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Polyphenol content in apricot fruits

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ABSTRACT

Apricot (*Prunus armeniaca* L.) species is one of the most important Mediterranean fruits. The fruits are important in the diet of Asian and Mediterranean countries in which the apricot is used as fresh and dried fruit, being an important source of nutrients. Despite of the amount of genetic resources and diversity studies available into the species, there are a few studies focused on fruit quality. Among the different compounds of fruit quality, polyphenols are classified as the most abundant antioxidants in nature, being important as a source of health benefits as well as a potential source of natural products for the food industry. The important role of polyphenols in human nutrition, outline these compounds as the most relevant for defining fruit quality. In this study, the polyphenol content on fruits from different apricot varieties included elite cultivars and hybrids from the IVIA breeding program have been compared for identifying the genotypes with relevant contribution to fruit quality. The most important compounds obtained in terms of quantity were: phenolic acids and flavonoids. Results identified the PPV resistant cultivar 'Goldrich' as the best cultivar for increasing the content of antioxidants in the varieties of the breeding program.

1. Introduction

Apricot (*Prunus armeniaca* L.) species is one of the most important Mediterranean fruits. Its center of origin is located in China and later it spread to Europe and the rest of Asian countries generating different ecological diversification centers in which the Mediterranean basin is one of them (Bailey and Hough, 1975). The long domestication history provided a wide genetic diversity in pomological characteristics and adaptability to different environments. The fruits are important in the diet of Asian countries in which the apricot is used as fresh and dried fruit, being an important source of sugar. Despite the genetic diversity of apricot species has been very well studied (Martínez-Mora et al., 2009; Romero et al., 2003; Wang et al., 2014) there are few studies focused on compounds related to fruit quality (Camps and Christen, 2009; Socquet-Juglard et al., 2013; Ruiz et al., 2005). Among the different compounds polyphenols are one of the most important as a source of health benefits as well as a potential source of natural products for the food industry. Polyphenols represent a group of chemical substances common in plants being the different parts of the plants the main provider of these important compounds in the human diets. Polyphenols are positively correlated with antioxidant capacity of fruits (Almeida et al., 2011; Gan et al., 2016; Mokrani et al., 2016). Hence, one of the most important benefits of fruit consumption is attributed to their high antioxidant content. Research studies supports the role of antioxidants in the prevention of several diseases (Ginter and Simko, 2012; Manach et al., 2005; Rodriguez-Mateos et al., 2014; Scalbert et al., 2005).

The involvement of reactive oxygen species (ROS) in the etiology of many diseases suggested that phytochemicals showing antioxidant activity may contribute to the prevention of these pathologies. In this sense polyphenols provide health benefits by elimination of free radicals, by the protection and regeneration of other dietary antioxidants (e.g. vitamin E) and the chelation of pro-oxidant metals (Lima et al., 2014). Their antioxidant potential provides other health benefits reported such as an antimutagenic activity, reduction of the risk of cardiovascular diseases, atherosclerosis protection (Yao et al., 2004). Dietary polyphenols contribute to epigenetic changes at cell level and have emerged as potential drugs for therapeutic uses.

In the food industry, preservation of food requires the addition of antioxidant compounds. Some plant extracts may represent an alternative source of natural antioxidants, that can be included in the human diet of being an important source for synthesis of these compounds as natural additives of the food industry. Polyphenol concentrations in foods vary according to numerous genetics and environmental factors (Manach et al., 2004). Differences on polyphenol content among cultivars from different species have been reported, pointing out the genetic diversity (Andre et al., 2007; Tabart et al., 2006). In temperate fruit

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crops, polyphenol content is relevant and arise as one of the main contributor to fruit quality (Veberic and Stampar, 2005). Polyphenol content and antioxidant activity of fruits have been very well referenced (Wolfe et al., 2003). For instance, the role on health benefits of phenolic compounds from apple was studied by Boyer and Liu (2004). The polyphenolic content varied among apple cultivars, remaining relatively stable during cold storage (Matthes and Schmitz-Eiberger, 2009) being an important feature for apple consumption. The studies of polyphenols in stone fruits are scarce and focused on antioxidant capacity, in nectarines and plums (Gil et al., 2002; Kim et al., 2003) and apricot (Erdogan-Orhan and Kartal, 2011; Fan et al., 2018). Besides of the antioxidant capacity, polyphenols fruit content are becoming an important component of fruit quality because affect the color, flavor and taste of the fruits, impacting the fruit consumption (Crisosto, 2003).

Polyphenols have been related to colour of fruits and anthocyanin accumulation (Jin et al., 2016; Luo et al., 2016). Several genes have been identified in the metabolic pathways, such as dihydroflavonol 4-reductase *(DFR)* and flavonol synthase (*FLS*), associated with anthocyanin pathway. On the other hand, in *Prunus* genus, *MYB10* gene has been proposed as the best candidate for skin colour in peach (Jiao et al., 2014; Rahim et al., 2014; Tuan et al., 2015) and apricot fruit (García-Gómez et al., 2019). In addition, some candidate genes have been reported for skin pigmentation in peach, such as a beta-carotene hydroxylase (*BCH*), a zeaxanthin epoxidase (*ZXE2*) and a leucoanthocyanidin dioxygenase (*PpLDOX*) (Ogundiwin et al., 2009, 2008). All the genes identified in the polyphenols pathways represent new strategies for increasing fruit quality by means of conventional and molecular breeding.

