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Highlights

- Southern Italy is the place of origin of several bean landraces;
- 'Cerato' and 'Curniciello' are bean landraces from Campania region, Italy;
- Both landraces show different chemometric traits;
- AFLP profiles are important for authentication/traceability of these beans.

Nutritional, metabolic and genetic profiling of 'Cerato' and 'Curniciello' bean landraces from Caserta, Southern Italy

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Short title:

'Cerato' and 'Curniciello' dry beans nutritional characterization

ABSTRACT

Italy represents a territory rich in common bean landraces, most of which have not yet been characterized. Therefore, the proximal composition and metabolic profiles (fatty acid composition, total and free amino acids) as well as total polyphenols and antioxidant capacities of both 'Cerato' and 'Curniciello' dry beans, cultivated in Caserta's rural areas (Southern Italy) were evaluated, in comparison with other local known dry beans. 'Cerato' dry beans have a lower content of crude proteins (21.18 vs 23.41 g/100 g), lipids (1.27 vs 2.08 g/100 g) and total amino acids (16.01 vs. 17.89 g/100 g) with respect to 'Curniciello' dry beans, considering the average values of two different harvest years (2020-2021), although slight statistical differences were found when the two harvest years were analysed separately. Two essential fatty acids (n-6 linoleic and n-3 alpha-linolenic) and oleic acid were the most abundant fatty acids in both dry beans, (~90% of the total). Subsequently, the trypsin and chymotrypsin inhibitory activities in raw and boiled (2 h) dry beans, as well as the α-amylase and α-glucosidase inhibitory activities were investigated, considering their capability to reduce crude protein and carbohydrates intake and assimilation. In addition, AFLP analysis of two landraces shows different polymorphic patterns useful for their authentication and traceability. Overall, our data provide a starting point for promoting the cultivation and consumption of 'Cerato' and 'Curniciello' dry beans, thus contributing to the preservation of local culinary traditions and Italian biodiversity.

Keywords: AFLP analysis; amino acids; antioxidants; food quality; *Phaseolus vulgaris* L.; pulses.

1. Introduction

The common bean (*Phaseolus vulgaris* L.; family Fabaceae) is an annual herbaceous plant that produces pods and edible seeds (Azarpazhooh and Boye, 2012). The plant was independently domesticated in Andean South America and Mesoamerica about 7 millennia ago (Bellucci et al., 2014; Bitocchi et al., 2012; Frascarelli et al., 2023). Indeed, native forms of common bean contain two major gene pools, although a novel pool, the Peru-Ecuador pool, derived from a small area on the western slopes of Andes, has recently been discovered (Debouck et al., 1993; Nadeem et al., 2021). Subsequently, common bean was introduced to Europe by the Spaniard and Portuguese, where the specific pedo-climatic conditions favoured the selection of different landraces (Di Vittori et al., 2017; Nadeem et al., 2021).

More recently, the reassessment by public opinion of the nutritional value of dry beans, considered a good source of crude proteins, starch, fibers, vitamins (especially folate), and minerals (Azarpazhooh and Boye, 2012), has determined the increase in dry bean production (27,71 million tons in 2021) at an annual rate of 1.8% (Knoema-group, 2021). Indeed, consumers, seeking a healthy lifestyle, are reconsidering the consumption of crude proteins from plant-based foods such as dry beans, leading to a decrease in red meat consumption considering its negative effects on human health, because high red meat intake is associated with cardiovascular diseases and cancer (De Oliveira Mota et al., 2019; Fletcher, 2021). In addition, several secondary metabolites found in dry beans have antioxidant and anti-inflammatory effects with important health benefits (Ciudad-Mulero et al., 2020; Ganesan and Xu, 2017). In this context, modern nutritional recommendations, inspired by the Mediterranean diet (Trichopoulou, 2021) encourage consumers to increase the intake of dry beans, despite their limited content of sulphur amino acids (i.e.: methionine and cysteine) content (Azarpazhooh and Boye, 2012).

On the other hand, ongoing climate change (Sampath et al., 2023) is leading to an increase in the susceptibility of common bean crops to many pests and diseases (Meziadi et al., 2016; Mukankusi et al., 2019; OECD, 2016) as well as a decrease in drought stress yield (Papathanasiou et al., 2022; Santos et al., 2021), affecting dry bean production.

Therefore, it is of interest to study local *P. vulgaris* populations, which are landraces locally adapted to the specific pedo-climatic conditions of limited areas. Indeed, natural genetic variation of *P. vulgaris* provides an opportunity to preserve the genetic diversity of this species to improve breeding programs and protect biodiversity (Rivera et al., 2018; Zander et al., 2016).

In this scenario, the Italian territory represents a rich reservoir of common bean landraces, most of them with a higher frequency for the Andean type (Bellucci et al., 2014), contributing to the

biodiversity richness of this territory (Florek and Gazda, 2021; Guarrera and Lucia, 2007). These landraces are cultivated for the local production of typical dry beans (Piergiovanni and Lioi, 2010; Zeven, 1997), consumed as a traditional food (Corrado, 2022).

In previous works, our research group has been involved in the characterization of several legume landraces [chickpea (*Cicer arietinum* L.) (Landi et al., 2021a), lentil (*Lens culinaris* Medik.) (Landi et al., 2015) and grass peas (*Lathyrus sativus* L.) (Tamburino et al., 2012) from Valle Agricola; bean (*P. vulgaris*) from Gallo Matese (Landi et al., 2017)] in the rural areas of Caserta (Southern Italy, Campania region), and still cultivated, resisting intensive cultivation, due to the unique characteristics of these places and the steadfastness of local farmers (Landi et al., 2021b). Therefore, in order to increase knowledge about legume landraces grown in Caserta' territories the objective of this work was to evaluate the biochemical and genetic traits of 'Cerato' and 'Curniciello' bean landraces (**Figure 1**), which are currently still cultivated and appreciated by local consumers.

'Cerato' beans (known as 'fagiolo Cerato' in Italian) are characteristic light-brown beans, cultivated in Alife, a town located in the Volturno valley in the province of Caserta, Campania region. 'Cerato' beans have a very thin skin, which makes them easier to cook. They are mainly used for soups because of their sweet, delicate, and persistent flavour. The 'Cerato' bean is listed in the Campania region's "Agro Biodiversità Campana" project as a local legume to be preserved (CREA, 2021). On the other hand, 'Curniciello' beans, known as 'fagiolo Curniciello' in Italian, are rare and small beans of ancient origin, ochre brown in colour and with a white eye. They are still cultivated in the municipality of Caiazzo located in the transition area between the middle and lower Volturno valley, province of Caserta, Campania region. These beans are protected by the Slow Food Campania organization (Sloow_Food_Campania, 2016).

In light of this, the proximate composition (crude proteins, total lipids, ashes, moisture and carbohydrates), total and free amino acids as well as fatty acids were determined for two consecutive years (2020 and 2021). Moreover, as common beans are known to contain anti-nutritional factors such as trypsin and chymotrypsin inhibitors, we analysed the presence of these protease inhibitors in raw and boiled dry beans. In addition, α-amylase and α-glucosidase inhibitory activities have been investigated, considering their ability to decrease carbohydrates intake and assimilation with potential benefits in controlled dietary plan (Peddio et al., 2022).

Finally, considering the peculiar adaptation of 'Cerato' and 'Curniciello' beans to the local territory as a potential genetic resource for the improvement of breeding programs and the search of possible molecular marker in supply chain traceability protocols, we carried out a molecular characterization of local 'Cerato' and 'Curniciello' landraces by using the Amplified Fragment Length Polymorphism (AFLP) technique to study their genetic diversity.

2. Material and methods

2.1. Chemicals and reagents

The sources of the chemicals and solvents have been described previously (Landi et al., 2021a; Landi et al., 2017) and most of them were obtained from Sigma-Aldrich Solutions (Merk Life Science, Milan, Italy). Standard fatty acid methyl esters (Supelco^{TM} 37 Component FAME Mix) were obtained from Supelco (Bellefonte, PA, USA).

