Comparative evaluation of phosphorus accumulation and partitioning in seeds of common bean (*Phaseolus vulgaris* L.)

Angela Rosa Piergiovanni, Lucia Lioi, Vincenzo Montesano, Giulio Sarli

Institute of Biosciences and Bioresources, National Research Council, Bari, Italy

Abstract

There is an increasing attention towards common bean due to its health benefits, prevention to human diseases and as ingredient for functional or fortified foods. Phosphorus, an essential element for plant growth, is mainly stored in seeds as phytic acid (Phy). Phy is negatively associated with mineral bioavailability, but, at the same time, is a natural antioxidant. Accumulation and partitioning of phosphorus were analysed in seeds of ten Italian common bean landraces for three subsequent growing seasons. Some important seed quality traits were also evaluated. For comparative purposes, the landrace harvests of two growing locations were analysed. A wide variation of total and phytic phosphorus contents was recorded among the landraces. Moreover, P accumulation and partitioning between Phy and inorganic P, as well as seed quality traits, resulted strongly affected by growing location. Statistically significant increases of Phy levels were recorded for harvests obtained outside the traditional area of cultivation. These results highlight how the cultivation of a landrace outside of its traditional area will appreciably affect harvest quality.

Correspondence: Angela Rosa Piergiovanni, Institute of Biosciences and Bioresources, National Research Council, via Amendola 165/A, 70124 Bari, Italy.
Tel: +39.080.5583400.
E-mail: angelarosa.piergiovanni@ibbr.cnr.it

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Introduction

Grain legumes are an important staple food due to their high nutritional value, long time of conservation of seeds, and low cost. Among legumes, common bean (*Phaseolus vulgaris* L.) has an important role in the human diet at worldwide level. Its consumption pattern varies dramatically by geographic regions and among cultures, with Latin America being the leading producer and consumer (FAO, 2016). Common bean is an integral part of the Mediterranean diet, though its cultivation in Mediterranean countries is progressively declined, hence presently the extent of importation in the area is increasing (Rubiales, 2015). In spite of the regressive pattern of production, farmers, food industries, and consumers are increasingly demanding autochthonous common beans. Italian consumers often prefer their own landraces, being the major component of many traditional dishes and an integral part of their culture and traditions (Piergiovanni and Lioi, 2010; Montesano *et al.,* 2012). Efforts to promote the *on farm* maintenance of the autochthonous landraces superior for seed composition and technological quality are in progress in Italy. Common bean seeds are not only an important source of vegetable proteins, starch, dietary fiber, and minerals, but also of bioactive and antinutritional compounds that can affect the metabolism in humans and animals (Sparvoli *et al.,* 2015). For these reasons, there is an increasing attention toward the potential health benefits of this pulse (Campos-Vega *et al.,* 2011; Blair, 2013; Sparvoli *et al.,* 2016). Several studies link common beanconsumption to reduced risk of several diseases (Bazzano *et al.,* 2001; Bourdon *et al.,* 2001). Protease inhibitors (Kennedy 1998, 2006), phenolic compounds, anthocyanins, flavonoids, flavones, tannins (Cardador-Martines *et al.,* 2006; Golam Masum Akond *et al.,* 2011; Chávez-Santoscoy *et al.,* 2013), saponins, and non-digestible fiber (Feregrino-Perez *et al.,* 2014) are responsible of the health properties.

