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Research article

Nutritional characterization of Italian common bean landraces (*Phaseolus vulgaris L.*): fatty acid profiles for "genotype-niche diversity" fingerprints

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Abstract: Major problems facing common bean production in the European Union include the significant and consistent decrease in legume consumption and the potential risk to local landraces by commercial cultivars. With the need to both increase local *Phaseolus vulgaris L.* ecotype production and to expand studies on potential genetic diversity impacts on nutritional components, the aim was to investigate a range of nutritional constituents in the Italian landraces, "Zolfino del Pratomagno" (Tuscany), "Fagiolo di Sarconi" (Basilicata) and "Fagiolo di Lamon (Veneto). Zolfino landraces were distinctive for significantly higher levels of amino acids, G2 protein fraction (lectin), ash, as well as total lipid and Monounsaturated Fatty Acid (MUFA) content, with Linear Discriminant Analysis (LDA) depicting a divergence of Zolfino from the Sarconi and Lamon landraces, respectively. Fatty acid profiles were distinctive for landrace. An equivalent ratio of Polyunsaturated Fatty Acids (PUFA) to MUFA was evident for Zolfino. LDA showed distinctive, separate cluster groupings for the landraces, with Zolfino differentiated by the combined increased levels of oleic and palmitoleic acids, and the presence of heptadecanoic acid. The Sarconi landraces were characterized by the combined higher palmitic and linolenic acids and the absence of both myristic and tridecanoic acids, whereas the Lamon landraces were characterized by combination of higher linolenic acid, lower palmitic acid and the presence of both myristic and tridecanoic acids. The potential of expanding studies to include fatty acid profiles as possible sources of "genotype-niche diversity" fingerprints for common bean is shown to be feasible.

Keywords: *Phaseolus vulgaris L*; Italian ecotypes; nutritional composition; fatty acid profiles; diversity fingerprints

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is an important nutritional source of protein, carbohydrates, minerals and vitamins, as a subsistence staple food along with cereals in developing countries, and as a healthy food choice in Western diets [1–3]. Problems facing common bean diversity and therefore, future production on a global scale are numerous, and include the following: a narrowing of the genetic base in selected commercial market classes (designated on the basis of size, color and pattern), increased endangerment (risk of extinction) of local landraces due to the use of higher-producing commercial varieties, and a reduced research input into common bean diversity in certain areas, leading to loss of information and resources [1,4–6].

Domestication of the common bean occurred independently in Middle America (Mesoamerica) and Andean South America, giving rise to two highly differentiated domesticated gene pools ("pure accessions") as well as inter-gene pool hybrids, currently detectable in the global collection of both commercial varieties and landraces following multiple introduction events and subsequent, complex distribution pathways into Africa, Europe and Asia [5]. Following the initial characterization of Middle American and Andean gene pools in common bean collections across the globe, based predominantly on morphological traits and phaseolin storage seed proteins [7,8], ongoing research focus has been primarily centered on investigating genetic variation and degree of relatedness using ever-increasing, sophisticated molecular techniques [4,6,9]. The primary research objectives included investigating diversity for a more efficient conservation of national germplasm and/or reintroducing landraces into breeding programs [4–6].

The evaluation of phenotypic and nutritional variation, nonetheless, remain crucial in determining agronomic potential, necessary in safeguarding landraces and/or reintroducing the latter into breeding programs [3,4,10,11]. Hence, in an attempt at extending knowledge on available resources, landraces have also been analyzed for possible trait associations between nutritional or chemical composition, Middle American or Andean gene provenance (plus respective geographical subdivisions or races), and/or seed color. The majority of studies have focused on mineral nutrient and protein content [3,10–13]. Reflecting the trend within the entire European Union [2], the two major problems facing common bean production in Italy include the significant and consistent decrease in legume consumption from 1961 to 2015 [14], and the potential risk to local landraces by commercial cultivars [5,15]. With the need to both increase local ecotype production and to expand studies on potential genetic diversity impacts on nutritional/functional components [2,14,15], the aim of the present study was to investigate a range of nutritional constituents, required for a healthy and balanced diet, in three Italian local landraces for potential differences between the latter, and any associations potentially based on provenance and seed color. The landraces (designated by "Indication of Geographical Provenance" [IGP]) included, "Zolfino del Pratomagno" (Tuscany), previously characterized as a Middle American genotype, as well as "Fagiolo di Sarconi" (various ecotypes in Sarconi, Basilicata) and "Fagiolo di Lamon" (various ecotypes from Veneto), both previously characterized as Andean genotypes, respectively [8,9]. Overall contents of the respective constituents were also compared to those in commercial cultivars. An additional aspect of the present study involved analyzing the fatty acid profiles of the Italian landraces, investigating the potential of including fatty acid profiles in “genotype-environmental niche” fingerprint characterization studies.

2. Materials and methods

2.1. Plant material

Different ecotypes of common bean (*Phaseolus vulgaris* L.) were collected from various rural communities in Veneto, Tuscany and Basilicata regions of the Italian peninsula (Figure 1). Seeds of “Zolfino del Pratomagno” were obtained from the locations, Loro Ciuffenna (LC) and Terranuova Bracciolini (TB), within the natural habitat of the Pratomagno region of Tuscany. The seeds were obtained from local famers in four locations in LC and five in TB, respectively, as was previously reported by Dinelli et al. [16] and Marotti et al. [9] The “Fagiolo di Sarconi” (Basilicata) is comprised of various ecotypes, which were obtained for the present study and included: Ciuoto (C), Cannellino Nano (CN), Munachedda (M), Nasieddu (N), Riso Bianco (RB), San Michele (SM), Tabacchino Nano (TN), Tuvagliedda Rossa (TR) and Verdolino Nano (VN). Information regarding the local farmers and producers was similarly provided previously [9,16]. The “Fagiolo di Lamon” (Veneto) included the ecotypes: Calonega (CA), Spagnol (SP1) and Spagnolit (SP2). The commercial varieties, selected for comparative purposes included Sanilac, Contender and Tendergreen, as well as Borlotto “Lingua di Fuoco” (commercial variety widely consumed in Italy), which were obtained from private seed trade companies. In the results section provided, the described analyses were conducted on a minimum of three replicates.