2.2. Material and sampling of 'Cerato' and 'Curniciello' bean seeds

'Cerato' and 'Curniciello' dry beans, harvested in 2020 and 2021, were obtained from custodian farmers, residing in the municipalities of Alife (Coordinates: 41°20'N 14°20'E) and Caiazzo (Coordinates: 41°10'40"N 14°21'50"E), respectively. In particular, three different pools of 'Cerato' dry beans for each year were kindly donated by the farm "Abbazia della Ferrara" (https://www.informazione-aziende.it/Azienda_AZIENDA-AGRICOLA-ABBAZIA-DELLA-FERRARA-DI-ANTONIO-TESTA), while three different pools of 'Curniciello' dry beans for each year by the farm "La Sbecciatrice" (https://www.lasbecciatrice.it/index.php/it/). These farmers have fields dedicated to the cultivation of these bean landraces for many years. Typically, after harvesting, for both pools the pods were sun-dried and then dry beans are separated from the pods and stored, respecting local traditions handed down.

For DNA extraction, dry seeds of both landraces were germinated to obtain seedlings. Specifically, the germination stage was considered completed after 10 days. The temperature and humidity conditions corresponded to an average temperature of 25±1 °C and ~26% humidity, light/dark period of 16 h/8 h (Bulgari et al., 2020). After the germination was completed, the seedlings were collected and subjected to nucleic acid extraction.

2.3. Proximate composition analysis

Crude protein content was measured by the Kjeldahl method [(AOAC 920.87, using nitrogen-to-protein conversion factor (N) = 6.25)] (Horwitz, 2000). Lipid content was obtained by using a Soxhlet apparatus (AOAC 948.22) (Horwitz, 2000) and $CHCl_3$ as extraction solvent, and carbohydrate content was determined as previously reported (FAO, 2003). Ash content (AOAC 923.03) and moisture levels (AOAC 925.10) were determined according to the official AOAC method (Horwitz, 2000).

2.4. Total and free amino acid determination

For the determination of free amino acids, two precipitation steps (80% ethanol followed by 3.0% sulfosalicylic acid) were carried out using 100 mg dry powder of bean samples in the presence of *nor*-Leu as an internal standard (Landi et al., 2021a). For the determination of total (free and protein) amino acids, ~10 mg dry powder of bean samples were hydrolysed in 0.5 mL of 6 M HCl containing 0.02% phenol and *nor*-Leu as internal standard at 110 °C for 20 h, according to AOAC 994.12 official method (Horwitz, 2000). Following hydrolysis, HCl was removed under vacuum and the samples were resuspended in 0.5 mL of 0.2 M lithium citrate buffer, pH 2.2. In order to determine the cysteine content, sample oxidation in the presence of performic acid was performed before the hydrolysis (Landi et al., 2021a).

Aliquots of hydrolysed and non-hydrolysed samples were directly analysed on a Biochrom30 amino acid analyzer (Biochrom, Cambridge, UK), equipped with a polyvinyl sulfonate cation exchange column for physiological fluids, a post-column ninhydrin derivatization system and a two-channel detection system set at 570 and 440 nm (to allow proline and hydroxyproline detection) (Moore and Stein, 1963).

Each sample was prepared individually and analysed in triplicate.

2.5. Gas chromatographic analysis of fatty acid methyl esters

Fatty acid methyl ester content analyses were performed as previously reported (Landi et al., 2017). Briefly, each crude $CHCl_3$ lipid extract (see paragraph 2.3) was dissolved in 0.2 mL of 2 M KOH in methanol. The solution was stirred for 30 min at 25 °C, mixed with heptane (0.8 mL) and then, centrifuged at 4,200 g for 10 min, 4 °C (Beckman GS-15R (Beckman Coulter, CA, USA). The upper organic phase (1.0 µL) was analysed by GC (100 m × 0.25 mm i.d., 0.2 µm SP2380, fused silica capillary column; Supelco, Sigma-Aldrich). The fatty acid methyl esters were identified by comparing their retention times with those of the standard fatty acid methyl esters (Supelco™ 37 Component FAME Mix). Metabolite quantitation was carried out using the internal standard method. A calibration curve was obtained by adding 50 µg of nonadecanoic acid, used as internal standard.

Each sample was prepared individually and analysed in triplicate.

2.6. Determination of trypsin and chymotrypsin inhibitory activities

The dry powders of bean samples (1.0 g each) were extracted overnight under stirring at 4 °C in 80 mM Tris•Cl, pH 7.8, containing 0.1 M $CaCl_2$ (1:4; w/v). The raw extracts were centrifuged at 24,000 g (Centrifuge Avant J-25, Beckman Coulter), 4 °C for 60 min. The supernatants were filtered on Miracloth (pore size: 22-25 µm) and stored at -80 °C before use. In addition, to evaluate the effect

of boiling treatment on the protease-inhibiting capacity, dry bean seeds (10 g) were first boiled in water (50 mL each for 120 min.). Subsequently, cooked and drained bean seeds were extracted as described above. The concentration of extracted proteins was determined with the Bio-Rad Protein Assay kit following the manufacturer's instructions and using bovine serum albumin (BSA) as the standard.

Trypsin and anti-trypsin activities, as well as chymotrypsin and anti-chymotrypsin activities, were determined using Nα-p-Tosyl-L-arginine methyl ester hydrochloride (TAME) and N-Benzoyl-L-tyrosine ethyl ester (BTEE) as substrates, respectively (Poerio et al., 2003). An inhibitory unit is defined as the amount of inhibitor that produce a 30% decrease in enzyme activity under assay conditions (Rocco et al., 2011). The IC_{50} values of trypsin and α-chymotrypsin activities (i.e.: the half maximal inhibitory concentration) of raw protein extract were calculated following a previously described procedure (Landi et al., 2021a; Landi et al., 2017).

Each sample was prepared individually and analysed in triplicate.

2.7. α-Amylase and α-glucosidase inhibitory properties

A synthetic chromogenic substrate (2-chloro-*p*-nitrophenyl-α-D-maltotrioside, CNP-G3) has been used to determine the inhibition of α-amylase enzymatic activity (Suganuma et al., 1997). α-amylase (5 E.U.) from porcine pancreas were incubated for 10 min at 37 °C in the presence of sodium phosphate buffer 250 mM, pH 6.5, NaCl 60 mM, $CaCl_2$ 5 mM, KSCN 500 mM. Then, 50 μL of substrate 9 mM solution were added. The enzymatic activity hydrolyzes CNP-G3 releasing 2-chloro-*p*-nitrophenol, that can be monitored at 405 nm in microplate spectrophotometer UV/VIS MultiskanGo (Thermo Fisher Scientific, Monza, Italy). Negative controls were performed in the absence of enzyme or substrate. The assay was repeated in the presence of adequate amounts of extracts to determine inhibition. As a positive control, a commercial dietary supplement extract (based on *P. vulgaris*) was purchased from a local market and analyzed as well. The supplement was based on a purified common bean extract. Each tablet contained 500 mg of extract and was processed using a protocol similar to that of *P. vulgaris* seeds.

One amylase E.U. was defined as the amount of enzyme capable of hydrolyze 1 μmol of CNP-G3 per minute at pH 6.5 and 37 °C (monitoring CNP formation, ε_{405} =14,580 M^{-1} cm^{-1}).

The amylase inhibitory unit (IAU) was defined as the number of amylase units inhibited under the assay conditions.

α-Glucosidase inhibition assay used a similar experimental protocol. The substrate used was 2.25 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG). One glucosidase E.U. was defined as the amount of

enzyme capable of hydrolyze 1 µmol of pNPG per minute at pH 6.5 and 37 °C (monitoring *p*-nitrophenol formation, ε_{405} =14,580 M^{-1} cm^{-1}).

The glucosidase inhibitory unit (IGU) was defined as the number of glucosidase units inhibited under the assay conditions.

Each sample was prepared individually and analysed in triplicate.

2.8. Total phenol content and antioxidant evaluation

Total phenol content (TPC): TPC was determined using the Folin-Ciocalteau procedure on aliquots of bean powder extracted in acetone/waters (80:20; v:v). TPC value was expressed as mg of gallic acid equivalents (GAE) per 100 g of seed powder (Landi et al., 2017).

ABTS radical cation scavenging capacity: $ABTS^{\bullet+}$ solution scavenging capacity of bean powder was estimated as previously reported (Landi et al., 2017; Tamburino et al., 2012). Results were expressed in terms of TEAC values (mmol Trolox® Equivalents per 100 g of seeds powders).

