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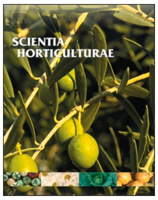

Understanding the phenotypic variability of the olive ‘Galega vulgar’ fruits and oil-related traits across environments

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ABSTRACT

‘Galega vulgar’, a genetically diverse variety, is the main olive tree variety across Portugal, being broadly cultivated across distinct environments. ‘Galega vulgar’ is presently being replaced by higher-yielding, although lower-quality, foreign varieties and efforts must be made to counteract this tendency, and to more efficient explore ‘Galega vulgar’ to the profit of olive tree producers, and the remaining market stakeholders. For that, in the present research, an evaluation of the influence of genetic and environmental factors and their interactions in the phenotypic variability of ‘Galega vulgar’ was performed, focused mainly on important traits to olive stakeholders (namely, fruit-related traits such as fruit and flesh weight and flesh-to-stone ratios and oil-related traits), towards the selection of the best representatives of the variability found. A three-year *in-situ* detailed agronomic characterization was conducted taking advantage of a previously defined ‘Galega vulgar’ collection composed of 595 trees from 95 clones, cultivated across all the Portuguese geographical districts where ‘Galega vulgar’ has representativeness (10 districts), considering thirteen phenotypic traits.

The environment was the main contributor to the phenotypic variability observed on the ‘Galega vulgar’ variety. Significant differences for almost all the analysed traits were detected across years, trees, and geographical districts, with significant district-by-clone interactions. Of the two most frequent clones, clone C002 was the most stable across the geographical districts under study. The present work provides information on the phenotypic variability of ‘Galega vulgar’ important fruit and oil related traits across different environments, of utmost importance from the conservation, breeding, resilience, and production perspectives, considering the pressing need to adapt to climate change effects. In addition, since a significant geographic district x clone interaction was observed, the generated knowledge may now contribute to a better adjustment of the right clone for the right region in what respects new orchards establishment.

A set of ‘Galega vulgar’ trees, primarily identified as belonging to the same clones, was selected due to their ‘residual’ phenotypic variability. These trees will contribute to broadening the genetic basis of a previously defined *ex-situ* conservation base ‘Galega vulgar’ collection of trees, to be trialled in a comparative field experiment, under the same environmental conditions. These experiments are needed for a more precise evaluation of the genetic potential of this variety, towards future genetic studies to clarify the complex genetic control of traditional olive breeding targeted traits and to develop molecular selection tools to support precision olive breeding.

1. Introduction

The olive tree (*Olea europaea* L. subsp. *europaea* var. *europaea*) is an ancient and typical Mediterranean Basin fruit crop, with a background of resilience and climate adaptation, characterized by an enormous genetic heritage, with more than 2600 traditional varieties described (di Rienzo et al., 2018). This vast genetic diversity has been preserved in each Mediterranean country, across different environments and growing systems (Belaj et al., 2016). The olive tree is a strategic crop from the socioeconomic point of view in Southern Europe, especially among

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Mediterranean countries, playing a major role in the bioeconomy sector of these countries, which jointly produce roughly 95 % of the world's supply of olive-based products (Fraga et al., 2021). These olive products are primary elements in the agricultural economy of the EU's southern countries, with about 5 million hectares of plantations and more than €7 000 million in production value every year (Rossi, 2017). In Portugal, the main national variety, widely spread across the country, is the traditional variety 'Galega vulgar', being mostly used for olive oil production (Cordeiro et al., 2014). It is characterized by high vigour, low-weight fruits (< 2 g), resistance to drought, and excellent quality olive oil (Cordeiro et al., 2010). However, 'Galega vulgar' is highly susceptible to several pests and diseases, namely caused by *Colletotrichum* spp. and *Bactrocera oleae* (also known as olive fruit fly and olive anthracnose, respectively), which induce important production losses. In particular, olive anthracnose may cause up to 100 % losses in 'Galega vulgar' production (Talhinhas et al., 2005). This variety is also known for possessing rigid branches, which difficult mechanical harvesting (Cordeiro et al., 2010), preventing its use in intensive production systems. From all the above-described disadvantages, 'Galega vulgar' is being replaced by higher-yielding, although lower quality, foreign varieties, more adapted to intensive systems (Sales et al., 2021; Linos et al., 2014). To counteract this replacement is of utmost importance to prospect and characterize the still available diversity and invest on its conservation, either *in-situ* or *ex-situ*, this last one, through the installation of the prospected diversity in comparative experimental fields.

Previously it was found that a fair amount of genetic diversity was still present in the 'Galega vulgar' variety (Sales et al., 2021). In that study, microsatellite markers were used in a universe of 595 'Galega vulgar' trees from different age groups growing in Portugal, where the authors verified the loss of some microsatellite marker allele combinations, from ancient to centenary 'Galega vulgar' trees, confirming the presence of genetic erosion across time. Despite this genetic diversity reduction in the more recently established 'Galega vulgar' groves (centenary trees), morphological variability was detected among trees in the 'Galega vulgar' endocarps (Sales et al., 2021). Nevertheless, since the analysed trees were growing in different environmental conditions (geographical districts) and the observed morphologic endocarp variability was measured for only one year, it was not possible to precisely discriminate between environmental and genotypic effects on the detected phenotypic variability. Indeed, in the traditional olive breeding targeted traits, such as oil quality and yield, the environmental influence has long been reported (Hamze et al., 2022; Serrano et al., 2021; Navas-López et al., 2020; Borges et al., 2017; León et al., 2016; Rondanini et al., 2014; R.J. Mailer et al., 2010). These breeding targeted traits are often complex, characterized by the contribution of several genes, with relatively small effects, influenced by the environment, and with strong genotype-by-environment interactions (GEI), which can constrain their breeding process (Podlich et al., 2004).

In a climate change context, the phenotypic stability of genotypes is becoming an extremely important breeding target (Sandro et al., 2022; Langridge et al., 2021). This target can only be efficiently tackled for the benefit of farmers, by a better understanding and use of all genotypic, environmental, and especially, GEI effects in the selection of interesting genotypes (Navas-López et al., 2019b; Hassani et al., 2018; Ebrahimi et al., 2016).

Different studies, using different statistical approaches, have been focusing on understanding both genetic and environmental effects on olive traits, to improve the design and effective operation of olive breeding schemes. For instance, in a two-year study, Mousavi et al. (2019) used the Residual Maximum Likelihood procedure (REML) and mixed models, to analyse the plasticity of fruit and oil traits of 113 different olive tree varieties, using five germplasm collections established in the Mediterranean Basin countries and Argentina. Navas-López et al. (2019a) used the Genotype main effects and Genotype × Environment interaction effects model (GGE), to evaluate, in 4 different agro-climatic conditions in Andalusia, the olive tree flowering phenology and flower quality, using data from two harvest seasons on 7 Spanish traditional olive tree varieties and 2 advanced breeding selections. The same authors (Navas-López et al., 2019b, 2020), used also the Additive Main effects and Multiplicative Interaction models (AMMI) to study the environmental effects and the stability and adaptability of genotypes in the chemical compound profiling and the oil accumulation pattern parameters in olive fruits using similar olive germplasm, under similar environments. More recently, Mousavi et al. (2022) applied the General Linear Model (multivariate test) to analyse the bioactive compound profiling of olive fruits from 61 olive tree varieties (representing 12 countries), during two consecutive years, to unravel the effects of genotype, environment, and their interaction on these traits' variation. Nonetheless, the availability of GEI studies on fruit and oil traits on diverse olive varieties, such as 'Galega vulgar', is still scarce.

To counteract the 'Galega vulgar' replacement in the Portuguese olive groves and to explore this variety more efficiently to the profit of olive tree producers and the remaining market stakeholders, we evaluated the influence of genetic and environmental factors and their interactions in the phenotypic variability of several fruit and oil-related traits in 'Galega vulgar' variety. The knowledge here generated will contribute not only to a better understanding of the phenotypic variability of this genetically diverse olive tree variety, but also to select the best representative trees of the diversity found to establish an experimental field as an *ex-situ* conservation strategy. For that, we took advantage of an existing *in-situ* collection of 595 'Galega vulgar' trees, clustered into 95 previously genetically identified clones (Sales et al., 2021). Due to the reduced number of microsatellite markers ($n = 14$) used previously to define clones among 'Galega vulgar' individuals (Sales et al., 2021), and knowing that they are molecular markers with reduced genomic abundance (Sion et al., 2021), individuals with different overall genotypes may have clustered in the same pre-defined genetic clone (i.e., some intra-clonal residual genotypic diversity could still exist). Therefore, the base collection established with 95 distinct clones, representing the 'Galega vulgar' genetic diversity existing at the Portuguese olive groves (Sales et al., 2021), might be still complemented with some more distinct genotypes (trees).