2.2. Extraction and measurement of protein constituents, and erythroagglutinating phytohemagglutinin activity

All results were expressed on a dry mass basis and dry mass was determined after drying seed material in an oven at 105 ℃. Amino acid content was determined with ninhydrin, as described previously [17]. Total nitrogen was determined according to the Kjeldahl method and converted to protein content according to AOAC [18]. Total proteins were extracted and measured by the method of Lowry et al. [19] using bovine serum albumin as a standard. Globulin 1 (G1) and Globulin 2 (G2) proteins were extracted according to Limongelli et al. [8] and measured according to Lowry et al. [19]. Erythroagglutinating phytohemagglutinin activity (PHA-E activity) was performed using the extracted G2 protein, which was serial diluted and added to human erythrocytes according to a modification of the method of Duranti et al. [20], as described in Bosi et al. [21].

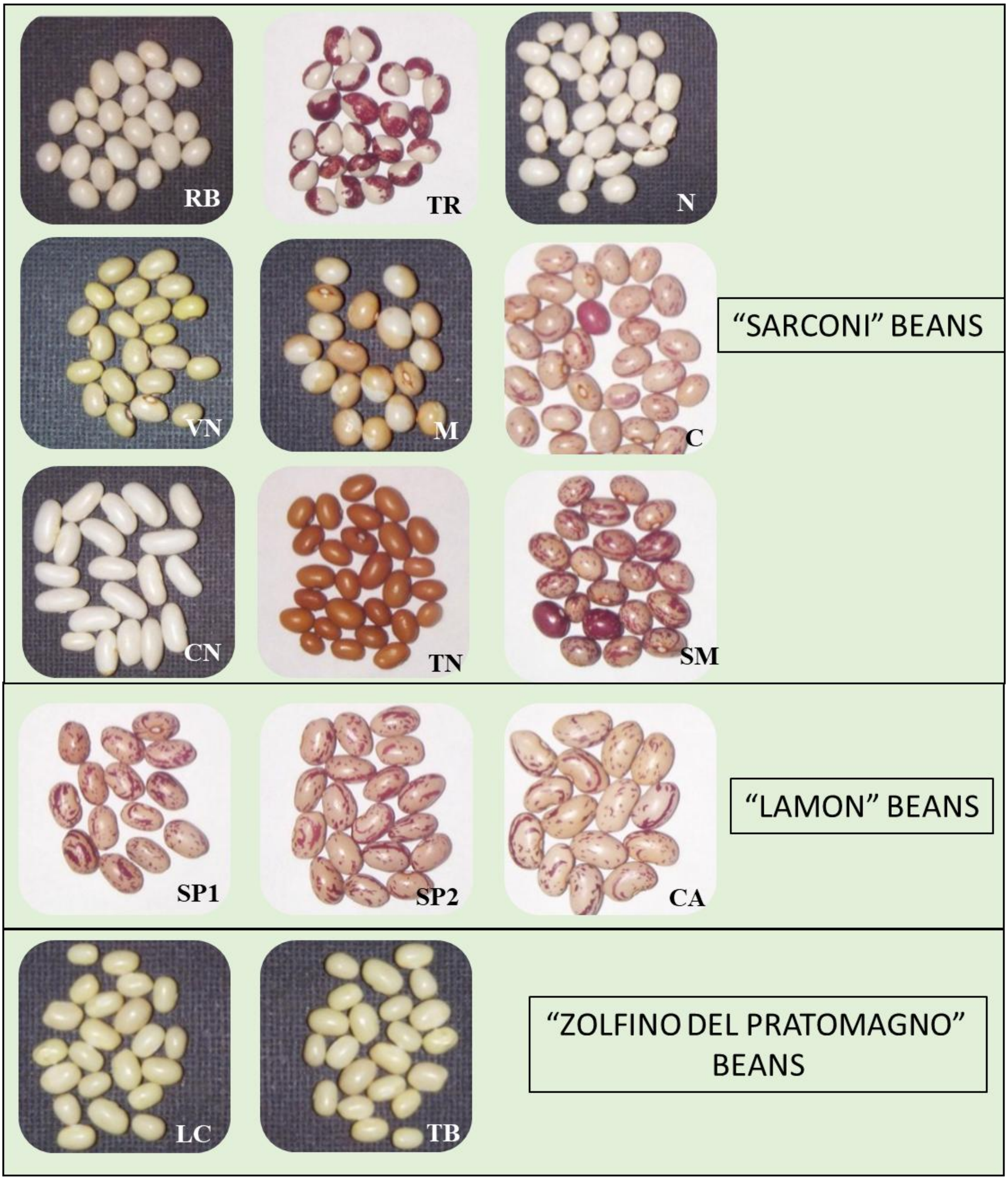

Figure 1. Photographs of the ecotypes of common bean (*Phaseolus vulgaris* L.) included in the study. “Sarconi” beans: Riso Bianco (RB), Tuvagliedda Rossa (TR), Nasieddu (N), Verdolino Nano (VN), Munachedda (M), Ciuoto (C), Cannellino Nano (CN), Tabacchino Nano (TN,), San Michele (SM). “Lamon” beans: Spagnol (SP1), Spagnolit (SP2), Calonega (CA). “Zolfino del Pratomagno” beans: Loro Ciuffenna (LC), Terranuova Bracciolini (TB).

