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Assessment of Phenotypic Diversity among Ethiopian Coriander Accessions (*Coriandrum sativum*) at Kulumsa, Southeastern Ethiopia

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Abstract

A field experiment was conducted at the Kulumsa Agricultural Research Center during the meher season (July to November 2019 and 2020) to evaluate traits that directly influence seed yield and contribute to overall phenotypic variation and classification. Twenty-five Ethiopian coriander accessions were laid out in a simple lattice design with two replications. The combined analysis of variance revealed highly significant differences ($p \leq 0.01$) among coriander accessions in eight morphological parameters. Plant height, the number of umbellets umbel^{-1}, the number of seeds umbel^{-1}, and the seed yield plant^{-1} all had a positive and direct effect on the seed yield ha^{-1}. The first two PCs contributed 62.6% of the total phenotypic variation, and the accessions were grouped into six clusters. The highest inter-cluster distances were observed between VI and III (D2=159.21), IV and III (D2=155.84), and VI and I (D2=113.26) clusters. Crossing between accessions included in those clusters could produce highly heterotic responses and segregants. In general, this study demonstrated significant phenotypic diversity among the tested accessions and could be used in improvement programs to develop desirable coriander varieties.

Keywords: Cluster; Coriander; Direct effect; Principal component

Introduction

Ethiopia is regarded as a center of primary diversification for coriander (*Coriandrum sativum L.*) [1]. Coriander seeds are widely available and sold at high prices in every market of the country as seed spice, while the leaves and immature fruits are used as an ingredient for the preparation of "data" in the southern parts of Ethiopia. It has various local names in Ethiopia, such as Dembilal (*Amharic*), debo, shucar (*Oromiffa*), tsagha, zagda (*Tigrignya*), and tibichota (*Konsonya*), reflecting its economic importance in the country's diverse cultures.

The mature seeds and fresh green leaves are the most valuable parts of coriander from an economic standpoint [2]. Green coriander leaves are rich in vitamins and minerals and are used in vegetables and salads, while the seeds can be used as a spice and contain linalool-rich essential oils. After processing, they can be used directly for consumption or indirectly for other purposes, although the two products have distinct smells and tastes. Coriander is used in cooking and medicine [3].

Numerous scientific studies have been conducted on coriander, including investigations into its botanical and chemical characteristics, as well as its genetic, phenotypic, and biochemical diversity. However, there have been limited studies on agronomic practices and evaluation activities specific to Ethiopian coriander accessions [4].

The huge diversity of coriander in Ethiopia remains largely unexplored, incomplete, and inconclusive, posing a significant challenge. To enable breeders to use Ethiopian coriander accessions directly or for other improvement efforts, it is essential to fully understand the key features that have contributed to the overall phenotypic variation, classification, and direct effect on seed yield. It is advantageous to exploit the richness of coriander diversity and fill existing knowledge and information gaps. Thus, the study aims to identify the key characters that have a direct effect on seed yield and contribute to total phenotypic variation and classification.

Materials and Methods

Description of experimental site

The experiment was carried out during 2019 and 2020 cropping seasons at Kulumsa Agricultural Research Center (KARC)., located at 8° 00' to 8° 02'N latitude and 39° 07' to 39° 10'E longitude at an elevation of 2210 m. a.s.l. in Tiyo district, Arsi Administrative Zone of the Oromia Regional State, 167 km Southeast of Addis Ababa. KARC is located on a very gently undulating topography with a gradient of 0 to 10% slope. It has a low relief difference, with an altitude ranging from 1980 to 2230 meters [5]. The agro-climatic condition of the area is wet, with 832 mm of mean annual rainfall and a uni-modal rainfall pattern with an extended rainy season from March to September. However, the peak season runs from July to August. The mean annual maximum and minimum temperatures are 23.2 and 10 °C, respectively (KARC metrological station, unpublished data). The coldest month is December, whereas March and May are the hottest months. KARC has three major soil types: Eutric Vertisol, Vertic Luvisol, and Vertic Cambisol [6].

