

Article

Toward the Development of Garlic Varieties: The First Attempts

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Abstract: Despite garlic (*Allium sativum* L.) being recognized as the second most important species in the *Allium* genus, a limited number of varieties are available worldwide. Aiming to develop new purple garlic varieties, a representative sample was used to determine the extent of phenotypic variability for commercially desirable characteristics. Initially, 360 bolting bulbs were selected based on their size and abundant external scales. After further assessment, 243 bulbs were discarded due to undesirable traits such as light purple color, numerous small cloves, or damage from *Fusarium* spp. and mites. Although garlic reproduces asexually, each bulb was considered to represent a distinct family. Thus, 117 families were planted in a randomized complete block design during the 2021–2022 growing season. Physiological, morphological, and phenological traits were assessed for each family, along with damages caused by pests and pathogens. As a result of these evaluations, 103 families were selected for a second trial the following season. Due to increased selection pressure, only 23 families remained in the program at the end of the second trial. High variability was observed for most traits, with a strong influence from family lineage. Environmental conditions significantly impacted the performance of the families, highlighting the need to evaluate them under diverse environments. The high variability within the selected sample indicates a strong potential for developing new garlic varieties.

Keywords: *Allium sativum* (L.); clonal selection; genotype–environment interaction; landrace; plant breeding; selection pressure



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1. Introduction

Garlic (*Allium sativum* L.) is a species belonging to the Amaryllidaceae family [1–5], cultivated from subtropical to boreal regions [6]. Its primary center of origin is located in Central Asia, extending through the Middle East and reaching the Mediterranean basin [5–7]. Garlic has been used since ancient times, from 5000 to 2000 years ago, by Babylonian, Chinese, Egyptian, and Indian cultures [8,9] for its medicinal properties, as well as its folkloric and culinary uses [9].

Although garlic is considered the second most important species in the *Allium* genus after onion, only a few thousand varieties have been used throughout history [10]. Today, the total number of cultivars available worldwide is estimated to be around 600 [11]. Biological, technological, and social factors may contribute to this limited diversity. Farmers and producers often make few distinctions, with the most recognizable characteristics being bolting/non-bolting, red/white, early/late, and local/exotic garlic [10]. This highlights the need to develop new varieties tailored for intensive agriculture, adapted to current environmental and legislative conditions, and possessing exceptional characteristics like a higher content of organosulfur compounds and other metabolites.

Garlic has been largely recalcitrant to conventional plant improvement techniques due to the near loss of its capacity for sexual reproduction [11]. While wild types can produce true seeds in some regions of Kazakhstan and Kyrgyzstan [9,12], garlic propagation is primarily accomplished using asexual propagules, mainly cloves. This poses a significant limitation for combining traits through the mating of outstanding parents. Under these

circumstances, one might expect high homogeneity in commercial fields and germplasm collections. However, wide variations have been documented for morphological, phenological, biochemical, and physiological traits, as well as tolerance to biotic stresses [13–20]. The main cause of this phenotypic and genetic heterogeneity remains unclear, but it has been suggested that the accumulation of changes from natural mutations, intercrossing between ancient ancestors [9,10], and epigenetic events may contribute to this unexpected variability [21,22]. Thus, these naturally occurring variations offer a valuable opportunity for selecting superior genotypes.

The focus of this research is on the violet or purple (morado) garlic from the Protected Geographical Indication (PGI) “Las Pedroñeras” (Spain). This landrace is characterized by the color of its cloves’ tunics, which gives it its name [23]. Bulbs are generally medium-sized with a spherical shape, bearing an average of 8–12 cloves. Unlike the cloves, their scales are mostly white. This violet garlic has a high content of organosulfur substances and iodide, contributing to its spicy flavor and facilitating its preservation. These characteristics make “Ajo Morado de Las Pedroñeras” a valuable landrace for selection. Although some efforts have been made to create varieties by selecting from this PGI [24,25] and other ecotypes [11,16], the scarcity of varieties suitable for modern agriculture remains a global issue.

Therefore, this research aims to determine (1) if there is sufficient phenotypic variability within this landrace to enable effective selection; (2) which traits exhibit the greatest variability; (3) if families can be differentiated based on physiological, morphological, and phenological characters; and (4) if there is potential to select superior clonal varieties.

2. Materials and Methods

2.1. Plant Material and Statements for the Field Trials

Violet or purple (morado) garlic, belonging to the Protected Geographical Indication (PGI) “Las Pedroñeras” (Spain), from commercial fields owned by SAT Peregrin, Spain (<https://peregrin.es/en/>, accessed on 6 June 2024), were used as starting material. To eliminate viruses, meristems from outstanding plants were cultured in vitro following the protocol described by Bhojwani et al. [26]. The first clonal generation (G0), consisting of mixed materials, was planted under an insect-proof net house (NHI), owned by SAT Peregrin (Spain), at 781 m AMSL [coordinates 38.007285, −1.912556, Caravaca, Murcia, Spain] in the 2019–2020 growing season. The second clonal generation (G1) was cultivated under similar conditions during the 2020–2021 season. After harvest, 360 bolting bulbs were randomly selected based on their weight (>50 g) and the presence of abundant external scales (>5). After removing the external scales, only bulbs with healthy purple cloves, a low proportion of small cloves (<25%), and no pest damage were selected. This resulted in 117 bulbs meeting these criteria, which were used for the first field trial. As garlic is only propagated asexually, high genotypic and phenotypic homogeneity would be expected. However, initial evaluations of the target sample revealed variations in all assessed characters, consistent with previous reports. Therefore, each bulb was considered a distinct family and was properly identified to preserve its individuality. The 117 families (5 cloves each family) were planted in an insect-proof net house using a randomized complete block design. After the first assessment at the end of the growing cycle, 14 families were discarded, and the remaining 103 were planted randomly (35 to 65 cloves per family) during the 2022–2023 season.

2.2. Phenotypic Selection

A clonal selection protocol was followed for family assessment. The first trial (117 families) was established on 3 December 2021, in an insect-proof net house (NHII), also owned by SAT Peregrin, at 191 m AMSL (above mean sea level) [coordinates 37.396960, −1.741961, Pulpí, Almería, Spain]. The initiation (SI, weeks) and duration (SP, weeks) of sprouting, as well as the initiation (DIF, weeks) and duration (DFF, weeks) of flowering, were recorded. After 26 weeks of cultivation, the number of active leaves (LN₂₆), plant height (PH, mm),

pseudo-stem height (PSH, mm), and pseudo-stem diameter (PSD, mm) were measured. Following harvest (24 June 2022), bulbs were stored under controlled conditions (19–21 °C and 50–60% relative humidity). Five months post-harvest, bulb weight (BW, g), bulb diameter (BD, mm), and the number of cloves (C) were recorded.

The second trial (103 families) was planted on 25 November 2022 in NHI. Due to adverse weather conditions during week 26, DIF, DFF, LN_26, PH, PSH, and PSD could not be evaluated. However, with a larger sample size of bulbs per family, the number of small (SC) and large (LC) cloves was counted; the weight of small (SW, g) and all (CW, g) cloves per bulb, and the unit weight of small (UWSC, g) and large (UWLC, g) cloves were measured; and the potential yield (Y , kg ha⁻¹) was estimated.

PSD and BD values represent the average of two perpendicular measurements. Bulb sphericity (Φ) was calculated as the ratio of the smaller BD to the larger BD. Active leaves were defined as those with green portions, regardless of their degree of wilting. PH was measured from the base of the pseudo-stem to the tip of the longest leaf and PSH from the base to the last visible point of sheath insertion with the leaf blade (Figure 1). The potential yield (Y) was calculated by multiplying the average BW by the planting density (270,000 plants ha⁻¹). The percentage of loss due to reduced clove size (WtP, %) was determined as the ratio of the number of small cloves to the total number of cloves. Other traits, such as premature sprouting of cloves, the percentage of plants affected by the formation of external cloves and/or bulbs (P), bursting of lateral shoots, and sprouting of multiple plants per planted clove, were also considered. Families exhibiting signs of damage caused by pathogens (mainly *Fusarium* spp.) and pests (mainly eriophyid mites) were discarded.