Oxygen radical absorbance capacity assay (ORAC): The antioxidant potential of bean powder was measured by ORAC assay as previously reported (Landi et al., 2015; Landi et al., 2021a). ORAC value was calculated by using the Trolox® calibration curve and was expressed as Trolox® equivalents (mmol Trolox® per 100 g of seeds powders).

Each sample was prepared individually and analysed in triplicate.

2.9. DNA extraction from seedlings of 'Cerato' and 'Curniciello' beans

Total DNA was isolated from three different biological samples, each of which constituted a pool of 10 plants. DNA was extracted from fresh leaf tissues using the CTAB procedure (Doyle and Doyle, 1987) followed by RNase A treatment. DNA quality and quantity were determined using the NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel (1%) electrophoresis stained with ethidium bromide.

2.10. AFLP assay

AFLP analyses were performed as previously reported (Vos et al., 1995) with some modifications. However, the specific procedures, buffers and adapters for AFLP study as well as statistical analysis have been extensively reported in the Supplementary materials (paragraph S1.1., Supplementary methods) (Jaccard, 1912; Swofford, 2002).

2.11. Statistical analysis

All analyses were repeated three times and data are expressed as mean ± standard deviation (SD). Data analysis was performed with Excel Office 2016 (Microsoft Corporation, Redmond, WA, USA). The IC_{50} values were calculated based on inhibition curves: residual enzyme activities were plotted *versus* different concentrations of protein extract by fitting the data with a semilogarithmic scale nonlinear regression analysis using GraphPad Prism 8.4.2 software (GraphPad Software Inc., San Diego, CA, USA). Bonferroni post-test was used to determine significant differences. The test was performed using a $p < 0.05$.

3. Results and discussion

3.1. Nutritional values

The proximate composition of 'Cerato' and 'Curniciello' dry beans obtained from the analysis of two different harvest years (2020 and 2021) is shown in **Table 1**. Statistical analysis between the two harvest years of each dry bean analysed shows the absence of statistical differences, except for crude proteins and ash content with a p value <0.0001 and <0.05, respectively. In view of this, different studies have reported that the nutritional quality of bean seeds can be affected by climatic changes such as variations in temperature or rainfall amount during the period of seed growth and formation (Hummel et al., 2018; Sita et al., 2017). Indeed, considering the weather data for the years 2020 and 2021 related to the cultivation period of the two local bean landraces (April-August), the amount of precipitation in May and June 2020 was respectively ~2- and 3-folds higher than in the same months of 2021 (Campania agrometeorological centre at http://www.agricoltura.regione.campania.it/meteo/archivio_meteo.html).

Overall, the average nutritional composition of 'Cerato' and 'Curniciello' dry beans revealed that they are a rich source of crude proteins and carbohydrates. Indeed, carbohydrates are ~64.83 and 62.72 g *per* 100 g of 'Cerato' and 'Curniciello' dry beans, respectively. These findings reveal that both have a high total carbohydrates content with an amount 1.03-fold higher in 'Cerato' dry beans with respect to 'Curniciello' dry beans, confirming that carbohydrates represent the main component of beans with a range value between 55% and 65.0% (Azarpazhooh and Boye, 2012).

On the other hand, crude proteins are 21.19 g and 23.40 g *per* 100 g of 'Cerato' and 'Curniciello' dry beans, respectively; these values are in good agreement with the range 16-33% reported in the literature (Azarpazhooh and Boye, 2012). In particular, the crude protein content of 'Curniciello' dry beans was ~1.10-fold higher than that of 'Cerato' dry beans. Moreover, considering the crude protein content in other dry bean seeds landraces of Campania region, 'Cerato' and 'Curniciello' beans have similar content to that of 'Gallo Matese' beans (22.64 g/100g) (Landi et al., 2017), 'Fagiolo occhio nero di Oliveto Citra' beans (22.84 g/100g) (Zaccardelli et al., 2013), 'Tondino di Villa Ricca' beans (22.8 g/100g) and 'Mustacciello d'Ischia' beans (23.4 g/100g) (Piergiovanni et al., 2015). Moreover, this content for both bean landraces was higher than that of 'Controne' beans (17.4 g/100g) and lower than 'Dente di Morto' beans (27.3 g/100g) (Piergiovanni et al., 2015). In addition, the total lipid content of 'Curniciello' dry beans is higher than that of 'Cerato' dry beans, with an average amount of 2.08 and 1.27 g *per* 100 g, respectively. These data were in good agreement with the range of values (1.0-3.0%) reported in the literature (Azarpazhooh and Boye, 2012). Furthermore, the lipid

content was ~2- and 1.2-fold lower in 'Cerato' and 'Curniciello' beans than in 'Gallo Matese' beans (Landi et al., 2017), another bean landrace from the Campania region, confirming that dry beans are a low source of lipids. Finally, the average contents of moisture and ash are similar for both dry bean seeds landraces analysed, with a value of 8 and 4 g/100g respectively, in good agreement with was reported for 'Gallo Matese' dry beans (Landi et al., 2017).

3.2. Amino acid content

To assess the protein quality of the two dry bean seeds landraces, the total amino acid composition (free plus protein) of 'Cerato' and 'Curniciello' dry beans was determined by acid hydrolysis for both harvest years.

As shown in **Table S1**, significant differences were found between the two years for both dry beans analysed, in agreement with total crude proteins (see paragraph 3.1). Subsequently, the average total amino acid content (free plus protein) of 'Cerato' and 'Curniciello' dry beans was compared with 'Gallo Mallo' dry beans, another typical landrace of Campania region. Data reported in **Table 2**, showed no significant differences between 'Cerato' and 'Curniciello' dry beans by using Bonferroni's multiple comparisons test. However, quantitative differences were found compared to 'Gallo Matese' beans (Landi et al., 2017). Glx (glutamic acid + glutamine; ~16% of total amino acids) was the most abundant in all three dry beans, followed by Asx (aspartic acid + asparagine), which accounted for 11% of total amino acids in 'Cerato' and 'Curniciello' dry beans, while only 8% in 'Gallo Matese' dry beans. Leucine was ∼8%, while serine and lysine ∼7% of total amino acids in 'Cerato' and 'Curniciello', with respect to 'Gallo Matese' beans (each of three amino acids is ∼6%). Overall, these amino acids account for ∼49% of total amino acids in 'Cerato' and 'Curniciello' beans or ∼42% in 'Gallo Matese' beans.

In addition, the amount of essential amino acids [His, Ile, Leu, Lys, Met, Phe, Thr, Val; Trp (tryptophan is not included as it was not determined in the total hydrolysed samples - see **Table 2**)] in 'Cerato', 'Curniciello' and 'Gallo Matese' dry beans was ~40% of total. In all cases, the most abundant essential amino acids were leucine, lysine and phenylalanine representing respectively the 20%, 18% and 16% of total essential amino acid in 'Cerato' and 'Curniciello' beans, while 16% (both leucine and lysine) or 18% (phenylalanine) in 'Gallo Matese' beans.

The methionine *plus* cysteine content of 'Cerato' and 'Curniciello' dry beans was 0.58 and 0.62 g *per* 100 g, respectively, confirming the low content of sulphur amino acids in legumes (Azarpazhooh and Boye, 2012; Landi et al., 2017).

Finally, the free amino acid profile of 'Cerato' and 'Curniciello' dry beans is shown in **Figure 2**, although their content is ~77-fold lower than the total amino acids (**Table S2)**, not exceeding 1.5% (for more details, see Supplementary Results, paragraph S2.1). Overall, the free amino acid content of the 'Cerato' and 'Curniciello' dry beans analysed shows quali-quantitative differences (**Table S2)**.