The important role of polyphenols in different plant mechanisms as well as their increasing importance in human nutrition, outline these compounds as the most relevant for defining fruit quality. In apricot the outbreak of the sharka diseases caused by the plum pox virus or PPV (García et al., 2014), point out the need of introgression of resistance as the unique solution. Only a few cultivars from the Ontario region of Canada were identified as resistance to PPV ((Soriano et al., 2012). Apricot as a temperate fruit crop needs to accomplish an amount of chilling during winter for spring budbreak. The resistant cultivars available have high chilling requirements. This mechanism of adaptability gathered during evolution results in bad adaptability to warmer winters as those of the Mediterranean area. Beside of the bad adaptability, the resistant cultivars provided other inconvenient characteristics as floral self-incompatibility and worse fruit quality. The introgression of resistance to PPV in apricot may have important consequences in the new obtained resistant cultivar as compromised adaptability and worse fruit quality.

Our hypothesis is that among the group of cultivars resistant to PPV, 'Goldrich' is the better adapted to the Mediterranean conditions. This cultivar has been used as the main donor of resistance in the IVIA breeding program(Badenes et al., 2018). In this study, we test the potential effect on fruit quality of the main donor of resistance to PPV and their suitability for increasing fruit quality in the program. Due to the important role of polyphenols in fruit quality we focused the study in these compounds. The relationship between phenolic components and the genotypes and structure of the data were analyzed using principal component analysis (PCA).

The study presents and compares the polyphenol content on fruits from different apricot varieties that included the main donor of resistance to PPV, traditional varieties adapted to the Mediterranean and the first generation of hybrids from the IVIA breeding program aimed at identifying the best genitors for increasing the content of antioxidants in the elite varieties.

2. Material and methods

2.1. Plant material

The plant material consisted in a set of cultivar and selections from the IVIA's breeding program (Badenes et al., 2006; Martínez-Calvo et al., 2009) that aims to obtain new varieties resistant to PPV (plum pox virus) the most important disease affecting *Prunus* genus species worldwide (Garcia and Cambra, 2007; García et al., 2014). A set of 4 well-known cultivars (group 1) and 9 selections (group 2) from the IVIA's breeding program were analysed (Table 1). First group includes' Canino', 'Mitger' and 'Tadeo', all three cultivars from the Mediterranean Basin, and 'Goldrich' a variety from North America, used as a donor of resistance to PPV. Second group includes 2 cultivars already registered 'Dama Rosa' and 'Dama Taronja' and other 7 preselected accessions All of them are selected seedlings resistant to PPV and self-compatible. The trees are maintained at the IVIA's germplasm collection located in Moncada (latitude 37°45′31.5″ N., longitude 1°01′35.1″ W.), near Valencia (Spain). The genotypes were characterized for agronomic and pomology traits for further selection. The pomological characterization of the genotypes studied was made following Martínez-Calvo et al. (2010). Variables related to fruit size and firmness were indicated in Table 2.

For polyphenols analysis, five fruits per tree were harvested at the ripening stage during 3 growing seasons (2016, 2017 and 2018). For each fruit, the peel was separated from the flesh with a peeler. Two samples consisted in a mix of the peel from 5 fruits and a mix of flesh from 5 fruits per genotype and crop year were frozen with liquid nitrogen and kept at −80 °C until processing. Tissue homogenization was carried out using a Polytrom 3100 (Kinematica AG, Switzerland) and a vortex for the flesh and peel samples, respectively.

2.2. Extraction and HPLC of phenolic compounds

Phenolics were extracted and determined according to the procedure described by Cano et al. (2008) and Cano and Bermejo (2011). Briefly, 5 mg of freeze-dried peel or flesh were mixed with 1 mL of DMSO/MeOH (1:1, v/v). Then the sample was centrifuged (Eppendorf 5810R centrifuge; Eppendorf Iberica, Madrid, Spain) at 4 °C for 20 min at 8.050×g. The supernatant was filtered through a 0.45 μm nylon filter and analysed by HPLC-DAD and HPLC-MS in a reverse-phase column C18 Tracer Excel 5 μm 120 OSDB (250 mm x 4.6 mm) (Teknokroma, Barcelona, Spain). An Alliance liquid chromatographic system (Waters, Barcelona, Spain) equipped with a 2695 separation module, coupled to a 2996 photodiode array detector and a ZQ2000 mass detector was used. A gradient mobile phase consisting of acetonitrile (solvent A) and 0.6 % acetic acid (solvent B) was used at a flow rate of 1 mL/min, with an injection volume of 10 μL. The gradient change was as follows: 10 % 2 min, 10–75 % 28 min, 75−10% 1 min, and hold at 10 % 5 min. An HPLC-MS analysis was performed and worked under electrospray ion positive (flavonoids) and negative (phenolic acids) conditions. Capillary voltage was 3.50 kV, cone voltage was 20 V, source temperature was 100 °C, desolvation temperature was 225 °C, cone gas flow was 70 L/h

Table 1
Plant material.

