Research Article

European cowpea landraces for a more sustainable agriculture system and novel foods

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Abstract

BACKGROUND: Genetic diversity is fundamental to breeding programs and consequently has an important role in obtaining new varieties. To properly use the genetic diversity present in germplasm collections, a good knowledge of the agro-morphological traits of each accession is needed. The aim of this study was to explore the production capacity of 24 cowpea landraces from southern Europe, through phenotypic characterization and evaluation in three different locations in Greece and Portugal.

RESULTS: Most qualitative parameters tested showed a high stability among the three locations. A wide difference was observed among the three locations with respect to number of days to flowering, ranging from 55 to 99 days. Quantitative traits showed a higher genotype $\times$ environment than genetic variance component. In general, an inverse relationship between σ^2_{ge}/σ^2_g ratio (where σ^2_{ge} is genotype $\times$ genotype interaction and σ^2_g is genotype impact) and heritability value was observed. Principal component analysis was able to group accessions based on their origin. The first two principal components explained 97.52% of variation, being the number of seeds per plant, plant height and seed protein content, the traits which contributed most to variability.

CONCLUSION: The results show that sufficient variation exists in different traits within landraces in the studied cowpea germplasm to pursue a breeding program. However, the quantitative traits showed a higher genotype $\times$ environment component.
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Keywords: *Vigna unguiculata* L. Walp; G $\times$ E interaction; landraces; qualitative traits; quantitative traits

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a primarily self-pollinated species of the genus *Vigna*, a member of the Leguminosae family. Different areas have been proposed as cowpea domestication centers,[1,2] although it is unquestionably of African origin.[3] Introduction of cowpea in Europe has been reported to occur throughout the eastern part of the Mediterranean Basin, as it was certainly cultivated by the Romans in the first century AD.[4,5] This grain legume is cultivated in many tropical and subtropical regions of the world.[6]

Nowadays, cowpea is cultivated on a small scale in southern European countries, representing only 0.43% of the total cowpea seed production, amounting to 5.59 million tonnes in 2014.[7] Cowpea is mainly used in the human diet but also as forage for animal feeding. It is mainly cultivated for its dry grain, although in some regions young leaves, fresh pods and fresh seeds are also consumed,[8] constituting a significant source of proteins, essential amino acids, minerals, vitamins and fiber.[9,10]

Agricultural productivity of food legumes, grown in semi-arid areas or drylands, e.g. the Mediterranean Basin, is usually characterized by instability, as it is influenced by several environmental constraints, such as water scarcity and extreme temperatures[11,12] that prevail in these areas.[13] Tolerance to low water regimes and

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Table 1. Collection code, geographical data and breeding status of the 24 cowpea accessions

Country of origin	Code	Latitude	Longitude	Altitude (m)	Breeding status
Portugal	Cp4906	40° 00′ 28″ N	8° 27′ 04″ W	198	Landrace
	Cp5128	39° 59′ 11″ N	7° 26′ 39″ W	402	Landrace
	Cp5129	39° 59′ 11″ N	7° 26′ 39″ W	402	Landrace
	Cp5131	39° 59′ 11″ N	7° 26′ 39″ W	402	Landrace
	Cp5553	39° 48′ 02″ N	8° 06′ 03″ W	226	Landrace
	Cp5556	37° 47′ 15″ N	7° 43′ 32″ W	160	Landrace
	Cp5647	39° 27′ 58″ N	7° 56′ 14″ W	281	Landrace
	Cp5648	39° 27′ 53″ N	8° 02′ 44″ W	45	Landrace
	Vg50	40° 51′ 15″ N	7° 08′ 22″ W	523	Landrace
	Vg52	40° 48′ 45″ N	7° 23′ 26″ W	770	Landrace
	Vg56	41° 44′ 38″ N	7° 38′ 57″ W	673	Landrace
	Vg59	40° 14′ 57″ N	7° 17′ 22″ W	507	Landrace
	Vg60	40° 22′ 00″ N	7° 15′ 32″ W	633	Landrace
	Vg65	41° 19′ 25″ N	7° 28′ 04″ W	766	Landrace
	Vg67	41° 17′ 52″ N	7° 05′ 53″ W	247	Landrace
	Vg72	41° 16′ 57″ N	6° 35′ 06″ W	726	Landrace
	Vg73	41° 27′ 19″ N	7° 00′ 30″ W	750	Landrace
	Fradel				Variety
Spain	BGE022146	37° 00′ 35″ N	3° 00′ 26″ W	1082	Landrace
	BGE038474	36° 31′ 47″ N	5° 15′ 26″ W	225	Landrace
	BGE038477	36° 36′ 51″ N	5° 08′ 53″ W	769	Landrace
	BGE038478	36° 37′ 37″ N	5° 10′ 11″ W	622	Landrace
	BGE038479	36° 37′ 37″ N	5° 10′ 11″ W	622	Landrace
Nigeria	IT97K-499-35				Breeding line

adaptation to high temperatures make cowpea an important crop for southern European countries; thus it is considered one of the most drought-tolerant crops.[11,14] Furthermore, cowpea capacity to establish symbiosis with rhizobia and mycorrhizal fungi allows it to grow in low-fertility soils, reducing or even eliminating the need for application of inorganic fertilizers, thus resulting in a more environmentally sustainable culture as well as rendering it one of the soil fertility-restoring crops.[9,15]

