2004; Robbins, 2003).

The purpose of the present study was the discovery, recovery, and characterization of five Sicilian winter pumpkin local populations by using morphological and chemical characteristics such as water content, pH, acidity, soluble solid, carotenoid, polyphenol, dietary fiber, ascorbic acid and colour, to reveal trait significant differences between them.

2. Materials and methods

2.1.Plants material and cultivation technique

The experimental field was carried out in open field during spring-summer 2012 and repeated during spring-summer 2013 at the experimental farm of the Palermo University (Italy). Five landraces were taken (G1), (G2), (G3), (G4), and (G5) from the Buonfornello area (37°98'39''N, 13°69'63''E) (Palermo, Sicily). In addition, one variety 'Butternut' (G6) was also tested as control sample.

Thirty seeds per genotype were seeded into 66 cells plug trays, containing a substrate mix characterized by blond peat moss and black peat moss (1:1, v/v). After sowing, the seeds were covered with a layer of the same substrate, and the trays were placed in greenhouse with a target of air temperature 25/18 °C (day/night). All trays were sub-irrigated using a nutrient solution containing the following elements in $mg \cdot L^{-1}$: 178.5 N (164.5 NO_3-N, 14 NH_4), 38.71 P, 254.15 K, 110 Ca, 24.31 Mg, 32.07 S, 0.84 Fe, 0.55 Mn, 0.33 Zn, 0.27 B, and 0.048 Mo (Sonneveld and Straver, 1994; De Kreij et al., 1999). After 15 days from the sowing, the seedlings achieved the plant phenological stage. Planting of all the genotypes took place on 20th April 2014, and repeated on 25th April 2015. In our study, a typical Sicilian cultivation technique in open field was used for the growth of winter pumpkin. Seedling bed was prepared through medium-deep ploughing (35 cm) and declodding using a rotary harrow. Aged manure was added as a soil amendment at a rate of 40 t ha^{-1}. Seedlings with their root ball were used and planted with a 2 m planting distance, and a 3.5 minter-row distance layout was adopted. A drip irrigation system was installed to provide water and nutrient solution containing

the following elements in mg L^{-1} 241.5 N (224 NO_3^-, 17.5 NH_4^+), 38.71 P, 312.8 K, 160 Ca, 33.42 Mg, 44.09 S, 0.84 Fe, 0.55 Mn, 0.33 Zn, 0.27 B, and 0.048 Mo (Sonneveld and Straver, 1994; De Kreij et al., 1999). All genotypes were planted with a distance between the plots containing the different genotypes of 25 m. At the flowering moment, all female flowers were submitted to the manual pollination and were applied one clip insulator to avoid the crossing between the genotypes.

2.2. Morphological characterization and data production

The morphological characterization was carried out upon 30 plants per accession, using over the IPGRI descriptor for *Cucurbitaceae* (Esquinas-Alcázar and Gulick, 1983). In addition, other morphological characters for a more complete description were evaluated. Twenty characters were evaluated: average total production per plant (kg), average number fruits per plant, average weight fruit (kg), thickness epicarp (cm), thickness mesocarp (apical, median, and distal part) (cm), leaf longitudinal length (cm), leaf transverse length (cm), petiole length (cm), thickness petiole (cm), peduncle length (cm), thickness peduncle (cm), fruit length (cm), fruit width (cm), fruit length/width ratio, scar flower diameter (cm), flesh colour (apical, median, and distal part)(L*, a*, b*). The flesh colour of raw pumpkin samples was assessed by a tristimulus colorimeter (Chroma Meter - CR-400, Konica Minolta) which is suggested by Mendoza et al. (2006), and evaluated along the longitudinal section of fruits (apical, median, and distal part).