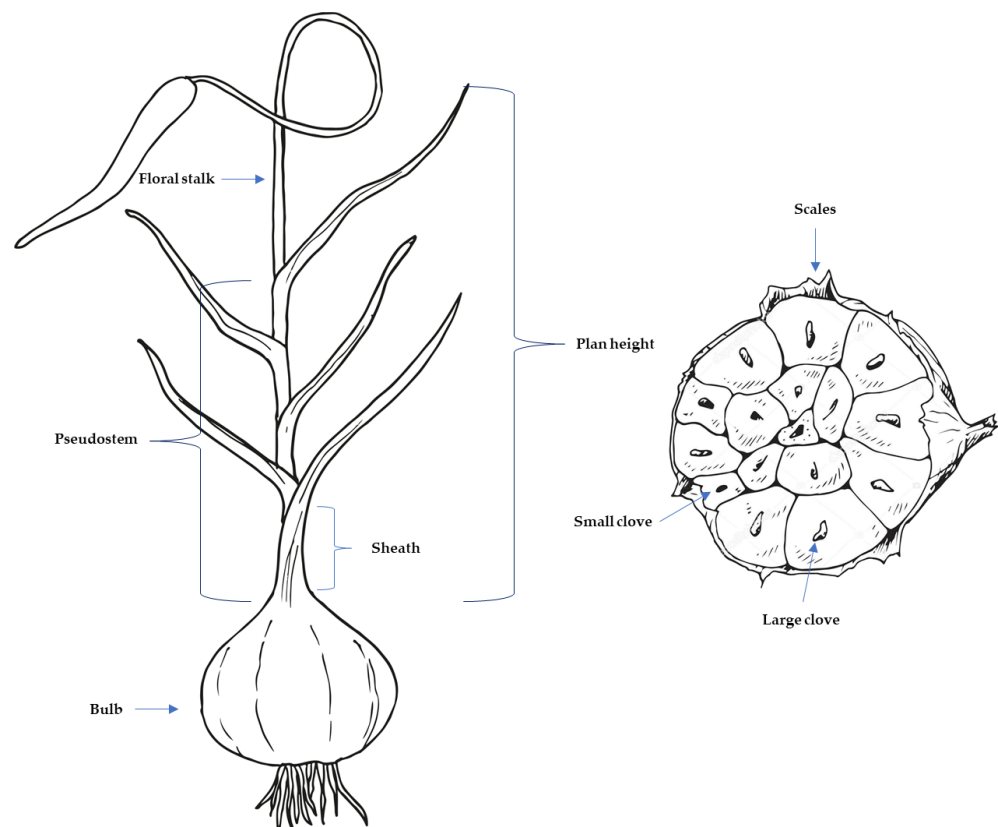


Figure 1. Representation of a garlic plant and morphological descriptions of some of its traits.

2.3. Statistical Analysis

Statistical data processing was performed using the R language [27] and its integrated development environment (IDE) RStudio. The aov() function was used for analysis of variance (ANOVA). ANOVA assumptions were assessed using the gvlma() function [28].

For dependent variables that did not meet ANOVA assumptions, a Kruskal–Wallis test (`kruskal.test()`) was employed. Effect sizes for parametric and non-parametric analyses were determined using omega squared (ω^2) and epsilon squared (ϵ^2) [29], respectively, following the interpretation guidelines of Sawilowsky [30]. Post hoc tests and figures were generated using the `posthoc()` and `barplot()` functions, respectively. Pearson’s correlations among variables were performed using `apa.cor.table()`, and graphs were produced using the `pairs.panels()` function from the `{psych}` package. Principal component analysis (PCA) was conducted using the `PCA()` function from the `{FactoMineR}` package [31] to determine the contribution of traits to the overall variance. Graphical representation of components and variables was achieved using the `fviz_pca_biplot()` function. A Euclidean distance matrix among families was calculated (`get_dist()`) for constructing dendrograms. The optimal number of clusters was determined using the `NbClust()` function with the ‘kmeans’ method. Each family was assigned to the suggested clusters using the `kmeans()` function with 30 starts. Dendrograms were generated using the `fviz_dend()` function.

3. Results

3.1. The First Trial: Season 2021–2022

During cultivation, some undesirable physiological disorders were observed. Consequently, 14 out of the 117 families were discarded: 11 due to early sprouting of cloves, 2 with multiple plants sprouting per planted clove, and 1 exhibiting multiple lateral shoots.

Regarding quantitative characters, substantial variability was observed for all assessed traits (Table 1), with medium effect sizes of families on most traits, except for leaf number (LN_26) and bulb sphericity (Φ).

Table 1. Results of ANOVA for 8 dependent variables and the effect sizes of families on them. Resume of the first trial (2021–2022 season).

Dependent Variables	F Value	df	Signif. (p)	ω^2	95% CI ω^2 **	Interpretation of Effect Size
PSH	6.631	102	<0.001	0.54	(0.41–1.00)	Medium
PSD	8.216	102	<0.001	0.60	(0.50–1.00)	Medium
LN_26	5.030	102	<0.001	0.45	(0.29–1.00)	Small
PH	7.843	102	<0.001	0.58	(0.48–1.00)	Medium
BD	12.230	102	<0.001	0.70	(0.63–1.00)	Medium
Φ *	148.100	102	<0.01	0.27	(0.36–0.48)	Small
BW	12.540	102	<0.001	0.70	(0.64–1.00)	Medium
C	6.296	102	<0.001	0.52	(0.39–1.00)	Medium

* Result of Kruskal–Wallis test. The size effect was calculated for ϵ^2 . ** One-sided CIs: upper bound fixed at (1.00), except for Φ . Legend: df, degree of freedom; p, probability; ω^2 , omega squared; CI, confidence interval; ϵ^2 , epsilon squared; PSH, pseudo-stem height; PSD, pseudo-stem diameter; LN-26, number of active leaves on the 26th week; PH, plant height; BD, bulb diameter; Φ , sphericity of bulb; BW, bulb weight; C, number of cloves.

Correlation analysis revealed significant relationships among several traits (Figure 2). As expected, bulb weight (BW) and diameter (BD) were highly interdependent ($r = 0.99$ ***). Similarly, BW and BD were strongly correlated with pseudo-stem diameter (PSD), number of active leaves (LN_26), and plant height (PH). A positive correlation was also found between PSD and LN_26 ($r = 0.71$ ***). Notably, only a moderate positive association was observed between the number of cloves (C) and BW ($r = 0.56$ ***) and BD ($r = 0.56$ ***). Conversely, moderate negative correlations were found between the initiation of flowering (DIF) and PSH ($r = -0.49$ ***) and PH ($r = -0.30$ **). No relationship was found between the initiation (SI) and duration (SP) of sprouting ($r = 0.09$ n.s.).