Cowpea cultivation in southern Europe depends to an extent on a remarkable number of cowpea landraces that constitute a valuable genetic material for breeding programs.[16,17] They possess significant phenotypic variability and some have developed the capacity to tolerate biotic and abiotic stresses, and thus are used in agricultural systems with low inputs and high yield stability.[18,19] Based on the landraces that still preserve high genetic variability in traits related to tolerance/resistance to certain abiotic and biotic factors, and high nutritional value, it is possible to establish a cowpea breeding strategy to obtain more productive and nutritious varieties. The implementation of these breeding programs will be of great importance for Europe, which is a major importer of grain legumes such as cowpea, 10 501 tonnes of dry cowpea having been imported in 2015 by the European Union.[20]

Availability, identification and characterization of plant genetic resources are fundamental to knowing the diversity present in the original material and the best way of undertaking a breeding program. Traditionally, the first step in studies of diversity and genetic relationships is to measure the variation in qualitative traits (such as growth habit and pattern, flower and seed color) and quantitative agronomic traits (such as number of pods per plant, number of seeds per plant and seed weight). Regarding cowpea, in recent years several studies have been carried out on morphological and agronomical characterization.[4,21−25] In these

studies a high level of variability between and even within cowpea landraces has been verified, which may be useful for breeding programs. However, a large amount of the European cowpea genetic material remains unexplored and unutilized by breeding programs. For this purpose, the main objective of this study was to explore, characterize and evaluate cowpea landraces originating from two southern European countries, grown in three different locations, aiming to enlarge the genetic diversity used in modern breeding programs.

MATERIALS AND METHODS

Plant material and experimental design

Twenty-two landraces, one variety and a reference breeding line (IT97K-499-35) of *Vigna unguiculata* cv.-gr. *unguiculata* (Table 1) were subjected to agronomical and morphological characterization in three different locations in southern Europe: the Agricultural University of Athens (AUA), Athens, Greece (37° 59′ N, 23° 42′ E, 24 m); the National Institute for Agrarian and Veterinarian Research (INIAV), Elvas, Portugal (38° 53′ N, 07° 09′ W, 208 m); and the University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal (41°17′ N, 07° 44′ W, 465 m), during spring–summer 2014. Sowing took place on 23 May in AUA, on 29 April in INIAV and on 9 May in UTAD.

In AUA the soil was clay loam of pH (H_2O) 7.7 and humus content of 6.3 g kg^{-1}. In INIAV, the soil was classified as sandy clay loam with a medium texture and presented 1.0 g kg^{-1} humus content, >200 mg kg^{-1} P_2O_5, >200 mg kg − 1 K_2O_2 and pH (H_2O) 5.2. The soil in UTAD was classified as lime with a medium texture and presented 1.3 g kg^{-1} humus content, 91.0 mg kg^{-1} P_2O_5, 158.0 mg kg^{-1} K_2O_2 and pH (H_2O) 4.7. Before sowing, the experimental field was ploughed with a rotary tiller and supplied with

Table 2. Temperature (°C) and precipitation (mm) occurring in the three locations during the cultivation period

	Temperature (°C)			
Location/month	Mean	Max.	Min.	Precipitation (mm)
AUA				
April	15.2	26.9	9.1	39.0
May	17.1	32.5	12.7	2.0
June	21.5	38.8	16.8	10.6
July	24.7	36.6	21.6	0.0
August	24.2	38.6	21.9	0.0
September	24.5	33.8	15.7	20.8
INIAV				
April	16.3	22.7	9.9	90.0
May	19.7	28.1	11.4	23.8
June	19.2	26.9	11.6	1.5
July	24.9	34.5	15.3	4.3
August	25.0	34.7	15.3	0.0
September	22.5	29.5	15.5	80.4
UTAD				
April	13.9	19.9	8.9	44.7
May	14.8	21.6	8.8	28.5
June	17.5	24.4	11.9	26.5
July	20.6	27.8	14.5	29.3
August	20.3	27.9	14.2	0.4
September	17.8	24.0	13.6	89.3

Table 3. Frequencies (%) of the five qualitative traits studied, presented separately in the three locations, for the 24 cowpea accessions