Genotype	Pedigree	Harvest date			
		Origin	2016	2017	2018
Canino	Unknown	Spain	June 3	May31	June11
Dama Rosa	Goldrich x Ginesta	IVIA	June 6	June 9	June 7
Dama Taronja	Goldrich x Katy	IVIA	June 10	June 9	June11
GG9310	Goldrich x Ginesta	IVIA	June 6	June 9	June 5
GG979	Goldrich x Ginesta	IVIA	June 13	June 9	June14
Goldrich	Sunglo x Perfection	USA	June 22	June 9	June11
GP9817	Goldrich x Palau	IVIA	June 13	June 9	June11
HG9821	Harcot X Ginesta	IVIA	June 8	May25	June 5
HG9850	Harcot x Ginesta	IVIA	June 3	May25	June 7
HM964	Harcot x Mitger	IVIA	June 1	June 2	May30
Mitger	Unknown	Spain	June 3	May25	May30
SEOP934	Seo x Palau	IVIA	June 8	June 2	June 5
Tadeo	Unknown	Spain	June 15	June 9	June18

Table 2
Pomological traits measured in the genotypes studied related to fruit size and firmness. *3-years average ± standard deviation*. Different letter means significative differences among genotypes.

Genotype	Height(mm)	Diameter (mm)	Ratio $\frac{\text{Height}}{\text{ventral width}}$	Weight (g)	Weight (stone)(g)	Ratio $\frac{\text{weight(fruit)}}{\text{weight(stone)}}$	Firmness (kgf/cm^2)
Canino	44.9 ± 6.9 def	45.9 ± 7.9 b	1.3 ± 0.3 ef	61.4 ± 21.5 d	3.5 ± 0.4 fg	17.2 ± 4.9 abc	2.8 ± 1.7 cde
Dama Rosa	41.7 ± 2.1 bcd	46.5 ± 2.3 b	1.1 ± 0.1 bc	49.3 ± 6.5 bc	3.2 ± 0.2 def	15.7 ± 2.6 a	1.5 ± 0.5 abc
Dama Taronja	52.5 ± 5.6 h	52.5 ± 6.4 d	1.6 ± 0.3 g	85.5 ± 25.2 f	5.5 ± 1.4 h	16.2 ± 6.3 ab	1.5 ± 1.4 abc
GG9310	43.1 ± 3.8 cde	46.8 ± 4.7 b	1.2 ± 0.2 cde	57.8 ± 13.3 bcd	2.7 ± 0.4 bcd	21.4 ± 4.0 d	0.6 ± 0.3 a
GG979	46.0 ± 5.1 efg	50.8 ± 6.5 cd	1.4 ± 0.2 f	73.4 ± 18.7 e	3.8 ± 0.7 g	19.4 ± 4.1 bcd	1.1 ± 0.6 ab
Goldrich	49.2 ± 4.0 gh	46.9 ± 3.2 b	1.3 ± 0.1 ef	60.6 ± 10.8 cd	3.8 ± 0.5 g	16.4 ± 4.0 abc	2.2 ± 1.4 bcde
GP9817	41.9 ± 3.5 bcd	48.5 ± 4.0 bc	1.1 ± 0.2 bcd	54.5 ± 13.1 bcd	3.2 ± 0.5 ef	17.2 ± 2.8 abc	1.5 ± 1.2 ab
HG9821	47.4 ± 3.4 fg	53.4 ± 4.7 d	1.4 ± 0.2 fg	77.1 ± 12.4 ef	3.1 ± 0.5 cde	25.7 ± 5.1 e	2.9 ± 3.4 de
HG9850	43.6 ± 2.9 cde	47.8 ± 3.1 bc	1.3 ± 0.2 de	60.2 ± 12.2 cd	3.0 ± 0.5 bcde	20.5 ± 3.8 d	3.1 ± 2.7 e
HM964	37.5 ± 4.2 a	45.4 ± 4.5 b	1.0 ± 0.2 b	48.4 ± 15.7 b	2.6 ± 0.3 bc	19.1 ± 5.3 abcd	1.7 ± 0.9 abcd
Mitger	42.3 ± 3.5 cd	46.8 ± 4.7 b	1.1 ± 0.2 bc	51.7 ± 15.6 bcd	2.6 ± 0.4 bc	19.6 ± 4.6 cd	2.9 ± 1.3 de
SEOP934	38.9 ± 3.0 ab	47.2 ± 1.9 bc	1.1 ± 0.1 bcd	52.7 ± 5.3 bcd	2.6 ± 0.3 b	20.5 ± 2.2 d	1.0 ± 0.6 ab
Tadeo	36.8 ± 2.9 a	40.1 ± 3.3 a	0.8 ± 0.1 a	33.0 ± 8.5 a	1.6 ± 0.3 a	20.8 ± 4.0 d	3.1 ± 1.2 e

and desolvation gas flow was 500 L/h. Full data acquisition was performed by scanning 200–800 uma in the centroid mode. Compounds were identified on the basis of comparing their retention times, UV–vis spectra and mass spectrum data with authentic standards from Sigma-Aldrich using an external calibration curve. All the solvents used were of LC–MS grade. Three samples per cultivar were analysed.

