CSIRO PUBLISHING

Crop & Pasture Science, 2017, **68**, 924–931
https://doi.org/10.1071/CP17071

Genotype by environment interactions in cowpea (*Vigna unguiculata* L. Walp.) grown in the Iberian Peninsula

Marina Martos-Fuentes[A], *Juan A. Fernández*[A], *Jesús Ochoa*[A], *Márcia Carvalho*[B], *Valdemar Carnide*[B,C], *Eduardo Rosa*[B], *Graça Pereira*[D], *Carina Barcelos*[D], *Penelope J. Bebeli*[E], *and Catalina Egea-Gilabert*[F,G]

[A]Departamento de Producción Vegetal, ETSIA, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, Spain.

[B]Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal.

[C]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal.

[D]National Institute for Agrarian and Veterinary Research (INIAV), Estrada de Gil Vaz, Apartado 6, 7351-901 Elvas, Portugal.

[E]Laboratory of Plant Breeding and Biometry, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.

[F]Departamento de Ciencia y Tecnología Agraria, ETSIA, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, Spain.

[G]Corresponding author. Email: catalina.egea@upct.es

Abstract. The aim of this work was to determine the variance components and genetic and environmental stability of 12 cowpea genotypes at three locations (South-east of Spain: Cartagena, South and North of Portugal: Elvas and Vila Real, respectively) in the Iberian Peninsula in two consecutive years (2015 and 2016). The genotype, the environment and the genotype × environment interaction significantly influenced all the morphological and agronomical parameters evaluated. For both years, the highest yields were observed at Elvas, whereas Cartagena and Vila real were the most suitable places to obtain crop precocity. Cartagena was the place where the filling of the seed was the fastest, probably due to the higher temperatures and radiation. The thermal time model (effective degree-days) could be used to predict the period of cowpea development, therefore predict flowering and pod maturity date. Correlation analysis showed that days to flowering, days to maturity and the seed yield vs protein content exhibited negative correlations. The highest heritability was found for plant height and pod length at Cartagena and for 100-seed weight at Elvas and Vila Real. In conclusion, the variations that exist in the studied accessions could give rise to a breeding program to develop cowpea cultivars with interesting agronomic traits.

Additional keywords: degree-days, heritability, legumes, protein content, yield.

Received 16 February 2017, accepted 22 June 2017, published online 11 August 2017

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is originated in Southern Africa and belongs to the family *Fabaceae*, tribe *Phaseoleae* and genus *Vigna*, which comprises several species, subspecies and varieties depending on morphology and domestication (Padulosi and Ng 1997). Cultivated cowpea belongs to *V. unguiculata*, spp. *unguiculata*, which contains the cultigroups Unguiculata, Biflora, Sesquipedalis and Textilis (Ng and Marechal 1985). This annual warm-season legume is one of the most widely adapted, versatile, and nutritious grain legumes (Ehlers and Hall 1997). During the 2010–2014 period, the world cowpea planting area was 58.1 million hectares and the production was 33.5 million tonnes. Africa has been responsible for 95.8% of worldwide cowpea production (FAOStat 2017). Nigeria and Niger are the largest producers with 3.4 and 1.6 million tonnes, respectively. In contrast, Europe is only responsible for 0.4% of worldwide cowpea production (FAOStat 2017). Now-a-days, cowpea is mainly grown by subsistence farmers in west and central sub-Saharan Africa, but also is an important food source in the rest of Africa, Central and South America, South-east Asia and in the southern United States (Davis *et al*. 1991; Timko and Singh 2008). In addition, cowpea is being cultivated at a small scale in many parts of Southern Europe and countries around the Mediterranean Basin (Domínguez-Perles *et al*. 2015), providing these countries a considerable income through exports to Northern European and non-European countries (EC 2016). Like other grain legumes, cowpea has the capacity to establish association with nitrogen-fixing bacteria (like rhizobia) and vesicular-arbuscular mycorrhizal fungi that make this crop interesting for predicted climatic changes. Cowpea can be used for human food and for fodder livestock (Tarawali *et al*. 1997). For human food, dry grain is the most important part, but leaves and immature pods are also consumed. Dry grains provide a significant amount of dietary protein (18–35%), as well as a source of calories, vitamins, minerals and essential amino acids as lysine and tryptophan (Singh 2002). For all this proprieties, this is an attractive crop with which many research is being done to promote it and include it in diets, not only because of its protein content but also because other functional properties, such as chlorophylls, carotenoids and phenolic contents, and high antioxidant activity (Khalid *et al*. 2012; Campbell *et al*. 2016; Karapanos *et al*. 2017). However, the value of grain legumes as a source of nutrients depends on a plethora of factors, including genetic characteristics, agro-climatic conditions, and postharvest management (Gonçalves *et al*. 2016).

The environment plays a very important role in the development and growth of plants. The major driving force that pushes crop growth and development is temperature although there are other environmental factors that can modify the effect of temperature such as photosynthetically active radiation (PAR) or photoperiod. Locations, growing seasons, rainfall, may have positive or negative impacts on several plant species as well as in cowpea genotypes. The thermal time concept or the accumulation of temperature for a life cycle or a particular phase of plant development, in contrast to the chronological time, has been used frequently to study the cowpea development, with the advantage to be independent of location and time of sowing. Craufurd *et al*. (1997) have described the effects of photoperiod and temperature on several development stages. Thus, the base temperature for development of seed germination, seedling emergence, leaf appearance, and days from sowing to first flowering is 8–11°C and the optimum temperature for most rapid reproductive development is close to 28°C. In addition, inclusion of radiation will allow describing development when temperature is not the only environmental variable affecting the process (Jones 2014). Thus, the ‘effective degree-days’ can be used to combine both temperature and radiation effects on plant development (Scaife *et al*. 1987). To our knowledge, no previous information exists on the effects of both temperature and radiation on cowpea development.