		Frequency (%)		
Qualitative trait	Class	AUA	INIAV	UTAD
Growth habit	Erect	47.40	16.67	86.90
	Semi-erect	12.30	79.17	13.10
	Intermediate	7.80	0.00	0.00
	Semi-prostate	32.50	4.16	0.00
Flower color	White	78.90	78.20	78.20
	Violet	9.20	21.80	21.20
	Mauve-pink	11.90	0.00	0.00
Seed color	Beige	11.50	17.40	13.10
	Brown	14.00	0.00	8.70
	Cream	63.00	78.30	73.90
	Other	11.50	4.30	4.30
Eye color	Eye absent	24.00	26.10	13.10
	Black	44.00	43.50	43.50
	Brown splash or gray	0.00	0.00	4.30
	Green	0.00	0.00	8.70
	Tan brown	24.50	30.40	30.40
	Other	7.50	0.00	0.00
Seed shape	Crowder	0.00	0.00	0.00
	Globose	4.00	4.30	13.10
	Kidney	67.50	82.60	56.50
	Ovoid	12.00	0.00	8.70
	Rhomboid	16.50	13.10	21.70

mineral fertilizer 600 kg ha^{-1} NPK 11:15:15 in AUA, 250 kg ha^{-1} NPK 15:15:15 in INIAV and 5700 kg ha^{-1} limestone in UTAD.

Twelve plants per accession were grown in a greenhouse for 2 weeks in AUA. The seedlings were then transplanted in the field and the plants were spaced at 50 cm from row to row and 20 cm apart within the row and drip irrigated. In INIAV and UTAD, 20 seeds per accession were directly sown in plots of 3.75 m^2 and plants were spaced at 75 cm from row to row and 25 cm apart within the row. In INIAV the accessions were drip irrigated, whereas in UTAD they were irrigated along grooves.

A randomized complete block experimental design (RCBD) was used in AUA with four replicates and three plants per replicate per accession. In INIAV and UTAD a completely randomized experimental design was implemented and 12 plants of each accession were randomly selected. During the growing season, weeds were hand-controlled and incidences of pests and diseases were handled through chemical management in all locations.

Climate data

Altitude of locations ranged from 24 m (AUA) to 465 m (UTAD), and differed mainly regarding their average mean air temperature (°C) and precipitation (mm). The average maximum (T_{max}) and minimum (T_{min}) air temperature (°C) and total rainfall (mm) per month (from April to September) were recorded at weather stations located at each experimental location (Table 2).

Morphological and agronomical traits

A total number of 14 qualitative and quantitative traits were analyzed in the three experimental locations according to IBPGR descriptors.[26] Regarding qualitative traits, growth habit, flower color, seed color and shape, and eye color were recorded in all plants of each accession used in each location. Regarding

quantitative traits studied, plant height (cm), first pod height (cm), number of pods, number of seeds and seed weight per plant (g) were recorded in 12 plants per accession. To analyze the number of days to flowering only the average for each accession was recorded. The average yield per accession per location was calculated (g m^{-2}), while 100-seed weight (g) was determined by weighing two random samples of each accession. Protein content (%) was determined by the Kjeldahl method[27] and calculated by multiplying the nitrogen content by 6.25.

Data analysis

All the traits were compared per accession across all and for each one of the three locations (AUA, INIAV and UTAD). Per accession and location, 12 plants were considered as replicates. The evaluation of qualitative traits was determined by the frequencies of each trait. Descriptive statistics per quantitative trait and location were obtained using the summary statistics procedure in SPSS program version 8.0 (IBM SPSS, Inc., Chicago, IL, USA). For each trait, the minimum, maximum, mean and standard deviation, and coefficients of variation (CV) were calculated.

To estimate variance components of traits, a complete linear mixed model was used in the analysis of all the quantitative traits within and across the accessions and locations using the restricted maximum likelihood (REML) algorithm of SPSS program version 8.0. The heritability of each quantitative trait was calculated using the following equation: $H^2 = (s_g^2) / [s_g^2 + (s_e^2/r)]$, where s_g^2 and s_e^2 represent the genetic and residual variance for each trait and r is the number of replicates of each accession.[28]

Pearson correlation coefficients between the different quantitative traits and locations were determined through SPSS program version 8.0. To quantify the variation size due to

Table 4. Quantitative traits: average values obtained for the 24 cowpea accessions in each location, and *F*-value and Tukey's test (significance level of 0.05) for the traits with replications

