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Morphometric and phytochemical characterization and elevation effect on yield of three potato landraces of the Ligurian Apennines (Northern Italy)

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Summary

The adaptability, productivity and importance to the human diet of the potato (*Solanum tuberosum* L.) have conditioned the crop to diffusion across very different areas, leading to the formation of several landraces through anthropic or natural selection. The mountainous inland of Genoa, Italy is a historical centre for potato cultivation, and landraces have been preserved in this area by farmers' associations, such as the *Consorzio della Quarantina*. This study aims to provide phytochemical and morphometric characterization of three potato landraces from this consortium (Quarantina Bianca, Quarantina Prugnona and Rubra Spes) by analysing their bio-agronomical performance at different elevations. The commercial cultivar Kennebec was used as a control. For the morphometric analysis, only Quarantina Bianca did not present significant differences from Kennebec in the mean shape results of a pairwise MANOVA. The four potato varieties showed significantly different results for most of the considered phytochemical parameters, among them the content in ash, total flavonoids, total phenols, chlorogenic acid, caffeic acid, ferulic acid and radical scavenging activity. Only starch and solanine content were not statistically different. A remarkable result was a more consistent yield for all of the varieties with the increase of elevation in the agronomical trials. The results indicate that Genovese landraces are a good choice to make use of marginal lands in the Genovese mountains.

Keywords: *Solanum tuberosum*, mountain agriculture, agro-biodiversity, geometric morphometric analysis, HPLC

Introduction

Potatoes (*Solanum tuberosum* L.) are perennial, herbaceous plants with an annual growing cycle belonging to the Solanaceae family. Potato tubers are an important dietary component of human nutrition (De Masi et al., 2020). They are considered a good source of energy due to their high percentage of digestible carbohydrates and low fat content, their high-quality proteins and minerals (Burlingame et al., 2009) and, even though potatoes are not a high fibre food, their possible role in providing a significant source of fibre for those who eat them regularly (De Masi et al., 2020; Seijo-Rodríguez et al., 2018). Water is the most abundant component in these tubers, with a moisture content ranging from 70 to 80% (Burlingame et al., 2009), and the dry matter content influences the final use of the product: transformation into chips, mashed potatoes, salads and so on (Benvenuti et al., 2001). Potatoes are mainly composed of carbohydrates (about 80% of dry matter), primarily in the form of starch; due to the size of the starch granules, cooked potatoes are considered a highly digestible food. This food is a source of vitamin C, which plays an important antioxidant function, and potassium (the mineral contained in greatest quantity together with phosphorus and magnesium); this tuber also contains small amounts of high-quality proteins (Seijo-Rodríguez et al., 2018). The nutritional composition of potatoes is influenced by a number of factors (cultivar, agricultural practices, agroclimatic factors or type of soils, ripening stage and storage conditions), but the variety is the most important factor affecting nutritional composition (Rodríguez-Galdón et al., 2012).

As part of its high adaptability to growing in diverse environments, this tuber also contains phytonutrients or bioactive compounds, which are the largest groups of secondary metabolites that occur as a reaction to the environment. Some secondary metabolites are synthesized by the plant as a mechanism of defence, such as glycoalkaloids; one of the main glycoalkaloids present in potatoes is α-solanine. The concentration of α-solanine in potatoes depends on the variety, agronomic practices, pesticide use, stress, light exposure and conservation of the harvest. In particular, a-solanine is mainly concentrated in the skin and may pass from the skin to the pulp after cooking; the average content of solanine in the potatoes is 7.5 mg $100g^{-1}$ of fresh weight (Fogelman et al., 2019). Also, potato is a source of antioxidant compounds, including phenols, flavonoids, folates, amino acids, anthocyanins, vitamins and carotenoids (Ezekiel et al., 2013; Valcarcel et al., 2015). Phenolic compounds are secondary metabolites that are synthesized during normal plant development (Cheynier, 2012) and in response to stress conditions such as infection, wounding and UV radiation, among others (Beckman, 2000). Chlorogenic acid with its isomers is the main phenolic acid in potatoes, and caffeic acid together with ferulic acid are important precursors (Friedman et al., 1997).

The potato is native to the Andes of South America where it was already cultivated two thousand or more years before the conquest of the pre-Columbian civilizations (Benvenuti et al., 2001). This "ancient crop" is grown to produce tubers used for human consumption (it is a staple food in some countries) but also for industrial and zootechnical uses (Luthra et al., 2018; Şanli, 2016). Potato cultivation provides the highest production according to the energy to protein ratio per hectare compared to all other crops, and it is the most cultivated species after the cereals (corn, wheat, rice) (Abbasi et al., 2016; Luthra et al., 2018). Due to its nutritional value and its adaptability to environmental conditions, this tuber is cultivated all over the world (Fogelman et al., 2019; Seijo-Rodríguez et al., 2018).

The high adaptability and productivity of this crop have conditioned its diffusion across different regions, allowing its cultivation both in the plains and at high elevation and latitudes (Benvenuti et al., 2001). This has led to the formation of numerous varieties, landraces (Camacho Villa et al., 2005) and ecotypes of this Solanaceae through anthropic or natural selection; there are close to 5000 varieties of potato worldwide (Burlingame et al., 2009; Ezekiel et al., 2013; Seijo-Rodríguez et al., 2018). They are classified according to the intended use (consumption potatoes, potato processing industry), cycle time, and physical characteristics of the tubers, including

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shape, colour, and appearance of the skin or colour of the flesh. Potato landraces, varieties and morpho-ecotypes constitute a large portion of plant agrobiodiversity, which is the result of natural selection processes and the careful selection and inventive developments of culturally diverse farmers, herders and fishers over millennia (FAO, 2019). This biological and cultural heritage has been preserved by farmers for centuries, but today it is severely endangered (GIUPPONI et al., 2020), largely due to the utilization of few commercial cultivars, principally the most productive, in intensive agriculture.

The cultural information or historical memory and the multiplication and conservation of landraces conducted by local farmers and exchange of seeds between them has always been informal and has never been regulated by written rules (MIPAAF, 2013). In this sense, strategies to preserve landraces nearing disappearance are important (GIUPPONI et al., 2020) to the extent that Italy is taking steps to create a national system of conservation and enhancement of agrobiodiversity aimed at protecting local genetic resources of food and agricultural interest from the risk of extinction and genetic erosion (Law 2015/194) (SANTAMARIA et al., 2016). This protection system provides for the constitution of the Agro-biodiversity National Register that has been recently undertaken (DM 2019/39407; https://rica.crea.gov.it/APP/anb/) and that contains all the available information regarding landraces/varieties present in the various Italian regions. Nonetheless, a large portion of the agro-biodiversity of many regions remains uncharacterized (GIUPPONI et al., 2020), and thus, has not been numbered in the Register.

