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RESEARCH ARTICLE

Diversity in cowpea (*Vigna unguiculata* (L.) Walp.) local populations from Greece

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Abstract Cowpea cultivation in many countries around the Mediterranean Basin depends on a number of locally adapted populations conserved on-farm at a small scale, rather than on the use of modern varieties. Documentation, characterization and exploitation of traditional local populations could contribute to their conservation and utilization as sources of desirable characteristics. Therefore, a study was conducted to (a) characterize, (b) assess diversity and (c) classify 23 on-farm conserved local cowpea populations based on 32 agro-morphological traits. Investigations on diversity of characteristics related to seed yield, mineral and seed crude protein content as well as on correlations among them were carried out. A relatively high phenotypic diversity was observed. In particular, a high level of within population diversity was found ($\bar{H}s = 0.34$) exceeding that among populations' diversity ($Gst = 0.27$). Principal component analysis classified the majority of local populations into two groups (mainly according to populations' seed coat color and eye color), further divided into six subgroups regardless of the populations' geographical origin. Significant differences were also observed among the populations studied for potassium and calcium, as well as for their seed crude protein content which ranged from 22.14 to 28.37 %. The results show appreciable levels of intra- and inter-phenotypic diversity in on-farm conserved cowpea populations, which indicates the existence of a valuable gene pool for future exploitation in breeding programs.

Keywords Breeding · Descriptors · Landraces · Mineral content · Phenotypic characterization · Protein content · *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the most important food and forage legumes in the semi-arid tropics (Timko and Singh 2008). The domestication of cowpea is presumed to have occurred in Africa, but there are divergent views regarding the exact center in the relevant literature (Coulibaly et al. 2002; Smykal et al. 2015). Nowadays, cultivation of cowpea extends worldwide, including developing countries from tropical and subtropical areas, especially sub-Saharan Africa, Asia, Central and South America,

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Mediterranean region and southern United States (Timko et al. 2007). The evolution of cowpea over time and space has resulted in cultivation of numerous local populations throughout the world. These have been spread over short or even long distances, brought into competition with other autochthonous landraces, and gradually adapted to different climatic and soil conditions by changing the phenotypes and genotypes frequencies (Zeven 1998).

As it can be concluded by texts of Theophrastus, cowpea was probably cultivated by the Greeks in the third century B.C. at which time it was called ‘‘phaseolus’’, while Plinius also reports the cultivation of cowpea in the first century A.D. by Romans (Tosti and Negri 2005). Cowpea existence in Europe is also mentioned as “Smilax Kipaia” (garden’s Smilax) by Dioskouridis, who describes a species that produces long, thin, cylindrical fresh pods, having leaves like ivy’s and kidney shaped seeds characterized by a lack of uniformity in color, a description that matches cowpea (Kavvadas 2015). The discovery of America triggered a rapid exchange of crop species, with strong evidence that common bean (*Phaseolus vulgaris* L.) has been introduced in France since 1508 (Zeven 1997), and gradually replaced cowpea cultivation in the Mediterranean region (Piergiovanni and Lioi 2010; Bitocchi et al. 2012). Despite the great extent of common bean cultivation and its key role in human nutrition, even now, a remarkable number of cowpea local populations are cultivated in Mediterranean Basin, primarily for human consumption of the grains, which are rich in protein, carbohydrates and nourishing minerals (Boukar et al. 2011), with a cowpea dry seed production in Europe amounting to 24 Kt for 2014 (FAO 2015). Moreover, cowpea populations are also cultivated for their young, tender pods as well as green-shelled seeds which are consumed fresh or frozen, or cooked (boiled).

Due to their widespread use in many geographically isolated areas for many centuries, on-farm conserved local cowpea populations preserve diversity of the species thereby constituting a precious genetic material for selection and breeding. Breeders are keen in utilizing local cowpea populations from different locations in order to develop improved varieties with high nutritional value and yield potential, as well as stable resistance to diseases and pests. Conventional breeding methods are primarily used for cowpea, identifying parents with important traits, generating genetically variable populations and selecting for agronomic performance and quality characteristics (Boukar et al. 2015). Landraces are adapted to specific agro-climatic conditions while still maintaining considerable among and within population diversity (Negri 2005). These populations are often reproduced concurrently by more than one farmer (Bellucci et al. 2013) and therefore, preserve the bulk of genetic diversity (Camacho Villa et al. 2005). The extent of genetic variation in a species and its distribution among and within landraces is determined by many factors, such as breeding system, habitat availability, migration among different landraces or landrace populations and their populations’ sizes, as well as many biotic and abiotic stresses (Nybom et al. 2014). Characterization and knowledge of the existing levels of within or intra- and among or inter-population diversity are fundamental for establishing suitable on-farm conservation practices while it is considered as a first step for using these populations in future breeding programs (Tosti and Negri 2005).

Diversity in cowpea has been assessed by numerous studies using morphological and agronomical characteristics, molecular markers and nutritional traits. Morphological characteristics seem to play an important role in the discrimination of these different populations by farmers and consumers as many local names, based on different cowpea characteristics, are often used in order to distinguish different cowpea types. Thus, for instance, local cowpea populations in Tanzania are named according to their growth habit, seed color, pod shape and coloration of the plant (Keding et al. 2007). Many names have been also provided in many European countries for cowpea, such as “Chicharo de Vaca” and “Judia de Vaca” (black-eyed) in Spain, “Feijão-Frade” (black-eyed), and “Feijão-Pequeno” (small beans) in Portugal (Lim 2012), “mavromatika” (black-eyed), “psilofasoula” (small beans), “velonakia” (needle beans), “ampelofasoula” (vine beans), and “arapofasoula” (black beans) in Greece (Kavvadas 2015), “crnookica” (black-eyed), “kravlji pasulj” (cowpea), and “mletacki grasak” (Venice pea) in Serbia (Mikić et al. 2010) and “Fagiolino Piccolo” (small bean), “Fagiolino dall’ occhio” (eye-shaped) (Lim 2012) and “Fagiolino pinto” (painted bean) in Italy and mainly referring to its seed and pod characteristics.

Different levels of diversity among cowpea accessions, varieties and landraces were displayed through phenotypic characterization and estimation of protein and mineral content. High variability was found among Portuguese cowpea landraces using morphological characteristics (Stoilova and Pereira 2013). Variability was observed also for grain yield and protein content in Brazilian cowpea genotypes by Ddamulira et al. (2015) and among cowpea cultivars by the use of agro-morphological traits and evaluation of nutritional composition (Animasaun et al. 2015). Furthermore, Perrino et al. (1993) reported a high phenotypic variability among cowpea landraces originated from Mediterranean region.

Observation of different levels of intra-population variation in various leguminous species has been reported in relation to their allogamy frequencies (Terzopoulos et al. 2008; Foschiani et al. 2009; Scarano et al. 2014). Despite numerous studies referring to inter-accessions/varieties diversity in cowpea, the ones referring to cowpea intra-accessions/varieties are rather few and those referring to an intra-landrace/population level are limited. Ghalmi et al. (2010), determining genetic diversity of twenty cowpea landraces collected from Algeria, observed low within population diversity, after using both morphological traits and molecular markers. Tosti and Negri (2005) determined genetic diversity among and within three local populations that are still cultivated and maintained on-farm, and found a relatively high level of within population diversity.

Given the limited number of studies concerning diversity and structure within cowpea populations and the significance of knowledge of populations' diversity towards their use in breeding programs, a study was designed to determine the presence of phenotypic diversity within individual cowpea local populations in Greece, where a wealth of cowpea landraces are still cultivated. This aim was achieved by analyzing an appropriate number of individuals per population and, for the first time to our knowledge, by using a sufficient number of agro-morphological descriptors in order to characterize, assess among and within diversity and classify on-farm conserved cowpea local populations. Investigation of variability of traits related to yield, mineral and protein content of seeds as well as their correlations constituted an additional purpose of this study, regarding their importance in cowpea breeding.

Materials and methods

Plant material and experimental design

The experiment was conducted at an experimental field of Agricultural University of Athens (N37°59′10″, E23°42′29″, altitude 24 m), during spring-summer 2014. One commercial cowpea variety 'dall' occhio' (VAR) (Agrogen SA, Athens, Greece), and twenty-three cowpea local populations collected from different locations of Greece (Fig. 1) in various expeditions, carried out by the Laboratory of Plant Breeding and Biometry (Thomas et al. 2012; unpublished data), were subjected to morphological and agronomical characterization, as well as to determination of their dry seed mineral elements and protein content. The origin and geographical data of the plant material are given in Online Resource 1. Forty-eight plants per population were grown in a greenhouse and transplanted to the field two weeks after emergence following a randomized complete block experimental design, with four replicates and twelve plants per replicate per population. The soil was clay with a loamy texture and a pH value of 8.1. Plants were spaced at a distance of 50 cm from row to row and 20 cm apart within the row. Plants were drip irrigated and supplied with 1000 kg ha^{-1} of a mineral fertilizer (NPK 11-15-15) as base dressing. During the growing season, weeds were hand-controlled, while pests were handled through chemical management.