The association between the environment and the phenotypic expression of a genotype constitute the genotype (G) × environment (E) interaction, which determines if a genotype is widely adapted for an entire range of environmental conditions or separate genotypes must be selected for different sub-environments. Presence of the G × E interaction indicates that the phenotypic expression of one genotype might be superior to another genotype in one environment but inferior in a different environment (Falconer and Mackay 1996). Most of the studies in cowpea have been carried out on the genotypic variability and stability of some grain yield components (e.g. Akande 2007; Adewale *et al*. 2010; Shiringani and Shimelis 2011), showing generally significant G × E interactions. In addition, the protein content in seeds is also influenced by environmental and genotypic factors, being negatively correlated with yield (Oluwatosin 1997). Therefore, G × E should be taken into account in any breeding program.

Thus, the aim of this work was to determine the variance components and genetic and environmental stability of 12 selected cowpea genotypes at three locations of the Iberian Peninsula in two consecutive years. The results of this study may assist cowpea breeders in the manipulation of interested traits.

Material and methods

Plant material

Ten cowpea landraces (five from Portugal, three from Spain and two from Greece), one commercial variety from Portugal and one advanced line from Nigeria (Table 1) were used in three field experiments in 2015 and in 2016. The accessions were selected based on previously studies that were developed in the three locations where morphological and agronomical characteristics were evaluated. The agronomic characterisation of the 12 genotypes was done at: Technical University of Cartagena (UPCT), Cartagena, Spain (N 37°36′, W 00°58′; 40 m) – field experiment 1; National Institute for Agrarian and Veterinarian Research (INIAV), Elvas, Portugal (N 38°53′, W 07°09′, 208 m) – field experiment 2; University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal (N 41°17′51′, W 07°44′12′, 465 m) – field experiment 3.

Field experiment 1

Cultivars were planted on 29 May 2015 and 15 June 2016 in a randomised complete block design with four replications. One row per plot with 8-m length, 0.9-m row spacing and 7 m^2 were used. Seeds were sown by hand and seed rate was 10 seeds/m^2. The topsoil (0–20 cm) was classified as clay loam with a medium texture in both growing seasons, and presented 1.97 organic matter, 78 mg/kg of P_2O_5, 354 mg/kg of K_2O_2 and pH (KCl) 8.4 in 2015 growing season, and 2.18 organic matter, 80.13 mg/kg of P_2O_5, 415.82 mg/kg of K_2O_2 and a pH (KCl) 8.3 in 2016. Before sowing, in both growing seasons, the experimental field was ploughed with a rotary tiller and fertilised with 30 kg/ha of ammonium nitrate, 170 kg/ha of potassium nitrate and 250 kg/ha

Table 1. Cowpea accessions, origin and breeding status

Accession	Origin	Status of accession
IT 97K-499-35	Nigeria	Advanced line
AUA1	Greece	Landrace
AUA2	Greece	Landrace
Cp 4877	Portugal	Landrace
Cp 5051	Portugal	Variety
Cp 5553	Portugal	Landrace
Vg 59	Portugal	Landrace
Vg 60	Portugal	Landrace
Vg 73	Portugal	Landrace
BGE038479	Spain	Landrace
BGE038474	Spain	Landrace
BGE038478	Spain	Landrace

of monoammonium phosphate. The trails were drip irrigated from the beginning of June until the end of September.

Field experiment 2

Cultivars were planted on 28 April 2015 and 24 May 2016 in a randomised complete block design with four replications. Two row plots with 3-m length, 0.6-m row spacing and 3.6 m^2 were used. Seeds were sown by hand and seed rate was 11 seeds/m^2. The topsoil (0–20 cm) was classified as sandy clay loam with a medium texture in both growing seasons, and presented 1.3 g/kg organic matter, >200 mg/kg of P_2O_5, 153 mg/kg of K_2O_2 and pH (KCl) 6.9 in 2015 growing season, and 0.80 g/kg organic matter, >200 mg/kg of P_2O_5, >200 mg/kg of K_2O_2 and pH (KCl) 6.4 in 2016. Before sowing, the experimental fields were ploughed with a rotary tiller and fertilised with 200 kg/ha of 15 : 15 : 15. The trials were drip irrigated from the beginning of May until the end of August.

Field experiment 3

Cultivars were planted on 11 May 2015 and 3 June 2016 in a randomised complete block design with four replications. Three row plots with 3-m length, 0.75-m row spacing and 6.7 m^2 were used. Seeds were sown by hand and seed rate was 11 seeds/m^2. The topsoil (0–20 cm) was classified as gleyic fluvisol with a medium texture in both growing seasons, and presented in 2015 1.29 g/kg organic matter, 36 mg/kg of P_2O_5, 103 mg/kg of K_2O_2 and a pH (KCl) 4.2, whereas in 2016 1.61 g/kg humus content, 44 mg/kg of P_2O_5, 110 mg/kg of K_2O_2 and a pH (KCl) 5.2. Before sowing in both growing seasons, the experimental field was ploughed with a rotary tiller and fertilised with 250 kg/ha of nitromagnesium 27 and 200 kg/ha of NPK (Ca-Mg-S) 8–12–12 (2–2–14). The trials were drip irrigated from the beginning of July until the end of August.

Climatic data and calculation of accumulated degree-days and effective degree-days

The mean daily air temperature, total rainfall (mm) and accumulated global radiation (MJ/m^2) from April to September for each experiment are presented in Table 2.

Summations of heat units were determined based on base temperature using the coefficient of variation model (CV) to identify the accurate base temperature to adjust the method, according to Ochoa *et al*. (2011). The base temperatures tested ranged from 0°C to 16°C. The following methods were used:

Method 1. Standard degree-days method: $DD = \Sigma\,(T_m - T_b)$, where T_m and T_b are the daily mean and base temperatures respectively.

Method 2. Use of maximum instead mean temperature: $DD = \Sigma\,(T_M - T_b)$, where T_M and T_b are the daily maximum and base temperatures respectively.

Method 3. The degree-days method modified by the effect of the daily photosynthetic radiation input or effective degree-days (EDD), calculated according to following equation: $EDD^{-1} = DD^{-1} + f\ PAR^{-1}$, where PAR is photosynthetically active radiation (MJ/m^2 day) and f is a constant that defines the relative importance of radiation and temperature (m^2/MJ).

