Research Article

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Phenotypic diversity and evaluation of fresh pods of cowpea landraces from Southern Europe

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Abstract

BACKGROUND: Cowpea fresh pods are consumed as green vegetables in many countries in Southern Europe. Among cowpea cultivated material, a relatively large number of landraces can be found, maintaining species genetic diversity. To assess the value of these landraces in breeding programs, the characterization and estimation of diversity levels is therefore required. In the present study, an estimation of the diversity and evaluation of cowpea landraces originating from Portugal, Spain and Greece, in terms of their fresh pod traits, was performed, aiming to assist with the problem of low cowpea production in Southern Europe.

RESULTS: A notable mean total phenotypic diversity ($H_T = 0.57$) was observed in the whole collection. The Spanish cv. - gr. *unguiculata* collection exhibited the highest value of total phenotypic diversity (0.56). Landraces did not differ significantly from each other regarding the three countries of origin. Landraces such as Cp4906, Vg60 and BGE038478 presenting higher values of some traits studied could contribute to the breeding of new cowpea varieties for fresh pod production. Positive correlations were observed, indicating the feasibility of breeding for preferable traits regarding fresh pod consumption.

CONCLUSION: The present study has revealed a wide diversity among and within cowpea landraces that could enhance fresh pod production in South European countries.

Supporting information may be found in the online version of this article.

Keywords: *Vigna unguiculata*; breeding; characterization; Mediterranean Basin; vegetable

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is considered as an important multi-purpose legume cultivated not only for its dry seed, but also for its immature seeds, leaves and fresh pods that are consumed as green vegetables.[1] Cultivation of cowpea extends worldwide, especially in sub-Saharan Africa, Asia, Central and South America, Mediterranean region and the Southern USA.[2]

To classify all domesticated cowpea forms, four cultivar groups (cv. - gr.) based on seed and fresh pod traits[3] are widely used, with cv. - gr. *unguiculata* and *sesquipedalis* constituting the two most important groups from an economical point of view.[4] The route of these cv. - gr. in Europe was different because cv. - gr. *unguiculata* was introduced directly from Africa, in contrast to cv. - gr. *sesquipedalis*, which was initially introduced to India during the Neolithic Period[1] and then to Europe. These diverged pathways, followed by different levels of human selection pressure for fresh pod traits, resulted in the formation of longer and more succulent pods of cv. - gr. *sesquipedalis* compared to cv. - gr. *unguiculata*.[5]

At present, both cv. - gr. are well adapted and cultivated in a small scale in many parts of Southern Europe and countries around the Mediterranean Basin,[6,7] where they are consumed as dry seed and fresh pod. Cowpea constitutes an important crop of Southern European countries as a result of its better adaptation to drought, high temperatures and other stresses compared to other crop plant species that prevail in the area. Its cultivation provides these countries with a considerable income through exports to Northern European and non-European countries.[8] Moreover, in recent years, consumer demands in the European Union for cowpea and beans

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as vegetables are outweighing production, resulting in an increase of imports, mainly from Morocco, Egypt, Kenya and Senegal, amounting to 188 287 tonnes and accounting for 96% of the total imports.[8] Therefore, efforts to enhance production in South European countries are considered as being of great importance with respect to covering these increased requirements.

Among the cultivated cowpea material in Southern Europe, a relatively large number of landraces that have not yet been collected can be found, in addition to 827 landraces accessions of European origin that are preserved in genebanks worldwide.[9] These landraces, which reflect the gastronomic preferences of people living in the region, could contribute to an alleviation of the lack of improved cultivars in terms of fresh pod production, as well as the poor marginal economical returns of cowpea production that are considered as the main limited factors of the crop in Southern European countries.[7]

Knowledge of the existing levels of diversity among and within cowpea landraces, originating from and adapted to the South European agro-climatic conditions, is considered to be of crucial importance for their use in breeding programs and the attainment of new bred/improved lines. High variability has been reported previously among and within cowpea landraces originating from countries around the Mediterranean Basin, based on plant and seed agro-morphological traits,[6,10−12] indicating the large amount of genetic diversity that is being maintained.[12] By contrast, only few studies have been based on fresh pod traits[13,14] of cowpea with Mediterranean origin. Worldwide, different levels of variability were reported for cowpea fresh pod traits,[15−17] many of them referring to cv. - gr. *sesquipedalis*.[18,19]

Taking into account the lack of improved lines, the possibility of increasing incomes from cowpea cultivation and the growing consumer demands for vegetable cowpea in Europe, the present study aimed to: (i) determine diversity in fresh pod traits; (ii) define the most promising landraces regarding fresh pod yield; and (iii) identify significant correlations among fresh pod traits of a collection of cowpea landraces originating from Portugal, Spain and Greece.