2.3. Preparation of sample and chemical analysis

All reagents were analytical grade Sigma of Fluke products used without any purification. Pumpkin fruits were peeled, the seeds were removed, then chopped into small sections with a kitchen knife, and the juice was extracted using a blender. All the samples of winter pumpkin juices were immediately analyzed within the extraction day for the both years production 2012 and 2013. pH were performed on the homogenized samples using a pH meter (METTLER TOLEDO mod. MP 220) following NMKL 179 Method (2005), the samples temperature was standardized at 25 °C. Pumpkin's water content is calculated as the ratio of (wet - dry) relative to the dry winter pumpkins. The dry matter content is then calculated as (100 – moisture content). It was obtained by placing 2 g of sample into a small china bowl of known weight, and putting all samples inside of oven set at 70 °C for 3 days. Determination of the soluble solids was made by homogenizing and filtering the juice of pumpkin samples. Then, the filtered samples were subjecting at the reading with an Optical Refractometer (ATAGO Hand Refractometer N-50 E). In order to evaluate total acidity, the samples were diluted with 250 ml of distilled water, and titrated with a sodium hydroxide solution (0.1 M) in presence of the indicator (alcoholic solution of phenolphthalein: 1% in ethyl alcohol at 95%), until the colour indicator changes (the pink colour of phenolphthalein persist for 30 seconds). The results were expressed as grams of citric acid monohydrate per 100 g of product, following the conversion index. The total carotenoids content was determined according to the method of Lee and Castle (2001) which was modified for pumpkin (Nur and Gülsah, 2012). Two ml of a winter pumpkin sample was mixed with 38 ml extraction solvent (hexane-acetone-ethanol (50:25:25 v/v/v)) with an agitator vortex mod. RX3 (constant speed 2400 rpm). Then, centrifuged (4000 rpm, 10 min) (HERAEUS, MULTIFUGE 3S+ Centrifuge), the supernatant was collected, and the absorption was measured with a SHIMADZU UV mini- 1240 spectrophotometer at 450 nm. Total carotenoid content was calculated as milligrams of carotene per gram of sample by using the following equation:

$$A = (\varepsilon bc)/1000 \quad (1)$$

were A is the absorbance value, **ε** = molar absorbance coefficient **ε** (1cm) = 2505, b = extent of unit light way, 1 cm, c = mg g^{-1} total carotenoid content.

To determine the total phenolic content, two grams of homogenized sample were added with 10 ml of pure ethanol. The extraction was done by using a vortex mixer mod. RX3 for 60 seconds. The mixture was filtered and the filtrate was taken into a test tube. The Folin-Ciocalteau micro method of Waterhouse (Brand-William et al., 1995) was used to determinate the total phenolic content (TPC). Three hundred µl of the filtrate were diluted in 4.8 mL of Milli-Q grade water, and 300 µl of Folin-Ciocalteau reagent was added and shaken. After 8 min, 900 µl of 20 % sodium carbonate solution was added with mixing. After reaction at 40 °C for 30 min, absorbance was measured at 765nm using SHIMADZU UV mini- 1240 spectrophotometer. A calibration curve of gallic acid (3, 4, 5- trihydroxybenzoic acid) was prepared (0-50 µg) and used as standards. The results were given as mg gallic acid equivalent per gram of fresh weight.

The measurement of ascorbic acid content was determined as described by Lee and Coates (1999) using an HPLC photodiode array detection approach. About 10 g of winter pumpkin sample was homogenized with 10 mL of 10 % metaphosphoric acid and sea sand in a mortar. The slurry obtained was pipetted and was transferred into a centrifuge tubes containing 20 mL of 5 % metaphosphoric acid and centrifuged (HERAEUS, MULTIFUGE 3S+ Centrifuge) for 20 min at 3000 rpm. The supernatant was filtered through 0.45 mm PTFE syringe filters and diluted 20 fold with distilled water. Ten mL of diluted supernatant were diluted with 0.13 mL of 2.5 M K_2HP0_4 to give a final pH of 7.0, and 20 µl of this solution was injected into C18 (15 cm 4.6 cm, pore size 5 mm) coupled with HyperODS guard column. The mobile phase was 8 mM phosphate buffer, pH 6.8, containing 3 mM tetra-n-butylammonium bromide with a flow rate of 1 mL min^{-1}. Eluate was monitored by UV detection at 245 nm. Chromatograms were recorded and processed with EZ Start Chromatography Software V.7.2.1. The identification of peak corresponding to ascorbic acid detected by their co-elution with standard.

Total dietary fibre was determined using an enzymatic-chemical method (Willem et al., 2010). Samples and blanks used to be tested for dietary fibres content were quadruplicate. One gram amounts of the sample being analysed was incubated in 250 mL Duran bottles in a shaking incubation bath at 150 rev/min in orbital mode, after adding 50 mL of pH 6 phosphate buffer to each bottle with 0,1 mL α- amylase (Product code A 3306) for 15 min at 95 °C. After pH correction at 8 by adding 10 mL of 0.275 N NaOH with 0.1 mL (5 mg Protease) (Product Code P 3910). The solutions reaction were incubated at 60 °C for 30 min to obtain the inactivation of α-amylase and denaturation proteins after adjusting the pH at 4.5 with 10 mL of 0.325 M HCL. In addition, 0.1 mL of amyloglucosidase (Product Code A 9913) was added and incubated for 30 min at 60 °C. One mL of diethylene glycol was added followed by four volumes of ethanol with mixing to precipitate soluble polymeric dietary fibre. The suspension was filtered and washed sequentially with 76% ethanol, 96% ethanol and acetone. Furthermore, it was dried and weighed. One duplicate was used to determine the proteins Kjeldahl nitrogen analysis as specified in the Official Methods of Analysis of AOAC 1997, and the other was incubated at 525 °C to determine ash. Finally, the total dietary fibre was calculated as the difference between the weight of the filtered and dried residue, and the weight of the proteins and ash.