2.3. Nutritional components and technological properties

Ash content was obtained from the inorganic mass after 3 h exposure to 550 ℃. Total carbohydrates were determined according to the Fehling test, and fiber constituents according to Perez-Hidalgo et al. [22]. Extraction of total fat was performed by Soxhlet and analyses were performed according to AOAC [18].

Fatty acids were extracted and methylated simultaneously according to Ulberth and Henninger [23]. Bean seed extracts were subjected to gas chromatography with a Carlo Erba series Mega 5160 Gas Chromatograph with silica-fused column SP-2560, 30 m × 0.25 mm × 0.20 mm. Following sample injection, profile determination was conducted as follows: 140 ℃ to 180 ℃ at 6 ℃·min^{-1} for 3 min, from 180 ℃ to 240 ℃ at 10 ℃·min^{-1} with a helium flux of 20 cm·sec^{-1}, 240 injector. Quali-quantitative identification of fatty acids was performed by employing a FAME Mix Supelco made from 37 standard fatty acids.

Seed coat percentage, calculated in relation to the whole seed, was measured on 30 soaked seeds by separating the coats of the cotyledons after the imbibition test and drying the latter at 105 ℃ for 24 h. Water adsorption or percentage imbibition was determined after soaking 50 seeds for 3, 8 and 24 h, respectively [24].

2.4. Statistical analysis

All statistical analyses were conducted using Statistica 7.1 software (2005, StatSoft, Tulsa, OK, USA). Differences between mean values were compared by least significant difference (LSD) in a one-way analysis of variance (ANOVA).

Linear discriminant analysis (LDA), a multivariate technique permitting the scoring of the cases as a function of the first two roots (canonical discriminant functions) was used to visualize similarities and differences among the different bean accessions [25]. LDA was also used to determine a linear combination of features (discriminant functions) that characterize or separate two or more classes of objects or events [26]. LDA was performed on two different data sets. The first data set included the standardized matrix of the fatty acid content (21 ecotypes and 10 variables, including tridecanoic, myristic, palmitic, palmitoleic, heptadecanoic, stearic, oleic, linoleic, linolenic and nervonic acids). The second data set included the standardized matrix of the nutrient and phytochemical content of bean accessions (21 ecotypes and 14 variables, including free amino acids, total protein, G2 protein fractions, ash, total carbohydrate, hemicellulose, cellulose, lignin, total lipids, unsaturated lipids, nutritional content, tegument percentage and 8 h imbibition).

Pearson’s correlation tests were also conducted to determine the linear correlations between the principle nutritional components (proteins, lipids, carbohydrates, fibers) and fatty acid composition. All statements of significance were based on the 0.05 and 0.01 probability levels.

3. Results

Three distinct types of electrophoretic banding patterns of the principle G1 storage protein, phaseolin (PHAS) have been shown to predominate in Italian bean landraces [5]. These include the Middle America “S” type pattern and the Andean “C” and “T” patterns, respectively designated after the commercial varieties Sanilac (S), Contender (C) and Tendergreen (T) [7]. The two populations of “Zolfino del Pratomagno” (Tuscany) were previously identified as Middle American “S” type [9], whereas the “Fagiolo di Sarconi” (Basilicata) and “Fagiolo di Lamon” (Veneto) based landraces were shown to be comprised of Andean “C” and “T” types [8,9]. Aside from the provenance designations, the landraces were also divided into color differences and comparisons made on the basis of nutritional components, including protein constituents

3.1. Protein constituents, PHA-E activity, nutritional components and technological properties

Significantly higher levels of amino acids were evident for Zolfino in both LC and TB compared to the Sarconi and Lamon accessions (Table 1). Total protein content was not significantly different between the landraces (Table 1). Though the G1 (PHAS) content was marginally higher in the Zolfino populations, no significant differences between Zolfino and the Sarconi accessions were reported (Table 1). There were significantly higher levels of G2 (equated with phytohemogglutinin [PHA] or lectin) in Zofino than in the Sarconi and Lamon landraces, resulting in the latter displaying a significantly higher G1/G2 ratio to that of Zolfino. No association for protein constituents was found for either T or C types or color, respectively (Table 1). PHA-E activity was investigated as a test of lectin capacity to agglutinate red blood cells. Though G2 is equated with lectin content, no positive correlation between G2 protein content and PHA-E activity was observed (Table 1). Lamon landraces were highly variable for PHA-E activity.

Ash content was significantly higher in Zolfino, than in the Sarconi and Lamon landraces (Table 2). No significant difference in total fiber, and respective cellulose and hemicellulose components, was observed between the landraces. Values obtained were subject to internal variation, as was evident for the lignin content (Table 2). Total carbohydrate content did not vary (Table 2). The total lipid content was significantly higher only for Zolfino cultivated in LC, whereas the percentage composition of both saturated and unsaturated fatty acids was not significantly different between the landraces analyzed (Table 2). The nutritional values of the landraces investigated were equivalent (Table 2). Overall, nutritional constituents did not vary for landrace, and hence no association was found for either T or C types or color, respectively.

An important technological feature in the adoption of a bean cultivar by consumers and, consequently, by producers, is related to cooking time, and the percentage imbibition represents a useful and rapid indirect selection method to screen germplasm for short cooking time. In the present study water absorption after 3, 8 and 24 hours, as well as seed coat percentage, was investigated (Table 3). After 8 h, imbibition was *ca* 80–90% complete. No association between seed coat percentage and imbibition capacity was evident in the present study, and after 8 h, all landraces were adequately imbibed.

Linear Discriminant Analysis (LDA) was used to further expound the variability in the bean accessions. LDA is a linear transformation technique that is used for dimensionality reduction, similar to more common algorithms such as Principal Component Analysis (PCA). PCA can be described as an “unsupervised” algorithm, since it “ignores” class labels and its goal is to find the directions (the so-called principal components) that maximize the variance in a dataset. In contrast to PCA, LDA is “supervised” and computes the directions (“linear discriminants”) that represent the axes that maximize the separation between multiple classes. Consequently, LDA was used in our study as it performs better when using a large dataset with multiple classes, and where class separation is an important factor while reducing dimensionality.

