



Exploring grass pea (*Lathyrus sativus* L.) genetic diversity in Mediterranean changing climate conditions

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ABSTRACT

Grass pea (*Lathyrus sativus* L.) is an annual cool-season grain legume, recognized as a stress-resilient crop, able to thrive in unfavourable growing conditions. It is widely cultivated in different world regions, with two main ecotypes based on seed traits. Particularly, in the Mediterranean region, climate change has amplified environmental instability, creating a fundamental need for producing more resilient or adaptable plant varieties. With the present study, we aimed to identify, among grass pea germplasm, new sources of interesting agronomic traits that can be used in breeding programs for adaptation to climate change. Hence, we trialled a worldwide grass pea collection of 182 accessions in Alvaiázere, Portugal, a Mediterranean climate region, across four growing seasons. We characterized the accessions for plant growth-, inflorescence-, seed-, and yield-related traits. By applying linear mixed models analysis, we assessed the diversity among accessions, as well as the genotype, the environment, and the genotype-by-environment effects. A principal component analysis was performed to summarize multivariate similarities among grass pea accessions and/or growing seasons. Additionally, we estimated the main ecotype clustering parameters, seed coat colour, seed size, and geographical origin, effects on the measured traits. Great diversity among accessions for all traits was observed. For most traits, the effect of the growing season was the most prominent, although significant genotype and genotype-by-environment interaction effects were also observed. Yield was higher on large and light seed accessions, with no significant yield differences detected across geographical origins. Nevertheless, South Asian accessions showed the tallest plants at the first flower and the highest number of seeds per plant (although with the smallest seed sizes), two of the most important yield components in the study. The Sub-Saharan African accessions showed the shortest growth cycles and mainly dark coat colours, trait associated with a reduced infestation by *Bruchus* weevils. The last two traits are of great interest as breeding targets. The Sub-Saharan accessions may thus be of significant value as potential donor material in breeding programs.

1. Introduction

Climate change will affect agriculture differently, depending on the region and crop (Anderson et al., 2020). In the Mediterranean region the most significant effects are predicted to be due to variable rainfall, reduced water availability, rising temperatures, frequent occurrence of extreme events, and the spread of pests and disease outbreaks (Arora, 2019; Langridge et al., 2021). The subsequent increasing climatic instability will pose new production challenges in this region where cool-season rainfed legumes are traditionally grown. As a result, two primary scenarios may unfold: rainfed cool-season legume production

shift to northern locations, characterized by lower temperatures and increased water availability. Alternatively, and to continue cultivation in the Mediterranean region, the development of new climate resilient varieties will be imperative (Gonçalves et al., 2022; Hillocks and Maruthi, 2012; Milczak et al., 2001; Vaz Patto et al., 2006).

Lathyrus sativus L. (diploid, $2n = 14$), commonly known as grass pea, is an annual cool season legume crop, originated from southwest and central Asia, and subsequently spreading into the eastern Mediterranean (Kislev, 1989). Its cultivation is widespread in the rainfed dry regions of the Indian Subcontinent, Sub-Saharan Africa, and in the Mediterranean region, where drought and waterlogging occur frequently (Kumar et al.,

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2013; Lambein et al., 2019). Worldwide, the grass pea cultivation area was recently estimated at 0.70 million ha, with 0.79 million ton production (Kumar et al., 2021). Although the major grass pea-producing countries are in Asia and Sub-Saharan Africa (India, Bangladesh, Ethiopia, Nepal), in the Mediterranean region farmers show a renewed interest in this crop production (Rubiales et al., 2020; Vaz Patto et al., 2006).

Grass pea is a model crop for sustainable agriculture (Vaz Patto et al., 2006). It is a resilient crop, tolerant to extreme temperatures, drought and flood, and considerably resistant to several pests and diseases, which allows its cultivation in different production systems and environments (Campbell et al., 1993; Gonçalves et al., 2022; Rath et al., 2021; Sarkar et al., 2019; Vaz Patto and Rubiales, 2014).

However, even in this hardish crop, the new production challenges caused by increased Mediterranean climate instability require a redirection of grass pea breeding. Ensuring the development of grass pea stress-resistant varieties that can withstand drought, flood, heat, and various diseases and pests, while simultaneously maintaining yield and quality stability is now paramount (Gonçalves et al., 2022).

In grain legumes like grass pea, breeding programs are scarce and hampered by insufficient diversity in specific emerging traits (Rubiales et al., 2021). However, great diversity can still be found within old varieties, traditional landraces, or wild populations, and so the characterization of these genetic resources is fundamental for facing climate change.

Until now, grass pea germplasm has been mainly characterized in limited regional contexts, and covering only its respective geographical origin growing conditions (Abate et al., 2018; Benková and Žáková, 2001; De la Rosa and Martín, 2001; Gixhari et al., 2016; Lioi et al., 2011; Mahapatra et al., 2020; Tadesse and Bekele, 2002; Tavoletti et al., 2005). Some more diverse grass pea germplasm collections have been studied under Mediterranean conditions (Arslan et al., 2022; Hanbury et al., 1999). However, there is still a notable gap concerning the knowledge of the full genetic potential of grass pea to face climate change under Mediterranean conditions. Neither is fully known, in this species, the potential effect of the Mediterranean environment on the expression of important climate change adaptive traits.

In order to address this lack of knowledge we characterised in detail an extensive grass pea germplasm collection, including accessions from diverse areas of the world.

2. Materials and methods

2.1. Plant material

A grass pea (*L. sativus*) collection of 182 accessions, composed of traditional landraces, old varieties and breeding lines, representative of the worldwide-cultivated diversity, was used in this study. Among these, 96 accessions had a Mediterranean origin (Portugal, Spain, France, Italy, Turkey, Montenegro, Cyprus, Morocco, Greece, Libya, Algeria, Tunisia, and Albania), 17 were from East Europe (Hungary, former Czechoslovakia, Poland, Bulgaria, and Serbia), 10 from North and Central Asia (Russia, Tajikistan, and Georgia), 38 from South Asia (India, Pakistan, Iran, Afghanistan, and Nepal), 15 from Sub-Saharan Africa (Ethiopia and Sudan), one had a North America (Canada) origin and 5 had an unknown geographical origin. Moreover, 99 accessions had light and 83 dark seed coat colours (based on a visual score), while 66 accessions had large (100 seeds weight > 18 g) and 116 small (100 seeds weight < 18 g) seed sizes (Sampaio et al., 2021).

Most of the accessions (167) were retrieved from different Germplasm Banks and the authors, in addition, collected 15 accessions from farmers within a traditional grass pea production region, Alvaizere, in Portugal. More details about the origin and morphological seed characteristics are shown in Supplementary files - Table S1.

2.2. Field trials

The grass pea collection was field evaluated, in participatory research, during four growing seasons: 2015–16, 2016–17, 2017–18, and 2018–19. The field trial was located in Alvaizere (Portugal), at 39°49′09.1″ N; 8°22′23.7″ W, 249 m above sea level. There the soil is classified as chromic cambisols, with a pH(H₂O) of 6.5, silty clay loam, and 1.9 % organic substance. Traditional grass pea growing season in this region comprises the period from December until June. Considering each year climatic particularities (Table S2), field trials sowing and harvesting dates were, 8th February and 3rd July 2016; 10th February and 2nd June 2017; 31st January and 10th July 2018 and 27th November 2018 and 18th July 2019.

2.2.1. Experimental design

In each year, grass pea accessions were evaluated using a randomized row-column alpha lattice design, with two plot replications. CropStat for Windows Version 7.2.2007.3 was used for field trial randomization. Each plot (0.90 m × 0.90 m) consisted of two plant rows with an inter-row distance of 0.50 m. Each row was hand sowed with 10 seeds, with an inter-seed distance of 0.10 m, and a total of 20 seeds per plot. The inter-plot distance was 0.5 m. Plots were hand-weeded when needed and managed following common low-input agricultural practices for grass pea in the region. During the driest 2017–18 and 2018–19 growing seasons, a supplementary irrigation was applied during five weeks including the flowering stage of the experiment to avoid plants to dry out. A sprinkler irrigation system was used, at a rate of one hour/week (corresponding to 10.5 mm/week).

2.2.2. Field trials meteorological characterization

Alvaizere is recognized as a representative Mediterranean environment (Joffre and Rambal, 2001), with a long-term (30 years) mean annual rainfall of 686 mm, mainly concentrated between October and April. To monitor the climatic conditions during field trials, meteorological data maximum and minimum temperature (T_{max}, T_{min}), rainfall (R), evapotranspiration (ET), wind speed (WS) and mean relative humidity (RH) were obtained from Leiria weather station (the closest from Alvaizere), IPMA – Instituto Português do Mar e Atmosfera. Considering sowing and harvesting dates, meteorological data collected consisted of the monthly weather reports for agriculture from December to June in each growing season. Monthly mean temperature (T) corresponded to a simple mean value calculated from T_{max} and T_{min} values. The water supplied by the supplemental irrigation (a total of 52.5 mm/growing season), in both growing seasons 2017–18 and 2018–19, was taken into account by adding it to the respective monthly rainfall (Table S2).