MATERIALS AND METHODS

Characterization and estimation of fresh cowpea pod traits diversity

Plant material and growth conditions

A field experiment was conducted at the Agricultural University of Athens (AUA), Greece (37°58′10′′N, 23°42′29′′E, altitude 24 m) during spring/summer 2014. Thirty-one cowpea landraces and one variety of *Vigna unguiculata* cv. - gr. *unguiculata*, nineteen originating from Portugal (INIAV, UTAD), four from Spain (CRF-INIA) and nine from Greece (AUA), and five of *Vigna unguiculata* cv. - gr. *sesquipedalis*, originating from Spain (CRF-INIA), were subjected to morphological and agronomical characterization, as well as estimation of their phenotypic diversity of fresh pod traits (see Supporting information, Table S1). Seeds were sown in trays on May 23 and twelve plants per landrace were grown in a greenhouse compartment for 2 weeks. Afterwards, the seedlings were transplanted to the field following a randomized complete block experimental design, with four replicates and three plants per replicate per landrace. The soil was clay with a loamy texture (pH 7.7). Plants were spaced at a distance of 50 cm from row to row and 20 cm apart within the row, drip irrigated and supplied with 600 kg ha^{-1} of a mineral fertilizer (NPK 11-15-15). During the growing season, weeds were hand-controlled and incidences of pests and diseases were handled via chemical management. Plants were grown under an insect proof net to avoid cross-pollination.[20]

Fresh pod traits used for characterization and estimation of diversity

The market acceptable harvesting stage for the consumption of fresh pods for each landrace was defined as the time interval from flowering to the stage by which the pods had the maximum possible length but sufficiently retained their tenderness. To determine this stage, pods from each landrace were harvested at different days after flowering and thus the time that the pods started to harden and develop fiber was estimated (Karapanos I, Skouloudi M and Lazaridi E; unpublished data). After identification of the appropriate harvesting stage of fresh pods, pods from one plant per landrace per replicate were collected and landraces were characterized according to their height to first pod, flowering duration, fresh pod pigmentation, fresh pod color, fresh pod weight, fresh pod length, fresh pod diameter and number of locules per fresh pod, whereas days to pod bearing, days to harvest from sowing and days to harvest from pod bearing were determined for each one of the fresh pods collected from each one of the four plants per landrace to the appropriate harvesting stage (see Supporting information, Table S2).

Evaluation of landraces for fresh pod traits

Plant material and growth conditions

A second year experiment was conducted at AUA, during spring/summer 2015, with fifteen selected cowpea landraces (*Vigna unguiculata* cv. - gr. *unguiculata*) of the above mentioned collection, marked with an asterisk (*) (see Supporting information, Table S1) being subjected to evaluation regarding their quantitative fresh pod traits. Breeding line IT97K-499-35 was used as a reference. Twenty-four seeds per landrace were sown in a greenhouse on May 4, grown for 2 weeks in seed trays and transplanted to the field following a randomized complete block experimental design, with four replicates and six plants per replicate per landrace. The soil was clay with a loamy texture (pH 8.2). Plants spaced at a distance of 50 cm from row to row and 40 cm apart within the row. Cultivation management was similar to that of the first experimental year, whereas herbicide Stomp 330 E (pendimethalin), with a volume of spray 200 L ha^{-1} was applied.

Fresh pod traits for evaluation of landraces

Ten quantitative traits related to fresh pod yield were used to evaluate pods from twelve plants per landrace: height to first pod, days to pod bearing, days to harvest from sowing, days to harvest from pod bearing, number of fresh pods per plant, weight of fresh pods per plant, fresh pod weight, fresh pod length, fresh pod diameter and number of locules per fresh pod.

Statistical analysis

To characterize cowpea landraces and estimate phenotypic diversities, all quantitative traits were transformed to ordinal by dividing their range into five equal classes.[21] Phenotypic variation across landraces was calculated using Nei's genetic diversity (H_E) statistics.[22] For each trait, total phenotypic diversity (H_T), intra-landrace diversity (H_S), its average across all landraces ($\overline{H}_S$) and inter- landrace diversity (G_{ST}) were calculated for the collection, for each cv. - gr. and for each country of origin with respect to cv. - gr. *unguiculata*. Mean phenotypic diversity within each landrace across all traits ($\overline{H}_P$) was also calculated.[23] Comparisons of $\overline{H}_P$ of all landraces belonging to each of the two cv. - gr. and to each of the three different countries of origin were carried out using Tukey's mean comparison method[24] with JMP, version 8.0.[25] Principal component analysis (PCA) was performed using all traits to classify landraces of each cv. - gr. and examine the contribution of each trait to the total diversity using JMP, version 8.0.[25]