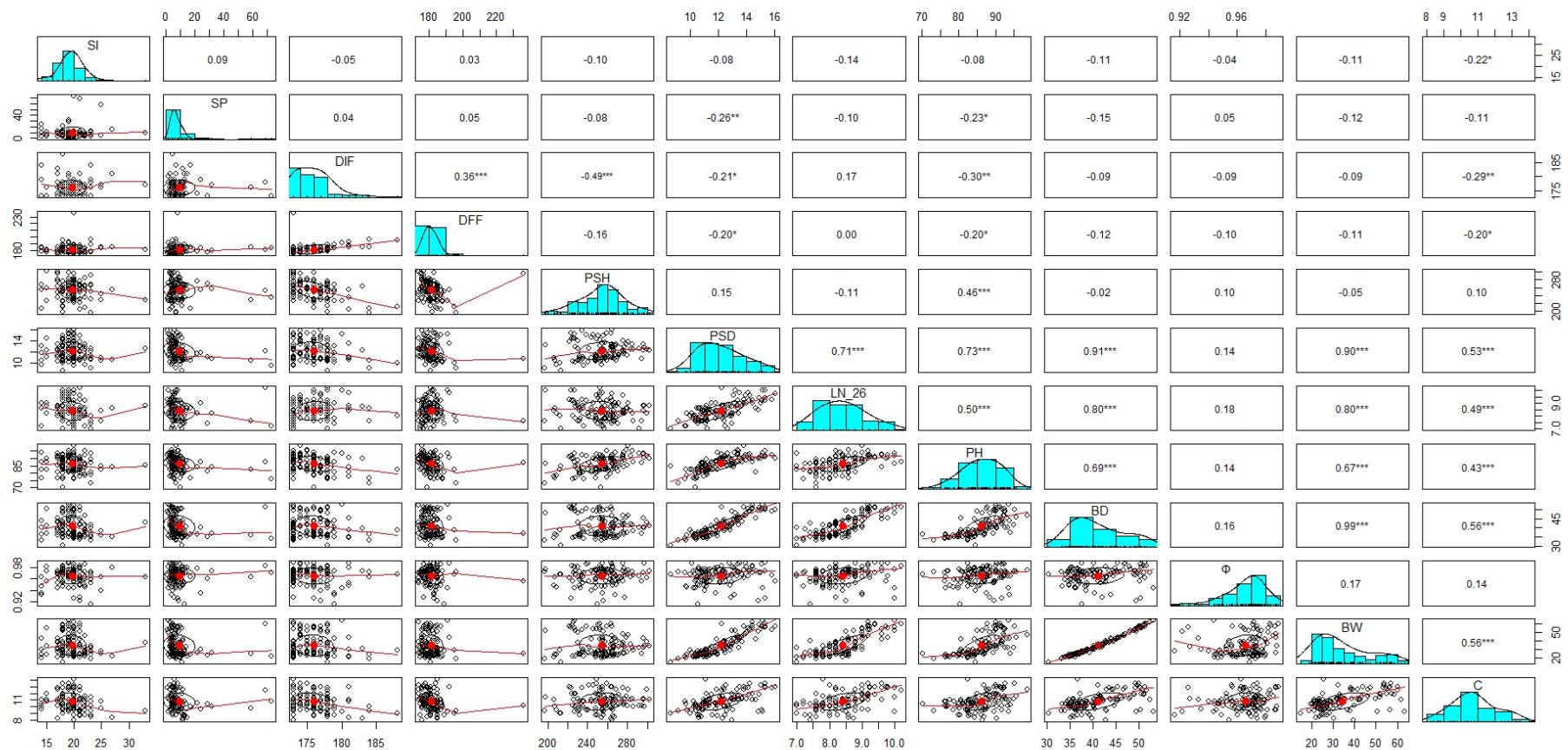


Figure 2. Pearson's correlation among characters, showing the histograms of correlation and the scatterplots for the assessed traits. For scatterplots, red dots are the average of traits. First trial (season 2021–2022). * Indicate signification for $p < 0.05$. ** Indicate signification for $p < 0.01$. *** Indicate signification for $p < 0.001$. Legend: SI, sprouting initiation; SP, length of sprouting; DIF, flowering initiation; DFF, length of flowering; PSH, pseudo-stem height; PSD, pseudo-stem diameter; LN-26, number of active leaves; PH, plant height; BD, bulb diameter; Φ , sphericity of bulb; BW, bulb weight; C, number of cloves.

According to the principal component analysis (PCA) for 12 traits, components 1 and 2 explained 54.7% of the total variance. For PC1, the traits with the highest contributions were BD, BW, PSD, PH, LN₂₆, and C, while for PC2, PSH and the phenological characters SI, SP, DIF, and DFF were the most important (Figure 3). The biplot representation of families on PC1 and PC2 suggests the formation of three main groups. Families with higher values associated with productivity (BW, BD, and C) were clustered on the right side of PC1, while less productive families were on the left, and those with average values were in the center.

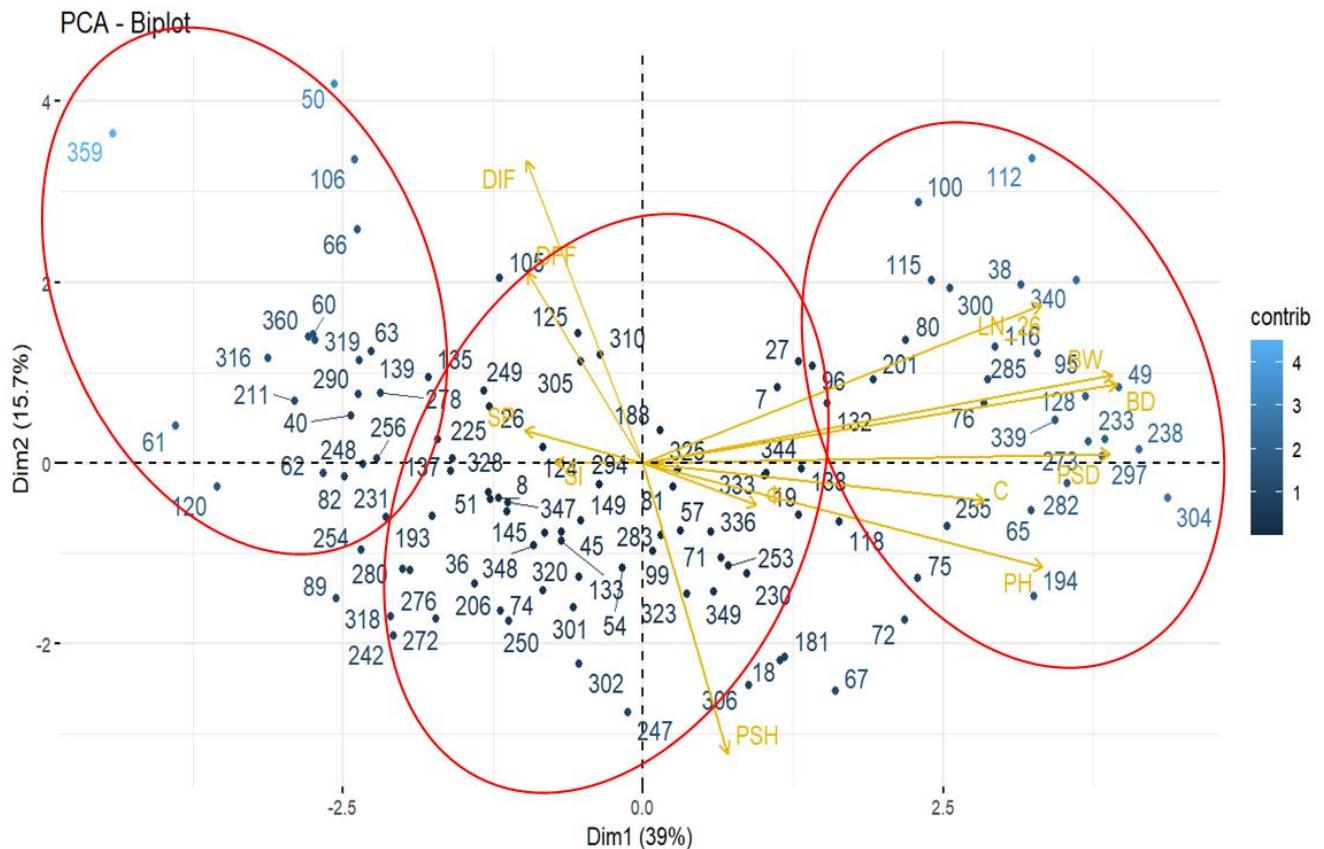


Figure 3. Scatterplot of bidimensional representation of 111 out 117 families and 12 traits on the first (Dim 1), and the second (Dim 2) components. For 6 families, it was not possible to complete the assessment because of abundant early sprouting of shoots. Legend: SI, sprouting initiation; SP, length of sprouting; DIF, flowering initiation; DFF, length of flowering; PSH, pseudo-stem height; PSD, pseudo-stem diameter; LN-26, number of active leaves; PH, plant height; BD, bulb diameter; Φ , sphericity of bulb; BW, bulb weight; C, number of cloves.

Among the 30 indices used by the NbClust function, the majority (9) recommended two as the optimal number of clusters, although 8 indices suggested three clusters. Following the majority rule, the dendrogram was constructed using two clusters. Cluster I (blue, on the right) contained 44 families, while the remaining 67 were in Cluster II (red, on the left) (Figure 4). Cluster I predominantly contained families with the highest productive potential ($BW > 50$ g, $BD > 48$ mm), while Cluster II comprised those with the lowest BW and BD values.