Phosphorus (P) is an essential element for plant growth, although grain legumes show different ability to use P contained in soil, and respond to fertilisers. In common bean, as well as in all legumes, a large proportion of phosphorus is present as phytic acid (Phy), *myo*-inositol hexakisphosphate (Lott *et al.,* 2000). The complex of phytic acid and some microelements in the form of phytate is negatively associated with mineral bioavailability, leading to a reduction in assimilation of bivalent cations (Frossard *et al.,* 2000; Petry *et al.,* 2014; Bohn *et al.,* 2008). At the same time, phytates have antioxidant properties, and have a role in the reduction of risk of cancer and other pathologies (Vucenik and Shamsuddin, 2006; Silva and Bracarense, 2016). One of the goals of bean breeders has been to reduce phytate accumulation increasing the proportion of inorganic phosphorus. Low Phy mutants have been obtained for most important crops and recently also for common bean (Campion *et al.,* 2009). However, the reduction of phytates must take into account various issues related to their role in making common bean a functional food (Panzeri *et al.,* 2011; Sparvoli *et al.,* 2016). Therefore, if common bean has to become a competitive source of phytonutrients, to look for good genotypes combined with optimal growing conditions, are critical points. It is well known that seed traits (*i.e.*: protein and ash contents, cooking time, *etc*.) are affected by genetic and environmental factors, such as genotype, growing location, climatic conditions, and cultivation year (Kumar *et al*., 2006; Nikolopoulou *et al*., 2007; Piergiovanni *et al*., 2000).

The objective of the present study was to compare ten common bean landraces with regard to grain content of total and inorganic phosphorus, and phytates. The effect of growing location, in and out the traditional cultivation area was evaluated. Moreover, in order to acquire additional information about seed quality, protein and ash contents, coat percentage, and cooking time. were investigated.

Materials and methods

Seed material

The ten common bean landraces listed in Table 1, were gathered from a local farmer traditionally growing them at Sarconi (Basilicata region, Southern Italy). The six landraces, Ciuoto, Verdolino, Tabacchino, Munachedda, Tuvagliedda, and San Michele, obtained in 1996 the PGI mark of European Community (dossier number IT/PGI/0017/1531).

Field trials

The landraces were grown for three subsequent growing seasons (2012-2014) in the traditional area of cultivation at Sarconi (40.2486, 15.8865). This location, at about 638 m asl, is dominated by a Mediterranean climate, with mild winters, and dry and warm summers. The mean rainfall during the cultural cycle was 224 mm, the mean temperatures were 12.6 and 27.0°C (minimum and maximum, respectively) over the three growing seasons. The soil is sand and loam-clay (51 and 49%, respectively), neutral in reaction (pH 7.1), with traces of limestone, a medium-high level of total nitrogen (1.8‰) and 1.8% of organic matter.

To evaluate the influence of growing location, field trials were performed for two years (2013 and 2014) also at Policoro (40.1907, 16.6446 Basilicata region, Italy). This location, placed at about 15 m asl, and characterised by a sub-humid climate, is outside the traditional area of cultivation of the landraces. The mean rainfall during the cultural cycle was 166 mm, the mean temperatures were 17.4 and 31.0°C (minimum and maximum, respectively) over the two growing seasons. The soil is classified as alluvial silty-clay (40, 37, and 23% of sand, silt, and clay, respectively), sub-alkaline in reaction (pH 7.9). Furthermore, it is highly fertile, well equipped with nitrogen and rich in phosphorus (Negro *et al.,* 2016).

Field trials were carried out according to the production protocol of common beans from Sarconi (http://ec.europa.eu/agriculture/quality/door/registeredName.html?denominationId=301)

Sowing was performed on the first and third decades of June at Policoro and Sarconi, respectively. A complete randomised block design was adopted with four repetitions. Seed density was 80-100 kg ha^{-1}, and weed control was mechanically performed. Harvesting occurred on the first decade of October at Policoro and the third decade of September at Sarconi.

Seed quality traits

For each landrace, equal amounts of seeds randomly selected from the each of the four field repetitions were mixed to obtain the bulk used for the analyses. About 50 g of dry seeds were ground in a Cyclotec 1093 mill (Tecator, Hillerød, Denmark) to obtain a fine meal used for the following measurements. Moisture was determined by loss of weight after meal drying in an oven (AOAC method 930.15); protein contents were determined by micro-Kjeldahl method (N x 6.25), following the AOAC method 979.09 (AOAC, 1970). Ash content was measured according to AOAC (method 923.03). Total P was determined by the colorimetric method of Fiske and Subbarow (1925). Inorganic phosphorus was measured on aliquots of acidic extracts according to Campion *et al.* (2009), whereas phytic acid was determined on aliquots of acidic extracts subjected to ferric precipitation as described by Pilu *et al.* (2003).