For the evaluation of fresh pod traits of the fifteen selected cowpea cv. - gr. *unguiculata* landraces used in the second experiment, an analysis of variance followed by Tukey's mean comparison method ($P < 0.05$) was performed using JMP, version 8.0.[25] Moreover, Pearson correlation coefficients were used to investigate possible correlations among fresh pod traits, using Statistica, version 8.0.[26]

RESULTS AND DISCUSSION

Characterization of the cowpea collection based on fresh pod traits

A relatively large variability was revealed among cowpea landraces regarding the different fresh pod traits examined (see Supporting information, Table S2). In the present study, most landraces (35.70%) were characterized by a short time interval (9–10 days) from pod bearing until harvest, whereas very few landraces (3.50%) required 13 days to reach the market acceptable pod harvesting stage for fresh consumption. The number of marketable produced fresh pods is considered as one of the main yield components for cowpea fresh pod production; therefore, the rate of fresh pods development has a direct impact on the overall crop performance.[27] As observed in the present study, the interval period from pod bearing to harvest is in agreement with Deshmukh *et al.*[27] who reported that the highest fresh pod growth rates were recorded 6–9 days after flowering and the maximum fresh pod length and diameter were measured 12 days after flowering. In addition, Omueti *et al.*[28] suggested that the appropriate stage and that most preferred by consumers for harvesting cowpea fresh pods ranged from 7 to 10 days after pod bearing.

All landraces of this collection were characterized by either an absence of fresh pod pigmentation (54.10%) or by a pigmented tip (45.90%), whereas fresh pod color was mainly recorded as medium green (64.90%). These findings indicate that green cowpea pods with an absence of pigmentation are preferred when consumed as vegetables in Southern Europe, as in many other parts of the world.[29] Indeed, the green pod color with an absence of pigmentation has been found to be the most prevalent type in other cowpea collections worldwide,[19,21] as well as in common bean.[30,31]

Cowpea fresh pods were mostly characterized by a very small pod weight (1.41–5.89 g) and pod length (8.55–22.08 cm), medium diameter (0.68–0.76 mm) and medium number of locules (10–12) (see Supporting information, Table S2). Similar values for pod length and number of locules per pod have been reported previously for landraces belonging to three cowpea cv. - gr. originating from countries around the Mediterranean Basin,[13] whereas variability has been also previously reported by Oliveira *et al.*,[32] among Brazilian landraces.

Estimation of fresh pod traits diversity

Total phenotypic diversity of the present collection for each trait (H_T) varied between 0.30 and 0.75, with an average of 0.57 (Table 1), indicating a notable diversity regarding fresh pod traits, despite the genetic diversity in cowpea considered to be presumably low as a result of the severe bottleneck during its domestication.[33] Intra- landrace diversity ($\overline{H}_S$) varied between 0.08 and 0.50, with fresh pod length and fresh pod weight exhibiting the lowest values ($\overline{H}_S \leq 0.18$). By contrast, flowering duration, number of locules per fresh pod and height to first pod presented the highest values ($\overline{H}_S \geq 0.41$), thereby indicating a need for selection for these traits regarding breeding, especially for height to first pod, which is an important trait for mechanical harvesting.[34] Phenotypic diversity among landraces (G_{ST}) ranged between 0.32 and 0.74. Fresh pod length and fresh pod pigmentation exhibited the highest G_{ST} values, whereas the lowest G_{ST} value was recorded for height to first pod (0.32) (Table 2). Mean phenotypic diversity within each landrace across all traits ($\overline{H}_P$) ranged between 0.17 and 0.54, with an average of 0.32 (Table 2; see also Supporting information, Table S3). Based on Tukey's mean comparison method, the landraces did not differ significantly from each other (see Supporting information, Table S3).

Differences were observed regarding total (H_T), among (G_{ST}) and within ($\overline{H}_S$ diversity, as well as $\overline{H}_P$ values of the two cv. - gr. Landraces belonging to cv. - gr. *unguiculata* presented lower *Ht* and $\overline{H}_S$ values and higher G_{ST} than cv. - gr. *sesquipedalis*, indicating possible differences in the outcrossing rates in each of the two cv. - gr.[20,35] Moreover, a greater environmental effect on landraces of cv. - gr. *sesquipedalis* became evident because most of the traits recorded were quantitative, being controlled by multiple genes and highly influenced by environmental conditions.[36] This differentiation between the two cv. - gr. could also be attributed to a stronger human selection pressure in the Mediterranean Basin for cv. - gr. *unguiculata* regarding some fresh pod traits such as fresh pod length and weight that exhibited lower H_T values than cv. - gr. *sesquipedalis*.