3.3. Fatty acid composition

In order to evaluate the fatty acid composition, the lipids from 'Cerato' and 'Curniciello' dry beans were extracted and analysed by GC-MS. The data obtained (**Table 3**) showed that there were no significant differences between the two years for both dry beans analysed, except for the linolenic acid content of 'Cerato' dry beans. On the other hand, considering the average value of fatty acids in both 'Cerato' and 'Curniciello' dry beans, it was found that the most abundant were linolenic (C18:3) and linoleic (C18:2) acids. In particular, the average amounts of linolenic and linoleic acid were respectively ∼702 and 293 mg *per* 100 g of 'Cerato' dry beans or ∼1166 and 487 mg *per* 100g of 'Curniciello' dry beans. The total content of two polyunsaturated fatty acids (PUFA) accounted for ∼80% of the total fatty acids in both 'Cerato' and 'Curniciello' dry beans analysed; this value was ∼1.13-fold higher than the percentage of PUFA retrieved in 'Gallo Matese' dry beans (Landi et al., 2017) and other dry beans with values ranging from 40.33% to 55.32% (Celmeli et al., 2018). Moreover, other fatty acids present in both dry beans analysed were monounsaturated fatty acids (MUFA; ∼11%) and saturated fatty acids (SFA; ∼9%). Considering the MUFA content of both dry seeds from two landraces, the most abundant fatty acid was oleic acid (C18:1) with an average value of 134 and 217 mg/100g of 'Cerato' and 'Curniciello' dry beans, respectively. These values were ∼1.4 and 2.2-fold higher in 'Cerato' and 'Curniciello' dry beans with respect to 'Gallo Matese' dry beans (Landi et al., 2017). In addition, very few amount of palmitoleic acid (C16:1; 3.38 mg/100g on dry-weight basis) was retrieved only in 'Curniciello' dry beans.

Finally, looking at the SFA content for both 'Cerato' and 'Curniciello' dry beans, the most abundant was palmitic acid (C16:2), whit an average amount of ~90 and 148 mg *per* 100 g of 'Cerato' and 'Curniciello' dry beans, respectively. These values were respectively ∼2.10 and 1.30-fold lower for 'Cerato' and 'Curniciello' dry beans than for 'Gallo Matese' dry beans (Landi et al., 2017).

3.4. Protease inhibitory activity

Although beans are a good source of proteins, carbohydrates, dietary fibers, and vitamins, they are also rich in anti-nutrients such as tannins, lectins, phytic acid, oligosaccharides, and protease

inhibitors. These components are anti-nutritional factors that can negatively affect the bioavailability, digestibility and assimilation of nutrients and minerals (Carbas et al., 2020). Among them, protease inhibitors reduce the proteolytic activity of pancreatic enzymes (trypsin and chymotrypsin), affecting the digestion and absorption of dietary proteins (Avilés-Gaxiola et al., 2018). Therefore, prior to consumption, pulses are subjected to heat treatments, aimed at inactivating or reducing the activity of protease inhibitors, thus facilitating nutrient absorption. In this context, the protease inhibitory activity of soluble proteins extracted from 'Cerato' and 'Curniciello' dry beans, considering both harvest years, was evaluated on raw and boiled beans and the IC_{50} values obtained are reported in **Table 4**. The average amount of soluble proteins extracted from 'Cerato' and 'Curniciello' dry beans, before the boiling process, was ∼32 and 44 mg of protein extract *per* g of seeds, respectively, while, after boiling, the amount was ∼32 and 37-fold lower than the extract of the raw 'Cerato' and 'Curniciello' dry beans, respectively.

Then, increasing concentrations of raw or cooked protein extracts were added to constant concentrations of proteolytic enzymes (trypsin and chymotrypsin) to evaluate their inhibitory activity. In this context, a comparison of the trypsin inhibitory action revealed a significant difference between the two different harvest years for both 'Cerato' and 'Curniciello' dry beans analysed only after cooking treatment. On the other hand, chymotrypsin inhibitory activity revealed a significant difference between the two harvest years only for raw and cooked 'Curniciello' dry beans.

In addition, raw protein extracts from both dry beans showed higher chymotrypsin inhibitory activity (IC_{50} average values of 0.12 and 0.23 µg of protein *per* mL of extract for 'Cerato' and 'Curniciello' dry beans, respectively) with respect to trypsin inhibitory activity (IC_{50} average values of 0.21 and 0.31 µg of protein *per* mL of extract for 'Cerato' and 'Curniciello' dry beans, respectively). This finding was consistent with that previously reported for 'Gallo Matese' dry beans, where the chymotrypsin inhibitory activity was approximately 26% higher than trypsin inhibitory activity (Landi et al., 2017).

A decrease in the protease inhibitory activity was observed for both cooked dry beans. Trypsin inhibitory activity of cooked 'Cerato' and 'Curniciello' dry beans was respectively ∼10- and 6-fold lower (corresponding to IC_{50} average values of 2.08 and 1.76 µg of protein *per* mL of extract, respectively) than that of raw beans; while the chymotrypsin inhibitory activity of cooked 'Cerato' and 'Curniciello' dry beans was respectively ∼5- and 4-fold lower (corresponding to IC_{50} average values of 0.62 and 0.96 µg of protein *per* mL of extract, respectively) compared to raw dry beans.

These data confirm the effectiveness of heat treatment in inactivating protease inhibitors in legumes. On the other hand, a residual protease inhibitory activity has been also detected, even after cooking treatment. However, it is known that the residual heat-resistant protease inhibitory activity

of legumes has anti-cancer properties, being implicated in carcinogenesis suppression and proteolytic activities inhibition and/or expression of some proto-oncogenes in colorectal cancer (Lima et al., 2016).

3.5. α-Amylase and α-glucosidase inhibitory properties

Among the lectins of common beans, proteinaceous α-amylase inhibitors (α-AIs) are a well-known class of proteins, that are gaining increasing interest in nutraceutical markets. α-AIs represent a plant defense mechanism against biotic stress. These proteins do not affect plant, bacterial or fungal amylases, but specifically interfere with digestive enzymatic activities of mammals and some insects (Singh et al., 2022). This biological effect has allowed the commercialization of bean extracts to ameliorate the conditions of type-2 diabetes patients and overweight subjects (Feng et al., 2022), as confirmed by several clinical trials that also pointed out only limited adverse effects (Peddio et al., 2022).

The ability to inhibit porcine pancreatic α-amylase and α-glucosidase was tested in 'Cerato' and 'Curniciello' dry beans extracts in both 2020 and 2021 harvest years. The differences between the samples were then evaluated by using Bonferroni's multiple comparisons test (**Table 5**). α-Glucosidase inhibiting activity was present in all the samples (IGU/g ranging 84-119), without any statistical difference. On the other hand, α-amylase inhibition power was detected in 'Cerato' dry beans both in 2020 and 2021 harvest years (118 ± 13 and 155 ± 18 IAU/g, respectively), whereas in 'Curniciello' dry beans the activity was detected only in 2020 sample (149 ± 14 IAU/g). 2021 'Curniciello' dry beans showed an almost negligible activity, confirming the fact that the inhibition of the two digestive enzymes could be related to different compounds (Bosi et al., 2019).

Several other dry beans have been studied for their anti-amylase and anti-glucosidase activities, including Mediterranean beans (Bosi et al., 2019; Ombra et al., 2018), white beans (Shi et al., 2021), and red kidney beans (Alizadeh et al., 2010). Our data seem to confirm the ability of legumes to affect digestive enzymes, but the drugs responsible for these activities have not always been identified (besides α-AIs, several polyphenols could also be responsible).

In addition, the difference in α-amylase inhibition activity detected for the two different harvest years of 'Curniciello' dry beans could be attributed to the different amount of precipitation in May and June 2020 with respect to the same months of 2021 (see above).

Overall, our data show that both 'Cerato' and 'Curniciello' dry beans have the ability to inhibit digestive enzymes and could also be suitable for nutraceutical formulations in this context. However,

the occurrence of differences between harvest years, requires further studies to confirm and/or explain this variability.

3.6. Total phenol content and antioxidant capability of 'Cerato' and 'Curniciello' beans

In order to assess the total phenol content (TPC) of 'Cerato' and 'Curniciello' dry beans for both harvest years, total phenolics were extracted by ultrasound-assisted maceration using 80% acetone as extractant (average yields retrieved 4.6% w/w) and data obtained were reported in **Figure 3a**. Statistical analysis between the two years show a significant difference only for 'Curniciello' dry beans with $p < 0.01$. In particular, TPC of 'Curniciello' dry beans was ∼23% higher in samples 2021 with respect to 2020 harvest year. Furthermore, considering the average TPC, data obtained showed a higher TPC in 'Curniciello' dry beans (~587 mg GAE *per* 100 g) compared to 'Cerato' dry beans (~548 mg GAE *per* 100 g). On the other hand, by comparing their TPC with other dry bean landraces from Campania region, it was found that 'Cerato' and 'Curniciello' dry beans had a TPC of ~2- and 4-fold higher than other dry bean landraces (Landi et al., 2017; Ombra et al., 2016; Zaccardelli et al., 2013). These differences may be due to the different landraces, growing conditions, and extraction method.