	Plant height (cm)			1st pod height (cm)			No. of pods/plant			No. of seeds/plant			Seed weight/plant (g)			100-seed weight (g)			Days to flowering			Yield (g m^{-2})			Protein (%)		
	AUA	INIAV[a]	UTAD	AUA	INIAV	UTAD	AUA	INIAV	UTAD	AUA	INIAV	UTAD	AUA	INIAV	UTAD	AUA	INIAV[a]	UTAD	AUA	INIAV	UTAD	AUA	INIAV	UTAD	AUA	INIAV	UTAD
Cp4906	107.17	81.00	28.50	36.67	35.50	31.08	57.83	29.25	16.50	337.75	171.42	49.42	88.84	36.83	23.36	25.36	21.70	29.45	45	63	95	236.90	122.78	74.75	25.99	23.67	26.21
Cp5128	34.83	37.00	32.42	22.75	33.33	24.46	35.75	28.00	31.50	427.88	230.00	194.58	39.25	21.18	31.81	12.6	9.70	11.80	56	66	95	104.67	70.58	101.79	26.72	25.00	27.21
Cp5129	69.55	94.00	86.50	35.75	36.75	28.17	34.00	19.08	18.25	267.25	143.25	101.25	49.88	22.64	29.93	20.06	16.00	22.20	48	63	77	133.00	75.47	95.79	25.6	22.02	25.99
Cp5131	78.75	56.00	51.00	37.67	36.92	25.67	34.17	30.17	20.17	327.50	214.00	130.08	47.50	29.63	29.82	17.49	14.70	18.20	42	60	77	126.67	98.78	95.41	25.52	23.26	26.99
Cp5553	84.08	78.00	54.17	34.78	37.00	28.33	32.25	18.67	18.50	245.00	134.92	108.08	48.88	24.26	29.03	19.86	19.10	21.40	46	57	91	130.33	80.86	92.88	25.65	24.8	25.43
Cp5556	66.92	77.00	44.58	26.46	43.12	27.92	19.67	20.50	8.08	150.25	119.08	26.50	34.48	21.50	12.80	19.09	17.90	26.75	44	63	97	91.93	71.67	40.96	25.63	26.97	27.08
Cp5647	91.58	67.00	67.83	34.17	39.42	27.25	30.92	12.00	20.08	256.00	79.08	94.42	53.28	12.90	29.25	21.16	13.10	20.10	45	63	105	142.07	43.00	93.60	26.76	22.67	26.98
Cp5648	57.67	62.00	94.17	28.08	40.00	25.67	31.00	14.25	15.17	201.50	100.17	85.92	41.09	18.35	21.78	22.25	17.20	21.00	50	65	81	109.57	61.17	69.71	26.56	24.64	27.77
Vg50	103.17	66.00	47.67	39.58	34.58	24.42	29.00	17.17	12.67	221.50	120.25	48.67	50.88	20.26	16.36	21.99	15.90	24.85	45	63	109	135.67	67.53	52.35	24.12	20.2	26.14
Vg52	77.17	70.00	84.92	33.33	36.92	28.08	35.17	11.67	17.67	349.75	70.92	94.92	64.50	13.51	21.23	21.21	20.00	20.10	47	74	105	172.00	45.03	67.92	24.31	26.24	27.54
Vg56	132.5	76.00	89.50	42.50	38.67	29.08	66.64	18.83	12.08	361.38	122.83	51.92	68.50	23.39	20.69	20.51	20.30	21.80	45	63	102	182.67	77.97	66.21	24.68	24.55	26.03
Vg59	69.25	62.00	35.58	35.33	32.08	24.25	42.17	32.33	16.50	465.13	214.00	35.92	67.75	29.08	16.09	16.00	12.80	16.15	46	60	105	180.67	96.92	51.49	24.66	20.92	27.43
Vg60	92.17	61.00	35.42	36.96	36.92	17.92	57.08	21.17	15.25	574.25	146.67	68.00	85.25	23.46	23.48	18.33	15.30	18.15	44	60	81	227.33	78.19	75.12	24.87	21.31	24.35
Vg65	78.17	39.00	82.17	34.50	38.42	25.00	31.83	9.67	11.75	269.25	69.92	43.75	57.81	14.05	21.34	25.15	20.90	28.90	44	57	91	154.17	46.83	68.29	25.06	21.56	27.73
Vg67	73.25	67.00	131.08	42.75	38.25	21.50	40.00	12.92	8.17	314.38	88.33	40.58	83.00	20.28	15.43	23.95	22.80	30.85	47	57	81	221.33	67.61	49.36	25.34	20.98	27.62
Vg72	89.42	84.00	91.50	31.04	40.58	25.33	25.58	12.5	13.92	168.88	97.42	67.67	39.38	15.90	21.84	20.00	18.80	24.45	45	57	102	105.00	53.00	69.89	24.64	20.93	27.27
Vg73	57.63	48.00	113.42	33.26	38.92	28.42	40.83	19.25	18.83	291.58	155.50	129.75	45.08	21.50	31.73	16.89	15.50	20.95	61	57	91	180.33	71.67	101.52	24.37	20.75	26.41
Fradel	80.23	73.00	48.25	34.42	36.08	22.75	29.50	69.42	16.58	216.00	500.33	97.50	43.25	84.17	28.35	14.21	19.70	25.50	45	73	112	115.33	280.56	90.72	25.08	23.89	28.45
BGE022146	105.50	200.00	136.00	49.00	42.13	33.42	31.00	33.00	11.58	273.38	270.00	75.92	71.50	54.00	17.71	12.75	18.70	20.65	60	72	112	190.67	14.94	56.67	23.47	27.96	27.97
BGE038474	48.84	200.00	106.00	28.58	50.00	38.33	44.17	19.00	15.42	552.38	149.00	107.25	78.75	18.00	23.79	12.50	11.60	14.90	68	77	116	210.00	4.99	76.13	23.97	26.84	26.93
BGE038477	28.57	200.00	107.83	37.29	42.00	42.33	32.25	19.00	30.67	268.33	149.00	267.17	36.17	21.00	41.49	16.89	12.00	14.35	66	76	112	144.67	5.81	132.77	MD	29.34	29.51
BGE038478	50.92	200.00	129.58	42.33	46.30	45.83	49.33	43.00	22.83	607.88	364.00	142.92	77.00	49.00	26.55	13.42	13.60	13.55	71	76	123	273.67	13.64	115.79	27.16	27.98	29.48
BGE038479	44.13	200.00	160.60	43.92	44.00	41.40	53.42	26.00	25.60	662.63	219.00	273.20	102.63	28.00	33.40	18.88	14.70	11.75	68	78	119	205.33	7.64	84.96	28.07	29.34	31.19
IT97K-499-35	35.89	66.00	33.38	33.82	35.00	24.13	94.22	36.00	18.38	906.22	284.00	216.38	169.67	52.00	22.73	12.20	18.50	10.25	71	75	109	529.00	14.44	60.91	23.28	21.09	22.75
F	5.17**	–	13.37**	2.08**	5.03**	8.73**	2.18**	12.70**	4.93**	3.76**	12.19**	12.73**	3.85**	12.49**	3.03**	7.73**	–	196.87**									
Tukey$_{0.05}$	51.53	–	44.94	21.66	6.51	10.24	53.23	15.37	12.01	448.63	114.96	80.46	72.91	18.27	17.53	6.88	–	2.02									