As a consequence of all the above, the detailed objectives of this study were to: (i) determine the contribution of genetic, environmental, and GEI effects in the fruits and oil phenotypic variability of the 'Galega vulgar' variety, (ii) evaluate the stability of the most frequent clones of 'Galega vulgar' across different environments, and (iii) identify, within each previously defined clone, morphologically different trees to complement the 'Galega vulgar' base collection. This extended base collection will be established in an experimental field, under the same environmental conditions, as an *ex-situ* conservation strategy and as the optimal experimental design for an effective selection of high performing genotypes and for the identification of important breeding traits associated molecular markers to support precision olive breeding.

2. Material and methods

2.1. Plant material and environments definition

In this work, we took advantage of a 'Galega vulgar' *in-situ* collection previously defined across ten geographical districts (Fig. 1), which embody the different Portuguese traditional regions of olive production where 'Galega vulgar' has a major representativeness (Sales et al., 2021).

The *in-situ* collection comprised 595 trees (260 ancient and 335 centennial olive trees) from the 'Galega vulgar' variety, primarily clustered into 95 genetically distinct clones (Sales et al., 2021). Due to the alternate bearing, wildfires, and the elderly age of some olive trees, some trees had no production in some of the three years of this study (harvest seasons of 2016–2017, 2017–2018, and 2018–2019). Therefore, a first data set, data set 1, was defined comprising 375 of the initial 595 prospected trees (with production in at least two of the three

Fig. 1. The geographical location of the ten Portuguese districts (all the Portuguese geographical districts where 'Galega vulgar' has a major representativeness), considered in this study (Google Earth, 2023). The pink points represent the capital city of each geographical district: 1- Beja, 2- Castelo Branco, 3- Coimbra, 4- Évora, 5- Lisboa, 6- Leiria, 7- Portalegre, 8- Santarém, 9- Setúbal, 10- Viseu.

monitored years; Supplementary Table S1). These 375 trees clustered in 72 of the previously genetically distinct clones defined by Sales et al. (2021). Eleven of these clones, within this data set 1, were constituted by at least four trees, and the two most frequent clones (C001 and C002), had in this data set more than 68 trees. For the case of the oil content trait, from the initial data set with 595 trees, only 212 had oil-related data. Therefore, a second data set, data set 2, was defined comprising 212 trees with data available for at least one year of monitoring (Supplementary Table S2). These 212 trees were clustered in 44 clones, being 7 of them constituted by at least four trees, and the two most frequent clones (C001 and C002) had in this data set more than 29 trees. Given this data constraint, and to integrate both morphological and oil traits, a combined data set (data set 3) was also defined overlapping data sets 1 and 2, with trees with data for all the analysed traits (181 trees) in at least one monitored year (Supplementary Table S3). This more comprising data set was used for the multivariate analysis and all the analysis run in batches. These 181 trees were clustered in 42 clones. Six of these clones were constituted by at least four trees, and the two most frequent clones (C001 and C002) had in this data set more than 21 trees.

For an edaphic-climatic characterization of each of the ten geographical districts, data for the main meteorological parameters – mean, maximum and minimum temperature, rainfall, mean and total evapotranspiration, mean wind speed, and available water storage capacity – were obtained from weather stations located in each of the analysed districts (annual values) and managed by the Portuguese Institute for Sea and Atmosphere, I.P. (IPMA IP). Using the global position system (GPS) coordinates of each analysed tree (Sales et al., 2021), the respective soil parameters (topsoil and subsoil pH, organic carbon, and salinity) were retrieved through the Harmonized World Soil Database v 1.2 (Nachtergaele et al., 2012). A complete list of soil and meteorological data, in the three monitoring years, from the ten geographical districts is available in Supplementary Table S1. To summarize multivariate similarities among geographical districts, and to identify the most relevant weather and soil parameters to discriminate among them, a multivariate principal component analysis (PCA), based on standardized meteorological and soil data, operated on a correlation matrix formed from the data variates, was performed.

2.2. *Phenotypic data acquisition*

2.2.1. *Fresh fruits*

In each harvest season, 100 mature fruits *per* tree were randomly collected across the tree canopy and stored in a refrigerated cooler box until transferred to the laboratory. In the laboratory, samples were standardized by fruit colour and size. Using 50 fruits *per* tree, three morphological traits were quantified on fresh fruits using a laboratory precision scale (Radwag, PS 4500/C/2): the mean fruit mass (**F**ruit **W**eight (FW)), the flesh and stone mean mass (**F**lesh **F**resh **W**eight (FFW), and **S**tone **F**resh **W**eight (SFW)), on a wet weight basis. The flesh-to-stone ratio on a wet weight basis was also calculated (**F**lesh to **S**tone ratio **F**resh **W**eight (F/S FW)). The percentage of fruits affected by diseases and pests, namely olive anthracnose (***Col***letotrichum spp.) (COL) and olive fruit fly (***Bact***rocera oleae) (BACT) was also recorded considering 100 fruits/tree. The total percentage of the affected fruits (**Pest**s) (PEST) was calculated as the sum of the traits COL and BACT.

2.2.2. *Dried fruits, oil extraction, and quantification*

After drying the olives in a forced-air oven at 105 °C, for around 42 h (i.e., until reaching constant mass) (del Río and Romero, 1999), four morphological traits were quantified on a dry weight basis: the flesh and stone masses (**F**lesh **D**ry **W**eight (FDW), **S**tone **D**ry **W**eight (SDW)), and the flesh and stone moistures (**F**lesh **M**oisture (FM), **S**tone **M**oisture (SM)). The flesh-to-stone ratio on a dry weight basis was also calculated (**F**lesh to **S**tone ratio **D**ry **W**eight (F/S DW)).

For total oil extraction and quantification purposes, only the dry flesh mass from olives was considered, since more than 95 % of the total oil is present in the flesh of the fruits (Frías et al., 2001). The olives' flesh was milled in a laboratory mill (Ivymen, 5810000) and the samples were then stored at −20 °C until further analysis. Since the total oil extraction and quantification was intended in this study (the oil quality and its components were not under analysis), the extraction was performed by Microwave-Assisted Extraction (MAE) methodology, using an Advanced Microwave Digestion and Extraction System SK-15 medium pressure rotor ETHOS EASY (Milestone, Italy), in three replications *per* tree, following the procedure described by Virot et al. (2007), optimized for the equipment of MAE extraction: 3 g of olive dried flesh were mixed with 40 mL *n*-hexane of analytical grade (VWR International, Darmstadt, Germany) and placed in modified polytetrafluoroethylene (TFM) vessels together with Weflon stirring bars. This mixture was subjected to a microwave treatment at 90 °C for 10 min of extraction time, with a 60 % stirring speed and pulsate power up to 1000 W. The heating ramp-up time was 10 min with a cooling time of 15 min (exclusive of the extraction time). After the extraction, the miscella was removed from the oil through decanting followed by centrifugation (Centrifuge K241, Centurion Scientific, United Kingdom) at 4427 × *g* for 10 min. The solvent was evaporated using a rotary vacuum evaporator (Heidolph, Hei-VAP Value, Heidolph Instruments GmbH & Co. KG, Germany) set to 40 °C and 200 rpm. For oil quantification (fat content), the formula given in AOAC 920.39B (AOAC, 2000) was adopted. The results were expressed as a percentage of dry fruit weight (OIL).

2.3. Phenotypic data analysis

2.3.1. Data quality control

Data quality control was performed on data set 1 (375 trees), for the 12 measured traits (BACT, COL, FW, FDW, FFW, FM, F/S DW, F/S FW, PEST, SDW, SFW, SM) and on data set 2 (212 trees) for the OIL trait. Graphical inspection of residuals was used to evaluate normality (quantile-quantile (QQ) plot), homogeneity of variance (residuals vs fitted values) and to identify outliers. To confirm the data normality, a Shapiro-Wilk test was performed and when needed, the BOX-COX transformation procedure, as implemented in Genstat® software, was carried out (Genstat® for Windows 20th edition, Hemel Hempstead, UK) (VSN, 2020). Data set 3 was defined after quality control of data set 1 and 2.

