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Molecular and Biochemical Characterization of the Greek Pepper (*Capsicum annuum*) Cultivars 'Florinis' and 'Karatzova'

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Peppers are the fourth most important vegetable in the global food economy. Greek pepper cultivars 'Florinis' and 'Karatzova' are especially popular because of the signature red color and sweet taste of the fruits. The economic interest in 'Florinis' peppers has led to many adulteration events. In that aspect, genetic profiles of 'Florinis', a 'Florinis'-type and 'Karatzova' peppers, were studied using Inter Simple Sequence Repeats (ISSR) molecular markers and an automated fragment detection system. Biochemical parameters, such as total dietary fiber, total phenolic and lycopene contents, and sugar profile that affect the fruit organoleptic and nutritional properties were evaluated. The molecular protocol established during this study may successfully discriminate the original 'Florinis' cultivar from the 'Florinis'-type peppers. 'Karatzova' cultivar, which fruits are similar to 'Florinis', presented also a unique profile. The biochemical evaluation revealed that 'Florinis' peppers had the highest sweetness index and total phenolic content. Such an analysis could be used for the discrimination of pepper cultivars sharing common morphological traits ensuring the unique identity of each cultivar and protecting farmers and consumers from fraud.

ABBREVIATIONS

PDO – protected designation of origin; ISSR – inter simple sequence repeats; RFU – relative fluorescence units; PCA – principal component analysis; PIC – polymorphic information content; MI – marker index; RP – resolving power; d.w. – dry weight.

INTRODUCTION

Pepper, *Capsicum sp.*, belongs to the *Solanaceae* family together with potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), and tobacco (*Nicotiniana tabacum*). The *Capsicum* genus includes about 30 species, 5 of which are domesticated; *Capsicum annuum*, *Capsicum pubescens, Capsicum baccatum, Capsicum frutescens*, and *Capsicum chinense*. *Capsicum annuum* is the widest cultivated species of pepper and includes hot and sweet varieties. Peppers are popular, not only for their distinct taste and aroma but also for the health benefits they offer upon consumption [Bagetta *et al.*, 2020; Sinisgalli *et al.*, 2020; Thuphairo *et al.,* 2019; Yokoyama *et al.*, 2020].

Studies have shown that peppers are an excellent source of ascorbic acid and phenolic compounds such as phenolic acids and flavonoids [Marín *et al.*, 2004]. Phenolics have antioxidant, anti-inflammatory, and antimicrobial properties [Shotorbani *et al.*, 2013]. In addition to phenolics, red peppers are rich in carotenoids, which are also considered to have antioxidant, cancer risk-reducing, and immune response-enhancing properties [Hornero-Méndez *et al.*, 2002]. One of the red pepper's carotenoids, lycopene, serves as the precursor of β-carotene and as a substrate for the biosynthesis of other carotenoids [Gómez-García & Ochoa-Alejo, 2013]. Furthermore, capsaicin, responsible for the pungent, spicy taste of hot peppers, has been tested for the treatment of migraine, chronic cough, diabetes, and as potent analgesic, anti-inflammatory, and anti-carcinogenic agent [Malagarie-Cazenave *et al.*, 2009]. Sweet peppers contain the non-pungent ester isostere of capsaicin, capsiate, that maintains the anti-inflammatory properties [Macho *et al*., 2003] and the same bio-potency as capsaicin without the sensory irritation [Sasahara *et al.*, 2010].

In Greece, 10–15 pepper varieties are cultivated [Thanopoulos, 2008]; in Northern Greece cultivar 'Florinis' is grown in the area of Florina, Region of Western Macedonia, greatly contributing to the economic activity of local farmers. The cultivar is very popular all over Greece and is in the process of getting a protected designation of origin (PDO) certificate. In another area of the Region of Central Macedonia, Aridea, local farmers grow the pepper cultivar 'Karatzova' which shares many common fruit traits with 'Florinis' cultivar. Fruits of both cultivars have a bright red color upon matura-

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tion, shiny and smooth surface, and sweet taste, while their size varies at around 20 cm for 'Karatzova' and 12–14 cm for 'Florinis' [Thanopoulos, 2008]. In the area of Drama, a 'Florinis'-type pepper variety is cultivated, used mainly for canned products. In many cases, it is falsely branded under the name 'Florinis' as it shares many common fruit traits with these peppers, like the bright red color, the shape, and sweet taste. The 'Florinis'-type peppers have thicker skin, slightly bigger fruit, and offer greater yield, thus farmers choose to use them over original 'Florinis' peppers to gain extra profit.