The DD and EDD were calculated considering the climatic conditions from sowing to flowering and sowing to maturity.

Morphological and agronomical traits

Phenotypic data for days to flowering and maturation were collected when 50% of the plants begin to flower and have mature pods, respectively. Plant height, first pod height, pod length and width and number of seeds per pod were measured in 10 plants per plot randomly selected. Yield, adjusted to 12% moisture, and 100-seed weight were evaluated per plot. Protein content (AOAC 1990) was derived from the estimated nitrogen (N) content, which was determined by the Kjeldahl method (Bremmer 1960), by the following formula: protein content (%) = N content (%) × 6.25.

Data analysis

Analysis of variance (ANOVA) of the three factors (genotype, location and year) followed by the Tukey's test was performed for each parameter in each environment and in the assembly of the three environments using the IBM SPSS Statistics 20 software.

A complete linear mixed model was used to estimate variance components of parameters in the analysis of all the quantitative parameters within and across the accessions and locations using Restricted Maximum Likelihood (REML) algorithm of SPSS program version 8.0. The heritability of each quantitative parameter was calculated for each environment using the following equation:

Table 2. Mean temperatures, precipitation and global radiation from April to September 2015 and 2016 in each location

Environment/	Year	Cartagena			Elvas			Vila Real		
Month		T (°C)	R (mm)	Solar radiation (MJ/m^2)	T (°C)	R (mm)	Solar radiation (MJ/m^2)	T (°C)	R (mm)	Solar radiation (MJ/m^2)
April	2015	16.0	10.2	596.02	16.6	110.3	630.57	13.5	48.8	453.97
	2016	16.1	14.6	627.72	14.3	80.6	648.39	11.0	193.0	456.62
May	2015	20.2	0.0	825.38	22.0	2.8	813.55	17.5	69.6	713.86
	2016	18.6	3.0	791.06	17.2	119.7	717.96	14.2	124.4	536.18
June	2015	23.1	1.6	876.83	25.6	37.9	820.66	20.9	2.2	729.61
	2016	22.8	0.0	853.27	23.7	0.0	953.87	19.1	25.2	759.73
July	2015	27.2	0.6	852.9	26.5	0.0	847.19	22.5	0.4	781.33
	2016	25.4	0.0	825.15	28.5	0.1	956.20	23.8	0.2	821.62
August	2015	27.2	1.0	693.22	25.4	0.9	809.93	20.9	0.6	647.58
	2016	25.5	1.2	759.95	27.2	0.1	858.85	23.3	0.2	657.18
September	2015	22.8	72.6	519.29	22.1	32.8	604.26	17.4	1.2	489.99
	2016	23.7	25.0	587.09	24.0	0.0	671.23	19.6	28.4	515.56

$$h^2 = V_g{}^2/[V_g{}^2 + (V^2/r)]$$

where $V_g{}^2$ and V^2 represent genotypic and error variance for each parameter and r the number of replications. For the three environments, the heritabilities were calculated using the equation:

$$h^2 = V_g{}^2/[V_g{}^2 + (V_{ge}{}^2/e) + (V^2/re)]$$

where $V_{ge}{}^2$ is the G × E interaction variance and e is the number of environments (Mendes-Moreira *et al*. 2015).

Pearson correlation coefficients between the different quantitative parameters and environments were determined through SPSS program version 8.0.

The principal components analysis (PCA) was performed using the MVSP program version 3.22.

Results and discussion

The plant genetic resources collections provide genetic variants, genes or genotypes that allow breeders to respond to new challenges based on systems of high production, high nutritional quality and disease and environmental resistance/tolerance. In the present study, we evaluated 12 cowpea accessions growing in three locations in the Iberian Peninsula (South-east of Spain: Cartagena, South of Portugal: Elvas and North of Portugal: Vila Real) during 2 years (2015, 2016) to identify morphological and agronomical parameters and the interactions among genotypes, environment and year.

In general, Vila Real registered the lowest temperatures and the lowest solar radiation. It is worth to highlights that the rainfall in Vila Real was 4-fold in 2016 than in 2015, whereas Cartagena had the driest conditions during the studied period (Table 2).

ANOVA to determine the effects of genotype, environment, year (Y) and their reciprocal interactions (G × E; G × Y; G × E; G × E × Y) on 10 morphological and agronomical parameters showed that all the factors had a high influence on the majority of the parameters (Table 3). These findings are according to those obtained by Shimelis and Shiringani (2010), who showed significant interactions among genotypes, locations and planting dates in cowpea. The genotype and the environment significantly influenced all the parameters evaluated. Year effect was also an important factor affecting all parameter except first pod height, pod length and number of seed per pod. The G × E interaction was significant for all parameter, but G × Y interaction was only significant for days to flowering and to maturity, first pod height, seed yield and number of seeds per pod. The E × Y interaction affected all parameters, except first pod height and pod width. Finally, the G × E × Y interaction was significant for all parameters, except 100-seed weight, pod length and protein content (Table 3). This high variability among the cowpea accessions indicates their utility in breeding programs.

The duration of the periods sowing to flowering and sowing to maturation were affected by the three factors and their interactions. In Cartagena, the days from sowing to maturity were the shortest, whereas in Elvas were the longest in both years (Table 4). Also in Cartagena the time from flowering to maturity was the shortest in both years, probably due to the effects of high temperature and radiation in this period (Tables 2 and 4).

The analysis of the three methods showed the least CV was obtained with Method 3 (Table 5), demonstrating that PAR had an important effect on the duration of crop cycles in all accessions. The best fit for *f* ranged from 0.11 to 0.12. The accurate base temperature for all methods and accessions ranged from 2°C to 14°C, varying in some accessions for each calculation method and period. This temperature range differed to that proposed by Craufurd *et al*. (1997), who fixed 8−11°C for development of cowpea cultivated in Nigeria.