Characterization using morphological and agronomical traits

Data on twenty-six morphological and agronomical traits, followed the International Board for Plant Genetic Resources descriptor list (IBPGR 1983), were recorded. Additionally, six agro-morphological traits were measured, namely plant height (cm), immature pod color, height to first pod (cm), number of seeds per pod, number of seeds per plant and seed weight per plant (g). All traits were recorded for each one of the forty-eight plants per population. The measurements taken referred to twelve vegetative traits, namely plant vigor, number of nodes on main stem, number of main branches, leaf color, terminal leaflet shape, twinning tendency, growth habit, growth pattern, plant height (cm), plant pigmentation (recorded separately for the nodal region of their main stem, base of tertiary

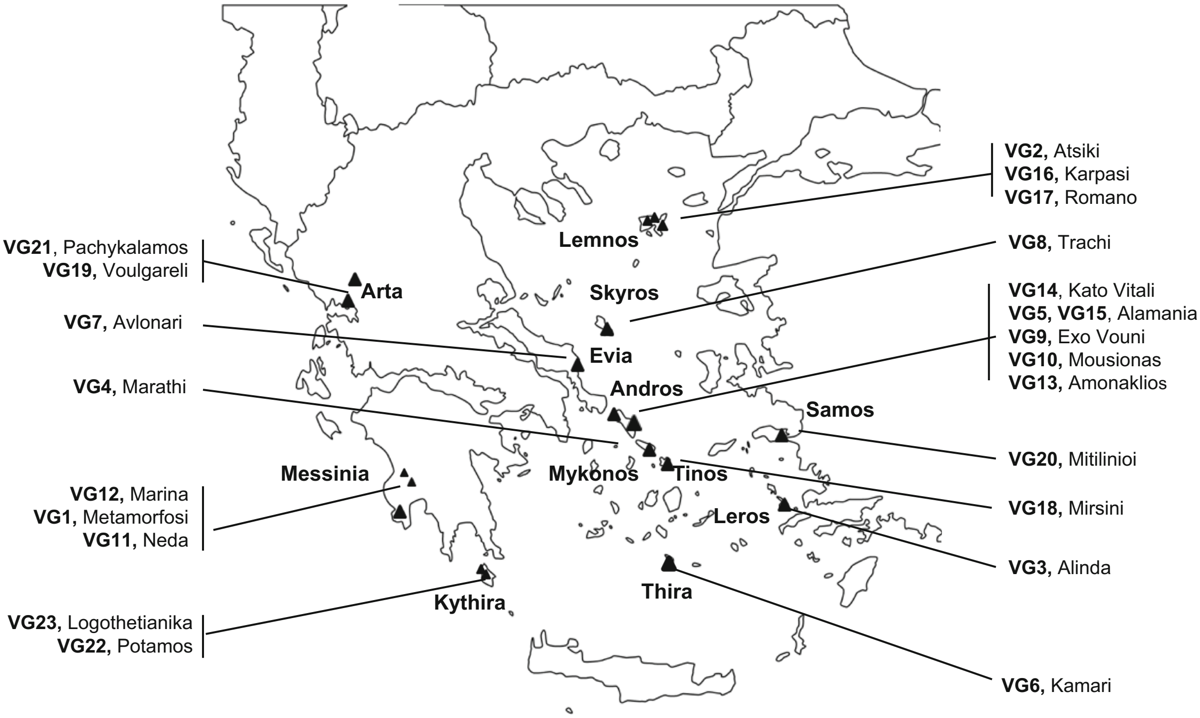

Fig. 1 Codes, collection sites and province or island of cowpea local populations studied

branches and base of the stalk of the trifoliate) and nine to reproductive stage, namely days to first flower, days to first mature pod, flowering duration, flower color, flower pigment pattern, immature pod pigmentation, immature pod color, height to first pod (cm) and mature pod color. In addition, six traits related to yield were recorded, namely number of pods per plant, pod length (cm), number of seeds per pod, number of seeds per plant, seed weight per plant (g), hundred seed weight (g) and five traits referring to seed morphology, namely seed shape, seed coat color, eye color, testa texture and splitting of testa (Table 1).

Determination of mineral elements and seed protein content

Three replications, of fifty seeds each, were used per population. Each sample was powdered using a ball mill, passed through a 40-mesh sieve and 0.50 g of each sample was subjected to dry ashing in a muffle furnace at 550 °C for 5 h. Seed samples were used to extract K, Ca, Mg, Fe, Mn, and Zn by means of 1 N HCl. The concentrations of Ca, Mg, Fe, Mn and Zn in the aqueous extracts were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA), while K was determined by flame photometry (Sherwood Model 410, Cambridge, UK). Kjeldhal-N method was used to determine the nitrogen content of cowpea populations in dry seed samples (Labtec^{TM} Digestor Basic, FOSS). Protein content was calculated by multiplying N by the factor 6.25 (2300 Kjeltec Analyzer unit, Tesco).

Data analysis

For statistical reasons, data from all quantitative traits were transformed to ordinal by dividing their range into four equal classes (Terzopoulos et al. 2008). The frequency of each rank within each trait was calculated for the entire collection and for each landrace separately in order to characterize the populations. The data were used to calculate phenotypic diversities. Phenotypic variation across local populations was calculated using Nei's genetic diversity (*He*) statistics (Nei 1973). For each trait, total phenotypic diversity (*Ht*), intra-population diversity (*Hs*), its average across

Table 1 Morphological and agronomical traits, class partition and frequencies of traits of the entire cowpea collection

Trait	Remarks	Classes	Frequencies		
Vegetative traits					
Plant vigor	Recorded in the 4th week after sowing	3: Non-vigorous	0.786		
		5: Intermediate	0.213		
		7: Vigorous	0.001		
		9: Very vigorous	0.000		
Number of nodes on main stem	Recorded in the 4th week after sowing	1: Small (one node)	0.714		
		2: Medium (2 nodes)	0.218		
		3: Large (3 nodes)	0.060		
		4: Very large (4 nodes)	0.008		
Number of main branches	Recorded in the 6th week after sowing	1: Small (0–2 branches)	0.595		
		2: Medium (3–5 branches)	0.350		
		3: Large (6–8 branches)	0.053		
		4: Very large (9–11 branches)	0.002		
Leaf color	Recorded in the 6th week after sowing	3: Pale green	0.000		
		5: Intermediate green	0.952		
		7: Dark green	0.048		
Terminal leaflet shape	Recorded in the 6th week after sowing	1: Globose	0.004		
		2: Sub-globose	0.443		
		3: Sub-hastate	0.453		
		4: Hastate	0.100		
Twinning tendency	Recorded in the beginning of flowering	0: Absence	0.737		
		3: Slight	0.111		
		5: Intermediate	0.074		
		7: Pronounced	0.078		
Growth habit	Recorded in the 6th week after sowing	1: Acute erect	0.006		
		2: Erect	0.370		
		3: Semi-erect	0.452		
		4: Intermediate	0.052		
		5: Semi-prostrate	0.047		
		6: Prostrate	0.016		
		7: Climbing	0.057		
Growth pattern		1: Determinate	0.000		
		2: Indeterminate	1.000		
Plant height (cm)	Recorded in the beginning of flowering	1: Low (10–50.5 cm)	0.700		
		2: Medium (50.6–91.1 cm)	0.173		
		3: High (91.2–131.7 cm)	0.110		
		4: Very high (131.8–172.3 cm)	0.017		
Plant pigmentation/stem/branches/petioles	Recorded in the 4th week after sowing	0: Absence	0.780	0.456	0.169
		1: Very slight	0.080	0.074	0.049
		3: Moderate	0.050	0.425	0.745
		5: Intermediate	0.060	0.042	0.036
		7: Solid	0.030	0.003	0.001

Table 1 continued

Trait	Remarks	Classes	Frequencies
Reproductive traits			
Days to first mature pod	Recorded from sowing to stage when a plant produced its first mature pod	1: Early (59–76 days)	0.220
		2: Medium early (77–94 days)	0.633
		3: Medium late (95–112 days)	0.139
		4: Late (113–130 days)	0.008
Flowering duration	Recorded from the beginning of flowering to stage when a plant is totally stopped to flower	1: Small (32–51 days)	0.154
		2: Medium (52–71 days)	0.765
		3: Large (72–91 days)	0.071
		4: Very large (92–111 days)	0.010
Flower color	Recorded of newly opened flowers, at the same hours of the day 9:00–11:00 a.m.	1: White	0.658
		2: Violet	0.243
		3: Mauve-pink	0.099
Flower pigment pattern	Recorded on newly opened flowers, at the same hours of the day 9:00–11:00 a.m.	0: Not pigmented (white)	0.478
		1: Wing pigmented; standard with light V-shaped pattern of pigment at top center	0.230
		2: Pigmented margins on wing and standard	0.000
		3: Wing pigmented; standard lightly pigmented	0.289
		4: Wing with pigmented upper margin; standard is pigmented	0.001
		5: Completely pigmented	0.002
Immature pod pigmentation	Recorded on ten full grown immature pods per plant	0: Non pigmented	0.487
		1: Pigmented tip	0.351
		2: Pigmented sutures	0.064
		3: Pigmented valves, green sutures	0.003
		4: Splashes of pigment	0.077
		5: Uniformly pigmented	0.018
Immature pod color	Recorded on ten full grown immature pods per plant	3: Pale green	0.431
		5: Medium green	0.569
		7: Dark green	0.000
Height to first pod (cm)	Recorded from the ground to the first pod set, at the beginning of maturity	1: Small (5–17.8 cm)	0.099
		2: Moderate (17.9–30.7 cm)	0.472
		3: High (30.8–43.6 cm)	0.345
		4: Very high (43.7–56.5 cm)	0.084
Mature pod color	Recorded for ten mature pods per plant	1: Pale tan or straw	0.615
		2: Dark tan	0.276
		3: Dark brown	0.001
		4: Black or dark purple	0.000
		99: Other	0.108