2.3. Data analysis

All the data analysis and graphics were made using R-studio software (Version1.1.463, 2009–2018, Rstudio, Inc.) with '*stats', grDevices'*, and *'graphics'* (R Core Team), '*dplyr*' (Wickham, et al., 2020), *'readxl'* (Wickham, et al., 2019)), '*plyr'* (Wickham, 2020), '*scales'* (Wickham and Seidel, 2019), '*grid'* (Murrell, 2005), '*ggbiplot'* (Vu, 2011.), 'FSA' (Ogle et al., 2020), 'DescTools' (Signorell, et al., 2020), 'rcompanion' (Mangiafico, 2020), 'multcompView' (Graves, et al., 2019), and '*ggplot2'* (Wickham, 2016) packages.

Polyphenol content from all compounds and accessions were statistically tested by Kruskal-Wallis test ($P \leq 0.05$) and averages were compared with the Pairwise Wilcoxon-Mann-Whitney test at 95 % confidence level ($P \leq 0.05$), using the Statgraphics XVI.I software (Statpoint Technologies, Warrenton, VA, USA). Significant different samples were labeled with different letters. Data of the accessions were analysed by multivariate analysis, applying the method of Principal Components Analysis (PCA) (Eriksson et al., 1999). PCA and correlogram were carried out using R (v.3.6.1, R Core Team, 2019) with R-studio software (v.3.5.3) with the '*stats' (*R Core Team), '*ggplot2'* (Wickham, 2016), '*GGally*' (Schloerke, et al., 2020), '*dplyr*' (Wickham, et al., 2020), and '*factoextra'* (Kassambara, 2020). Previously, data was centred and scaled to have unit variance. The variables included were the compounds analyzed. A biplot of individual scores and loadings was obtained.

For testing the contribution of 'Goldrich' to the parameters of quality in the studied population, we performed a regression of the data to a linear model as described by Gómez and Ligarreto (2012). In the model, the phenotype is linearly explained as follows:

$$[Phenotype = C + G_{Goldrich} + Year + G_{Goldrich*}\ Year + Residual]$$

Where C is the general average of the population (constant), $G_{Goldrich}$ is the genetic effect of 'Goldrich', *Year* is the environmental effect due to the year and *Residual* is the residual effect.

The model was calculated using the Statgraphics XVI.I software (Statpoint Technologies, Warrenton, VA, USA). A quantitative variable for evaluating the genetic effect of 'Goldrich' was included with a value of 1 for 'Goldrich', 0.5 value for 'Goldrich x X' hybrids and null value for the other genotypes non-related to 'Goldrich'. Model parameters were estimated with a 95 % confidence level ($P \leq 0.05$).

3. Results

3.1. Total polyphenols content

The polyphenol content in plants varies depending on the part of the plant and the tissue. In the first year of the study, we analysed the polyphenol content on flesh and peel. Results showed the content in peel was about 8–10 fold than flesh (Fig. 1). From the results obtained, in the next crop years the analysis was focused on peel, since there is the main contributor on polyphenols of the fruit. Taking into account that fresh and dried apricots are consumed with peel, this is the part of the fruit most important for assessing antioxidant capacity.

The total polyphenol content of the varieties and selections studied varied among genotypes and years (Table 3 and Fig. S1). Interestingly, the variety 'Goldrich', used in the breeding program as donor of resistance to PPV, has the highest content of total polyphenols, followed by 'Dama Rosa', a seedling from 'Goldrich' registered from the program and characterized by more than 80 % of red blush peel. Both varieties showed an average of total polyphenols higher than 850 mg/100 g DW. A second group with more than 700 mg/100 DW on average included the variety 'Canino' and the hybrids GG9310, GP9817, both seedlings from 'Goldrich' and the hybrids SEOP934 and HM966, this group resulted very rich in polyphenols. The year effect was relevant in the total content of polyphenols being the 3rd year the one in which the content was lower in 70 % of the varieties studied (Table S1)

3.2. Polyphenols compounds

Fruits present complex mixtures of polyphenols. The phenolics substances in fruits are mainly phenolic acids and flavonoids. The most important compounds obtained in terms of quantity were: neochlorogenic acid, chlorogenic acid and flavonoids, as rutin and quecertin-3 glucuronide.

3.2.1. Neochlorogenic acid

Neochlorogenic acid concentration results revealed significant differences among accessions (Table 3). 'Goldrich' showed one of the highest concentrations on average and during the three years of sampling. The accessions with higher neochlorogenic acid content were the same that those with maximum polyphenol content. Neochlorogenic acid is one of the most relevant components of the total polyphenols according to quantity, being the most contributors to the polyphenol content in apricot. Neochlorogenic concentration within accessions was year dependent. A trend observed was a general lower concentration in all genotypes during the crop year 2018. Only 2 hybrids, HG9821 and HG9850 present the lowest content in 2016 year. Both hybrids are siblings from the same cross (Table S2)