Similar levels of mean ($\overline{H}_P$) phenotypic diversity were observed for landraces of cv. - gr. *unguiculata*, regardless of their geographical origin, indicating equal amounts of variability in the three collections landraces, with a slightly increased amount for the Spanish collection (Table 1). Although no significant differences were observed among $\overline{H}_P$ values of landraces with different origin, different levels of total diversity were observed, with the Spanish collection exhibiting higher values ($H_T = 0.56$) compared to the Portuguese ($H_T = 0.48$) and Greek ($H_T = 0.49$) collections. The highest value of within diversity was also observed in the Spanish collection ($\overline{H}_S = 0.37$), exceeding the among landraces diversity ($G_{ST} = 0.33$), in contrast to the Portuguese and Greek collections that presented higher or equal among and within landraces diversity, respectively (Table 1).

In all three collections with a different country of origin, flowering duration exhibited the highest H_T value (0.73, 0.74 and 0.71 for Portugal, Spain and Greece, respectively). Remarkable similarities within and among the diversity of fresh pod pigmentation and color were also observed among Portuguese and Greek landraces; thus, both presented low $\overline{H}_S$ and high G_{ST} values for these traits, whereas Spanish landraces exhibited low amounts of among landraces diversity for both traits (0.14 and 0.17, respectively) (Table 1). The low G_{ST} values observed in Spanish collection combined with the low within diversity observed for these two qualitative traits probably reveals a more specific consumer preference for these traits regarding their fresh pod consumption.

A low total phenotypic diversity was observed for fresh pod weight in Portuguese and Greek collections ($H_T = 0.16$ and 0.18, respectively), whereas both collections exhibited low $\overline{H}_S$ and G_{ST} values. Moreover, there was no diversity present regarding fresh pod length in the Portuguese collection ($H_T = 0.00$), whereas the Greek collection had the lowest H_T value (0.06). Furthermore, both Portuguese and Greek collections presented no intra- and inter-

Table 1. Total phenotypic diversity (H_T), mean intra- landrace diversity ($\overline{H}_S$), inter- landrace diversity (G_{ST}) and mean phenotypic diversity within each landrace across all traits ($\overline{H}_P$) recorded in the collection, for each cv. - gr. and for each country of origin studied

	Collection				cv.- *gr. unguiculata*				cv.- *gr. sesquipedalis*			
Trait	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$
Height to first pod	0.60	0.41	0.32		0.53	0.39	0.28		0.76	0.53	0.30	
Flowering duration	0.75	0.50	0.34		0.74	0.48	0.35		0.74	0.60	0.19	
Days to pod bearing	0.59	0.36	0.39		0.56	0.34	0.40		0.67	0.49	0.27	
Days to harvest from sowing	0.58	0.39	0.33		0.56	0.36	0.35		0.67	0.56	0.16	
Days to harvest from pod bearing	0.69	0.38	0.44		0.66	0.38	0.42		0.53	0.39	0.27	
Fresh pod pigmentation	0.50	0.22	0.56		0.49	0.21	0.58		0.40	0.28	0.30	
Fresh pod color	0.49	0.22	0.55		0.43	0.21	0.50		0.32	0.25	0.22	
Fresh pod weight	0.41	0.18	0.55		0.22	0.15	0.34		0.66	0.41	0.37	
Fresh pod length	0.30	0.08	0.74		0.08	0.03	0.65		0.74	0.40	0.46	
Fresh pod diameter	0.65	0.40	0.39		0.65	0.40	0.38		0.53	0.36	0.32	
Number of locules per fresh pod	0.71	0.44	0.37		0.69	0.43	0.37		0.71	0.51	0.28	
Mean	0.57	0.32	0.45	0.32	0.51	0.31	0.42	0.31 a	0.61	0.43	0.29	0.44 b

	Portuguese collection cv.- gr. *unguiculata*				Spanish collection cv.- gr. *unguiculata*				Greek collection cv.- gr. *unguiculata*			
Trait	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$	H_T	$\overline{H}_S$	G_{ST}	$\overline{H}_P$
Height to first pod	0.52	0.36	0.30		0.63	0.41	0.17		0.51	0.43	0.15	
Flowering duration	0.73	0.48	0.34		0.74	0.56	0.24		0.71	0.44	0.37	
Days to pod bearing	0.53	0.29	0.45		0.70	0.47	0.33		0.50	0.37	0.25	
Days to harvest from sowing	0.53	0.37	0.30		0.62	0.35	0.44		0.48	0.36	0.26	
Days to harvest from pod bearing	0.64	0.34	0.47		0.62	0.53	0.15		0.65	0.40	0.38	
Fresh pod pigmentation	0.50	0.19	0.62		0.22	0.19	0.14		0.48	0.25	0.47	
Fresh pod color	0.46	0.24	0.49		0.19	0.23	0.17		0.42	0.18	0.57	
Fresh pod weight	0.16	0.11	0.28		0.62	0.11	0.53		0.18	0.15	0.14	
Fresh pod length	0.00	0.00	0.00		0.46	0.47	0.76		0.06	0.05	0.23	
Fresh pod diameter	0.62	0.39	0.37		0.62	0.47	0.24		0.61	0.40	0.35	
Number of locules per fresh pod	0.60	0.38	0.37		0.70	0.47	0.33		0.76	0.52	0.31	
Mean	0.48	0.29	0.36	0.29 A	0.56	0.37	0.33	0.37 A	0.49	0.32	0.32	0.32 A