Table 1 continued

Trait	Remarks	Classes	Frequencies
Yield traits			
Number of pods per plant	Recorded after harvesting	1: Small (1–19 pods)	0.737
		2: Medium (20–38 pods)	0.249
		3: Large (39–57 pods)	0.012
		4: Very large (58–76 pods)	0.002
Pod length (cm)	Recorded after harvesting for ten mature pods per plant	1: Small (3–8.21 cm)	0.098
		2: Medium (8.22–13.43 cm)	0.649
		3: Large (13.44–18.65 cm)	0.252
		4: Very large (18.66–23.87 cm)	0.001
Number of seeds per pod	Recorded after harvesting for ten mature pods per plant	1: Small (1–4 pods)	0.215
		2: Medium (5–8 pods)	0.533
		3: Large (9–12 pods)	0.232
		4: Very large (13–16 pods)	0.020
Number of seeds per plant		1: Small (1–120 seeds)	0.836
		2: Medium (121–240 seeds)	0.089
		3: Large (241–360 seeds)	0.065
		4: Very large (361–480 seeds)	0.010
Seed weight per plant (g)		1: Small (0.1–17.3 g)	0.642
		2: Medium (17.4–34.6 g)	0.276
		3: Large (34.7–51.9 g)	0.071
		4: Very large (52–69.2 g)	0.011
Hundred seed weight (g)	Average of two random samples per plant	1: Small (6–12.2 g)	0.207
		2: Medium (12.3–18.5 g)	0.503
		3: Large (18.6–24.8 g)	0.221
		4: Very large (24.9–31.1 g)	0.069
Seed traits			
Seed shape	Recorded visually of a sample of ten seeds per plant	1: Kidney	0.826
		2: Ovoid	0.090
		3: Crowder	0.000
		4: Globose	0.001
		5: Rhomboid	0.083
Seed coat color	Recorded visually of a sample of ten seeds per plant	1: White	0.324
		2: Cream	0.361
		3: Brown	0.202
		4: Red	0.000
		5: Purple	0.000
		6: Black	0.005
		99: Other	0.108

Table 1 continued

Trait	Remarks	Classes	Frequencies
Eye color	Recorded visually of a sample of ten seeds per plant	0: Eye absent	0.219
		1: Brown splash or gray	0.095
		2: Tan brown	0.409
		3: Red	0.000
		4: Green	0.000
		5: Blue to black	0.251
		6: Blue to black spots or mottle	0.000
		7: Speckled	0.011
		8: Mottled	0.000
		9: Mottled and speckled	0.000
		99: Other	0.015
Testa texture	Recorded visually of a sample of ten seeds per plant	1: Smooth	0.249
		3: Smooth to rough	0.247
		5: Rough	0.381
		7: Rough to wrinkled	0.123
		9: Wrinkled	0.000
Splitting of testa	Recorded visually of a sample of ten seeds per plant	0: Absence	0.700
		1: Presence	0.300

all populations ($\bar{H}s$) and inter-population (Gst) were calculated. Mean phenotypic diversity within each population across all traits ($\bar{H}p$) was also calculated (Terzopoulos and Bebeli 2010). Comparisons among all populations' $\bar{H}p$ were conducted using Tukey's mean comparison method (Kuehl 2000) with the statistical software JMP-8 (SAS Institute Inc. 2008). To identify the traits with the highest value of phenotypic diversity within populations, a Monte Carlo sampling (Weir 1990) was carried out by creating 100 samples of 40 randomly chosen plants for each landrace with the resultant samples being used as replications for the Tukey's mean comparison method.

Principal Component Analysis (PCA) was performed using all characteristics in order to examine the contribution of each trait to the total diversity and classify the local populations using statistical programs JMP-8 (SAS Institute Inc. 2008) and NTSYS-pc (Rohlf 1998). Furthermore, a Mantel test was performed, based on Manhattan distance coefficient, to define possible correlation between agro-morphological data and geographical Euclidean distances, using NTSYS-pc software (Rohlf 1998).

Mean values of all quantitative traits were calculated and compared using Tukey–Kramer comparison method. Coefficients of variation (CV) across all populations and for each population separately were also calculated. Analysis of variance (ANOVA), followed by Duncan's multiple range ($p < 0.05$), was performed in order to analyze populations' seed crude protein content and mineral elements through STATISTICA 8.0 for Windows software (Copyright © StatSoft, Inc. 1984–2007). Moreover, Pearson Correlation Coefficients were used to investigate possible correlations between traits related to yield and seed mineral and protein content, using SPSS 20 of IBM (IBM SPSS Statistics 20.0, 2011).

Results

Characterization of the entire collection

Vegetative traits

The collection of cowpea populations was characterized by low plant vigor (78.6 %), one node on main stem (71.4 %) and small number of branches (not more than two branches, ≤2, 59.5 %), intermediate green leaf color (95.2 %), sub-hastate terminal leaflet shape (45.3 %), absence of twinning tendency (73.7 %), semi-erect (45.2 %) to erect growth habit (37 %), indeterminate growth pattern (100 %), low plant height (10–50.5 cm, 70 %), absence of plant pigmentation in stem (78 %), presence of plant pigmentation in branches (54.4 %) and limited plant pigmentation in the base and the tips of petioles (74.5 %) (Table 1).

Reproductive traits

Most populations were characterized as medium early according to days to first flower (56–77 days, 77.5 %) and days to first mature pod (77–94 days, 63.3 %), while their flowering duration was characterized as medium (52–71 days, 76.5 %). White was the main flower color (65.8 %) with presence of flower pigment pattern (52.2 %). Most of the immature pods were pigmented (51.3 %) presenting medium green color (56.9 %), while the height to first pod was characterized as moderate, with most of plants having deployed their first pod at a height of 17.9–30.7 cm (47.2 %), presenting pale tan/straw mature pod color (61.5 %) (Table 1).

Traits related to yield

Most populations were characterized by small number of pods per plant (≤19, 73.7 %), medium pod length (8.22–13.43 cm, 64.9 %), medium number of seeds per pod (5–8, 53.3 %) and small number of seeds per plant (≤120, 83.6 %). Seed weight per plant was characterized as small (≤17.3 g, 64.2 %), while the hundred seed weight was characterized as medium (12.3–18.5 g, 50.3 %) (Table 1).

Seed traits

Seeds were mainly recorded as kidney-shaped (82.6 %), while 32.4 and 36.1 % of the plants were characterized by white and cream seed coat color, respectively, while 40.9 % by tan brown eye color. Testa texture fluctuated particularly, with 24.9 and 38.1 % of seeds being characterized as smooth and rough, respectively, while most of seeds characterized by absence of splitting of testa (70 %) (Table 1).

Phenotypic diversity of cowpea populations

Total phenotypic diversity for each trait (Ht) varied between 0.00 and 0.72 with an average of 0.48. Seed coat color, testa texture, and eye color showed the highest Ht values with 0.72, 0.72 and 0.71 respectively, while some traits such as leaf color and growth pattern had extremely low values (0.09 and 0.00, respectively) and therefore they did not contribute to phenotypic diversity of the collection (Table 2).

Intra-population diversity ($\bar{H}s$) varied between 0.00 and 0.58, with an average of 0.34. Leaf color, flower color, flower pigment pattern and seed shape had $\bar{H}s \leq 0.19$, while growth habit, height to first pod, number of seeds per pod, seed weight per plant and hundred seed weight had $\bar{H}s$ values ≥0.48. Most of traits exhibited a wide range of $\bar{H}s$ values across populations, while traits that contributed most to within populations' heterogeneity were quite variable between the studied populations (Table 2). Seed traits studied, revealed that some of the populations, expressed uniformity (VG2, Atsiki, Lemnos). However some other populations appeared to be cultivated by farmers as mixtures, like VG13 (Amonaklios, Andros) and VG18 (Mirsini, Tinos) that show a relatively large number of seed morphotypes, six and four different types, respectively (Fig. 2).

Phenotypic diversity among populations (Gst) ranged between 0.00 and 0.81. Flower color, flower pigment pattern and eye color exhibited the biggest Gst values, while low Gst values were recorded for number of nodes on main stem (0.08), number of branches (0.08), leaf color (0.08), number of seeds per plant (0.09) and seed weight per plant (0.05) (Table 2).