In addition, to evaluate the antioxidant activity of acetonic extracts for both dry bean landraces, ABTS and ORAC assay have been carried out, and the results are shown in **Figure 3b** and **2c**, respectively. In particular, ABTS method showed a significant difference in antiradical activity for both 'Cerato' and 'Curniciello' dry beans analysed, considering the two harvest years. Conversely, ORAC method showed a significant difference only in 'Curniciello' dry beans, considering the two harvest years. The presence of higher antioxidant activity in the year 2021 could be justified by the different climatic trend in the two years analysed. Indeed, common beans are legumes with very rapid growth and sensitive to water stress and soil conditions (Abd El-Wahed et al., 2017). In particular, under water stress, it has been observed an increase of radical species (ROS) produced by plants, which are harmful to cell membranes.

Therefore, the plant responds to stress by increasing the level of enzymes and secondary metabolites (phenolic compounds, vitamins, and terpenoids) that can eliminate the ROS produced (Gaafar et al., 2020). Moreover, the average antioxidant activity evaluated by the ORAC method, showed that 'Curniciello' dry beans exhibited an antioxidant power ∼1.70-fold higher than 'Cerato' dry beans (8.91 and 5.31 mmol TE *per* 100 g of 'Cerato' and Curniciello' dry beans, respectively). Conversely, the average antioxidant activity evaluated by ABTS method, showed similar values for both dry bean landraces (4.06 and 5.04 mmol TE *per* 100 g of 'Cerato' and Curniciello' dry beans,

respectively). Finally, the average antioxidant activity of 'Cerato' and 'Curniciello' dry beans was respectively 4- and 6-fold higher (according to the ORAC assay) or 3- and 4-fold higher (according to the ABTS assay) than that of 'Gallo Matese' dry beans, another landrace of Campania region (Landi et al., 2017).

3.7. Study of bean genetic diversity

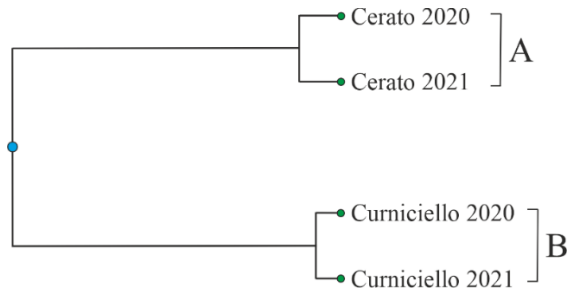
In recent years, food safety and quality have attracted considerable interest from producers, retailers and consumers (Gentile et al., 2023). The authenticity and detection of food adulteration is therefore an important objective. In this context, the use of molecular traceability and agri-food authentication techniques is essential. Food traceability techniques are methods based on the use of molecular markers. A molecular marker is defined as a specific DNA sequence that identify a specific genome (Fanelli et al., 2021). Molecular markers are stable and detectable in all tissues regardless of the growth, differentiation, and development of the cells and are not affected by the environment. The detection and identification of molecular markers are performed by hybridization and PCR-based techniques (Galimberti et al., 2019; Lo and Shaw, 2018). The aim of the present research was to evaluate the genetic diversity of two 'Cerato' and 'Curniciello' *P. vulgaris* landraces using amplified fragment length polymorphism (AFLP) analysis. Five primer pair combinations were used for the final AFLP assay after a pre-screening to select the best primer combinations for genotypes characterization. Each primer combination was tested on DNA samples collected in 2020 and 2021.

A total of 253 bands (loci) were identified (**Table 6**) ranging in size from 250 bp to 2500 bp, of which 89 (35%) were monomorphic bands. An average of bands for each primer combination was 21 and all exhibited a unique banding pattern. The monomorphic bands ranged from 2 (MseI/ MseI+A) to 14 (MseI+G) in the 'Curniciello' genome, and from 3 (MseI/ MseI+A) to 12 (EcoR1/ EcoR1+C) in the 'Cerato' genome. The results are shown in **Figure 4, S1 and S2** and summarized in the **Table 6**.

All polymorphic bands were detected in a reproducible AFLP experiment performed with DNA from three different biological samples from a pool of 10 plants for each bean landrace. The results showed identical AFLP patterns, indicating the absence of intra-genotype polymorphisms. Each primer combination produced a different polymorphic pattern (**Figure 4, S1 and S2**). All primer combinations revealed the presence of monomorphic bands for both bean landraces with high resolution and reproducibility. The EcoRI/ EcoRI +C and MseI/ MseI +G primer combinations produced the highest number of monomorphic bands for 'Cerato' and 'Curniciello' landraces, respectively (**Table 6, Figure 4**). On the other hand, the MseI/ MseI +A combination produced a low

number of monomorphic AFLP bands. Monomorphic bands identified within a specific landrace represented genotype-specific markers. Similarity matrix, based on Jaccard's coefficient (Jaccard, 1912), was constructed including AFLP profiles. Bands were considered monomorphic if they were present in only one bean landrace. Each bean landrace showed a higher genetic similarity in 2020 and 2021 (0.936 and 0.960 for 'Cerato' and 'Curniciello', respectively) within the same landrace, on the contrary, a lower similarity was observed comparing 'Cerato' and 'Curniciello' landraces (0.461) that showed high level of genetic diversity (**Table 6**). Usually, species growing in small geographical areas show a low genetic diversity compared to those distributed over larger geographical areas (Brütting et al., 2012; Hamrick and Godt, 1990). The high variability observed between the two bean landraces could be explained by the self-fertilisation (selfing) of their breeding strategy. It is also worth noting that excessive human exploitation reduced the size of the populations over the years. One of the consequences of this reduction is the genetic differentiation among isolated populations, and the decrease in genetic variation within populations with loss of heterozygosity and allele fixation (Ellstrand and Elam, 1993).

The dendrogram reliability of the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method with AFLP genetic similarity matrix was confirmed by a high cophenetic correlation coefficient rAFLP=0.998 (**Scheme 1**).



Scheme 1. UPGMA-based dendrogram of 'Cerato' and 'Curniciello' landraces, harvested in 2020 and 2021. Cophenetic Correlation Coefficient (CP) = 0.999.

The dendrogram showed two clusters, in which the two different harvest years of 'Cerato' and 'Curniciello' landraces clustered together, indicating no genetic variability within the same landrace between the two years (**Scheme 1**). These results are confirmed by the presence of the same number of bands obtained in the different AFLP experimental replicates (data not shown). The evaluation of variations in two bean landraces is crucial to establish their geographical origin due to intraspecific genetic variability in natural populations and provides valuable information for their conservation. AFLP is a widely used marker for the study of heredity of agronomical traits in plants, parentage

analysis, pedigree analysis, genetic traits etc. (Sheeja et al., 2021). It is recognized as a universal DNA fingerprinting technique to study small sequence variations using a low concentration of DNA without prior knowledge of the genomic sequence. In addition, AFLP showed greater diversity compared to RAPD, ISSR, SSR, RFLP markers (Sheeja et al., 2021).

4. Conclusions

In recent years, interest in local bean landraces has increased, and this phenomenon is based on their cultural value as well as the conservation of local agrobiodiversity. In this context, the dry bean characterisation of local landraces, increases local consumption and promotes a self-sustaining economy of local communities.

In light of this, a plethora of approaches were used to characterize the dry seeds of 'Cerato' and 'Curniciello', two bean landraces, well-known in the rural areas of Caserta and still cultivated by local farmers in the Alife and Caiazzo territories, respectively. Our results confirm that 'Cerato' and 'Curniciello' dry beans are a good source of essential nutrients (e.g.: crude proteins and fatty acids) as well as micronutrients and nutraceuticals. Indeed, the residual protease inhibitor activity (anticancer properties) and antioxidant capability (defence against oxidative stress) detected in these two dry beans highlight their potential health benefits. In addition, AFLP analyses show that the two landraces have reproducible polymorphic bands, indicating no genetic variability within the same landrace and a different genetic pool despite the proximity of the two cultivation areas (20 km). This finding could be useful for the authentication and traceability of 'Cerato' and 'Curniciello' dry beans.