Figure 2 shows a scatter plot of bean landraces defined by the first two canonical functions, Root 1 and Root 2, which respectively explained 95.2 and 3.2% of the total variability, respectively. The multivariate technique showed a high discrimination power as indicated by the Wilks lambda value (0.00205) significant at $P < 0.0013$. The Zolfino ecotypes were divergent from the Sarconi and Lamon landraces on the positive branch of Root 1 (Figure 2), influenced by the tegument percentage, unsaturated fatty acids, total lipids, and protein fraction G2, with canonical discriminant function values of 3.39, 3.44, 3.84, and 4.89, respectively. The distinct grouping between ecotypes from LC (positive Root 2) and TB (negative Root 2) was based on the free amino acid content (canonical function value of 1.08 on Root 2). Clustering of both Sarconi and Lamon ecotypes on the negative branch of Root 1 branch was associated with seed imbibition (8 h), hemagglutination, total carbohydrates, protein, lignin, cellulose, nutritional value and hemicellulose with canonical function values of −2.41, −2.92, −3.68, −4.25, −4.76, −4.83, −5.90, −7.85, respectively.

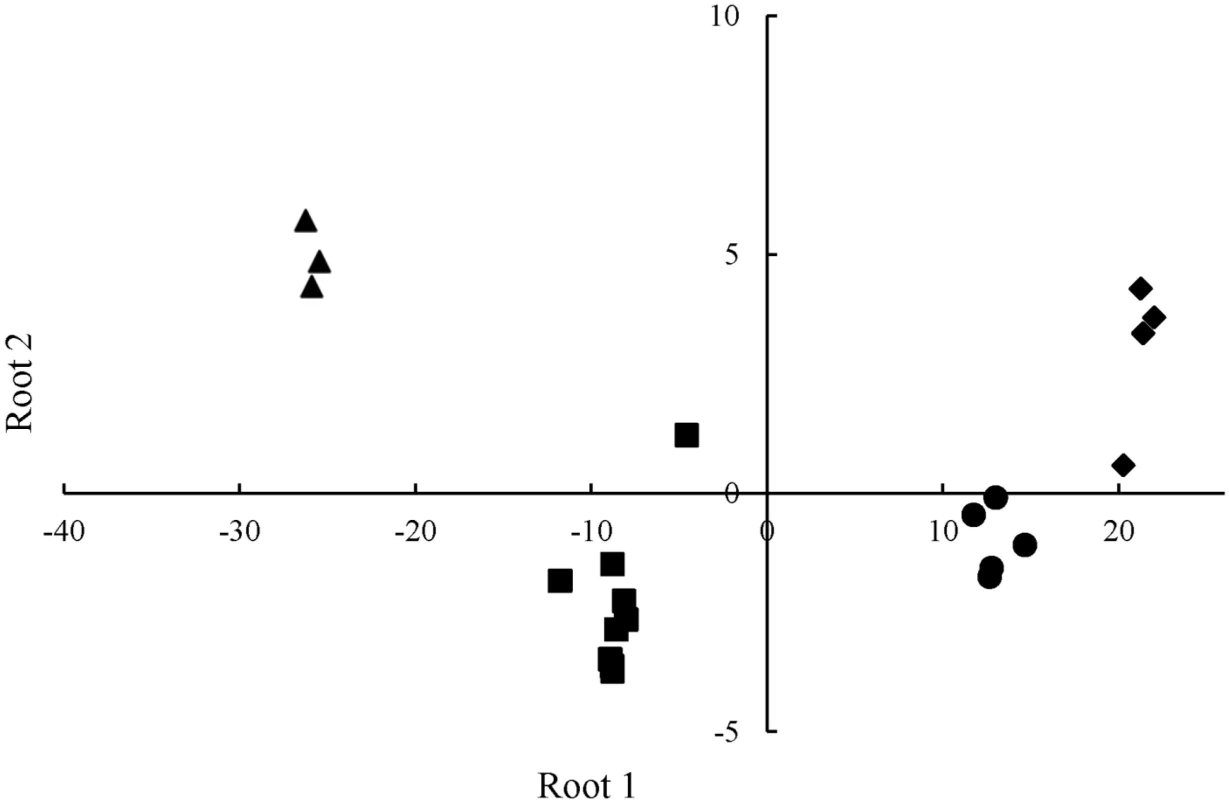

Figure 2. Least Discriminant Analysis Scatter plot based on 14 nutritional constituent variables for the common bean accessions, Zolfino Loro Ciuffenna (♦), Zolfino Terranuova Bracciolini (●), Sarconi (■) and Lamon (▲).

Table 1. Comparison among G1 (PHAS) profile, color, protein constituents and hemogglutination index in Zolfino, Sarconi and Lamon ecotypes.

	Zolfino LC (4)	Zolfino TB (5)	CN,N,RB Sarconi	M,TN,TR Sarconi	C,SM,VN Sarconi	CA,SP1,SP2 Lamon
G1 Profile	S	S	C	C	T	C + T
Color	Light yellow	Light yellow	White	White-brown	Green/Brown	Brown speckled
Hemogglutination index ns	39.8	34.6	27.3	33.3	30.5	66.1
Free AA (mg g^{-1}) **	28.2 a	23.6 ab	13.2 bc	8.8 c	10.6 bc	8.4 c
Protein (%) ns	25.8	24.9	23.9	24.8	23.4	25.3
G1 (%) ns	14.3	14.6	10.5	10.7	10.5	11.0
G2 (%) ***	10.1 a	10.1 a	5.4 b	6.2 b	5.8 b	5.3 b
G1/G2 **	1.4 b	1.4 b	2.0 a	1.7 ab	1.8 ab	2.1 a

Notes: Locations: LC: Loro Ciuffenna (4); TB: Terranuova Bracciolini (5). Ecotypes: C: Ciuoto; CA: Calonega; CN: Cannellino Nano; M: Munachedda; N: Nasieddu; RB: Riso Bianco; SM: San Michele; SP1: Spagnol; SP2: Spagnolit; TN: Tabacchino Nano; TR: Tuvagliedda Rossa; VN: Verdolino Nano. In a row, different letters indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and ns is not significant.