Mean phenotypic diversity within each population across all traits ($\bar{H}p$) ranged between 0.25 and 0.48 with an average of 0.34 (Online Resource 2). The populations with the highest $\bar{H}p$ values (>0.40) were VG23 (Logothetianika, Kythira), VG21 (Pachykalamos, Arta), and VG13 (Amonaklios, Andros), while VG9 (Exo Vouni, Andros), VG2 (Atsiki, Lemnos) and VG16 (Karpasi, Lemnos), showed the lowest $\bar{H}p$ values (0.26, 0.25 and 0.25, respectively). Tukey's mean comparison method showed that the populations did not significantly differ with the exception of VG2, VG9, and VG16 with VG23 (0.48) (HSD = 0.16) (Online Resource 2).

Table 2 Total phenotypic diversity (*Ht*), mean intra-population diversity ($\bar{H}s$, *Hs* range in each trait in parenthesis) and among populations diversity (*Gst*) recorded in the collection

Trait	*Ht*	$\bar{H}s$	*Gst*
Plant vigor	0.34	0.29 (0.04–0.50)	0.14
Number of nodes on main stem	0.44	0.41 (0.19–0.64)	0.08
Number of main branches	0.52	0.48 (0.19–0.65)	0.08
Leaf color	0.09	0.08 (0.00–0.35)	0.08
Terminal leaflet shape	0.59	0.46 (0.04–0.66)	0.22
Twinning tendency	0.43	0.34 (0.00–0.72)	0.22
Growth habit	0.65	0.51 (0.12–0.77)	0.21
Growth pattern	0.00	0.00	0.00
Plant height (cm)	0.47	0.40 (0.00–0.70)	0.15
Plant pigmentation/stem	0.38	0.32 (0.00–0.76)	0.18
Plant pigmentation/branches	0.60	0.43 (0.00–0.71)	0.28
Plant pigmentation/petioles	0.41	0.20 (0.00–0.58)	0.52
Days to first flower	0.37	0.32 (0.08–0.57)	0.14
Days to first mature pod	0.53	0.45 (0.19–0.64)	0.16
Flowering duration	0.39	0.32 (0.04–0.62)	0.16
Flower color	0.50	0.15 (0.00–0.48)	0.70
Flower pigment pattern	0.64	0.12 (0.00–0.61)	0.81
Immature pod color	0.49	0.28 (0.00–0.49)	0.43
Immature pod pigmentation	0.63	0.26 (0.00–0.72)	0.58
Height to first pod (cm)	0.64	0.58 (0.43–0.67)	0.09
Mature pod color	0.54	0.48 (0.08–0.73)	0.12
Number of pods per plant	0.39	0.36 (0.10–0.59)	0.08
Pod length (cm)	0.51	0.43 (0.08–0.59)	0.15
Number of seeds per pod	0.62	0.55 (0.42–0.64)	0.11
Number of seeds per plant	0.29	0.26 (0.00–0.60)	0.09
Seed weight per plant (g)	0.51	0.48 (0.31–0.65)	0.05
Hundred seed weight (g)	0.65	0.50 (0.28–0.61)	0.23
Seed shape	0.30	0.19 (0.00–0.50)	0.36
Seed coat color	0.72	0.32 (0.00–0.67)	0.56
Eye color	0.71	0.27 (0.00–0.64)	0.62
Testa texture	0.72	0.32 (0.00–0.62)	0.55
Splitting of testa	0.42	0.22 (0.00–0.50)	0.48
Mean	0.48	0.34	0.27

Populations' classification

The first three axes of PCA, including all studied characteristics, explained 45.28 % of the total variation (Fig. 3). Seed traits as well as flower color were related to the first principal component (PC1, 19.08 %), while characteristics related to yield were correlated with the second principal component (PC2, 13.92 %). The third principal component (PC3, 12.28 %) was related mainly to seed morphology and number of pods per plant (data demonstrating relation with PCA components are not shown).

PCA showed that all studied populations, except population VG2 from Lemnos, could be classified into two main groups, one made of populations with brown seed coat color, without splitting of testa (group A) and one made of local populations with cream/white seed coat color and brown or black eye color, with splitting of testa (group B) (Fig. 3). Six subgroups were also formed, three in each main group, regardless of the

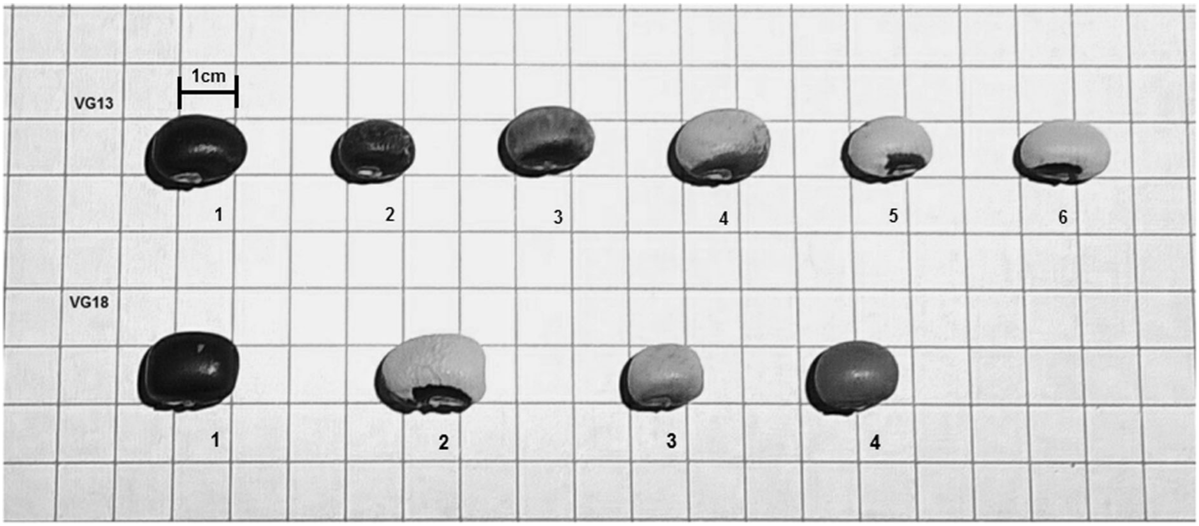

Fig. 2 Seed morphotypes observed in studied populations; (*a*) VG13 (Amonaklios, Andros): *1* eye absent, black testa; *2* black eye, dark grey-speckled testa; *3* black eye, light grey-speckled testa; *4* brown eye, cream/brown-speckled testa; *5* tan brown eye, white testa; *6* black eye, white testa, and (*b*) VG18 (Mirsini, Tinos): *1* eye absent, black testa; *2* black eye, white testa; *3* tan brown eye, cream/brown speckled testa; *4* eye absent, brown testa

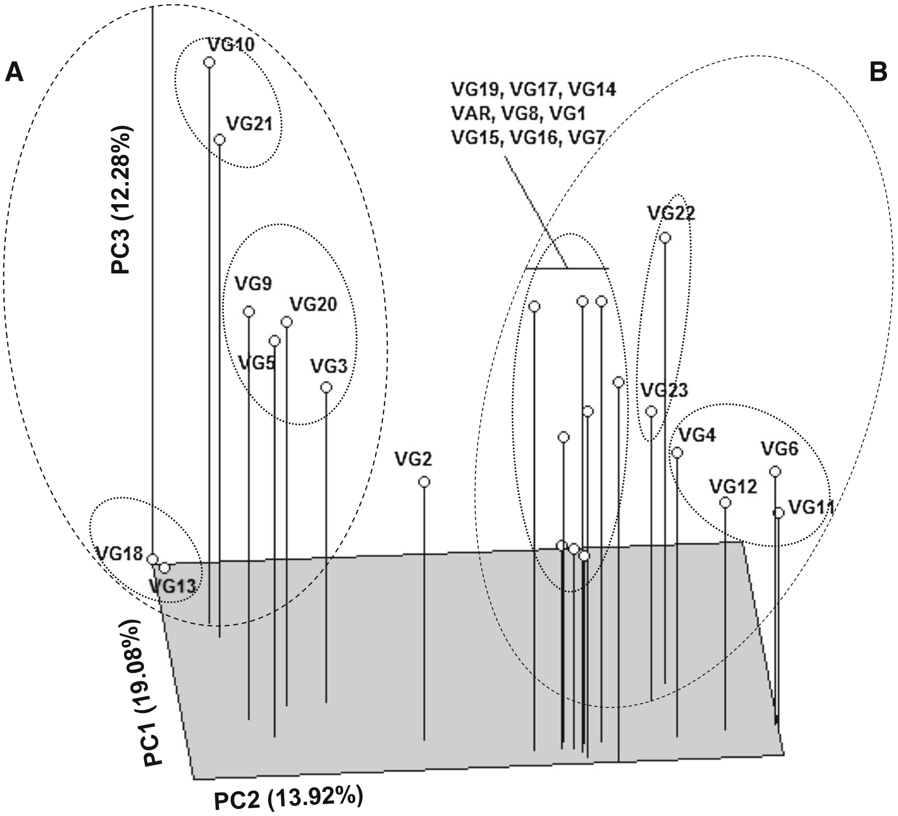

Fig. 3 Principal component analysis (PCA) of cowpea populations based on studied morphological and agronomical characteristics. The two resulted groups and the six subgroups formed are indicated in separate circles

populations' geographical origin. The Mantel test suggested that no significant correlation between morphological data and geographical origin was obtained (r = −0.21, $p > 0.05$). One large subgroup consisting of nine populations was formed within group A, while a small subgroup formed by four populations, VG4, VG6, VG11, and VG12, differentiated from the previous one due to populations' hundred seed weight (Fig. 3). Another subgroup consisting of two populations, VG22 and VG23, was formed mainly according to differences observed in eye color and number of seeds per pod. Two small subgroups including two populations each, VG13, VG18 and VG10, VG21, were formed within group B, and differentiated according to their testa texture and eye color. Another distinct subgroup was formed within this group consisting of four populations VG3, VG5, VG9, and VG20 discriminated from the other two subgroups due to eye color, growth habit and seed coat color.

