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Influence of environmental conditions on the production of nutraceuticals in Italian edible plant landraces

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ABSTRACT

Autochthonous plant varieties, also referred to as landraces, represent an important genetic resource, being well-adapted to the environment in which they have been selected. Landraces usually show profiles rich in nutraceuticals, making them an effective and valuable alternative to commercial agri-products, as well as potential candidates for crop improvement programs. Basilicata region is recognized as an Italian hotspot for agrobiodiversity, due to its complex orography. Thus, this work aimed to characterize and monitor, for two successive years, the content of secondary metabolites and related antioxidant properties of seven different species, four officinal (i.e., wild fennel - *Feoniculum vulgare* Mill.; oregano - *Origanum vulgare* L.; thyme - *Thymus vulgaris* L.; valerian - *Valeriana officinalis* L.) and three fruit species (i.e., fig - *Ficus carica* L. cv. Dottato; sweet cherry *Prunus avium* L. cv. Majatica; plum - *Prunus domestica* L. cv. Cascavella Gialla), collected in three different sites of this region. In detail, spectrophotometric tests were performed to assess the concentration of phenolic compounds, flavonoids, and – for officinal plants – also terpenoids, together with the antiradical activity (FRAP assays). In addition, to better typify the phytocomplexes of these landraces, HPLC-DAD and GC–MS analyses were carried out. In general, officinal plants showed higher values of nutraceutical compounds and related bioactivity with respect to fruit species. The data showed how different accessions of the same species had different phytochemical profiles, according to the sampling area and the year of collection, suggesting a role for both genetic and environmental factors in determining the observed results. Therefore, the final goal of this research was also to find a possible correlation between environmental factors and nutraceutics. The greatest correlation was found in valerian, where a lower water intake seemed to lead to a higher accumulation of antioxidants, and in plum, where the flavonoid content correlated positively with high temperatures. All these outcomes contribute at valorising Basilicata landraces for their aptitude to be high-quality foods and, at the same time, promoting the preservation of the agrobiodiversity for this region.

1. Introduction

The Mediterranean region is one of the most responsive and potentially vulnerable areas to global change and for this reason it has been identified as a climate change hot spot (Giorgi, 2006; Giorgi & Lionello, 2008). According to the last report of the Intergovernmental Panel on Climate Change (IPCC, 2021), the Mediterranean basin is facing rising temperatures and altered precipitation patterns, resulting in increased periods of drought and, in general, of extreme weather events. Agriculture is a sector that is already suffering from these shifts in climate conditions, being dependent and influenced by them. Nowadays, international agreements and political decisions regarding this issue are focusing on the protection of agricultural biodiversity, or agrobiodiversity, which is a subset of biodiversity and refers to the diversity of agroecosystems (FAO, 2004). Indeed, the safeguard of the variety and variability of plants, animals, and microorganisms allows to preserve agroecosystem structure, functions, and processes, which are key elements for food production and security. It must be noticed that the conservation of many components of the agrobiodiversity is reliant on human activity; therefore, local knowledge, culture, and practices can be considered as integral parts of it (FAO, 2004). In this context, plant landraces represent an important genetic resource, since they have "an

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actual or potential value for food and agriculture" (FAO, 2009). Indeed, autochthonous horticultural varieties stand out for being well-adapted to the environmental and cultivation conditions of the area in which they have been selected, making them good candidates for crop improvement programs (Giupponi, Pedrali, Leoni, Rodari, & Giorgi, 2021; Zeven, 1998). These species have been documented to be richer in nutraceuticals, that is bioactive phytochemicals, compared to their commercial counterparts, because their major adaptation capacity to environmental pressures depends precisely on secondary metabolite content (Bonasia, Conversa, Lazzizera, Gambacorta, & Elia, 2021; Gismondi, Di Marco, Canuti, & Canini, 2017). Consequently, they can be recognized as excellent sources of beneficial compounds for human health (Martínez-Ispizua et al., 2021).

Laghetti, Bisignano, and Urbano (2018) have reported that plant landraces are still abundant in inland territories of southern Italy. In particular, the major richness of landraces is found in mountainous and hilly areas of the Apennine chain. Indeed, Basilicata region is recognized as a crucial center for agrobiodiversity (Montesano, Negro, Sarli, Logozzo, & Spagnoletti Zeuli, 2012), considering that the plains represent a small portion of the territory (8 %) (Gizzi, Proto, & Potenza, 2019; Lanorte et al., 2019) and its complex orography determines a varied climate: Mediterranean along the coasts (i.e., cool wet winters and hot dry summers), continental near the mountains (i.e., cold snowy winters and mild summers), while the central area is subjected to severe drought (Lanfredi et al., 2015).

According to this premise, the aim of the present study was to investigate the influence of the environmental conditions on the composition in secondary metabolites of autochthonous officinal and fruit plant species collected from Basilicata region, in three different locations and during two subsequent years. In detail, the phytochemical profile of the landraces was characterized, in quantitative and qualitative terms, by spectrophotometry and chromatographic analyses, and their potential antiradical activity was estimated by ferric reducing antioxidant power (FRAP) assay. Moreover, the existence of a correlation between content of nutraceuticals (i.e., phenols, flavonoids, and terpenoids) and meteorological parameters was evaluated. The results of this work are part of a research project funded by Regione Basilicata, whose final purposes are the valorisation and the preservation of ancient autochthonous plant varieties.

2. Materials and methods

2.1. Chemicals

Standards of analytical grade (ascorbic acid; *p*-coumaric acid; caffeic acid; 1,1-dimethylallyl caffeate; caffeic acid phenethyl ester; chlorogenic acid; rosmarinic acid; 4-hydroxybenzoic acid; syringic acid; salicylic acid; gallic acid; vanillic acid; quercetin dihydrate; quercetin-3-*O*-glucoside; myricetin; kaempferol; genistein; chrysin; epicatechin; resveratrol; 5,7-dimethoxycoumarin; 3-hydroxytyrosol), 2,4,6-tripyridyl-s-triazine (TPTZ), anhydrous ferric (III) chloride, and potassium acetate were purchased from Sigma Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent, aluminium chloride, sodium carbonate, and hydrochloric acid were bought from PanReac Applichem (Barcelona, Spain).

HPLC grade methanol and formic acid were purchased from Carlo Erba (Milan, Italy), while hexane from Riedel-de Haën (Seelze, Germany). Ultrapure water was obtained by a Millipore Milli-Q purification system (Merck, Darmstadt, Germany).