2.3. Agronomic performance evaluation

For the grass pea accessions agronomic performance evaluation, 16 quantitative traits were scored according to the “Descriptors for *Lathyrus* spp.” (IPGRI, 2000). Among these, one was a *Bruchus* infestation-related trait, three were plant-growth-related traits, four were yield-related traits, five were seed-related traits, and three were considered inflorescence-related traits. For traits detailed information check Table 1.

All 16 traits were recorded during the four growing seasons, except for the inflorescence-related traits that were recorded only during three growing seasons (2015–16, 2017–18 and 2018–19).

For the traits recorded during four growing seasons, for each growing season three different data sets were obtained: (A) one comprising the 6 traits recorded by plot, meaning one single value per accession, per plot (SWT100, PDP, SPD, SP, SYP, BCS), (B) a second comprising the 3 traits recorded in all plants in each plot (PH1F, PH50F, PHM) and (C) a third comprising the 4 traits recorded in 10 random seeds per plot (SL, SW, ST, SFI). The traits recorded during only three growing seasons composed

Table 1
Morphological and agronomic traits accessed.

Related trait	Acronyms	Name	Units
Bruchus infestation-	BCS	Seeds infested with <i>Bruchus</i>	%
Plant-growth-	PH1F	Plant height at the beginning of flowering	cm
	PH50F	Plant height at 50% flowering	cm
	PHF	Plant height at maturity	cm
Yield-	PDP	Pods per plant	n°
	SPD	Seeds per pod	n°
	SP	Seeds per plant	n°
	SYP	Seed yield per plant	g
Seed-	SFI	Seed flatness index ^a	-
	ST	Seed thickness	mm
	SW	Seed width	mm
	SL	Seed length	mm
	SWT100	100-seeds weight	g
Inflorescence-	DD1F	Degree days to first flower ^b	°C
	DD50F	Degree days to 50% flowering ^b	°C
	DD1MP	Degree days to first mature pod ^b	°C

^a) Calculated with the formula $SFI = (SL+SW)/2*ST$

^b) Calculated according to the formula ((Maximum Temperature- Minimum Temperature/2) – Temperature Base). The base temperature was set at 5°C: if Minimum temperature < Temperature Base => the day temperature was excluded from the calculation.

one last data set (D) obtained for each growing season with the three inflorescence-related traits (DD1F, DD50F, DD1MP) recorded as, one single value per plot. More details about the measurement procedures can be found in Supplementary files –Figure S1.

2.4. Meteorological data analysis

To evaluate how different the four growing seasons / environments (2015–16, 2016–17, 2017–18, and 2018–19) were from each other, a Principal Component Analysis, based on the monthly standardized climatological data was performed and biplots generated. This analysis allowed also to identify which were the most discriminant meteorological factors among seasons. All analyses were performed with Genstat (Genstat® for Windows 21th edition, VSN International, Hemel Hempstead, UK).

2.5. Phenotypic data analysis

2.5.1. Data quality control

Data quality control was performed individually for each trait, on the four data sets described above (A, B, C and D), in each growing season. A graphical inspection of residuals was used to assess normality (Q-Q plot), homogeneity of variance (residuals versus fitted values) and to identify outliers. Potential influential observations exceeding 3 times the interquartile range identified in the raw data, were considered outliers and removed from the analysis. Afterward, in the data sets B and C, a simple mean was calculated to obtain one single value per accession / growing season/ plot. Following this, data sets A, B and C were merged into a single file that comprised 13 traits evaluated in four growing seasons, and data sets A, B, C and D were merged into another single file that comprised 16 traits evaluated in three growing seasons. Next, a second round of data quality control was performed. To confirm data normality, a Shapiro-Wilk test was performed and when needed, a BOX-COX transformation procedure, as implemented in Genstat® software, was applied (Genstat® for Windows 21th edition, VSN International, Hemel Hempstead, UK). As a result of the BOX-COX procedure, some traits (PDP, SP, SYP, ST, and SWT100) were transformed and re-coded by adding a suffix “t” to the initial code label. After transformation, the same control of data quality was performed again.

2.5.2. Diversity analysis

To assess the diversity among the 182 grass pea accessions, linear mixed models were applied to the 16 evaluated traits, using the maximum likelihood (REML) procedure (Genstat® software). Linear mixed models provide a valuable platform to better fit the several random terms that need to be included in the statistical analysis to correct for external variation, like fluctuation growing conditions (Smith et al., 2005). These models were defined as *trait* (BCS, PH1F, PH50F, PHM, PDPt, SPD, SPt, SYPt, SFI, STt, SW, SL, SWT100t, DD1F, DD50F, and DD1MP) = *environment* + *genotype* + *genotype.environment* + *environment.repetition* + *environment.repetition.row* + *environment.repetition.column*. Here *environment* refers to the growing seasons (one to four), *repetition*, *row*, and *column* identify the experimental design within each *environment*, *genotype* identifies the grass pea accessions, and *genotype.environment* is the interaction between the grass pea accessions and growing season.

In a first step, all terms were fitted as random and the model was used to obtain the best linear unbiased predictors (BLUPs) used to estimate the variance components (environment variance component - σ_E^2 , genotype variance component - σ_G^2 and genotype x environment variance component - σ_{GE}^2) and broad sense heritability, as the proportion of phenotypic variance that is due to genetic factors. For broad sense heritability calculation, the VHERITABILITY procedure in Genstat software was applied. This procedure uses the formula $h^2 = 1 - \bar{v}BLUP / 2\sigma_g^2$ where $\bar{v}BLUP$ is the average estimated breeding value obtained using the best linear unbiased predictors, and the $2\sigma_g^2$ is the estimated genetic variance (Cullis et al., 2006).

In a second step, the best linear unbiased estimates (BLUEs) were calculated while setting the terms environment, genotype, and genotype x environment interaction as fixed and all the other terms as random. A Wald test for the significance of the fixed effects was performed using the generated BLUEs dataset. The phenotypic values (range and mean \pm standard deviation) were obtained, and Pearson's correlation coefficients calculated among traits. Due to a significant genotype x environment interaction (GxE) effect for all measured traits, as estimated by REML, Pearson's correlations were also calculated based on the BLUEs obtained for each trait, independently in each growing season, for comparison. Fisher's Least Significant Differences (LSD) tests were applied to compare the growing season means of the measured traits, at P-value < 0.05. All analyses were performed in Genstat (Genstat® for Windows 21th edition, VSN International, Hemel Hempstead, UK).

To summarize multivariate similarities among grass pea accessions, and to identify the most discriminant agronomic traits among grass pea accessions and growing seasons, a principal component analysis (PCA) on standardized data, using the traits adjusted means (BLUEs) was performed. Similarly, to Pearson's correlations, also the PCA was performed based on the BLUEs calculated for each trait, in each growing season independently. The number of principal components was determined by checking the eigenvalues of each component (Kaiser Criterion that retains components with eigenvalues greater than one) and the cumulative proportion of variance explained. Due to the existence of a few traits measured only during three growing seasons, two PCA analysis were performed, one comprising the 13 traits (all traits except DD1F, DD50F, and DD1MP) measured during four growing seasons, and another comprising all 16 traits measured during only three growing seasons.

2.5.3. Variation explained by ecotype clustering parameters

Linear mixed models were additionally used to estimate how much of the agronomic variation could be explained by the clustering parameters defining grass pea ecotypes, i.e. seed morphology (coat colour and size) or accessions geographical origin. The following liner mixed model was applied to investigate differences among seed coat colour classes: *trait* (BCS, PH1F, PH50F, PHM, PDPt, SPD, SPt, SYPt, SFI, STt, SW, SL, SWT100t, DD1F, DD50F, and DD1MP) = *environment* + *seed coat colour*

+ genotype within seed coat colour + genotype within seed coat colour. environment (as fixed terms) + environment.repetition + environment.repetition.row + environment.repetition.column (as random terms). A similar model was used to estimate how much of the accessions variation was explained by seed size replacing seed coat colour with this term. For geographical origin, the model used was trait (BCS, PH1F, PH50F, PHM, PDPt, SPD, SPt, SYPt, SFI, STt, SW, SL, SWT100t, DD1F, DD50F, and DD1MP) = environment + geographic origin (as fixed terms) + environment.repetition + environment.repetition.row + environment.repetition.column (as random terms). A Wald test for the significance of the fixed effects was performed. Fisher's Least Significant Differences (LSD) tests were applied to the means of the measured traits among the geographical origin classes, at P -value < 0.05.

To visualize any potential relation between the grass pea phenotypic variation and grass pea ecotype clustering parameters, the PCA biplots with 1st and 2nd PCs obtained previously for the four and the three growing seasons data, were coded based on seed size, coat colour and geographical origin.