2.4. Experimental design and statistical analysis

Treatments were defined by a completely randomized design with three replicates per

treatment, each consisting of 10 plants. Statistical analysis were performed using ANOVA and mean separation was carried out by Duncan multiple range test by the statistical program SPSS 14.0 (StatSoft, Inc., Chicago, USA). Percentages were subjected to angular transformation prior to perform statistical analysis ($\Phi = \arcsin(p/100)^{1/2}$). Investigation of multi-character variation was conducted by Principal Component Analysis (PCA).

3. Results and Discussion

The results demonstrated that winter pumpkin genotypes differed in respect of physical and chemical characteristics which are confirmed in literature (Sztangret et al., 2004; Gajc-Wolska et al., 2005; Paulauskiene et al., 2006). Recently, in other vegetable crops, such as in eggplant, the chromosomal region and QTL (Quantitave Trait Loci) associated to the content of anthocyanin, dry matter, solamargine glycoalkaloid, chlorogenic and other organic acid have been identified (Gramazio et al, 2014; Toppino et al, 2016).

For the colorimetric evaluation, significant differences ($P \leq 0.001$) were observed among genotypes for flesh colour represented by the three color space values: L*, a*, b*. This demonstrates that genetic variation for flesh color is present among the tested samples. The lowest value of L* (lightness) and highest value of a* were found in G2 and G6 ('Butternet' variety) genotype samples respectively (Table 1). Along the longitudinal section, from apical to distal part, the lowest L*, a* coordinates values were obtained in the median part, therefore, seem to be a longitudinal flesh colour gradient.

Table 2 indicates that the dry matter content in the pumpkin fruit samples differed significantly ($P \leq 0.001$) between the samples. It ranged from 5.93 to 10.35 %. The highest and the lowest proportion of dry matter was measured in the sample G1 and G3, respectively. Such high variation in dry matter content of winter pumpkin fruit has also been reported by Paulauskiene et al. (2006). This response could be due to variation in starch content of the genotype (Hazzard, 2006). The changes in the pH of the fruit flesh influences the activities of ripening related enzymes and antioxidant system, ultimately affects the sensory quality (McCollum et al., 1988). The pH value of the sixth fresh winter pumpkin fruit samples was significantly different ($P<0.001$). It ranged between 6.12 and 6.48. The highest pH value was recorded in winter pumpkin sample G1 and the lowest in sample G4. These results are similar to those reported by Paulauskiene et al. (2006).

Significant ($P \leq 0.001$) difference in fruit total soluble solids content was obtained among the winter pumpkin samples with a range varying between 4.89 and 9.28^{o}Brix (Tab. 2). Pumpkin sample G1 had the highest TSS (9.28^{o}Brix.), followed by sample G2 (7.26 ^{o}Brix) while, sample G3 had significantly lower TSS (4.89^{o}Brix). These variations in TSS are due to the genetic differences among the genotypes that affect fruit quality (Stepansky et al., 1999; Burger et al., 2006, Burger and Schaffer, 2007). More than 85% of pumpkin fruit TSS content is sugar and it is highly related to the sensory quality of pumpkin fruit and it is used to screen pumpkin fruit (Cantwell and Suslow, 1998).

The biosynthesis and metabolism of carotenoids in vegetables can significantly be affected by the differences in growing environment, such as temperature, nutrient availability, soil, intensity of sunlight, ripening stage, and post harvesting (Rodriguez-Amaya, 1999; Cazzonelli and Pogson, 2010). According to Murkovic et al. (2002); Gajc-Wolska et al. (2005) the genotypes significantly influence carotenoids content in pumpkin fruits. The content of total carotenoids in the samples examined varied between 0.25 and 0.48 mg g^{-1}, the highest value being measured in the control 'Butternut' sample G6 (Table 2).

From the nutritional point of view polyphenols are important determinant factors for the quality trait of winter pumpkin. The content of polyphenols was found to differ considerably among the samples, ranging from 28.57 to 93.70 mg/100g (Table 2). The lowest value was measured in the control 'Butternut' sample G6 (28.57 mg/100 g).

Table 1. Flesh colour measurement of winter pumpkin samples.