Table 3. Analysis of variance for the 10 morphological and agronomical parameters evaluated in 12 cowpea accessions at three environments (Cartagena, Elvas, Vila Real) during 2 years (2015, 2016)

*$P<0.05$; **$P<0.01$; ***$P<0.001$; n.s., not significant

Parameters	ANOVA						
	G	E	Y	G × E	G × Y	E × Y	G × E × Y
Days to flowering	***	***	***	***	***	***	***
Days to maturity	***	***	***	***	***	***	***
Plant height	***	***	***	***	n.s.	***	*
First pod height	***	*	n.s.	***	**	n.s.	*
Seed yield	***	***	***	***	**	***	***
100-seed weight	***	***	***	***	n.s.	**	n.s.
Pod length	***	**	n.s.	***	n.s.	**	n.s.
Pod width	***	***	***	***	n.s.	n.s.	*
Number of seeds/pod	***	***	n.s.	***	*	**	***
Protein content	***	*	***	***	n.s.	***	n.s.

Table 4. Means and standard deviation of the 10 morphological and agronomical parameters evaluated in 12 cowpea accessions at three environments (Cartagena, Elvas, Vila Real) during 2 years (2015, 2016)

For each year, means followed by the same letter in the row are not significantly different at the 0.05 level using Tukey test, $n = 4$

Parameters	2015			2016		
	Cartagena	Elvas	Vila Real	Cartagena	Elvas	Vila Real
Days to flowering	75.58 ± 10.34a	66.44 ± 6.28c	70.00 ± 9.21b	59.21 ± 9.13b	67.94 ± 13.54a	69.98 ± 3.56a
Days to maturity	86.42 ± 10.08c	101.29 ± 4.60a	89.04 ± 9.01b	69.58 ± 8.88b	89.27 ± 11.53a	89.25 ± 3.9a
Plant height (cm)	212.47 ± 63.26a	123.87 ± 61.36b	57.07 ± 32.58c	212.72 ± 62.33a	146.38 ± 60.67b	80.05 ± 37.39c
First pod height (cm)	37.48 ± 7.54a	38.70 ± 9.15a	39.22 ± 10.55a	38.88 ± 7.24ab	36.97 ± 4.78b	41.84 ± 8.59a
Seed yield (g/m^2)	89.84 ± 33.24b	197.69 ± 103.21a	95.28 ± 43.39b	102.43 ± 44.18b	312.06 ± 122.89a	65.68 ± 26.56b
100-seed weight (g)	15.91 ± 2.26c	17.27 ± 4.52b	18.63 ± 5.39a	15.90 ± 2.32c	17.92 ± 5.10b	19.93 ± 5.43a
Pod length (cm)	17.16 ± 3.75a	16.94 ± 2.82a	16.63 ± 1.81a	17.10 ± 3.89a	16.38 ± 3.21b	17.14 ± 1.94a
Pod width (cm)	0.88 ± 0.09a	0.78 ± 0.08b	0.46 ± 0.09c	0.89 ± 0.08a	0.80 ± 0.10b	0.47 ± 0.09c
Number of seeds/pod	11.60 ± 1.26a	11.63 ± 1.03a	11.19 ± 1.13a	11.88 ± 0.92a	10.87 ± 1.02b	11.30 ± 1.17b
Protein content (%)	21.71 ± 2.52a	21.69 ± 1.78a	22.41 ± 0.98a	23.88 ± 2.26a	22.44 ± 1.14b	22.39 ± 1.15b

An explanation of our different findings could be due to the base temperature drops with the increase of the daily thermal amplitude (Bonhomme 2000), higher in our conditions than in Nigeria.

Table 5. The base temperature for each cowpea accession and over growing periods incorporating PAR radiation

Tbase is the base temperature. *f* is a constant that defines the relative importance of radiation and temperature in Method 3 (EDD calculation) as described before. CV is the coefficient of variation expressed as a percentage. S-F is the growing period from sowing to 50% of flowering. S-M is the growing period from sowing to maturity of pods

Accession	Tbase (°C)		*f*		CV (%)	
	S-F	S-M	S-F	S-M	S-F	S-M
IT-97K-499-35	5	5	0.12	0.11	10.61	12.80
AUA1	2	2	0.12	0.11	11.18	07.80
AUA2	2	9	0.12	0.11	06.27	10.24
Vg 59	2	2	0.12	0.11	20.39	13.07
Vg 60	10	10	0.12	0.11	17.83	12.85
Vg 70	2	2	0.12	0.11	10.87	10.28
Cp 4487	2	2	0.12	0.11	10.33	09.80
Cp 5051	14	14	0.12	0.11	09.73	12.20
Cp 5553	2	2	0.12	0.12	10.83	09.49
BGE038479	7	11	0.12	0.12	04.97	04.83
BGE038474	12	12	0.12	0.11	10.59	02.25
BGE038478	2	2	0.12	0.11	10.94	08.63

The seed yield was also affected by the three factors and their interactions (Table 3). For both years, the highest yields were observed in the trials located in Elvas (Table 4). In the second year (2016), the seed yield average increased in Cartagena and Elvas, while decreased in Vila Real (data not shown). At Cartagena, the most productive accessions were BGE038474 and IT97K-499–35 in 2015 and BGE038474 in 2016 (Table 6). In this location, the yield ranged from 52 to 165.4 g/m^2 and from 63.8 to 226.5 g/m^2 in 2015 and in 2016, respectively. At Elvas, Cp 5051 and Vg73 were the most productive in 2015, whereas in 2016 the most productive were Cp5553 and Vg73. The yield ranged from 35.98 to 329.6 g/m^2 in 2015 and from 152.32 to 514.4 g/m^2 in 2016. The commercial variety Cp 5051 revealed to be one of the well adapted accessions to this environment, this result could be expected due to this variety was selected at the INIAV Breeding Station in Elvas. Finally, in the first year, the most promising accessions in Vila Real were Cp 5553, Vg 60 and Vg 73, whereas in 2016 the most productive was AUA1. The seed yield varied from 34.3 to 167.2 and from 39.4 to 123.0 g/m^2 in 2015 and 2016, respectively. In general, the most productive accessions in each location were those that originally came from their own country, due to they are better adapted to their environmental conditions.