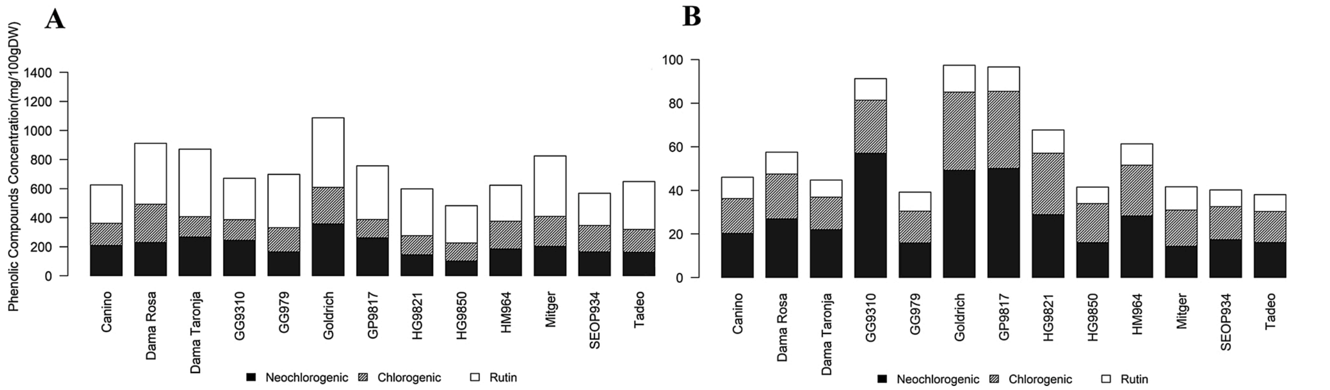

Fig. 1. Polyphenol compounds concentration: Neochlorogenic acid, chlorogenic acid and Rutin,. Data from 2016. A) Concentration in peel. B) Concentration in flesh.

Table 3
Phenolic compounds: Neochlorogenic, chlorogenic, rutine and quercetin-3-glucuronide. *3-years average* ± *standard deviation*. Different letter means significative differences among genotypes.

Genotype	Neochlorogenic acid	Chlorogenic acid	Rutin	Quercetin-3-glucuronide
Canino	174.43 ± 53.13 abc	110.28 ± 38.94 a	420.16 ± 238.55 a	73.34 ± 13.62a
Dama Rosa	242.59 ± 68.12 bcd	264.24 ± 117.33 b	316.02 ± 134.03 a	57.26 ± 22.74 a
Dama Taronja	216.28 ± 77.50 abcd	166.22 ± 96.88 ab	257.27 ± 141.18 a	75.06 ± 41.35 a
GG9310	278.97 ± 44.54 cd	131.47 ± 15.22 a	324.27 ± 140.95 a	53.53 ± 17.59 a
GG979	160.31 ± 19.75 ab	165.08 ± 31.40 ab	241.45 ± 134.84 a	51.14 ± 25.47 a
Goldrich	297.43 ± 111.09 d	263.97 ± 109.64 b	388.92 ± 85.30 a	79.11 ± 26.37 a
GP9817	236.79 ± 73.99 bcd	175.80 ± 84.08 ab	293.97 ± 67.30 a	48.33 ± 16.59 a
HG9821	162.66 ± 16.52 ab	126.27 ± 31.78 a	289.51 ± 117.55 a	53.17 ± 25.79 a
HG9850	110.92 ± 8.69 a	130.46 ± 11.03 a	212.63 ± 52.60 a	33.60 ± 30.95 a
HM964	237.58 ± 109.86 bcd	203.72 ± 92.04 ab	243.43 ± 46.39 a	60.71 ± 12.51 a
Mitger	164.16 ± 38.00 ab	134.59 ± 61.62 a	268.43 ± 130.97 a	53.18 ± 23.73 a
SEOP934	207.65 ± 88.31 abcd	224.15 ± 107.90 ab	255.74 ± 71.70 a	78.35 ± 58.47 a
Tadeo	139.32 ± 20.76 ab	123.23 ± 29.97 a	375.03 ± 127.49 a	71.13 ± 12.35 a

3.2.2. Chlorogenic acid

Results of chlorogenic acid content average of the three crop years studied ranged between 110–277 mg/100 g DW (Table 3). The variety ‘Goldrich’ shows the maximum content. The variety ‘Dama Rosa’ and the hybrids GP9817, HM964 and SEOP934 showed content higher of 200 mg/100 g DW. Results into the different crop years showed differences among varieties and a similar trend than the observed in neochlorogenic acid (Table S3). The crop year 2018 resulted in the lower content of the 3 crop years studied in most of the varieties, except two hybrids HG9821 and HG9850, similarly to the results on neochlorogenic content.

3.2.3. Rutin

Results of rutin from the 3 crop years showed the variety ‘Canino’ a traditional Mediterranean variety, with the highest content on average. The varieties in which the content was higher than 300 mg/100 g DW were ‘Goldrich’, ‘Dama Rosa’ and ‘Tadeo’. Rutin concentration was no year-dependent (Table3, Table S4). The trend detected of lower phenolic acids content in 2018 crop year was not observed in the content of rutin.

3.2.4. Quercetin-3-glucuronide

Results of quercetin-3-glucuronide average content in the three crop years analysed ranged between 33, 7–78,6 from the hybrid HG9850 and ‘Goldrich’ respectively (Table 3). On the other hand, no significant differences were detected among years. The variety ‘Goldrich’ is one of the varieties with higher content among the set during the 3 crop years, which indicates it can be good parental for increasing the content of this compound in apricot by breeding.