Genotype	L apical part	a apical part	b apical part	L median part	a median part	b median part	L distal part	a distal part	b distal part
G1	59.13 bc	15.36 d	56.70 n.s.	60.23 a	15.89 c	59.77 a	62.1 a	16.55 cd	59.56 ab
G2	66.98 a	16.60 cd	55.47 n.s.	62.00 a	13.99 d	53.51 ab	63.2 a	15.65 d	53.53 d
G3	63.85 a	21.23 b	55.81 n.s.	61.51 a	20.82 ab	54.96 a	63.1 a	21.76 b	56.64 c
G4	55.92 c	17.80 c	50.77 n.s.	53.56 b	16.47 c	46.90 b	51.7 c	17.20 c	49.70 e
G5	57.87 c	17.50 cd	54.56 n.s.	58.37 ab	20.26 b	59.44 a	57.3 b	20.61 b	60.20 a
G6	63.35 ab	24.04 a	57.67 n.s.	61.98 a	22.53 a	55.88 a	59.7 ab	25.86 a	57.76 bc

Results indicate mean value of two years (2014 and 2015). In each column, figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 2. Physico-chemical characteristics of winter pumpkin samples.

Genotype	Dry matter [%]	pH	Total acidity [g/100g citric acid monohydrate]	Soluble solid [°Brix]	Carotenoids [mg g^{-1}]	Total phenolics [mg/100]	Total dietary fibre [g/100g]	Ascorbic acid [mg/Kg]
G1	10.35 a	6.48 a	0.09 n.s.	9.28 a	0.27 de	66.36 d	1.50 a	30.00 a
G2	8.18 c	6.45 a	0.10 n.s.	7.26 b	0.29 d	66.67 c	1.40 b	26.50 b
G3	5.93 f	6.27 c	0.10 n.s.	4.89 e	0.38 c	41.52 e	1.40 b	23.00 d
G4	7.07 e	6.12 d	0.09 n.s.	6.20 c	0.25 e	76.20 b	1.20 d	24.90 c
G5	9.27 b	6.15 d	0.09 n.s.	5.30 d	0.43 b	93.70 a	1.30 c	15.63 f
G6	7.49 d	6.39 b	0.15 n.s.	6.39 c	0.48 a	28.57 f	1.40 b	21.30 e

Results indicate mean value of two years (2014 and 2015). In each column, figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 3. Factor loadings, eigen values, and percentages of variance and cumulative variance that can be explained by the first 3 PCs.

Variable	PC1	PC2	PC3
Average total production/plant	0.799	-0.015	-0.316
Average number fruits/plant	-0.682	0.453	-0.166
Average weight fruit	0.695	-0.086	-0.214
Thick epicarp	-0.172	-0.479	-0.409
Thick mesocarp (apical part)	0.224	0.079	0.856
Thick mesocarp (median part)	0.709	0.096	-0.603
Thick mesocarp (distal part)	0.320	0.328	-0.011
Average weight fruit	0.695	-0.086	-0.214
Leaf transverse length	0.004	-0.701	0.008
Petiole length	0.209	-0.750	0.024
Thickness petiole	0.095	-0.502	0.519
Peduncle length	0.538	0.032	-0.379

Thickness peduncle	0.526	0.277	-0.276
Fruit length	0.220	0.382	0.687
Fruit width	0.934	-0.099	-0.128
Fruit length/width ratio	-0.510	0.550	0.593
Scar flower diameter	0.819	0.004	0.097
Humidity	0.608	-0.631	-0.439
pH	0.183	0.838	-0.307
Total acidity	0.676	-0.013	0.051
Soluble solid	-0.501	0.736	-0.262
Total carotenoid	0.700	-0.105	0.644
Total phenolic content	-0.783	-0.147	0.361
Total dietary fiber	0.299	0.933	-0.072
Ascorbic acid content	-0.317	0.504	-0.771
Flesh colour L* (apical part)	0.637	0.321	-0.291
Flesh colour a* (apical part)	0.774	-0.297	0.041
Flesh colour b* (apical part)	0.390	0.511	0.124
Flesh colour L* (median part)	0.598	0.555	0.049
Flesh colour a* (median part)	0.654	-0.234	0.512
Flesh colour b* (median part)	0.153	0.610	0.587
Flesh colour L* (distal part)	0.495	0.736	-0.119
Flesh colour a* (distal part)	0.727	-0.209	0.390
Flesh colour b* (distal part)	0.218	0.584	0.716
Eigen value	9.955	7.863	5.689
Variance/%	29.278	23.126	16.731
Cumulative/%	29.278	52.405	69.136

Results indicate mean value of two years (2014 and 2015).