Variability of characteristics related to yield

Mean values of all quantitative traits were calculated for all populations examined, followed by a comparison using Tukey–Kramer's method. Coefficients of variation (CV) across all populations and for each population separately were also calculated (Table 3). VG9 and VG20 presented the longest periods for days to first flower and mature pod, while VG14 and VG17 proved to be the earliest ones. VG19 presented the greatest height to first pod (42 cm) among populations, while VG18 produced the highest number of pods per plant with an average of 21.57. VG19 and VG20 presented the longest pods (14.18 cm) and produced the highest number of seeds per plant (196.88), respectively. Seed weight per plant fluctuated among populations with VG19 showing the highest value (23.06 g) and VG11 the lowest one (10.6 g). Hundred seed weight ranged from 12.27 to 24.21 g. Different CV values were calculated for different traits, with the highest values recorded for number of branches, total seed weight and number of seeds per plant, and days to first mature pod, days to first flower, flowering duration with the lowest ones (Table 3).

Variability of seed mineral and protein content

Significant differences in seed crude protein content were observed ($p < 0.01$) among the tested populations, with an average of 24.37 % (Table 4). Population VG20 (Mitilinioi, Samos) exhibited the highest protein content (28.37 %) and VG5 the lowest (22.14 %). With respect to mineral nutrients, significant differences were observed among populations studied only for K ($p < 0.05$) and Ca ($p < 0.01$) (Table 4). VG7 showed the highest (7.83 mg g^{-1}) and VG12 the lowest (4.67 mg g^{-1}) content of K among the studied populations. Ca concentration ranged from 0.72 mg g^{-1} (VAR, VG17) to 1.10 mg g^{-1} (VG9) with an average of 0.91 mg g^{-1}.

Investigation of correlations among yield traits and seed mineral and protein content

No significant correlations were observed among traits related to seed yield, and nutrient and protein content (Online Resource 3). Plant height was negatively correlated with days to first flower (r = −0.58, $p < 0.01$), flowering duration (r = −0.54, $p < 0.01$), days to first mature pod (r = −0.61, $p < 0.01$), Ca (r = −0.73, $p < 0.01$) and Mg (r = −0.52, $p < 0.01$) concentrations, while was positively correlated with height to first pod, pod length, number of seeds per plant and seed weight per plant ($p < 0.01$). Days to first flower were positively correlated with flowering duration (r = 0.97, $p < 0.01$), days to first mature pod (r = 0.93, $p < 0.01$) and Ca seed concentration (r = 0.69, $p < 0.01$) with a negative correlation to height to first pod (r = −0.59, $p < 0.01$) and pod length (r = −0.47, $p < 0.05$) (Online Resource 3). Height to first pod was positively correlated with number of pods per plant (r = 0.42, $p < 0.05$), pod length (r = 0.53, $p < 0.01$), number of seeds per pod (r = 0.63, $p < 0.01$), number of seeds per plant (r = 0.58, $p < 0.01$) and seed weight per plant (r = 0.54, $p < 0.01$), while the correlation with Ca seed content was negative (r = −0.63, $p < 0.01$). The number of pods per plant was significantly correlated with number of seeds per plant (r = 0.80, $p < 0.01$) and seed weight per plant (r = 0.81, $p < 0.01$). Pod length was also associated with number of seeds per pod (r = 0.50, $p < 0.05$), number of seeds per plant (r = 0.42, $p < 0.05$), seed weight per plant (r = 0.47, $p < 0.05$), including seed concentrations of Ca (r = −0.72, $p < 0.01$) and Mg (r = −0.49, $p < 0.05$). Number of seeds per pod was also negatively correlated with Ca concentration (r = −0.56, $p < 0.01$) as well as with hundred seed weight

Table 3 Mean values and coefficients of variation (CV) for each quantitative characteristic within each population

Population		NN	NBR	PH	DFL	DMAT	DUFL	H1POD	NPOD	PODL	SPOD	SPL	SW	HSW
VG1	Mean	1.42a–d	2.10a–g	50.84c–e	77.79ij	56.33g–i	54.23fg	35.26bc	19.43ab	12.69a–c	7.34b–g	137.91a–e	18.72a–e	13.70ij
	CV%	45.7	74.8	66.9	10.6	12.6	12.7	28.0	47.7	15.8	26.5	62.7	71.2	16.2
VG2	Mean	1.60ab	2.92a–d	26.69gh	82.52d–i	60.46d–h	57.54d–g	26.27f	15.00b–e	9.47gh	5.68g–k	88.27d–i	12.23de	13.50ij
	CV%	44.1	63.6	40.4	9.5	12.1	11.9	28.7	54.1	21.2	36.7	69.2	71.0	19.0
VG3	Mean	1.77a	3.23ab	18.83h	88.24b–e	66.75bc	63.52bc	23.81f	11.35c–e	12.95a–c	6.47d–k	80.92e–i	12.31c–e	14.78g–i
	CV%	45.5	63.6	25.2	10.9	14.9	16.7	36.9	53.1	21.3	42.6	81.9	82.9	16.6
VG4	Mean	1.13d	1.75d–g	51.06b–e	81.52f–i	59.85d–i	58.10c–g	36.45ab	13.96b–e	11.00d–g	5.60g–k	75.08f–i	15.55a–e	20.33bc
	CV%	34.9	78.6	66.9	7.6	8.5	9.1	25.3	48.0	15.9	36.1	65.1	66.1	15.0
VG5	Mean	1.27b–d	1.63e–g	34.94e–h	80.77g–j	58.40f–i	56.77d–g	27.17ef	13.56b–e	9.41gh	6.59d–i	95.04c–i	12.28de	13.52ij
	CV%	42.1	85.7	64.1	8.8	11.3	11.6	25.9	57.5	21.0	38.1	80.7	74.5	15.6
VG6	Mean	1.25b–d	1.58e–g	38.43e–h	81.13f–i	59.56e–i	57.98c–g	25.16f	12.98c–e	11.81c–e	5.83g–k	80.06f–i	15.05a–e	18.00d–f
	CV%	35.0	87.2	58.6	8.8	11.4	11.3	34.1	55.2	22.4	39.2	81.5	85.1	19.6
VG7	Mean	1.33a–d	1.33fg	39.45e–h	84.25d–h	60.23d–i	58.90c–g	27.26ef	15.58a–e	10.63e–g	4.58k	67.94g–i	16.77a–e	23.79a
	CV%	41.9	88.0	63.1	9.7	11.9	12.2	27.1	53.0	18.2	31.3	69.2	79.3	15.4
VG8	Mean	1.29b–d	2.76a–e	37.22e–h	91.63bc	65.34c–e	62.52cd	27.74d–f	15.86a–e	9.62gh	5.74g–k	92.42d–i	15.37a–e	16.95e–g
	CV%	35.6	90.6	57.9	12.6	16.7	17.1	26.3	62.2	18.8	33.3	79.9	79.5	16.8
VG9	Mean	1.13cd	2.52a–f	26.19gh	102.67a	80.65a	78.13a	25.77f	14.60b–e	8.94h	5.53g–k	83.49d–i	11.37de	15.00g–i
	CV%	35.4	85.6	36.0	8.1	10.1	10.9	28.5	87.8	18.2	32.5	95.5	92.2	14.5
VG10	Mean	1.44a–d	3.19a–c	48.04c–f	88.38b–d	65.64cd	62.45cd	24.15f	14.76b–e	11.35c–f	6.74c–h	97.89c–i	12.15de	13.38ij
	CV%	51.6	63.6	52.7	10.8	15.3	16.6	33.0	65.7	21.4	40.9	89.8	82.7	19.6
VG11	Mean	1.15cd	2.06a–g	53.89b–e	84.19d–h	60.88d–h	58.81c–g	26.37ef	10.75e	11.63c–f	4.70jk	48.73i	10.60e	22.33ab
	CV%	36.0	77.7	65.3	8.6	8.1	8.6	37.0	56.8	18.3	46.9	80.0	82.3	15.2
VG12	Mean	1.60ab	1.71d–g	64.13a–d	82.00e–i	58.35f–i	56.65d–g	26.62ef	16.90a–e	12.55b–d	5.09h–k	82.08f–i	19.96a–d	24.21a
	CV%	49.4	88.7	71.3	11.2	13.1	13.5	37.3	68.1	21.2	37.3	85.9	83.1	14.8
VG13	Mean	1.40a–d	2.56a–f	63.9a–d	80.21g–j	59.38f–i	57.06d–g	35.96ab	17.45a–c	12.38b–d	8.38a–c	140.68a–d	19.57a–d	14.87g–i
	CV%	54.8	66.3	60.4	8.0	11.8	11.6	25.5	49.3	13.6	27.8	68.3	63.4	17.3
VG14	Mean	1.63ab	1.13a–f	44.96d–g	74.60j	54.50i	53.38g	33.91b–d	13.58b–e	12.66a–c	8.18b–d	106.04c–h	13.48b–e	12.79ij
	CV%	41.4	94.6	73.0	8.5	11.7	12.1	25.4	43.2	16.3	31.6	63.5	65.7	18.5
VG15	Mean	1.38a–d	2.79a–e	29.44f–h	84.50d–h	62.15c–f	59.35c–f	24.68f	12.67c–e	9.68gh	5.96f–k	72.07f–i	11.76de	16.20f–h
	CV%	44.1	66.1	41.8	9.9	13.6	13.5	28.9	45.6	21.5	37.6	64.3	69.5	17.4
VG16	Mean	1.19b–d	2.17a–g	35.3e–h	87.26c–f	63.59c–f	61.37c–e	28.81d–f	14.91b–e	10.07f–h	4.90i–k	66.46g–i	13.39b–e	20.09cd
	CV%	37.5	75.2	36.7	6.4	6.3	6.9	23.9	43.4	17.2	34.1	65.1	67.3	20.5