Table 2. Nutritional components in Zolfino, Sarconi and Lamon ecotypes.

	Zolfino LC (4)	Zolfino TB (5)	CN,N,RB Sarconi	M,TN,TR Sarconi	C,SM,VN Sarconi	CA,SP1,SP2 Lamon
G1 Profile	S	S	C	C	T	C + T
Color	Light yellow	Light yellow	White	White-brown	Green/Brown	Brown speckled
Ash (%) ***	4.7 a	4.8 a	4.0 b	3.9 b	3.8 b	4.0 b
Hemicellulose (%) ns	7.9	14.7	6.3	6.1	13.4	8.6
Cellulose (%) ns	6.8	6.6	6.6	5.4	5.3	6.7
Lignin (%) *	0.2 ab	0.1 b	0.4 ab	0.3 ab	0.9 a	0.6 ab
Total Fiber (%) ns	17.1	21.4	13.2	14.4	19.7	15.9
Saturated FA (%) ns	0.28	0.24	0.20	0.20	0.15	0.23
Unsaturated FA (%) ns	1.3	1.1	1.1	1.0	1.1	1.0
Total lipid (%) **	1.7 a	1.4 b	1.4 b	1.3 b	1.4 b	1.3 b
Total carbohydrate (%) ns	51.0	47.7	57.6	56.9	52.3	53.5
Nutritional value (Kcal)	320.8	302.2	338.7	333.3	321.3	327.0

Notes: Locations: LC: Loro Ciuffenna (4); TB: Terranuova Bracciolini (5). Ecotypes: C: Ciuoto; CA: Calonega; CN: Cannellino Nano; M: Munachedda; N: Nasieddu; RB: Riso Bianco; SM: San Michele; SP1: Spagnol; SP2: Spagnolit; TN: Tabacchino Nano; TR: Tuvagliedda Rossa; VN: Verdolino Nano. In a row, different letters indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and ns is not significant.

Table 3. Seed coat proportion and water absorption in Zolfino, Sarconi and Lamon ecotypes.

Ecotype Location	Zolfino LC (4)	Zolfino TB (5)	CN,N,RB Sarconi	M,TB,TN, TR Sarconi	C, SM,VN Sarconi	CA,SP1,SP2 Lamon
Tegument (%) ns	6.4	7.8	7.6	7.2	7.5	6.7
Imbibition % (3 h) ns	64.0	54.9	75.7	62.6	61.7	54.8
Imbibition % (8 h) ns	83.9	90.6	92.8	83.3	76.2	77.0
Imbibition % (24 h) **	95.8 ab	101.5 a	92 ab	95.5 ab	89.4 b	104.3 a

Notes: Locations: LC: Loro Ciuffenna (4); TB–Terranuova Bracciolini (5). Ecotypes: C: Ciuoto; CA: Calonega; CN: Cannellino Nano; M: Munachedda; N: Nasieddu; RB: Riso Bianco; SM: San Michele; SP1: Spagnol; SP2: Spagnolit; TN: Tabacchino Nano; TR: Tuvagliedda Rossa; VN: Verdolino Nano. In a row, different letters indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and ns is not significant.

Import of commercial common beans at a lower cost than local ecotypes, and a predominant use of a few selected varieties such as Borlotto and Cannellino in Italy has resulted in a decreased use of local ecotypes [15]. To establish whether the commercial varieties were superior in protein, and nutritional constituents compared to the local ecotypes, comparisons were made between the combined Zolfino and Sarconi accessions, and four commercial varieties, including Sanilac, Contender and Tendergreen, as well as Borlotto "Lingua di Fuoco". There were no significant differences in the nutritional/technological components between the ecotypes and commercial varieties (Table 4), with the exception of significantly higher levels of ash content, G2 protein and amino acids for Zolfino.

Table 4. Comparisons between protein-related parameters, nutritional components and technological features in commercial cultivars and the Zolfino and Sarconi ecotypes.

	Commercial	Zolfino	Sarconi
Hemogglutination index	32.0	37.2	30.4
Free AA (mg g^{-1}) ***	8.6 b	25.9 a	10.9 b
Protein (%)	24.9	25.3	24.1
G1 (%)	11.0	14.5	10.6
G2 (%) ***	6.1 b	10.1 a	5.8 b
Ash (%) **	4.2 b	4.8 a	3.9 b
Hemicellulose (%)	10.1	11.3	8.6
Cellulose (%)	6.8	6.7	5.8
Lignin (%)	1.0	0.1	0.5
Total Fiber (%)	17.9	19.2	15.8
Saturated FA (%)	0.2	0.3	0.2
Unsaturated FA (%)	0.9	1.2	1.0
Total lipid (%)	1.4	1.5	1.4
Total carbohydrate (%)	51.7	49.4	55.6
Nutritional value (Kcal)	318.3	311.5	331.1
Tegument (%)	8.9	7.1	7.4
Imbibition % (3 h)	78.7	59.4	66.7
Imbibition % (8 h)	89.5	87.3	84.1
Imbibition % (24 h)	98.4	98.6	94.3

Notes: Commercial varieties: Borlotto "Lingua di Fuoco", Contender, Sanilac. and Tendergreen. Ecotypes: Zolfino (2 locations). Sarconi (all landraces). In a row, different letters indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.2. *Fatty acid profiles*

Extensive genotype as well as genotype-environmental "niche" variation in fatty acid profiles of common bean landraces have recently been reported [10,15]. Given that there is little work investigating fatty acid profiles in common bean landraces [10], and that it is well known that genotype and environment affect the composition of fatty acid profiles, we investigated the potential feasibility of a fatty acid profile in contributing partly towards a "landrace-environmental niche fingerprint" in common bean.