2.2. Plant material and climate data

Four officinal (i.e., wild fennel - *Feoniculum vulgare* Mill.; oregano - *Origanum vulgare* L.; thyme - *Thymus vulgaris* L.; valerian - *Valeriana officinalis* L.) and three fruit plant species (i.e., fig - *Ficus carica* L. cv. Dottato; sweet cherry *Prunus avium* L. cv. Majatica; plum - *Prunus domestica* L. cv. Cascavella Gialla) without symptoms of pathology or physiological disorders were collected, in two subsequent years (i.e., 2020 and 2021), from three different Basilicata farms (i.e., *Az. Agr. Agri-Mecca* located in Filiano, Potenza; *Az. Agr. Lorenzo* in Tricarico, Matera; and *Az. Agr. Officina Sammaurese* in San Mauro Forte, Matera) (Supplementary Material 1) out of seven, which participated to the research project funded by Basilicata Region. After the harvesting, which occurred in September for officinal species and at the ripening of the fruits for the other species (i.e., August-September for fig, May-June for sweet cherry and August for plum), the samples were dried, stored at room temperature in dry and dark conditions, and then transferred to the Laboratory of Botany (Dept. of Biology, University of Rome Tor Vergata) for the analyses. For all collection sites (Supplementary Material 2), geographic and meteorological data were reported in detail in Supplementary Material 1.

2.3. Sample preparation

Above ground parts of wild fennel, oregano, thyme, and valerian and fruits of plum, sweet cherry and fig were powdered with mortar and pestle in liquid nitrogen, to obtain a fine powder then sieved with a mesh of size 0.5 mm. For the determination of total phenolic and flavonoid content, high-performance liquid chromatography-diode array detector (HPLC-DAD) analysis, and FRAP assay, 500 mg of each sample were dissolved in 7.5 mL of extraction solvent (methanol:water, 50:50; *v:v*) for 48 h, under agitation, in the dark. For gas-chromatography mass-spectrometry (GC–MS) investigation, instead, 100 mg of plant material were incubated with 1 mL of extraction solvent (methanol:hexane, 90:10; *v:v*) for 48 h, under agitation, in the dark. In all cases, after centrifugation at 11,000 *g* for 10 min, the supernatant was recovered, filtered, and diluted (1:3; *v:v*) with the respective extraction solvent.

2.4. Total phenolic content

Simple phenolic compounds were determined according to the Folin-Ciocalteu spectrophotometric method, as reported in Impei, Gismondi, Canuti, and Canini (2015). The absorbance of the samples was read at 760 nm by an ELISA microplate reader (Sunrise, Tecan, Austria). The quantitation of the phenolic content was estimated by a calibration curve (0–3 mg/L, $R^2 = 0.9975$), adequately prepared using gallic acid (GA) as standard. Results were expressed as milligrams of gallic acid equivalent per gram of plant dry weight (mg GAE/g DW).

2.5. Total flavonoid content

The concentration of flavonoids was assessed by the aluminum chloride colorimetric assay, as described in Di Marco et al. (2014). The absorbance of the reaction mixtures was measured at 415 nm, using an ELISA microplate reader (Sunrise, Tecan, Austria). Total flavonoid content was calculated as milligrams of quercetin equivalent per gram of plant dry weight (mg QE/g DW), based on a standard curve of quercetin (0–5 mg/L, $R^2 = 0.9975$).

2.6. Total terpenoid content

For officinal species, total terpenoids were extracted and estimated according to the method reported in Ghorai, Chakraborty, Gucchait, Saha, and Biswas (2012), with some modifications. The absorbance of the samples was read at 538 nm by an ELISA microplate reader (Sunrise, Tecan, Austria). The quantitation was evaluated by a calibration curve (0–4 mg/mL, $R^2 = 0.9763$), adequately prepared using linalool as standard. Results were expressed as milligrams of linalool equivalent per gram of plant dry weight (mg LE/g DW).

2.7. In vitro antiradical assay

The ferric reducing antioxidant power (FRAP) assay was performed

according to the method of Benzie and Strain (1996), with some modifications as described in Gismondi et al. (2013). The absorbance of the samples was read at 593 nm, using an ELISA microplate reader (Sunrise, Tecan, Austria). Ascorbate was used as standard to obtain a calibration curve (0–1 mM, $R^2 = 0.9933$) and results were expressed as molarity (M) of ascorbate equivalent per gram of plant dry weight (M AE/g DW).

2.8. Metabolomic approach

Using an HPLC system equipped with SPD-M20A diode array detector (DAD; Shimadzu, Kyoto, Japan), twenty-one metabolites were detected and quantified in the plant extracts. Chromatographic separation was achieved by a Phenomenex Luna 3u C18(2) column (3 μm × 4.6 mm × 150 mm), applying 1 % formic acid (*v:v*) (phase A) and methanol (phase B) as solvents and an elution gradient (flow rate: 0.95 mL/min) set as follows: t_{0min} (A 85 %, B 15 %); t_{20min} (A 65 %, B 35 %); t_{55min} (A 10 %, B 90 %); t_{68min} (A 85 %, B 15 %); t_{70min} (end run) (Gismondi et al., 2018). The injection volume was 20 μL and column temperature was fixed at 40 °C. Identification and quantitation of each molecule were carried out as reported by Gismondi et al. (2018) and Nanni, Canuti, Gismondi, and Canini (2018). Results were expressed as micrograms of respective standard equivalent per gram of plant dry weight (μg SE/g DW). The phytocomplex of the officinal plants was characterized by GC–MS. In detail, 2 μl of extract were injected at the temperature of 280 °C (splitless modality) into a QP2010 GC System (Shimadzu) equipped with an SH-Rtx-5MS capillary column (Shimadzu; length 30 m × diameter 0.25 mm × thickness 0.25 μm). The carrier gas was helium, employed at a constant flow of 1 mL/min. The temperature gradient was set as follows: 50 °C for 2 min, 200 °C for 2 min (reached at a rate of 3 °C/min), and 300 °C for 5 min (reached at a rate of 4 °C/min); to obtain a better resolution. An electron impact of 70 eV (scanning from 100 to 700 *m/z*) was used for the ionization (ion source temperature 230 °C; interface temperature 320 °C; solvent cut time 6 min). The molecular markers were identified by comparing their mass spectra with those registered in the software database NIST (National Institute of Standards and Technology) Library 14, loaded into the detection software of the instrument (Solution software; Shimadzu). Percentage similarity values were considered acceptable only if higher than 85 %. The relative abundance of each identified compound was expressed as percentage with respect to the total area of all peaks (100 %).