Table 3 continued

Population		NN	NBR	PH	DFL	DMAT	DUFL	H1POD	NPOD	PODL	SPOD	SPL	SW	HSW
VG17	Mean	1.30b–d	1.94c–g	53.22b–e	77.38ij	55.15hi	53.21g	32.6b–e	12.23c–e	12.34b–d	7.80b–e	88.53d–i	12.39de	14.60hi
	CV%	48.0	96.0	56.4	9.5	15.0	15.1	26.4	54.4	20.0	33.8	67.6	63.3	14.7
VG18	Mean	1.15cd	1.96b–g	52.72b–e	85.34c–g	61.96c–g	60.00c–f	27.24ef	21.57a	10.19e–h	7.83b–e	164.17d–i	21.11a–c	13.09ij
	CV%	36.0	59.5	53.3	9.5	8.9	9.2	27.3	52.2	14.7	27.1	67.0	65.8	14.2
VG19	Mean	1.15cd	2.77a–e	77.62a	77.81ij	58.25f–i	55.48fg	42a	19.40ab	14.18a	10.10a	183.71ab	23.06a	12.27j
	CV%	40.2	51.7	47.0	5.6	6.3	7.1	17.9	35.5	10.9	21.0	50.0	51.9	13.8
VG20	Mean	1.56a–c	3.31a	24.25gh	94.85b	72.40b	69.02b	26.52ef	13.94b–e	12.63a–d	6.61c–i	196.88a	15.41a–e	16.30e–h
	CV%	52.7	79.3	25.6	9.0	17.7	19.7	30.7	76.1	19.2	37.3	95.9	94.6	17.4
VG21	Mean	1.77a	2.17a–g	38.83e–h	82.24d–i	59.89d–i	57.68d–g	25.36f	15.04b–e	11.70c–f	7.94b–e	126.09b–f	15.91a–e	12.80ij
	CV%	46.9	107.9	69.9	17.8	21.2	21.5	31.2	65.1	24.3	36.4	87.2	78.0	21.7
VG22	Mean	1.27b–d	2.25a–g	52.28b–e	81.35f–i	59.27f–i	57.02d–g	29.26c–f	11.00de	12.57a–d	6.41e–j	66.35hi	12.56c–e	17.85ef
	CV%	42.1	56.1	66.0	9.5	9.5	9.5	27.1	58.3	17.4	40.8	89.8	95.9	22.2
VG23	Mean	1.29b–d	2.92a–d	71.99b–e	82.17d–i	58.94f–i	56.02e–g	29.21c–f	15.65a–e	12.55b–d	7.74b–f	122.31b–g	21.58ab	18.58c–e
	CV%	47.8	56.5	55.3	12.8	11.1	11.5	32.2	54.3	22.3	35.9	63.7	63.8	19.2
VAR	Mean	1.25b–d	1.81d–g	67.52a–c	78.29h–j	58.29f–i	56.48e–g	36.48ab	17.17a–d	13.76ab	8.96ab	150.08a–c	19.36a–d	13.90ij
	CV%	42.1	60.9	45.4	7.9	8.4	8.5	25.8	31.1	18.8	31.4	49.8	42.9	22.6
	Total CV%	42.95	75.74	54.13	9.65	11.98	12.43	28.77	54.90	18.75	34.84	73.90	73.83	17.41

Means in columns with different letters are significantly different at the 0.05 level, by Tukey's comparison method

NN number of nodes on main stem, *NBR* number of main branches, *PH* plant height, *DFL* days to 1st flower, *DMAT* days to 1st mature pod, *DUFL* flowering duration, *H1POD* height to 1st pod, *NPOD* number of pods per plant, *PODL* pod length, *SPOD* number of seeds per pod, *SPL* number of seeds per plant, *SW* seed weight per plant, *HSW* hundred seed weight

($r = -0.72, p < 0.01$). Number of seeds per plant was correlated with seed weight per plant ($r = 0.77$, $p < 0.01$) and hundred seed weight ($r = -0.62$, $p < 0.01$). Finally, seed K and Ca concentrations were correlated positively to Mg ($r = 0.41, p < 0.05$ and $r = 0.44, p < 0.05$ respectively), while seed crude protein content was positively correlated with number of branches ($r = 0.54$, $p < 0.01$) and days to first mature pod ($r = 0.41, p < 0.05$) (Online Resource 3).

Discussion

Characterization based on morphological and agronomical traits

Although cowpea has been reported to have a narrow genetic base (Asare et al. 2010), the characterization of twenty-three cowpea local populations revealed that a relatively high amount of phenotypic variation, observed in most studied traits, has been maintained through on-farm conservation. This variation is presumably a result of evolutionary processes that took place by natural and human selection pressure. In Greece, farmers have been traditionally maintaining seeds of landraces, at least for their own use and consumption, implementing various agricultural practices, where landraces are growing under different agro-climatic conditions. Several cowpea landraces are consumed as a vegetable salad and their cultivation in vegetable gardens has been consistent over the years.

Pigmentation in different plant parts is one of the most important morphological traits used by breeders to characterize cowpea varieties, because of their simple inheritance (Othman et al. 2006). Pigmentation on stem, branches and petioles that were recorded in our collection separately, indicated differences in intensity between different parts of cowpea plants, while not all plants presented pigmentation simultaneously in all studied parts (Table 1). This finding does not agree with previous statements that any cowpea plant pigmented on the nodal region of the main stem to be uniformly pigmented (Ishiyaku and Singh 2004). This discrepancy may be ascribed to phenotypic differences arising from the impact of light and temperature on accumulation of anthocyanins, which are responsible for the presence of color in cowpea plants (Biesiada and Tomzak 2012). Purple flower color has also been suggested to be dominant over white color (Othman et al. 2006) with evidence of linkage between flower color, immature pod pigmentation and seed coat color in cowpea (Egbadzor et al. 2012). In the collection of populations tested in this study, the main flower color was white (65.8 %). Differences observed in flower color among different cowpea collections (Negri et al. 2000; Cobbinah et al. 2011; Gbaguidi et al. 2015) points to diverse consumer preferences among countries with respect to pod color pigmentation, seed coat color, and eye color and pattern.

In many countries around the world fresh green cowpea pods are preferred to dry seed consumption (Timko and Singh 2008), where long, tender and fibreless pods, having green color are considered as market appealing characteristics (Ehlers et al. 2002; Peksen 2004; Pandey et al. 2006). The relatively frequent appearance of preferable characteristics, like the absence of immature pod pigmentation (48.7 %) or pods with only pigmented tips (35.1 %) renders the twenty-three local cowpea populations tested in the present study an interesting germplasm source for future breeding programs. A high variability in seed coat color and eye color was also observed, in accordance with Egbadzor et al. (2014). Many different combinations of cowpea seed coat color and eye color are available, with cream or white seeds to occur when all genes are recessive as proposed by Drabo et al. (1988). Different preferences for various cowpea seed characteristics can be found worldwide (Mashi 2006) and even within different cultivation areas of the same country (Fulton et al. 2007). These traits that have been under the conscious or unconscious selection of the consumers or/and the farmers, were found to correlate with seed nutritional properties, like anti-oxidant activity (Nzaramba et al. 2005), tannin content (Asante et al. 2004) and physical and cooking cowpea characteristics (Hamid et al. 2016). Taking all these preferences into consideration, seed coat color and eye color are considered to be the most important traits for dry seed consumption. The present collection included populations with preferable traits in accordance with the traditional cooking habits in Greece. These include cream/white seed coat color with black eye color when intended to be consumed as dry seed and absence of immature pod pigmentation when consumed as fresh pod.