a For this location only had the average for the quantitative trait;
** significant at level of 0.01.

Table 5. Descriptive statistics for the nine quantitative traits studied, for each and all the three locations, for the 24 cowpea accessions

Location	Trait	Min.	Max.	Mean	SD	CV (%)	H^2
AUA	Plant height (cm)	17.00	187.00	73.62	44.69	60.71	0.27
	1st pod height (cm)	4.00	125.00	35.64	14.89	41.78	0.10
	No. of pods/plant	1.00	166.00	39.14	30.47	77.80	0.10
	No. of seeds/plant	13.00	1454.00	362.56	313.01	86.33	0.27
	Seed weight/plant (g)	2.00	255.00	63.92	51.06	79.88	0.27
	100-seed weight (g)	5.00	33.30	18.55	5.57	30.05	0.49
	Days to flowering	42	71	52.04	10.04	19.29	
	Yield (g m^{-2})	91.93	529.00	179.29	88.59	49.41	
	Protein (%)	23.28	28.07	25.28	1.19	4.75	
INIAV	Plant height (cm)	37.46	200.00	94.22	56.88	60.36	MD
	1st pod height (cm)	24.00	55.00	38.12	5.75	15.08	0.29
	No. of pods/plant	3.00	111.00	22.24	17.16	77.14	0.56
	No. of seeds/plant	13.00	865.00	156.63	126.80	80.96	0.55
	Seed weight/plant (g)	2.40	140.00	25.47	20.28	79.61	0.55
	100-seed weight (g)	9.70	22.80	16.68	3.48	20.88	MD
	Days to flowering	57	78	65.62	7.40	11.28	
	Yield (g m^{-2})	4.99	280.56	65.46	56.05	85.52	
	Protein (%)	20.20	29.34	24.17	2.89	11.99	
UTAD	Plant height (cm)	10.00	200	77.43	40.03	62.31	0.51
	1st pod height (cm)	8.00	58	28.53	9.85	34.53	0.43
	No. of pods/plant	1.00	58	17.12	10.38	60.63	0.25
	No. of seeds/plant	4.00	548	100.52	84.84	84.39	0.50
	Seed weight/plant (g)	1.30	97.30	24.39	14.21	58.29	0.15
	100-seed weight (g)	10.00	31.00	20.33	5.73	28.16	0.99
	Days to flowering	77	123	99.50	13.61	13.68	
	Yield (g m^{-2})	40.96	132.77	78.54	22.48	28.63	
	Protein (%)	24.35	31.90	27.32	1.54	5.64	
Total	Plant height (cm)	10.00	200.00	76.26	46.93	61.54	0.15
	1st pod height (cm)	4.00	125.00	33.93	11.76	34.66	0.09
	No. of pods/plant	1.00	166.00	26.56	23.49	88.29	0.10
	No. of seeds/plant	4.00	1454.00	193.56	219.17	99.90	0.17
	Seed weight/plant (g)	1.00	255.00	36.09	25.62	98.70	0.12
	100-seed weight (g)	5.00	33.30	14.33	9.26	64.62	0.54
	Days to flowering	42	123	72.39	22.68	31.33	
	Yield (g m^{-2})	4.99	529.00	107.76	79.67	73.91	
	Protein (%)	20.20	31.90	25.52	2.38	9.32	