Coat percentage, calculated respect to the whole seed weight, was measured on 30 seeds randomly selected. After 24 h of seed soaking, coats were manually separated and oven dried overnight. Fifty seeds, soaked overnight at room temperature, were used to carry out the cooking test. After 20 min of cooking, the seed softness was measured every 2 min on three seeds until complete cooking.

Statistical analysis

Mean values of recorded traits were subjected to the Duncan's test. Associations among the traits were established by correlation analysis. The analysis of variance (one-way ANOVA) was performed to evaluate the role of season, genotype, and growing location. To perceive the differences among the harvests of the two locations, normalised data were submitted to the Principal Component Analysis (PCA). STATISTICA software package, version 7.1 (StatSoft, Tulsa, OK, USA) was used for all statistical analyses.

Results and discussion

Phosphorus accumulation and partitioning in seeds

The contents of total and inorganic phosphorus (P_{tot} and P_i, respectively), and phytate (Phy) recorded at Sarconi, the traditional growing location of the landraces, are reported in Table 1. Overall, the mean values of total and partitioned phosphorus (Phy and P_i), were not significantly different among the three growing seasons. The values of P_{tot} cover a very broad range (2.62-5.17 g kg^{-1}). The extreme values were recorded in different years for Munachedda and Nasieddu nero in 2012 and 2013, respectively. A so wide variation is in agreement with previous studies available in the literature. For example, Blair *et al.* (2009), who analysed recombinant inbred lines, reported P_{tot} amounts ranging from to 2.8 to 6.8 g kg^{-1}, while Beebe *et al.* (2000), screening 29 common bean varieties, found values comprised from 3.7 to 7.1 g kg^{-1}.

The quantification of the fraction of phosphorus in the form of Phy provides an important information on how much P is committed to Phy. Although Phy content of seeds largely depends on environmental factors, previous studies demonstrated that a natural pronounced variability is present in both wild and cultivated common beans (Ariza-Nieto *et al.,* 2007; Guzmán-Maldonado *et al.,* 2000; Piergiovanni *et al.,* 2000). The results of the Phy determinations carried out in the present investigation confirm the variation of this parameter within the cultivated common bean. The highest observed value was about twice the lowest one (5.68 *vs* 10.94 g kg^{-1}), and both these extremes were recorded in 2014 (Table 1). Moreover, our variation partially overlaps to the range 9.8-22.6 g kg^{-1} reported by Guzmán-Maldonado *et al.* (2000).

The primary functions of Phy in seeds are phosphate storage as energy source and antioxidant for germinating seeds. Current literature suggests that Phy content in bean seeds explains from 53% to 81% of total P, and that more than 95% of seed phytic acid is accumulated into the cotyledons (Lott *et al.,* 2000; Ariza-Nieto *et al.,* 2007). In good agreement with the literature, we found Phy/P_{tot} ratios ranging from 44% to 75%, with a mean value over the three years equal to 57.0%±5.78. The extreme values were recorded in different years and for diverse landraces (44.0% for Tuvagliedda in 2014 and 75.5% for Munachedda in 2012), but no significant differences were observed among the Phy/P_{tot} mean values (Table 1).

Inorganic P is another important kind of phosphorus accumulation. Although it is present into the grains at low concentrations, high levels are regarded as desirable from a nutritional point of view. Over the three growing seasons, an appreciable variation was observed also for P_i, being the recorded values comprised from 0.518 to 0.894 g kg^{-1} for Tuvagliedda rossa and Munachedda, in 2013 and 2014, respectively (Table 1).