The aim of this study was to establish a protocol for the genetic characterization of the cultivars 'Florinis' and 'Karatzova', as well as for the discrimination between original 'Florinis' and 'Florinis'-type peppers, using inter simple sequence repeats (ISSR) molecular markers. The identification of the fragments was performed using an automated capillary electrophoresis system, which is a novelty of this study. In order to obtain a unique identity of each cultivar, the results of the genetic analysis were correlated with quantitative biochemical parameters.

MATERIALS AND METHODS

Plant material

The pepper (*Capsicum annuum*) samples analyzed in this study included 60 samples of 'Karatzova' cultivar (KAR1 – KAR60), collected from 6 different fields from the area of Aridea, Central Macedonia, Greece; 40 samples of 'Florinis' (FL1-FL40) cultivar collected from 5 different fields from the area Florina, Western Macedonia, Greece and 27 samples of 'Florinis'-type (DR1-DR27) collected from 7 different fields from Drama, Eastern Macedonia, Greece. All pepper plants were planted in greenhouses in February, 2020; plantlets where moved in open fields in April, 2020; and samples for analysis were collected in October, 2020, when fruits had reached full maturation.

For the biochemical analysis, three replicates of a pool of mature fruits from five plants per field was used. For total phenolic and lycopene contents determination, the fruits were freeze-dried (LyoQuest, Telstar, Terrassa, Spain), and milled to a fine powder. For sugar and total dietary fiber analysis, a pool of peppers was milled to a fine pulp.

Preparation of genomic DNA and PCR amplification

DNA was extracted from young leaves and/or mature dried fruits of 'Karatzova', 'Florinis', and 'Florinis'-type peppers using the NucleoSpin Plant II DNA extraction kit (Macherey- Nagel, Duren, Germany). Prior to extraction, the tissue was mechanically lysed in TissueLyser (Qiagen, Hilden, Germany) using zirconia beads (Biospec, Bartlesville, OK, USA). Five primers were used for ISSR amplification: UBC807 [Sequence (5'-3'): $(AG)_8$T, Tm: 47°C], UBC825 [Sequence (5'-3'): $(AC)_8$T, Tm: 50°C] [Lijun & Xuexiao, 2012], UBC811 [Sequence (5'-3'): $(GA)_8$C, Tm: 47°C], UBC823 [Sequence (5'-3'): $(TC)_8$C, Tm: 47°C], and UBC810 [Sequence (5'-3'): $(GA)_8$T, Tm: 47°C]. ISSR amplification was performed in PCR reactions of a total volume of 20 μL using 1 unit of MyTaq DNA polymerase (Bioline, London, UK), 15 ng of DNA template, 0.6 μM primer, and the following cycling program: 95°C for 2 min, 40 cycles of 95°C for 15 s, 20 s at annealing temperature (Tm), 72°C for 1 min, and one final extension step at 72°C for 7 min on a Thermocycler (Thermo Fisher Scientific, Waltham, MA, USA). ISSR fragments were separated in the Fragment Analyzer 5200 (Agilent Technologies, Santa Clara, CA, USA) genetic analyzer using the DNF-915 dsDNA reagent kit (Agilent Technologies). The results were acquired using the software ProSize 3.0 (Agilent Technologies).

ISSR data analysis

ISSR sharp and discrete fragments with a minimum peak height of 500 relative fluorescent units (RFU) and peak width above 5 s, were scored as present (1) or absent (0), creating a binary matrix for downstream analysis. The matrix was then analyzed using the GeneAlex 6.5 software [Peakall & Smouse, 2012]. Principal component analysis (PCA) was performed using the covariance-standardized method, and Neighbor-Joining phylogenetic trees [Saitou & Nei, 1987] were constructed using MEGAX software [Tamura *et al.*, 2007]. The information content of each primer was calculated based on the formula $PIC_i = 2f_i\ (1 - f_i)$ [Roldán-Ruiz *et al.*, 2000], where PIC_i is the polymorphic information content of marker 'i', f_i is the frequency of the amplified allele (band present), and $1 - f_i$ is the frequency of the null allele (band absent). Marker index (MI), also used as a measure of the utility of the markers, was calculated as the product of PIC and EMR for the specific marker. EMR was defined as the product of the fraction of polymorphic loci (n_p) and the number of polymorphic loci for an individual assay, meaning $EMR = n_p(n_p/n)$ [Milbourne *et al.*, 1997]. The resolving power (RP) of each primer was calculated as $RP = \Sigma Ib$, where Ib represents the informative fragments as $Ib = 1 - (2 \times |0.5 - p_i|)$, where p_i is the proportion of accessions containing the *i*th band [Prevost & Wilkinson, 1999]. Nei's gene diversity (H) and Shannon information index (I) were calculated using the software PopGene32 [Yeh & Boylet, 1997]. The average number of different alleles (Na), effective number of alleles (Ne), Shannon information index (I), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) per locus were calculated using the GeneAlex 6.5 software [Peakall & Smouse, 2012].