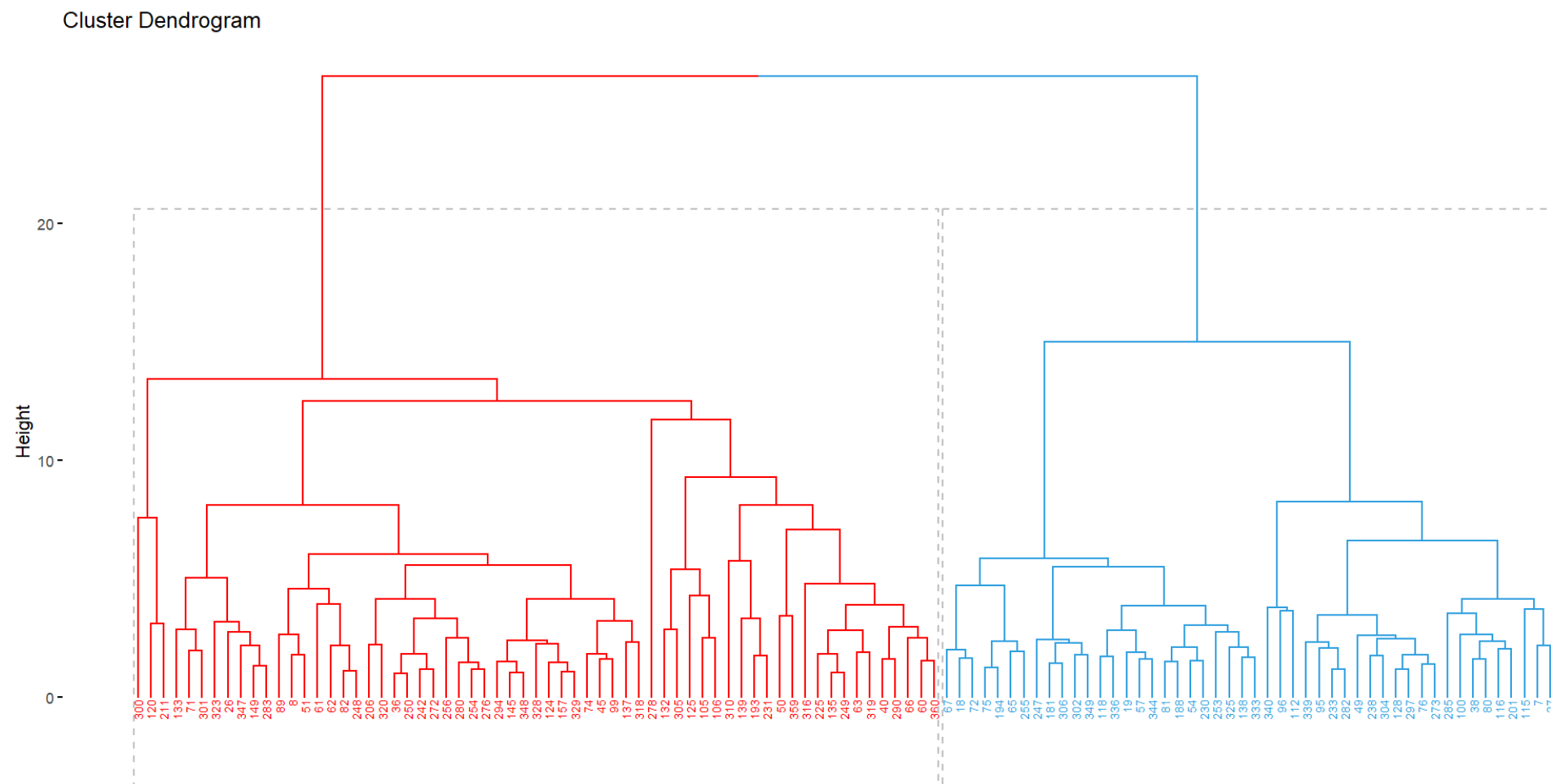


Figure 4. Clustering of 111 out 117 families based on the Euclidean distance for 12 morphological and phenological traits. For 6 families, it was not possible to complete the assessment because of abundant early sprouting of shoots. First trial (season 2021–2022).

3.2. The Second Trial: Season 2022–2023

Unlike the first trial, 80 out of the 103 families (77.7%) were discarded in this trial: 41 (51.25%) due to low yield, 34 (42.5%) because they were affected by physiological disorders, and 5 (6.25%) because they exhibited damage from *Fusarium* spp. and/or mite infestations.

A high incidence of abnormal clove sprouting was observed during this season. Therefore, a threshold of 20% of plants affected by this physiological disorder per family was established for positive selection. Consequently, 25 families were eliminated from the selection program during the cultivation period. After bulb assessment, another nine families affected by the formation of external bulbs/cloves were also discarded.

The calculation of potential yield revealed substantial differences among families, ranging from 8874 to 19,800 kg ha^{−1}, with an average of 13,353.9 kg ha^{−1} for the 69 assessed families. As the minimum yield for selection was set at 14,000 kg ha^{−1}, 41 families were removed from the program.

Similar to the first trial, significant differences were observed for all traits (Figure 5 and Table 2), although the effect sizes of families were reduced. However, relatively high variability remained for bulb diameter, total clove number, and the number of small cloves. Notably, greater variation (F-value: 8.018 vs. 3.225) and effect size (ω^2 : 0.31 vs. 0.12) were observed for the number of small cloves compared to large cloves.

Table 2. Results of ANOVA for 6 dependent variables and the effect sizes of families on them. Resume of the second trial (2022–2023 season).

Dependent Variables	F Value	df	Signif. (p)	ω^2	95% CI ω^2 *	Interpretation of Effect Size
BD	8.254	23	<0.001	0.32	(0.22, 1.00)	Small
Φ	2.326	23	<0.001	0.08	(0.00, 1.00)	Very Small
BW	5.330	23	<0.001	0.22	(0.11, 1.00)	Small
C	8.883	23	<0.001	0.33	(0.24, 1.00)	Small
SC	8.018	23	<0.001	0.31	(0.21, 1.00)	Small
LC	3.225	23	<0.001	0.12	(0.02, 1.00)	Small

* One-sided CIs: upper bound fixed at (1.00). Legend: df, degree of freedom; p, probability; ω^2 , omega squared; CI, confidence interval; BD, bulb diameter; Φ , sphericity of bulb; BW, bulb weight; C, number of cloves; SC, number of small cloves; LC, number of large cloves.

Despite a decrease in the calculated correlation coefficient (r) between BD and BW compared to the first trial, it remained high ($r = 0.78^{***}$). Correlations between the number of cloves (C) and bulb diameter and weight were similar to the previous trial: 0.53^{**} and 0.55^{**} , respectively (Figure 6). As expected, the total number of cloves was positively correlated with both the number of small cloves ($r = 0.88^{***}$) and large cloves ($r = 0.74^{***}$). The unit weight of large cloves (UWLC) was closely related to BW ($r = 0.84^{***}$) and BD ($r = 0.64^{***}$). High correlations were also found between yield and bulb diameter and weight, clove number, and the unit weight of large cloves. The percentage of loss (WtP) showed high positive correlations with the number ($r = 0.83^{***}$) and weight ($r = 0.93^{***}$) of small cloves. However, no association was found between WtP and yield ($r = 0.06$ n.s.). Bulb sphericity had a negative correlation ($r = -0.51^{**}$) with the sprouting of lateral shoots and/or the formation of external cloves and/or bulbs (P).

Despite a reduction in overall variability, it was still possible to separate families into clusters (Figure 7). Again, most indices (13 out of 30) used by the NbClust function suggested the formation of two groups. Families 54, 75, 233, and 344 were grouped into a separate cluster (right blue cluster). These families generally exhibited the highest yields (averaging 18,418.5 kg ha^{−1}) and the longest sprouting periods (2–3 weeks).



Figure 5. Phenotype of bulbs (a–d) and cloves (e,f) from families (a) 89, (b) 40, (c,f) 344, (d) 26, and (e) 157. Families 26 and 89 were discarded from the selection program because of the formation of external cloves (signaled with red arrows) and low yield, respectively. Although selection was performed for the PGI “Morado de las Pedroñeras” (purple or violet garlic), important differences in color can be observed. Second trial (season 2022–2023).