For the BACT trait, which contained several observations with zero values, 0.005 was added to the original measured value before the transformation. The traits whose values were transformed were re-coded by adding the suffix 'Tran' (BOX-COX transformed) to the initial code label.

2.3.2. Diversity analysis

To determine the contribution of genetic (tree), environmental (year), and GEI (year by tree interaction) effects in the fruits and oil phenotypic variability of the 'Galega vulgar' variety, a linear mixed model was applied to the more comprising data set (data set 3), using the restricted maximum likelihood (REML) procedure (Genstat® software). The model was defined as *Trait* = *year* + *tree* + *year.tree*. A two-step approach was employed as explained below. In a first step, all terms were fitted as random, and the model was used to obtain the best linear unbiased predictors (BLUPs) to estimate the variance components of each term and the broad-sense heritability. The broad-sense heritability (H^2) was estimated as the ratio between genotypic and phenotypic variances: $\sigma^2G/(\sigma^2G + \sigma^2GE + \sigma^2\varepsilon)$ (accordingly to Mousavi et al., 2019). In a second step, the best linear unbiased estimators (BLUEs) were calculated while setting the terms *tree* and *year* as fixed and the other terms as random. A Wald test for the significance of the fixed effects was performed using the generated BLUEs data set, and Pearsoń's correlation coefficients were calculated among traits.

Next, considering the primarily genetically defined clones, within the screened geographical districts (Sales et al., 2021), a linear mixed model was applied also to data set 3, using the REML procedure (using Genstat® software) to test for significant phenotypic differences among clones, among districts and for significant interaction between clones and districts. For each trait, the model applied was *Trait* = *year* + *district* + *clone* + *year.district* + *year.clone* + *district.clone* with terms *district, clone*, and *district.clone* fitted as fixed and terms *year, year.district*, and *year.clone* fitted as random. A Wald test for the significance of fixed effects was applied.

2.3.3. Environment adaptation of the most frequent clones

After testing the interaction between districts and clones, the level of stability of the most frequent clones on the detailed data sets (data set 1 and 2) were assessed, here defined as the clones present in more than two districts and with more than one tree per district (only two clones, C001 and C002, complied with these criteria). The following linear model (REML procedure) was applied to each clone data separately using the Genstat® software: *Trait* = *year* + *district* + *year.district*, with all terms fitted as fixed in the model. A Wald test for the significance of fixed effects was applied. Moreover, for multiple comparison of means, Fisher's least significant difference (LSD) post-hoc tests, at a 95 % confidence level, were applied to identify the geographical districts in which each clone behaved differently.

2.3.4. Identification of phenotypically diverse 'Galega vulgar' trees within clones

To assess if there were significant differences among trees, within the primarily defined clones (Sales et al., 2021) and using only the clones with available agronomic data from a minimum number of individuals (here defined as having at least data from four individuals), a linear mixed model (REML procedure, using Genstat® software) was applied separately for each of the 11 clones that complied with this numerical criterion on data sets 1 and 2. The applied model to each clone data was *Trait* = *year* + *tree* + *year.tree* (*tree* as fixed term and *year* and *year.tree* as random terms). A Wald test for the significance of fixed effects was applied.

To visualize the within-clone phenotypic variability and support the identification of the more contrasting trees within the previously assessed clones, a multivariate PCA based on the best linear unbiased estimators (BLUEs) for each tree per clone, on the clones with at least significant differences in three traits (7 clones complied with this criterion), was performed. 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 SCREE plot) and the cumulative proportion of variance explained by them.

To complement the selection of the most contrasting trees, within the clones with more than four and less than 21 individuals, (8 clones) a Fisher's LSD post-hoc test at 95 % confidence level was applied to compare the trees adjusted means for the different traits within each clone.

The selection of the most contrasting trees focused mainly on the most important traits for future genetic studies (namely, fruit-related traits such as fruit and flesh weight and flesh-to-stone ratios).

3. Results and discussion

3.1. Defining environments

The PCA on standardized meteorological and soil data of each tree location showed that several of the analysed trees were grouped by district and year, defining particular geographical district/year combinations as sufficiently differentiated environments (Fig. 2).

By inspecting the PCA biplot, we could clearly define "Leiria", "Coimbra", and "Viseu" environments (all belonging to Portugal' Central region) as the most distinct ones among each other (separated mainly by soil-related parameters) in comparison with the remaining districts, with "Lisboa" and, to a certain extent, "Portalegre", with differentiated, but intermediate positions. These five districts were separated from the other more similar districts ("Évora", "Beja", "Castelo Branco", "Setúbal", and "Santarém") based mainly on the rainfall and temperatures. The first and the second PC together explained 59.8 % of the observed variance.

3.2. Phenotypic diversity analysis

In the data quality control, no phenotypic variation was detected for the traits COL and PEST, possibly because of a reduced natural infestation in the studied years, not allowing the evaluation with the necessary reliability of the responses of the trees. Therefore, these two traits were not included in the subsequent analysis.

From the linear mixed model analysis and as shown in Table 1, environment was the main contributor to the phenotypic diversity observed on the 'Galega vulgar' variety for the majority of the traits, since the highest percentages of the phenotypic variance were due to environmental (year) effects ($\sigma 2$ *year*) for almost all the traits.

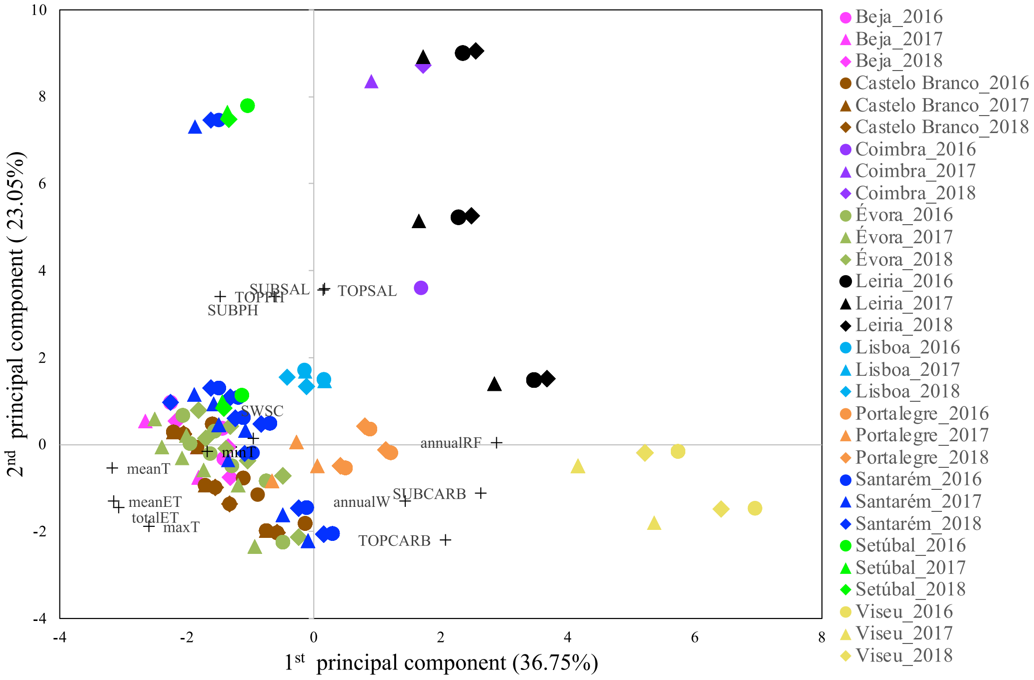

Fig. 2. PCA biplot based on the meteorological and soil data recorded in three different years, on ten geographical districts, using 375 'Galega vulgar' trees locations. Different colours represent different districts and different shapes (circles, triangles, and rhombi) represent different years (2016, 2017 and 2018, respectively). Meteorological and soil parameters' abbreviations: annualRF – annual rainfall (mm/year); annualW – annual wind speed mean (Km/h); maxT - maximum annual temperature (°C); meanET – annual evapotranspiration mean (mm); meanT – annual temperature mean (°C); minT – minimum annual temperature (°C); SUBCARB – subsoil organic carbon (% weight); SUBPH – subsoil pH; SUBSAL – subsoil salinity (dS/ m); SWSC – soil available water storage capacity (mm); TOPCARB – topsoil organic carbon (% weight); TOPPH – topsoil pH; TOPSAL – topsoil salinity (dS/m); totalET – total annual evapotranspiration (mm).