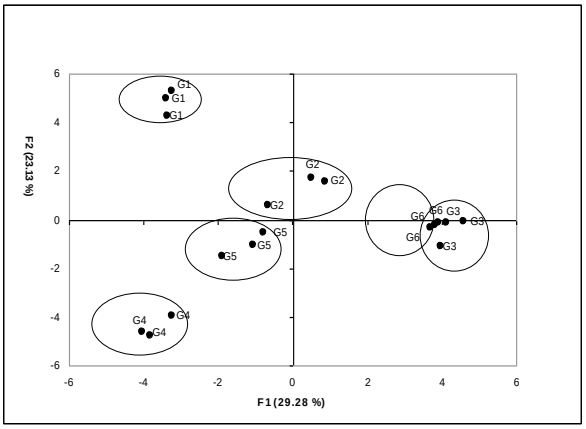

Figure 1. Results indicate mean value of two years (2014 and 2015). Principal component score plots of the separation of winter pumpkin samples (PC1 and PC2). Abbreviated variables are explained in Table 3

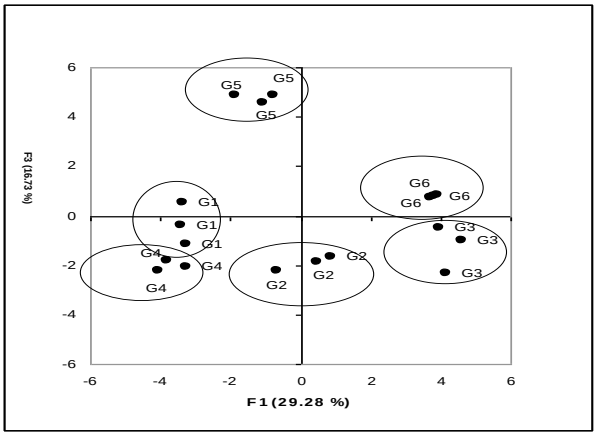

Figure 2. Results indicate mean value of two years (2014 and 2015). Principal component score plots of the separation of winter pumpkin samples (PC1 and PC3). Abbreviated variables are explained in Table 3.

The highest total phenol content (93.70 mg/ 100g) was recorded in G5. These results are in accord to those reported by Sharma and Ramana Rao (2013).

Winter pumpkin fruit samples dietary fibre content showed variations among landraces. The sample G1 showed the highest value (1.5 g/100 g), and the sample G4 showed the lowest value (1.2 g/100 g). These variations reflect the genotype effect on dietary fibre content. This variability was already detected from other fruits by Mrabet et al. (2013). Furthermore, both factors (genotype and environment) have been shown to contribute significantly to the dietary fibre content of cereals such as wheat (Gebruers et al., 2010) and leguminous like peas (Stoughton-Ens et al., 2010).

Many researchers reported that pumpkin provides a valuable source of ascorbic acid that have a major role in nutrition in the form of vitamin C as antioxidants (Duke and Ayensu, 1985; Sudhakar et al., 2003). Winter pumpkin fruit samples examined showed highly significant ($P < 0.001$) variation in ascorbic acid content (Tab. 2). The ascorbic acid content ranged between 15.63 mg/Kg for the sample G5 and 30 mg/Kg for the sample G1. Our results confirmed those obtained by Sudhakar et al. (2003) who indicated that ascorbic acid content of pumpkin varies among genotypes.

Principal Component Analyses was performed to evaluate the global effect of agronomical, morphological, and physicochemical properties on the winter pumpkin landraces (Tab. 3). The factor loadings are shown in the Table 3. The first axis accounted for 29.28 % of the variance, the second 23.13 % and the third 16.73 %, making a total of 69.14% of variance with the three axis together.

The first two factors (PC1 and PC2) represent 52.40 % of the initial data variability. However, some information still might be hidden behind the third factor, PC3, 16.73 %. Figure 1 represents the clustering differences between the sixth winter pumpkin genotypes; all of the G3 samples are positioned on the right side of the PC1, and other samples are positioned on its left side, with G2 samples situated in the up-right position. The first principal component (PC1) explains 29.28 % of the total variance, and was positively correlated with average total production per plant, thickness mesocarp (apical, median and distal part), average weight fruit, leaf transverse length, petiole length, thickness petiole, peduncle length, thickness peduncle, fruit (length, width), scar flower diameter, humidity, pH, total acidity, total, carotenoids, total dietary fibers, and