Experimental material and design

The two coriander cultivars registered in Ethiopia, Denkinesh and Indium 01, which have varied yield potentials and oil contents, were chosen as standard checks for this experiment (Table 1).

EIAR: Ethiopian Institute of Agricultural Research, KARC: Kulumsa Agricultural Research Center, DZARC: Debre Zeit Agricultural Research Center, TNSRC: Tepi National Spice Research Center, a: dry based. A total of 23 coriander accessions, representing the crop germplasm along with their source is given in Table 2. The

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Table 1: Description of the released coriander varieties used as standard checks for this experiment.

Variety Name	Released		Altitude (m.a.s.l)	Maturity (days)	Productivity (t ha^{-1})		Essential oil content
	Year	*Breeder			Research	Farmer	
Indium 01	2008	DZARC/EIAR	1600-2300	114-125	1.25	0.95	0.5
Denkinesh (Brazil)	2017	TNSRC and KARC/EIAR			1.8-2.6	1.5	0.6

Table 2: List of coriander accessions included in the experiment.

No	Accession	Source	No	Accession	Source	No	Accession	Source
1	229714	EBI	11	212225	EBI	21	229811	EBI
2	212115	EBI	12	240547	EBI	22	212950	EBI
3	202734	EBI	13	20662	EBI	23	202518	EBI
4	240546	EBI	14	240577	EBI	24	Denkinesh	KARC
5	212830	EBI	15	211568	EBI	25	X1	EBI
6	240574	EBI	16	240552	EBI			
7	211471	EBI	17	90309	EBI			
8	90305	EBI	18	90451	EBI			
9	329702	EBI	19	229716	EBI			
10	INDIUM 01	KARC	20	90444	EBI			

EBI: Ethiopian Biodiversity Institute, KARC: Kulumsa Agricultural Research Center

experiment was laid out in a simple lattice design, with each genotype replicated twice.

Data collection and statistical analysis

The analysis of variance (ANOVA) was performed on the data collected for the eight morphological parameters using R software [7], in accordance with the steps specified in Gomez and Gomez (1984). To determine the characteristics that directly influenced seed yield, the factors that contributed most to overall variation, and the grouping of Ethiopian coriander accessions, bivariate and multivariate analyses were carried out.

Path coefficient analysis

Simple correlation analysis was unable to adequately explain the association between yield and the other features. However, path coefficient analysis, which is a more efficient method according to Singh and Chaudhary (1979), helps breeders comprehend the direct and indirect effects of independent variables on the dependent variable. It has been used by several academics, including Vidhyavathi et al. (2005) and Goksoy & Turan (2007). The path analysis was carried out by adhering to the procedures described by Dewey & Lu (1959). The direct and indirect effects at the genotypic level for the accessions were calculated using the path coefficient method proposed by Wright (1921) and Dewey & Lu (1959), with seed yield as the dependent variable.

Rij=Pij+ Σrikpkj Where: - rij=Mutual association between the independent trait (i) and dependent trait (j) as measured by the correlation coefficient. Pij=Component of direct effects of the independent trait (i) on the dependent variable (j) as measured by the path coefficient and, Σrikpkj=Summation of components of an indirect effect of a given independent trait (i) on the given dependent trait (j) via all other independent traits (k). The residual effect is estimated by the following formula.

√(1-R^2); Where: - R2=Σpijrij, R2 is the residual factor, Pij is the direct effect of yield by ith trait, and rij is the correlation of yield with the ith trait.

Principal component analysis

To examine the relationships among the quantitative traits that were correlated with each other, principal component analysis was performed using a correlation matrix. This analysis converted the correlated variables into uncorrelated components, called principal components, using R software (R Core Team, 2020).