The quality of common bean grains depends on many seed features at harvest time. For this reason, some of the most relevant parameters related to common bean economic value (*i.e.*: hundred-seed weight, protein and ash contents, coat, cooking time) were determined (Table 2). Overall, the seed quality was good and the mean value of scored traits resulted not significantly affected by growing season, with the exception of 100 seed weight and coat content. The results of the correlation analysis are summarised in Table 3. According to Blair *et al.* (2012), the correlations of 100-seed weight with P_{tot}, Phy or P_i resulted not statistically significant, whereasa significant correlation ($P<0.05$) between Phy and ash was found. This is attributable to the interdependence of ash amount from mineral elements accumulated in grains. Raboy *et al.* (1991) found a correlation between Phy and protein content. In the present study, the correlation between these traits resulted positive but not statistically significant (Table 3). Several studies point out that phytic acid metabolism in grains or leaves is sensitive to cellular level of P_i, which leads to increased translocation of P towards grain (Raboy and Dickinson, 1993). In this investigation, a positive correlation statistically significant ($P<0.01$) between P_{tot} and Phy was found (Table 3).

Table 1. Phosphorus partitioning in seed cultivated at Sarconi for three subsequent growing seasons.

Work code	Landrace	P_{tot} (g kg^{-1})			Phytate (g kg^{-1})			P_i (g kg^{-1})			P_{Phy}/P_{tot} (%)		
		2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
1	Riso	3.87	4.21	3.58	7.98	8.64	6.54	0.677	0.758	0.792	58.1	57.8	51.4
2	Ciuoto	3.61	3.96	4.61	7.34	7.51	9.18	0.536	0.741	0.710	57.3	53.3	56.2
3	Verdolino	4.80	4.72	4.44	9.10	9.19	10.42	0.689	0.785	0.711	53.3	54.9	66.2
4	Tabacchino	3.40	4.65	5.23	8.02	9.84	10.94	0.789	0.849	0.772	66.5	59.5	58.9
5	Munachedda	2.62	4.25	4.16	7.03	8.39	8.21	0.676	0.588	0.894	75.5	55.5	55.5
6	Munachedda nera	4.62	4.20	3.89	7.78	8.28	7.18	0.671	0.658	0.716	47.4	55.4	51.9
7	Tuvagliedda	4.36	4.50	3.64	9.45	9.32	5.68	0.668	0.666	0.641	61.0	58.2	44.0
8	Tuvagliedda rossa	3.60	3.95	3.58	7.93	7.79	6.76	0.754	0.518	0.544	62.0	55.7	53.1
9	San Michele	3.88	4.16	4.00	7.68	8.41	8.39	0.557	0.667	0.826	55.7	57.0	59.0
10	Nasieddu nero	4.16	5.17	3.47	8.85	10.46	6.63	0.641	0.598	0.624	59.8	56.9	53.9
Mean		3.89^{a}	4.38^{a}	4.06^{a}	8.12^{a}	8.78^{a}	7.99^{a}	0.666^{a}	0.683^{a}	0.723^{a}	59.7^{a}	56.4^{a}	55.0^{a}
SD		0.639	0.382	0.562	0.777	0.916	1.754	0.077	0.101	0.103	7.597	1.813	5.838

P, phosphorus; SD, standard deviation. Values expressed on dry matter basis. ^{a}Values followed by the same letter are not significantly different ($P>0.05$).

Table 2. Quality traits of seed cultivated at Sarconi for three subsequent growing seasons.