Table 4 Mineral and seed crude protein content (CP (%)) of cowpea populations

Population	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	CP (%)
VG1	5.67 ± 0.44a–d	0.81 ± 0.02d–g	0.97 ± 0.01	39.53 ± 14.94	42.07 ± 7.16	42.33 ± 3.70	26.15 ± 0.16bc
VG2	6.50 ± 0.76a–d	1.08 ± 0.05a	0.98 ± 0.04	26.40 ± 2.62	40.33 ± 6.49	42.93 ± 0.85	25.40 ± 0.15b–d
VG3	7.67 ± 1.76ab	1.04 ± 0.04ab	1.04 ± 0.17	29.73 ± 4.76	44.20 ± 6.33	40.13 ± 0.93	25.97 ± 0.42bc
VG4	6.17 ± 0.17a–d	0.98 ± 0.01ac	1.04 ± 0.01	26.80 ± 3.07	40.07 ± 6.78	38.27 ± 0.71	22.69 ± 0.10gh
VG5	6.17 ± 0.44a–d	0.91 ± 0.01b–e	1.05 ± 0.06	21.80 ± 2.23	38.60 ± 6.77	33.73 ± 1.92	22.14 ± 0.25h
VG6	5.67 ± 0.60a–d	0.95 ± 0.08a–d	0.95 ± 0.05	36.53 ± 15.31	38.87 ± 8.62	36.13 ± 5.38	24.00 ± 0.36d–g
VG7	7.83 ± 0.33a	0.99 ± 0.03ac	0.12 ± 0.07	23.87 ± 3.37	37.33 ± 5.62	39.60 ± 1.51	25.07 ± 0.53c–e
VG8	5.50 ± 0.29bd	0.92 ± 0.02b–e	1.04 ± 0.10	17.07 ± 1.39	35.00 ± 7.66	33.27 ± 3.37	24.54 ± 0.24c–f
VG9	5.67 ± 0.33a–d	1.10 ± 0.10a	0.93 ± 0.06	26.07 ± 5.13	37.07 ± 7.72	38.27 ± 2.63	23.45 ± 0.14e–h
VG10	5.17 ± 0.60d	1.02 ± 0.03ab	0.87 ± 0.08	26.47 ± 0.52	37.33 ± 7.85	39.40 ± 2.91	27.02 ± 0.39ab
VG11	5.33 ± 0.33cd	0.91 ± 0.06c–g	0.95 ± 0.05	31.07 ± 3.14	35.93 ± 7.51	41.40 ± 1.75	22.59 ± 0.65gh
VG12	4.67 ± 0.60d	0.81 ± 0.08d–g	0.84 ± 0.05	27.47 ± 2.05	34.33 ± 8.14	37.00 ± 3.38	23.55 ± 0.57e–h
VG13	6.50 ± 0.58a–d	0.85 ± 0.04c–g	1.07 ± 0.09	29.60 ± 3.75	35.00 ± 7.40	42.93 ± 3.06	24.40 ± 0.96c–f
VG14	6.67 ± 0.44a–d	0.84 ± 0.05c–g	1.00 ± 0.01	30.47 ± 2.19	35.73 ± 7.80	43.87 ± 2.76	22.45 ± 0.16gh
VG15	5.67 ± 0.60a–d	1.04 ± 0.04ab	1.09 ± 0.09	30.80 ± 3.58	33.47 ± 7.40	43.73 ± 2.77	25.32 ± 0.60b–d
VG16	6.00 ± 0.87a–d	0.91 ± 0.02b–e	1.03 ± 0.06	30.13 ± 2.94	38.07 ± 9.17	70.27 ± 27.77	24.48 ± 0.87c–f
VG17	5.50 ± 0.76bd	0.72 ± 0.01g	0.93 ± 0.09	29.47 ± 2.56	33.93 ± 7.86	44.00 ± 4.01	23.06 ± 0.33f–h
VG18	6.67 ± 0.44a–d	1.02 ± 0.06ab	1.04 ± 0.02	22.00 ± 2.39	31.60 ± 7.89	35.40 ± 3.82	22.66 ± 0.15gh
VG19	7.50 ± 0.29ac	0.76 ± 0.01fg	0.80 ± 0.26	29.33 ± 0.66	32.40 ± 8.49	42.00 ± 1.45	24.65 ± 0.47c–f
VG20	7.67 ± 1.17ab	0.92 ± 0.07b–e	1.04 ± 0.23	25.27 ± 3.20	33.20 ± 9.34	36.33 ± 7.25	28.37 ± 0.60a
VG21	6.67 ± 0.33a–d	0.93 ± 0.03b–e	0.98 ± 0.02	26.87 ± 1.97	30.07 ± 8.26	37.87 ± 3.04	25.68 ± 0.95b–d
VG22	5.33 ± 0.17cd	0.77 ± 0.03eg	0.85 ± 0.02	28.67 ± 2.45	29.20 ± 7.62	31.60 ± 6.07	25.45 ± 0.46b–d
VG23	6.50 ± 0.29a–d	0.78 ± 0.02eg	1.02 ± 0.04	21.67 ± 1.16	28.20 ± 7.70	33.13 ± 5.44	23.25 ± 0.82f–h
VAR	5.00 ± 0.29d	0.72 ± 0.02g	0.95 ± 0.05	23.93 ± 0.94	36.73 ± 6.72	38.60 ± 3.47	26.13 ± 0.50bc
Mean	6.15	0.91	0.98	27.54	35.78	40.09	24.37
	*	**	NS	NS	NS	NS	**

Values are given as mean ± SE of three replicates, means in columns with different letters are significantly different at $p < 0.05$ by Duncan's multiple range test

NS non significant at 0.05 or 0.01 level

* Significant at the 0.05 level

** Significant at the 0.01 level

Diversity in cowpea populations

In the present study, a relatively high average of total variation was observed ($Ht = 0.48$). Data indicated that there is a notable diversity in seed morphology, with the exception of seed shape ($Ht = 0.30$), the other traits, namely seed coat color, eye color, testa texture and splitting of testa, exhibited Ht values $\geq$0.42. High variability in seed color pattern, among and within thirteen cowpea landraces, was also observed by Negri et al. (2000), who reported nine and seven different combinations, respectively. Furthermore, a high level of within population diversity was present ($\bar{H}s = 0.34$) which exceeded among populations' diversity ($Gst = 0.27$), although *Vigna unguiculata* (L.) Walp. is a predominantly self-pollinated species. These results are in contrast to those reported by Ghalmi et al. (2010), who observed no intra-landrace variation for qualitative and low inter-landrace diversity for quantitative traits in Algerian cowpea landraces collected on-farm. This differentiation can be ascribed to on-farm collected populations used in this study that are originating mainly from Greek islands that constitute physically isolated barriers or isolated mountainous areas. A second reason for this discrepancy may be the different number of plants, about fifty individuals per population, analyzed in the present study in order to estimate phenotypic diversity within each population. This number was considered sufficient for this type of analysis (Brown and Marshall 1995). Disputes observed in levels of diversity regarding morphological traits and yield of cowpea plants among studies could be also attributed to different physical and chemical soil properties of experimental fields (Ndema et al. 2010), like soil alkalinity (pH 8.1) observed in this study, that can develop Fe, Zn and Mn deficiencies, resulting in stunted plant growth and reduction of yield (Goenaga et al. 2010). Rhizosphere microbes are considered also as a substantial factor, as many of them are able to suppress some soil-borne plant pathogens, induce plant growth and enhance the bioavailability of mineral nutrients (Valencia-Cantero et al. 2007). Different root microbes grown in the same soil can be found among and even within a plant host species (Berendsen et al. 2012).

Gst mean value (0.27) estimated for the collection tested in the present study was equivalent to *Gst* values estimated for other mainly self-pollinating species (Nybom 2004). The *Gst* average calculated in the present study was higher than the values reported in other studies which addressed the genetic diversity of cowpea using AFLPs (Coulibaly et al. 2002; Polegri and Negri 2010). Nwofia et al. (2012) also observed a higher phenotypic coefficient of variation than the corresponding genotypic coefficient of variation for traits associated with cowpea dry seed yield. This divergence of morphological and genetic variation demonstrates the great impact of environment and genotype x environment interactions that affect quantitative traits of economic importance and display phenotypic variation (Kumar and Ali 2006), as well as the possibility of the number of molecular markers used in the analysis not being sufficient to assess genetic diversity. Epigenetic effects, which do not involve alterations to the nucleotide sequence, could also contribute to the different levels observed among morphological and genetic diversity. Epigenetic changes have occurred frequently in plants causing variation within species (Fujimoto et al. 2012), affecting their structure, phenotypic traits (Cubas et al. 1999; Manning et al. 2006; Johannes et al. 2009; Miura et al. 2009) and phenotypic plasticity (Zhang et al. 2013; Pikaard and Scheid 2014) and therefore increase the phenotypic diversity observed, suggesting that there may become a valuable resource in plant breeding (Richards 2011).

The mean $\bar{H}p$ value (0.34) did not differ significantly among populations with the exception of VG23 (0.48) that presented the highest value and three populations namely VG9 (0.26), VG2 (0.25), and VG16 (0.25) that showed low values. This indicates that VG23 includes a remarkable variability in contrast to VG9, VG2 and VG16, that characterized by a uniformity in expression of many characteristics. A landrace can therefore be characterized by various levels of variability which can be estimated by measuring different traits. Some of the traits can express high variability within a landrace and therefore cannot be used as discrimination characters. However several traits of a landrace present uniformity within it and can be used for its identification.