C1= b11(X1)+b12 +…b1p(Xp) Where: C1=the subject's score on principal component 1 (the first component extracted); b1p=the regression coefficient (or weight) for observed variable p, as used in creating principal component 1; Xp=the subject's score on observed variable.

Clustering of accessions and Euclidean distance

Based on eight quantitative variables, the genotypes were sorted into appropriate clusters using the ward linkage method. The number of clusters was calculated using the pseudo-F and pseudo-T values. To calculate the genetic distance between and within clusters, Mahalanobis' (1936) generalized distance statistics were used. The values calculated between cluster pairs were considered chi-square values and were tested for significance using p degrees of freedom, where ''p'' represents the number of traits used.

Results and Discussion

Analysis of variance

To test for homogeneity of error variance, we used the maximum F-ratio as a shortcut, as recommended by Hartley (1950), given that the experiment was conducted over two seasons. We confirmed the homogeneity of error variances, which allowed us to combine the data across both years for analysis.

Analysis of variance showed highly significant differences (P≤0.01) in plant height, umbel number per plant, number of umbellets per umbel, number of seeds per umbel and umbellets, seed yield per plant, and hectare among the different coriander accessions studied (Table 3).

***Dij* 2=Xi − Xj `S −1 Xi − Xj** Where: - Dij2 is the distance between groups i and j; Xi and Xj are the vectors of the means of the traits for groups i and j and S-1 is the pooled within groups variance-covariance matrix.

Consistent with our findings, previous studies have also reported

Table 3: Combined mean squares for quantitative traits of coriander accessions in 2019 and 2020 at Kulumsa.

Traits	Source of variation								
	Replication (DF=1)	Block (Rep) (D=8)	Year (DF=1)	Acc (DF=24)	Year*Acc (DF=)	Error (DF=41)	CV (%)	R^2	Means
PH	154.77	63.72	18455.96**	936.77**	37.25	47.84	6.14	0.95	114.95
NUPP	134.33	14.21	4155.74**	513.06**	19.09**	7.35	5.36	0.98	54.82
NULTSPU	4.87	4.2	387.22**	33.14**	0.83**	1.16	11.47	0.94	11.37
NSPU	12.11	16.97	8.44	90.9**	4.25	9.12	12.62	0.82	25.7
NSPULTS	7.67	0.41	64.87**	1.48**	0.021	0.22	7.54	0.9	6.68
SYLDPP	24.21	6.29	234.28**	79.57**	2.19	5.32	17.02	0.89	13.88
TSW	8.3	2.18	43.91**	6.62**	0.137**	0.99	8.79	0.79	12.52
SYLDHA	0.19	0.21	9.76**	5.39**	0.078	0.058	8.96	0.97	3.24

PH: Plant height at maturity (cm), NUPP: Umbels number per plant, NULTSPU: Umbellets number per umbel, NSPU: number of seeds per umbel, NSPULTS: number of seeds per umbellet, SYLDPP: Seed yield per plant (g), TSW: thousand seed weight (g), SYLDHA: Seed yield per hectare (t ha^{-1}). *, and ** significance at the 0.05, and 0.01 probability levels, respectively.

Table 4: Path coefficients of direct (main diagonal) and indirect effects of the quantitative characters of coriander studied at Kulumsa in 2019 and 2020.

	PH	NULTSPU	NSPU	SYLDPP	rg
PH	**0.564669**	0.000584	0.057531	-0.01868	0.6041**
NULTSPU	0.001694	**0.194516**	0.10736	0.12363	0.4272*
NSPU	0.097857	0.062906	**0.331974**	0.064063	0.7083**
SYLDPP	-0.06149	0.140168	0.123959	**0.171565**	0.342*

PH: plant height at maturity (cm), NULTSPU: Umbellets number per umbel, NSPU: number of seeds per umbel, SYLDPP: Seed yield per plant (g), rg: genotypic correlation coefficients * and ** significance at the 0.05 and 0.01 probability levels.

significant variability and differences in these traits among Ethiopian coriander accessions [8,9].