flesh color (Table 3). The second principal component (PC2) explains 23.13 % of the variance, and was positively correlated with average fruits number per plant, thickness mesocarp (apical, medium and distal part), peduncle length, thickness peduncle, fruit (length and length/width ratio), scarf flower diameter, pH, soluble solid, total dietary fibre, ascorbic acid content, flesh color except flesh color a, and negatively correlated with average total production per plant, thickness epicarp, average weight fruit, leaf (longitudinal and transversal length), petiole (length and thickness), fruit width, humidity, total acidity, total carotenoids, total phenolic content (Table 3). The second two factors (PC1 and PC3) represent 69.14 % of the initial data variability. In the figure 2 all of the G2, G3 and G6 samples are positioned on the right side of the PC1 expect one sample (G2), and other samples (G1, G4 and G5) are located on its left side, particularly G5 samples is situated in the up-left position, and G4 sample in the down-left position. While G3 samples is located in down-right position and G6 in the up-right position. The first and the third factor (PC1 and PC3) were also presented graphically (Fig. 2), they stand for the cumulative 69.14 % of the initial data variability. Thick epicarp and mesocarp (median part), humidity and ascorbic acid content were strongly negatively correlated with PC3, while the thickness petiole, fruit (length and length/width ratio), total carotenoid, flesh color a, and b (median and distal part) were strongly positively correlated with PC3 (Table 3). It is possible to observe that for the PC1, the average total production per plant, thickness mesocarp (median part), flesh color a* (apical and distal part), and total carotenoids content were the most important variables that explain the separation in the winter pumpkin samples. The loading in PC2 the most important variables that explain the separation between the winter pumpkin samples were the average number fruits per plant, the fruit length/width ratio, the flesh colour L* and b* (median and distal part), pH, and total dietary fibre, while in PC3 the most important variables were both the thickness mesocarp (apical part) and total carotenoids content. For most of the variations it is more likely that flesh colour and total carotenoids content parameters had a high influence in explaining the separation among samples. The correlation between colour parameters in CIE Lab system and carotenoids content in vegetables were found also in some other works (Paulauskiene et al., 2006).

4. Conclusions

Our results demonstrated the possible differentiation among the chosen pumpkin genotypes by their agronomical, morphological, and physico-chemical characteristics. The data reveal great deal of diversity for morphological traits. Among the sixth tested pumpkin genotypes, sample G3 showed the highest total production per plant followed by both samples G2 and G6 (control sample) which represent the biggest fruit sizes. By contrast, the sample G1 demonstrated small fruit sizes but with the highest fruit number per plant, soluble solids, and ascorbic acid content. Growers must realize that greater fruit number will result in a smaller average fruit size. The sample G5 showed the highest carotenoid and total phenolic compounds contents. In terms of nutritional value both samples G1 and G5 were the most valuable genotypes. Qualitative characteristics showed also variation in flesh color. The results of this study demonstrate a wide range of genotype variability among Sicilian winter pumpkin populations and might represent a valuable source of information for future *Cucurbita maxima* breeding improvement.

5.References

Achouri, A., Boye, J.I., Belanger, D. (2005). Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. *Food Research International*, 38, 1199-1204.

Antolovich, M., Prenzler, P., Robards, K., Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. *Analyst*, 125, 989-1009.

Astorg, P. (1997). Food carotenoids and cancer prevention: an overview of current research. *Trends in Food Science and Technology*, 8, 406-413.

Brand-Williams, W., Cuvelier, M., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissens-chaft-und-Technologie*, 28, 25-30.

Burger, Y., Sa'ar, U., Paris, H.S., Lewinsohn, E., Katzir, N., Tadmor, Y., Schaffer, A.A. (2006). Genetic variability for valuable fruit quality traits in Cucumis melo. *Isreal Journal of Plant Science*, 54, 233-242.

Burger, Y., Schaffer, A.A. (2007). The contribution of sucrose metabolism enzymes to sucrose accumulation in Cucumis melo. *Journal of the American Society for Horticultural Science*, 132, 704-712.

Cantwell, M., Suslow, T.V. (1998). Pumpkins and Winter squashes. Recommendations for maintaining postharvest quality. *Perishables Handling Quarterly*, 94, 15-16.

Cazzonelli, C.I., Pogson, B.J. (2010). Source to sink: Regulation of carotenoid biosynthesis in plants. *Trends in Plant Science*, 15, 266–274.

D'Anna, F., Sabatino, L. (2013). Morphological and agronomical characterization of eggplant genetic resources from the Sicily area. *Journal of Food, Agriculture & Environment* 11(1), 401-404.

De Kreij, C., Voogt, W., Van den Bos, A.L., Baas, R. (1999). Bemestringsdviesbasis Substraten. Proefstation voor Bloemistreij en Glasgtoente Naaldwijk The Netherlands, 145 pp.

Dragsted, L.O., Krath, B., Ravn-Haren, G., Vogel, U.B., Vinggaard, A.M., Jensen, P.B. (2006). Biological effects of fruits and vegetables. *Proceedings of the Nutrition Society*, 65, 61-67.

Duke, J.A., Ayensu, E.S. (1985). Medicinal Plants of China Reference Publications. Inc. ISBN 0-917256-20-4.