Many varieties and landraces are grown and preserved by farmers, hobbyists or consortiums of protection such as the *Consorzio della Quarantina – Associazione per la Terra e la Cultura Rurale*, whose aim is to support the rural world in order to protect and promote the cultivation of local varieties and landraces in mountain areas. This consortium carries out the cultivation, production and sale of numerous potato varieties in the Ligurian Apennines of Italy (ANGELINI, 2001; Consorzio della Quarantina, http://www.quarantina.it).

These types of consortiums or associations for the protection and exploitation of local landraces can receive great benefit from the capabilities of research centres to study and promote the biological and cultural heritage that they grow and preserve on-farm.

Landraces are characterized by a lack of formal crop improvement, and their peculiarity is not being the most productive varieties but the ones linked to the territory and the traditional farming systems (CAMACHO VILLA et al., 2005). Thus, a great part of their value is the possibility to distinguish them from other varieties and investigate whether they have unique nutritional characteristics that can be used to promote their cultivation (CASSANI et al., 2017) or if they have a unique adaptation to grow in particular environments, such as mountains (GIUPPONI et al., 2018).

The aim of the present research is to provide phytochemical and morphometric characterization of three potato landraces from the Ligurian Apennines (Quarantina Bianca, Quarantina Prugnona and Rubra Spes), analysing also their bio-agronomical performance at different elevations through the cultivation of field trials in the Ligurian Apennines. The geometric morphometric analysis of tubers was conducted according to modern scientific methods (GIUPPONI et al., 2019; GIUPPONI, 2020), while the phytochemical analysis assessed different parameters to characterize the pulp flesh (ash, starch, solanine, total flavonoids, total phenols, chlorogenic acid, caffeic acid, ferulic acid content and radical scavenging activity).

Materials and methods

Plant material

The three potato landraces considered in this research are:

- Quarantina Bianca – This potato variety derives its name from its short agricultural cycle that is particularly suitable for mountain areas. There is evidence of its presence in the area of Genoa in 1880, but its characteristics suggest possible derivation from Patraque Blanche, a French variety cultivated at the end of eighteenth century, or Institut de Beauvais, a variety which was probably selected from the former in the middle of nineteenth century. Quarantina Bianca has an irregular round/round-oval tuber with smooth, yellow skin and white flesh, blue-violet base of sprouts, pink shade of medium-depth sprouts and white flowers.
- Quarantina Prugnona – The name of this potato variety comes from its short agricultural cycle that is particularly suitable for mountain areas and its dark skin. It is similar to the French variety Institute de Beauvais Violette selected in the middle of nineteenth century. It has an irregular round/round-oval tuber with smooth, yellow-violet skin and white flesh violet base of sprouts, dark violet medium-depth sprouts, and white flowers with an indigo-violet central shade. Quarantina Prugnona is considered by farmers to be rustic and adapted to higher elevation.
- Rubra Spes – This potato was probably derived from a mutation of Quarantina Bianca. It is a more rustic and productive variety with the organoleptic features of Quarantina Bianca. It has red skin, white flesh and deep sprouts with a pink base. Flowers and leaves are typically shades of violet/red.

In addition to the three landraces mentioned above, a commercial variety (Kennebec) was included as a control (Fig. 1). Kennebec was developed in 1948 in the US from the crossing (Chippewa × Kathadin) × (Earlaine × W-ras), and it is the most popular cultivar in Italy, where it arrived in the 1960s, especially in mountain regions due to its relatively short cycle. It has a round-oval tuber, with deep sprouts, light skin and white flesh.

Tubers of the four genotypes were obtained from the *Consorzio della Quarantina – Associazione per la Terra e la Cultura Rurale* (http://www.quarantina.it/category/il-consorzio/). The consortium was created in 2000 from the initiative of 20 farmers from the province of Genoa, Italy and as a result of the research by Massimo Angelini and Co.Re.Pa. (committee for the valorization and recovery of Genovese mountain potato heritage, which in 2003 expanded its activity to all ancient landraces and breeds of the Genovese mountains and recovery of rural mountain activities) (ANGELINI, 2001).

The representative sampling for morphometric, phytochemical and agronomical evaluations was performed using 50 kg of each variety provided by the consortium from the members' previous harvest (September 2018). The samples were divided for the morphometric and phytochemical characterization and field trials at different elevations. Sample material for the exploratory phytonutrient analysis was prepared immediately: about 30 tubers of a similar size of each variety were bulked together. Whole potatoes were then individually washed with water and dried with absorbent paper tissue, and half of them were preserved in an unaltered state at a cool temperature in the dark. The other half was lyophilized in the following way: tuber flesh and skin were manually separated, flesh was cut into small pieces, shock frozen under liquid nitrogen and stored at -80 °C until lyophilization in a freeze dryer; the lyophilized samples were then hermetically sealed and kept refrigerated.

Geometric morphometric analysis

Thirty tubers for each potato landrace (Bianca, Prugnona and Rubra Spes), randomly collected from the *Consorzio della Quarantina* and thirty tubers of Kennebec were used for the outline analysis (elliptical Fourier descriptors analysis). Each tuber was placed on a white support table and photographed using a Canon EOS 2000D digital camera positioned perpendicular to the support surface. The images of the tubers were processed using Adobe Photoshop software: the shadows were removed, and the images were transformed to black and white. The outline coordinates were extracted with Momocs

1.3.0 (Bonhomme et al., 2014; Claude, 2008) in an R environment (R Development Core Team, 2019) and converted into Fourier coefficients considering 12 harmonics that gathered at least 99% of the total harmonic power (Bonhomme et al., 2014). In order to control left/right asymmetry, the tubers were flipped in the same direction (considering as apex the most acute part of the tuber), and then a landmark was defined at their base as a starting point for importing outline coordinates. The contours were centred, and the outline analysis was performed without numerical normalization. Principal component analysis (PCA) was conducted on the matrix of coefficients in order to reduce dimensionality, and the samples were plotted on the first two axes (principal components). Linear discriminant analysis of principal components (DAPC) (Jombart et al., 2010) was performed, retaining 15 principal components (PCs). The mean shape of the tubers of each potato genotype was reconstructed using the MSHAPES function of Momocs and multivariate analysis of variance (MANOVA) was performed to evaluate the significance of tuber shape differences between the four genotypes. Finally, pairwise MANOVA was used to highlight the differences between each pair of genotypes.