3.3. Principal components analysis

Principal components analysis (PCA) was performed. (Table 4). Data revealed that 81.78 % of variance was explained by the two first principal components. All the studied variables had positive scores for PC1. Distribution of varieties and hybrids studied plotted in the space of the first two PC is showed in Fig. 2. The accessions with higher polyphenol acid content are located in the positive scores of PC1 and negative of PC2. The variety with higher scores is ‘Goldrich’ which indicates that might be a good candidate for increasing the polyphenols acids in a breeding program. On the other hand, the content of polyphenols from the flavonoid group (rutin and quercetin-3-glucuronide) has positive values in PC1 and PC2. The varieties with higher PC2 scores are two traditional varieties well known ‘Canino’ and ‘Tadeo’.

Table 4
Variable contribution to Principal Components, eigenvalues, and cumulative variance in the PCA.

Variable	PCA			
	PC1	PC2	PC3	PC4
Neochlorogenic acid	0.55	−0.40	0.35	0.64
Chlorogenic acid	0.47	−0.55	−0.52	−0.46
Rutin	0.42	0.62	−0.57	0.34
Quercetin-3-glucuronide	0.54	0.40	0.53	−0.51
Eigenvalue	2.06	1.22	0.40	0.32
Variance(%)	51.38	30.40	10.10	8.12
Cumulative Variance(%)	51.38	81.78	91.88	100.00

3.4. Contribution of the resistant cultivar ‘Goldrich’ to the quality traits studied

In the frame of the breeding program all the genotypes studied were characterised according to the main pomological characteristics during the procedure of selection. Among the pomological traits we selected

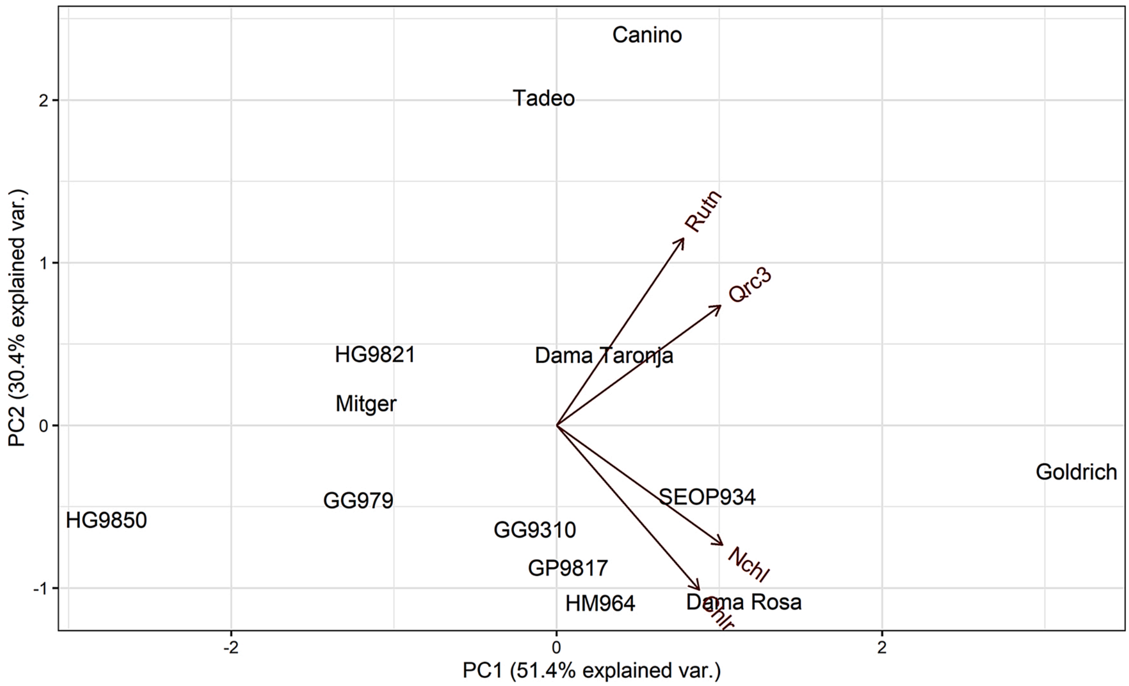

Fig. 2. Plot of the variables studied and accessions in the space defined by the two first PC.

size and firmness of the fruit as traits that contribute to the quality. Table 5 indicates the obtained coefficients of the linear model related to the contribution of 'Goldrich' in the variables studied, being C: general average (constant); $G_{Goldrich}$: genetic main effect by 'Goldrich'. and the relative effect $G_{Goldrich}$/C.

Among the phenolic compounds, neochlorogenic and chlorogenic acids showed a significant genetic effect of 'Goldrich' (contribution of 25 and 12.2 % of total sum of squares, respectively). However, non-significative contribution was observed in rutin and quercetin-3-glucuronide. The linear model coefficients were calculated for 'Goldrich' genetic effect in the accumulation of the studied phenolic compounds (Table 6). The value for neochlorogenic was 121.8 mg/100 (71 % of general average) and for chlorogenic acid 92.6 mg/100 gDW (63.5 % of general average) These results indicate an important contribution of this variety to these polyphenol acids.