Ekaterini, T.M., Metaxia, K.S., Pritsa, T. (2000). Response of squash (Cucurbita spp.) as rootstock for melon (Cucumis melo L.). *Scientia Horticulturae*, 83, 353-362.

Esquinas-Alcázar, J.T., Gulick, P.J. (1983). Genetic resources of Cucurbitaceae a global report. IBPGR Secretariat, Rome.

Ferriol, M., Beleén Picò, M., Nuez, F. (2003). Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SBAP markers. *Genetic Resources and Crop Evolution*, 50, 227-238.

Gajc-Wolska, J., Gajewski, M., Radzanowska, J., Niemirowicz Szczytt, K., Korzeniewska, A. (2005). The fruit quality of chosen hybrids and cultivars of pumpkin (Cucurbita maxima Duch.). *Vegetable Crops Research Bullettin*, 63, 34-43.

Gebruers, K., Dornez, E., Bedõ, Z., Rakszegi, M., Fras, A., Boros, D. (2010). Environment and genotype effects on the content of dietary fiber and its components in wheat in the Health Grain Diversity Screen. *Journal of Agricultural and Food Chemistry*, 58, 9353-9361.

Gramazio, P., Prohens, J., Plazas, M., Andújar, I., Herraiz, F.J., Castillo, E., Knapp, S., Meyer, R.S., Vilanova, S. (2014). Location of chlorogenic acid biosynthesis pathway and polyphenol oxidase genes in a new interspecific anchored linkage map of eggplant. *BMC Plant Biology* 14, 350. doi: 10.1186/s12870-014-0350-z.

Griffith, A.P., Collison, M.W. (2001). Improved methods for the extraction and analysis of isoflavones from soy-containing foods and nutritional supplements by reversed-phase high performance liquid chromatography and

liquid chromatography - mass spectrometry.*Journal of Chromatography A*, 913, 397-413.

Hazzard, R. (2006). Pumpkin crop. *Vegetable Notes for Vegetable Farmers in Massachusetts*, 17(20), 20-32.

Lee, H.S., Castle, W.S. (2001). Seasonal changes of carotenoid pigments and color in Hamlin, Early gold, and Budd Blood orange juices. *Journal of Agricultural and Food Chemistry*, 49, 877-882.

Lee, J.H., Coates, G.A. (1999). Vitamin C in frozen, fresh squeezed, unpasteurized, polyethylene-bottled orange juice: A storage study. *Food Chemistry*, 65, 165-168.

Lee, J.H., Renita, M., Fioritto, R.J., Martin, S.K., Schwartz, S.J., Vodovotz, Y. (2004). Isoflavone characterization and antioxidant activity of Ohio soybeans. *Journal of Agricultural and Food Chemistry*, 52, 2647-2651.

Lira-Saade, R. (1995). Estudios taxónomicos y ecogeográficos de las Cucurbitaceae latinoamericanas de importancia económica Systematic and ecogeographic studies on crop genepools. International Plant Genetic Resources Institute, Roma.

Luthria, D.L. (2006). Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. *Journal of Science of Food and Agriculture*, 86, 2266-2272.

McCollum, T.G., Huber, D.J., Cantliffe, D.J. (1988). Soluble sugar accumulation and activity of related enzymes during muskmelon fruit development. *Journal of the American Society for Horticultural Science*, 113(3), 399-403.

Mendoza, F., Dejmek, P., Aguilera, J.M. (2006). Calibrated color measurements of agricultural foods using image analysis. *Postharvest Biology and Technology*, 41, 285-295.

Mrabet, A., Rodríguez-Arcos, R., Guillén-Bejarano, R., Chaira, N., Ferchichi, A., Jiménez-Araujo, A. (2013). Dietary fiber from Tunisian common date cultivars (Phoenix dactylifera L.): Chemical composition, functional properties, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 60, 3658-3664.

Murkovic, M., Mülleder, U., Neunteuflw, H. (2002). Carotenoid Content in Different Varieties of Pumpkins. *Journal of Food Composition and Analysis*, 15, 633-638.

Naczk, M., Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054, 95-111.

NMKL 179 Method (2005). Newsletter for The Nordic Committee on Food Analysis NMKL n 58.

Nur, D.S., Gülsah, Ç. (2012). Determination of the effect of freeze drying process on the production of pumpkin (*Cucurbita moschata)* puree powder and the powder properties. *GIDA/The Journal of Food*, 37(4), 203-210.

Official Methods of Analysis of AOAC (1997). International,16th Edition, Volume I, Section 12.1.07, Method 960.52.

Paulauskiene, A., Danilcenko, H., Jariene, E., Gajewski, M., Seroczyńska, A., Szymczak, P., Korzeniewska, A. (2006). Quality of pumpkin fruits in relation to electrochemical and antioxidative properties. *Vegetable crops research bulletin*, 65, 137-144.