The highest plant height and pod width were observed in Cartagena in both years (Table 4). In 2015, the first pod height,

Table 6. Seed yield (g/m^2) for the 12 cowpea accessions evaluated at three environments (Cartagena, Elvas, Vila Real) during 2 years (2015, 2016)

Means followed by the same letter within the column for each year are not significantly different at the 0.05 level using Tukey test, *n* = 4

Accessions	2015			2016		
	Cartagena	Elvas	Vila Real	Cartagena	Elvas	Vila Real
IT 97K-499-35	142.43 ± 26.51a	247.17 ± 93.29abc	49.25 ± 20.91b	130.02 ± 45.87b	152.32 ± 20.68d	40.05 ± 24.21b
AUA1	52.00 ± 13.89c	84.14 ± 17.41de	101.05 ± 55.20ab	86.65 ± 28.95b	263.75 ± 99.86cd	123.03 ± 47.77a
AUA2	94.40 ± 13.22b	258.20 ± 56.64abc	76.73 ± 28.21ab	86.77 ± 26.71b	292.35 ± 55.32bcd	45.90 ± 27.41ab
Cp 4877	64.98 ± 11.41bc	169.89 ± 37.26bcd	89.50 ± 31.05ab	88.98 ± 18.69b	237.05 ± 46.19cd	39.35 ± 16.62b
Cp 5051	77.15 ± 18.93bc	329.56 ± 45.06a	79.45 ± 17.04ab	82.65 ± 39.32b	310.90 ± 80.93bcd	46.53 ± 33.97ab
Cp 5553	97.53 ± 26.03b	279.93 ± 81.56ab	167.15 ± 71.96a	122.23 ± 40.51b	506.82 ± 91.66a	82.58 ± 51.01ab
Vg 59	67.85 ± 12.81bc	231.72 ± 41.56abc	57.10 ± 14.26b	70.20 ± 13.59b	465.75 ± 137.68ab	57.05 ± 22.51ab
Vg 60	64.53 ± 11.09bc	257.20 ± 58.07abc	153.58 ± 58.14a	83.58 ± 9.88b	352.35 ± 53.57abc	103.80 ± 9.51ab
Vg 73	75.20 ± 4.38bc	310.67 ± 95.24a	157.78 ± 38.44a	63.83 ± 17.34b	514.37 ± 85.65a	61.80 ± 13.58ab
BGE038479	83.55 ± 9.77bc	40.66 ± 2.36de	34.33 ± 15.44b	73.43 ± 20.33b	197.82 ± 41.13cd	44.40 ± 15.05ab
BGE038474	165.35 ± 1.11a	35.98 ± 9.42e	78.68 ± 27.45ab	226.45 ± 60.19a	217.12 ± 43.74cd	71.65 ± 31.79ab
BGE038478	91.23 ± 2.66b	127.12 ± 18.31cde	98.75 ± 12.80ab	114.38 ± 15.77b	234.11 ± 4.71cd	72.18 ± 51.72ab

Table 7. Pearson correlation coefficients for 10 morphological and agronomical parameters for 12 cowpea accessions in the three environments (Cartagena, Elvas, Vila Real) and 2 years (2015, 2016)

**P* < 0.05; ***P* < 0.01

	Days to flowering	Days to maturity	Plant height	First pod height	Seed yield	100-seed weight	Pod length	Pod width	Number of seeds per Pod	Protein content
Days to flowering	1		–	–	–	–	–	–	–	–
Days to maturity	0.737**	1	–	–	–	–	–	–	–	–
Plant height	0.089	–0.178**	1							
First pod height	0.118*	0.084	0.210**	1	–	–	–	–	–	–
Seed yield	–0.277**	0.018	–0.006	0.029	1	–	–	–	–	–
100-seed weight	–0.142*	–0.031	–0.360**	–0.092	0.092	1		–	–	–
Pod length	–0.220**	–0.231**	0.167**	0.186**	–0.033	0.176**	1		–	–
Pod width	–0.150*	–0.234**	0.488**	–0.149*	0.240**	0.077	0.055	1		
Number of seeds/pod	–0.108	–0.116*	0.160**	0.142*	0.076	–0.216**	0.227**	0.047	1	–
Protein content	–0.148*	–0.255**	0.115	0.105	–0.172**	–0.106	0.196**	0.025	0.081	1

pod length and number of seeds per pod did not differ among the trial places. Vila Real was the location in which the seeds reached the highest 100-seed weight in both years. The seed size, measured as 100-seed weight, is one of the most important parameter for the consumer's preference.

As regards protein content, it was influenced by genotype, environment and their interaction (Table 3), in agreement with the results obtained by Oluwatosin (1997) with 15 cowpea cultivars grown in three locations in Nigeria and Ravelombola *et al*. (2016) who grew 11 cowpea breeding lines in three locations in Arkansas. The highest percentage was found in Cartagena in 2016 (~24% in average) (Table 4). The values of protein content obtained in this study are in agreement with the results found in literature (Singh 2002; Timko *et al*. 2007).

In general, correlation coefficients between the 10 parameters in the three environments and 2 years were not too high (Table 7). The highest correlation coefficient was between days to flowering and days to maturity ($r = 0.737$, $P = 0.01$)

Table 8. Heritability for the 10 morphological and agronomical parameters evaluated for UPCT, UTAD and INIAV and across the three environments in 12 cowpea accessions