3. Results

3.1. Climate environment characterization

The two first components of the PCA on the monthly-standardized climatological data, explained about 81.4 % of the observed variability, clearly discriminating the four growing seasons from each other (Fig. 1).

Higher precipitation, stronger wind and higher minimum temperatures characterized the 2015–16 growing season (Fig. 1 and Table S2). Pointing in the opposite direction, the 2018–19 season exhibited lower precipitation, reduced wind speed and lower minimum temperatures. A

high relative humidity and precipitation, and a low evapotranspiration, wind speed and temperatures distinguished the 2017–18 growing season. In contrast, at the opposing position, along the same principal component, the 2016–17 season exhibited higher temperatures, evapotranspiration, wind speed, and lower relative humidity and rain.

Therefore, the field trials started with a more typical Mediterranean mild wet Winter-Spring (2015–16), but with very different climatic conditions in the following growing seasons. The 2016–17 growing season showed higher temperatures and lower precipitation, while the following 2017–18 growing season exhibited cooler and wetter conditions. Finally, the 2018–19 growing season was defined as cooler and drier.

3.2. Phenotypic diversity

Significant differences across genotypes (G) (grass pea accessions), environments (E) (growing seasons) and genotype by environment interaction (GXE) effects were detected for the majority of the 16 evaluated traits, except for PH1F, PH50F, and SWT100t, across environments (Table S3, in Supplementary files). Additional information on the grass pea accessions phenotypic values for the 16 agronomic traits can be found in Supplementary files Table S4.

3.2.1. Variance components

From the analysis of the variance components (Fig. 2), the highest percentage of variance was due to differences among genotypes (σ_G^2) in the case of the seed-related traits SFI, SW, and SL, the yield-related traits SPD and SWT100t, and the plant growth-related traits PH1F and PH50F.

The environment variance component (σ_E^2) was the highest for all the inflorescence-related traits DD1F, DD50F, and DD1MP, the *Bruchus* infestation-related trait BCS, the yield-related traits PDPt, SYPt, SPt, and

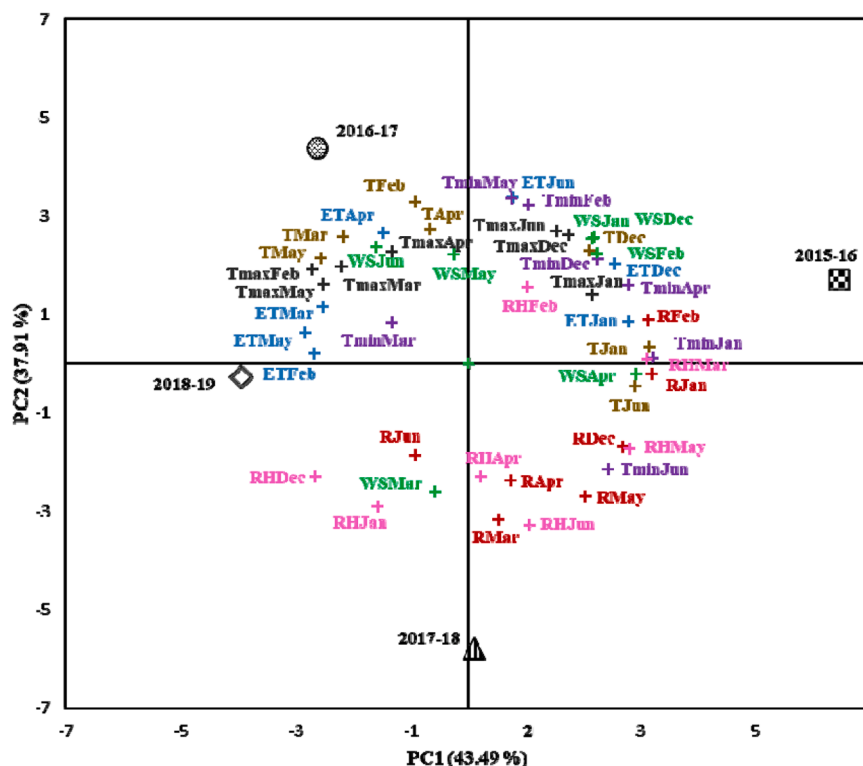


Fig. 1. PCA biplot based on the monthly meteorological data recorded from December until June on each growing season/ environment. Different geometric figures depict the four growing seasons / environments (2015–16, 2016–17, 2017–18, and 2018–19). Different colours depict the seven meteorological parameters (Tmin in purple, Tmax in black, T in brown, ET in light blue, RH in pink, WS in green and R in red). TminDec, TminJan, TminFeb, TminMar, TminApr, TminMay, TminJun – minimum temperature from December until June (°C); Tmaxxxx – maximum temperature from December until June (°C); Txxx – average temperature from December until June (°C); ETxxx – evapotranspiration from December, until June (mm); RHxxx – relative humidity from December until June (%); WSxxx – the wind speed from December until June (Km/h); Rxxx – rainfall from December until June (mm).

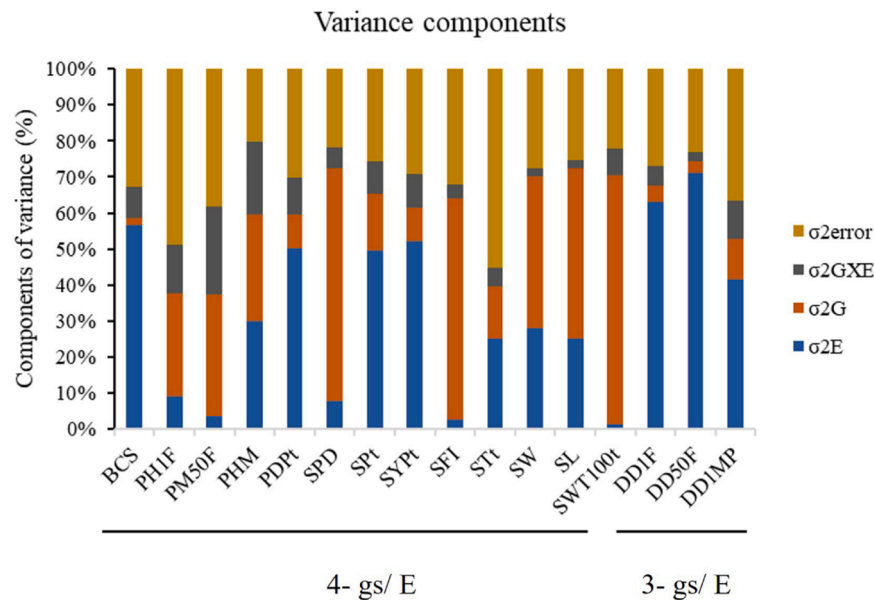


Fig. 2. Variance components, in percentage, for 16 traits evaluated in 182 grass pea accessions during four or three growing seasons / environments (gs/ E). BCS – seeds infested with *Bruchus*, in percentage; PH1F – plant height at first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPT – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to the first mature pod, in °C. σ^2_{E} (%): percentage of variance attributed to environments; σ^2_{G} (%): percentage of variance attributed to genotypes; σ^2_{GXE} (%): percentage of variance attributed to the interaction between genotypes and environments; σ^2_{error} (%): percentage of variance attributed to the block, row, column, and residual terms which altogether compose the error variance. t – BOX-COX transformed trait suffix.

the seed-related trait STt. On the other hand, the genotype variance component (σ^2_{G}) was the highest for the plant growth-related traits PH1F, PH50F, the yield-related traits SPD, and for all the seed-related traits except for STt. Both, the environment variance component (σ^2_{E}) and the genotype variance component (σ^2_{G}) were similar ($\sigma^2_{\text{E}} \cong \sigma^2_{\text{G}}$) for the plant growth-related trait PHM. In general, the variance associated with differences among genotypes was higher than the variance attributed to the GXE effect ($\sigma^2_{\text{G}} / \sigma^2_{\text{GXE}} > 1$), except for the *Bruchus* infestation-related trait, BCS, the inflorescence-related trait DD1F and, for the yield-related trait PDPt. Detailed values of variance components are shown in Supplementary files - Table S5.

3.2.2. Broad sense heritability

As displayed in Fig. 3, broad sense heritability (h^2) were higher for the seed-related traits SW (89.6 %), SL (88.3 %), and SFI (81.9 %), for the yield-related traits SWT100t (88.7 %) and SPD (88 %), and the plant growth-related traits PHM (72.2 %), PH1F (69 %), and PH50F (68.6 %). The lowest heritabilities were observed in the inflorescence-related traits DD1F (36.4 %), DD50F (38.6 %) and DD1MP (45.2 %), and in the *Bruchus* infestation-related trait BCS (21.5 %). Detailed values for broad sense heritability (h^2) are shown in Supplementary files - Table S5.

3.2.3. Phenotypic variation across growing seasons

Grass pea phenotypic means and respective comparison for the traits in which significant differences were previously detected (Wald test) across the three or four growing seasons / environments (gs/E) are reported in Table 2.