Biochemical analysis

Total dietary fiber content of pepper fruits were measured with the Association of Official Analytical Chemists (AOAC) method 2009.01 [McCleary, 2007; McCleary *et al.*, 2010]. The results were expressed as g of dietary fiber per 100 g of fresh fruit.

Total phenolic content of the peppers was determined with a Folin-Denis reagent (Sigma–Aldrich, St. Louis, MO, USA) according to the method described by Lanza *et al.* [2010] with minor modifications. Briefly, 0.05 g of a dried fruit powder was extracted with 2 mL of methanol. The extraction was repeated five times and the extracts were combined. A small amount (50 μL) of the extract was mixed with an equal volume of the Folin-Denis reagent. In the mixture, 300 μL of a Na_2CO_3 saturated solution was added and brought to 1 mL volume with d.H_2O. The reaction was preceded for 60 min at room temperature. The solution was centrifuged

at 3000×*g* for 10 min and the absorbance of the supernatant was measured at 725 nm (UV-2600 spectrophotometer, Shimadzu, Kyoto, Japan). The quantification of total phenolics was determined by a calibration curve of caffeic acid (Fluorochem Ltd, Hadfield, U.K.) ranging from 20 to 100 μg/mL with a regression coefficient value (R) of 0.9982. Total phenolic contents were expressed as mg of caffeic acid equivalents (CAE) per g of dry weight (d.w.) of pepper fruits.

Lycopene content was measured with a modified method of Barrett *et al.* [2007]. Briefly, 0.05 g of a dried pepper powder was incubated in the dark for 1 h with occasional vortexing with 7.0 mL of a 4:3 (*v/v*) ethanol:hexane mixture. After 60 min, 1.0 mL of H_2O was added to each sample and shaken briefly. The samples were centrifuged at 3000×*g* for 2 min to allow a phase separation and dissipation of air bubbles. The organic layer was collected and the extraction was repeated with the addition of 3 mL of hexane. The extraction was repeated for 4 times. All hexane layers were combined, and the absorbance at 503 nm (A_{503}) was recorded. Lycopene content of pepper fruits d.w. was then calculated according to the following equation:

$$\text{Lycopene content (mg/g d.w.)} = \\ = (A_{503} \times 537 \times V_{extract})/(W_{sample} \times 172)$$

where: 537 M is the molecular weight of lycopene, $V_{extract}$ in mL is the volume of the hexane layer, W_{sample} in g is the weight of the extracted sample, and 172 1/(M·cm) is the extinction coefficient for lycopene in hexane.

Sugar analysis (glucose, fructose, saccharose) in pepper fruits was performed with a Dionex HPLC Ultimate 3000 equipped with a refractive index detector (Thermo Fisher Scientific), using a LiChrospher 100 NH_2 5 μm column (Sigma-Aldrich). The analysis was carried out according to Navarro *et al.* [2006] protocol. Briefly, a pool of peppers was milled to a fine pulp, centrifuged at 5000×*g* for 5 min, and in the supernatant the chromatographic determination of sugar profile was carried out with HPLC. Samples pretreatment was carried out according to Navarro *et al.* [2000]. The mobile phase was 85% (*v/v*) acetonitrile, with a flow rate of 0.9 mL/min. Solvent of HPLC grade was purchased from PanReac Applichem (Barcelona, Spain). Sweetness index was expressed as a sum of fructose and glucose.

Statistical analysis

For each chemical analysis triplicate measurements were conducted and data are expressed as mean value ± standard error (n=3). Statistical analysis was performed using paired t-test (GraphPad, San Diego, CA, USA).