In conclusion, although further studies are needed, the biochemical/nutritional data of 'Cerato' and 'Curniciello' dry beans are of interest for increasing local consumption, motivating their cultivation and preserving culinary traditions, useful for attracting a flourishing gastronomic tourism.

CRediT authorship contribution statement

Nicola Landi, Laura Alberico, Angela Clemente, Stefania Peddio, Hafiza Z. F. Hussain and Sara Ragucci: Investigation; Data curation; Software. Pasqualina Woodrow and Paolo Zucca: Methodology; Validation, Formal analysis, Writing-review & editing. Antimo Di Maro: Conceptualization; Methodology; Validation; Funding acquisition; Supervision; Roles/Writing-original draft; Writing-review & editing.

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Declarations 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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Data availability

Raw data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at

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Figure legends

Figure 1. Seeds of the *Phaseolus vulgaris* landraces compared in the study.

Figure 2. (**a**) and (**b**) stacked bar graph showing the relative abundance percentage of proteinogenic and non-proteinogenic free amino acids from 'Cerato' and 'Curniciello' bean seeds compared to 'Gallo Matese' beans. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article).

Figure 3. Total phenol content and antioxidant capabilities of 'Cerato' and 'Curniciello' bean landraces in two consecutive harvest years (2020 and 2021, blue and red, respectively). (**a**) Total phenol content (TPC), antioxidant capacity determined by: (**b**) ABTS, (**c**) ORAC. TPC was expressed as mg of gallic acid equivalents (GAE); ORAC and ABTS are expressed as mmol of Trolox equivalents (TE) per 100 g on a dry-weight basis of each sample ±SD. For each landrace, different symbols indicate statistically significant differences according to Bonferroni's multiple comparisons test ($p < 0.05$), between the two considered years. ** and *** indicate significant differences with $p < 0.01$ and $p < 0.001$, respectively. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article).

Figure 4. AFLP profiles of 'Cerato' and 'Curniciello' landraces with different selective primer combinations: EcoRI/EcoRI+ C; MseI/MseI + G. Lanes: M1 GeneRuler 100 bp Plus DNA Ladder, M2 DNA Molecular Weight Marker 20 bp (Sigma-Aldrich Solutions, Merk Life Science), M3 Molecular Weight Marker 50 bp (Sigma-Aldrich Solutions), 1, 2, 3 and 4 genomic DNA from 'Cerato' 2020, 'Cerato' 2021, 'Curniciello' 2020 and 'Curniciello' 2021, respectively.