Min, average minimum; Max., average maximum; Mean, average; SD, standard deviation; CV, coefficient of variation; H^2, heritability; MD, missing data.

genotype × environment (location) interaction relative to main genotype variation, the quantitative parameters over locations were analyzed using a linear mixed model with the REML procedure of SPSS program version 8.0. The genotypes and genotype × environment interaction (G × E) were considered as random effects and the locations as fixed effects. The results of this mixed model quantify the size of the G × E interaction relative to the genetic variance using the ratio $\sigma^2_{ge} / \sigma^2_g$, where σ^2_{ge} and σ^2_g represent the genotype × genotype interaction and the genotype impact, respectively. Principal component analysis (PCA) was performed using MVSP version 3.22 statistical software.[29]

RESULTS AND DISCUSSION
The environmental parameters or climatic data recorded in 2014 comparative to historical averages at the three locations (AUA, INIAV and UTAD) can be considered normal, suggesting that the

data observed in this study reflect the plant performance in each location.

Qualitative traits are considered the most appropriate to determine a specific cultivar/variety because they are mostly genetically controlled, being independent from the environment. In this present study, the frequencies for each five qualitative traits studied were determined in regard to the three different locations (Table 3).

Growth habit presented some variation among locations, erect growth being the most common (47.4% and 86.9%, respectively) in AUA and UTAD, whereas in INIAV semi-erect (79.17%) was the most prevalent growth habit regarding the total accessions studied. For consumers and farmers, seed traits such as color seed and eye, seed size and seed coat are considered the most important traits of cowpea.[30,31] In all locations, seeds had a predominant cream color, kidney shape and black eye (Table 3), in accordance with consumer preferences.[22] These findings are in contrast to the results obtained by Negri et al.[4] and Egbadzor et al.,[24] who observed a higher

Table 6. Estimates of variance components for genotypic variance and variance for genotype × environment and ratio of genotype × environment interaction variance to genetic variance for the five quantitative traits in 24 cowpea accessions

Trait	Source of variance		
	Genotype ($\sigma^2 g$)	Genotype × environment (σ^2_{ge})	σ^2_{ge}/σ^2_g
Plant height	134	902.22	6.70
1st pod height	10.87	12.69	1.17
No. of pods/plant	85.64	23.19	0.27
No. of seeds/plant	5423.78	8104.40	1.49
Seed weight/plant	20.97	290.57	13.86

variability in these two traits in cowpea accessions from Italy and Ghana.

Regarding the nine quantitative traits (Table 4), a high variability was observed in the number of days to flowering among the three locations. The average number of days to flowering was 52 (AUA), 65 (INIAV) and 99 (UTAD), with an average of 73.39 for the three environments (Table 5). This differentiation could be explained by the different temperature range observed in the three locations and also the different sowing dates in each location (Table 2). The INIAV sowing date was earlier than AUA and UTAD because among the three locations INIAV is the warmest, so it is important to sow early (Table 2). In general, cowpea accessions with the higher and the lower values were concordant; namely, accession BGE038478 presented the latest flowering in all locations whereas accession Cp5131 presented the earliest one (Table 4). The beginning of flowering has been considered an important trait in genotype selection for cowpea improvement. Indeed, Silva *et al.*[32] referred to its negative correlation with seed production. Moreover, accessions with earlier flowering dates would be more interesting because this way cowpea plants are more likely to escape high temperatures, long water stress periods and low relative humidity.[22] In fact, Hamidou *et al.*[33] verified that there is a higher drought susceptibility in the flowering stage than in the vegetative stage of cowpea.

Variance analysis revealed significant differences, at a level of 1%, between accessions for six quantitative traits (plant height, first pod height, number pods per plant, number seeds per plant, seed weight and 100-seed weight (Table 4).

Plant height of the accessions fluctuated particularly in each tested location, ranging from 10 to 200 cm, with a mean value of 77.43 (Table 5). De Souza *et al.*[34] previously reported similar maximum values for plant height in cowpea populations and a mean value of 164 cm, whereas a mean value of 113.7 cm was reported by Basaran *et al.*[35] In comparison, Abayomi *et al.*[36] reported a maximum plant height of 59.12 cm. A higher CV value was calculated for plant height (61.54%) than that reported by de de Souza *et al.*,[34] indicating the high variability of this trait among the accessions tested in this study.

The average value for the first pod height in the three environments was 33.93 cm.

The extreme values of the three locations were observed in Cp5556 (4 cm) and BGE022146 (125 cm) accessions at the AUA location; at INIAV, the values ranged from 24 cm (Cp4906 and Vg 59 accessions) to 55 cm (BGE038478 accession), with an average of 38.12 cm; and at UTAD from 8 cm (Vg 59 accession) to 58 cm (BGE038474 and BGE038478 accessions), with an average of 28.53 cm (Table 5).