Work code	Landrace	100 seed weight (g)			Protein (g kg^{-1})			Ash (g kg^{-1})			Coat (g kg^{-1})			Cooking time (min)		
		2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014			
1	Riso	49.2	58.6	47.9	218.5	222.8	217.7	39.6	39.7	37.3	56.4	47.1	51.5	42	34	36
2	Ciuoto	38.0	43.4	37.7	186.6	186.5	193.4	37.4	39.3	45.0	67.0	59.0	58.1	32	34	33
3	Verdolino	37.5	50.4	39.4	216.0	225.4	208.4	39.5	40.2	40.7	61.6	50.6	53.5	34	36	32
4	Tabacchino	37.9	43.8	42.9	223.5	265.3	267.3	39.1	39.4	39.5	62.0	57.4	57.4	40	30	36
5	Munachedda	48.5	59.4	62.8	222.2	213.5	250.8	39.2	42.7	40.0	67.1	54.7	56.0	34	34	42
6	Munachedda nera	46.4	60.4	51.1	264.2	255.0	238.6	41.8	41.1	43.3	69.6	60.1	61.7	40	38	40
7	Tuvagliedda	58.2	68.3	69.0	246.2	248.9	219.7	47.3	41.1	38.3	55.3	47.8	54.4	44	38	41
8	Tuvagliedda rossa	52.2	59.0	59.9	241.2	237.2	245.1	39.7	42.5	38.3	77.1	59.3	61.3	53	38	46
9	San Michele	34.3	60.5	38.7	224.6	229.8	253.4	45.8	40.1	45.6	72.7	54.5	68.8	34	38	36
10	Nasieddu nero	53.2	52.9	61.6	240.6	235.5	239.0	41.9	48.6	38.9	75.0	61.5	64.0	44	34	39
Mean		45.5^{a}	55.7^{bc}	51.1^{ac}	228.4^{a}	232.0^{a}	233.3a	41.1^{a}	41.5^{a}	40.7^{a}	66.4^{a}	55.2^{bc}	58.7^{bc}	39.7^{a}	35.4^{a}	38.1^{a}
SD		8.113	7.928	11.511	21.048	22.381	22.868	3157	2.775	2.935	7.490	5.186	5.297	6.464	2.675	4.306

SD, standard deviation. Values expressed on dry matter basis. $^{a\text{-}c}$Values followed by the same letter are not significantly different ($P>0.05$).

Comparative evaluation of landraces

Common bean genotypes vary in their efficiency of P uptake from soil, capability to store and partitioning it among different forms (Coelho *et al.,* 2002). Experimental data collected in the present study are in good agreement with literature. Analysing in detail the data recorded for the three sequential harvests, differences among the landraces in the extent of year-to-year variation of the P forms investigated can be observed (Table 1). For example, Tabacchino, Munachedda and Nasieddu nero showed the higher year-to-year variation of P_{tot} content. Conversely, Tuvagliedda rossa and S. Michele, showing very close P_{tot} amounts in the three years, appear scarcely affected by growing season.

Tabacchino, Tuvagliedda and Nasieddu nero showed the higher year-to-year variations of Phy level. For these landraces, the highest value was from 36 to 66% greater than the lowest one. As expected, the highest Phy amounts were recorded in 2013 or 2014 being it significantly correlated to P_{tot} (Table 1). Although the metabolism of P in common bean grains cannot entirely explained by differences in phytate content, these results suggest that the surplus of P accumulated was mainly stored in form of phytate. It is interesting to underline that S. Michele presented the lowest variation of both P_{tot} and Phy levels among the three harvests (Table 1). This suggests that the capacity of P accumulation and partitioning of this genotype is scarcely influenced by the year of cultivation. Finally, Munachedda, Tuvagliedda rossa and S. Michele showed very broad ranges of variation for P_i over the three growing seasons. This suggests that for these landraces the different quantities of P accumulated were mainly converted in P_i.

Table 3. Correlation coefficients among the traits recorded at Sarconi in the growing seasons 2012-2014.

	Phy	P_i	Ash	100 seed weight	Protein	Coat	Cooking time
P_{tot}	0.81**	0.16	0.40	-0.09	-0.30	0.27	-0.28
Phy	-	0.25	0.41*	-0.24	-0.22	0.24	-0.30
P_i		-	-0.18	-0.19	-0.23	0.23	-0.03
Ash			-	-0.09	0.17	0.17	-0.07
100 seeds weight				-	-0.37*	0.32	0.44*
Coat					-	0.13	0.32
Protein						-	0.36*

P, phosphorus. *P<0.05; **P<0.01.

Table 4. Seed traits of landraces cultivated at Policoro for two subsequent growing seasons. Upper section: phosphorus accumulation and partitioning; lower section: seed quality traits.