	Cartagena	Elvas	Vila Real	Cartagena × Elvas × Vila Real
Days to flowering	0.80	0.94	0.80	0.59
Days to maturity	0.79	0.84	0.77	0.60
Plant height (cm)	0.99	0.96	0.90	0.78
First pod height (cm)	0.98	0.52	0.92	0.77
Seed yield (g/m^2)	0.93	0.82	0.78	0.29
100-seed weight (g)	0.94	0.99	0.99	0.89
Pod length (cm)	0.99	0.97	0.93	0.81
Pod width (cm)	0.98	0.97	0.97	0.91
Number of seeds/pod	0.84	0.64	0.88	0.65
Protein content (%)	0.89	0.65	0.36	0.53

and between plant height and pod width ($r = 0.488$, $P = 0.01$). The correlation between days to flowering and days to maturity was expected because they are closer in the plant development. Plant height and 100-seed weight showed the highest negative correlation ($r = -0.360$, $P = 0.01$) (Table 7), which shows that selection for the increase of plant height can induce a reduction in the 100-seed weight. There was also a negative correlation between the beginning of flowering and seed production in 2016 as it was reported by Silva *et al*. (2014). The seed yield and protein content exhibited negative correlations, which is agreement to the results obtained by Oluwatosin (1997) in cowpea and by Simmonds (1995) in cereals, and consequently indicates some restrictions in breeding alongside for high-yielding and high-protein genotypes. For the first pod height and seed yield, a positive correlation was registered. And for number of seeds per pod and 100-seed weight it was negative in agreement with the result obtained by Silva *et al*. (2014). The correlation between pod length and 100-seed weight was positive as the results obtained by Peksen and Artik (2004).

Heritability reflects the genetic variability that is transmitted from parents to their offspring (Robinson *et al*. 1949). Heritability, in broad-sense, estimates across environments, ranged from 0.29 for seed yield to 0.91 for pod width (Table 8). In general, it was higher at the Cartagena than at the other environments, with the exception of days to flowering, days to maturity, 100-seed weight and number of seeds per pod. The parameters plant height and pod length had the highest heritability at UPCT (0.99), whereas at Elvas and Vila Real was 100-seed weight that had the highest heritability (0.99) (Table 8). A hundred had high values of heritability in the three environments (0.94 at UPCT, 0.99 at Elvas and 0.99 at Vila Real) and across the three environments (0.89). These values are very close to the ones obtained in other studies with cowpea, which were always higher than 0.83 (Drabo *et al*. 1984; Omoigui *et al*. 2006; Manggoel *et al*. 2012;

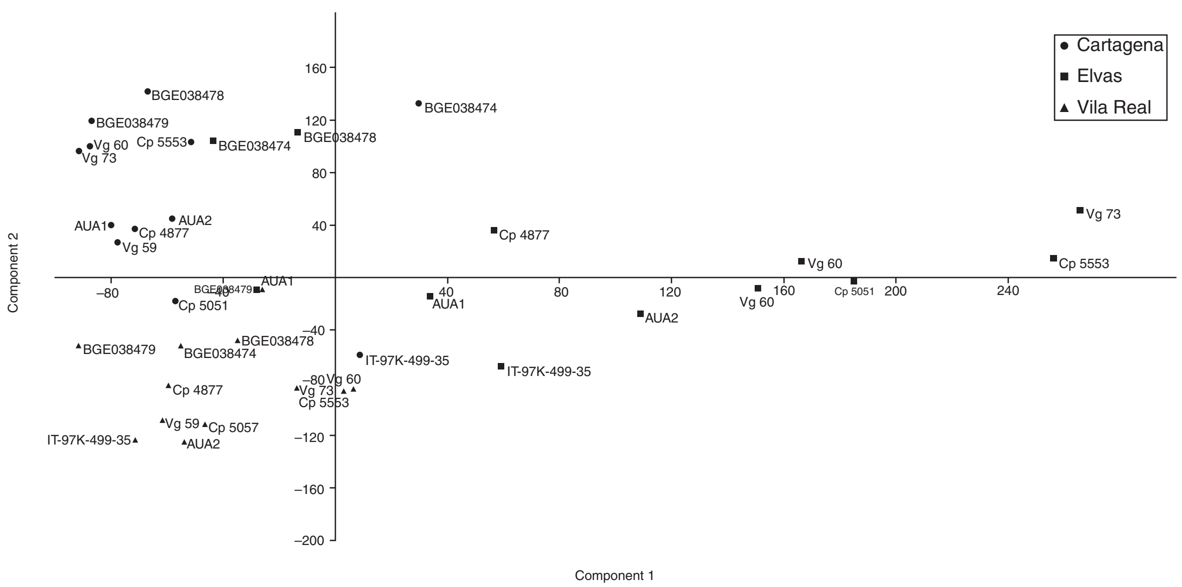

Fig. 1. Principal component analysis of 12 cowpea accessions (average of 2 years) and the three environments based on 10 quantitative traits. The data are the mean of 2 years.

Table 9. Eigen value, factor scores and contribution of the first two principal component axis to variation in the 10 morphological and agronomical parameters of 12 cowpea accessions

	Axis 1	Axis 2
Eigenvalues	9456.529	6147.711
Percentage	59.747	38.842
Cumulative percentage	59.747	98.589
Days to flowering	–0.034	0.001
Days to maturity	0.027	–0.022
Plant height	–0.201	0.979
First pod height	–0.009	0.018
Seed yield	0.978	0.202
100-seed weight	0.008	–0.021
Pod length	–0.003	0.006
Pod width	0	0
Number of seeds/pod	0	0.003
Protein content	–0.005	0.002

Egbadzor *et al*. 2013). These parameters with high heritabilities can be used in future breeding programs and for further quantitative genetic studies. However, it is important to refer that a high heritability alone is not enough to perform an efficient selection in advanced generations unless that it is accompanied by substantial genetic gains (Johnson *et al*. 1955; Mishra and Singh 2014). In the three environments (Cartagena*Elvas*Vila Real), the lowest values of heritability were estimated in seed yield (0.29) and protein content (0.53) (Table 8). The days to maturity (0.79), first pod height (0.52) and protein content (0.36) were the parameters with lowest values of heritability in Cartagena, Elvas and Vila Real, respectively. The low seed yield heritability was also reported by Omoigui *et al*. (2006) in cowpea. The value obtained in protein content is in agreement with the value reported by Ravelombola *et al*. (2016), who estimated a protein content of 0.58, and pointed out that this parameter can be inherited and can be selected for in the progeny.