2.9. Statistical analyses

All experiments were carried out in triplicate (i.e., independent biological measurement) and the results were reported as means ± standard error. Data were subjected to one-way analysis of variance (ANOVA) and the differences were evaluated by the post-hoc lowest standard deviations (LSD) test, through PAST software (*p* values: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$). Multivariate analyses were performed on HPLC-DAD and GC–MS data. In particular, hierarchical clustering was carried out using Python software (v. 3.9.7). Scripts used to analyse and plot the results are available on GitHub at the following link: (https://github.com/lorenzo-bioinfo/basilicata). Before performing clustering, data were normalized using the function *MinMaxScaler()* from the *preprocessing* module of the *Scikit-learn* library (Buitinck et al., 2013). The function applies the following transformation to data:

$$y = (x - x_{min})/(x_{max} - x_{min})$$

where y is the new scaled value, x is the original unscaled value, and x_{min} and x_{max} are the least and highest values recorded for each feature. In brief, the highest value becomes 1 and the least becomes 0, while the others are scaled accordingly. Hierarchical clustering was performed using the function *hierarchy.linkage()* from the *cluster* module of the *Scipy* library (Virtanen et al., 2020). Ward's algorithm for variance minimization was used as linkage criterion. The resulting dendrograms and heat-maps were plotted with the *clustermap()* function from *Seaborn* library (Waskom, 2021). Pearson's coefficients (r), measured by MS Excel software (CORREL statistical function), were used to evaluate the eventual correlations between environmental factors (i.e., temperature, precipitation, and elevation) and antioxidant characteristics (i.e., total phenolics, flavonoids, terpenoids, and FRAP).

3. Results and discussion

Over the last decades, the intensification of agriculture and the simplification of cropping systems have allowed high yields in large-scale production worldwide, at the expense of agrobiodiversity, soil health, and ecosystem services. Nowadays, climate change imposes further serious limits on the achievement of food security for an ever-growing world population. Thus, an alternative to conventional agriculture is needed, in order to reduce the impact of this sector on the environment and, at the same time, to continue in sustaining high levels of crop production (Hufnagel, Reckling, & Ewert, 2020; Roberts & Mattoo, 2019). Moreover, consumer's demand for high-quality and sustainable food is rising, driving research and market towards the recovery of autochthonous varieties, the so-called landraces (Casals Missio et al., 2018; Petropoulos, Barros, & Ferreira, 2019). These varieties possess a genotype well-adapted to the specific environmental contexts in which they live, making themselves resistant and/or tolerant to peculiar habitats. Since they continue to evolve in this direction, they should be considered as the most adequate representatives for their species in a determined area. The resilience demonstrated by landraces is partially based on the production of secondary metabolites, in both qualitative and quantitative terms, playing a crucial role in plant defense and acclimation strategies (Balestrini et al., 2021; Bonasia, Conversa, Lazzizera, Gambacorta, & Elia, 2021). Consequently, this enriched chemical profile confers an added value on food products, which show high nutritional and nutraceutical content with beneficial effect on human health (Botonaki, Polymeros, Tsakiridou, & Mattas, 2006; Dillard & German, 2000).

According to all this premise, the present contribution aimed at characterizing the content in secondary metabolites and the antioxidant power of selected edible officinal and fruit species autochthonous from Basilicata, to valorise the agrobiodiversity of this Italian region.

3.1. Total phenolics, flavonoids, terpenoids, and antioxidant activity

For all samples, the quantitation of total phenolic and flavonoid compounds, together with FRAP, were determined by spectrophotometric assays. Moreover, for officinal plants, a colorimetric method was performed to assess the amount of terpenoids, since presence and quantity of this class of metabolites is directly related to their nutraceutical properties. In Fig. 1, for all specimens, the data of 2020 were compared with those of 2021.

In general, a significant variation in values was always observed between the two years, for almost all samples, with some exceptions for terpenoids. Concerning officinal plants, in both years, total phenolic levels were highest in *Orivul2* (17.32 ± 0.08 and 35.92 ± 0.07 mg GAE/g DW in 2020 and 2021, respectively), while the lowest values were registered in *Foevul1* (3.55 ± 0.05 and 4.73 ± 0.08 mg GAE/g DW in 2020 and 2021, respectively). In the analysis for flavonoids, *Orivul2* resulted again the extract with the highest levels (13.36 ± 0.02 mg QE/g DW) but exclusively in 2021; in fact, in 2020 the highest flavonoid content was found in thyme (*Thyvul1*, 6.04 ± 0.02 mg QE/g DW). Instead, *Valoff2* was the extract with the lowest flavonoid values, both in 2020 and 2021 (0.96 ± 0.01 and 2.41 ± 0.04 mg QE/g DW, respectively). The terpenoid levels, in both years, resulted lower in wild fennel, precisely 3.95 ± 1.43 mg LE/g DW (*Foevul1* in 2020) and 6.25 ± 2.08 mg LE/g DW (*Foevul2* in 2021), but it must be noticed that these two samples showed no significant variation between the two years, while *Foevul3* was 4-fold higher in 2021 with respect to 2020. *Valoff1* was found to be the sample with the highest terpenoid content in 2020