Growing seasons averages for inflorescence-related traits (DD1F, DD50F, and DD1MP) were different, with smaller values for the beginning of flowering and pod maturation (shorter growth cycles) on the cooler and dryer 2018–19 season, and higher values (longer growth cycles) on the wetter 2015–16 and 2017–18. However, the 2018–19 season presented the highest DD50F values.

The sole plant growth-related trait revealing significant differences

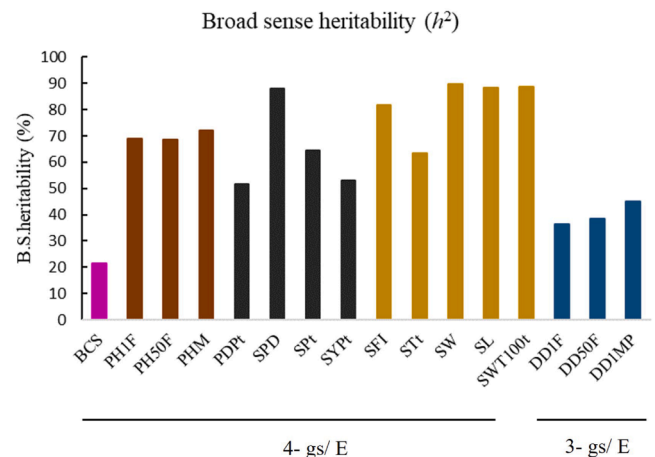


Fig. 3. Broad sense heritability (h^2) for the 16 traits evaluated in 182 grass pea accessions during four or three growing seasons / environments (gs/ E). BCS – seeds infested with *Bruchus*, in percentage; PH1F – plant height at first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPT – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C. h^2 – broad sense heritability was obtained using the formula $h^2 = 1 - \bar{v}BLUP / 2\hat{\sigma}_g^2$ in Genstat software. t – BOX-COX transformed trait suffix.

across growing seasons was the main branch height (PHM). It depicted higher values in the warmer and drier season (2016–17), intermediate values during the milder and wetter (2015–16), and lower values during the cooler, wetter and drier seasons (2017–18 and 2018–19).

Table 2

Fisher's Least Significant Difference (LSD) tests ($p \leq 0.05$) for 16 agronomic traits, accessed in 182 grass pea accessions, over three to four consecutive growing seasons / environments.

	Trait	gs/E ¹	LSD ²	
3 growing seasons	DD1F	2015-16	60.57	c
		2017-18	49.62	b
		2018-19	44.12	a
	DD50F	2015-16	73.67	a
		2017-18	71.89	a
		2018-19	93.09	b
	DD1MP	2015-16	128.8	b
		2017-18	124.7	b
		2018-19	110.6	a
4 growing seasons	PHM	2015-16	50.93	b
		2016-17	59.68	c
		2017-18	37.17	a
		2018-19	37.43	a
	PDPt	2015-16	5.22	c
		2016-17	5.28	c
		2017-18	2.31	a
		2018-19	3.41	b
	SPD	2015-16	2.39	b
		2016-17	2.28	b
		2017-18	2.04	a
		2018-19	2.01	a
	SPt	2015-16	7.39	c
		2016-17	7.39	c
		2017-18	3.52	a
		2018-19	4.71	b
	SYPt	2015-16	3.25	b
		2016-17	2.98	b
		2017-18	0.5	a
		2018-19	1.21	a
	SFI	2015-16	1.55	a
		2016-17	1.49	a
		2017-18	1.54	a
		2018-19	1.62	b
	STt	2015-16	13.07	a
		2016-17	26.42	b
		2017-18	22.05	b
		2018-19	22.53	b
	SW	2015-16	4.25	a
		2016-17	7.37	b
		2017-18	6.19	b
		2018-19	7.09	b
	SL	2015-16	5.13	a
		2016-17	7.27	b
		2017-18	7.43	b
		2018-19	7.2	b
	BCS	2015-16	1.52	a
		2016-17	5.21	a
		2017-18	3.19	a
		2018-19	13.13	b

DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPt – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; BCS – seeds infested with *Bruchus*, in percentage.¹ Growing seasons / Environments | ² Means with the same letter are not significantly different at 0.05 significance level (Fisher's multi-comparison test).t – BOX-COX transformed trait suffix.

When considering the yield-related traits, the n° of seeds/pod (SPD), n° of pods/plant (PDPt) and n° of seeds/plant (SPt) all decreased from the two first seasons with milder and warmer growing conditions, to the second cooler growing seasons (2017–18 and 2018–19). The same trend was observed for the seed weight/plant (SYPt), but with an extreme decrease.

Seed length, width and thickness (SL, SW and STt) increased from the first growing season (2015–16) to the next studied growing seasons. However, the seed flatness index (SFI) showed distinct values solely in the final season of trials (2018–19). *Bruchus* infestation (BCS) was high only during the last growing season (2018–19).

3.3. Correlations between measured phenotypic traits

As shown in Fig. 4A, when considering the traits measured during four growing seasons, two groups of high and positively and one group of high and negatively correlated traits ($r > 0.900$, P -value < 0.001) were detected.

The first group of highly positively correlated traits was composed of the three plant growth-related traits (PH1F, PH50F and PHM) and the four yield-related traits (SPD, PDPt, SPt and SYPt). Exception to these high paired correlations was observed between SPD and SYPt, which, despite being positive, did not exhibit a strong correlation ($r = 0.14$). The second group of highly and positively correlated traits was composed of the five seed-related traits (SL, SW, STt, SFI and SWT100t) and the *Bruchus*-infestation related trait (BCS). Finally, a distinct group of highly and negatively correlated traits was identified, particularly involving the yield-related trait SPD (and, to a lesser extent, SPt), and the four seed-related traits SFI, SW, SL and SWT100t. The inflorescence-related traits measured only during three growing seasons were high and positively correlated only among themselves (Fig. 4B). Detailed trait correlation values can be found in Supplementary files – Tables S6 and S7.

Considering the correlations obtained in each of the four growing seasons, the highly positively correlated group of three plant growth-related traits (PH1F, PH50F and PHM) and the four yield-related traits (SPD, PDPt, SPt and SYPt) was identified solely in the two last growing seasons (2017–18 and 2018–19). Heat maps of Pearson's correlations calculated for each of the four and three growing seasons can be found in Supplementary files – Figures S2 and S3.

3.4. Multivariate analysis

3.4.1. Grass pea accession phenotypic relatedness and environments discrimination

Although there was overlap among grass pea accessions, some discrimination between growing seasons / environments (gs/ E) was observed, in both, the 4gs/E and the 3gs/E multivariate Principal Component biplots (Fig. 5A and B). The first three principal components explained 80.10 % and 74.48 % of the variance observed for the 4gs/E and the 3gs/E, respectively.

The most discriminant traits among growing seasons were clearly the inflorescence-related traits, although they were only measured during three of the four growing seasons. Based on these traits, longer growth cycles were observed on the 2015–16 season and the shortest on 2018–19. This last growing season was also clearly separated by the percentage of *Bruchus* infestation (Fig. 5B – PC1/PC3). Discrimination within and among growing seasons was feasible through the evaluation of plant growth and yield-related traits, specifically considering PHM, SYPt, SPt and PDPt traits. On the other hand, seed-related traits as STt, SW, SL, SFI and SWT100t contributed the most for the observed within growing season variation. It was possible to identify some grass pea accessions behaving similarly well, in terms of yield potential, across all the experiment (Fig. 5A, different coloured squares with accessions numbers identification).

More detailed trait eigenvalues per principal component can be found in Supplementary files – Table S8.

3.4.2. Variation explained by ecotype clustering parameters

From the analysis of the effect of the grass pea ecotype clustering parameter *seed colour*, significant differences were detected between light and darker seed coat accessions for all the accessed traits except for