In pomological traits related to size and weight of the fruit, the genetic contribution of 'Goldrich' was significant as well (Tables 5 and 6). However, the contribution in firmness is negative, being −1.4 kgf/cm^2 (57 % less of general average). This result indicates that 'Goldrich' might decrease the firmness of the fruits in the progenies.

4. Discussion

4.1. Total polyphenol content

Recent studies pointed out the antioxidant content of fruits as one of the main attributes to promote fruit consumption. Breeding for fruit quality should take into account the increase of those compounds with antioxidant activity.

Several studies shown phenolic compounds distribution depends on tissues, being higher in peel than in pulp (Campbell and Padilla-Zakour, 2013). In fruits polyphenols have been located in flesh and peel. In many fruits analysed the content in peel is higher than in flesh. In the present study the content of all compounds analysed was more than 10 fold in peel than in flesh, in agreement with results in other studies focused in plum, peach and apricot (Veberic and Stampar, 2005). This fact has been explained because of their role in defence against ultraviolet radiation, protection in front of pathogens and environmental stress (Manach

Table 5
General Linear Model for phenolic compounds and pomological traits to test the Goldrich effect and interaction. SS_i: Sum of Squares; SS relative: SS_i/SS_{total}; *Year*: environmental effect due to the year; $G_{Goldrich}$: genetic main effect of Goldrich; Year x $G_{Goldrich}$: interaction; *Residual*: residual effect; R^2: variance explained by the model.

Parameter	Year		$G_{Goldrich}$		Year x $G_{Goldrich}$		Residual		SS_{total}	R^2
	SS_Y	SS relative	SS_G	SS_G relative	$SS_{Y x G}$	SS_{YxG} relative	SS_R	SS_R relative		
Neochlorogenic	50,558**	0.073	174,553**	0.250	32397.6**	0.046	316,370	0.454	696,869	0.546
Chlorogenic	30924.2*	0.037	101,004**	0.122	71525.1**	0.086	473,383	0.571	828,517	0.428
Rutin	4083.31 NS	0.002	61965.9 NS	0.028	68295.8 NS	0.031	$1.98{\cdot}10^6$	0.900	$2.20{\cdot}10^6$	0.100
Quercetin-3-glucurunide	14236.6**	0.164	594.246 NS	0.007	1772.51 NS	0.020	54330.4	0.627	86715.6	0.373
Height (mm)	472.148**	0.085	628.233**	0.113	11.936 NS	0.00214	3951.410	0.709	5572.190	0.291
Diameter(mm)	667.365**	0.133	31.518 NS	0.006	67.731NS	0.01346	3578.150	0.711	5032.970	0.289
Ratio $\frac{\text{Height}}{\text{Diameter}}$	1341**	0104	0338*	0026	0055 NS	0004	9909	0770	12,870	0230
Weight (fruit) (g)	6867.690**	0.113	1044.740 NS	0.017	557.715 NS	0.00918	44907.600	0.739	60771.600	0.261
Weight(stone) (g)	3.094 NS	0.018	26.946**	0.157	1.733 NS	0.01009	132.377	0.771	171.720	0.229
Ratio $\frac{\text{weight(fruit)}}{\text{weight(stone)}}$	379.157**	0.102	392.408**	0.106	2.493NS	0.00067	2787.390	0.750	3716.600	0.250
Firmness (kgf/cm^2)	85.560**	0.173	29.321**	0.059	13.592 NS	0.02752	359.382	0.728	493.834	0.272

* Significant differences ($P \leq 0.05$); **Significant differences($P \leq 0.01$); NS: non-significant.

Table 6
Variables studied and Goldrich contribution. C: General average value of the population studied. $\mathbf{G_{Goldrich}}$: Goldrich contribution. $\mathbf{G_{Goldrich}}$ **relative** : Relative contribution of Goldrich to the general average. Confidence intervals at 95 %.

Parameter	C	$G_{Goldrich}$	$G_{Goldrich}$ relative
Neochlogenic	170.2 ± 12.8	121.8 ± 30.8 **	0.72
Chlorogenic	145.8 ± 15.7	92.6 ± 37.7**	0.64
Rutin	284.6 ± 32.1	72.6 ± 77.1	0.25
Quercetin-3-glucurunide	58.7 ± 5.3	7.1 ± 12.8	0.12
Height (mm)	41.5 ± 1.0	6.3 ± 2.5**	0.15
Diameter(mm)	47.0 ± 1.0	1.4 ± 2.4 NS	0.03
Ratio $\frac{\text{Height}}{\text{Diameter}}$	1.2 ± 0.1	0.1 ± 0.1*	0.13
Weight (fruit) (g)	55.6 ± 3.4	8.2 ± 8.4 NS	0.15
Weight(stone) (g)	2.8 ± 0.2	1.3 ± 0.5**	0.48
Ratio $\frac{\text{weight(fruit)}}{\text{weight(stone)}}$	20.4 ± 0.9	−5.1 ± 2.2**	−0.25
Firmness (kgf/cm²)	2.5 ± 0.3	−1.4 ± 0.8**	−0.57

* Significant differences ($P \leq 0.05$); **Significant differences($P \leq 0.01$); NS: non-significant parameter.

et al., 2004). Since apricot is consumed with peel in all ways of consumption, fresh, dried and canning, the content of polyphenols of apricot becomes one of the most important attributes of fruit quality. The fruit consumption is decreasing in the EU 28, hence the apricots fruits as a source of antioxidants, could be used for encouraging their consumption.