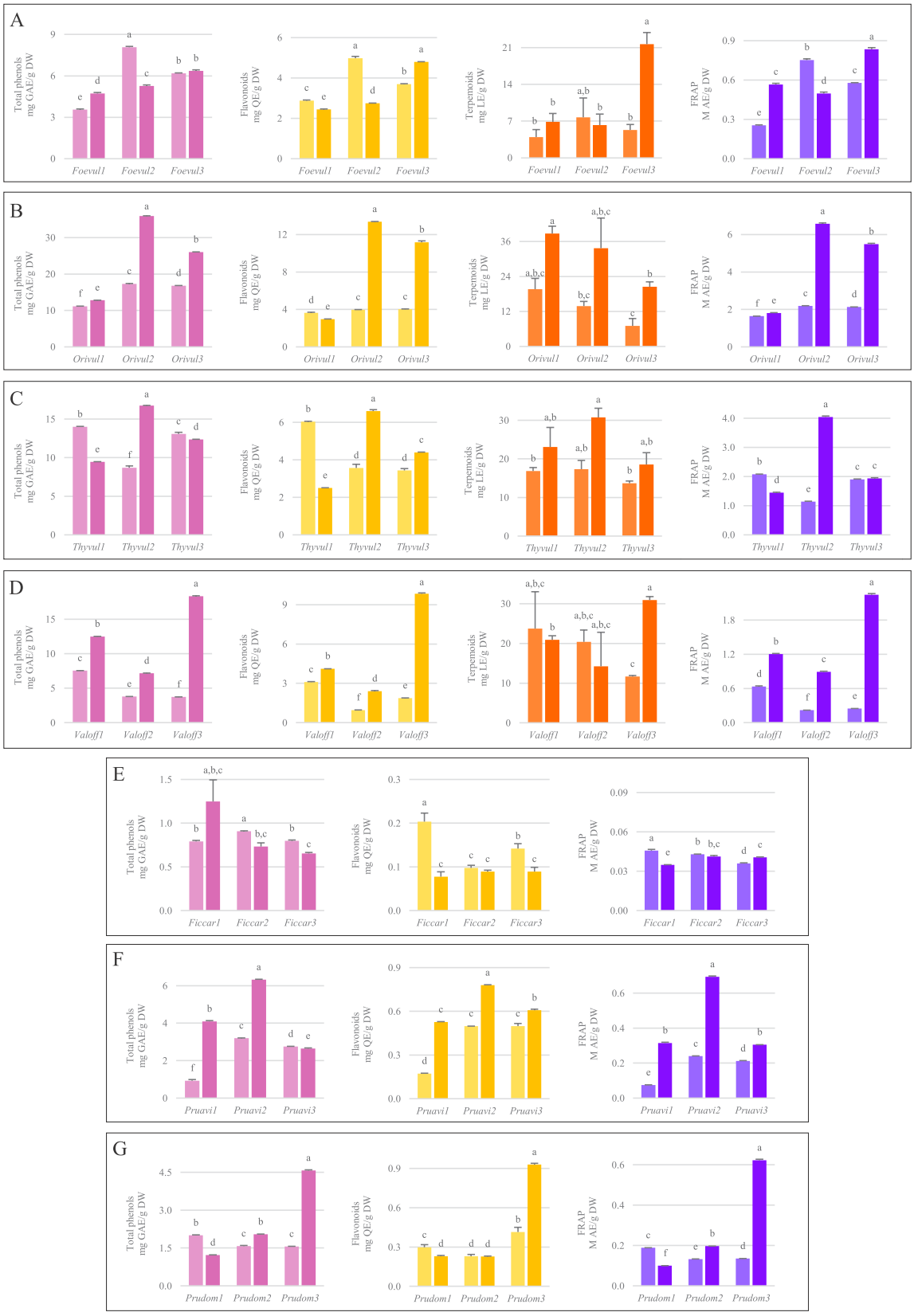

Fig. 1. Spectrophotometric assays. The total content of phenolics (pink), flavonoids (yellow), and terpenoids (orange), together with FRAP level (violet), are reported for each species: wild fennel (A); oregano (B); thyme (C); valerian (D); fig (E); sweet cherry (F); plum (G). Sample labelling reflects the names specified in Supplementary Material 1. Light colours refer to year 2020, while dark ones to year 2021. The unit of measure for each test was indicated near the respective graph. *p*-values relative to these data were shown in Supplementary Material 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(23.74 ± 9.27 mg LE/g DW), while in 2021 this record was observed in *Orivul1* (38.71 ± 2.52 mg LE/g DW). In the same year, the terpenoids levels in *Orivul3* (20.50 ± 1.64 mg LE/g DW) were significantly lower by nearly half than those in *Orivul1*. Curiously, in 2021, the opposite trend was registered for valerian samples: *Valoff3* (30.92 ± 0.88 mg LE/g DW) was significantly higher than *Valoff1* (20.89 ± 1.04 mg LE/g DW). Similarly, in 2021, *Foevul3* showed significantly 3-fold higher terpenoid levels with respect to *Foevul1* and *Foevul2*. The FRAP results largely overlapped with observations given for phenolics, since in both 2020 and 2021, the highest values were found in *Orivul2* (2.19 ± 0.01 and 6.57 ± 0.05 M AE/g DW, respectively). In 2020, in line with the flavonoid results, FRAP was lower in *Valoff2* (0.22 ± 0.01 M AE/g DW), while in 2021 the lower values were founded in *Foevul2* (0.50 ± 0.01 M AE/g DW), in agreement with the phenolic and terpenoid data.

In fruit extracts, figs revealed the lowest levels of phenolic compounds, precisely 0.79 ± 0.01 mg GAE/g DW in 2020 (*Ficcar1*) and 0.65 ± 0.01 mg GAE/g DW in 2021 (*Ficcar3*). Similarly, also the flavonoid content resulted the lowest in fig samples, in both years (*Ficcar2* in 2020, 0.10 ± 0.01 mg QE/g DW; *Ficcar1* in 2021, 0.08 ± 0.01 mg QE/g DW). The highest phenolic values recorded were 3.20 ± 0.01 (*Pruavi2*) and 4.57 ± 0.03 (*Prudom3*) mg GAE/g DW in 2020 and 2021, respectively. These same samples also showed the highest flavonoid contents, which were 0.50 mg QE/g DW in *Pruavi2* and *Pruavi3* (2020) and 0.93 ± 0.01 mg QE/g DW in *Prudom3* (2021). In 2020 the maximum and minimum values of FRAP reflected what was observed for phenolics and flavonoids: the highest levels in sweet cherry (*Pruavi2*, 0.24 ± 0.01 M AE/g DW) and the lowest ones in figs (*Ficcar2* and *Ficcar3*, both 0.04 ± 0.01 M AE/g DW). In 2021, the minimum value of FRAP registered for figs was in line with their phenolic and flavonoid content (*Ficcar1*, 0.03 ± 0.01 M AE/g DW), while the maximum was shown by *Pruavi2* (0.69 ± 0.01 M AE/g DW), as expected due to its phenolic and flavonoid concentrations.