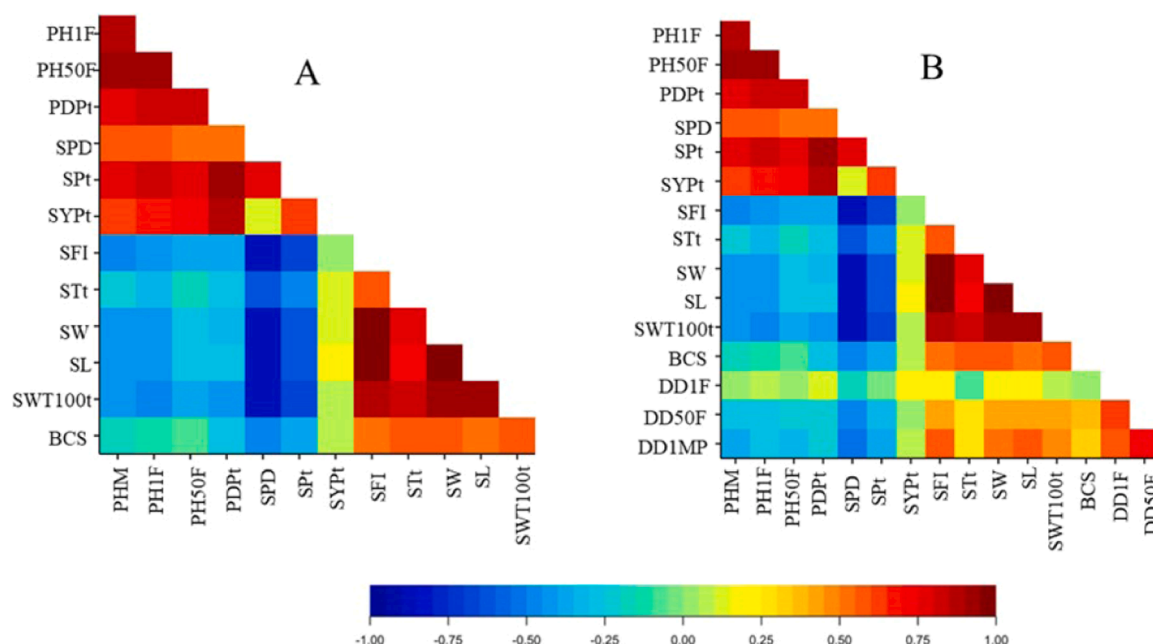


Fig. 4. Correlation matrix heat maps with Pearson correlation coefficients based on phenotypic traits adjusted means (BLUES) of 182 grass pea accessions, coloured by a spectrum from blue (correlation = −1.0) to red (correlation = 1.0). **A** - 13 traits measured over four growing seasons / environments, **B** - 16 traits measured over three growing seasons / environments. PH1F – plant height at first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPt – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; BCS – seeds infested with *Bruchus*, in percentage; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C. t – BOX-COX transformed trait suffix.

the plant growth-related trait PH1F, and the yield-related trait PDPt. Also, significant differences were detected between large and small seed accessions for all the accessed traits except for the same plant growth-related trait PH1F, and yield-related trait PDPt, and inflorescence-related trait DD1MP (Supplementary files – Table S9).

Indeed, from the superimposition of the grass pea ecotypes clustering parameters, seed colour and seed size, into the PCA biplots obtained for the three and the four growing seasons, two main groups were detected (Fig. 6). The grass pea accessions characterized by dark coat colour and small size were clustered together with lower values for seed-related traits (SW, SL, SFI, STt and SWT100t), higher values for the yield-related trait SPD and lower *Bruchus* (BCS) infestation. On the other hand, the light coat colour and large size accessions were more scattered, but nevertheless, with the highest values for seed-related traits (SW, SL, SFI, STt and SWT100t) and lowest values for the yield-related trait SPD.

From the analysis of the effect of the grass pea ecotype clustering parameter geographic origin, significant differences were detected among the seven defined regions (P -value < 0.001) for all the accessed traits except for *Bruchus* infestation-related trait BCS, plant growth-related traits PHM and PH50F, yield-related traits PDPt and SYPt, and inflorescence-related traits DD1F and DD1MP (Table 3 and Supplementary file – Table S9).

Particularly, as showed in Table 3 for the inflorescence-related trait DD50F the Sub-Saharan African accessions were the earliest to reach 50 % flowering, whereas the North and Central Asian, East European and Mediterranean accessions were the latest.

Concerning the growth-related trait PH1F, Sub-Saharan African and Mediterranean accessions showed similarities and were slightly shorter than South Asian, North Asian, and East European accessions, which, in turn, displayed similarities among themselves.

When considering the yield-related traits, seeds per pod (SPD) and seeds per plant (SPt), Mediterranean, Sub-Saharan African and South Asian accessions were the most contrasting. Those from Mediterranean

and Sub-Saharan African origin showed the lowest number of seeds per plant. In addition, Mediterranean accessions showed the lowest number of seeds per pod. On the contrary, South Asian accessions exhibited the highest number of, both, seeds per pod and seeds per plant. Regarding the yield-related traits (SPD, SPt), North and Central Asian and East European accessions were similar among themselves and exhibited intermediate values.

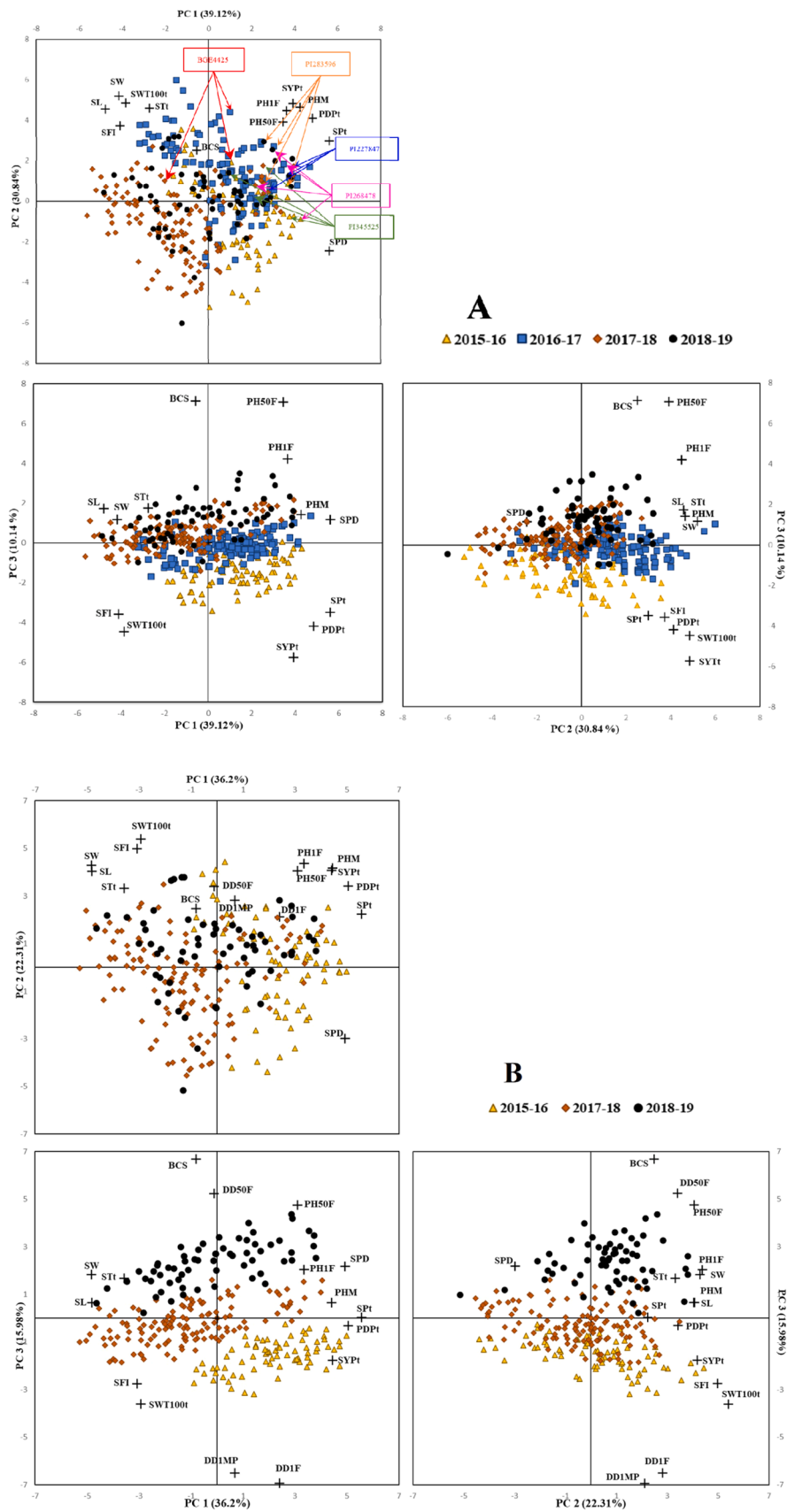
Finally, concerning all the seed-related traits (SFI, SW, SL, STt, and SWT100t) the Mediterranean accessions were the largest, flattened and heaviest, whereas South Asian and Sub-Saharan African accessions were smaller, rounded and the lightest.

The Mediterranean had larger and heavy seeds than the accessions of South Asia and Sub-Saharan African origin (Fig. 7). In addition, the South Asia and Sub-Saharan African accessions had a higher number of seeds per pod than the Mediterranean accessions. South Asian accessions were slightly taller and yielded more than those of Mediterranean origin. The accessions of East Europe and North and Central Asia origin had similar behaviour, showing intermediate values in what concerned the seed-related traits (SFI, SW, SL and SWT100t), and the yield-related traits (SPD, SPt), but were different from the accession of Mediterranean, South Asia, and Sub-Saharan African origin.

4. Discussion

In an increasingly unstable Mediterranean climate, characterized more and more by extreme weather events and with an increasing frequency of pests' outbreaks (Joffre and Rambal, 2001; Palutikof and Wigley, 1996), grass pea, due to its hardish nature, is a promising source of traits to breed for climate change adaptation.