Significant differences of $\overline{H}_P$ for the two cv. - gr. and for the three countries of origin at $P < 0.05$ by Tukey's mean comparison method are indicated by different lowercase and uppercase letters, respectively.

landrace diversity ($\overline{H}_S = 0.00$) and low G_{ST} values (0.05 and 0.23) for fresh pod length, respectively. Therefore, both collections were characterized as being quite homogeneous regarding these two traits, as well as dissimilar to Spanish collection that presented the highest H_T values: 0.62 and 0.46 for these two traits, respectively (Table 1).

Classification of landraces based on fresh pod traits

The first two axes of PCA explained 44.26% of the total fresh pod traits variation of landraces belonging to cv. - gr. *unguiculata* (Fig. 1). Fresh pod weight, fresh pod length and days to harvest from sowing were related to PC1 (26.94%). Height to first pod, fresh pod pigmentation and fresh pod color were related to PC2 (17.32%). Similar proportions of phenotypic diversity explained by the first two axes have been reported previously for various cowpea traits by Ahamed *et al.*[37] and Gerrano *et al.*,[38] and for fresh pod traits of Turkish common bean genotypes.[39] PCA classified all cv. - gr. *unguiculata* landraces in one group regardless of their country of origin, with the exception of BGE038478 that differentiated from the others mainly as a result of its larger fresh pod weight and length, as well as its longer duration to harvest from sowing (Fig. 1).

Regarding cv. - gr. *sesquipedalis*, the first two axes of PCA explained 77.48% of the total diversity (Fig. 2). Traits related to PC1 (53.32%) were height to first pod, days to pod bearing, days to harvest from sowing and from pod bearing, and number of locules per fresh pod, whereas fresh pod weight, length and diameter of pods were related to PC2 (24.16%). Height to first pod, days to harvest from sowing, fresh pod weight and length therefore contributed significantly to phenotypic diversity of both cv. - gr., whereas a higher level of total diversity was explained by the two first principal axes for cv. - gr. *sesquipedalis* regarding the fresh pod traits measured. Vi4 was differentiated mainly according to later days to pod bearing, days to harvesting from sowing and pod bearing (Fig. 2). Despite their common Spanish origin, all landraces of cv. - gr. *sesquipedalis* were slightly differentiated.

Evaluation of landraces for fresh pod traits

Long, fresh, succulent, tender pods, early or late flowering and maturity are considered as the most important traits when breeding cowpea varieties for fresh pod consumption.[40] In the present study, significant differences were found among the fifteen Southern European cowpea landraces evaluated for all quantitative fresh pod traits recorded in terms of breeding for fresh pod cowpea consumption (Table 2). The smallest average period of days from sowing to pod harvesting was recorded for Cp4906 (77.32 days), whereas the highest was for BGE038478 (87.37 days). Moreover, Cp5128 produced the highest average number of fresh pods per

Table 2. Differences in fresh pod traits among fifteen cowpea landraces evaluated in 2015