The observed high variation between the tested coriander accessions suggests that they could be used as a valuable source of genetic materials for further crop breeding programs. We found a significant interaction between accessions and year, indicating that the accessions exhibited a differential response across the two years for traits such as umbel number per plant, number of umbellets per umbel, and thousand seed weight. These findings underscore the importance of selecting appropriate coriander accessions for specific breeding programs, taking into account potential variation across different growing conditions and environments.

Path coefficient analysis

Coriander seed yield was positively and significantly associated with plant height, number of umbellets per umbel, number of seeds per umbel, and seed yield per plant (Table 4). The yield of seeds is influenced by a wide range of variables. However, assessing the direct and indirect contributions of each trait to seed yield requires a more sophisticated approach than simple correlation estimations, which do not fully capture the complex interrelationships between yield components and seed yield [10]. Path coefficient analysis is a more suitable approach for determining the specific effects of different traits on seed yield and can provide valuable information for selecting the most promising components to improve seed yield [11].

Table 4 displays the direct and indirect effects of eight yield-related traits on seed yield. We found that plant height, number of umbellets per umbel, number of seeds per umbel, and seed yield per plant had a positive direct effect on seed yield. Among these parameters, plant height had the highest positive degree of favorable influence on seed yield (0.56), followed by number of seeds per umbel (0.33) and number of umbellets per umbel (0.19). In addition, the highest positive indirect effect was exerted by seed yield per plant, number of seeds per umbellet, and umbel. According to Awas et al. (2015), there are both positive and negative associations between seed yield and the number of seeds per umbel, as well as between seed yield and plant height and the number of umbellets per plant. In contrast, we found that plant height and the number of umbellets in each umbel had a positive direct effect on seed yield.

Similar results have also been found in other scholars [12,13]. These results suggest that to enhance coriander seed yield, it may be desirable to focus on traits that have a higher positive association and a direct influence on seed yield.

The magnitude of the residual effect (0.33) indicates that the traits included in the path analysis explain 67% of the variation in seed yield, while 33% of the variation is due to other parameters not included in our study. Our findings suggest that primary components for improving seed yield include plant height, number of umbellets per umbel, number of seeds per umbel, and seed yield per plant, as indicated by both the genotypic correlation and path coefficient analysis. Characters other than those are secondary components that are relevant to yield improvement. By focusing on these primary components, breeders can improve coriander.

Principal component analysis

The first two principal components (PCs) within an eigenvalue greater than unity explained 62.6% of the total variation among the examined accessions (Figure 1A). With eigenvalues of 3.42 and 1.59, respectively, the overall variances of the eight variables evaluated among the 25 accessions accounted for 42.7% and 19.9% for PC1 and PC2, respectively. The number of umbels per plant, number of umbellets per umbel, seed yield per plant, and seed yield per hectare were the main contributing features to higher loading effects in the pc1 and Plant height and seed yield per hectare are the highest contributors to the total variation in the pc2 (Figure 1B). The maximum variability (variable PCA) was seen from parameters which are displayed in the right two quadrants (Figure 1C) and accessions, which are extremes from the origin (PCA-Biplot) signifies phenotypic diversity of the genotypes compared with those near to the origin (Figure 1D).

The first principal component accounted for 42.7% of the variability of the data with respect to subsequent components, indicating the independent impact of plant height, number of secondary branches, days to maturity, biomass per plant, biomass per plot, grain yield per plant, and grain yield per hectare on the total variation, as reported by Syafii et al. (2015) and Tadesse et al. (2018). The higher coefficients (eigenvectors) for some traits designated the relatedness of that