With the focus on the development of climate change resilient and adaptable multi-stress resistant/tolerant varieties, we explored a representative collection of the worldwide grass pea genetic diversity. Significant genotypic diversity on seed yield potential, plant size and development, plant growth cycle duration and pest infestation was



(caption on next page)

Fig. 5. Principal component analysis (PCA) based on the BLUEs values for 13 and 16 agronomic traits, using 182 grass pea accessions. **A** - 13 traits measured over four growing seasons / environments, **B** - 16 traits measured over three growing seasons / environments. Different coloured squares with numbers in 5 A identified accessions behaving similarly well in terms of yield potential across different growing seasons. BCS – seeds infested with *Bruchus*, in percentage; PH1F – plant height at the first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPT – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C. t – BOX-COX transformed trait suffix.

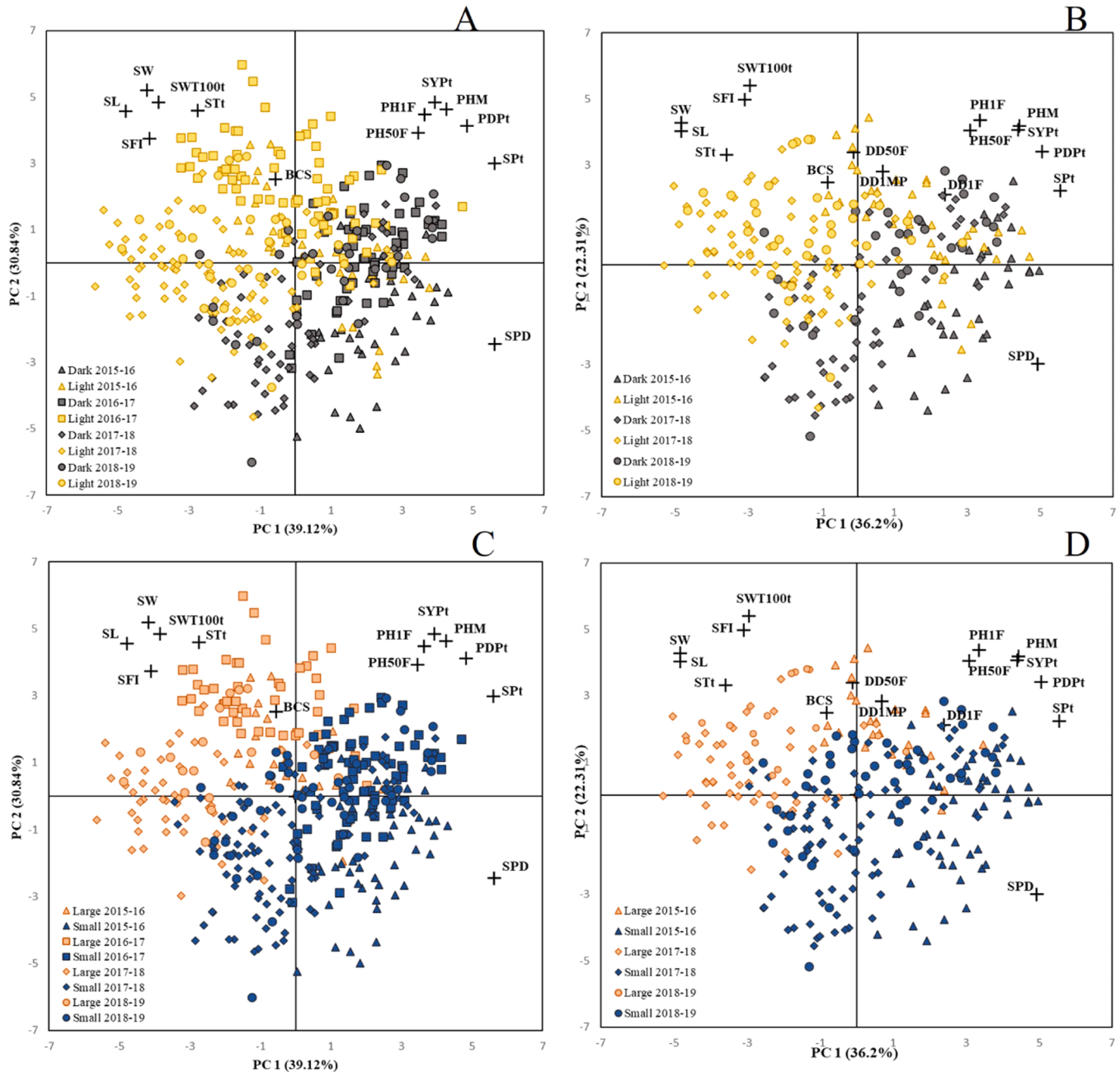


Fig. 6. A biplot of principal component analysis (PCA) shows the phenotypic correlations between 13 and 16 analysed traits and the seed colour (6 A, 6B) and the seed size (6 C, 6D), using 182 grass pea accessions. **A** and **C** – 13 traits measured across four growing seasons / environments, **B** and **D** – 16 traits measured across three growing seasons / environments. BCS – seeds infested with *Bruchus*, in percentage; PH1F – plant height at first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPT – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C. t – BOX-COX transformed trait suffix.

Table 3

Mean comparison for *geographical origin* of 13 and 16 traits evaluated in four or three growing seasons/ environments, in 182 grass pea accessions.

Trait	Geographical origin	LSD ^{1,a}	
3 growing seasons	Sub-Saharan Africa	72.94	a
	South Asia	76.58	b
	East Europe	79.85	c
	North and Central Asia	80.19	c
	Unknown	81.05	c
	Mediterranean	81.10	c
4 growing seasons	North America	83.85	c
	Sub-Saharan Africa	12.43	a
	Unknown	12.57	a
	Mediterranean	13.26	a
	North America	14.16	ab
	North and Central Asia	14.83	b
	East Europe	14.85	b
	South Asia	15.05	b
	Mediterranean	1.89	a
	Unknown	2.05	b
	North and Central Asia	2.22	c
	East Europe	2.25	c
	North America	2.41	cd
	Sub-Saharan Africa	2.52	d
	South Asia	2.76	e
	Unknown	4.95	a
	Mediterranean	5.28	ab
	Sub-Saharan Africa	5.62	bc
	North and Central Asia	5.82	c
	East Europe	6.07	c
	North America	6.24	abcd
	South Asia	6.73	d
	South Asia	1.71	a
	Sub-Saharan Africa	1.73	a
	North and Central Asia	1.95	b
	Unknown	1.96	b
	East Europe	2.00	b
	North America	2.06	bc
	Mediterranean	2.11	c
SFI	South Asia	1.33	a
	Sub-Saharan Africa	1.35	a
	North America	1.49	ab
	North and Central Asia	1.51	bc
	Unknown	1.52	bcd
	East Europe	1.58	bd
STt	Mediterranean	1.67	e
	South Asia	16.55	a
	Sub-Saharan Africa	17.28	a
	North America	19.53	ab
	Unknown	19.72	bc
	North and Central Asia	20.13	bc
SW	East Europe	20.36	bc
	Mediterranean	23.54	b
	South Asia	4.62	a
	Sub-Saharan Africa	4.76	ab
	North America	5.83	bc
	North and Central Asia	5.86	c
SL	East Europe	6.11	c
	Unknown	6.12	c
	Mediterranean	7.19	d
	Sub-Saharan Africa	5.23	a
	South Asia	5.35	a
	North America	6.44	b
SPD	North and Central Asia	6.47	b
	East Europe	6.74	b
	Unknown	6.77	b
	Mediterranean	7.65	c

DD50F – degree days from sowing to 50 % flowering, in °C; PH1F – plant height at first flower, in cm; SPD – number of seeds per pod; SPt – number of seeds per plant; SWT100t – weight of 100 seeds, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm.¹ Mean with the same letter are not significantly different at 0.05 significance level (Fisher's multi-comparison test).t – BOX-COX transformed trait suffix.

identified among the studied accessions, anticipating great potential to breed for adaptation to the changing Mediterranean climate.

Significant grass pea genotypic diversity on seed yield has been also reported by Hanbury et al. (1999) studying a collection of 407 Indian sub-continent and Mediterranean/Europe accessions, under a Mediterranean-type environment. Diversity on seed yield, plant height and growth cycle duration was also described by Arslan et al. (2022) and Parihar et al. (2013), studying a diverse collection of 94 grass pea accessions under lowland and highland conditions in Turkey, and, in a diverse collection of 358 grass pea accessions, evaluated in India, respectively. In more regional germplasm evaluations, genetic diversity and variability for seed yield, plant size and growth cycle duration has also been found. This was the case of the studies of Abate et al. (2018) with Ethiopian germplasm, Gixhari et al. (2016) with Albanian germplasm, and De la Rosa and Martín (2001) with Spanish germplasm. When analysing the phenotypic diversity of 50 Ethiopian grass pea accessions, Tadesse and Bekele (2003) reported variability in seed yield and growth cycle duration, but not in plant height. This variability was observed both within altitude and within regions, suggesting that the traits variability expression may differ across environments.