Figure 1

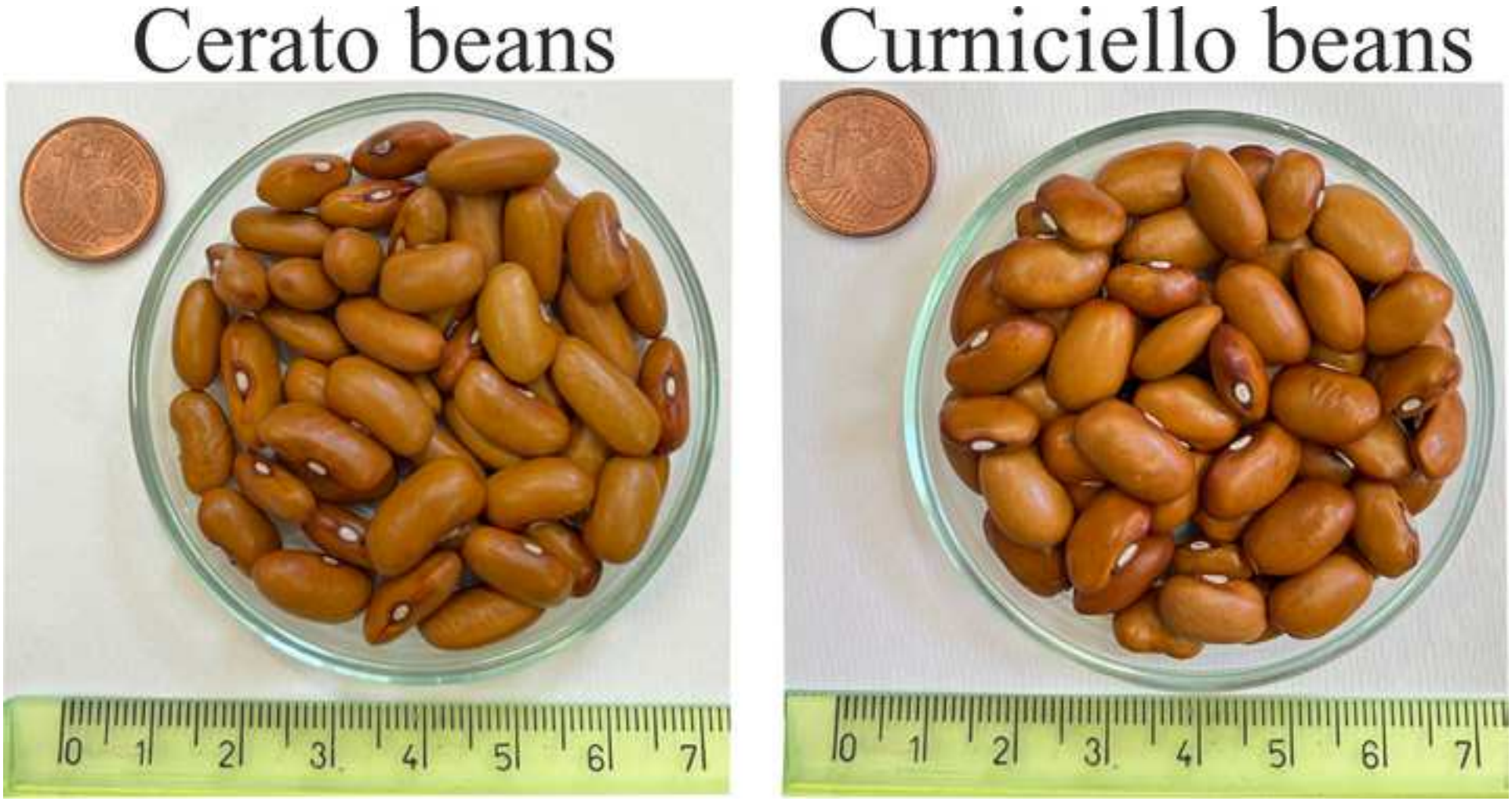

Figure 2

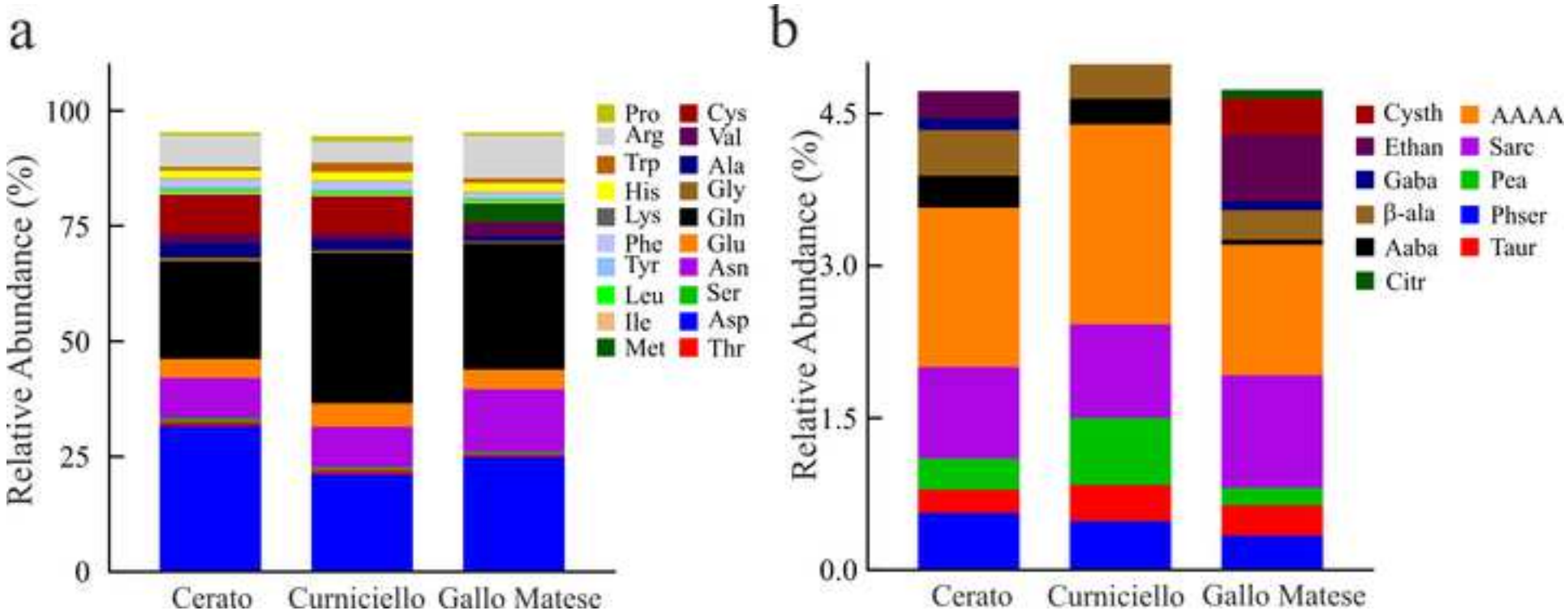

Figure 3

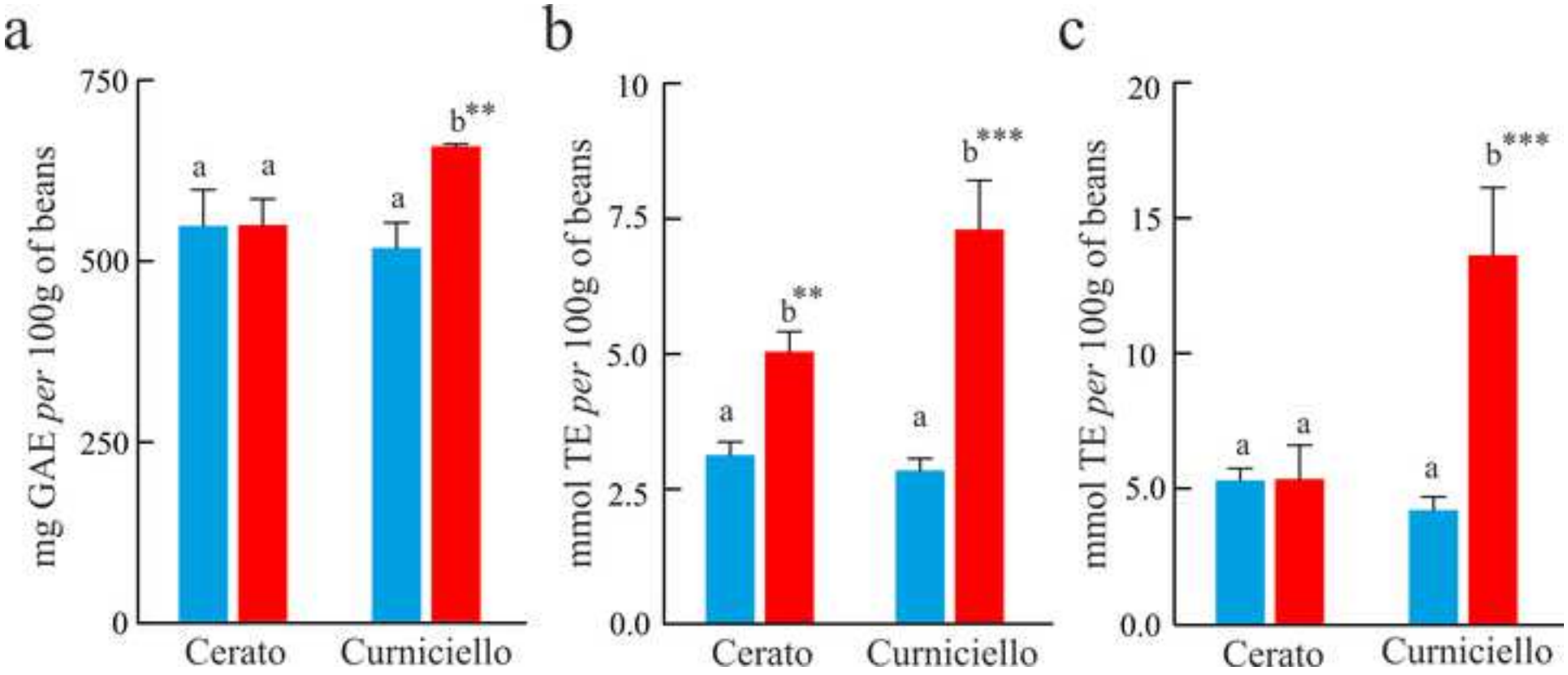

Figure 4

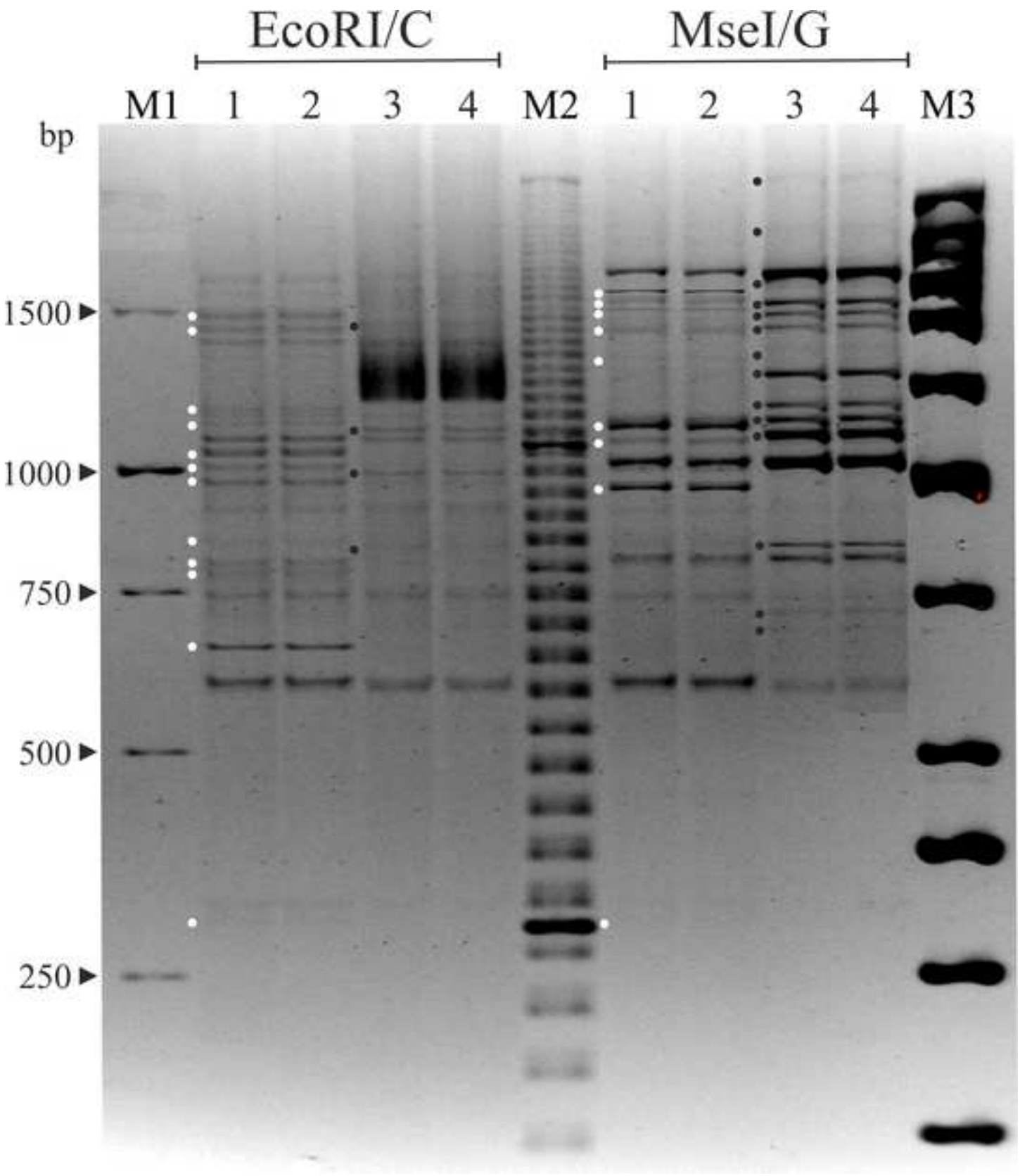

Table 1. Proximate composition of 'Cerato' and 'Curniciello' bean seeds collected in the years 2020 and 2021. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on dry-weight basis (g/100 g).