Phytochemical analysis

Ash and starch were determined using fresh potatoes, while the following indices were determined using the dried potatoes samples: α-solanine, total phenols, phenolic acids (ferulic, caffeic and chlorogenic), total flavonoids and radical scavenging activity as 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition. For each cultivar, four biological replications of each analysis were considered.

All the reagents, solvents and standards used were purchased from Sigma-Aldrich (Milan, Italy). All reagents and standards used were HPLC grade, and purified water from a Milli Q system was used throughout the experiments. The SPE cartridges (GracePureTM SPE C18-Max) were purchased from SepaChrom (Milan, Italy).

The ash content was determined according to Association of Official Agricultural Chemists (AOAC) protocol (AOAC, 1990), while starch quantification was estimated by Ewers polarimetric method in accordance with protocol IS0 10520, 1997(E).

Solanine content in the flesh was determined by high-performance liquid chromatography (HPLC), which was conducted on a Varian ProStar series ternary LC system with a UV-VIS detector Varian Model 345. An RP 18 (Lichrosphere, Phenomenex, 5 μm 250x 4.60 mm i.d.) column was used with acetonitrile:potassium dihydrogen phosphate 0.01M 36:64 as the mobile phase. The isocratic elution was performed at a flow of 0.9 ml min^{-1} and monitored at 200 nm. The retention time for α-solanine was approximately 8.5 min. The extraction for solanine quantification was performed according to the method of Hossain (Hossain et al., 2014), and an SPE clean-up for the crude extracts was used. This procedure includes an equilibration step using 6 ml of methanol followed by an equilibration step with water (3 ml). The crude extract was evaporated and re-dissolved in water, 1 ml of sample was loaded, washed with water (3 ml) and eluted with 3 ml of water. The last eluate was evaporated in vacuum and re-dissolved in methanol (0.1 or 0.5 ml) for the analysis. A linear correlation between solanine concentration and area of peak was obtained for the covered concentration ranges from 0.11 to 1.75 μg/ml, sustained by the regression coefficient ($R^2 = 0.9979$).

Total phenolic compounds were determined by the Folin-Ciocalteu photo-colorimetric method (Slinkard et al., 1977) and measured as equivalent of gallic acid.

The extraction followed the method of Seijo-Rodríguez et al. (2018), modified. The lyophilized samples were ground to a fine powder using a mortar. The ethanol extracts were obtained by mixing 2 g of potato freeze-dried powder with ethanol 80% (v/v) to a final volume of 20 ml. This mixture was stirred in darkness at 70 rpm for 5 hours. After centrifugation at 4,000 rpm for 10 min, the supernatant was collected, filtrated with a Whatman® syringe filter pore size 0.2 μm and evaporated to dryness on a rotary evaporator under reduced pressure at 40 °C. Then, the dry extract was mixed with 2 ml ethanol HPLC grade and re-filtered.

The spectrophotometric method used was that described by Singleton and Rossi (1965), modified. The potato extract (0.2 ml) was mixed with 2 ml of double distilled water and 0.2 ml of Folin-Ciocalteu reagent. This mixture was gently shaken and left to rest for 2 min. Then, 0.8 mL of 5% (w/v) Na_2CO_3 was added, and finally 5 ml of distilled water was added. Ethanol 80% was used as a control. Finally, the solution was incubated in darkness for 1 h at room temperature, and the absorbance was read at 765 nm in a UV/Vis spectrophotometer Cary® 50. The results were expressed as μg gallic acid equivalent per mg of dry weight of potato (μg GAE/mg of dry weight) derived from the standard curve (10-250 μg/ml; $y = 0.0017x + 0.006$, $R^2 = 0.9997$).

The same extracts were used to determine the main potato phenolic acids (ferulic, chlorogenic and caffeic) following the method of Friedman et al. (2017), modified. The HPLC system used was an LC Agilent series 1200 (Waldbronn, Germany), consisting of a degasser, a quaternary gradient pump, an auto-sampler and a UV-Vis detector (Waldbronn, Germany). A Phenomenex Lichrospher C18, 4.6 × 250 mm, 5 μm column (Torrance, CA, USA) was used for this analysis with a column flow of 1 ml. Sample injections were made at 20 μl for all samples and standards. The run time was 40 min, with 5 min post-run time. The method was conducted with the details that follow: column oven (20 °C), mobile phase A (0.1% formic acid), mobile phase B (acetonitrile), flowrate (1 mL/min), needle wash (100% acetonitrile), injection volume (20 μL) and detection at 325 nm. The gradient applied was 0 min (5% B), 9 min (15% B), 15 min (20% B), 20 min (30% B), 25 min (40% B), 27 min (70% B), 29 min (90% B), 35 min (90% B), 37 min (5% B) and 40 min (5% B). Calibration curves were constructed with the commercial standards. The working standard mixture solutions were made by diluting the appropriate amount of each stock standard solution to obtain 4 calibration levels (final concentrations of 250, 100, 50 and 5 μg/mL; chlorogenic acid: $y = 15.287x - 69.556$, $R^2 = 0.9993$; caffeic acid: $y = 29.956x - 198.83$, $R^2 = 0.9919$; ferulic acid: $y = 42.837x - 110.72$, $R^2 = 0.9995$). The retention time was approximately 13.6 min for chlorogenic acid, 16.3 for caffeic acid and 21.7 for ferulic acid. The limit of detection (LOD, 1 μg/ml) was calculated according to the S/N ratio 3.

Total flavonoid contents of the potato cultivars, in contrast, were determined using the $AlCl_3$ method (Dewanto et al., 2002). Quercetin was used as the standard and the amount of total flavonoid compounds was reported as μg quercetin equivalent/mg dry weight (DW). Briefly, 1 ml of extract was added to 8 ml of ethanol 80% and 200 μl of $NaNO_2$ solution (5%); after 6 min, 200 μl of $AlCl_3$ (10%) was added, and after 5 min, 600 μl of NaOH solution at 4% was added. The absorbance of solution was measured at 415 nm and 80% ethanol was used as a control. Total flavonoid content was expressed as quercetin equivalents derived from the standard curve (22-220 μg/ml).

Finally, the ability of phenolic compounds to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. In this assay, activity is measured as the relative decrease in absorbance at 520 nm as the reaction between DPPH and antioxidant progresses. The DPPH radical scavenging activity is plotted as a function of μg/ml sample concentration. Percent activity was observed to increase with sample extract concentration between 0.05-0.25 μg/ml. Radical scavenger activity was reported as IC50 and Trolox was used as a control sample.