The existence of diversity reinforces the ability of a crop to resist changes in climate, diseases or pests, besides being the basis of any breeding program (Chowdhury and Slinkard, 2000; Das et al., 2021; Gonçalves et al., 2022; Litrico and Violle, 2015). Therefore, breeding efforts of any cultivated crop rely on the identification and characterization of the respective germplasm resources diversity (Vaz Patto and Rubiales, 2014). However, for a proper selection of a genotype, for a target trait, in a target environment, it is most relevant to understand the effects of factors such as the genotype (G), the environment (E), and the genotype by environment interaction (GxE) (Rubiales et al., 2020).

From the multivariate analysis, the Mediterranean accessions formed overall the most diverse agro-morphological group, overlapping, on the lower seed related traits plot side, with a much more clustered group of Sub-Saharan African and South Asian accessions. The North and Central Asian and Eastern European accessions group occupied an intermediate position, also with overlap with the broader Mediterranean group. Interestingly, the Mediterranean accessions yield potential (measured here by seed weight/plant (SYPt), our best yield proxy) was not significantly different from the potential of the other tested grass pea geographical origins. Overall, there was no significant differences across geographical origins in what concerned the number of pods/plant (PDpt), the plant size and development related traits (PHM and PH50F), the inflorescence related traits (DD1F and DD1MP) or the seed *Bruchus* infestation (BCS).

Based on the grass pea PCA seed coat colour and seed size codes imposition, the presently analysed accessions were grouped into two main agro-morphological clusters. The first one grouped the accessions with a light coat colour and large seed size and corresponded to the Mediterranean and East European accessions. The second one grouped the accessions with dark coat colours and small seed sizes and corresponded to the Asian and sub-Saharan African origins. Hanbury et al. (1999) found similar clusters when studying 407 grass pea lines, primarily obtained from a wide range of geographical origins. These authors identified an Indian subcontinent and a Mediterranean / European clusters that corresponded to the two main grass pea ecotypes and suggested that the higher yields of the Mediterranean accessions may be due to their larger seed size. In the present study, and as already mentioned, we did not find significant yield differences across the six different geographical origins under analysis (one of them Mediterranean), but we found significant yield differences between light and dark seeds accessions or between large and small seed accessions, with light and large seed accessions showing higher yields.

Overall, in agro-morphological terms, the South Asian and the Sub-Saharan African accessions were the most differentiated from the Mediterranean accessions, but still carriers of interesting traits. The South Asian accessions showed the highest number of seeds/pod (SPD),

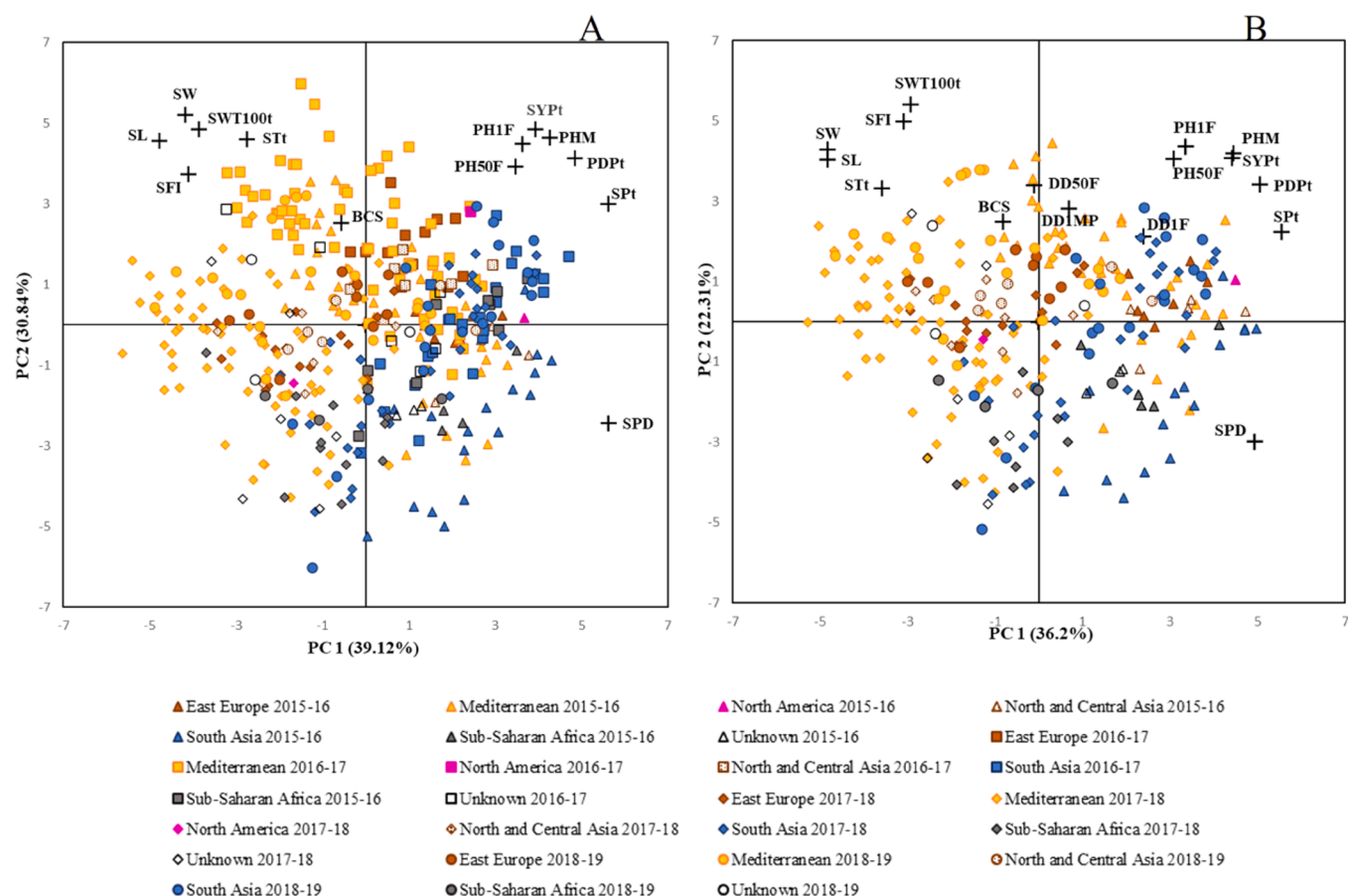


Fig. 7. A biplot of principal component analysis (PCA) shows the phenotypic correlations between 13 and 16 analysed traits and the geographical origin, using 182 grass pea accessions. **A** – 13 traits measured across four years/ environments, **B** – 16 traits measured across three years/ environments. BCS – seeds infested with *Bruchus*, in percentage; PH1F – plant height at first flower, in cm; PH50F – plant height at 50 % flowering, in cm; PHM – plant height at maturity, in cm; PDPt – number of pods per plant; SPD – number of seeds per pod; SPT – number of seeds per plant; SYPt – seeds weight per plant, in grams; SFI – seed flatness index, in mm/mm; STt – seed thickness, in mm; SW – seed width, in mm; SL – seed length, in mm; SWT100t – weight of 100 seeds, in grams; DD1F – degree days from sowing to first flower, in °C; DD50F – degree days from sowing to 50 % flowering, in °C; DD1MP – degree days from sowing to first mature pod, in °C. t – BOX-COX transformed trait suffix.

highest number of seeds/plant (SPT) and the taller plants at first flower (PH1F). The Sub-Saharan African accessions were the earliest to reach 50 % flowering. In a climate change context, a shorter growth cycle might be an advantage when a terminal drought is more expected to occur. Additionally, considering the high correlation found in the tested Mediterranean environment between yield potential (SYPt), number of seeds/plant (SPT) and plant height at first flower (PH1F), bigger plants at flowering and higher number of seeds/plant would be a way to potentiate yield in any grass pea breeding program for the region. In a comparison, performed by Grela et al. (2010) in Poland, between grass pea accessions originated from West-central Europe and the Mediterranean region, it was reported that the taller plants, with smaller seeds, had West-central European origin. On the other hand, Campbell (1997) indicated that the European and Mediterranean grass pea lines, with larger seeds, usually were taller than the South East Asian lines (India, Bangladesh and Pakistan) with smaller seeds, under Canadian conditions. This difference in plant growth, compared with our results, may be due to the different locations where the field studies were carried out.