Significance of the differences observed for the data obtained from the same specimens in the two years was estimated, together with the changes observed among samples of the same species for the same year (Supplementary Material 3). In 2021 compared to 2020, all valerian samples showed an increase in the content of total phenolics and flavonoids, as well as for FRAP values; however, this trend was not observed for terpenoids. About the latter, an increase of their concentration was noticed between 2020 and 2021 in all samples from San Mauro Forte (labelled by number *3*). For oregano and thyme collected in Filiano (labelled by number *1*) and Tricarico (labelled by number *2*), the same tendency was found. It was also detected that officinal plants from San Mauro Forte and Filiano always showed higher FRAP values in 2021 compared to 2020. Moreover, with the exception of *Ficcar3*, flavonoid content of 2020 resulted always lower than 2021 in all the samples from San Mauro Forte. The sweet cherry of Tricarico (*Pruavi2*) harvested in 2021 revealed higher values for all assays compared to the fruits of 2020; a similar trend was observed for *Prudom3*, whose levels of phenolic compounds, flavonoids, and FRAP significantly increased in 2021 with respect to 2020. Similarly to what observed for officinal plants, in 2021, FRAP values of all San Mauro Forte fruits were higher than those of 2020.

Overall, officinal plants and fruits are rich in nutraceuticals, such as phenolic acids, flavonoids, and terpenoids, especially for officinal plants; all these secondary metabolites are the basis of their antioxidant quality, making them an excellent source of antiradicals (Chrysargyris, Mikallou, Petropoulos, & Tzortzakis, 2020). For this reason, the existence of possible correlations between content of secondary metabolites and antioxidant properties of the samples, as well as the levels of specific classes of plant compounds, were evaluated (Supplementary Material 4). Although no correlation was found in figs, in all other samples the antioxidant activity was positively correlated with the levels of phenolic compounds ($r \geq 0.9$), except for wild fennels that presented r values equal to 0.838. Similarly, a positive correlation between FRAP and flavonoid content seemed to exist in most of the samples, reaching the highest values in oregano, valerian, and plum ($r > 0.946$). In these same plant species, total phenolics and flavonoids showed positive correlation with r values > 0.933. On the contrary, terpenoids were not always correlated to the other parameters, excepting valerian whose correlation coefficient was always > 0.7.

Although it is tricky to make comparisons between our own data and literature evidence, due to different variables (e.g., extraction procedure, analytical method), we observed that Basilicata samples generally exhibited elevated values of antioxidants. For instance, regarding oregano (which showed the highest phenolic content and the strongest antiradical activity among all samples examined in this study), several works have documented that both commercial and wild *O. vulgare* extracts obtained from different geographical areas of the world presented lower nutraceutical properties than those registered in the current research (Capecka, Mareczek, & Leja, 2005; Kruma et al., 2008; Slimestad, Fossen, & Brede, 2020; Su et al., 2007; Vallverdú-Queralt et al., 2014; Wojdyło, Oszmiański, & Czemerys, 2007). Taking into account that landraces are well-adapted to their own peculiar environments, it is not surprising to detect such variability in phytocomplex composition among specimens living in different geographical areas.

The content of secondary metabolites does not exclusively affect the antioxidant properties of food plants but also other nutraceutical aspects. For instance, some works have reported that phytochemicals may exert anti-inflammatory and immunomodulatory functions (Ambriz-Pérez, Leyva-López, Gutiérrez-Grijalva, & Heredia, 2016; de las Heras & Hortelano, 2009; Jiang & Dusting, 2003; Venkatalakshmi, Vadivel, & Brindha, 2016). Consequently, the increase of these bioactive compounds in Basilicata samples, as a function of genetics and geographical parameters, would suggest a potential use for them as possible food supplements and preventive treatment, beyond the dietary purposes.

Moreover, a high level of phenols and flavonoids has been correlated with taste and flavour of edible fruits and seeds (Bucalossi et al., 2020; de Oliveira, de Carvalho, & Melo, 2014; Ku et al., 2020; Roland, Pouvreau, Curran, van de Velde, & de Kok, 2017). The same scholars have also reported that, during the evolution, the palatability associated to these molecules has probably played a central role in the anthropic selection of crops. Therefore, it could be very interesting to expand the present research with a *citizen science* program aimed at investigating the correlation between content of secondary metabolites in the studied landraces and consumers' food preferences.

3.2. Metabolomics analysis

The chromatographic approach, performed by HPLC-DAD and GC–MS methods, allowed to obtain a fine characterization of the phytochemical profiles from the Basilicata landraces investigated in this study. Twenty-one phenolic and flavonoid molecules were monitored, detected, and quantified by liquid chromatography and listed, for each sample, in Supplementary Material 5. In order to facilitate understanding of the huge amount of data, a clustergram and a heatmap based on the concentrations of compounds obtained by HPLC analysis were generated (Fig. 2). Two main clusters were identified: the first at the top of the graph, while the second, immediately below, further divided into three subgroups. The latter can be recognized by their different shades of colour, from darker (upper part) to lighter (lower part), depending on the increase in concentration of metabolites.

The first cluster grouped all oregano and thyme samples; according to the chromatic scale, they showed the richest and most varied phenolic and flavonoid profiles. In particular, among all samples, the highest genistein levels were recorded in oregano (e.g., *Orivul2-21*). Similarly, *Orivul3-20* was the richest in vanillic acid and caffeic acid (555.41 and 250.08 μg SE/g DW, respectively), followed by *Orivul2-21* for myricetin and quercetin 3-*O*-glucoside (4740.66 and 5268.60 μg SE/g DW, respectively). At the same time, *Orivul1-20* was characterized by the most elevated concentrations of quercetin, resveratrol, and gallic acid (641.09, 2483.49, and 410.16 μg SE/g DW, respectively). On the other hand, thyme profiles appeared to be the richest in syringic acid and 5,7-