Phytochemical data were analysed using a one-way ANOVA test (once the assumptions of normality of group data and homogeneity of variances had been verified) in order to highlight significant differences ($P < 0.01$) in the chemical parameters of the four potato genotypes. When significant phytochemical differences were found between genotypes, the Tukey post-hoc test was carried out to detect the differences between each pair of cultivars. Statistical analyses were performed using R 3.5.2 (R DEVELOPMENT CORE TEAM, 2019).

Experimental fields and biological-agronomic analysis

Potato tubers for the biological and agronomical evaluations were field grown according to best agricultural practices established by the consortium: ploughing and fertilization with 35 t/ha of manure in autumn, harrowing in spring, and furrowing before potato planting. The potatoes were planted at three experimental fields located in the Ligurian Apennines of Italy (Genoa province) at different elevations (Tab. 1, Fig. 1). The chemical-physical characteristics of the soil of each experimental field are reported in Tab. 2. The distance between the rows was 70 cm, the distance between tubers was 30 cm, and the depth of planting was about 7 cm. In each farming system, the experiment was laid out in a randomized design, and 50 seedling tubers (size 35-45 mm, sprouting) of each variety were planted at each field station on the 22nd of June 2019 and harvested at plant senescence (approximately 110 days after planting). The following agronomical data were collected from each experimental field where the potatoes were cultivated:

- Plant height – the distance between the apex of the shoot and the ground level was measured on 10 randomly chosen plants. Plant height was measured during the period of full flowering using a measuring rod.
- Tuber length – measured with a calibre, utilizing the larger dimension of the tuber. This data was collected from 30 randomly selected tubers for each genotype.
- Tuber width – measured with a calibre, utilizing the second largest dimension (orthogonal to the first) of 30 tubers for each genotype randomly selected.
- Yield – calculated as the ratio between the weight of the harvested tubers and the weight of the tubers planted in a known area unit (4 m^2) within the experimental fields. This data was collected in triplicate in each field.

Tab. 2: Physico-chemical properties of soil of each experimental field (data source: CRC Ge.S.Di.Mont., 2019). The identification code of each field is the same as that used in Tab. 1.

Parameter	Experimental field 1	Experimental field 2	Experimental field 3
Skeleton (%)	26.15 ± 9.36	37.02 ± 2.05	37.63 ± 3.20
Sand (%)	60.62 ± 2.26	51.90 ± 1.57	62.48 ± 1.10
Silt (%)	30.33 ± 1.98	38.87 ± 2.11	25.67 ± 0.63
Clay (%)	9.05 ± 1.98	9.23 ± 0.72	11.85 ± 0.58
Texture (USDA)	Sandy loam	Loam	Sandy loam
pH (H_2O)	6.56 ± 0.03	5.62 ± 0.03	5.64 ± 0.02
Organic matter (%)	7.12 ± 0.74	7.23 ± 0.35	6.74 ± 0.47
NO_3 (mg kg^{-1})	49.50 ± 2.12	29.00 ± 0.71	39.50 ± 1.77
NH_4^+(mg kg^{-1})	1.50 ± 0.14	3.65 ± 0.04	3.25 ± 0.11
K (mg kg^{-1})	100.5 ± 4.95	43.5 ± 1.06	121.5 ± 3.89
P available (mg kg^{-1})	8.5 ± 0.71	6.5 ± 0.35	8.0 ± 0.71

Tab. 1: Characteristics of the three experimental fields.

Code	Municipality	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	Slope (°)	Exposure	Farm
1	Rovegno (GE)	44° 34' 40''	9° 16' 48''	660	4	West	Az. Agr. Michele Ravera
2	Rezzoaglio (GE)	44° 31' 10''	9° 24' 30''	800	2	North-West	Az. Agr. Villa Rocca
3	S. Stefano D'Aveto (GE)	44° 33' 00''	9° 25' 39''	900	2	South	Az. Agr. I Moretti

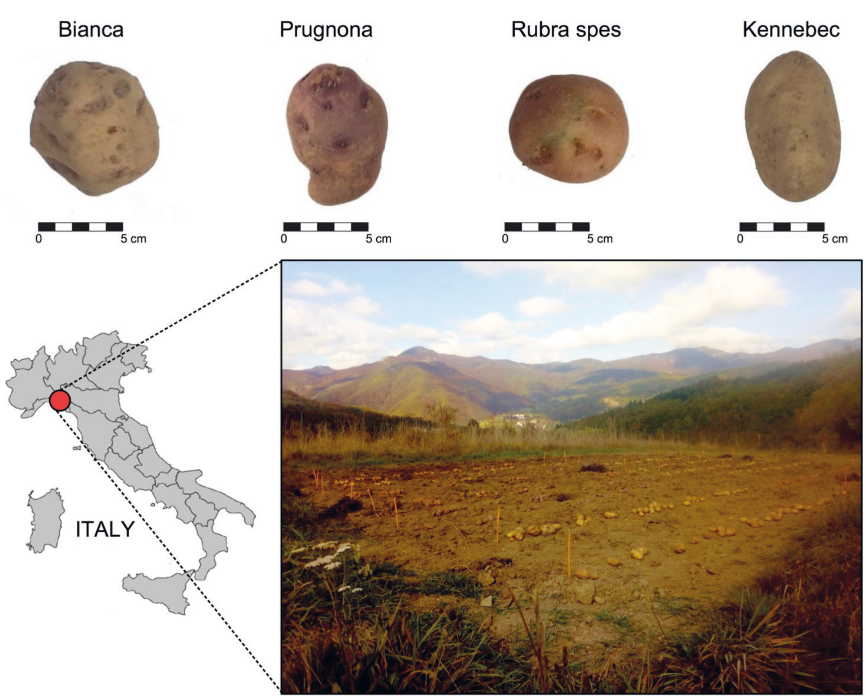

Fig. 1: Tubers of the four potato genotypes considered in this research, three are landraces of the Ligurian Apennines (Bianca, Prugnona and Rubra Spes), and one is a commercial cultivar (Kennebec). An experimental field located in Ligurian Apennines is shown.

Biologic-agronomic data were analysed using a one-way ANOVA test to highlight the significant differences ($P < 0.05$) attributable to experimental field effects on each potato genotype. In addition, PCA was performed on agronomical data to highlight the factors contributing to these differences, particularly elevation and soil properties. Statistical analyses were performed using R 3.5.2 (R Development Core Team, 2019).