Landrace	H1STPOD	DPB	DHS	DHPB	NFPOD	WFPOD	FPW	FPL	FPD	FPNL
Cp4906	28.50 ± 1.86 ab	67.68 ± 0.56 g	77.32 ± 0.54 fg	9.64 ± 0.16 a–d	23.33 ± 4.41 de	59.74 ± 12.97 cd	2.56 ± 0.07 b–e	12.18 ± 0.12 d–g	5.68 ± 0.07 a	8.97 ± 0.18 g
Cp5128	23.30 ± 2.19 ab	74.01 ± 0.30 c	83.56 ± 0.33 d	9.55 ± 0.08 abc	98.83 ± 8.27 a	232.38 ± 22.85a	2.27 ± 0.02 f	12.54 ± 0.10 gh	5.13 ± 0.09 e	11.46 ± 0.0 9 ab
Cp5131	24.37 ± 2.95 ab	68.96 ± 0.46 fg	78.03 ± 0.47 fg	9.19 ± 0.22 b–e	52.55 ± 8.52 b–e	124.14 ± 20.95 bcd	2.29 ± 0.16 ef	12.75 ± 0.09 def	5.12 ± 0.05 de	10.91 ± 0.12 cde
Vg52	21.37 ± 2.08 ab	70.30 ± 0.47 efg	79.82 ± 0.51 efg	9.52 ± 0.12 a–d	32.50 ± 6.98 cde	99.12 ± 25.88 bcd	2.67 ± 0.06 abc	12.50 ± 0.12 cde	5.48 ± 0.06 ac	10.86 ± 0.14 b–e
Vg56	24.91 ± 2.92 ab	69.09 ± 0.39 fg	78.23 ± 0.39 fg	9.14 ± 0.10 cde	58.00 ± 9.26 bcd	147.91 ± 26.21 bcd	2.71 ± 0.05 ab	13.33 ± 0.08 ab	5.52 ± 0.15 bc	11.46 ± 0.10 abc
Vg59	16.95 ± 3.47 b	69.04 ± 0.38 fg	78.54 ± 0.36 efg	9.49 ± 0.11 a–d	47.18 ± 4.02 b–e	113.44 ± 11.42 bcd	2.23 ± 0.04 f	11.77 ± 0.08 gh	5.04 ± 0.05 e	10.46 ± 0.12 df
Vg60	30.05 ± 3.91 a	69.11 ± 0.32 fg	77.92 ± 0.33 g	8.82 ± 0.08 e	76.09 ± 11.06 ab	193.93 ± 28.96 ab	2.52 ± 0.04 bcd	13.48 ± 0.08 a	5.33 ± 0.04 bcd	11.83 ± 0.10 a
BGE038474	25.74 ± 1.65 ab	78.42 ± 0.89 a	87.23 ± 0.93 ab	8.81 ± 0.16 cde	29.14 ± 7.04 cde	64.58 ± 16.66 cd	2.04 ± 0.06 f	11.54 ± 0.13 gh	4.92 ± 0.08 e	11.37 ± 0.18 abc
BGE038478	22.44 ± 1.62 ab	77.52 ± 0.70 a	87.37 ± 0.72 a	9.85 ± 0.13 ab	26.80 ± 4.80 cde	72.83 ± 16.79 cd	2.37 ± 0.05 c–f	12.54 ± 0.10 cde	5.15 ± 0.07 cde	11.87 ± 0.13 a
BGE038479	24.84 ± 1.80 ab	76.49 ± 0.58 ab	86.00 ± 0.62 abc	9.51 ± 0.13 a–d	35.80 ± 6.26 cde	99.31 ± 21.16 bcd	2.59 ± 0.05 bcd	12.75 ± 0.09 bcd	5.47 ± 0.23 b–e	11.89 ± 0.12 a
AUA2	31.25 ± 2.44 a	68.83 ± 0.38 fg	78.14 ± 0.39 fg	9.30 ± 0.10 a–e	59.58 ± 8.79 bc	151.03 ± 23.11 bcd	2.53 ± 0.04 bcd	13.02 ± 0.19 abc	5.44 ± 0.06 ab	11.13 ± 0.10 bcd
AUA6	23.51 ± 2.86 ab	69.77 ± 0.58 efg	79.70 ± 0.57 efg	9.93 ± 0.12 a	32.40 ± 7.19 cde	90.93 ± 22.83 cd	2.37 ± 0.06 def	11.92 ± 0.15 e–h	5.34 ± 0.07 bcd	9.94 ± 0.15 f
AUA18	21.62 ± 1.63 ab	71.39 ± 0.37 de	80.39 ± 0.38 e	8.99 ± 0.08 de	58.84 ± 9.86 bc	126.22 ± 20.53 bcd	2.12 ± 0.03 f	10.47 ± 0.06 i	5.27 ± 0.07 bcd	10.17 ± 0.08 f
AUA20	22.33 ± 0.91 ab	70.61 ± 0.41 ef	80.05 ± 0.45 ef	9.43 ± 0.10 a–d	42.75 ± 6.07 b–e	124.87 ± 19.64 bcd	2.92 ± 0.06 a	12.71 ± 0.10 cd	5.45 ± 0.05 ab	11.84 ± 0.12 a
AUA21	27.25 ± 3.06 ab	73.42 ± 0.73 cd	83.44 ± 0.76 cd	10.01 ± 0.16 a	20.08 ± 3.61 e	43.72 ± 8.19 d	2.17 ± 0.06 f	11.73 ± 0.13 fgh	5.12 ± 0.07 cde	10.60 ± 0.50 def
IT97K-499-35	26.30 ± 1.15 ab	74.51 ± 0.51 bc	84.04 ± 0.55 bcd	9.53 ± 0.14 a–d	27.25 ± 6.40 cde	63.56 ± 13.26 cd	2.33 ± 0.06 def	11.27 ± 0.10 h	4.99 ± 0.06 e	10.45 ± 0.14 def
Statistical significance										
	**	***	***	***	***	***	***	***	***	***