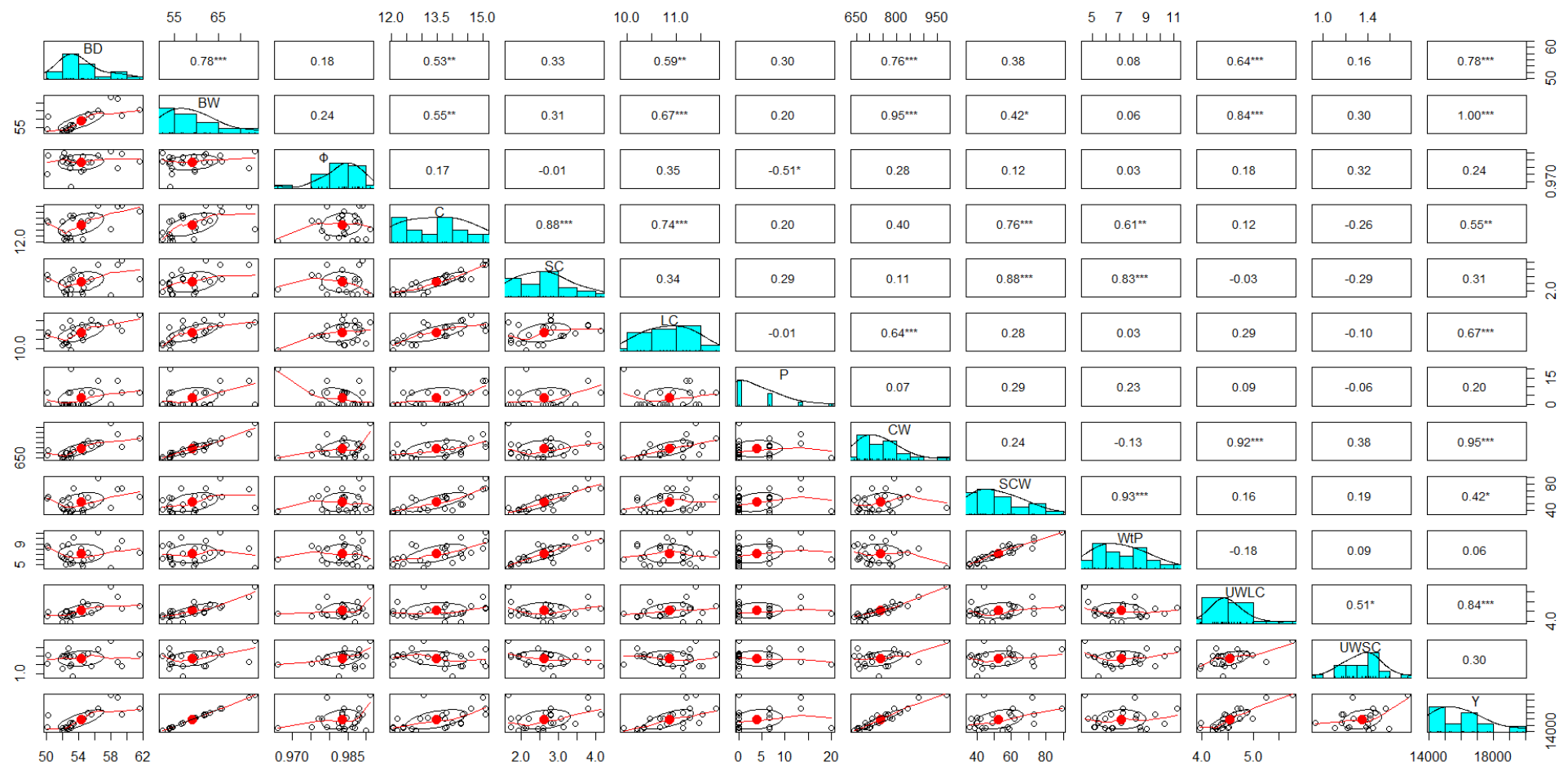


Figure 6. Pearson's correlation among phenotypic characters, showing the histograms of correlation and the scatterplots for assessed traits. For scatterplots, red dots are the average of traits. Second trial (season 2022–2023). * indicate signification for $p < 0.05$. ** indicate signification for $p < 0.01$. *** indicate signification for $p < 0.001$. Legend: BD, bulb diameter; Φ , sphericity of bulb; BW, bulb weight; C, number of cloves; SC, number of small cloves; LC, number of large cloves; CW, total clove weight; SCW, weight of small cloves; P, percentage of bulbs with external cloves; WtP, percentage of lost cause for reduced size of cloves; UWLC, unitary weight of large cloves; UWSC, unitary weight of small cloves; Y, potential yield.

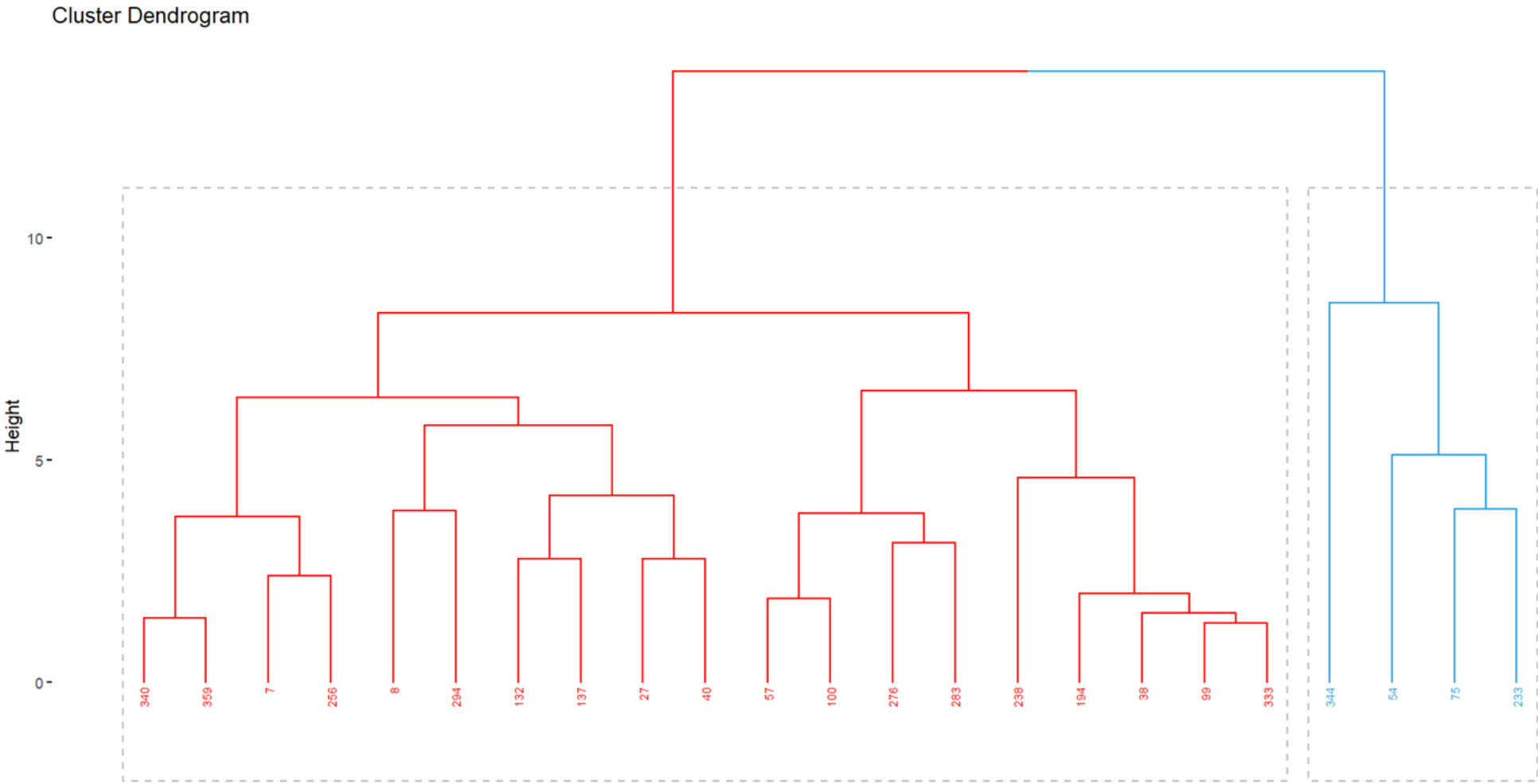


Figure 7. Clustering of 23 families based on the Euclidean distance for 14 morphological and phenological traits. Second trial (season 2022–2023).

For the 23 families remaining in the program, a classification into different commercial categories based on BD was performed (Table 3). It is expected that a higher yield would correspond to a higher percentage of bulbs in the superior categories (A–C) and vice versa. Therefore, four groups were created based on their potential yield to avoid biased comparisons among families with significantly different yields. While most bulbs in Group I fell into category C, the other three groups primarily produced category B bulbs. However, striking differences were observed within the same group. For example, unlike most families in Group I, family 283 produced more category D bulbs (53.3%) than category C (26.7%). In contrast, families 100 and 256 showed a more balanced distribution across categories B to D. Within Groups II and IV, families 233 and 75 stood out, producing the highest percentages of the largest bulbs (category A) at 46.7% and 33.3%, respectively.