Table 1
Variance components and broad-sense heritability for the eleven traits measured in 181 'Galega vulgar' trees (data set 3).

Trait	Variance components				H^2
	σ^2_{year}	σ^2_{tree}	$\sigma^2_{year.tree}$	$\sigma^2_{residual}$	
BACTTran	5.13E+00	1.64E-01	3.41E+00	4.83E-02	0.05
FWTran	5.99E-01	8.50E-02	9.53E-02	1.93E-09	0.47
FDWTran	4.39E-01	1.68E-02	2.48E-02	7.17E-10	0.40
FFWTran	1.38E+02	4.08E-02	7.69E-02	7.07E-07	0.35
FMTran	1.65E-01	4.60E-04	5.46E-04	1.84E-10	0.46
F/S DWTran	1.86E+00	4.48E-02	5.59E-02	4.15E-09	0.45
F/S FWTran	3.38E-01	5.91E-01	5.92E-01	8.92E-06	0.50
SDWTran	1.07E-07	1.16E-06	4.51E-05	2.25E-12	0.03
SFWTran	2.06E-01	5.60E-05	1.26E-04	3.43E-11	0.31
SMTran	1.43E-04	2.60E-05	1.13E-04	9.88E-07	0.19
OILTran	3.00E-04	5.29E-04	1.15E-03	–	–

Note: BACTTran, olive fruit fly transformed; FWTran, mean fruit mass transformed; FDWTran, flesh mass on dry weight basis transformed; FFWTran, flesh mean mass on wet weight basis transformed; FMTran, flesh moisture transformed; F/S DWTran, flesh to stone ratio on dry weight basis transformed; F/S FWTran – flesh to stone ratio on wet weight basis transformed; SDWTran, stone mass on dry weight basis transformed; SFWTran, stone mean mass on wet weight basis transformed; SMTran, stone moisture transformed; OILTran, oil content transformed.

Exceptions were F/S FWTran, SDWTran, and OILTran, where the highest percentage of phenotypic variance was due to the year-by-tree interaction effect ($\sigma 2$ *year.tree*). These results concur with previous observations made across different years/locations on fruits from different olive varieties (Navas-López et al., 2019a,b, 2020; Borges et al., 2017; Rondanini et al., 2014; Mailer et al., 2007, 2010; Beltran et al., 2004) or across artificial environments accomplished by manipulating field temperatures (Garcia-Inza et al., 2014). Notwithstanding, in some of these studies the genotypic effect has been documented as the main source of variation for olive traits, especially oil composition-related traits (Navas-López et al., 2020; Salazar-García et al., 2018; Beltran et al., 2016) and for the fruit fresh weight trait (Mousavi et al., 2019). In the present study, the stronger contribution of the environment to the 'Galega vulgar' phenotypic variability could be explained by the fact that all the trees in the present study belong to the same olive variety. Indeed, according to Sales et al. (2021), these 'Galega vulgar' trees differ only in up to eight alleles (considering 14 SSRs), contrary to the above-mentioned studies, where different olive tree varieties were compared, potentially with higher allelic differentiation among them. Navas-López et al. (2020), compared seven different traditional varieties, plus two advanced breeding selections; the study of Mousavi et al. (2019) analysed 408 olive accessions, belonging to 113 different varieties, from 16 countries; Salazar-García et al. (2018) compared 31 olive tree varieties from 11 different municipalities from Valencian Community in Spain; and Beltran et al. (2016) evaluated 28 different varieties from the World Olive Germplasm Collection of IFAPA in Córdoba. In this last study, most of the analysed varieties come from the Worldwide Olive Germplasm Banks, namely WOGB from Córdoba and from Marrakech, which characterized their accessions with SSR molecular markers in previous studies, reporting allelic differences on average of 40.74 and 27 alleles, respectively (Trujillo et al., 2014; Haouane et al., 2011). These are indeed much bigger average allelic differences than the ones reported in the 'Galega vulgar' variety (Sales et al., 2021).

The broad-sense heritability (H^2) ranged from 0.03 to 0.50, over the analysed traits. F/S FWTran had the highest H^2 estimates (0.50); other traits, especially FWTran, FMTran, and F/S DWTran, had also high values (0.47, 0.46, 0.45), while the lowest value was calculated for SDWTran (0.03).

As shown in Table S4, significant differences for almost all the analysed traits (P-value < 0.05, Wald test) were detected in the different years and among trees. These significant differences detected among the evaluated trees point to an interesting breeding potential within this collection of trees, considering that the existence of genetic diversity is the prime basis for breeding (Shilpashree et al., 2021). No significant differences were detected among years only for OILTran trait (Table S4).

Pearson's correlation coefficients between the eleven traits assessed were calculated. Some expected strong correlations ($r > 0.900$, $P < 0.001$), were identified among several traits. Overall, the flesh mean mass on wet weight basis (FFWTran) was strongly and positively correlated with fresh mean fruit mass (FWTran) ($r = 0.9847$), and flesh to stone ratio on wet weight basis (F/S FWTran) ($r = 0.9130$). Moreover, strong and positive correlations were detected between flesh to stone ratio on dry weight basis (F/S DWTran) and the flesh mass on dry weight basis (FDWTran) ($r = 0.9015$) and between stone mean mass on wet weight basis (SFWTran) and stone mass on dry weight basis (SDWTran) ($r = 0.9678$) (Fig. 3). To the best of our knowledge, previous studies provide limited information on comparing these fruit and oil-related traits, hampering a direct comparison with the results obtained in this work. Nevertheless, due to the strong correlations found, although all these traits are important selection targets in olive breeding, in future large screenings of individuals, with limited resources, we propose the use of only the less time-consuming parameters (like flesh and stone mass in wet basis) to characterize the fruits.

From the liner mixed model analysis used to test for trait differences among clones and geographical districts, no significant differences (P-value < 0.05, Wald test) were detected among clones for all the analysed traits (Table S5). These results indicate a lack of accordance between the genetic and phenotypic diversity analysed. This situation may in part be due to a partial overlap/incomplete separation of clones, based on just a few SSR molecular markers ($n = 14$) (Sales et al., 2021). Indeed, this type of marker is known to have reduced genomic abundance (Sion et al., 2021), and although microsatellite markers present an efficient way to identify varieties, in contrast, they generally fail to distinguish between highly similar clones and to detect intra-varietal differences (Zombardo et al., 2022; Pelsy, 2010).

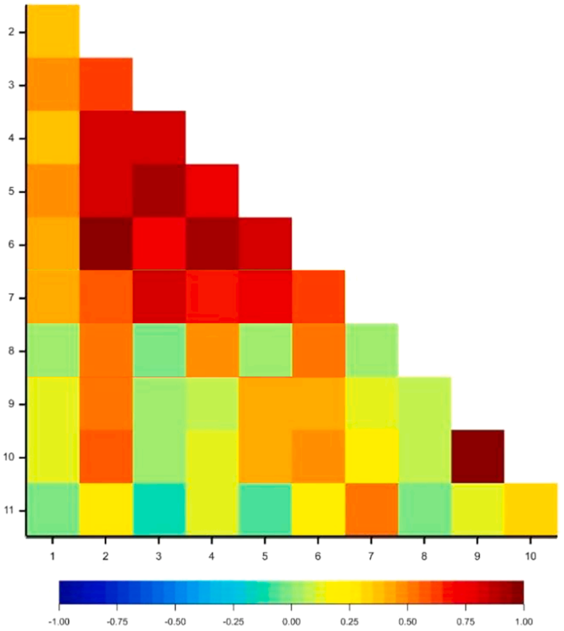

Fig. 3. Heatmap of Pearson's pairwise correlations, between eleven traits, measured in the 181 'Galega vulgar' trees (data set 3). Traits' key: 1 - BACTTran, olive fruit fly transformed; 2 – FWTran, mean fruit mass transformed; 3 - F/S DWTran, flesh to stone ratio on dry weight basis transformed; 4 - F/S FWTran – flesh to stone ratio on wet weight basis transformed; 5 - FDWTran, flesh mass on dry weight basis transformed; 6 – FFWTran, flesh mean mass on wet weight basis transformed; 7 – OILTran, oil content transformed; 8 – FMTran, flesh moisture transformed; 9 – SDWTran, stone mass on dry weight basis transformed; 10 – SFWTran, stone mean mass on wet weight basis transformed; 11 – SMTran, stone moisture transformed.