The Polyunsaturated Fatty Acids (PUFA), were composed predominantly of the omega-3 linolenic acid, followed by the omega-6 linoleic acid in all landraces (Table 5). Monounsaturated Fatty Acids (MUFA) in the landraces were comprised of the omega-9 fatty acids, oleic and nervonic, as well as the omega-7, palmitoleic, respectively. Interestingly, Zolfino also contained trace levels of the MUFA, heptadecanoic acid, which was undetectable in both the Sarconi and Lamon accessions (Table 5). The significantly higher MUFA content in Zolfino, compared to the Sarconi and Lamon landraces, was primarily attributable to palmitoleic and nervonic acid levels (Table 5). As consequence, the PUFA:MUFA ratio was *ca* 50:50% in Zolfino for both LC and TB (Table 5), whereas the Sarconi landraces all had a PUFA:MUFA ratio of *ca* 70:30%. An intermediate PUFA:MUFA ratio of *ca* 60:40% was evident for the Lamon landraces (Table 5). No significant differences in the polysaturated fatty acids, palmitic and stearic acids, were evident.

Myristic acid was only detected in the Lamon landraces, whereas tridecanoic acid was present only in the Zolfino and Lamon landraces, respectively (Table 5). The predominant fatty acids extracted from Zolfino were linolenic > palmitoleic > nervonic ~ linoleic, respectively, whereas those within Sarconi were linolenic > linoleic > palmitic, respectively (Table 5). In turn, the predominant fatty acids extracted from Lamon were, respectively, linolenic > linoleic > palmitoleic ~ palmitic ~ nervonic acids. In order to better evaluate the relationships between the landraces based on the seed content of various fatty acids, LDA was performed to explain the variability in bean accessions.

Root 1 and Root 2 canonical functions, respectively explained 74.7 and 21.4% of the total fatty acid variability (Figure 3). The multivariate technique showed a good discrimination power as indicated by the Wilks lambda value (0.00205), significant at $p < 0.00001$. The Zolfino landraces were clustered, indiscriminately for location on the positive branch of Root 1 (Figure 3), strongly influenced by heptadecanoic and oleic acid content with canonical discriminant function values of 0.85 and 2.14, respectively. The positive branch of Root 2 was associated with palmitoleic and palmitic acid content with canonical function values of 0.64 and 0.88, respectively. Hence, overall Zolfino accessions were distinguished by the combined increased levels of oleic and palmitoleic acids, and the presence of heptadecanoic acid. The Sarconi and Lamon landrances formed separate clusters on the negative branch of Root 1, influenced by myristic and linolenic acid content, with canonical discriminant function values of −0.75, and −1.21, respectively. The Sarconi accessions (negative Root 1, positive Root 2) were characterized by combined higher levels of linolenic and palmitic acids, and the absence of both myristic and tridecanoic acids. In turn, Lamon accessions (negative Root 1, negative Root 2) were characterized by the combined higher linolenic acid, lower palmitic acid and the presence of both myristic acid as well as tridecanoic acid (canonical function value of −1.21 on Root 2).

3.3. *Correlation analysis*

In order to evaluate the relationship between fatty acid composition and the principle nutritional components (proteins, carbohydrates, lipids, fibers) of bean seeds, Pearson's correlation coefficients were calculated (Table 6). Fibers were negatively correlated with palmitic acid ($r = -0.59$, $p < 0.01$), as well as proteins with linoleic acid ($r = -0.53$, $p < 0.05$). Carbohydrate content was positively correlated with palmitic (r = 0.49, $p < 0.05$), and linoleic (r = 0.45, $p < 0.05$) acids, and positive correlations were also observed between lipids and oleic acid (r = 0.68, $p < 0.01$), linoleic acid (r = 0.48, $p < 0.05$), and nervonic acid (r = 0.53, $p < 0.05$).