RESULTS AND DISCUSSION

ISSR molecular markers have been widely used for the differentiation of *Capsicum* species and the genetic characterization of *Capsicum annuum* cultivars [Ibarra-Torres *et al.*, 2015; Lijun & Xuexiao, 2012; Tsaballa *et al.*, 2015]. The genetic analysis presented here resulted in a total of 53 amplicons in 127 pepper plants using 5 ISSR primers. To capture all information derived from ISSR fragments, the PCR products were analyzed in an automated capillary electrophoresis system. Studies have shown that the amount of data obtained using automated detection systems exceeds that obtained using the more conventional methods of agarose electrophoresis, eliminating also factors affecting the results originating from gel preparation, imaging and analysis as well as the subjectivity of each user at scoring [Bentley *et al.*, 2015].

To the best of our knowledge, this is the first time that ISSR bands were evaluated in a Fragment Analyzer system in plant studies, revealing an average of 10.6 bands amplified per primer. Primer UBC811 generated the maximum number of fragments (N=13) and primer UBC823 the lowest (N=8). The number of polymorphic loci where 31 out of 53, resulting in 58.5% mean percentage of polymorphic bands (Table 1). In fact, more alleles were scored in our research compared with the use of the same markers in other studies. For instance the use of UBC811 in other studies has resulted in 10 bands [Tsaballa *et al.*, 2015] or 6 bands [Hatami Maleki *et al.*, 2019], in contrast to 13 scored in our study. Moreover, Hatami Maleki *et al.* [2019] referred to 7 bands using the marker UBC823 and 6 using the UBC825, while in the present study these markers resulted

TABLE 1. Polymorphic information content and genetic diversity indices calculated for each primer used in the analysis of 'Florinis', 'Florinis'-type, and 'Karatzova' pepper cultivars.

Primer	No. of bands			%P[a]	Range (bp)	PIC[b]	MI[c]	RP[d]	H[e]	I[f]
	Total	Monomorphic	Polymorphic							
UBC807	12	4	8	66.7	202–833	0.13	0.71	21.1	0.15	0.24
UBC810	11	3	8	72.7	327–1159	0.18	1.05	18.66	0.20	0.30
UBC811	13	4	9	69.2	328–1615	0.12	0.73	22.48	0.15	0.25
UBC823	8	6	2	25.0	390–980	0.09	0.04	14.68	0.09	0.23
UBC825	9	5	4	44.4	369–1433	0.09	0.16	16.97	0.12	0.19
Total	53	22	31							
Average	10.6	4.4	6.2	58.5		0.12	0.54	18.75	0.14	0.22

[a]percentage of polymorphism; [b]polymorphic information content; [c]marker index; [d]resolving power; [e]Nei's gene diversity; [f]Shannon information index.

TABLE 2. Genetic diversity indices of pepper cultivars.

Cultivar	%P[a]	N[b]	Na[c]	Ne[d]	I[e]	He[f]	uHe[g]
'Karatzova'	18.87	58.64	6.75	6.81	0.66	0.45	0.48
'Florinis'	15.47	38.25	5.64	5.56	0.45	0.31	0.33
'Florinis'-type	4.31	23.70	6.83	7.20	0.17	0.12	0.14

[a]percentage of polymorphic loci; [b]sample size; [c]number of different alleles; [d]number of effective alleles; [e]Shannon information index; [f]expected heterozygosity; [g]unbiased expected heterozygosity.

in 8 and 9 bands, respectively. The percentage of polymorphic loci identified by the primers used in this study (58.5%) was lower than other relative studies in Greek *Capsicum annuum* landraces (83.6%) [Tsaballa *et al.*, 2015] or for the differentiation of pepper species (75%) [Lijun & Xuexiao, 2012]; however was sufficient for the discrimination of the cultivars of interest. The relatively small percentage of detected polymorphism indicates the narrow genetic pool of the tested samples.

Marker parameters regarding the information content and resolution power of each primer were calculated (Table 1). The mean PIC, MI, and RP values observed for all 5 primers were 0.12, 0.54, and 18.75, respectively. The primers that showed higher polymorphism had higher MI values, as expected [Najaphy *et al.*, 2011]. PIC reflects the discriminating ability of the marker and depends on the number of known alleles and their frequency distribution, thus representing genetic diversity. The average PIC value of the primers used in our study (0.12) was slightly lower than the PIC value of other studies, for instance regarding Bulgarian pepper cultivars (0.177) [Tsonev *et al.*, 2017] or Greek pepper cultivars (0.242) [Tsaballa *et al.*, 2015]. This suggests that there are few unique alleles detected by each primer, highlighting once again the genetic proximity of the cultivars under investigation.