Nonetheless, significant differences were observed among districts in the traits BACTTran, FWTran, F/S DWTran, and F/S FWTran (P-value $<$ 0.05, Wald test). Regarding the interaction between district x clone, significant effects were detected for BACTTran, FWTran, FFWTran, FMTran, and SFWTran (Table S5). According to Navas-López et al. (2019a), high interaction between genotype and environment indicates that it is very difficult to predict the behaviour of a particular genotype in a given location. Furthermore, this behaviour might change if the climatic conditions of a specific geographic region changes. Nevertheless, the detected GEI might be also explored in the future through resorting to olive local experimentation for a better adaptation/choice of the best clonal material to be used in particular environments, allowing higher quality/production in the new orchards.

3.3. *Environment adaptation of the most frequent clones*

When analysing the environmental stability of the two most frequent clones (C001 and C002) and considering the six districts where the two clones were simultaneously found ("Santarém", "Castelo Branco", "Beja", "Portalegre", "Leiria" and "Évora"), clone C002 was more stable across years and districts, when compared with clone C001. Significant differences (P-value < 0.05, Wald test) between years, districts, and year x districts were observed for all traits in clone C001, except for FDWTran and OILTran (year), FMTran (district), and F/S DWTran (year.district) (Table S6). In clone C002 significant differences (P-value < 0.05, Wald test) between years and districts were detected for all traits, except for BACTTran, FDWTran, and OILTran (year) and SMTran and OILTran (district) (Table S7). Regarding year x district interaction, significant differences were only observed for SFWTran in clone C002 (Table S7).

The adoption of strategies that allow investigating the stability of different varieties is crucial in a climate-changing world scenario. These approaches will be useful when choosing the more adapted trees to counteract the consequences of climate changes (Zelasco et al., 2021), but also when considering the introduction of olive trees into new crop environments (Borges et al., 2017; Torres et al., 2017). Clone C002 may benefit a breeding program with trees of greater stability given the ongoing climate change. Furthermore, in the breeding process, it is fundamental to assess the genotype's performance in different environments due to environmental variations or to the GEI. These differences might affect the comparative behaviour of different genotypes in several environments, and the best approach to manage GEI is to select highly adaptable and stable genotypes (Sousa et al., 2019). Nevertheless, since this clone was only present in 6 of the 10 different environments that represent the Portuguese olive growing regions, future testing should include a more diverse set of environments to prove a wider stability of this 'Galega vulgar' clone. It will be relevant to test it namely in "Viseu", "Setúbal" and "Coimbra" districts, considered quite distinct at an edaphic-climatic level.

Within clone C001, the districts of "Viseu", "Beja" and "Évora" showed to be the most differentiated from each other, with more traits with significant differences among them (significant differences in at least 5 traits). There were several pairs of districts between which clone C001 trees behaved similarly, with only one or two significantly different traits ("Santarém"/"Castelo Branco", with significant differences in BACTTran; "Viseu"/"Santarém", with differences in SDWTran and SMTran; and "Castelo Branco"/ "Beja", with differences in BACTTran and FDWTran) (Table 2). Nevertheless, this differentiation only partially concurs with the edaphic-climatic differentiation of the same environments. As an example, differences in rainfall and average temperatures might partially explain the distinct behaviour of C001 trees grown in "Viseu" (higher rainfall, lower temperatures) compared to C001 trees grown in "Évora" or "Beja" districts (lower rainfall, higher temperatures). However, the significant differences detected also among C001 trees grown in "Évora" when compared with the ones grown in "Beja" could not be readily explained by the previous meteorological parameters, since these parameters do not differentiate these two

Table 2
Mean comparisons for the most frequent ‘Galega vulgar’ clones (C001, C002), for the eleven studied traits among districts.

District Trait	Viseu	Santarém	Castelo Branco	Beja	Portalegre	Leiria	Évora
C001[1]							
BACTTran	-1.705^{a}	-2.146^{a}	-4.125^{bc}	-4.380^{c}	-4.194^{bc}	-2.23^{a}	-3.593^{b}
FWTran	0.829^{a}	0.829^{a}	0.797^{a}	0.614^{b}	0.595^{b}	0.485^{bc}	0.436^{c}
FDWTran	-0.689^{a}	-0.710^{a}	-0.727^{ab}	-0.821^{cd}	-0.752^{abc}	-0.823^{bcd}	-0.857^{d}
FFWTran	0.359^{a}	0.348^{a}	0.312^{ab}	0.177^{bc}	0.169^{c}	0.044^{cd}	0.004^{d}
FMTran	na						
F/S DWTran	0.505^{a}	0.492^{a}	0.388^{ab}	0.249^{bd}	0.454^{a}	0.404^{ab}	0.155^{d}
F/S FWTran	3.259^{a}	3.068^{ab}	2.843^{bc}	2.437^{c}	2.757^{bc}	2.620^{bc}	1.832^{d}
SDWTran	-0.421^{bc}	-0.419^{a}	-0.418^{a}	-0.419^{ab}	-0.422^{c}	-0.426^{d}	-0.417^{a}
SFWTran	-0.397^{bc}	-0.395^{ab}	-0.392^{ab}	-0.395^{ab}	-0.402^{cd}	-0.408^{d}	-0.391^{a}
SMTran	-0.642^{a}	-0.653^{bc}	-0.649^{b}	-0.653^{bc}	-0.655^{c}	-0.648^{ab}	-0.652^{bc}
OILTran	-0.491^{a}	—	—	—	-0.522^{b}	—	-0.582^{c}
C002[1]							
BACTTran	—	-2.346^{a}	-2.427^{ab}	-5.298^{d}	-3.772^{bcd}	-2.967^{abc}	-4.009^{cd}
FWTran	—	0.784^{ab}	0.647^{bc}	1.174^{a}	0.655^{bc}	0.416^{cd}	0.358^{d}
FDWTran	—	-0.730^{a}	-0.787^{a}	-0.610^{a}	-0.718^{a}	-0.7780^{a}	-0.935^{b}
FFWTran	—	0.301^{b}	0.228^{bc}	0.647^{a}	0.218^{bc}	0.035^{cd}	-0.051^{d}
FMTran	—	-0.262^{a}	-0.254^{a}	-0.246^{a}	-0.273^{ab}	-0.296^{b}	-0.254^{a}
F/S DWTran	—	0.389^{a}	0.377^{a}	0.556^{a}	0.491^{a}	0.444^{a}	0.078^{b}
F/S FWTran	—	2.779^{ab}	3.034^{ab}	3.770^{a}	2.822^{ab}	2.391^{bc}	1.833^{c}
SDWTran	—	Na					
SFWTran	—	-0.392^{ab}	-0.400^{abc}	-0.388^{a}	-0.3985^{abc}	-0.405^{c}	-0.398^{ac}
SMTran	— na						
OILTran	— na						

Note: BACTTran, olive fruit fly transformed; FWTran, mean fruit mass transformed; FDWTran, flesh mass on dry weight basis transformed; FFWTran, flesh mean mass on wet weight basis transformed; FMTran, flesh moisture transformed; F/S DWTran, flesh to stone ratio on dry weight basis transformed; F/S FWTran – flesh to stone ratio on wet weight basis transformed; SDWTran, stone mass on dry weight basis transformed; SFWTran, stone mean mass on wet weight basis transformed; SMTran, stone moisture transformed; OILTran, oil content transformed.

[1] Fisher's LSD test at 95 % confidence level - mean values in each row followed by the same letter are not significantly different at P-value < 0.05.

na: non-available. Fisher's LSD – comparisons were not calculated as the variance ratio for the district was not significant.

geographical districts.