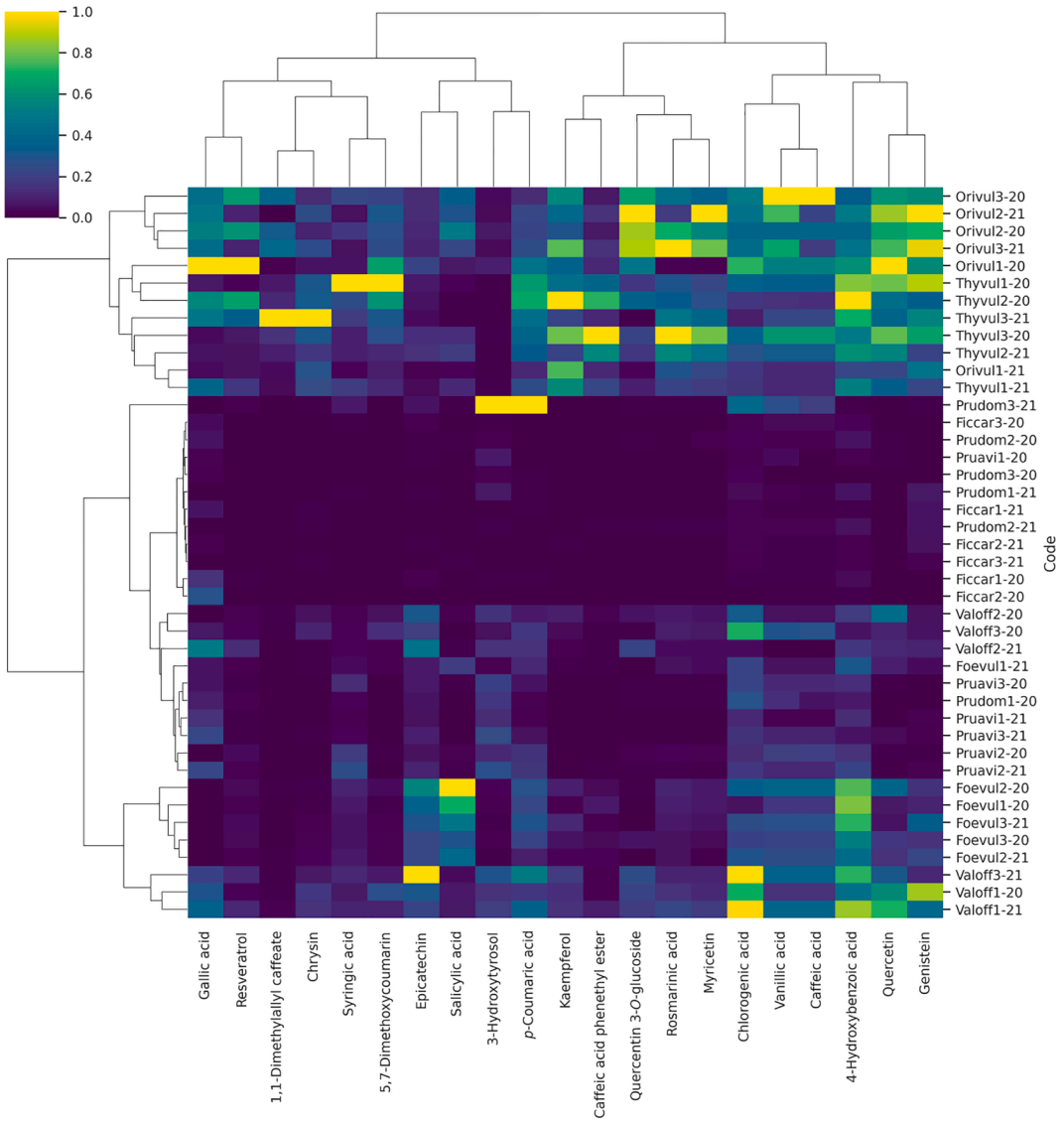

Fig. 2. Clustergram and heatmap of metabolites detected by HPLC-DAD. Visualization of twenty-one secondary metabolites detected by liquid chromatography in Basilicata samples. Minimum and maximum values were set as 0 (dark blue) and 1 (yellow), respectively. Each row is labelled with a sample code, while columns represent molecules. Hierarchical clustering was also performed and reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dimethoxycoumarin (*Thyvul1-20*), kaempferol and 4-hydroxybenzoic acid (*Thyvul2-20*), chrysin and 1,1-dimethylallyl caffeate *(Thyvul3-21*), caffeic acid phenethyl ester and rosmarinic acid (*Thyvul3-20*).

About the second cluster, the first subgroup emerged as the poorest in secondary metabolites detected by HPLC-DAD method, as evidenced by darker shades in this part of the heatmap. All fig and plum specimens (except *Prudom1-20*) and a single sweet cherry sample (*Pruavi1-20*) belonged to this subdivision. Interestingly, *Pruavi1-20* clustered so far from other sweet cherries, even from its 2021 counterpart. It should be noted that only *Prudom3-21* stands out in this subgroup, showing the highest levels of 3-hydroxytyrosol (5755.85 μg SE/g DW) and *p*-coumaric acid (238.70 μg SE/g DW), compared to the whole collection. The second subgroup was characterized by intermediate values of secondary metabolite and included the remaining samples of sweet cherry, one fennel (*Foevul1-21*), the last plum (*Prudom1-20*), and three valerians. None of these specimens reached maximum values for the investigated molecules. The last subsection presented the most abundant contents of phytochemicals, with respect to the other two previously described. There, the remaining samples of fennel and valerian grouped. In detail, the highest values were found in *Foevul2-20* for salicylic acid (6715.37 μg SE/g DW), in *Valoff3-21* for epicatechin (2166.47 μg SE/g DW), and in *Valoff3-21* and *Valoff1-21* for chlorogenic acid (424.73 and 416.09 μg SE/g DW, respectively).

In conclusion, no peculiar association of molecules, representing a species-specific fingerprint, was highlighted. As already observed in the spectrophotometric assays, also in this case, samples showed significant differences between the two sampling years, most probably due to environmental factors.

In this study, the lipophilic fraction of the phytocomplex purified from the officinal landraces was typified by GC–MS analysis, monitoring, identifying, and quantifying forty-seven metabolites (Supplementary Material 6). These results, including the volatilome, allowed the construction of a clustergram and a heatmap (Fig. 3). In this graph, samples formed four main clusters, which generally reflected their different membership to the analysed species, or rather specimens of the same species tended to group together.