Raimondo, F.M., Gianguzzi, L., Ilardi, V. (1992). Inventario delle specie "a rischio" nella flora vascolare della Sicilia. *Quaderni di botanica ambientale applicata*, 3, 65-132.

Robbins, R.J. (2003). Phenolic acids in foods: An overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, 51, 2886-2887.

Rodriguez-Amaya, D.B. (1999). A guide to carotenoid analysis in foods. Washington: *International Life Sciences Institute (ILSI)*, Press.

Sabatino, L., Palazzolo, E., D'Anna, F. (2013). Grafting suitability of Sicilian

eggplant landraces onto Solanum torvum: Fruit composition, production and phenology. *Journal of Food, Agriculture & Environment*, 11(3&4), 1195-1200.

Sabatino, L., Iapichino, G., Vetrano, F., D'Anna, F. (2014). Morphological and agronomical characterisation of Sicilian bottle gourd Lagenaria siceraria (Mol.) Standley. *Journal of Food, Agriculture & Environment*, 12(2), 587-590.

Sabatino, L., Iapichino, G., Maggio, A., D'Anna, E., Bruno, M., D'Anna, F. (2016). Grafting affects yield and phenolic profile of Solanum melongena L. landraces. *Journal of Integrative Agriculture*, 15(5), 1017-1024.

Schiavi, M., Masera, R., Lorenzoni, C., Soressi, G.P. (1991). Metodi utilizzati per il miglioramento genetico della Dorata di Parma. Agricoltura Ricerca n 82. *Ismea*. Roma.

Schippmann, U., Leaman, D.J., Cunningham, A.B. (2002). Impact of cultivation and gathering of Medicinal Plants on biodiversity: global trends and issues. "Biodiversity and the ecosystem approach in Agriculture. Foresty and Fisheries". *FAO*. Rome: pp 21.

Sharma, S., Ramana Rao, T.V. (2013). Nutritional quality characteristics of pumpkin fruit as revealed by its biochemical analysis. *International Food Research Journal*, 20(5), 2309-2316.

Sonneveld, C., Straver, N. (1994). Nutrient solution for Vegetables and Flowers grown in water or substrates. Research Station for Floriculture and Glasshouse Vegetables, Aalsmeer/Naaldwijk, The Netherlands, Series: Voedingsoplossingen Glastuinbouw 8, 45.

Stepansky, A., Kovalski, I., Schaffer, A.A., Perl-Treves, A. (1999). Interspecific classification of melons (C. melo L.) in view of their phenotypic and molecular variation. *Plant Systematics and Evaluation*, 217, 313-332.

Stoughton-Ens, M.D., Hatcher, D.W., Warkentin, T.D. (2010). Influence of genotype and environment on the dietary fiber content of fiela pea (Pisum sativum L.) grown in Canada. *Food Research International*, 43, 547-552.

Sudhakar, P.S., Jagdish, A.K., Upadhyay, D.R., Mathura, R. (2003). Ascorbate and carotenoid content in an Indian collection of pumpkin (Cucurbita moschata Duch. ex Poir). *Cucurbit Genetics Cooperative Report*, 26, 51-53.

Sztangret J, Korzeniewska A, Horbowicz M, Niemirowicz-Szczyyy K (2004). Comparison of fruit yields and carotenoids content in new winter squash hybrids (*Cucurbita maxima* Duch.). *Vegetable Crops Research Bullettin*, 61, 51-60.

Taylor, M.J., Brant, J. (2002). Trends in world cucurbit production, 1991 to 2001. In: Maynard DN (ed), Cucurbitaceae. Alexandria, VA: ASHS Press:373-379.

Toppino, L., Barchi, L., Lo Scalzo, R., Palazzolo, E., Francese, G., Fibiani, M., D'Alessandro, A., Papa, V., Laudicina, V.A., Sabatino, L., Pulcini, L., Sala, T., Acciarri, N., Portis, E., Lanteri, S., Mennella, G., Rotino, G.L. (2016). Mapping Quantitative Trait Loci Affecting Biochemical and Morphological Fruit Properties in Eggplant (Solanum melongena L.). *Frontiers in Plant Science*, 7.doi: 10.3389/fpls.2016.00256.

Torunn, S., Remberg, S.F., Kare, A.L. (2009). Total antioxidant activity in 35 Ugandan fruits and vegetables. *Food Chemistry*, 113, 85-91.

Willem, J., Jones, J., McCleary, B., Topping, D. (2010). Dietary fibre: new frontiers for food and health. Wageningen Academin Publishers, Netherlands pp 583.