In the case that a landrace is characterized by high level of within variability for some traits then cannot comply with uniformity levels (10 %) required for registration according to the legislation enforced by European Commission Directives 2008/62/EC and 2009/145/EC. Therefore the European Directives should be revised and changed accordingly since landraces are by definition genetically rich heterogeneous, poly-genotypic populations that enclose valuable genetic diversity (Newton et al. 2010; Terzopoulos and Bebeli 2010) that should be conserved. Thus landraces should be treated differently than improved varieties (mono-genotypic) when their registration is needed.

Height to first pod rendered the highest $\bar{H}s$ value (0.58), pointing to a need for selection for this trait which is of high importance for mechanical harvesting (Basaran et al. 2011). Traits with the lowest phenotypic diversity within individual populations mainly referred to reproductive phase. These included pod and seed morphology traits and flower color. Furthermore, growth pattern did not contribute to the diversity of the collection as all plants were characterized by indeterminate growth pattern. These low phenotypic diversity values could be due to the monogenic inheritance with complete dominance reported for traits such as growth pattern (Ribeiro et al. 2014) and flower color (Padi 2003), given that cowpea is a primarily self-pollinated plant. A strong selection pressure by farmers on pod and seed traits of cowpea, in order to suit consumers' preferences and therefore enhance local market development, becomes evident from these findings. Although a high uniformity of seed traits characterized many of the populations studied, some of them showed extreme phenotypic differences in seed coat and eye color, which indicates that they are cultivated as mixtures of populations by the same farmer.

Populations' classification

The first three axes of PCA explained only 45.28 % of the total variation, possibly due to low levels of inter-population and high levels of intra-population diversity of the collection (Terzopoulos and Bebeli 2010). Similar proportions of phenotypic variation have been reported for cowpea by Aremu et al. (2007), who found that the total variation accounted for the first three axes was 40.78 %, as well as for other *Vigna* species. For instance, Olukolu et al. (2012) explained 39.29 % of total phenotypic variability in *Vigna subterranea* (L.) Verdc. accessions.

PCA classified the majority of populations into two main groups, according to populations' seed coat color and eye color, while six subgroups were formed, not consistent with the populations' geographical origin. Indeed, only two of the three populations originating from Messinia (VG11, VG12) were grouped together, while the two populations originating from Arta (VG19, VG21) were not classified in the same group (Fig. 3), presumably because they were collected from areas with different altitudes and cultivated following different agricultural practices to which they are adapted. Regarding the three populations originating from Lemnos VG2, VG16, VG17 and taking also into account stable morphological traits, such as flower color and seed coat color, these populations seemed to act as subpopulations of a single landrace, although VG2 was not grouped together with the other two VG16 and VG17. Furthermore, only two pairs VG5, VG9 and VG14, VG15 of the six studied populations from Andros were classified to the same groups, presumably because of the rich geographical diversity of the island, which is characterized by many capes, mountainous sections, hills, valleys, ravines and streams. These results are in agreement with previous studies suggesting that a large crop genetic diversity is conserved on-farm in Greek islands (Laghetti et al. 2008; Thomas et al. 2012, 2013; Douma et al. 2016) indicating once again the delicate and highly variable ecosystems of these islands due to their physical isolation (Cronk 1997). The Mantel test performed, assured the existence of no correlation between agro-morphological data and geographical Euclidean distances ($r = -0.21$, $p > 0.05$). The lack of grouping populations according to their geographical distribution presumably reveals seed exchanges by farmers. Therefore, further investigation on the structure of these populations should be conducted, in order to design appropriate conservation approaches, as multiple processes seem to take place at each location.

Variability of characteristics related to yield

Coefficients of variation (CV) calculated for quantitative characteristics received medium (10–20) to high (>20) values almost for all studied characteristics. High CV values for Greek landraces have been previously reported by Perrino et al. (1993) for days to first flower (22.1), days to first mature pod (20.2), pod length (23) and hundred seed weight (26.8) who assessed diversity among Mediterranean cowpea landraces. Moreover, in the present study, higher values for many characteristics related to yield were reported when compared to breeding lines (Manggoel et al. 2012; Ajayi et al. 2014). The lowest CV values of number of pods, seeds and seed weight per plant, which amounted to 31.1, 49.8 and 42.9 respectively, were calculated for the commercial variety (VAR). In contrast, all local populations studied showed higher CV values, revealing the lack of uniformity in local populations when compared to modern, commercial varieties, which are characterized by a high uniformity (Gepts 2002).

Further investigation of variability of traits related to seed yield provided evidence that populations producing high number of pods per plant, such as VG18, VG19 and VG1, high number of seeds per plant, such as VG20, VG14 and VG13, and high seed weight per plant like VG19, VG23 and VG18, could be used in evaluation and selection programs with the aim to improve seed yield. Locally adapted populations, particularly VG14 and VG17, which exhibited the shortest days to first flower and first mature pod, could potentially be a good source for drought tolerance (Belko et al. 2014).

Variability of seed mineral and protein content

Cowpea plays an important role in human nutrition because of its high content in vitamins, minerals and especially proteins (Li et al. 2001). Due to its high protein content and its affordable cost, cowpea is commonly referred to as poor man's meat in sub-Saharan area (Boukar et al. 2015) as well as "gyfto-fasoula" (gypsy beans) in Greece (Kavvadas 2015). In this study, the seed protein content of twenty-three local populations, showed a high variability among populations (Omoigui et al. 2006; Nwosu et al. 2013). In particular, the amount of seed crude protein content, ranged from 22.14 to 28.37 %, similar to the values reported by Carvalho et al. (2012). These values are also consistent with those found in ten cowpea cultivars (23.18–26.78 %) by Animasaun et al. (2015). Although none of the studied populations exceeded the threshold of 30 %, which could characterize them as "high protein content populations" according to Afiukwa et al. (2013), VG20, VG1 and VG3, exceeded the 25 % level of protein content that is found in most of the cowpea varieties (Boukar et al. 2011). After further evaluation under different environmental conditions these populations could presumably contribute to the breeding of improved cowpea cultivars with good agronomic performance in combination with higher protein content (Lambot 2002; Santos et al. 2012), reaching even 34 %.

In the present study, significant differences were found only for K and Ca, among macronutrients. K concentrations were lower than those previously reported by Carvalho et al. (2012), while Ca concentrations were either equal or higher than those reported in previous studies (Belane and Dakora 2011; Carvalho et al. 2012; Madodé et al. 2011). The intrinsically high calcium carbonate content and alkalinity or high limestone content of most soils as well as the intensive application of herbicides (Hansen et al. 2004), intensifies micronutrient deficiencies, especially those of Fe and Zn, enhancing malnutrition. Non-significant differences were observed for Fe, Mn and Zn among the tested local populations, while their values ranged from 17.07–39.53, 28.20–44.20 and 31.60–70.27 mg kg^{-1}, respectively. Fe concentrations were lower than those reported in previous studies, while Zn and Mn were similar to them (Carvalho et al. 2012; Madodé et al. 2011). The low concentrations of these micronutrients could be attributed to the high soil alkalinity of the experimental field, as bioavailability of nutrients from soil to crop is influenced by many soil factors, such as pH and salinity (Shrivastava and Kumar 2015). Furthermore, different nutrient and seed protein contents of cowpea reported in previous studies disclosed the great impact of genotype, environment and their interactions on them (Ddamulira et al. 2015).

Correlations among yield and seed mineral and protein content

Correlations between K and Mg, as well as Ca and Mg displayed significantly positive values, indicating that it will be feasible to develop cowpea populations with increased concentrations of all these three nutrients. On the other hand, a negative correlation between Ca seed concentration and many agronomical traits like pod length, number of seeds per pod and seed weight per plant was found. A low-magnitude negative correlation was also reported by Ribeiro et al. (2013) in common bean segregating populations indicating that selection in advanced generations for higher Ca concentration would probably be the best strategy in a breeding program for cowpea. Although most Pearson correlation coefficients between nutrients or protein content and yield were not significant in this present study, many of them exhibited negative values, indicating restrictions in breeding alongside for high-yielding and high-protein genotypes. Considering that cowpea seed protein content is the major reason why cowpea is popularly consumed in many countries of the world, breeders should screen segregating generations in order to identify and breed for the desirable recombinant types which combine high seed yield and protein content (Som and Hazra 1993).

Conclusions

The characterization of twenty-three cowpea landraces, conserved on-farm revealed different population structures. A large number of descriptors including vegetative, reproductive, traits related to yield and seed morphology was used. The within population diversity exceeded that among populations' diversity, while most landraces had similar magnitude of phenotypic diversity. High variability within populations and lack of uniformity in some measured traits indicated the difficulties of application of the current legislation for their registration which will encourage their cultivation and conservation. PCA grouped all populations studied in two main groups, except population VG2 from Lemnos that was one of the populations that was characterized by low $\bar{H}p$ value. Additionally, some of the populations exhibited desirable traits related to yield (VG18, VG19, VG20), high protein content (VG10, VG20) and mineral concentrations (VG9, VG2, VG3) could be further used in evaluation and selection programs, aiming to contribute to biofortification and breeding of cowpea.

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