Table 3. Grouping of families according to their potential yield and distribution of percentages of the different commercial bulb size categories per family.

Family	Yield (kg ha ^{−1})	Grouping of Families by Yield	Bulb Size Category (%)					
			F < 37 mm	E 37–45 mm	D 45–50 mm	C 50–55 mm	B 55–60 mm	A >60 mm
38	14,112	I	0.0	0.0	20.0	60.0	20.0	0.0
57	14,490		0.0	0.0	20.0	53.3	26.7	0.0
99	14,724		0.0	6.7	13.3	66.7	13.3	0.0
100	14,112		0.0	13.3	26.7	26.7	26.7	6.7
194	14,652		0.0	0.0	13.3	73.3	6.7	6.7
238	14,562		0.0	0.0	26.7	53.3	13.3	6.7
256	15,372		0.0	0.0	20.0	46.7	33.3	0.0
276	15,228		0.0	0.0	6.7	60.0	33.3	0.0
283	14,670		0.0	6.7	53.3	26.7	13.3	0.0
333	14,544		0.0	0.0	6.7	73.3	20.0	0.0
340	15,336		0.0	0.0	13.3	46.7	40.0	0.0
359	14,508		0.0	6.7	13.3	66.7	13.3	0.0
7	16,668	II	0.0	0.0	20.0	20.0	53.3	6.7
8	16,092		0.0	0.0	20.0	26.7	46.7	6.7
27	16,650		0.0	13.3	26.7	53.3	6.7	0.0
40	17,028		0.0	0.0	6.7	40.0	46.7	6.7
132	16,056		0.0	0.0	13.3	46.7	26.7	13.3
137	16,146		0.0	0.0	6.7	53.3	40.0	0.0
233	16,776		0.0	0.0	0.0	20.0	33.3	46.7
294	16,092		0.0	0.0	0.0	53.3	46.7	0.0
54	17,640	III	0.0	0.0	13.3	20.0	40.0	26.7
75	19,458	IV	0.0	0.0	0.0	0.0	66.7	33.3
344	19,800		0.0	0.0	0.0	20.0	66.7	13.3

3.3. Interaction Genotype–Environment ($G \times E$)

Since clustering based on phenotype suggested the existence of genotypic differences, the influence of location (NHI–NHII) on family behavior and the family–location ($F \times L$) interaction were analyzed. Family (except for bulb sphericity), location, and the $F \times L$ interaction had significant effects on the assessed variables, although with varying magnitudes (Table 4). Location had the greatest influence on bulb diameter, followed by bulb weight, clove number, and sphericity. Although effect sizes for the $F \times L$ interaction were small or very small (for bulb sphericity), the interaction was significant.

Table 4. Results of 2-way ANOVA for 4 dependent variables, and the effect sizes of families (F), location of trials (L), and the interaction between them on phenotype (F × L).

Dependent Variable	Variation Factor	F Value	df	Signif. (p)	ω^2	95% CI ω^2 *	Interpretation of Effect Size
Bulb Diameter							
	F	8.750	22	<0.001	0.27	(0.18, 1.00)	Small
	L	824.089	1	<0.001	0.64	(0.60, 1.00)	Medium
	F × L	9.725	22	<0.001	0.30	(0.21, 1.00)	Small
Sphericity							
	F	1.507	22	n.s.	0.02	(0.00, 1.00)	Very small
	L	69.445	1	<0.001	0.13	(0.08, 1.00)	Small
	F × L	2.256	22	<0.01	0.06	(0.00, 1.00)	Very small
Bulb Weight							
	F	6.296	22	<0.001	0.20	(0.12, 1.00)	Small
	L	429.649	1	<0.001	0.49	(0.43, 1.00)	Small
	F × L	8.750	22	<0.001	0.27	(0.18, 1.00)	Small
Number of Cloves							
	F	7.859	22	<0.001	0.25	(0.16, 1.00)	Small
	L	256.415	1	<0.001	0.36	(0.30, 1.00)	Small
	F × L	5.255	22	<0.001	0.17	(0.08, 1.00)	Small

* One-sided CIs: upper bound fixed at (1.00). n.s.: non significant. Legend: df, degree of freedom; p, probability; ω^2 , omega squared; CI, confidence interval; F, family; L, location of trial.

4. Discussion

The first step in any selection program is to determine whether phenotypic variation exists within the target population for the traits of interest. This is particularly crucial for vegetatively propagated species like garlic, where the only sources of variation are natural mutations, the accumulation of pre-existing sexual variation, and epigenetic changes [22,32]. Given the asexual propagation of garlic, high phenotypic homogeneity would be expected within populations. However, significant differences were observed for all assessed variables in both trials. Traits closely related to yield (bulb weight and diameter and clove number) were among the most influenced by family lineage, as were plant height, pseudo-stem diameter and height, and the number of active leaves. Phenological characteristics, such as the initiation and duration of sprouting and flowering, also contributed significantly to the observed variation.

These findings align with previous reports documenting substantial phenotypic and genotypic variation in natural and commercial populations, as well as in germplasm collections [11,33–36]. Consistent with the present research, statistical differences among accessions have been reported for bulb diameter and weight [17,34–40], bulb color [34,38], clove number [34,37–39], plant height [38,39], pseudo-stem height and diameter [39], number of green leaves [38,39], plants forming multiple shoots [15], and number of external sheaths [34,38], among other traits. Additionally, moderate to high heritabilities have been calculated for most of these traits [33,35], supporting the feasibility of selecting stable clones. Therefore, the results presented here lay the groundwork for obtaining new violet garlic varieties from the PGI “Morado de las Pedroñeras,” as previously suggested for various local and national selection programs [11,15,16,18,20,38].

Establishing mathematical relationships among characters can help accelerate the selection of high-yielding genotypes. In this study, high positive correlations were found between yield components and plant height, pseudo-stem diameter and height, and the number of active leaves, consistent with previous reports [33,35].

The number of cloves is arguably the most contentious trait in garlic improvement programs. Often, a higher number of cloves correlates with increased bulb weight [34,41]. However, a higher clove number per bulb also leads to a greater number of small cloves, which is undesirable. In the assessed families, a high positive correlation was observed

between the percentage of loss due to the number and weight of small cloves. The development of varieties with large, uniform cloves is a critical objective for garlic improvement, with significant economic implications for both “seed” (clove) production and overall productivity.

Paradoxically, the yield was not influenced by the percentage of loss, likely because the selection process focused on bulbs/families with fewer small cloves. This suggests that selecting varieties with a low number of small cloves is essential to minimize economic losses, particularly during seed production and the processing of peeled cloves. However, the feasibility of selecting based on low small clove numbers needs further confirmation in subsequent years, as this trait appears to be highly influenced by environmental factors [41], agricultural practices [42], and biological factors [43]. This was partially confirmed by the ANOVA results, which revealed significant differences among families, locations, and their interaction for the total number of cloves per bulb.

Undoubtedly, achieving high yields is the ultimate goal of any improvement program. Substantial variation in yield was observed among families, ranging from 8874 to 19,800 kg ha⁻¹. Establishing a threshold of 14,000 kg ha⁻¹ enabled the positive selection of 23 out of 69 families, with an average potential yield of 15,857.2 kg ha⁻¹. These are promising results, considering that historical yields for this PGI range from 9000 to 10,000 kg ha⁻¹, reaching up to 13,000 kg ha⁻¹ under optimal conditions [23,44].

For species like garlic, yield alone does not fully express its true productivity. It is important to differentiate between agronomic and marketable yield, as the latter often determines the success of commercial operations [45]. While the occurrence of multiple external bulbs/cloves does not negatively affect bulb weight and may even lead to larger bulbs, this undesirable characteristic reduces its commercial value. The early sprouting of cloves is another significant issue that limits bulb usability. In fact, farmers in Syria do not favor new genotypes prone to multiple shoot formation [15]. Although sphericity has a limited impact on agronomic yield, it has been included in some selection programs [46,47] because it facilitates post-harvest bulb handling, packaging, and acceptance by both producers and consumers.