Resolving power (RP) index indicates the number of genotypes identified by a primer; thus the highest value shows the most informative marker [Najaphy *et al.*, 2011]. In our study, the most informative markers for distinguishing the genotypes were UBC807, UBC811, and UBC810, exhibiting values of 21.1, 22.48, and 18.66, respectively. The average Nei's gene diversity index was 0.14 while the average Shannon index was 0.22. The highest Nei's gene diversity value was observed for the primer UBC810 (0.20), suggesting that this primer detected the most polymorphic loci, which is in agreement with the highest percent of polymorphism (%P), while the lowest was observed for primer UBC823 (0.09). The average number of alleles per locus (Na) was 1.585 and the effective number of alleles per locus (Ne) was 1.245.

Evaluation of genetic diversity indices of each cultivar were calculated for each field separately and then averaged per cultivar (Table 2). The highest percentage of polymorphic loci was observed for 'Karatzova' cultivar, followed by 'Florinis', while 'Florinis'-type samples had a very low percentage of this index. The same trend was observed for all the indices calculated. The higher the percentage of polymorphic loci, the higher the genetic diversity observed within a cultivar [Jiang & Liu, 2011]. Thus, 'Karatzova' cultivar appeared more genetically diverse while the samples from 'Florinis'-type cultivar started from a very narrow genetic base. This is also verified by the higher I and He value of 'Karatzova' samples.

Principal component analysis of the binary matrix for the 53 loci of *Capsicum annuum* showed that the two primary components accounted for 29.95% of total genetic variation (Figure 1). The analysis differentiated mostly the 'Katatzova' peppers which formed a tight cluster enclosing all samples of this cultivar analyzed. Although 'Florinis' peppers also formed a tight cluster enclosing the majority of 'Florinis' samples, a small nucleus, consisting of four samples, generated a different genetic profile, clustering away from the other

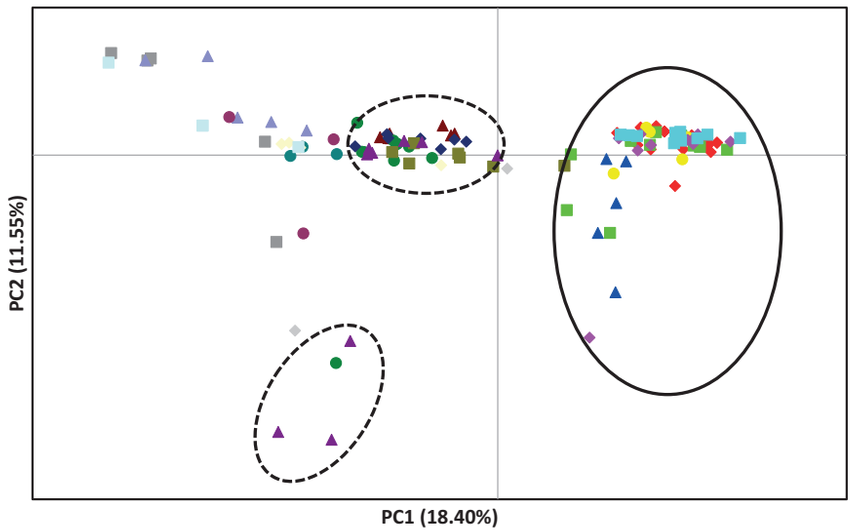

FIGURE 1. Principal component analysis based on the two first principal components derived from 53 bands amplified by five primers ISSR among 127 pepper samples. The samples in the black circle are 'Karatzova' peppers, those in the dashed circles are 'Florinis' peppers, and all the others are 'Florinis'-type samples.

TABLE 3. Sugar profile and contents of total dietary fiber, total phenolics, and lycopene of pepper cultivars.