Work code	Landrace	P_{tot} (g kg^{-1}) 2013	P_{tot} (g kg^{-1}) 2014	Phytate (g kg^{-1}) 2013	Phytate (g kg^{-1}) 2014	Pi (g kg^{-1}) 2013	Pi (g kg^{-1}) 2014	PP_{hy}/P_{tot} (%) 2013	PP_{hy}/P_{tot} (%) 2014
1	Riso	5.40	5.20	12.51	10.97	0.755	0.560	65.2	59.4
2	Ciuoto	4.93	5.15	10.43	11.00	1.116	0.575	59.6	60.1
3	Verdolino	5.20	5.80	11.38	12.10	0.675	0.653	61.6	58.8
4	Tabacchino	4.64	5.42	9.42	11.92	0.571	0.583	57.1	66.0
5	Munachedda	4.72	5.06	10.20	10.84	0.658	0.668	60.7	60.2
6	Munachedda nera	5.58	5.07	11.74	10.50	0.731	0.572	59.3	58.4
7	Tuvagliedda	4.92	4.66	9.86	9.04	0.600	0.526	56.5	54.7
8	Tuvagliedda rossa	4.91	4.95	9.41	11.32	0.535	0.570	53.9	64.5
9	San Michele	5.45	5.45	10.75	11.23	0.669	0.614	55.6	57.9
10	Nasieddu nero	5.40	4.28	10.59	9.98	0.621	0.523	55.2	65.6
Mean		5.12[a]	5.10[a]	10.63[a]	10.89[a]	0.693[a]	0.584[a]	58.7[a]	60.6[a]
SD		0.332	0.424	1.006	0.897	0.163	0.048	3.458	3.672

Work code	Landrace	100 seed weight (g) 2013	100 seed weight (g) 2014	Protein (g kg^{-1}) 2013	Protein (g kg^{-1}) 2014	Ash (g kg^{-1}) 2013	Ash (g kg^{-1}) 2014	Coat (g kg^{-1}) 2013	Coat (g kg^{-1}) 2014	Cooking time (min) 2013	Cooking time (min) 2014
1	Riso	43.0	38.9	298.1	244.2	44.0	47.8	53.5	59.6	32	35
2	Ciuoto	38.9	31.9	265.9	211.9	45.0	45.0	58.4	66.1	32	33
3	Verdolino	32.1	32.7	241.5	241.6	44.5	46.0	57.0	62.6	32	34
4	Tabacchino	36.1	34.5	235.9	240.0	45.2	47.9	60.7	61.9	34	38
5	Munachedda	38.3	34.4	232.3	235.3	49.6	51.6	61.1	67.9	30	36
6	Munachedda nera	38.5	39.5	257.9	251.8	52.6	47.7	72.0	65.9	32	32
7	Tuvagliedda	47.9	45.7	242.3	232.8	46.7	47.4	54.7	53.8	32	35
8	Tuvagliedda rossa	50.1	42.1	222.6	246.8	47.6	45.4	63.0	64.0	36	36
9	San Michele	31.8	43.9	242.0	251.4	48.9	47.1	70.4	68.6	34	37
10	Nasieddu nero	51.2	36.5	227.9	235.9	46.5	47.9	69.7	67.8	34	34
Mean		40.8[a]	38.0[a]	246.6[a]	239.2[a]	47.1[a]	47.4[a]	62.0[a]	63.8[a]	32.8[a]	35.0[b]
SD		7.022	4.793	22.268	11.591	2.685	1.832	6.641	4.570	1.687	1.826

P, phosphorus; SD, standard deviation. Values expressed on dry matter bases. [a,b]Values followed by the same letter are not significantly different (P>0.05).

As concerns nutritional traits (Table 2), the lowest protein content was recorded for Ciuoto confirming previous studies (Piergiovanni *et al.,* 2000). At the opposite, higher values were associated to Tabacchino and Munachedda. The cooking times showed an appreciable variation both among landraces as well as among growing seasons. Conversely, very a low variation was associated to ash amount.