After classifying bulbs by size categories, variations were also observed among families with similar yields. This is an aspect to consider in selection programs aiming to obtain higher proportions of large bulbs.

Phenotypic characterization enabled the clustering of families, suggesting the presence of genetic differences, which should be further confirmed through appropriate DNA-based tests. This aligns with previous reports demonstrating the influence of genetic factors on phenotype, allowing for the selection of new garlic varieties [11,16,18]. However, in some cases, genetic similarity among accessions does not correlate with observed phenotypic variations [48], suggesting other sources of variation beyond genetics.

Variable effect sizes of families on the evaluated traits were calculated for the first trial. However, due to increased selection pressure, these effects were drastically reduced in the second trial, although they were still sufficient to phenotypically separate the remaining 23 families at the end of the second trial (Figure 7). Similarly, in a 13-year experiment, a reduction in polymorphism was observed as a result of increased selection pressure, yet the remaining variability was adequate for the morphological classification of genotypes [32].

A critical step in selection programs is to determine the response of families to different environmental conditions. In the USA, it has been observed that bulb diameter and weight are influenced by location, likely due to variations in soil mineral composition [17]. For the Belltall landrace, variations in bulb size and clove heterogeneity were also observed depending on the cultivation site, with environmental factors determining its identity [41]. The evaluation of 14 garlic genotypes in six locations over two years under Ethiopian conditions revealed significant genotype–environment ($G \times E$) interactions for yield and other traits, primarily caused by varying weather conditions [49]. Similarly, in the present study, bulb size and weight, as well as clove number, were most influenced by family, location, and their interaction ($F \times L$). This emphasizes the importance of evaluating families

under the conditions in which they will be grown to provide reliable recommendations to producers.

5. Conclusions

In this initial approach to selecting high-yielding garlic varieties from the purple PGI “Morado de las Pedroñeras”, high variability was observed in the target population for most assessed traits. These traits are primarily associated with productivity, physiological, and phenological characteristics, ensuring a reasonably comprehensive characterization of each family. This allowed for the clustering of families based on their phenotypes. As $G \times E$ interactions were statistically significant for bulb weight, diameter, sphericity, and clove number, it is crucial to evaluate candidate varieties under different environments and management conditions. The next step should focus on assessing new sources of variation and characterizing phenotypes genetically and/or epigenetically. These results could serve as a reference for clonal selection from other ecotypes.

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References

1. APG. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot. J. Linn. Soc.* **2009**, *161*, 105–121. [\[CrossRef\]](#)
2. Chase, M.W.; Reveal, J.L.; Fay, M.F.A. Subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Bot. J. Linn. Soc.* **2009**, *161*, 132–136. [\[CrossRef\]](#)
3. Haston, E.; Richardson, J.E.; Stevens, P.F.; Chase, M.W.; Harris, D.J. The Linear Angiosperm Phylogeny Group (LAPG) III: A linear sequence of the families in APG III. *Bot. J. Linn. Soc.* **2009**, *161*, 128–131. [\[CrossRef\]](#)
4. Li, Q.Q.; Zhou, S.D.; He, X.J.; Yu, Y.; Zhang, Y.C.; Wei, X.Q. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. *Ann. Bot.* **2010**, *106*, 709–733. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Choi, H.J.; Giussani, L.M.; Jang, C.G.; Oh, B.U.; Cota-Sánchez, J.H. Systematics of disjunct northeastern Asian and northern north American *Allium* (Amaryllidaceae). *Botany* **2012**, *90*, 491–508. [\[CrossRef\]](#)
6. Fritsch, R.M.; Friesen, N. Evolution, domestication and taxonomy. In *Allium Crop Science: Recent Advances*; Rabinowitch, H.D., Currah, L., Eds.; CABI publishing: Wallingford, UK, 2002; pp. 5–30.
7. Kamenetsky, R.; Khassanov, F.; Rabinowitch, H.D.; Auger, J.; Kik, C. Garlic biodiversity and genetic resources. *Med. Aromat. Plant Sci. Biotechnol.* **2007**, *1*, 1–5.
8. Simon, P.W.; Jenderek, M.M. Flowering, seed production, and the genesis of garlic breeding. In *Plant Breeding Reviews*, 23; Janik, J., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2003; pp. 211–244.
9. Dhall, R.K.; Cavagnaro, P.F.; Singh, H.; Mandal, S. History, evolution and domestication of garlic: A review. *Plant Syst. Evol.* **2023**, *309*, 33. [\[CrossRef\]](#)
10. Simon, P.W. The Origins and Distribution of Garlic: How Many Garlics Are There? Available online: <https://www.ars.usda.gov/midwest-area/madison-wi/vegetable-crops-research/docs/simon-garlic-origins/> (accessed on 3 March 2020).
11. Shemesh-Mayer, E.; Kamenetsky-Goldstein, R. Traditional and Novel Approaches in Garlic (*Allium sativum* L.) Breeding. In *Advances in Plant Breeding Strategies: Vegetable Crops. Bulb, Roots and Tubers*; Al-Khairy, J.M., Mohan Jain, S., Jhonson, D.V., Eds.; Springer Nature Switzerland AG: Cham, Switzerland, 2021; Volume 8, pp. 3–49.
12. Etoh, T. Fertility of the garlic clones collected in Soviet Central Asia. *Hort. J.* **1986**, *55*, 312–319.