In modern agriculture, one of the most important characteristics in grain legumes is the first pod height. Plants with compact growth and a great distance of the first pod from the ground are highly desirable, allowing increased sowing density, facilitating mechanical harvesting and benefiting seed quality, since contact with soil and therefore rotting of pods and seeds are avoided. The importance of first pod height was also previously reported by Silva *et al.*,[32] who described a simple correlation between the first pod height and the number of seeds per plant.

High variability was presented for number of pods and seeds per plant. Specifically, number of pods per plant ranged from one (Fradel at AUA and Vg 67 at UTAD) to 166 (BGE038479 at AUA), with an average of 26.56 pods per plant; seeds per plant ranged from four (Cp5556 at UTAD) to 1454 (BGE038478 at AUA), with an average of 193.56 (Table 5). The mean number of pods per plant observed in this study, as well as the CV value, was higher than reported by de Souza *et al.*[34] for Brazilian local cultivars and by Oliveira *et al.*[37] Seed weight per plant was also characterized by high variability, ranging from 1 to 255 g. All three traits studied that are related to seed yield production presented high CV values, while CV values for number of pods and seeds per plant were higher than these reported by Ajayi *et al.*[38] for cowpea breeding lines. Hundred-seed weight ranged from to 5 to 33.3 g, with an average of 18.52 g, which was slightly higher than that reported by Perrino *et al.*[39] among cowpea landraces originating from the Mediterranean region.

Concerning yield, average values were calculated and for this reason it was not possible to perform statistical analysis. The average yield of the three locations was 107.76 g m^{-2}. In AUA, IT97K-499-35 had the highest yield (529 g m^{-2}) and Cp5128 the lowest (91.93 g m^{-2}), whereas in INIAV the yield varied between 4.99 g m^{-2} (BGE038474) and 280.56 g m^{-2} (Fradel). In UTAD yield ranged from 40.96 g m^{-2} (Cp5556) to 132.77 g m^{-2} (BGE038477). These results showed evidence of the good adaptation of some accessions to different environments, such as BGE038477 from Spain and Fradel from Portugal.

The parameters that presented higher heritability were different in the three locations: seed weight per plant (AUA), number of pods per plant (INIAV) and 100-seed weight (UTAD) (Table 5). The genetic variability transmitted from parents to their offspring is reflected by heritability.[40] This parameter is very important because it indicates the possibility and extent to which improvement can change a trait by selection.[40,41] A high heritability alone is not sufficient to perform an efficient selection in advanced generations unless accompanied by a substantial amount of genetic advance.[40,42] The different heritability observed could be explained by the behavior of the accessions in the different locations, allowing an understanding of how the environment affects these traits.

Regarding all three locations, the protein content varied between 20.20% (Vg50 in INIAV) and 31.90% (BGE038478 in UTAD), with an average of 25.69%. The lowest protein contents observed were 23.28% at AUA (IT97K-499-35), 20.20% at INIAV (Vg50) and 24.35% at UTAD (Vg60). BGE038478 showed the highest protein content in all three locations (28.07% at AUA, 29.34% at INIAV and 31.90% at UTAD). The values of protein content obtained are in agreement with the reference values that have been previously given for cowpea.[9,43,44]

Correlation coefficients between the six quantitative traits and the three locations together are presented in Table 6. The number of pods per plant was correlated with the number of seeds per plant ($r = 0.813$, $P = 0.01$) and with the seed weight per plant

Table 7. Pearson correlation coefficients for the six quantitative traits, in all three locations, for the 24 cowpea accessions

	Plant height	1st pod height	No. of pods/plant	No. of seeds/plant	Seed weight/plant	100-seed weight
Plant height	1					
1st pod height	0.274[**]	1				
No. of pods/plant	0.052	0.263[**]	1			
No. of seeds/plant	0.004	0.307[**]	0.813[**]	1		
Seed weight/plant	0.071	0.275[**]	0.809[**]	0.894[**]	1	
100-seed weight	0.189[**]	0.021	0.001	−0.144[**]	0.026	1