The phenolic acids studied as well the flavonoids derivates are secondary metabolites, they are related to different functions including pigments and antioxidant activity. Polyphenol genetic control have been studied in model plants and some relevant genes have been identified. In Arabidopsis, a phenylalanine ammonia-lyase (PAL) has been identified as involved in the first step of phenylpropanoid metabolism (Fraser and Chapple, 2011). Other genes associated to anthocyanin accumulation were dihydroflavonol 4-reductase *(DFR)* and flavonol synthase (*FLS*) (Jin et al., 2016; Luo et al., 2016). In apricot by means of a transcriptomic approach *MYB10* gene was proposed as the best candidate for skin colour (García-Gómez et al., 2019), however, there is still a lack of information of the genes and mechanisms involved in the anthocyanin pathway for using them in molecular breeding.

Their concentrations in foods vary according to numerous genetic and environmental factors (Manach et al., 2004; Mole et al., 1988). In this study, the genetic effect was indicated by the differences among genotypes and the environment effect was analysed by means of sampling in 3 crop years. An important effect of lower general content of polyphenols during crop year 2018 was observed. Since the polyphenols synthesis and accumulations occurs during maturity of the fruit, the ripening process is being close related to polyphenol accumulation (Kennedy et al., 2000). In our study since the varieties share the same location, crop management and laboratory conditions the differences observed between years might be due to differences in climatic conditions among years.

Several studies have shown that chlorogenic and neo-chlorogenic acids are related to some biological activities in which the antioxidant and antimicrobial properties are very relevant (Dillard and Bruce German, 2000; Jin et al., 2005; Sabu and Kuttan, 2002). The range of values obtained in apricot for both compounds was similar to those described in read plum skin (Stacewicz-Sapuntzakis et al., 2001), which indicates that apricot species is a good source of polyphenols acids. In apricot, a similar to plum range of concentrations of chlorogenic acid was found (Gündoğdu et al., 2013; Ruiz et al., 2005) in agreement with our results.

Concerning to the amount of rutin content in apricot, similar results were obtained by Fan et al. (2018) and Gündoğdu et al. (2013). Rutin is the glycoside form of quercetin and it has been related as well with antioxidant and antimicrobial properties and due to its chemical structures are related with others beneficial health processes. Due to the high content of these compounds in apricot, some studies suggested that apricot is a good source of phytochemicals with antioxidant potential (Fan et al., 2018). Concerning to quercetín-3-glucuronide, the range of content obtained was similar as described in other species (Nicolle et al., 2004). Additionally, this compound had the higher contribution to antioxidant activity in apricots (Fan et al., 2018).

4.2. Contribution of the PPV-resistant 'Goldrich' variety to fruit quality

Since the spread of sharka diseases, the production of apricot in the main producing areas of Europe and the Mediterranean Basin are based on varieties obtained by breeding (Bassi et al., 2010; Egea et al., 2010; Karayiannis, 2006;Martínez-Calvo et al., 2009; Pennone et al., 2010). In Central Europe the resistant varieties from Ontario, such as 'Henderson' and 'Harlayne' were well adapted (Polak et al., 2008) but it was not the case in the European Southern regions as Spain and Italy in which the crop needs medium chilling varieties. Among the different resistant cultivars 'Goldrich' was the less affected for the lack of chilling.

Results from this study showed that 'Goldrich' is a good contributor for increasing antioxidant content, its genetic effect represented up to 65–70 % of the total average, which indicated a relevant role in increasing polyphenolic compounds compared to the other cultivars studied. This fact pointed out that crosses involving this variety are even more relevant for increasing the polyphenol content of the seedlings than the other genotypes studied.

5. Conclusions

The set of apricot accessions analysed showed different contain in the polyphenols compounds. The content was genetic and environment dependent. Concentration of polyphenols in apricot peel is 10 fold higher than flesh, since this fruit is consumed with peel in the different ways, fresh and dried, this trait is relevant for increasing the apricot consumption. The cultivar 'Goldrich' used as a donor of resistance to sharka diseases at different breeding programs, including the IVIA's program, resulted the variety with highest contribution to the polyphenol content among the accessions studied. The genetic effect of 'Goldrich' in this trait indicated it was a good candidate for increasing both neochlorogenic and chlorogenic acid content of fruits in the breeding program. The comparison of the first generation of 'Goldrich' hybrids with other genotypes shows that 'Goldrich' remains as a good parental for increasing the antioxidant content of apricot by breeding, which would increase as well the fruit quality.

CRediT authorship contribution statement

H. Gómez-Martínez: Data curation, Writing - original draft, Formal analysis, Writing - review & editing. **A. Bermejo:** Data curation. **E. Zuriaga:** Supervision, Writing - review & editing. **M.L. Badenes:** Project administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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