Results

Tuber shape

Fig. 2 shows the PCA biplot of the Fourier coefficients calculated for tubers of the four potato genotypes and the results of DAPC. Along the first principal component (PC1), the reconstructions of the tuber shape become rounder, while along the PC2, the apexes become less acute. The samples are greatly overlapped in the two biplots of Fig. 2, while results of MANOVA test show significant shape differences between the tubers of the four genotypes ($F_{3,116} = 1.897$; $P < 0.01$). Fig. 3 shows the mean shape of tubers of each genotype. While the mean shape of Quarantina Prugnona, Quarantina Bianca and Kennebec is ovate, that of Rubra Spes is elliptical. The results of pairwise MANOVA (Tab. 3) demonstrate that only Bianca does not present significant differences from Kennebec. Furthermore, there are significant differences between the shape of the Rubra Spes and Quarantina Bianca tubers.

Phytochemical features

The four potato genotypes differed significantly in most of the phytochemical aspects measured (Tab. 4). In fact, the only two parameters that did not show significant differences were the content in starch and solanine. The ash content (in percentage) was similarly high in Rubra Spes (6.0%) and Quarantina Bianca (5.7%), while Kennebec had the lowest value (4.2%); the ash content of Quarantina Prugnona was 4.9% (Fig. 4). For the content in active metabolites, Kennebec had the highest content in total phenols (3.7 µg GAE mg^{-1}) and the lowest total flavonoid content (0.30 µg QE mg^{-1}), while Quarantina

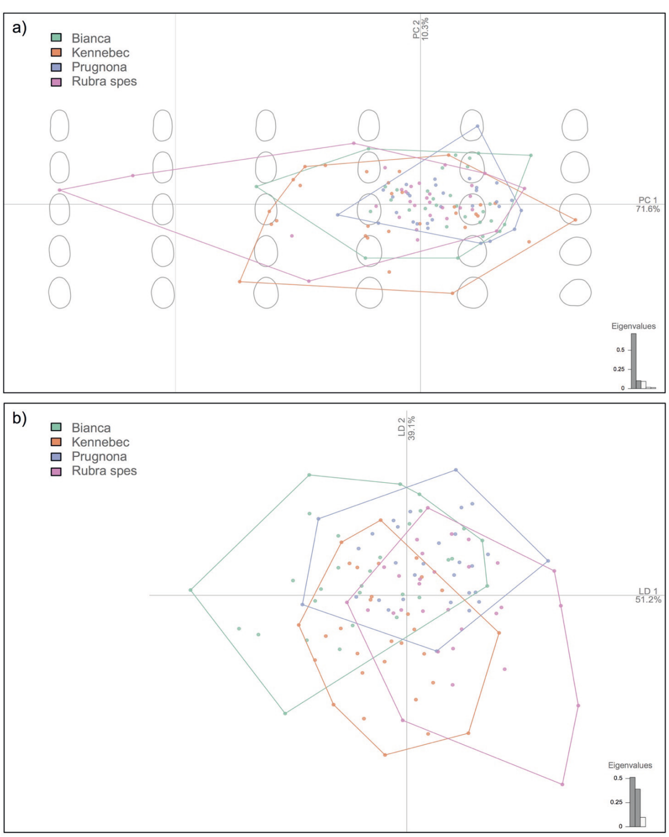

Fig. 2: PCA biplot (a) resulting from outline analysis of the tubers of the four genotypes and linear discriminant analysis of principal components (DAPC) biplot (b). Grey figures in the background (a) show reconstructions of tuber shape according to each position in the multidimensional space.

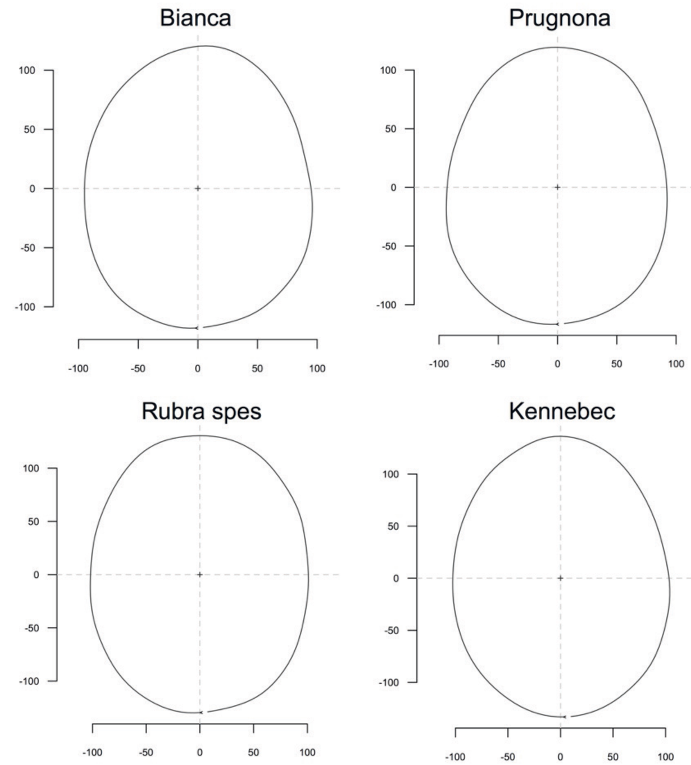

Fig. 3: Mean shape of tubers assigned to the four potato genotypes according to elliptical Fourier descriptors analysis.

Tab. 3: *P*-values returned by pairwise MANOVA performed on the Fourier coefficients of the tuber shape of each potato genotype. *P*-values < 0.05 are highlighted in bold.

Genotype	Bianca	Kennebec	Prugnona	Rubra spes
Bianca	-	0.1179	0.4035	**0.0167**
Kennebec		-	**0.0008**	**0.0193**
Prugnona			-	0.6271
Rubra spes				-

Tab. 4: One-way ANOVA results of potato genotype effect on phytochemical parameters. Key: *, significant ($P < 0.01$); ns, not significant.