Cultivar	Fructose (g/100 g fresh fruit)	Glucose (g/100 g fresh fruit)	Saccharose (g/100 g fresh fruit)	Total dietary fiber (g/100 g fresh fruit)	Total phenolic content (mg CAE/g d. w.)	Lycopene (mg/g d. w.)
'Karatzova'	1.78±0.16^{b}	1.36±0.20^{ab}	<0.5	2.20±0.13	1.11±0.18^{b}	1.14±0.27^{a}
'Florinis'	2.36±0.16^{a}	1.82±0.16^{a}	<0.5	2.93±0.53	7.26±0.39^{a}	0.33±0.06^{b}
'Florinis'-type	2.45±0.15^{a}	0.88±0.18^{b}	<0.5	2.04±0.16	7.48±0.25^{a}	0.60±0.08^{a}

Results are presented as means with their respective standard error (±SE). Different letters (a, b) in column indicate statistically significant differences ($p \leq 0.05$). CAE – caffeic acid equivalents; d.w. – dry weight.

samples. This could be an indicative that these are admixed samples and should not be included in 'Florinis' pepper breeding schemes. The PCA also highlights the genetic proximity of 'Florinis'-type peppers to 'Florinis' rather than 'Karatzova' cultivar. This confirms what was empirically known by some farmers that use them under the brand name 'Florinis'.

The phylogenetic tree constructed using the Neighbor-Joining method showed that the three cultivars formed distinct branches with the presence of some admixed samples (Figure 2). Three 'Florinis'-type samples, collected from two different populations, appeared genetically closer to 'Florinis' peppers than other plants of the same variety (DR4, DR5, DR13). *Vice versa*, the same was observed for one 'Florinis' sample (FL4). All the other samples clustered according to their cultivar. No particular grouping among populations (fields) of the same cultivar was observed.

Principal component analysis and phylogenetic tree highlighted the discriminatory power of the established protocol for the classification of 'Karatzova', original 'Florinis', and 'Florinis'-type samples. This is especially important for distinguishing between 'Florinis' and 'Florinis'-type as the latter is often branded under the PDO name 'Florinis' in canned products where it is difficult to identify it by the fruit morphology.

Since, the compositional quality and organoleptic properties of peppers have been shown to affect consumer preference [Bozokalfa & Eşiyok, 2011; Parisi *et al.*, 2017], the genetic characterization of the cultivars of interest was followed by the analysis of biochemical factors. Table 3 presents the results from the biochemical analysis including contents of total dietary fiber, total phenolics and lycopene, as well as sugar profile. The saccharose levels of all the samples were below 0.5 g/100 g of fresh fruit. Regarding the fructose values, 'Florinis'-type cultivar exhibited the highest value at 2.45 g/100 g of fresh fruit, slightly higher than 'Florinis' (2.36 g/100 g of fresh fruit), while 'Florinis' samples had higher glucose levels. As a result, 'Florinis' cultivar had the highest sweetness index, calculated as a sum of fructose and glucose, about 1.2-fold higher than the other two cultivars that were at the same level. 'Florinis' cultivar was also found rich in dietary fiber and phenolics, as along with 'Florinis'-type samples, exhibited 7-fold higher total phenolic content than 'Karatzova' samples. 'Karatzova' cultivar on the other hand, had the highest content of lycopene, 3.4 times higher than 'Florinis' that had the lowest.

The fact that 'Florinis' cultivar was characterized by high values in more than one biochemical trait was not a surprise as a similar phenomenon has been observed to Balkan cultivars [Nankar *et al.*, 2020]. Although there are several records on the biochemical traits or properties of various pepper cultivars of the Balkan region [Denev *et al.*, 2019; Nankar *et al.*, 2020], there is a limited number of studies about Greek peppers with emphasis on 'Florinis' cultivar [Niklis *et al.*, 2002; Papathanasiou *et al.*, 2020]. The high total phenolic content of 'Florinis' and 'Florinis'-type peppers was expected, as in full maturity the sweet peppers contain high amount of phenolic compounds [Papathanasiou *et al.*, 2020]. However, a direct comparison with previous studies might be controversial as either different measuring units were employed [Denev *et al.*, 2019; Papathanasiou *et al.*, 2020] or different cultivars were chosen [Denev *et al.*, 2019]. Nevertheless, considering that peppers contain 90–93% water, the values obtained in the present study are alike to previous measurements [Papathanasiou *et al.*, 2020]. Compared to other Balkan cultivars, 'Florinis' and 'Florinis'-type peppers had similar glucose contents to 'Pungent' and 'Sweet green' peppers [Denev *et al.*, 2019]. On the other hand, the contents of fructose were significantly higher to other Balkan varieties, while the high levels