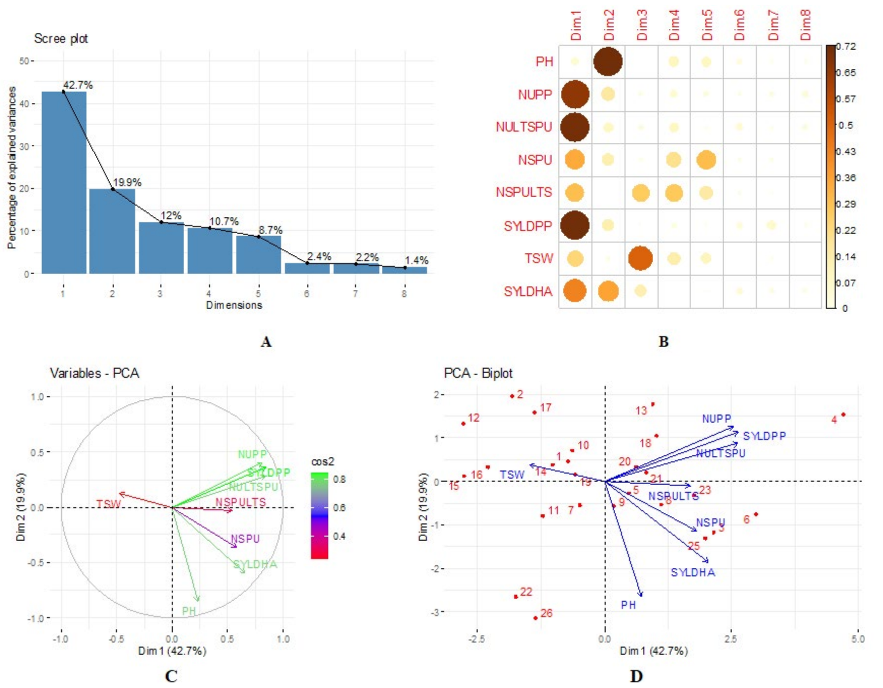

Figure 1: Total contribution, association of characters, and the accessions. (A) the scree plot; (B) the principal components of variables; (C) the total contribution of the variables, the principal component biplot of both the morphological characters and coriander accessions; (D) The numbers (1-25) in red show the accessions as indicated in Table 2. PH: plant height (cm), NUPP: number of umbels per plant, NULTSPU: umbellet number per umbel, NPU: number of seeds per umbel, NSPULTS: number of seeds per umbellets, SYLDPP: seed yield per plant (g), TSW: thousand seed weight (g), SYLDHA: seed yield per hectare (t ha^{-1}).

character to relevant PC axes [14].

Positive and negative correlations between components and variables were interpreted using positive and negative loading values. Components with the largest absolute values within the first PC exerted a stronger influence on the cluster than those with lower absolute values closer to zero [15]. The loading plot demonstrated the extent of variance and relationships among different variables, indicating the extent of association among measured variables . The loading plot of PC1 against PC2 showed the relationships among variables based on the pooled performances of the coriander accessions across the two seasons. Similar relationships were observed among the variables for the biplot analysis, where the position of each accession was plotted. While all variables had positive values for the first component, they were roughly equally divided into positive and negative values for the second component. The importance and relationship between variables within a component were determined by the magnitude and direction of factor loadings within a PC [16].

The sign of the loading indicates the direction of the relationship between the components and the variable. The greater the loading factors, the higher the contribution of the associated traits to the variance. Our analysis revealed relatively strong associations between plant height and seed yield per hectare, seed yield per plant, number of umbellets per umbel, number of umbellets per umbel, and number of seeds per umbel and umbellets. Moreover, there was a relatively strong association between the number of umbellets per umbel and seed yield per hectare. In contrast, thousand seed weight had relatively weak associations with the other parameters, except for the number of umbellets per umbel, which had a weak association with the rest of the traits [17].