Source of variance	Mean square	$F_{3,8}$	*P*	
Ash	1.91	8.339	<0.01	*
Starch	2.5	0.041	0.99	ns
Solanine	17.64	0.727	0.56	ns
Total flavonoids	0.014	96.34	<0.01	*
Total phenols	2.5922	20.35	<0.01	*
Chlorogenic acid	122.04	31.75	<0.01	*
Caffeic acid	11.64	32.24	<0.01	*
Ferulic acid	10.34	3130	<0.01	*
DPPH	0.243	50.06	<0.01	*

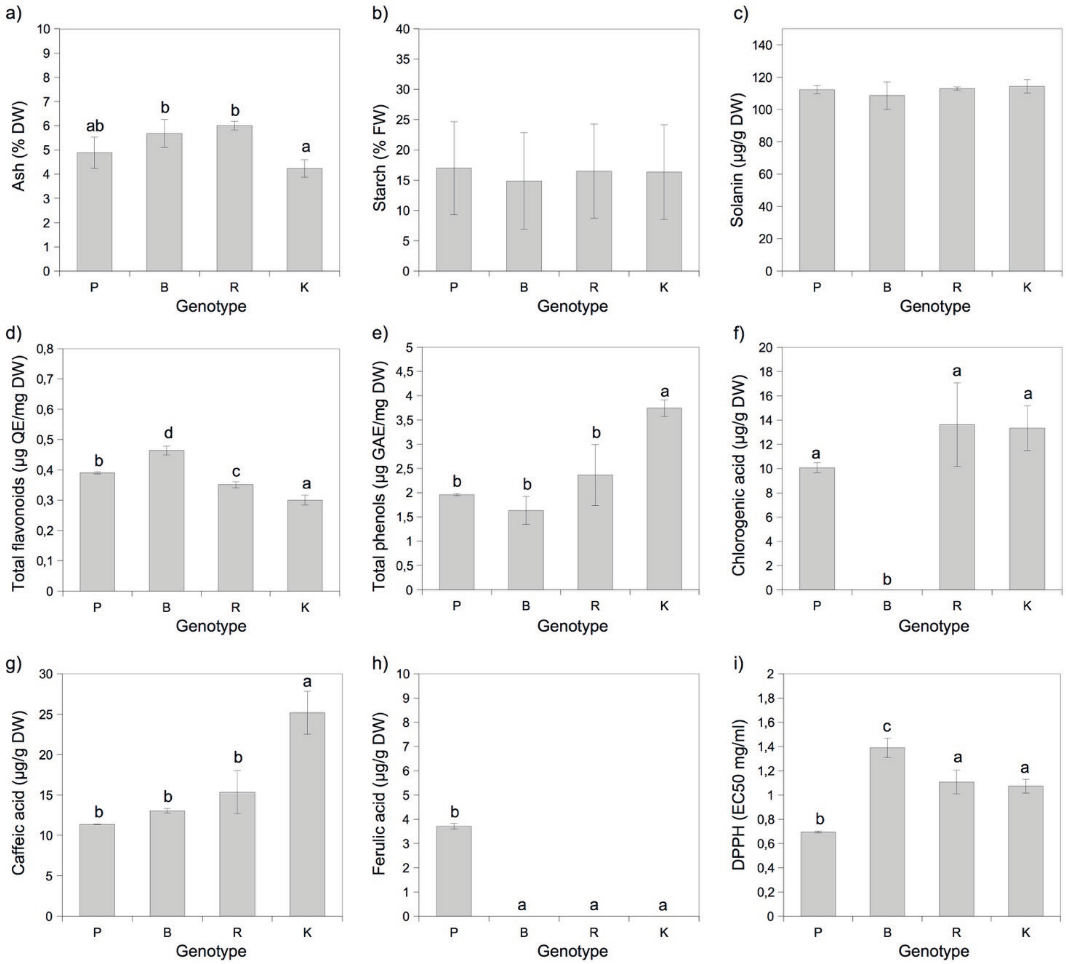

Fig. 4: Phytochemical features of the tubers of the four potato genotypes (P, Prugnona; B, Bianca; R, Rubra Spes; K, Kennebec): ash (a), starch content (b), solanine (c), total flavonoids (d), total phenols (e), chlorogenic acid (f), caffeic acid (g), ferulic acid (h), antioxidant activity (DPPH) (i). Different letters above the error bars indicate significant differences ($P < 0.05$) among genotypes according to the Tukey post-hoc test.

Bianca was the opposite, with the highest flavonoid content (0.46 µg QE mg^{-1}) and the lowest total phenols (3.7 µg GAE mg^{-1}) (Fig. 4). Quarantina Prugnona and Rubra Spes had similar total phenol content, in between Kennebec and Bianca, while the total flavone content of Prugnona was slightly higher than Rubra, but always in between Kennebec and Bianca (Fig. 4).

Concerning the qualitative analyses, the compounds were present in all the cultivars, but ferulic acid only exceeded the LOD value in Quarantina Prugnona, while chlorogenic acid was under the LOD in Quarantina Bianca, and the probable presence of isomers of this acid in Kennebec was clearly indicated, as previously demonstrated in Friedman et al. (2017). Chlorogenic acid content was similar in the three varieties where it was detected, while caffeic acid was significantly higher in Kennebec (Fig. 4). Finally, DPPH radical scavenging activity was the highest in Bianca (EC50 1.39 mg ml^{-1}) and lowest in Prugnona (EC50 0.01 mg ml^{-1}), while Rubra Spes and Kennebec had similar values (EC50 1.11 mg ml^{-1} and 1.18 mg ml^{-1}, respectively) (Fig. 4).

Mountain environment effects

In the field trials at different elevations, some features were significantly different for single varieties (Tab. 5): we observed taller plants at altitude for Quarantina Bianca and Prugnona, and longer and wider shaped tubers for Kennebec. A remarkable result was a more consistent yield for all the varieties with the increase of elevation (Tab.5; Fig. 5). It was possible to link the elevation trend and the skeleton content in the soil with the increase in yield as shown by the PCA biplots (Fig. 6): the yield increased significantly in fields located at elevations over 750 m a.s.l. with soils containing over 35% skeleton content (Fig. 6).

Discussion

Potatoes are an important source of nutrients for humankind, and they are widespread around the world due to their adaptability, which has given origin to many traditional varieties now at risk of being lost. In this research, the main features of this crop (starch, ash content and bioactive compounds such as solanine, phenols, and flavonoids, further explained in the introduction) were used to characterize cultivars from the Ligurian Apennines of Italy, an area where potato cultivation is very important and has a long historical tradition. Historically, Genoa was a well-known maritime republic, and the first potatoes probably arrived during the travels of the early modern period. However, potato cultivation expanded in the second part of eighteenth century, in part due to the actions of Michele Dondero (1744-1813), a priest from Chiavari, doctor and agronomist, who encouraged potato cultivation, inspired by the "Parmantier Treatise on the Culture and Use of the Potato, Sweet Potato, and Jerusalem Artichoke" (*Traité sur la Culture et les Usages des Pommes de Terre, de la Patate, et du Topinambour*) that was available in Genoa in 1779. Dondero brought potato cultivars from Switzerland and France, and they soon became

Tab. 5: ANOVA results of experimental field effect on biological and agronomic features of each potato genotype. Key: *, significant ($P < 0.05$); ns, not significant.