Several studies showed the existence of correlations between climatic conditions (*i.e.* monthly average temperature, rainfall quantity) during the plant development and levels of antinutritionals in seeds (Kumar *et al.,* 2006; Nikolopoulou *et al.,* 2007; Piergiovanni *et al.,* 2011). To shed light on the role of growing location climate on P accumulation and partitioning in common bean, monthly average temperature and rainfall quantity recorded at Sarconi in the years 2012-2014 from June to October, were examined. On monthly base, the maximum average temperatures recorded in 2012 during the complete crop cycle, were superior to those recorded in 2013 and 2014. The 2013 was the rainiest year with 316 mm of rain against 165 and 190 mm in 2012 and 2014, respectively. When these meteorological data are matched with P data, rainfall quantity seems to promote the accumulation in seeds of both P_{tot} and Phy (Table 1). Riso, Munachedda, Munachedda nera and Nasieddu nero appear to be the more sensitive to rainfall showing the highestP_{tot} and Phy values in 2013.

Influence of growing location

Due to the differences in P accumulation and partitioning recorded at Sarconi among the landraces in 2012, it was planned to grow them in another location. It is known that, the comparison of a group of genotypes grown for subsequent seasons in different locations, where the level of plant stress is different, can provide interesting information about the environmental effects on grain composition. To amplify the genotype x environment interaction, the trial was carried out at Policoro, a location of Basilicata region, outside the traditional area of cultivation of these landraces. Data collected at Policoro, summarised in Table 4, show similarities with those of Sarconi (Table 1) in P accumulation and partitioning. For example, Nasieddu nero showed a high P_{tot} variation between 2013 and 2014 at both locations, and the lowest values were recorded in 2014. Ciuoto, Verdolino and Tabacchino showed higher values of Phy at both locations in 2014. Finally, the lack of appreciable variation of Phy content for Munachedda and S. Michele was observed also at Policoro. It is interesting to underline that P accumulation and partitioning detected in S. Michele were scarcely affected by growing season at both locations (Tables 1 and 4). The comparison of data relative at the two locations is showed in Table 5. The P_{tot} and Phy mean values were significantly different ($P<0.05$) between the locations in 2013 and 2014. The higher values were always detected at Policoro. Conversely, P_i mean values were significantly different between the locations only in 2014, but the highest value was observed at Sarconi. As concern nutritional traits, ash and coat mean values were also significantly different between Sarconi and Policoro in both years.

Results of ANOVA analysis applied to experimental data collected in this study are resumed in Table 6. The effects of growing season and genotype resulted not significant in determining P accumulation and partitioning, whereas were significant on the other analysed traits (seed size, protein content, cooking time). Conversely, the growing location significantly affected all traits with the exception of 100 seed weight and protein content (Table 6). This is mainly attributable to differences in soil composition and environmental conditions between the locations. Actually, Sarconi's soil is not calcareous (less than 0.5%) and has a neutral pH, while at Policoro the carbonate in soil is about 25%, and the pH is alkaline. When data relative to Sarconi and Policoro harvests for both years, were submitted to principal component analysis (PCA) the scatter plot in Figure 1 was obtained. The first three components accounted for the 79.1% of observed variance. The first component (PCA1) has high negative loadings on P_i and 100 seed weight; the second one (PCA2) has high negative loadings on P_i and Phy. The effect of growing location is clear-cut being the landraces divided in two distinguishable subgroups associated to Sarconi and Policoro (S and P, respectively). Tabacchino appeared the less influenced by growing location, being the points representing the harvests at the two locations (4P and 4S) very close in the scatter plot.

Table 5. Comparison of mean values of seed traits recorded at Sarconi and Policoro locations.

	2013		2014	
	Sarconi	Policoro	Sarconi	Policoro
P_{tot} (g kg^{-1})	4.38^a	5.12^b	4.06^a	5.10^b
Phy (g kg^{-1})	8.78^a	10.63^b	7.99^a	10.89^b
P_i (g kg^{-1})	0.683^a	0.693^a	0.723^b	0.584^a
100 seed weight	55.7^a	40.8^b	51.1^a	38.0^b
Protein	232.0^a	246.6^a	233.3^a	239.2^a
Ash	41.5^a	47.1^b	40.7^a	47.4^b
Coat	55.2^a	62.0^b	58.7^a	63.8^b
Cooking time	35.4^a	32.8^b	38.1^a	35.0^a

^{a,b}Values followed by the same letter are not significantly different (P>0.05).