PCA of the 12 cowpea accessions in three different environments in two seasons is presented in Fig. 1. The first two principal components (PC) explained 98.59% (PC1 = 59.75 and PC2 = 38.84) of total variation. In PC1, the main contributing parameter was yield (0.98) and in PC2 plant height (0.98) (Table 9). Manggoel and Uguru (2011) and Doumbia *et al*. (2013) also obtained in their studies that yield and plant height were parameters that contribute to the divergence between accessions. In addition, they found another parameter such as number of peduncles and flowers per plant, the days to flowering and days to maturity, which also contributed to the divergence, although some of them were not analysed in the present study. The accessions characterised at Vila Real were grouped principally in the third quadrant, those characterised in Cartagena were mainly distributed in the second quadrant and the accessions at Elvas were dispersed for the four quadrants, although the majority were in first and fourth ones (Fig. 1).

Conclusion

The results indicate the existence of significant interactions among genotypes, locations and years, providing a useful knowledge about the breeding value of the genetic resources studied. INIAV could be the best place to grow these accessions because of the highest yield obtained. However, if we are looking for precocity, Cartagena and Vila Real are the most suitable places. Cartagena was the place where the filling of the seed was the fastest, probably due to the higher temperatures and radiation. The thermal time model (EDD) could be used to predict the period of cowpea development, therefore predict flowering and pod maturity dates, an important issue in harvest logistic and marketing strategies.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This study was supported by the EUROLEGUME project. This project has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no. 613781 and by European Investment Funds by FEDER/COMPETE/POCI – Project POCI-01-0145-FEDER-006958 and National Funds by FCT – under the project UID/AGR/04033/2013.We thank Timothy J. Close for supplying the seeds.

References

Adewale BD, Okonji C, Oyekanmi AA, Akintobi DAC, Aremu CO (2010) Genotypic variability and stability of some grain yield components of cowpea. *African Journal of Agricultural Research* **5**, 874–880.

Akande SR (2007) Genotype by environment interaction for cowpea seed yield and disease reactions in the forest and derived savanna agro-ecologies of south-west Nigeria. *American-Eurasian Journal of Agricultural & Environmental Science* **2**, 163–168.

AOAC (1990) 'Official methods of analysis.' 15th edn. (Ed. K Helrich) (AOAC: Arlington, VA, USA)

Bonhomme R (2000) Bases and limits to using 'degree.day' units. *European Journal of Agronomy* **13**, 1–10. doi:10.1016/S1161-0301(00)00058-7

Bremmer JM (1960) Determination of nitrogen in soil by the Kjeldahl method. *The Journal of Agricultural Science* **55**, 11–33.

Campbell L, Euston SR, Ahmed MA (2016) Effect of addition of thermally modified cowpea protein on sensory acceptability and textural properties of wheat bread and sponge cake. *Food Chemistry* **194**, 1230–1237. doi:10.1016/j.foodchem.2015.09.002

Craufurd PQ, Summerfield RJ, Ellis RH, Roberts EH (1997) Photoperiod, temperature, and the growth and development of cowpea. In 'Advances in cowpea research'. (Eds BB Singh, DR Mohan Raj, KE Dashiell, LEN Jackai) pp. 75–86. (IITA: Ibadan and JIRCAS: Tsukuba)

Davis DW, Oelke EA, Oplinger ES, Doll JD, Hanson CV, Putnam DH (1991) Cowpea Exploration. University of Wisconsin-Extension, Cooperative Extension and Alternative Field Crops Manual, Madison, WI, USA.

Domínguez-Perles R, Carnide V, Marques G, de Castro I, de Matos M, Carvalho M, Rosa E (2015) Relevance, constraints and perspectives of cowpea crops in the Mediterranean Basin. *Legume Perspectives* **10**, 40–42.

Doumbia IZ, Akromah R, Asibuo JY (2013) Comparative study of cowpea germplasms diversity from Ghana and Mali using morphological characteristics. *Journal of Plant Breeding and Genetics* **1**, 139–147.

Drabo I, Redden R, Smithson JB, Aggarwal VD (1984) Inheritance of seed size in cowpea (*Vigna unguiculata* (L.) Walp.). *Euphytica* **33**, 929–934. doi:10.1007/BF00021923

Egbadzor KF, Dadoza M, Danquah EY, Yeboah M, Offei SK, Ofori K (2013) Genetic control of seed size in cowpea (*Vigna unguiculata* (L.) Walp). *International Journal of Agriculture Science* **5**, 367–371. doi:10.9735/0975-3710.5.2.367-371

Ehlers JD, Hall AE (1997) Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research* **53**, 187–204. doi:10.1016/S0378-4290(97)00031-2

European Commission (2016) Trade: Export Helpdesk: EU Customs Union. European Commission 344. Available at: http://exporthelp.europa.eu/thdapp/index.htm (accessed 17 September 2016).

Falconer DS, Mackay TFC (1996) 'Introduction to quantitative genetics.' 4th edn. (Longmans Green: Harlow, Essex, UK)

FAOStat (2017) Food and Agriculture Organization (FAO), World Production. Available at: http://faostat3.fao.org/browse/Q/QC/E (accessed 2 May 2017).

Gonçalves A, Goufo P, Barros A, Domínguez-Perles R, Trindade H, Rosa EAS, Ferreira L, Rodrigues M (2016) Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *Journal of the Science of Food and Agriculture* **96**, 2941–2951. doi:10.1002/jsfa.7644

Johnson HW, Robinson HF, Comstock RE (1955) Estimates of genetic and environmental variability in soybean. *Agronomy Journal* **47**, 274–318.

Jones HG (2014) 'Plants and microclimate. A quantitative approach to environmental plant physiology.' 3rd edn. (Cambridge University Press: Cambridge, UK)

Karapanos I, Papandreou A, Skouloudi M, Makrogianni D, Fernández JA, Rosa E, Ntatsi G, Bebeli PJ, Savvas D (2017) Cowpea fresh pods – a new legume for the market. Assessment of their quality and dietary characteristics of 37 cowpea accessions grown in southern Europe. *Journal of the Science of Food and Agriculture*, doi:10.1002/jsfa.8418

Khalid II, Elhardallou SB, Elkhalifa EA (2012) Composition and functional properties of cowpea (*Vigna ungiculata* L. Walp) flour and protein isolates. *American Journal of Food Technology* **7**, 113–122. doi:10.3923/ajft.2012.113.122

Manggoel W, Uguru MI (2011) Comparative study on the phenology and yield components of two photoperiodic groups of cowpea (*Vigna unguiculata* (L.) Walp.) in two cropping seasons. *African Journal of Agricultural Research* **6**, 5232–5241.