Parameter	Genotype	Mean square	$F_{2,6}$	P	
Plant height	Bianca	585.70	18.61	0.002	*
	Prugnona	543.00	11.09	0.009	*
	Rubra spes	523.40	3.57	0.095	ns
	Kennebec	403.20	4.30	0.069	ns
Tuber length	Bianca	7.05	3.26	0.110	ns
	Prugnona	1.67	0.91	0.453	ns
	Rubra spes	4.84	2.09	0.250	ns
	Kennebec	23.02	30.44	<0.001	*
Tuber width	Bianca	3.12	7.63	0.022	*
	Prugnona	1.70	1.58	0.281	ns
	Rubra spes	4.01	4.63	0.060	ns
	Kennebec	7.04	9.45	0.014	*
Yield	Bianca	34.52	89.27	<0.001	*
	Prugnona	10.14	20.48	0.002	*
	Rubra spes	12.86	12.70	0.006	*
	Kennebec	16.01	15.41	0.004	*

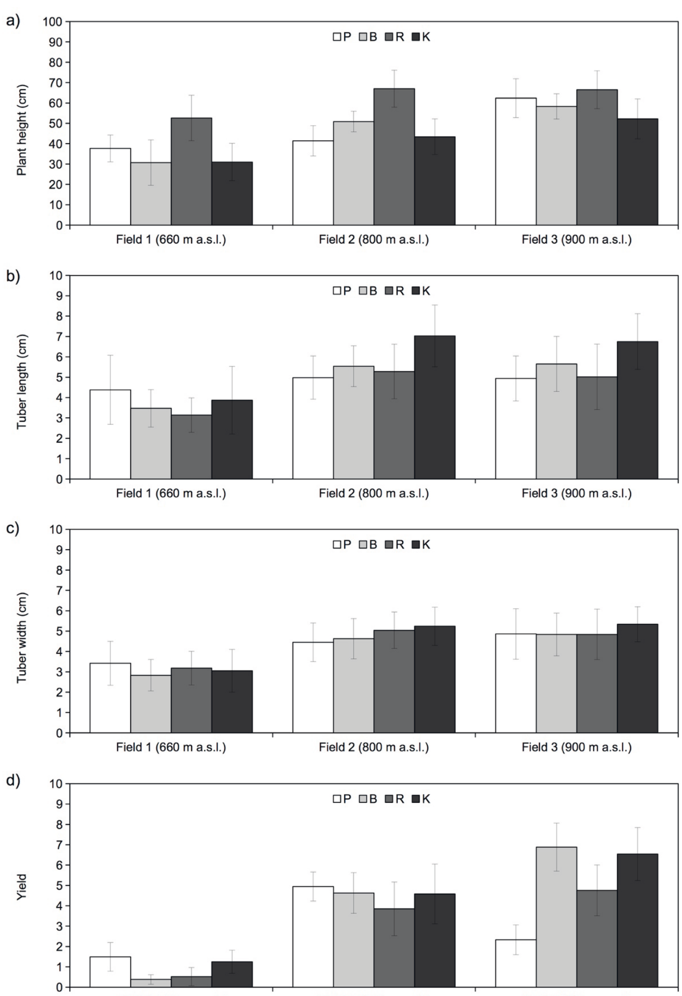

Fig. 5: Biological and agronomic features of the four potato genotypes (P, Prugnona; B, Bianca; R, Rubra Spes; K, Kennebec) cultivated in the three experimental fields.

a primary agri-food product in Roccatagliata. From the nineteenth century onward, they became an important product of the mountain territories surrounding Genoa, including Aveto, Fontanabuona and Graveglia Valley. The first records of local potato varieties, including Giana Riunda, Quarantina Bianca, Cannellina Nera and Morella, come from the final decades of the nineteenth century. Years later, during the economic boom following World War II, the local landraces were gradually replaced with new varieties, such as Kennebec (1948), Monalisa (1979), Desirèe (1962), Primura (1963) and Spunta (1969). These varieties are still popular today, and local landraces have now been limited to cultivation in the most marginal areas for many years; only recently have efforts begun to revitalize them through study, sanitation and replication (ANGELINI, 2001, 2011).

Whether they are new varieties or local landraces, shape, size and colour are important sensory attributes for potatoes. Shape, size and colour are important sensory attributes for potatoes. In this research, the tuber outline analyses demonstrated that there are differences among the four genotypes assessed, particularly between two of the landraces (Quarantina Prugnona and Rubra Spes) and the commercial variety (Kennebec). This result can be attributed to the consistency of Kennebec tubers, which have quite a consistent oval shape compared to the landraces, which present tubers with more variable shapes (even within the same genotype). This might be due to the fact that Kennebec, in contrast to the landraces, is a commercial variety that has been subjected to strong morphometric selection (and genetic depletion) to produce standardized tubers, without necessarily considering the nutritional point of view. The similarity of Kennebec and Quarantina Bianca (Tab. 3) could be due to a genetic similarity, but further genetic investigations should be conducted to affirm this observation.

In addition to sensory attributes, the phytochemical characterization of crop plant varieties is important for their commercial valorization. The phytochemical characteristics of the potato cultivars in this study were almost all statistically different, apart from solanine and amid content. The significantly higher ash content in the traditional varieties is interesting from a nutritional point of view for the supply of high quality minerals, while MONDY et al. (1971) report a positive correlation between phenolic content and bitterness and astringency of potatoes. It is well known that these metabolites play a decisive role in the sensory quality of fruits, vegetables and other plants, affecting characteristics such as bitterness, astringency, colour or smell (CHEYNIER, 2005). Thus, traditional varieties may offer a way to increase the "taste" availability associated with biodiversity. Today, food diversity is being promoted by entities such as Slow Food and, above all, knowledge of food nutrient properties is increasingly demanded by consumers. Furthermore, although some other vegetables and fruits have higher phenolic content, potatoes are the most consumed vegetable in many countries, and this makes their contribution to the intake of phenolic compounds in the human diet of particular interest (DEUSSER et al., 2012; VALCARCEL et al., 2015). These phytonutrients, in fact, promote cardiovascular health and have chemo-protective and anti-inflammatory properties (PARR et al., 2000). Our results demonstrated that the varieties assessed exhibit different phenolic profiles and a statistically different biological activity, confirming the importance of preserving this genetic heritage.