The first cluster included all oregano samples, characterized by caryophyllene, gamma-muurolene, germacrene D, humulene, and gamma-terpinene. The highest levels of these compounds were found in *Orivul2-20*, which also showed the most elevated concentration of

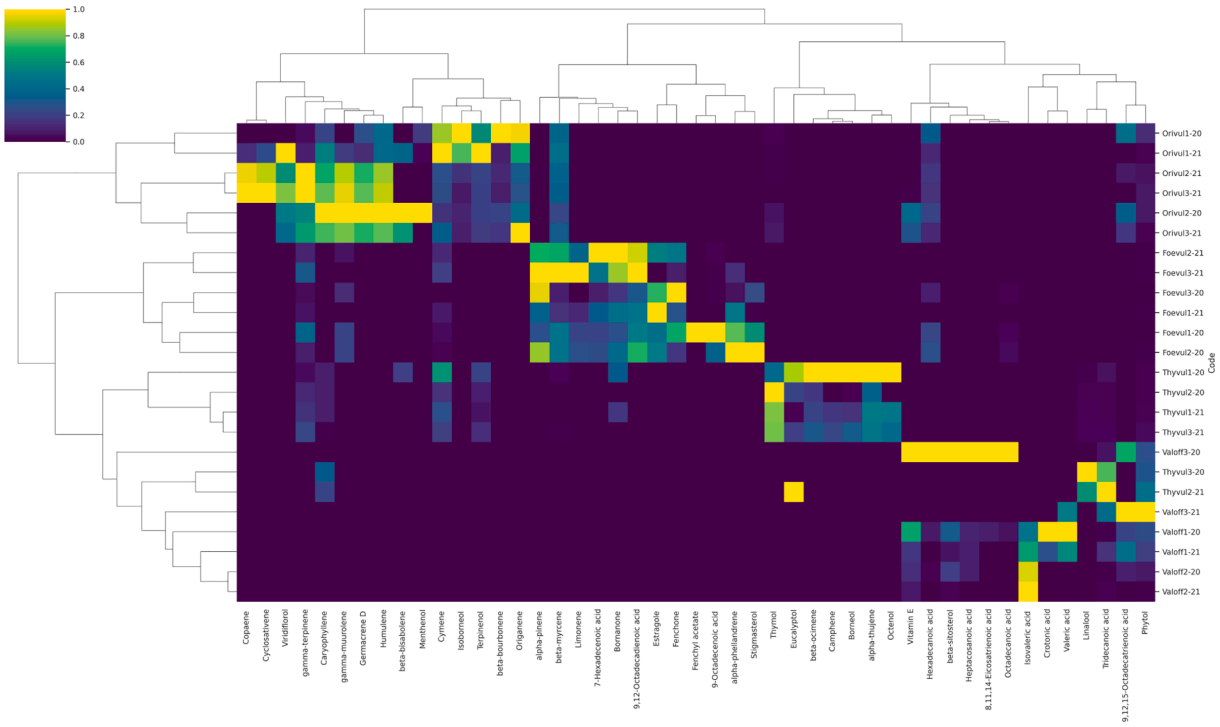

Fig. 3. Clustergram and heatmap of metabolites detected by GC–MS. Visualization of forty-seven compounds detected by gas chromatography in Basilicata samples. Minimum and maximum values were set as 0 (dark blue) and 1 (yellow), respectively. Each row is labelled with a sample code, while columns represent molecules. Hierarchical clustering was also performed and reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

menthenol. Curiously, *Orivul1,* in both years, presented a slightly different profile than the other oregano specimens, showing high amounts of cymene, isoborneol, terpinenol, origanene. Copaene and cyclosativene were detected almost exclusively in *Orivul2-21* and *Orivul3-21*.

All fennel specimens joined together in the second group, where alpha-pinene, 9,12-octadecadienoic acid, and bornanone resulted the most common compounds. The highest amounts of specific molecules were found in *Foevul3-21* (alpha-pinene, beta-myrcene, limonene, and 9,12-octadecadienoic acid), *Foevul1-20* (fenchone, and 9-octadecenoic acid), and *Foevul2-20* (alpha-phellandrene and stigmasterol).

Four thyme samples grouped in the third class; among them, *Thyvul1-20* stood out for the high concentrations of beta-ocimene, camphene, borneol, alpha-thujene, and octenol, while *Thyvul2-20* by high levels of thymol (78.85 %).

The remaining samples of thyme and all valerian extracts belonged to the fourth group. *Thyvul3-20* and *Thyvul2-21* presented a similar profile, rich in tridecanoic acid and linalool, although *Thyvul2-21* showed the highest concentration of eucalyptol (3.46 %). *Valoff3-20* differed from all samples for the elevated amounts of vitamin E, hexadecanoic acid, beta-sitosterol, heptacosanoic acid, 8,11,14-eicosatrienoic acid, and octadecanoic acid. The other valerian exemplars were typified by isovaleric acid, excepted *Valoff3-21*, which showed high levels of phytol (61.75 %) and 9,12,15-octadecatrienoic acid (12.70 %). Finally, in *Valoff1-20* valeric acid and crotonic acid were particularly abundant. Unlike what was observed for HPLC-DAD data, most of molecules detected by gas chromatography seemed to associate forming species-specific profiles. Although specific molecular markers have been identified for each species and/or specimen, it would not be appropriate to associate and restrict the bioactivity of plant products to single molecules, since it is known that the compounds present in the phytocomplex work in synergy (Ettorre, Frosali, Andreassi, & Di Stefano, 2010). Moreover, literature states that various plant metabolites can perform overlapping biological functions on human health, even if belonging to different chemical classes. For example, quercetin (flavonoid) and linalool and limonene (terpenes) have been proved to possess a similar anti-diabetic effect in streptozocin-induced diabetic rats (More, Kulkarni, Nalawade, & Arvindekar, 2014; Vessal, Hemmati, & Vasei, 2003). Thus, it would be arduous to assign a certain role to specific phytochemicals. On the other hand, profiling plant extracts can represent a very useful tool to understand how the environment modulates the accumulation of peculiar compounds and the plant genotype's role on the induced chemical patterns. The information obtained through our metabolomics analyses could be strengthened by increasing the number of specimens, in order to attenuate the interference of non-significant data due to intraspecific variability.