Nevertheless, both South Asian and Sub-Saharan African accessions had the smallest seed sizes (and frequent dark coat colours), seed traits that are normally not appreciated by the majority of the traditional Mediterranean grass pea farmers. Nevertheless, the organic markets seems to prefer smaller sizes (Tavoletti et al., 2005). Interestingly, smaller and darker seed accessions were also significantly less infested by *Bruchus* than large and light seed accessions. To the best of our

knowledge, there is no published study on *Bruchus*-resistance in grass pea. However, Grela et al. (2010) found a positive correlation between tannin seed content and flower colour intensity, with white or cream flower colours associated with low seed tannin content, while the colourful blooming accessions presented higher seed tannin contents. Jackson and Yunus (1984) found strong correlations between grass pea flower colour and seed coat colour, with white flower accessions giving raise only to white seeds, whereas blue flower accessions showed coloured seeds. The observations from both studies may suggest that the grass pea dark seed coat colour has higher seed tannin content. In fact, Deshpande and Campbell (1992) described a high correlation between condensed tannins content and coat pigmentation in grass pea seeds, with darker seeds exhibiting moderate to high tannin contents. This may hint for a higher tannins content as the basis of the observed lower *Bruchus*-infestation in darker seeds. Moreover, tannins could act as deterrents for feeding in natural conditions, since they are recognized as plant defence compounds against herbivore insects (Aznar-Fernández and Rubiales, 2019; Barbehenn and Constabel, 2011). *Bruchus* resistance, together with aphid resistance, are important traits for grass pea Mediterranean farmers concerned with the sustainability of their grass pea production systems (reduced pesticide applications) under the much more frequent pest outbreaks due to climate change (Skendžić et al., 2021; Yadav et al., 2010). From all the above, to cope with specific markets, genetic variation for seed size seems to be an important prerequisite for breeding. Significant variation was still found, in the

present study, for seed sizes within the Mediterranean accessions, allowing an effective selection within this germplasm group for the different consumer choices.

Great influence of the environment on yield has often been reported (Hanbury et al., 1999; Lioi et al., 2011; Polignano et al., 2009). In the present study, the seed weight per plant (SYPt), our best yield potential proxy, was also highly influenced by the growing season (high σ_E^2), showing an extreme decrease, from the first two, milder and hotter growing seasons (2015–16 and 2016–17), to the second two cooler growing seasons (2017–18 and 2018–19). The same decreasing trend across growing seasons was observed on the plant size (measured by PHM, where $\sigma_E^2 \cong \sigma_G^2$) and the other yield components (measured by PDPt, SPt, and SPD). In SPD, the decreasing trend was less extended. Besides temperature, also rainfall was considered one of the most differentiation parameters across the studied growing seasons. Nevertheless, we did not observe a clear effect of the drier growing seasons (2016–17 and 2018–19), as the one seen for the cooler seasons, on the agro-morphological traits variation. One hypothesis could be a higher resilience of grass pea to hot/drought conditions than to cold, making cold tolerance a target trait for breeding. Selection for grass pea cold tolerance is feasible as shown by Mikić et al. (2011). Grass pea resilience to hot/drought conditions seems to be, mainly due to tolerance, where the plant continues to grow and function at reduced water content (this feature was observed in grass pea through a delayed maturity and senescence) (Leport et al., 1998; Thomson et al., 1997) and/or avoidance, where the crop minimizes its water loss. Early phenology together with rapid ground cover will contribute for greater water use during grain filling. This, combined with tolerance to low temperatures during flowering and pod formation, would be of major importance to yield in short-season environments, such as the Mediterranean ones (Siddique et al., 2001). In addition, the emergency irrigation applied during the dryer conditions observed in 2017–18 and 2018–19 could have contributed for a lower effect of drought on yield than the lower temperatures.

In the Mediterranean region, earlier sowings of cool-season grain legumes are recommended, to profit from winter rains and escape to terminal heat and drought at the end of the spring (Rubiales et al., 2022; Singh et al., 1997), provided there is no cold event or disease that can compromise the crop during winter. A study performed by Singh et al. (1997) showed that chickpea seed yield can be increased by 70 % in an early winter sowing when compared with a traditional spring sown crop. This higher yield could be due to a longer period of vegetative growth, resulting in increased plant height and total biomass, together with a longer reproductive phase of winter chickpea.

The grass pea growth cycle duration (measured by DD1F, DD50F, and DD1MP) decreased from the typical Mediterranean milder and wet Winter-Spring first growing season to the cooler wet and dry following growing seasons. The inflorescence related traits were as expected highly influenced by the environment ($\sigma_E^2 = 69.5, 129.18$ and 93.4 , respectively) with low heritability ($h^2 = 36.43, 38.62$ and 45.16 , respectively). We also observed in this study, that a longer growth cycle duration corresponded to a higher yield (SYPt). Indeed, when the growth cycle became longer, the grass pea plants were better established, with taller plants (PHM), higher number of pods/plant (PDPt), seeds/pod (SPD), leading to higher yields, taking fully advantage of a longer vegetative stage. Nevertheless, seed-related traits (SFI, STt, SW, SL) increased from 2015 to 16–2018–19.

Gusmão et al. (2012) in a greenhouse pot experiment, using the grass pea line Ceora, imposed a water deficit, and observed that water-deficit plants were shorter and less yielding. The lower yield, probably, was due to the higher number of flower abortion that result in a lower number of pods per plant, seeds per pod, seeds per plant and, consequently, lower yield. Nevertheless, the seeds of the water deficit plants were slightly larger than those of control plants. Hanbury et al. (1999) on his study with a grass pea diverse collection (mainly from Mediterranean and Asian origin) conducted in a Mediterranean-type climate, in Australia,

suggested that grass pea seed weight seems to have a greater influence on yield than early phenology. Indeed, the grass pea hundred seeds' weight is an important feature contributing to yield.

Also, with a strong effect on production and breeding efforts was the presence of GxE in all of the measured traits. This implies a change in the accessions ranking from one growing season to another. Accordingly, Hanbury et al. (1999) described the presence of GxE effects on the grass pea yield. Namely, the author described clear differences between the grass pea lines from the two origins (Mediterranean and Indian sub-continent) in yield response to environment. Nevertheless, in the present study and for the yield proxy trait (SYPt), and as for the majority of the measured traits, the genotypic variance was bigger than the variance due to GxE, reassuring the possibility of a successful genetic selection within this germplasm (Polignano et al., 2009). In fact, it was still possible to identify a few accessions behaving similarly well in terms of yield potential across all the experiment, namely the accessions PI283596, PI268478, PI227847, and PI345525 from South Asian origin, and BGE4425 from Mediterranean origin (Fig. 5A and Table S1 for more details).

The plant size and development traits (height at the first flower (PH1F), and at 50 % flowering (PH50F) or the weight of 100 seeds (SWT100t)) were the most stable traits across growing seasons, following their larger genotypic effects. These traits were, together with the seed traits, seed width (SW), seed length (SL) and seed flatness index (SFI), and the number of seeds per pod (SPD), the traits with the highest heritabilities. The heritability reflects the degree of transmissibility of a trait to their offspring (Mahapatra et al., 2020). Heritability, together with genetic variability and selection intensity are the main factors that influence genetic advance (Abate et al., 2018), thus can be considered important selection parameters. Likewise, Abate et al. (2018) and Kumar and Dubey (2001) revealed high heritability estimates for plant height in grass pea. Polignano et al. (2009), in a study with eight grass pea elite lines, suggested that the seed size is a stable trait. Accordingly, Ahmadi et al. (2015), in a study with 14 grass pea lines, reported high heritability for 100 seeds weight and considered that this trait should be taken into consideration during selection for higher yield in grass pea.

The high heritability observed for plant size and development traits indicates that improvement can be achieved through selection in these traits. Thus, plant size as a general trait could be important to breed for due to its correlation with yield potential. Unfortunately, the interesting climate change adaptable traits related to the growth cycle duration (short growth cycles) and to the resistance to pests (*Bruchus* infestation) depicted the lowest heritabilities.

One limitation to this work is that we were unable to obtain a similar number of accessions from each geographical origin. This may have introduced some bias. We also did not characterize the diversity that might exist on underground traits, such as for instances, the root nodule formation with the local *Rhizobium* symbiont. Thus, we cannot conclude about the potential differences among grass pea accessions regarding this capability. This could be an interesting issue to further explore in future research. Nevertheless, this work provides useful information to support a more educated choice of grass pea accessions for production and breeding under Mediterranean growing conditions.

5. Conclusions

It is apparent from this study that the grass pea germplasm of Mediterranean origin still contains great genetic diversity, conferring it high potential for breeding progress. We might anticipate, by selection within Mediterranean germplasm, the development of light small and large seed varieties, with taller plants and greater yield potential, to answer present consumers and farmers concerns. However, under the present climate change threats in the region (with increase terminal drought events and increased frequency and intensity of pests' outbreaks), it seems that the Sub-Saharan African and South Asian accessions, could be the optimal breeding sources for Mediterranean grass pea

improvement. These accessions, showed the shorter plant growth cycles and mainly dark seed types, characterized by the lowest *Bruchus* infestation, as well as important yield components such as higher number of seeds per plant.

More stable productive varieties, with an earliest phenology and lower *Bruchus* infestation will be of great interest under climate change, being their donor accessions valuable resources for present Mediterranean grass pea breeding.

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CRediT authorship contribution statement

Leticia Gonçalves: Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Diego Rubiales:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Manuel Lourenço:** Methodology. **Maria Carlota Vaz Patto:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2024.127142.

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