Although the nutrient content of potatoes has been reported to be influenced by various factors (cultivar, agricultural practices, agroclimatic factors or type of soils), variety is in fact the most influential factor (RODRÍGUEZ-GALDÓN et al., 2012; TOLEDO et al., 2006). Numerous studies have demonstrated that the genetic makeup of a potato variety has more effect on its phenolic content than the environment (EZEKIEL et al., 2013; LOMBARDO et al., 2013; GIUPPONI et al., 2018), to the extent that active compounds have been proposed as a means of varietal recognition or a "fingerprint" for ancient varieties (ROJAS-PADILLA et al., 2016). In our research three important

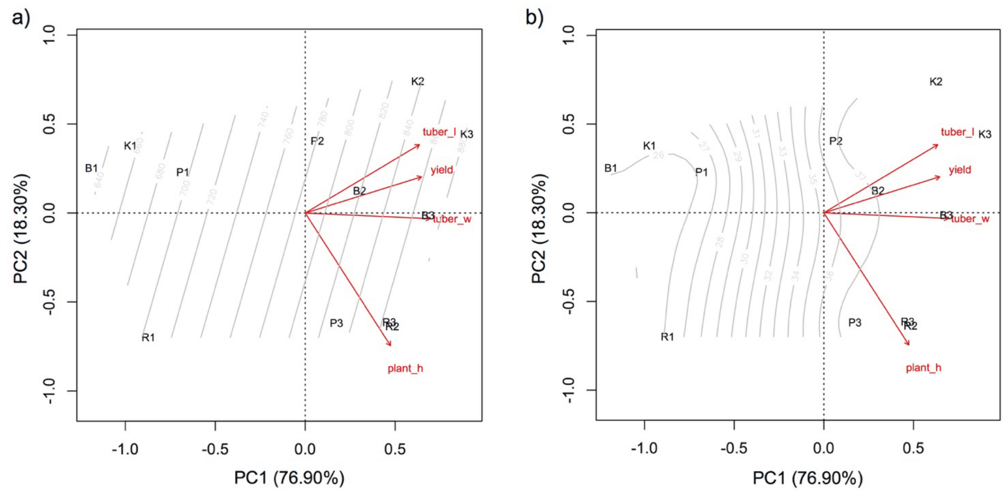

Fig. 6: PCA ordination biplots of potato genotypes (P, Prugnona; B, Bianca; R, Rubra Spes; K, Kennebec) cultivated in the three experimental fields (1, field 1; 2, field 2; 3, field 3), overlapped with elevation (a) and soil skeleton contour lines. Key: tuber_l, tuber length; tuber_w, tuber width; plant_h, plant height; yield, yield of tubers.

phenolic acids were differently expressed in the distinct varieties, and in Kennebec in particular, the peak overlapping around the chlorogenic acid retention time differed. Further investigations utilizing other instruments such as NMR could testify the presence of isomers of chlorogenic acid. Different studies have demonstrated the presence of several isomers of chlorogenic acid in potatoes (Friedman et al., 2017; López-Cobo et al., 2014). Different potato cultivars grown under the same conditions have been found to contain the following isomers: 3-O-caffeoylquinic (n-chlorogenic acid), 4-O-caffeoylquinic (crypto-chlorogenic acid), 5-O-caffeoylquinic (neo-chlorogenic acid), 3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic. Chlorogenic acid is the primary phenolic acid in potatoes, and caffeic acid together with ferulic acid are important precursors (Friedman et al., 1997). In our study, Kennebec was indeed the variety with the highest content in total phenols and the lowest total flavonoid content, while Quarantina Bianca was the opposite, with the highest flavonoid content and the lowest total phenols. The highest radical scavenging activity was associated with Quarantina Bianca, probably due to the low phenol content.

In general, our results indicate that potatoes are a crop suitable to the traditional area of Genovese, in particular the higher elevations, where it enables cultivation of marginal mountain areas. *S. tuberosum* is characterized by high adaptability, and it can be cultivated both on the plains and in the hills. Although the optimum average daily temperature is 15 °C for germination, 20 °C for flowering and 18 °C for the accumulation of carbohydrates, organic acids and other compounds in the tubers (which can halt growth at a temperature of 20 °C), daily thermal fluctuation also favours tuber development (Benvenuti et al., 2001). Thus, the Genovese mountain area, with hot days and cool nights in summer, is ideal for potato cultivation. Water stagnation and rapid changes in soil humidity conditions (drought alternating to saturation) are damaging for tubers, and the ideal soil conditions include optimal organic matter content, and a well-drained loamy, sandy texture (Benvenuti et al., 2001; Parisi, 2011). The presence of skeleton, characteristic of Apennine soils, could have also contributed to the superior development of the tubers at the higher elevation field stations. The soil of the experimental fields utilized in our research, in fact, could have been defined as loam or sandy loam with an increased presence of skeleton in the higher fields. Also, in hilly and mountainous areas farmers report that pest management is often easier. Thus, we can conclude that Genovese landraces are a good choice to make the best use of marginal lands in the Genovese mountains.

Conclusion

This research uses a multidisciplinary approach to characterize three little-known potato landraces of the Ligurian Appenines, namely Quarantina Bianca, Quarantina Prugnona and Rubra Spes. The results demonstrate morphometric and nutritional differences between the tubers of the genotypes assessed. This agro-biodiversity is a resource that could be converted into social and economic value if the genetic potential were adequately promoted through the creation of high-value, unique and sustainable mountain region production chains, following the example of other Italian landraces (Cassani et al., 2017; Giupponi et al., 2018, 2019). This study seeks to provide useful information for the *Consorzio della Quarantina* to preserve the three potato landraces, prevent the loss of their unique characteristics, promote their unique nutritional features and support their on-farm conservation and cultivation. This research also aims to provide the scientific basis to include the three considered landraces in the Italian Agrobiodiversity National Register and the official online Italian database of biodiversity of agricultural interest.

Considering that around the world, the production of many traditional cultivars is diminishing, largely due to their unknown yield potential and lack of information about them (Pazderů et al., 2015), we hope that this kind of research is incentivized, as the study of landraces is of paramount importance for conservation, alimentary, economic and socio-cultural purposes.

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Confict of interest

No potential confict of interest was reported by the authors.

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