Clustering analysis

The cluster analysis was used to assess the phenotypic diversity of 25

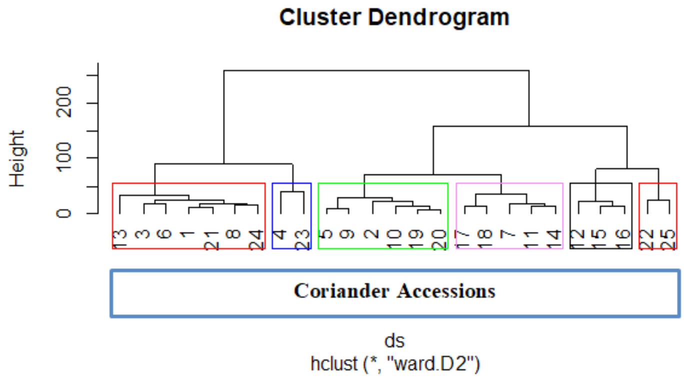

Figure 2: Dendrogram depicting genetic relationships among accessions using Ward's linkage method, and the Arabic numbers indicate the accessions under each of the six clusters, as shown in Table 2. Six clusters of coriander accessions were identified. The first, second, third, fourth, fifth, and sixth clusters, respectively, comprehend seven (1, 3, 6, 8, 13, 21, and 24), six (2, 5, 9, 10, 19, and 20), two (4 and 23), five (7, 11, 14, 17, and 18), three (12, 15, and 16), and two (22 and 25) accessions.

coriander accessions studied at Kulumsa, and the results are presented in Figure 2. The genotypes were grouped based on similarities in their morphological characters, allowing representative accessions from a particular cluster to be chosen for hybridization programs. The cluster diagram (*Dendrogram*) based on Euclidean distance, using the Ward method, categorized the accessions into six clusters. The number of accessions per cluster varied from two genotypes in clusters III and VI to seven genotypes in cluster I. Cluster II, IV, and V consisted of six, five, and three accessions, respectively. The distribution pattern of all the genotypes into various clusters showed the presence of considerable genetic divergence among the genotypes for most of the parameters studied [18-22].

Cluster one accounted for 28% of the total genotypes and was characterized by a higher number of seeds per umbel and medium plant height, which were peculiar characteristics that distinguished these genotypes from the rest. Cluster III accounted for 8% of the total genotypes and exhibited a large difference in seed yield per hectare compared to other clusters. Cluster II included 24% of the total genotypes, and seed yield and the number of seeds per umbel had limited cluster mean values. Cluster IV accounted for 20% of the total genotypes, and the number of umbels per plant, number of umbellets per umbel, number of seeds per umbel per plant, and seed yield per plant had maximum cluster mean values, with lower thousand seed weight [23,24].

The fourth cluster had the highest mean values of 1000 seed weights. Maximum plant height was one of the unusual characteristics of the accessions clustered in cluster VI, which accounted for 4% of the total genotypes. Therefore, accessions grouped in this cluster took longer to grow and were affected by the lodging and shattering of seeds. Among the clusters, clusters II and V had the lowest mean performance in yields and other important characteristics. This indicates that the opportunity to obtain high-yielding segregating individuals is limited by crossing with other clusters. In general, the mean values of each parameter in each cluster indicated that accessions could be selected for different breeding purposes. Hence, genotypes grouped under clusters III, IV, and VI were found to be high yielders, based on their mean values for seed yield per hectare and other important characteristics. These clusters could be targeted for further breeding programs to develop high-yielding coriander varieties [25,26].

Estimation of inter and intra cluster distances

Divergence analysis is often performed using Mahalanobis` D2 technique to classify different genotypes for hybridization purposes [27,28]. Genetic improvement through crossing and selection depends on the level of genetic diversity between the parents. The paired D2 value was calculated based on the pooled average of the accessions. Based on the D-square value, accessions were grouped into six (Table 5). Cluster-wise t-tests showed that there were statistically significant differences between paired clusters.

The chi-square (X2) tests for the six clusters indicate that there are statistically significant differences between all the clusters. The highest inter-cluster distance was recorded between cluster VI and cluster III (D2=159.21), followed by between cluster V and cluster III (D2=155.84), between cluster I and cluster VI (D2=113.26), between clusters III and IV (D2=112.5), and between clusters I and V (D2=106.08). These results revealed that these groups were more genetically distinct from each other (Table 6) [29,30].