	Cerato			Curniciello	
	2020	2021		2020	2021
Crude proteins	22.04±0.41a	20.33±0.14b****		24.95±0.64a	21.86±0.55b****
Lipids	1.32±0.05a	1.21±0.00a		2.06±0.21a	2.11±0.12a
Ash	3.90±0.02a	4.48±0.48b*		4.07±0.05a	3.28±0.04b*
Moisture	8.41±0.15a	8.28±0.08a		7.86±0.15a	8.37±0.10a
Carbohydrates	63.97	65.70		61.06	64.38

For each landrace, in each row, different letters indicate statistically significant differences according to Bonferroni's multiple comparisons test ($p < 0.05$). * and **** indicate significant difference with $p < 0.05$ and $p < 0.0001$, respectively.

Table 2. Total amino acid composition of 'Cerato' and 'Curniciello' bean seeds compared to 'Gallo Matese' beans (Landi et al., 2017) another typical landrace bean of Campania region. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on dry-weight basis (g/100 g).

Amino acid	Campania bean landraces		
	Cerato	Curniciello	Gallo Matese
essential amino acids			
His	0.48±0.04a	0.57±0.10a	0.72±0.06
Ile	0.65±0.04a	0.68±0.09a	0.82±0.05
Leu	1.30±0.02a	1.41±0.27a	1.30±0.09
Lys	1.16±0.02a	1.30±0.23a	1.30±0.10
Met	0.22±0.01a	0.28±0.02a	0.35±0.04
Phe	1.05±0.03a	1.13±0.23a	1.49±0.08
Thr	0.84±0.03a	0.91±0.22a	1.20±0.08
Val	0.75±0.05a	0.80±0.15a	1.07±0.07
non-essential amino acids			
Ala	0.83±0.04a	0.92±0.18a	0.94±0.07
Arg	0.96±0.05a	1.21±0.18a	1.76±0.21
Asx	1.78±0.15a	1.97±0.66a	1.65±0.11
Cys[§]	0.36±0.01a	0.34±0.01a	0.51±0.01
Glx	2.49±0.14a	2.85±0.29a	3.42±0.26
Gly	0.72±0.01a	0.81±0.11a	0.78±0.06
Pro	0.69±0.05a	0.81±0.07a	1.51±0.06
Ser	1.21±0.29a	1.24±0.28a	1.39±0.09
Tyr	0.55±0.06a	0.61±0.12a	0.91±0.05
Total	**16.01**	**17.84**	**21.08**

Values followed by different letters indicate statistically significant differences according to Bonferroni's multiple comparisons test ($p<0.05$). Protein amino acids. A three-letter code has been used: Asx – L-asparagine + L-aspartic acid, Arg – L-arginine, Cys – L-half cystine, Glx – L-glutamine + L-glutamic acid, Gly – glycine, His – L-histidine, Ile – L-isoleucine, Leu – L-leucine, Lys – L-lysine, Met – L-methionine, Phe – L-phenylalanine, Pro – L-proline, Ser – L-serine, Thr – L-threonine, Tyr – L-tyrosine, Val – L-valine.
[§] Cys amount was evaluated after performic acid oxidation.

Table 3. Fatty acid composition of 'Cerato' and 'Curniciello' bean seeds collected in the years 2020 and 2021. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on dry-weight basis (mg/100g).

		Cerato			Curniciello	
		2020	2021		2020	2021
Saturated fatty acids						
Palmitic	C16:0	92.10±6.25a	88.45±5.84a		145.69±2.10a	150.56±14.10a
Stearic	C18:0	26.38±1.83a	29.10±3.17a		35.90±2.43a	35.76±2.56a
Monounsaturated fatty acid						
Palmitoleic	C16:1	*n.d.*	*n.d.*		2.86±0.00a	3.90±0.37a
Oleic	C18:1	137.31±6.66a	130.56±1.71a		217.52±27.78a	217.11±10.34a
Polyunsaturated fatty acid						
Linoleic	C18:2 (n-6)	306.51±6.77a	279.75±8.38a		478.44±40.22a	496.29±47.96a
α-Linolenic	C18:3 (n-3)	736.97±40.47a	667.74±18.74b**		1153.01±130.38a	1179.28±69.42a

For each landrace, in each row, different letters indicate statistically significant differences according to Bonferroni's multiple comparisons test ($p < 0.05$). **indicate significant difference with $p < 0.01$. *n.d.*, not determined.

Table 4. Anti-proteinase inhibitory activity of raw and boiled (2 h) 'Cerato' and 'Curniciello' dry beans collected in the years 2020 and 2021. Inhibitory activities obtained from the protein extracts are reported as IC_{50} average values *per* year and expressed as µg of protein per mL of extract.

Antitrypsin activities of soluble protein extract						
Cerato	*2020*	*2021*		Curniciello	*2020*	*2021*
raw	0.19±0.01a	0.23±0.01a		*raw*	0.25±0.01a	0.36±0.02a
cooked	1.85±0.09a	2.32±0.12b***		*cooked*	2.12±0.11a	1.40±0.07b****
Anti-chymotrypsin activities of soluble protein extract						
raw	0.11±0.01a	0.13±0.01a		*raw*	0.16±0.01a	0.31±0.02b*
cooked	0.64±0.03a	0.60±0.03a		*cooked*	0.65±0.03a	1.27±0.06b****

For each landrace, in each row, different symbols indicate statistically significant differences according to Bonferroni's multiple comparisons test ($p < 0.05$). *, *** and **** indicate significant differences with $p < 0.05$, $p < 0.001$ and $p < 0.0001$, respectively.

Table 5. Anti-amylase and anti-glucosidase inhibitor activity of 'Cerato' and 'Curniciello' bean seeds collected in the years 2020 and 2021. Results are expressed as amylase inhibiting units (IAU) per gram of dried seed, and as glucosidase inhibiting units (IGU) per gram of dried seed. Data are expressed as mean ± SEM (n =6).

	IAU/g	**IGU/g**
Cerato 2020	$118 \pm 13^{a, b}$	108 ± 14^{a}
Cerato 2021	155 ± 18^{a}	97 ± 11^{a}
Curniciello 2020	149 ± 14^{a}	84 ± 8^{a}
Curniciello 2021	17 ± 5^{b}	119 ± 15^{a}

Mean values for the same analysis having different letters are significantly different ($p < 0.05$; One–way ANOVA followed by the Bonferroni Multiple Comparisons Test).

Table 6. Comparison between total bands, polymorphic bands and common bands obtained from each primer combination for 'Cerato' and 'Curniciello' bean landraces. Primer sequences for ARFP analysis are reported.

Primer sequence combinations	Cerato		Curniciello		
	Total bands	Polymorphic bands	Total bands	Polymorphic bands	Common bands
EcoRI 5′-CCCAAAGCCTATCCTCGAATTC-3′ EcoRI+C 5′-CCCAAAGCCTATCCTCGAATTCC-3′	22	12	14	4	10
MseI 5′-TCCTGAGTCCAACAGATCCGG-3′ MseI+G 5′-TCCTGAGTCCAACAGATCCGGG-3′	15	9	20	14	6
MseI 5′-TCCTGAGTCCAACAGATCCGG-3′ MseI+A 5′-TCCTGAGTCCAACAGATCCGGA-3′	20	3	19	2	17
MseI 5′-TCCTGAGTCCAACAGATCCGG-3′ MseI+C 5′-TCCTGAGTCCAACAGATCCGGC-3′	22	7	19	4	15
MseI 5′-TCCTGAGTCCAACAGATCCGG-3′ MseI+T 5′-TCCTGAGTCCAACAGATCCGGT-3′	24	8	27	11	16
MseI+G 5′-TCCTGAGTCCAACAGATCCGGG-3′	23	6	26	9	17