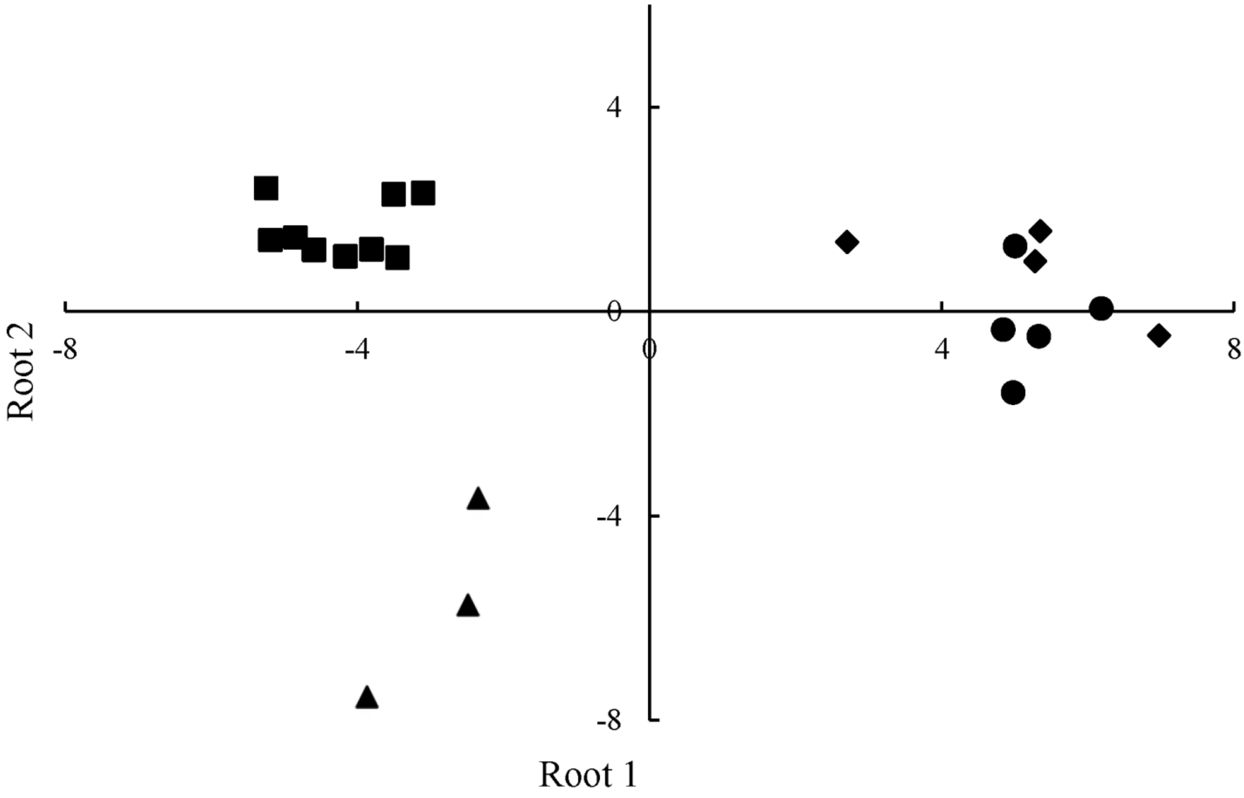

Figure 3. Least Discriminant Analysis scatter plot based on 10 fatty acid variables for the common bean accessions, Zolfino Loro Ciuffenna (♦), Zolfino Terranuova Bracciolini (●), Sarconi (■) and Lamon (▲).

Table 5. Percentage of fatty acid content in Zolfino, Sarconi and Lamon ecotypes.

Fatty acids	Zolfino LC (4)	Zolfino TB (5)	CN,N,RB Sarconi	M,TN,TR Sarconi	C,SM,VN Sarconi	CA,SP1,SP2 Lamon
MUFA (g 100 g^{-1}) ***	0.614 a	0.540 b	0.317 c	0.316 c	0.345 c	0.369 c
PUFA (g 100 g^{-1}) *	0.638 ab	0.488 b	0.751 a	0.651 ab	0.729 ab	0.580 ab
PUFA:MUFA (%)	50.9: 49.1	48.1 : 51.9	70.3 : 29.7	67.4 : 32.6	67.9 : 32.1	61.2 : 38.8
Tridecanoic (C13:0) ***	0.080 a	0.067 a	ND b	ND b	ND b	0.053 a
Myristic (C14:0) **	ND b	ND b	ND b	ND b	ND b	0.050 a
Palmitic (C16:0) ns	0.153	0.131	0.191	0.180	0.154	0.133
Palmitoleic (C16:1) *	0.290 a	0.240 ab	0.122 b	0.140 b	0.113 b	0.148 b
Heptadecanoic (C17:1) ***	0.015 a	0.013 a	ND b	ND b	ND b	ND b
Stearic (C18:0) ns	0.020	0.016	0.036	0.010	0.018	0.011
Oleic (C18:1) ns	0.106	0.091	0.080	0.062	0.063	0.075
Linoleic (C18:2) ns	0.225	0.179	0.285	0.223	0.261	0.198
Linolenic (C18:3) ns	0.393	0.309	0.465	0.428	0.467	0.383
Nervonic (C24:1) ***	0.222 a	0.196 ab	0.156 bcd	0.113 d	0.168 bc	0.146 cd

Notes: Locations: LC: Loro Ciuffenna (4); TB: Terranuova Bracciolini (5). Ecotypes: C: Ciuoto; CA: Calonega; CN: Cannellino Nano; M: Munachedda; N: Nasieddu; RB: Riso Bianco; SM: San Michele; SP1: Spagnol; SP2: Spagnolit; TN: Tabacchino Nano; TR: Tuvagliedda Rossa; VN: Verdolino Nano. In a row, different letters indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and ns is not significant. ND is not detected.

Table 6. Correlations among the principle nutritional parameters and fatty acid composition of the investigated common bean ecotypes.

Nutritional contents	Tridecanoic (C13:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmitoleic (C16:1)	Heptadecanoic (C17:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Nervonic (C24:1)
Proteins	0.24	−0.12	−0.10	0.24	0.02	0.04	0.32	−0.53*	−0.04	0.05
Lipids	0.37	−0.14	0.40	0.30	0.33	0.37	0.68**	0.48*	0.32	0.53*
Carbohydrates	−0.35	0.18	0.49*	−0.40	−0.42	0.10	−0.23	0.45*	0.21	−0.38
Fibers	0.15	−0.17	−0.59**	0.29	0.39	−0.17	−0.01	−0.37	−0.26	0.30

Notes: * and ** indicate that correlation is significant at the 0.05, 0.01 level, respectively.

4. Discussion

Common bean landraces are not only treasures that need to be safeguarded for the future, but are in need of extensive trait characterization as genetic resources [10]. "Zolfino del Pratomagno" is shown to be more divergent or distinctive, from the Sarconi and Lamon landraces. Noteworthy was the significantly higher MUFA content, the lower PUFA to MUFA ratio, as well as the presence of heptadecanoic fatty acid. Additional distinguishing factors include higher levels of amino acids, G2 protein, ash content and lipid contents.