Table 5: Cluster wise mean values of characters of coriander accessions, studied at 2019/2020 cropping season.

	Clusters					
Traits	**I**	**II**	**III**	**IV**	**V**	**VI**
PH	113.4	104	128.61	115.01	97.15	149.45
NUPP	51.13	62.16	65.8	85.19	40.58	39.15
NULTSPU	11.55	12.13	13.67	17.46	7.12	7.17
NSPU	27.58	21.52	27.63	31.13	22.77	24.53
NSPULTS	6.62	6.79	6.87	7.16	6.46	6.49
SYLDPP	13.17	14.78	16.11	28.79	10.44	8.47
TSW	12.2	13.02	11.81	11.44	13.59	13.19
SYLDHA	3.15	2.68	4.42	4.01	2	4.21

PH: plant height (cm), NUPP: number of umbels per plant, NULTSPU: number of umbellets per umbel, NPU: number of seeds per umbel, NSPULTS: number of seeds per umbellets, SYLDPP: seed yield per plant (g), TSW: thousand seed weight (g), SYLDHA: seed yield per hectare (t ha^{-1})

Table 6: Intra (bold diagonal) distance and inter cluster (off-diagonal) distance analysis among 25 coriander accessions in the six clusters.

Clusters						
	I	**II**	**III**	**IV**	**V**	**VI**
I	**13.23**					
II	32.86**	**11.15**				
III	50.23**	82.52**	**19.99**			
IV	63.30**	30.83**	112.50**	**13.56**		
V	106.08**	73.57**	155.84**	43.99**	**10.6**	
VI	113.26**	83.60**	159.21**	60.18**	51.09**	**11.84**

X^2 (0.05)=15.51, X^2 (0.01)=20.09

Crosses involving parents from the most divergent groups will exhibit maximum heterosis and higher variability in genetic architecture [31]. In the present study, clusters with the highest inter-cluster distances were the most dissimilar. However, the chances of obtaining segregants with higher yield yields are quite limited when one of the clusters has a very low yield level [32,33]. However, when one of the clusters has a very low yield level, the chance of getting segregants with a high yield level is quite limited [34]. Therefore, in breeding programs, it is important to consider not only the genetic distance between clusters but also their mean performance for important agronomic traits to maximize the chances of obtaining superior progenies [35]. The selection of parents for hybridization should also consider the specific advantages of each cluster and each accession within the cluster, depending on the specific goals of the breeding program [36]. Therefore, based on the present results, it can be proposed that crosses involving cluster III with clusters VI, V, and III exhibit high heterosis and may lead to segregation with respect to coriander genotypes [37,38]. The present study revealed significant genetic diversity among the accessions tested, suggesting opportunities to improve seed yield through the crossbreeding of accessions from different clusters and subsequent selection from segregating and advanced generations [39,40].

Conclusion

For the eight traits studied in this experiment, there was a wide range of phenotypic diversity among the accessions, with some features having a direct effect on seed yield, including plant height, number of umbellets per umbel, number of seeds per umbel, and seed yield per plant. The first two principal components accounted for 62.6% of the total variation, with the major contributing traits being number of umbels per plant, number of umbellets per umbel, seed yield per plant, seed yield per hectare, plant height, and seed yield per hectare. The highest inter-cluster distances were observed between clusters VI and III, followed by the distances between clusters V and III, clusters I and VI, clusters III and IV, and cluster I and cluster V, indicating greater genetic divergence among these clusters. Therefore, there is an excellent opportunity to improve coriander through direct selection and hybridization of accessions from different clusters. For further refinement, this research should be repeated across multiple locations and seasons.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be taken as a potential conflict of interest.

Author Contributions

Proposal writing, field and laboratory data collection, and research report write-up and editing.

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