Manggoel W, Uguru MI, Ndam ON, Daskak MA (2012) Genetic variability, correlation and path coefficient analysis of some yield components of tem cowpea [*Vigna unguiculata* (L.) Walp.] accessions. *Journal of Plant Breeding and Crop Science* **4**, 80–86. doi:10.5897/JPBCS12.007

Mendes-Moreira P, Alves ML, Satovic Z, Santos JP, Santos JN, Souza JC, Pêgo SE, Hallauer AR, Vaz Patto MC (2015) Genetic architecture of ear fasciation in maize (*Zea mays*) under QTL scrutiny. *PLoS One* **10**, e0124543. doi:10.1371/journal.pone.0124543

Mishra P, Singh P (2014) Genetic variability, heritability, genetic advance, correlation coefficient and path analysis in gladiolus. *IOSR Journal of Agriculture and Veterinary Science* **7**, 23–26. doi:10.9790/2380-07722326

Ng NQ, Marechal R (1985) Cowpea taxonomy, origin and germplasm. In 'Cowpea research, production and utilization'. (Eds SR Singh, KO Rachie) pp. 11–21. (John Wiley & Sons Ltd: London)

Ochoa J, Niñiirola D, Conesa E, Lara L, López-Marín J, Fernández JA (2011) Predicting purslane (*Portulaca oleracea* L.) harvest in a hydroponic floating system. *Acta Horticulturae* **898**, 205–209. doi:10.17660/ActaHortic.2011.898.25

Oluwatosin OB (1997) Genetic and environmental variation for seed yield, protein, lipid and amino acid composition in cowpea (*Vigna unguiculata* (L) Walp). *Journal of the Science of Food and Agriculture* **74**, 107–116. doi:10.1002/(SICI)1097-0010(199705)74:1<107::AID-JSFA776>3.0.CO;2-L

Omoigui LO, Ishiyaku MF, Kamara AY, Alabi SO, Mohammed SG (2006) Genetic variability and heritability studies of some reproductive traits in cowpea (*Vigna unguiculata* (L.) Walp.). *African Journal of Biotechnology* **5**, 1191–1195.

Padulosi S, Ng NQ (1997) Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. In 'Advances in cowpea research'. (Eds BB Singh, D MohanRaj, KE Dashiell, LEN Jackai) pp. 1–12. (IITA-JIRCAS: Ibadan, Nigeria)

Peksen E, Artik C (2004) Comparison of some cowpea (*Vigna unguiculata* L. Walp.) genotypes from Turkey for seed yield and yield-related characters. *Journal of Agronomy* **3**, 137–140. doi:10.3923/ja.2004.137.140

Ravelombola WS, Ainong S, Yuejin W, Dennis M, Pengyin C, Vibha S, Clay W (2016) Evaluation of total seed protein content in eleven Arkansas cowpea (*Vigna unguiculata* (L.) Walp.) lines. *American Journal of Plant Sciences* **07**, 2288–2296. doi:10.4236/ajps.2016.715201

Robinson HF, Comstock RE, Harvey PH (1949) Estimates of heritability and the degree of dominance in corn. *Agronomy Journal* **41**, 353–359. doi:10.2134/agronj1949.00021962004100080005x

Scaife A, Cox EF, Morris GEL (1987) The relationship between shoot weight, plant density and time during the propagation of four vegetable species. *Annals of Botany* **59**, 325–334. doi:10.1093/oxfordjournals.aob.a087321

Shimelis H, Shiringani R (2010) Variance components and heritabilities of yield and agronomic traits among cowpea genotypes. *Euphytica* **176**, 383–389. doi:10.1007/s10681-010-0222-z

Shiringani RP, Shimelis HA (2011) Yield response and stability among cowpea genotypes at three planting dates and test environments. *African Journal of Agricultural Research* **6**, 3259–3263.

Silva AC, Morais OM, Santos JL, D'Arede LO, Silva CJ, Rocha M (2014) Estimativa de parâmetros genéticos em *Vigna unguiculata*. *Sociedade de Ciências Agrárias de Portugal* **37**, 399–407.

Simmonds NC (1995) The relation between yield and protein in cereal grain. *Journal of the Science of Food and Agriculture* **67**, 309–315. doi:10.1002/jsfa.2740670306

Singh BB (2002) Recent genetic studies in cowpea. In 'Cowpea genetics and breeding'. (Eds CA Fatokun, SA Tarawali, BB Singh, PM Kormawa, M Tamò) pp. 3–13. (International Institute of Tropical Agriculture: Ibadan, Nigeria)

Tarawali SA, Singh BB, Fernandez-Rivera S, Peters M, Smith JW, Schultze-Kraft R, Ajeigbe HA (1997) Optimizing the contribution of cowpea to food and fodder production in crop–livestock systems in West Africa. In 'Grasslands 2000, Proceedings of the XVIII International Grassland Congress'. Winnipeg and Saskatoon, Canada. (Eds JG Buchanan-Smith, LD Bailey, P McCaughey) pp. 19–53. (Associate Management Centre: Calgary, Canada)

Timko MP, Singh BB (2008) Cowpea, a multifunctional legume. In 'Genomics of tropical crop plants'. (Eds PH Moore, R Ming) pp. 227–258. (Springer Science + Business Media LCC: New York)

Timko MP, Ehlers JD, Roberts PA (2007) Cowpea. In 'Genome mapping and molecular breeding in plants: Pulses, sugar and tuber crops'. (Ed. C Kole) pp. 49–67. (Springer-Verlag: Berlin)