Significantly higher levels of amino acids were evident for Zolfino in both locations. Previous research indicated a positive relationship between free amino acid content and total protein content in Brazilian landraces [27], however, this was not evident for Zolfino. The reason for the significantly higher amino acid content in Zolfino is not known, and further studies would be required to investigate this aspect. The possibility that a higher protease activity during maturation in the latter may have contributed to the higher amino acid content cannot be excluded. Zolfino in both locations showed significantly higher levels of G2 protein relative to the Sarconi and Lamon landraces. Though G2 is equated with lectin content, no positive correlation between G2 protein content and PHA-E activity was observed across the landrace groupings. This may be attributable to the presence of differential proportions of the lectins in the albumin protein fraction or variability in the PHA-E activity capacity of the lectins present in G2. Traditionally PHA content has long been perceived solely as an anti-nutritional factor. However, more recent research has focused on *in vitro* and *in vivo* biological activities of PHA from common beans, acting as health-promoting compounds in the prevention/treatment of non-communicable diseases [1,2]. A toxin in uncooked beans, effective processing is shown to be instrumental in reducing PHA-E activity to safe levels [28], as is evidenced by the ever-increasing documented health benefits of red kidney beans, which are widely reported to contain the highest reported PHA-E activities in common bean. More extensive research on the physiological properties of lectins after food consumption has been proposed [28]. Interestingly, using 1,072 landraces, obtained from the International Center for Tropical Agriculture (CIAT) genebank, Islam et al. [12] indicated a higher lectin content for the Middle American group in comparison to the Andean group. However, given that red kidney bean is of the Nueva Granada race (Colombia) of Andean origin, this classification reflects a general tendency. Wide variation in both lectin content and PHA-E activity has been reported both for genotype and location [12,29–31], and therefore, variation present in genotype and location must be taken into consideration when selecting and/or breeding specifically for either high or low lectin content and/or activity.

Ash content, significantly higher for Zolfino, in both locations, was evident. The results of the present study corroborate previous research showing a significantly higher ash content for Middle American landraces within the Spanish core collection [3]. Although individual mineral element composition comprising the ash component, was not measured in the present study, previous research indicated significantly higher levels of individual mineral elements in the landraces of Middle American origins [3,12]. In a study of 91 common bean varieties investigating chemical composition associations to seed coat color, higher mineral element content was associated with black beans of the Jamapa genotype which are Middle America in origin [13].

The present study reported distinctive differences in the fatty acid profiles for the Zolfino Sarconi and Lamon landraces. Fatty acid predominance in the order of linolenic > linoleic > palmitic was demonstrated within the Sarconi landraces (Basilicata), also evident for the landraces of Campania region (Italy) [15]. The latter order of fatty acid predominance is also reported in commercial varieties, cultivated and grown in Italy, Mexico and various African countries [32], giving rise to the widely reported higher PUFA to MUFA ratios for common bean landraces [10,15,32,33]. In contrast, Zolfino (Tuscany) was distinctive, due to a significantly higher MUFA content, and equivalent PUFA to MUFA ratios. Variation in fatty acid components was shown to be valuable towards creating an "ecological niche" fingerprint for common bean landraces in Campania [15]. In the present study, LDA further evidenced the potential for "genotype-niche diversity" fingerprints in Italian germplasm. Confirmation would necessitate wide-scale studies including a vast number of landraces and ecological niche environments. In the present, small-scale study, three distinctive cluster groupings were evident for the Zolfino, Sarconi and Lamon landraces. Zolfino was distinct for the combined presence of increased oleic and palmitoleic acids (MUFA) as well as the presence of heptadecanoic (MUFA) acid. Instead, Sarconi was characterized by the combined higher levels of linolenic and palmitic acids and the absence of both myristic and tridecanoic acids. Lamon was characterized by a higher level of linolenic acid, lower palmitic acid and the presence of both myristic and tridecanoic acids. Interestingly, recent models based on fingerprints derived from fatty acid profiles have been produced. These fingerprints were shown to be highly specific for authenticating the provenance of pistachio nuts, thereby showing that chromatographic fingerprints (in addition to mineral element composition) can be used as powerful tools in several application fields, including the identification of the geographical origin of the food products [34]. Though nutritional compounds are not recognized as "genotype-niche diversity" fingerprints, it was interesting that the landraces formed distinctive clusters for these attributes, predominantly attributable to the divergence of Zolfino from the Sarconi and Lamon landraces.

Correlation analysis between fatty acids and the principle nutritional components showed that lipid content was positively correlated with the amount of the unsaturated fatty acids oleic, linoleic and nervonic acids, respectively. On the other hand, carbohydrates were positively correlated with palmitic and linoleic acids. Instead, negative correlations were found between proteins and linoleic acid, as well as between fibers and palmitic acid. Our results differ from previous literature findings: Celmeli et al. [35] reported a negative correlation between fat content and oleic acid as well as an absence of correlations between proteins and fatty acid composition in Turkish common bean varieties. These differences may be attributable to the effect of growing conditions, genotype and environment x genotype interactions that have been shown to strongly affect the nutritional composition, including fatty acid contents, in various legume seeds [11,36].

5. Conclusions

"Zolfino del Pratomagno" was higher in amino acids, G2 protein, ash content and MUFA than the Sarconi and Lamon ecotypes. The landraces are sources of diversity, as is evidenced from protein constituents and fatty acid profiles. Provenance (based on G1 profile) and color had no effect on these properties. Landraces were not inferior in either nutritional components or technological features compared to the commercial varieties, which should serve an incentive to increase local ecotype production and consumption as well as extensive trait characterization of these unique genetic resources. Despite the small-scale size of the study, both nutritional and fatty acid profiles were very distinctive for the Zolfino, Sarconi and Lamon landraces. The potential of expanding studies to include fatty acid profiles as possible sources of "genotype-niche diversity" fingerprints for common bean in Italy appears feasible.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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