3.3. Correlation between environmental parameters and antioxidants

Levels of phytochemicals change according to genetic and exogenous factors. Indeed, although DNA contains information for secondary metabolite synthesis, their production is strongly influenced by developmental stage and biotic/abiotic pressures, such as temperature and humidity (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). Since the content of these compounds affects bioactivity and antioxidant properties of food plants, the environment in which these plants live play a key role in determining them as high-quality foods. For this reason, the correlations between meteorological factors and content of antioxidants (i.e., phenolics, flavonoids, and terpenoids) or bioactivity (i.e., FRAP) in Basilicata autochthonous samples were investigated. For each sampling site (i.e., Filiano, Tricarico, and San Mauro Forte), the meteorological data considered in this study were: minimum temperature (T_{min}), maximum temperature (T_{max}), mean temperature (T_{mean}), and total precipitation (P_{tot}) (Supplementary Material 7). However, to determine Pearson's correlations, a time frame of 90 days before the sampling was considered in acquiring meteorological variables (see Supplementary Material 1). Results of this statistical analysis were reported in Table 1. Particular attention should be paid to plum samples,

Table 1
Correlations between meteorological parameters and content of nutraceuticals. Pearson's correlations coefficients (r) estimated between content of secondary metabolites (phenolics, flavonoids, and terpenoids) or antioxidant properties (FRAP) and meteorological data (total precipitation, P_{tot}; minimum temperature, T_{min}; maximum temperature, T_{max}; mean temperature, T_{mean}) for each sample species were shown.

		P_{tot}	T_{min}	T_{max}	T_{mean}
Foevul	Phenolics	0.428	0.024	−0.179	−0.153
	Flavonoids	0.356	0.057	−0.034	−0.048
	FRAP	−0.105	0.405	0.262	0.297
	Terpenoids	−0.484	0.672	0.675	0.677
		P_{tot}	T_{min}	T_{max}	T_{mean}
Orivul	Phenolics	−0.479	0.790	0.533	0.570
	Flavonoids	−0.537	0.752	0.606	0.611
	FRAP	−0.558	0.795	0.634	0.648
	Terpenoids	−0.378	0.472	0.449	0.584
		P_{tot}	T_{min}	T_{max}	T_{mean}
Thyvul	Phenolics	−0.119	−0.007	−0.013	−0.018
	Flavonoids	0.054	0.153	0.127	0.136
	FRAP	−0.293	0.253	0.202	0.196
	Terpenoids	−0.638	0.604	0.548	0.540
		P_{tot}	T_{min}	T_{max}	T_{mean}
Valoff	Phenolics	−0.757	−0.323	−0.216	−0.311
	Flavonoids	−0.746	−0.170	−0.068	−0.162
	FRAP	−0.828	−0.270	−0.240	−0.335
	Terpenoids	−0.249	−0.483	−0.332	−0.391
		P_{tot}	T_{min}	T_{max}	T_{mean}
Ficcar	Phenolics	0.013	−0.416	−0.391	−0.349
	Flavonoids	0.637	−0.670	−0.591	−0.661
	FRAP	0.618	−0.358	−0.433	−0.444
		P_{tot}	T_{min}	T_{max}	T_{mean}
Pruavi	Phenolics	−0.088	0.366	−0.123	−0.095
	Flavonoids	−0.267	0.558	0.128	0.074
	FRAP	−0.221	0.267	−0.257	−0.263
		P_{tot}	T_{min}	T_{max}	T_{mean}
Prudom	Phenolics	−0.456	0.679	0.750	0.736
	Flavonoids	−0.481	0.703	0.807	0.779
	FRAP	−0.488	0.684	0.763	0.750

whose levels of antioxidants and FRAP values appeared to be affected by temperature variations (i.e., T_{min} and T_{max}); in particular, the highest value of correlation was measured between flavonoids and T_{max} ($r = 0.807$). In valerian, phenolic and flavonoid compounds, but especially their bioactivity (FRAP; $r = -0.828$), were negatively related with total precipitation, suggesting that low water input might have promoted the accumulation of such phytochemicals for this species. High positive correlations ($0.752 \geq r \geq 0.795$) were estimated in oregano between T_{min} and phenolics, flavonoids, and FRAP. Concerning figs, correlation values were < 0.7 in most cases, although flavonoids barely tended to correlate with P_{tot} (positively) and with T_{min} and T_{mean} (negatively). Similar slight positive correlations were observed in thyme and fennel for terpenoids content; in detail, in fennel, these volatile compounds positively correlated with all considered temperature parameters, while in thyme exclusively with T_{min} and negatively with precipitation. No significance was found for sweet cherry.

The influence of the collection sites on nutraceutical content and potential bioactivity of plant products is well-known, considering that diverse eco-geographical features may modulate in different ways gene expression and rate/activity of biochemical pathways involved in the production of secondary metabolites (Chrysargyris, Mikallou, Petropoulos, & Tzortzakis, 2020). However, the comprehension of the specific correlations existing between these factors is still partially unclear and sometimes contradictory, deserving further investigation. Indeed, for instance, although the negative correlation between antioxidant levels in *V. officinalis* and precipitation we observed is in line with similar evidence on both valerian and other herbs (Hassan & Ali, 2014; Mustafavi, Shekari, Hatami-Maleki, & Nasiri, 2016; Rebey et al., 2012), the positive association registered in this work between plum nutraceuticals and temperature cannot be considered absolute (Miletić, Nastasović, & Loos, 2012; Sahamishirazi, Moehring, Claupein, & Graeff-Hoenninger, 2017; Xu, Zhang, Zhu, Huang, & Lu, 2011).

It could be certainly informative to extend the present study on a wider time span, for a better comprehension of the side effects that the climate change has been inducing on crops and how autochthonous plant species deals with this phenomenon by modulating synthesis and activity of secondary metabolites.

4. Conclusions

In a climate-changing world, the preservation of local varieties well-adapted to the area in which they grow and/or have been selected is of pivotal importance with the perspective of guaranteeing food security for the world population. This work aimed to enhance several plant landraces of Basilicata, a region considered a hotspot for agro-biodiversity. The study provided new knowledge and insights about seven autochthonous varieties of officinal and fruit plants, supporting their recovery and introduction into local markets, and renewed the importance of agrobiodiversity conservation. For this purpose, the metabolome of four officinal and three fruit species and their potential antioxidant activity were characterized at biochemical level. In particular, the study of the lipophilic fraction of the phytocomplexes revealed peculiar species-specific fingerprints for the studied officinal species; they were found to be also the richest in total phytochemicals and antiradical capacity. The different results obtained from the several accessions of the same species, collected during two successive years, confirmed the significant role of the environment on the quality of food plants, beyond the basal role of their genetics. Analyses of correlations supported this hypothesis, showing for certain species positive and negative correlations with some abiotic parameters. This evidence would confirm how environmental factors modulate gene expression, via the well-known phenomenon of epigenetics.

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.

Data availability

All data are provided in the main text or in Supplementary Material files

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112483.

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