H1STPOD, Height to first pod; DPB, days to pod bearing; DHS, days to harvest from sowing; DHPB, days to harvest from pod bearing; NFPOD, number of fresh pods per plant; WFPOD, weight of fresh pods per plant; FPW, fresh pod weight; FPL, fresh pod length; FPD, fresh pod diameter; FPNL, number of locules per fresh pod. Values are given as the mean ± SE. Means in columns with different letters are significantly different at $P < 0.05$ level by Tukey's mean comparison method.
$^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

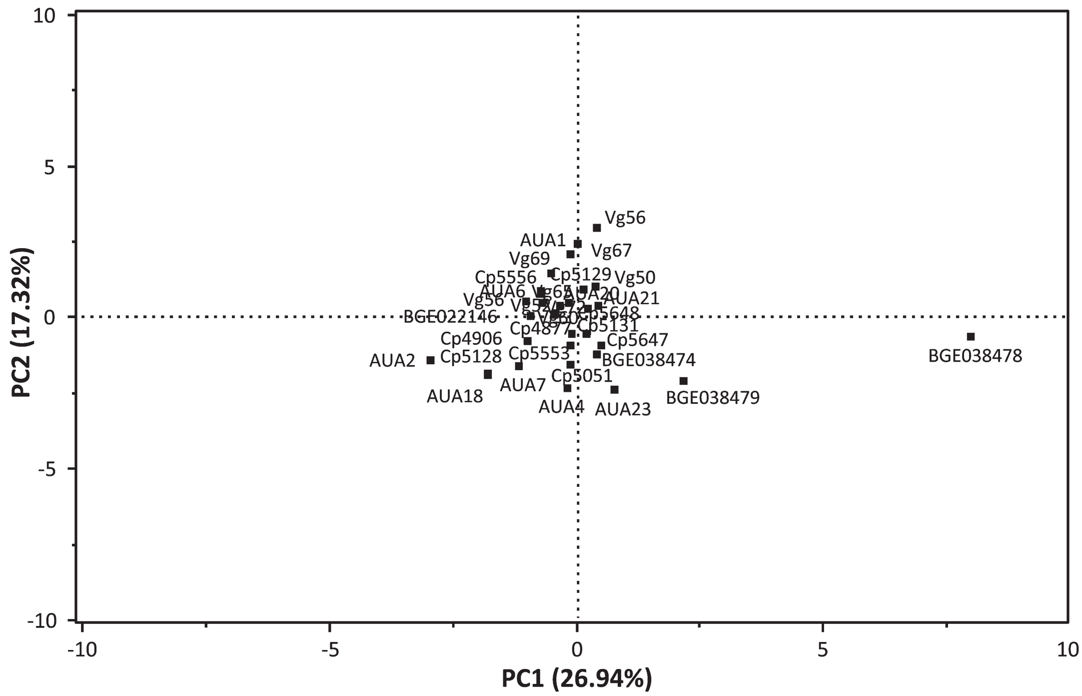

Figure 1. Principal component analysis (PCA) of cowpea landraces of cv. - gr. *unguiculata* based on studied fresh pod traits