Comparable situations were detected in the case of C002 trees. Overall, for clone C002, “Santarém”/“Castelo Branco” and “Santarém”/“Évora”, as well as “Beja”/“Leiria” proved to be the pairs of districts with more significant differences between each other when compared with the other districts (significant differences in at least six traits). Within the pairs “Santarém”/ “Castelo Branco”, “Castelo Branco”/ “Portalegre” and “Portalegre”/ “Leiria”, clone C002 trees behaved similarly, with no significant differences among all the measured traits. Similar meteorological environments (rainfall and temperatures) might in part explain the indistinguishable behaviour of C002 trees grown across “Santarém” and “Castelo Branco”. Nevertheless, “Portalegre” and “Leiria” were characterized as distinguishable meteorological and soil environments, but C002 trees behaved similarly across these two geographical districts. This lower accordance of results may in part be due to the simplified environment characterization that we used in the present study, based on a reduced set of meteorological and soil parameters. Moreover, the two first principal components of the environmental PCA (Fig. 2) explained only 59.8 % of the variation observed among geographical districts, which might also contribute to this lower accordance of results. Nevertheless, it is well known that specific environmental conditions and geographic locations may influence olive tree characteristics. The importance of the impact of several edaphic-climatic conditions, such as rainfall, soil properties, and temperature has already been discussed in previous studies. Rodrigo-Comino et al. (2021) reported that the impact of rainfall on olive production is variable and depends on drought intensity and the monthly rainfall distribution. Moreover, they also recognised that other key factors have also important influences on the fluctuations of olive production over the years, such as soil properties. In addition, abiotic factors such as temperature, precipitation, solar radiation, relative humidity and wind, directly interfere in the manifestation of olive pests and diseases (Caselli et al., 2021; Fraga et al., 2021).

For four other pairs of districts, with only one or two significantly different traits among them, similar situations occurred to C001 and C002 clones (“Santarém”/“Portalegre”, with BACTTran; “Castelo Branco”/“Leiria”, with FMTran; “Santarém”/“Beja”, with BACTTran and FFWTran; “Beja”/“Portalegre”, with FWTran and FFWTran) (Table 2).

3.4. Selection of phenotypically diverse ‘Galega vulgar’ trees within clones

A set of ‘Galega vulgar’ trees, primarily identified as belonging to the same clones (clones with at least four trees: clones C001 to C013, excluding the clones C011 and C012, that were only constituted by one and three trees, respectively), was selected due to their ‘residual’ phenotypic variability. Based on the linear mixed model analysis significant differences were detected (P-value < 0.05, Wald test) for several of the traits analysed. Exceptions were with no differences, for clone C001, the trait OILTran, for clone C002, SDWTran, and SFWTran, for clone C004, BACTTran, and SMTran and clone C005, BACTTran, FMTran, and SMTran (Table 3). In the case of clones C008, C009, and C010 significant differences were only detected in four of the analysed traits (Table 3). Furthermore, for clones C003, C006, and C013 significant differences were observed in only one trait, and in the case of clones C007, and C012, no significant differences were detected for any of the respective analysed traits (Table 3).

From the combined analysis of the multivariate PCA performed separately for each clone with significant differences in at least three traits (Figs. 4 and 5; Figures S1 to S5) and the clones' variable traits means comparison via Fisher's test (Table 4), a selection of the most contrasting trees within each clone, was accomplished (Table 5). For the two most frequent clones, the selection of trees was only based on the PCA analysis, and the 10 most contrasting trees (5 %) were selected based on the fruit and flesh weights and flesh-to-stone ratios traits. For the clones with less than 21 trees, the selection was based on both the PCA and Table 4 results. Only the two most contrasting trees, based on fruit and flesh weights and flesh-to-stone ratio traits, were selected. In particular, and as can be seen in Fig. 4, for clone C001, the first two components of the PCA explained 68.66 % of the variability. The phenotypic variability across the first principal component (PC1) was

Table 3

Mean comparison analysis for the eleven traits measured in 375 ‘Galega vulgar’ trees clustered in 11 clones with at least four trees (or 212 ‘Galega vulgar’ trees for the OILTran trait, clustered in 7 clones with at least four trees).

	Wald statistic (*P-value*)										
Clone	BACTTran	FW Tran	FDW Tran	FFW Tran	FMTran	F/S DW Tran	F/S FW Tran	SDW Tran	SFW Tran	SMTran	OILTran
C001	415.50 (< 0.001)	341.51 (< 0.001)	316.09 (< 0.001)	352.02 (< 0.001)	428.17 (< 0.001)	452.36 (< 0.001)	468.72 (< 0.001)	205.87 (0.038)	208.44 (0.032)	302.86 (< 0.001)	20.24 (0.063)
C002	123.40 (0.005)	178.64 (< 0.001)	123.49 (0.005)	189.37 (< 0.001)	174.03 (< 0.001)	171.87 (< 0.001)	252.54 (< 0.001)	91.10 (0.092)	87.99 (0.119)	98.53 (0.048)	–
C003	12.00 (0.338)	8.13 (0.557)	23.19 (0.081)	7.55 (0.600)	14.34 (0.245)	30.99 (0.034)	10.85 (0.389)	6.46 (0.685)	6.46 (0.685)	18.04 (0.153)	18.04 (0.153)
C004	15.04 (0.375)	108.62 (< 0.001)	79.33 (< 0.001)	109.58 (< 0.001)	29.91 (0.048)	56.56 (0.002)	43.92 (0.008)	40.17 (0.013)	40.06 (0.013)	9.79 (0.695)	–
C005	19.69 (0.051)	44.60 (0.004)	61.61 (0.001)	50.06 (0.003)	8.92 (0.284)	77.94 (< 0.001)	50.66 (0.002)	44.48 (0.004)	46.68 (0.003)	6.76 (0.418)	–
C006	16.29 (0.110)	12.89 (0.150)	26.99 (0.033)	9.23 (0.249)	12.05 (0.172)	12.18 (0.158)	5.85 (0.424)	8.24 (0.280)	6.71 (0.363)	2.98 (0.708)	–
C007	–	15.35 (0.183)	15.28 (0.181)	13.26 (0.252)	20.79 (0.082)	18.03 (0.122)	12.83 (0.270)	17.27 (0.138)	11.93 (0.312)	15.07 (0.193)	3.70 (0.842)
C008	–	69.34 (0.005)	267.48 (< 0.001)	91.77 (0.003)	78.34 (0.004)	31.68 (0.014)	12.26 (0.096)	15.10 (0.058)	11.45 (0.094)	6.17 (0.249)	–
C009	4.08 (0.575)	24.76 (0.032)	17.23 (0.071)	28.24 (0.024)	2.88 (0.719)	27.17 (0.026)	23.96 (0.036)	–	7.45 (0.304)	2.99 (0.705)	–
C010	1.47 (0.916)	20.00 (0.042)	10.00 (0.188)	21.48 (0.037)	8.78 (0.239)	23.32 (0.033)	30.37 (0.015)	3.47 (0.644)	3.18 (0.681)	5.08 (0.472)	–
C012	8.45 (0.197)	3.65 (0.313)	–	3.14 (0.362)	15.71 (0.102)	5.42 (0.213)	6.78 (0.189)	–	0.50 (0.793)	0.26 (0.885)	–
C013	4.28 (0.327)	11.74 (0.085)	–	11.90 (0.084)	17.83 (0.042)	–	14.32 (0.061)	2.15 (0.581)	2.47 (0.532)	9.91 (0.115)	–

Note: BACTTran, olive fruit fly transformed; FWTran, mean fruit mass transformed; FDWTran, flesh mass on dry weight basis transformed; FFWTran, flesh mean mass on wet weight basis transformed; FMTran, flesh moisture transformed; F/S DWTran, flesh to stone ratio on dry weight basis transformed; F/S FWTran – flesh to stone ratio on wet weight basis transformed; SDWTran, stone mass on dry weight basis transformed; SFWTran, stone mean mass on wet weight basis transformed; SMTran, stone moisture transformed; OILTran, oil content transformed.

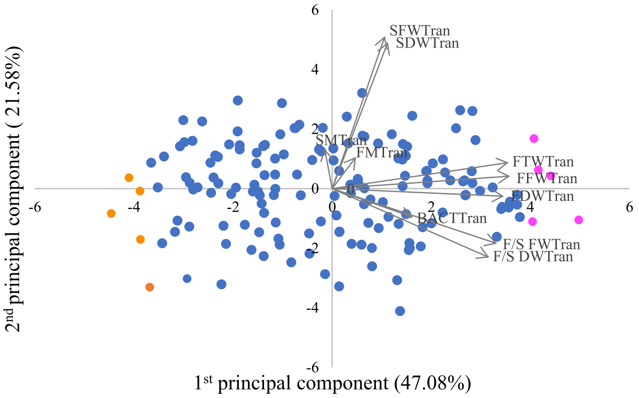

Fig. 4. PCA biplot based on the BLUEs for ten traits measured in clone C001 trees. The first two principal components represent 68.66 % of the total variance. The pink and orange circles represent the most contrasting trees that could be selected based on their higher and lower values (respectively) for fruit and flesh weight and flesh-to-stone ratio traits. Abbreviations: BACTTran – olive fruit fly transformed; FWTran – mean fruit mass transformed; FDWTran – flesh mass on dry weight basis transformed; FFWTran – flesh mean mass on wet weight basis transformed; FMTran – flesh moisture transformed; F/S DWTran – flesh to stone ratio on dry weight basis transformed; F/S FWTran – flesh to stone ratio on wet weight basis transformed; SDWTran – stone mass on dry weight basis transformed; SFWTran – stone mean mass on wet weight basis transformed; SMTran – stone moisture transformed.