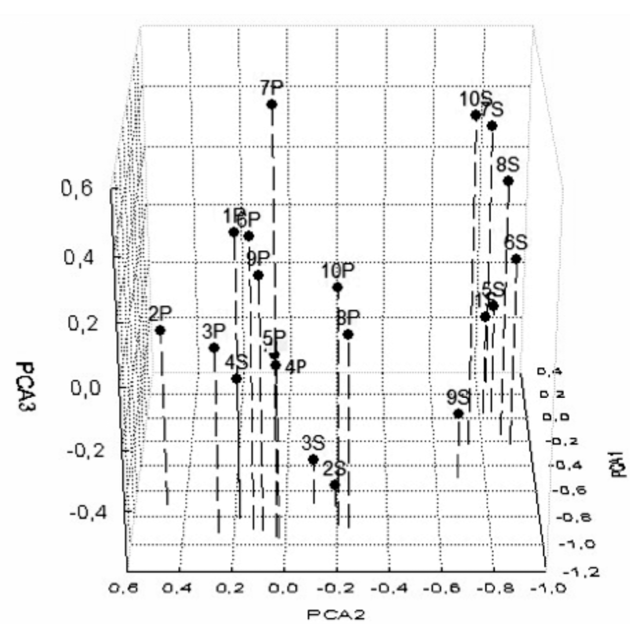

Figure 1. Three-dimensional scatter plot of the principal component analysis (PCA) based on data of the harvest of the two growing locations: Sarconi (S), Policoro (P). The landrace codes are as in Table 1.

Table 6. Analysis of variance over harvests at Sarconi and Policoro locations.

Source of variation		DF	P_{tot}	Phy	P_i	P_{Phy}/P_{tot}	Ash	100 seed weight	Coat	Protein	Cooking time
Sarconi: growing seasons 2012-2014	Year	2	0.607	1.807	0.009	56.847	1.529	257.359	3.275**	66.333	47.233
	Landrace	9	0.283	1.674	0.011	29.759	8.080	210.603**	0.921	1087.243**	41.393*
Policoro: growing seasons 2013-2014	Year	1	0.000	0.341	0.059	21.840	0.512	38.642	0.157	279.004	24.200*
	Landrace	9	0.137	0.949	0.015	8.367	7.148	47.896	0.544*	288.289	4.200
Sarconi and Policoro: growing seasons 2013-2014	Year	1	0.269	0.700	0.012	1.156	0.529	135.056	0.686	93.636	60.025*
	Landrace	9	0.193	1.878	0.020	16.452	8.466	169.431	0.946**	504.320	11.725
	Location	1	7.939**	66.240**	0.041	144.400**	376.996**	1955.802	3.600**	1048.576	81.225**

P, phosphorus; DF, degrees of freedom. Mean squares and significance are reported. *P<0.05; **P<0.01.

Conclusions

Many studies showed that some seed traits depend on genotype as well as environmental factors, such as soil and climate, during plant and seed development. Graham *et al.* (1999), analysing the micronutrient contents of some staple crops, observed that environmental effect appeared to be greater than genetic component. Kumar *et al.* (2006) who carried out a multi-location field trial, reported that phytic acid accumulation in soybean seeds is significantly affected by environment and genotype. The complexity of P metabolic pathway in plants depends on the interaction of several factors. So, present data related to aptitude to accumulate and partition phosphorus in seeds are preliminary indications about the differences among the studied landraces. The knowledge of performance of a common bean landrace in relation to cultivation area is important for its success and acceptance by farmers and consumers. The results collected in this study evidenced that cultivation outside the traditional environment appreciably affects the seed quality. These results confirm how the close link between landrace and a well-defined cultivation area, crucial for the attribution of European PGI mark, has a great importance not only to recognise the heritage of a local community, but also to safeguard the characteristics of the final product.

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