[**] Correlation is significant at 0.01 level.
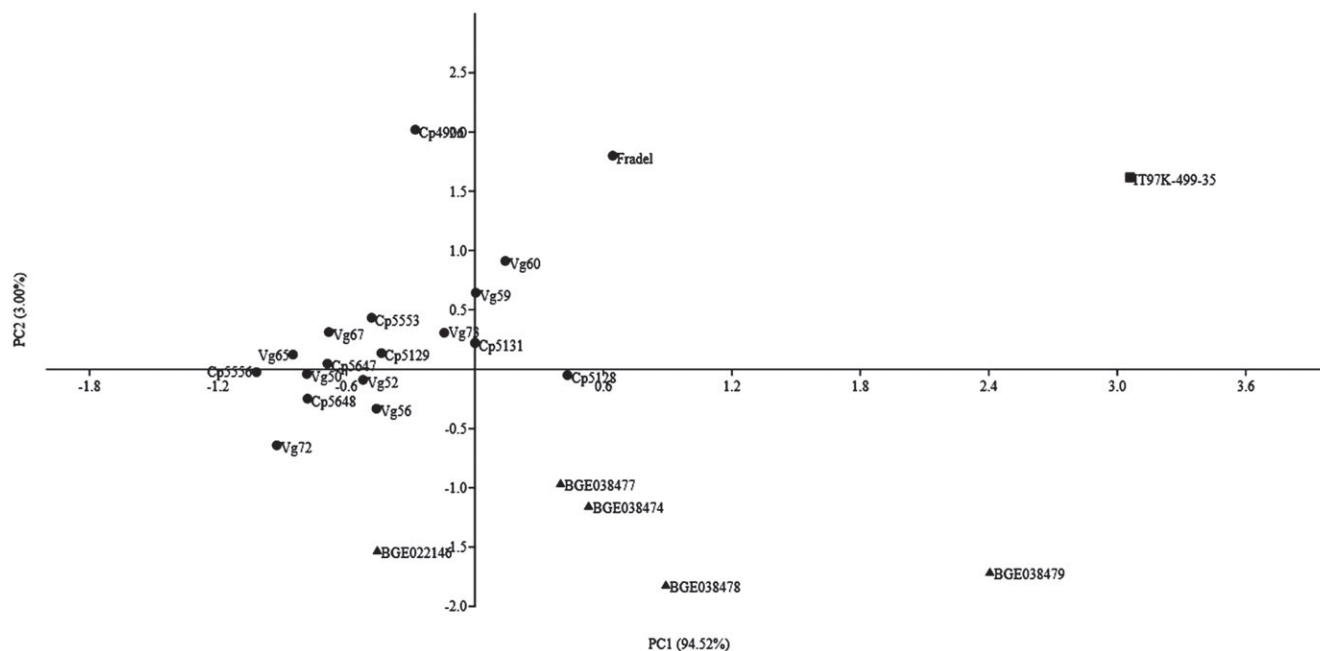
Figure 1. PCA of cowpea accessions in all three locations based on the eight quantitative traits measured (circles, Portuguese origin; triangles, Spanish origin; square, Nigerian origin).

($r = 0.809$, $P = 0.01$). This allows us to infer that the selection to increase the number of pods per plant favors seed weight and, consequently, productivity. These results confirm those obtained by Mohammed et al.,[45] Stoilova and Pereira[22] and Silva et al.,[32] who state that one of the most important components for seed production for cowpea is the number of pods per plant. Number of seeds per plant was negatively correlated with the 100-seed weight ($r = −0.144$, $P = 0.01$), which shows that selection for increased number of seeds can induce a reduction in the 100-seed weight.

Four of the five quantitative traits (plant height, first pod height, number of seeds per plant and seed weight) revealed a higher G × E component than genetic variance component (Table 7). In general, an inverse relationship between σ^2_{ge}/σ^2_g ratio and heritability value was observed.

The first two principal components of PCA explained 97.52% (PC1 = 94.52% and PC2 = 3.00%) of the total variation (Fig. 1 and Table 8). The major trait that contributed to the first component separation was the number of seeds per plant (0.971), and to the second component plant height (−0.548) and protein content (0.790) (Table 8). PCA allowed the discrimination of cowpea accessions based on their country of origin. Portuguese accessions were grouped mainly together in the second and third quadrant, while the Fradel variety and the reference line IT97K-499-35

Table 8. Eigen values, factor scores and contribution of the first two principal axes (PC1, PC2) to the variation of the 24 cowpea accessions

	PC1	PC2
Plant height	−0.056	−0.548
1st pod height	0.007	−0.102
No. of pods/plant	0.076	0.056
No. of seeds/plant	0.971	−0.198
Seed weight/plant	0.128	0.130
100-seed weight	−0.014	0.061
Yield	0.003	−0.038
Protein content	0.180	0.790
Eigen value	14 845.234	471.433
Percentage (%)	94.518	3.002
Cumulative (%)	94.518	97.519

were separated at higher distance (first quadrant). In addition, the Cp4906 accession, was separated from the other Portuguese accessions. This accession was the only one collected near the coast (Atlantic Ocean). Four of the five Spanish accessions were grouped in the fourth quadrant.

CONCLUSIONS

The present study highlights the high genetic diversity existing in the Iberian Peninsula cowpea genetic resources and useful knowledge about its breeding value. Seeds per plant is the trait that should be used primarily for plant selection. A clear distinction was observed between landraces and the reference samples, variety and breeding line. Moreover, the set of accessions with Spanish and Portuguese origin was discriminated in PCA, suggesting a specific gene pool structure.

G × E interaction, important yield components such as number of pods and seeds per plant and seed weight encompass variation between accessions. This variability reveals the potential of this germplasm for breeding programs to be conducted in different environments.

The accessions BGE038477 and BGE038478 from Spain and Cp5553 and Vg60 from Portugal have already been included in a cowpea breeding program.

ACKNOWLEDGEMENTS

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