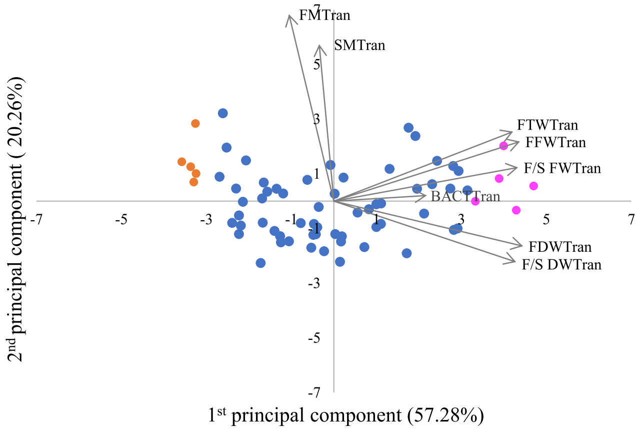

Fig. 5. PCA biplot based on the BLUEs for eight traits measured in clone C002 trees. The first two principal components represent 77.54 % of the total variance. The pink and orange circles represent the most contrasting trees that could be selected based on their higher and lower values (respectively) for fruit and flesh weight and flesh-to-stone ratio traits. Abbreviations: BACTTran – olive fruit fly transformed; FWTran – mean fruit mass transformed; FDWTran – flesh mass on dry weight basis transformed; FFWTran – flesh mean mass on wet weight basis transformed; FMTran – flesh moisture transformed; F/S DWTran – flesh to stone ratio on dry weight basis transformed; SDWTran – stone mass on dry weight basis transformed; SMTran – stone moisture transformed.

primarily set by the variation in fruit and flesh weight and flesh-to-stone ratios related traits. The stone weights related traits (SFWTran and SDWTran) set the variation across PC2. As illustrated in Fig. 4, it is possible to select contrasting trees, accordingly to the fruit and flesh weight, and flesh-to-stone ratios target traits. For example, trees *abbp05, pvfd03, abbp02, vlsq08* or *ctsm01* (in pink in Fig. 4) showed higher values for fruit-related traits. On the other hand, the trees *mopb01, mopb02, nlqf04, ctqp12* or *nlqf08* (in orange in Fig. 4) presented lower values for these same traits.

In the case of C002, the two first PCA components explained 77.54 %

Table 4
Means comparison analysis for the different traits within the clones with more than four and less than 21 trees for the selection of ‘Galega vulgar’ trees based on significant phenotypic differences.

Trait	Clone	Mean comparisons by Fisher’s test[1]
FWTran	C004	abbp10^{a}; abbp06^{a}; vlsq02^{a}; abbp01^{a}; abbp09^{ab}; abbp04^{ab}; absd10^{ab}; absd07^{b}; evjr05^{bc}; mceo04^{bcd}; sdvl01^{cd}; sdsm09^{d}; sdvl04^{d}; sdvl10^{d}
	C005	ptmv04^{a}; vbqq07^{b}; spql01^{b}; spql06^{b}; ptmv09^{b}; evce07^{b}; mvmn03^{c}
	C008	vlsq06^{a}; mnhf10^{b}; cbmb09^{c}; ezhg12^{c}
	C009	absd01^{a}; absd03^{a}; absd06^{a}; absd02^{a}; vlsq10^{ab}; sdsm07^{b}
	C010	sphm07^{a}; avmc08^{ab}; rdbq02^{b}; alcp06^{b}; rdbq05^{b}; ezhg04^{b}
FDWTran	C004	vls102^{a}; abbp06^{ab}; abbp10^{ab}; absd10^{abc}; abbp01^{abc}; abbp04^{abc}; mceo04^{abc}; absd07^{bc}; abbp09^{abcd}; evjr05^{cde}; sdsm09^{def}; sdvl01^{ef}; sdvl10^{f}; sdvl04^{f}
	C005	ptmv04^{a}; vbqq07^{ab}; ptmv09^{b}; spql06^{b}; spql01^{b}; evce07^{b}; mvmn03^{c}
	C006	vlsq03^{a}; semp03^{ab}; vrbf01^{ab}; alot01^{abc}; evjp01^{bc}; ezhg14^{c}
	C008	vlsq06^{a}; mnhf10^{b}; cbmb09^{c}; ezhg12^{d}
FFWTran	C004	abbp10^{a}; abbp06^{a}; vlsq02^{a}; abbp01^{a}; abbp04^{ab}; abbp09^{abc}; absd10^{abc}; absd07^{bc}; evjr05^{cd}; mceo04^{bcde}; sdsm09^{de}; sdvl01^{de}; sdvl10^{de}; sdvl04^{e}
	C005	ptmv04^{a}; vbqq07^{b}; spql01^{b}; spql06^{b}; ptmv09^{b}; evce07^{b}; mvmn03^{c}
	C008	vlsq06^{a}; mnhf10^{b}; cbmb09^{bc}; ezhg12^{c}
	C009	absd01^{a}; absd03^{a}; absd06^{a}; absd02^{a}; vlsq10^{ab}; sdsm07^{b}
	C010	sphm07^{a}; avmc08^{b}; rdbq02^{b}; alcp06^{b}; rdbq05^{b}; ezhg04^{b}
FMTran	C004	abbp09^{a}; sdvl01^{ab}; sdvl10^{abc}; abbp04^{abc}; sdvl04^{abcd}; abbp01^{abcde}; abbp10^{abcde}; evjr05^{bcde}; abbp06^{bcde}; absd07^{bcde}; absd10^{cde}; vlsq02^{de}; sdsm09^{de}; mceo04^{e}
	C008	ezhg12^{a}; cbmb09^{b}; mnhf10^{b}; vlsq06^{b}
	C013	ezhg03^{a}; rdbq07^{a}; sdsm10^{a}; sdsm02^{b}
F/S DWTran	C003	llqt02^{a}; alqb05^{ab}; vbqq03^{ab}; mnhf08^{abc}; nlqf14^{bc}; evce03^{bc}; sdvl09^{bc}; nlqf10^{c}; vdhg09^{c}; evce11^{c}
	C004	absd10^{a}; abbp10^{a}; vlsq02^{a}; abbp06^{ab}; abbp01^{abc}; abbp04^{abc}; absd07^{abc}; mceo04^{abcd}; abbp09^{abcd}; sdsm09^{bcde}; evjr05^{cde}; sdvl10^{de}; sdvl04^{e}; sdvl01^{e}
	C005	vbqq07^{a}; ptmv04^{a}; ptmv09^{ab}; spql06^{bc}; spql01^{bc}; evce07^{cd}; mvmn03^{d}
	C008	vlsq06^{a}; cbmb09^{ab}; mnhf10^{bc}; ezhg12^{c}
	C009	absd01^{a}; absd03^{a}; absd06^{a}; vlsq10^{a}; absd02^{a}; sdsm07^{b}
	C010	rdbq02^{a}; sphm07^{a}; avmc08^{a}; alcp06^{ab}; ezhg04^{b}; rdbq05^{b}
F/S FWTran	C004	abbp10^{a}; abbp04^{ab}; absd10^{ab}; vlsq02^{abc}; abbp06^{abcd}; abbp01^{abcd}; abbp09^{abcde}; absd07^{bcdef}; sdsm09^{bcdef}; evjr05^{ef}; mceo04^{bdef}; sdvl10^{ef}; sdvl01^{f}; sdvl04^{f}
	C005	vbqq07^{a}; ptmv04^{ab}; ptmv09^{abc}; spql01^{c}; spql06^{bc}; evce07^{cd}; mvmn03^{d}
	C009	absd01^{a}; absd03^{a}; absd02^{a}; absd06^{a}; vlsq10^{ab}; sdsm07^{b}
	C010	sphm07^{a}; avmc08^{b}; rdbq02^{b}; ezhg04^{b}; alcp06^{b}; rdbq05^{b}
SDWTran	C004	mceo04^{a}; abbp06^{a}; evjr05^{a}; abbp01^{ab}; vlsq02^{ab}; abbp09^{abc}; abbp10^{abc}; sdvl01^{abc}; abbp04^{abc}; absd07^{abc}; absd10^{bcd}; sdvl04^{cd}; sdvl10^{cd}; sdsm09^{d}
	C005	ptmv04^{a}; evce07^{ab}; spql06^{abc}; spql01^{bc}; ptmv09^{cd}; vbqq07^{cd}; mvmn03^{d}
SFWTran	C004	abbp06^{a}; evjr05^{a}; abbp01^{ab}; vlsq02^{ab}; mceo04^{ab}; abbp09^{abc}; sdvl01^{abc}; absd07^{abc}; abbp04^{abc}; abbp10^{abc}; absd10^{abcd}; sdvl04^{bcd}; sdvl10^{cd}; sdsm09^{d}
	C005	ptmv04^{a}; evce07^{ab}; spql01^{bc}; spql06^{bcd}; ptmv09^{cde}; vbqq07^{de}; mvmn03^{e}