13. Lallemand, J.; Messian, C.M.; Briand, F.; Etoh, T. Delimitation of varietal groups in garlic (*Allium sativum* L.) by morphological, physiological and biochemical characters. In Proceedings of the I International Symposium on Edible Alliaceae, Mendoza, Argentina, 14–18 March 1994; Burba, J.L., Galmarini, C.R., Eds.; ISHS Acta Horticulturae: Leuven, Belgium, 1997; Volume 433, pp. 123–132.
14. Chovelon, V.; Souche, S.; Delecolle, B.; Etoh, T.; Messiaen, C.M.; Lot, H. Resistance to onion yellow dwarf virus and leek yellow stripe virus found in a fertile garlic clone. In *Proceeding of the II International Symposium on Edible Alliaceae, Adelaide, Australia, 10–13 November 1997*; Armstrong, J., Ed.; ISHS Acta Horticulturae: Leuven, Belgium, 2001; Volume 555, pp. 243–246.
15. Al-Safadi, B.; Arabi, M.I.E.; Ayyoubi, Z. Differences in quantitative and qualitative characteristics of local and introduced cultivars and mutated lines of garlic. *J. Veg. Crop Prod.* **2003**, *9*, 21–31. [\[CrossRef\]](#)
16. Burba, J.L.; Portela, J.A.; Lanzavechia, S. Argentine garlic I: A wide offer of clonal cultivars. In Proceedings of the IV International Symposium on Edible Alliaceae, Beijing, China, 21–26 April 2004; Guangshu, L., Ed.; ISHS Acta Horticulturae: Leuven, Belgium, 2005; Volume 688, pp. 291–296.
17. Volk, G.M.; Stern, D. Phenotypic characteristics of ten garlic cultivars grown at different North American locations. *HortScience* **2009**, *44*, 1238–1247. [\[CrossRef\]](#)
18. García-Lampasona, S.; Asprelli, P.; Burba, J.L. Genetic analysis of a garlic (*Allium sativum* L.) germplasm collection from Argentina. *Sci. Hortic.* **2012**, *138*, 183–189. [\[CrossRef\]](#)
19. Wang, H.; Wu, Y.; Liu, X.; Du, Z.; Qiu, Y.; Song, J.; Zhang, X.; Li, X. Resistance and clonal selection among *Allium sativum* L. germplasm resources to *Delia antiqua* M. and its correlation with allicin content. *Pest. Manag. Sci.* **2019**, *75*, 2830–2839. [\[CrossRef\]](#)
20. Barboza, K.; Salinas, M.C.; Acuña, C.V.; Bannoud, F.; Beretta, V.; Garcia-Lampasona, S.; Burba, J.L.; Galmarini, C.R.; Cavagnaro, P.F. Assessment of genetic diversity and population structure in a garlic (*Allium sativum* L.) germplasm collection varying in bulb content of pyruvate, phenolics, and solids. *Sci. Hortic.* **2020**, *261*, 108900. [\[CrossRef\]](#)
21. Ferreira de Carvalho, J.; de Jager, V.; van Gurp, T.P.; Wagemaker, N.C.A.M.; Verhoeven, K.J.F. Recent and dynamic transposable elements contribute to genomic divergence under asexuality. *BMC Genom.* **2016**, *17*, 884. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Sun, X.; Zhu, S.; Li, N.; Cheng, Y.; Zhao, J.; Qiao, X.; Lu, L.; Liu, S.; Wang, Y.; Liu, C.; et al. A chromosome-level genome assembly of garlic (*Allium sativum*) provides insights into genome evolution and allicin biosynthesis. *Mol. Plant* **2020**, *13*, 1328–1339. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Ajo Morado de Las Pedroñeras. Description. EC No: ES/PGI/005/0228/12.03.2002. GI 1204. The International Appellations of Origin; World Intellectual Property Organization (WIPO): Geneva, Switzerland, 2023; pp. 1–18.
24. Peña-Iglesias, A. El ajo: Virosis, fisiopatías y selección clonal y sanitaria. II: Científico-experimental. *Boletín Sanid. Vegetal. Plagas* **1988**, *14*, 493–553.
25. RAEA. Ensayo de variedades comerciales de ajo. Campaña 2005–2006. Instituto de Investigación de Formación Agraria y Pesquera. Consejería de Innovación, Ciencia y Empresa. Consejería de Agricultura y Pesca. Junta de Andalucía, 2006; pp. 1–92. Available online: https://www.juntadeandalucia.es/export/drupaljda/1337163241RAEA_Ajo_2006.pdf (accessed on 16 April 2024).
26. Bhojwani, S.S.; Cohen, D.; Fry, P.R. Production of virus-free garlic and field performance of micropropagated plants. *Sci. Hortic.* **1982**, *18*, 39–43. [\[CrossRef\]](#)
27. R Core Team. R. A language and environment for statistical computing. In *R Foundation for Statistical Computing*; R Foundation: Vienna, Austria, 2013.
28. Peña, E.A.; Slate, E.H. Global validation of linear model assumptions. *J. Am. Stat. Assoc.* **2006**, *101*, 341–354. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Tomczak, M.; Tomczak, E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends Sport. Sci.* **2014**, *1*, 19–25.
30. Sawilowsky, S.S. New effect size rules of thumb. *J. Mod. Appl. Stat. Methods* **2009**, *8*, 26. [\[CrossRef\]](#)
31. Lê, S.; Josse, J.; Huisson, F. FactoMineR: A Package for Multivariate Analysis. *J. Stat. Softw.* **2008**, *25*, 1–18. [\[CrossRef\]](#)
32. Gimenez, M.D.; García Lampasona, S. Before-after analysis of genetic and epigenetic markers in garlic: A 13-year experiment. *Sci. Hortic.* **2018**, *240*, 23–28. [\[CrossRef\]](#)
33. Figliuolo, G.; Candido, V.; Logozzo, G.; Miccolis, V.; Spagnoletti Zeuli, P.L. Genetic evaluation of cultivated garlic germplasm (*Allium sativum* L. and *A. ampeloprasum* L.). *Euphytica* **2001**, *121*, 325–334. [\[CrossRef\]](#)
34. Ragas, R.E.G.; Padron, F.K.J.R.; Ruedas, M.Y.A.D. Analysis of the morpho-anatomical traits of four major garlic (*Allium sativum* L.) cultivars in the Philippines. *Appl. Ecol. Environ. Res.* **2019**, *17*, 1143–1157. [\[CrossRef\]](#)
35. Benke, A.P.; Khar, A.; Mahajan, V.; Gupta, A.; Singh, M. Study on dispersion of genetic variation among Indian garlic ecotypes using agro morphological traits. *Indian. J. Gen. Plant Breed.* **2020**, *80*, 94–102. [\[CrossRef\]](#)
36. Khar, A.; Hirata, S.; Abdelrahman, M.; Shigyo, M.; Singh, H. Breeding and genomic approaches for climate-resilient garlic. In *Genomic Designing of Climate-Smart Vegetable Crops*; Chittaranjan, K., Ed.; Springer Nature: Cham, Switzerland, 2020; pp. 359–383.
37. Baghalian, K.; Ziai, S.A.; Naghavi, M.R.; Badi, H.N.; Khalighi, A. Evaluation of allicin content and botanical traits in Iranian garlic (*Allium sativum* L.) ecotypes. *Sci. Hortic.* **2005**, *103*, 155–166. [\[CrossRef\]](#)
38. Panthee, D.R.; Kc, R.B.; Regmi, H.N.; Subedi, P.P.; Bhattarai, S.; Dhakal, J. Diversity analysis of garlic (*Allium sativum* L.) germplasms available in Nepal based on morphological characters. *Genet. Resour. Crop Evol.* **2006**, *53*, 205–212. [\[CrossRef\]](#)
39. Jabbes, N.; Arnault, I.; Auger, J.; Dridi, B.A.M.; Hannachi, C. Agro-morphological markers and organo-sulphur compounds to assess diversity in Tunisian garlic landraces. *Sci. Hortic.* **2012**, *148*, 47–54. [\[CrossRef\]](#)

40. Tchorzewska, D.; Bocianowski, J.; Najda, A.; Dąbrowska, A.; Winiarczyk, K. Effect of environment fluctuations on biomass and allicin level in *Allium sativum* (cv. Harnas, Arkus) and *Allium ampeloprasum* var. *ampeloprasum* (GHG-L). *J. Appl. Bot. Food Qual.* **2017**, *90*, 106–114.
41. Casals, J.; Rivera, A.; Campo, S.; Aymerich, E.; Isern, H.; Fenero, D.; Garriga, A.; Palou, A.; Monfort, A.; Howad, W.; et al. Phenotypic diversity and distinctiveness of the Belltall garlic landrace. *Front. Plant Sci.* **2023**, *13*, 1004069. [[CrossRef](#)]
42. Nasir, S.; Regasa, T.; Yirgu, M. Influence of clove weight and planting depth on yield and yield components of garlic (*Allium sativum* L.). *American-Eurasian J. Agric. Environ. Sci.* **2017**, *17*, 315–319.
43. IPGRI; ECP/GR; AVRDC. *Descriptors for Allium (Allium spp.)*; International Plant Genetic Resources Institute: Rome, Italy; European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR), Asian Vegetable Research and Development Center: Taiwan, China, 2001; pp. 1–42.
44. Quintero, J.J. *El Cultivo del Ajo*; Hojas Divulgadoras, Ministerio de Agricultura: Pesca y Alimentación, España, 1984; Volume 1/84, pp. 1–16. Available online: https://www.mapa.gob.es/ministerio/pags/biblioteca/hojas/hd_1984_01.pdf (accessed on 16 April 2024).
45. Hoopes, R.W.; Plaisted, R.L. Potato. In *Principles of Cultivar Development: Crop Species*; Fehr, W.R., Ed.; Macmillan Publishing Company: New York, NY, USA, 1987; Volume 2, pp. 385–436.
46. Tascón Rodríguez, C.; Morales, D.A.; Ríos Mesa, D.J. Caracterización morfológica preliminar de un grupo de ajos de la Isla de Tenerife. In *XXXVI Seminario de Técnicos y Especialistas en Horticultura: Ibiza, 2006*; Centro de Publicaciones Agrarias: Pesqueras y Alimentarias, España, 2007; pp. 53–58.
47. Chen, S.; Chen, W.; Shen, X.; Yang, Y.; Qi, F.; Liu, Y.; Meng, H. Analysis of the genetic diversity of garlic (*Allium sativum* L.) by simple sequence repeat and inter simple sequence repeat analysis and agro-morphological traits. *Biochem. Syst. Ecol.* **2014**, *55*, 260–267. [[CrossRef](#)]
48. Papaioannou, C.; Fassou, G.; Petropoulos, S.A.; Lamari, F.N.; Bebeli, P.J.; Papasotiropoulos, V. Evaluation of the genetic diversity of Greek garlic (*Allium sativum* L.) accessions using DNA markers and association with phenotypic and chemical variation. *Agriculture* **2023**, *13*, 1408. [[CrossRef](#)]
49. Mengistu, F.G.; Mossie, G.A.; Fita, G.T. Evaluation of garlic genotypes for yield performance and stability using GGE biplot analysis and genotype by environment interaction. *Plant Genet. Resour.* **2023**, *21*, 490–496. [[CrossRef](#)]

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