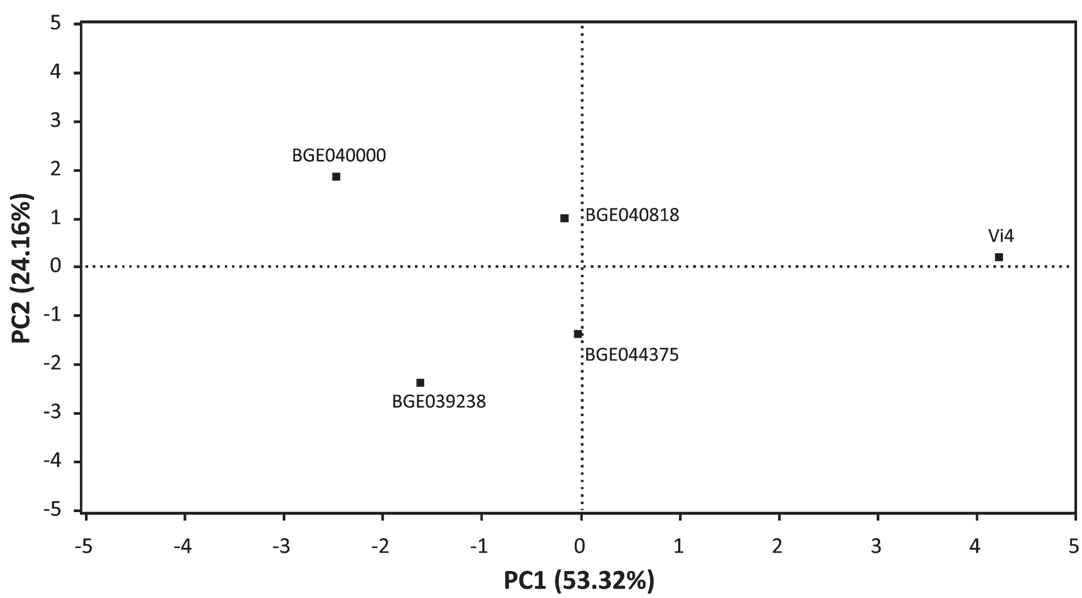

Figure 2. Principal component analysis (PCA) of cowpea landraces of cv. - gr. *sesquipedalis* based on studied fresh pod traits

plant (98.83) and fresh pod weight per plant (232.38 g). On average, AUA20 presented the highest fresh pod weight (2.92 g) and Vg60 had the longest pods (13.48 cm), whereas the lowest number of locules per fresh pod was observed in Cp4906 (8.97) (Table 2). Significant differences for fresh pod traits have been previously reported by Sapara *et al.*[16] among cowpea genotypes.

Furthermore, significant correlations were found among fresh pod traits (Table 3). Days from pod bearing to harvest were negatively correlated with days from sowing to pod bearing ($r = -0.34$, $P < 0.001$) and days from sowing to harvest ($r = -0.22$, $P < 0.01$), indicating that a delay of pod bearing reduces the required harvesting period of fresh pods. Days to pod bearing, as well as days to harvest from sowing, were also negatively correlated with all the other fresh pod traits studied. Given the different requirements among landraces for photoperiod and temperature,[38,41] the selection of cowpea genotypes regarding their photosensitivity[42] and appropriate sowing time[41] based on the different agro-climatic conditions of each region could contribute to increase fresh pod yield. Quantitative trait loci clusters for pod traits, such as length and width, have been found in cowpea and other legumes,[43–45] indicating additive and epistatic effects among these important agronomic traits. Positive correlations were found among fresh pod weight, length, diameter and number of locules per pod in the present study (Table 3), indicating the feasibility of including these traits in breeding programs.

Table 3. Pearson correlation coefficients among fresh pod traits

	H1STPOD	DPB	DHS	DHPB	NFPOD	WFPOD	FPW	FPL	FPD	FPNL
H1STPOD	1.00	-0.15^{*}	-0.14	0.13	-0.02	-0.05	0.02	0.14	0.17^{*}	-0.04
DPB		1.00	0.99^{***}	-0.34^{***}	-0.17^{*}	-0.21^{**}	-0.27^{***}	-0.43^{***}	-0.35^{***}	-0.24^{**}
DHS			1.00	-0.22^{**}	-0.20^{**}	-0.23^{**}	-0.24^{**}	-0.40^{***}	-0.31^{***}	-0.24^{**}
DHPB				1.00	-0.14	-0.13	0.27^{***}	0.33^{***}	0.42^{***}	0.05
NFPOD					1.00	0.93	0.08	0.18^{*}	-0.01	0.36^{***}
WFPOD						1.00	0.10	0.19^{*}	-0.02	0.38^{***}
FPW							1.00	0.55^{***}	0.40^{***}	0.12
FPL								1.00	0.32^{***}	0.65^{***}
FPD									1.00	-0.08
FPNL										1.00

H1STPOD, Height to first pod; DPB, days to pod bearing; DHS, days to harvest from sowing; DHPB, days to harvest from pod bearing; NFPOD, number of fresh pods per plant; WFPOD, weight of fresh pods per plant; FPW, fresh pod weight; FPL, fresh pod length; FPD, fresh pod diameter; FPNL, number of locules per fresh pod.
$^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

CONCLUSIONS

Characterization and estimation of diversity among and within landraces revealed that a notable phenotypic variability regarding fresh pod traits is maintained in Southern Europe. No significant differences were observed among the collections regarding their country of origin, as also indicated from the PCA, whereas different levels of diversity (i.e. total, among and within) were observed for each trait studied in each of the three collections, with the Portuguese and Greek collections presenting similar levels of diversity for many traits. The potential of some Southern European landraces, which exhibited desired traits, to contribute to breeding varieties for fresh pod production became evident, whereas the feasibility of developing cowpea genotypes with preferable fresh pod traits was indicated by significant positive correlations observed among the most studied traits.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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