Note: FWTran, mean fruit mass transformed; FDWTran, flesh mass on dry weight basis transformed; FFWTran, flesh mean mass on wet weight basis transformed; FMTran, flesh moisture transformed; F/S DWTran, flesh to stone ratio on dry weight basis transformed; F/S FWTran – flesh to stone ratio on wet weight basis transformed; SDWTran, stone mass on dry weight basis transformed; SFWTran, stone mean mass on wet weight basis transformed.

[1] Fisher’s LSD test at 95 % confidence level - mean values in each row followed by the same letter are not significantly different at P-value < 0.05.

Table 5
Selection of the most contrasting ‘Galega vulgar’ trees based on fruit and flesh weights and flesh-to-stone ratio traits within the clones with more than four and less than 21 trees.

Clone	Selected ‘Galega vulgar’ trees based on significant phenotypic differences
C001	*abbp05; pvfd03; abbp02; vlsq08; ctsm01; mopb01; mopb02; nlqf04; ctqp12; nlqf08*
C002	*elvb07; abbp03; abbp08; pvdf02; ctsm07; vdhg07, rdbq09, vdhg01, sdvl08; atsl03*
C003	*llqt02; evce11*
C004	*abbp10; sdsm09*
C005	*ptmv04; mvmn03*
C006	*vlsq03; ezhg14*
C008	*vlsq06; ezhg12*
C009	*absd01; sdsm07*
C010	*sphm07; ezhg04*
C013	*ezhg03; sdsm02*

of the phenotypic variability present in the data set (Fig. 5). Although trees from Clone C002 did not vary for stone weight-related traits (contrary to what happened to trees from C001), the phenotypic variability across the first principal component (PC1) was also set by fruit and flesh weight and flesh-to-stone ratios traits. In this clone if trees with higher values of fruit and flesh weight and flesh-to-stone ratios traits were desired, *elvb07, abbp03, abbp08, pvdf02* or *ctsm07* (pink in Fig. 5) could be selected. On the contrary, if trees with lower values of these traits were needed, the *vdhg07, rdbq09, vdhg01, sdvl08* or *atsl03* could be selected (in orange in Fig. 5).

Regarding C004 clone, the mean value of the *abbp10* tree was always significantly different from *sdsm09* values for FWTran, FDWTran, FFWTran, F/S DWTran, F/S FWTran, SDWTran, and SFWTran traits. In agreement with these results, in the respective PCA biplot (Supplementary Figure S1) tree *sdsm09* (pink circle, Figure S1) was plotted on the opposite side of tree *abbp10* (orange circle, Figure S1) and were both selected.

Within each of the clones C005, C008, C009 and C010, there was always a pair of trees that were significantly different for the entire clone varying traits, which positions in the respective PCA were always contrasting and due to that selected. These were for clone C005, *ptmv04* and *mvmn03*, for clone C008, *ezhg12* and *vlq06*, for clone C009, *sdsm07* and *absd01* and finally for clone C010, trees *sphm07* and *ezhg04* (Supplementary Figures S2-S5 and Tables 4 and 5).

For the clones varying in just one trait (C003, C006 and C013) (Table 4), and due to the lower variation found in them, also the two most extreme trees were selected (Table 5). Namely, trees *llqt02* and *evce11* in C003; trees *vlsq03* and *ezhg14* in C006; and trees *ezhg03* and *sdsm02* in C013.

The selected phenotypically contrasting trees will contribute to broadening the genetic basis of a previously defined base ‘Galega vulgar’ collection of trees. This base collection representative of the ‘Galega vulgar’ genetic diversity still under cultivation, created towards the genetic preservation of this resource and its efficient use in breeding programs, counted initially with only one tree per each of the 95 genetically predefined clones. This enlarged collection will be now trialled in comparative *ex-situ* field experiments, under the same environmental conditions, needed for a more precise evaluation of the genetic potential of this variety, towards future genetic studies to develop molecular selection tools to support precision olive breeding, as already proven successful in other perennial crops, such as walnuts (Arab et al., 2020).

One of the limitations of the present study was the very unbalanced data set, with a considerable number of missing values due to different factors out of our control (alternate bearing, wildfires, etc.). Nevertheless, we believe that the extension of the planned original data collection and its final numbers (thirteen fruit and oil-related traits, in 595 planned trees, during 3 growing seasons and across 10 geographical districts) and the obtained results, clearly support the presented results and discussion. Additionally, the availability of a very reduced genotypic characterization, only with 14 SSR markers, limited the outcomes of this study. A more integrated approach, combining phenotypic and genotypic highly dense data sets (like Single Nucleotide Polymorphisms), using the soil and climate characteristics as covariates, is still needed for a more comprehensive understanding of the ‘Galega vulgar’ behaviour in a certain environment or production system. The application of Genotype-to-phenotype (G2P) models, that combine these two types of data, may be a good future option. These models are highly relevant in breeding since they can help to predict the performance of new genotypes (from genome-wide marker profiles) under a given environment, or of a given variety under different climate conditions (van Eeuwijk et al., 2019).

4. Conclusions

From the present work, we conclude that although the ‘Galega vulgar’ fruit and oil-related traits are highly influenced by the environment, this diverse olive variety still harbours considerable genotypic variability, worthy to conserve. This genotypic variability contributes to an interesting phenotypic variability from which olive tree producers can still profit through the development of high-quality improved varieties, better adapted and resilient to a changing climate.

Differences in the ‘Galega vulgar’ clonal phenotypic stability were clarified, with the identification of the most stable genotypes. Since a significant geographic district x clone interaction was also observed in the present study, this new knowledge may now contribute to a better adjustment of the best clone, for the best region, in what respects new orchards establishment towards a resilient bioeconomy.

The enlarged ‘Galega vulgar’ base collection, here presented, with a broader representation of this variety phenotypic variability still under cultivation, may contribute to an efficient breeding, through future *ex-situ* genetic association analysis, fundamental for the development of the fruit and oil-related traits molecular selection tools.

These future investigations might involve other important traits for olive stakeholders such as yield or the trees vigour, and also determining factors in the quality of the olive variety, such as the oil composition. A wider range of environments should be tested, to better clarify the phenotypic stability and the identification of the most stable genotypes to confirm their direct utility in facing climate change. Additionally, a more integrated approach, combining phenotypic and genotypic highly dense data sets, using the soil and climate characteristics as covariates, can be used in these future studies to understand the ‘Galega vulgar’ behaviour in a certain environment or production system.

Overall, the generated information on the phenotypic variability of the genetically diverse olive ‘Galega vulgar’ fruits and oil-related traits across environments is of utmost importance from a conservation, breeding, resilience, and production perspective focusing on the more than ever needed adaptation to climate change effects. Nevertheless, to attain these purposes, a collaborative and synergistic effort involving olive producers, academia, and research institutes is required. The combined efforts of these players foster a holistic approach, by integrating local knowledge, academic rigor, and technological innovation to develop resilient, high-yielding, and quality ‘Galega vulgar’ individuals that address the challenges of a dynamic agricultural landscape.

CRediT authorship contribution statement

Hélia Sales: Conceptualization, Methodology, Investigation, Writing – original draft. **Mara Lisa Alves:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Ana Margarida Sampaio:** Methodology, Formal analysis, Investigation, Writing – review & editing. **João Nunes:** Supervision, Writing – review & editing. **Maria Carlota Vaz Patto:** Conceptualization, Supervision, Writing – review & editing.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112738.

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