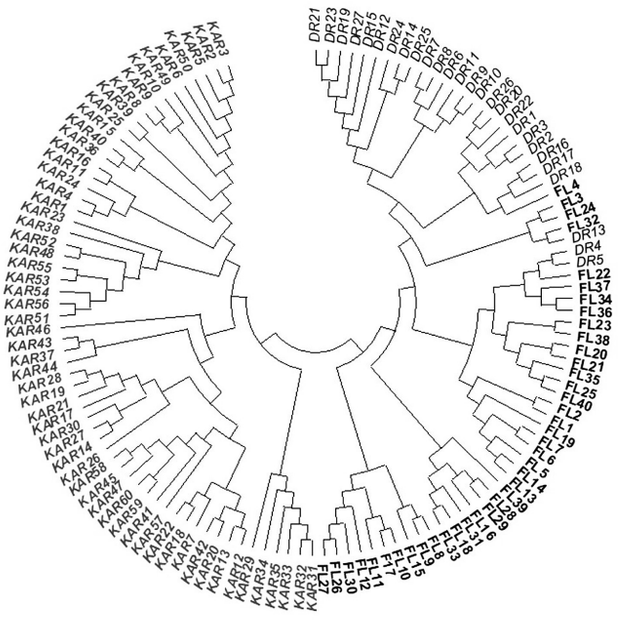

FIGURE 2. Neighbor-Joining phylogenetic tree representing the evolutionary relationships between the 127 pepper samples analyzed ('Karatzova' samples (KAR) in grey bold italic letters, 'Florinis' (FL) in black bold letters, 'Florinis'-type (DR) in black italic letters).

of total sugars resembled the values of 'Pumpkin' and 'Kapia' cultivars [Denev *et al.*, 2019]. Due to a lack of information regarding Greek cultivars, the biochemical parameters of total dietary fiber and lycopene content will be compared to other sweet peppers [Chávez-Mendoza *et al.*, 2015; Hernández--Carrión *et al.*, 2015]. Regarding total dietary fiber, the values of all three cultivars were significantly lower compared to red sweet Californian peppers [Hernández-Carrión *et al.*, 2015]. Furthermore, the lycopene contents of the three cultivars were lower than 'Sweet/Robusto' genotype that were harvested on September, still the fact that the sampling in the present study was carried out in October should be considered as it has been shown to influence the pigment content [Chávez-Mendoza *et al.*, 2015].

The biochemical profile of each cultivar underlined their differences in the sweetness value, the total dietary fiber, lycopene and total phenolic contents. 'Karatzova' and 'Florinis'--type cultivars are hard to distinguish based only on biochemical analysis, as their sweetness value and total dietary fiber content are similar and about 1–1.3-fold lower than 'Florinis' peppers. The quantification of both the total phenolics and lycopene level is important as studies have shown that the antioxidant properties of a plant are defined by multiple compounds [Chávez-Mendoza *et al.*, 2015]. It is well known that the biochemical properties of peppers are affected by various parameters, such as local environmental factors [Montalvo *et al.*, 2021], growth conditions [Ayodele *et al.*, 2015], and maturity stage [Bae *et al.*, 2014]. Therefore, in order to differentiate cultivars based solely on biochemical properties, it is important to cultivate them under identical conditions [Denev *et al.*, 2019; Nankar *et al.*, 2020]. However, the advantage of ISSR molecular markers is that they are not affected by such parameters, making the differentiation of phenotypically similar cultivars feasible.

CONCLUSIONS

Despite their broad presence in a human diet and their global economic importance, the taxonomic classification of *Capsicum* within and between species is still confusing. Until recently, their classification was based only on morphological traits of the fruit which requires effort and time for the full growth of the plant and is subject on the expertise of the agronomist. It is evident from this study that the cultivars 'Florinis' and 'Karatzova' can be characterized based on their DNA profile, following the established protocol, while the biochemical evaluation may further reinforce their identity. Moreover, it was shown that 'Florinis'-type peppers may be discriminated from the original cultivar, preventing consumers' fraud. The findings of this research project, the first one studying 'Florinis' and 'Karatzova' peppers combining genetic and biochemical methodologies, contribute to the effort made to monitor Greek pepper cultivars, not only as raw material but as processed food, throughout the food production chain. The developed protocols could be used in the protection of producers and consumers through the application of traceability systems that will protect PDO products and gain consumers' trust.

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CONFLICT OF INTERESTS

There is no conflict of interest to declare.

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